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dissertation entitled

NUTRITIONAL QUALITY OF LOW-COST SUPPLEMENTARY FOODS FOR SUPPORTING GROWTH AND REHABILITATION OF UNDERNOURISHED POPULATIONS IN TANZANIA

presented by

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has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Food Science and Human Nutrition

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07/21/04
Date

DATE DUE	DATE DUE	DATE DUE
07 1906 SEP 11 2006		
JUN 25 2012		

NUTRITIONAL QUALITY OF LOW-COST SUPPLEMENTARY FOODS FOR SUPPORTING GROWTH AND REHABILITATION OF UNDERNOURISHED POPULATIONS IN TANZANIA

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

2004

ABSTRACT

NUTRITIONAL QUALITY OF LOW-COST SUPPLEMENTARY FOODS FOR SUPPORTING GROWTH AND REHABILITATION OF UNDERNOURISHED POPULATIONS IN TANZANIA

By

Theobald Conrard Edward Mosha

Severe undernutrition during childhood remains a common health problem in many parts of the world and contributes immensely to childhood morbidity and mortality. According to WHO/UNICEF, producing low-cost, ready-to-feed, nutritious foods from locally produced ingredients by using low-to-medium level technologies in local settings can considerably help mitigate child undernutriton through increased access to food. The aim of this study was to formulate, process, and evaluate the quality of processed, ready-to-feed bean-based composite supplementary foods for pre-school age children in low-income populations in Tanzania.

Supplementary foods based on cereal-bean-sardine mixtures were formulated from ingredients produced locally in Tanzania. The products were formulated to maximize the amino acid score as recommended by the FAO/WHO/UNU for pre-school age children and to provide the desired amount of energy and fat as stated by the FAO/WHO Codex Alimentarius guidelines (CAC/GL 08-1991) for supplementary foods for older infants and young children. Red beans (*Phaseolus vulgaris*), corn/maize (*Zea mays*), rice (*Oryza sativa*), sardines (*Sardinops melanosticta*) and red palm oil (*Elaeis guineensis*) were formulated into single/multi-mix diets and processed into ready-to-feed powders by extrusion, drum-processing and conventional cooking. The processed

products were evaluated for true protein digestibility, net protein retention ratio, protein digestibility-corrected amino acid scores (PDCAAS), amino acid profile, residual phytohemagglutinins, trypsin, chymotrypsin, and α-amylase inhibitors. Foods were also evaluated for potential to support normal growth and for rehabilitation of undernourished children using a weanling rat model. Furthermore, the products were evaluated for storage stability at 38^0C.

The studies showed that corn-bean-sardine, sorghum-bean-sardine and rice-bean sardine products had superior nutritional value compared to individual cereals or cereal + bean blends. The composite products had high true protein digestibility, ranging from 82 – 93%, high ratio of net protein retention ranging from 0.86 – 0.92 and PDCAAS ranging from 77 – 89%. The composite products also showed a good potential to support growth and rehabilitation of undernourished animals. Extrusion and drum-processing thoroughly cooked the foods as characterized by high gelatinization rate (95 – 100%) and low residual urease activity levels ($\leq$ 0.05 units per 100 g food). They were also effective in inactivating the phytohemagglutinins (91 – 97%) and the anti-nutritional factors - trypsin, chymotrypsin and α-amylase inhibitors. Extrusion and drum-processing also resulted in products that had high protein digestibility and PDCAAS. During storage at 38^0C, the food pH and total acids did not change significantly ($p > 0.05$). The products were shelf-stable for at least 16 weeks.

Dedicated to my parents – Mzee Conrard Edward Mosha and Mama Lodwina Conrard Mosha who instilled in me the value of education and truly believed I could attain whatever education goal I set

ACKNOWLEDGEMENTS

I gratefully acknowledge the financial support from the United States Agency for International Development through the Bean/Cowpea Collaborative Research Support Program (CRSP), East, Central and Southern Africa region, which enabled me to undertake this program.

I would like to express my profound gratitude to my major Professor, Dr. Maurice R. Bennink for his tireless guidance, encouragement and advice throughout the period of this study.

I also would like to thank my graduate committee members - Drs. Mark Uebersax, Lorraine Weatherspoon and Anne Ferguson for offering professional advice, brilliant insights and constructive criticism.

I want to thank also my lab mates and my dear friends in and outside the department for providing social and moral support and interactions that made my stay at MSU exciting and memorable.

I am also grateful to the Sokoine University of Agriculture, Tanzania for granting me the study leave to undertake this Ph.D. program.

Finally, I would like to express my profound appreciation and thanks to my wife Esther and to my children Gloria and Collins for their patience, love and encouragement. Without their constant support and prayers, I would have never been able to achieve my educational goals at Michigan State University.

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ACRONYMS

CRNPR – Corrected relative net protein ratio

FAO – Food and Agriculture Organization of the United Nations

MCH – Mother and Child Health

NCHS - National Center for Health Statistics

NPR – Net protein ratio

NPU – Net protein utilization

PAHO – Pan American Health Organization

PDCAAS – Protein Digestibility-Corrected Amino Acid Score

PER – Protein efficiency ratio

RNPR – Relative net protein ratio

UNICEF – United Nations Fund for Children

UNU – United Nations University

WHO – World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Childhood malnutrition remains a common problem in much of the developing world today. A recent comprehensive report prepared by World Health Organization using data collected from 1980 to 1992 indicates that, more than one third of children less than five years of age in developed countries have a z-score height-for-age less than 2 standard deviations (SD) with respect to international reference data [1]. WHO report shows that the proportion of children who are undernourished does not appear to have changed very much during the past 20 years [2, 3, 4, 5]. Because of the considerable global population growth during this period, the number of malnourished children continued to escalate.

Childhood growth retardation is associated with a broad range of adverse functional consequences. For example, undernourished children have impaired immune function [6] and increased rates [7, 8] and severity of enteric [9, 10] and other infections [11] than children who are better nourished. Undernourished children also have a higher risk of dying prematurely, and recent data indicate that as much as one-third to one-half of childhood mortality can be attributed to undernutriton [12, 13]. In addition to this elevated mortality risk, delayed motor development [14] and impaired cognitive function and intellectual performance are also associated with the process of childhood undernutrition [15].

Inadequate consumption of protein, energy and micronutrients accounts for most of the nutrition-related ailments among children under the age of five years in the Sub-Saharan Africa [16]. In Tanzania, various forms of nutrient deficiencies such as protein, iron, zinc and vitamin A have been associated with the high rates of morbidity and mortality among children under the age of five years [17]. Young

children at the age of 6 – 60 months are the most affected with 31% of them having low weight-for-age and weight-for-height less than –2 SD compared to their reference peers [18].

The causes of under-nutrition are multifaceted by nature. The immediate cause is inadequate intake and poor utilization of nutrients due to poor quality foods, low feeding frequency, high dietary bulk and a high incidence of diseases. Underlying factors are insufficient household food security, an inadequate child-care system and insufficient health services, while deeper-rooted basic factors relate to socio-economic and political reasons. Factors directly related to the food component have been highlighted in various reports [19 - 22] and have been reported to account for more than 75% of the deaths of infants and young children in Tanzania [20]. Also, childhood diseases such as malaria, diarrhea, acute respiratory infections, and measles aggravate the nutritional problem by impairing intake and utilization of nutrients, and destroying the body's immune system thus predisposing the body to other infections and increasing requirements for other nutrients [20].

Various reports have indicated that, most children in Tanzania start their life in sound health as they are born with weights well above standard weight of 2.5 kg [20, 22]. However, weight and height growths start to falter during weaning and/or immediately after. Protein, energy and micronutrient deficiencies become a serious problem during this period, as most weaning foods consumed do no supply adequate amounts of these nutrients. Inadequate nutrient intake is caused not only by inherent low protein, energy and other nutrient densities of the gruels fed to the children, but also due to low frequency of feeding the child per day. Since most of the weaning foods are made from starchy staples with high dietary bulk, the children's small stomachs cannot handle enough food to last for 4 – 5 hours before they are offered

another meal [22 – 24]. Under such conditions, the child would require feeding of small portions 5 to 6 times per day [19]. Various studies in rural communities in Tanzania have revealed that, average young child feeding frequency per day is three to four times [22, 24]. Mothers who are traditionally responsible for child care do not have much time to prepare gruels and feed the child several times in a day due to their other competing roles such as agricultural production on farms, collecting fuel wood, fetching water, washing and cleaning houses and caring for the other members of the family [24 - 26]. Women's heavy workloads therefore reduce the time the mothers have to care for the children.

To cope up with the problem of low feeding frequency of young children, mothers prepare a large amount of porridge that is stored and used to feed the child several times during the day [22]. These gruels, when stored in hot tropical conditions, and probably under low sanitary conditions, cause proliferation of infective microorganisms that lead to increased incidences of childhood food-borne diseases. Children at the weaning stage (4 months – 3 years) are also more vulnerable to infections due to a decline in the immunity acquired from their mother [23]. The interaction between nutrient deficiencies and diseases during and/or immediately after the weaning period has been reported as the major cause of high morbidity rates and growth faltering among weaned infants and young children [20].

The traditional weaning foods of Tanzania, just like in other parts of sub-Saharan Africa are based on the local starchy staples, usually cereals such as maize (*Zea mays*), sorghum (*Sorghum vulagare*), finger millet (*Pennisetum typhoideum*) and rice (*Oryza sativa*) or non-cereals such as cassava (*Manihot esculenta*), potatoes (*Ipomea tuberosum*), and plantains (*Musa paradisica*). Food preparations based on these staples supply between 70 - 80% of the energy, protein and micronutrient intake

of the weaned infants and young children [20]. The use of maize for preparation of weaning foods has been extensive for the past two decades, but use of sorghum and millets has increased steadily in the recent years, due to the fact that, millets and sorghum are drought resistant, they have low average market prices and their supply in markets is stable compared to maize [27]. Sorghum, millets and cassava based weaning foods, however, have been pointed out to contribute immensely to the prevalence of protein energy undernutrition among young children [27, 28]. Conversely, some common roots/tubers such as sweet potato (*Ipomoea batatas*) and yams (*Dioscoria spp*) that are rich in micronutrients such as β-carotene are not utilized as weaning foods due to among others, lack of appropriate, low-cost processing technology [27].

The traditional methods of preparing the weaning/supplementary foods at household level involve cooking the flours made from cereals or root/ tubers in boiling water to a semi-liquid porridge (*Uji*) or by mashing boiled roots or tubers such as cassava, yams or potatoes and diluting with some water to form paps. The major problems associated with these porridges and paps include:

i) Bulkiness – foods have a stiff consistency that make them difficult for children to consume enough amount to meet their protein, energy and micronutrient requirements. With the exception of gruels prepared in some pastoral communities where butter and/or lard is used, fat content in the gruels is generally very low. When diluted to an edible consistency, the foods become too watery and hence are unable to supply sufficient nutrients and calories.

ii) Monotonous - foods lack variety and hence are unappetizing. This limits children from consuming enough food. Nutrient rich foods, which can add variety to the weaning foods such as meat, fish, legumes, fruits and vegetables, are unfortunately

not readily available or are too cumbersome to incorporate into children's foods. This limits their use in children's meals.

iii) Low protein and micronutrient density - these foods naturally are low in essential nutrients such as protein, vitamin A/pro-vitamin A, iron and zinc. The dilution made in the bid to attain the edible consistency reduces the nutrient density even further. Food low in protein or with imbalanced essential amino acids (EAA) have also been shown to suppress appetite [29, 30].

iv) Antinutritional factors – most cereals and legumes contain antinutritional such as α-amylase, trypsin and chymotrypsin inhibitors, tannins and phytate that impair the digestion of proteins and starch, and absorption of minerals such as iron, zinc and calcium. Some foods also contain natural toxins such as phytohemagglutinins, gossypols, cyanogens and allergens.

v) Contamination - foods are often contaminated due to unhygienic handling, lack of proper storage facilities and poor environmental sanitation.

vi) Low digestibility and bioavailability of essential nutrients such as protein, iron and zinc.

Several strategies were suggested to improve the quality of the weaning foods including multi-mix formulations [31, 32], reduction of anti-nutritional factors and natural toxins through processing and cleaning [33 - 35] and reduction of dietary bulk through malting/germination, fermentation, and enzyme treatment [23, 35]. Adoption of these strategies at household level caused an increase in mothers' workload due to increased chores such as fetching more water for soaking, malting, fermenting, collecting more fuel wood for roasting/toasting and spending more time and energy on manual dehulling or peeling. Increasing the mothers' chores constrained further the time that the mother could avail herself for feeding and caring

for the children. Increased demand for water in the processing of these foods also increased the risk for more contamination especially in some rural communities where water is not potable and may require boiling before use. These strategies were therefore not widely adopted in many households and the problem of low feeding frequency of young children remained as mothers had even less time to feed their children due to increased workloads [24, 25].

To address the problem of undernutrition among children, WHO [36], WHO/UNICEF [37], and Dewey and Brown [38] recommends preparation of low-cost, fortified supplementary foods from locally available ingredients using suitable small-to-medium scale production technologies in community settings. According to WHO/UNICEF [37] centrally processed, fortified ready-to-eat "instant" products would provide a reliable option for families and can thus contribute immensely in mitigating the problem of protein and energy undernutrition among young children in developing countries. Fully cooked, fortified ready-to-feed foods produced from locally produced ingredients have several advantages: i) the foods contain high a nutrient density because they are enriched with high protein foods e.g. legumes and fish and fortified with multivitamins and multi-minerals. ii) they relieve mothers of some workload which avail them more time to care for the children and feed them more frequently. They also reduce the need for the mothers to collect fuel wood and cook frequently because the weaning/supplementary foods require only reconstitution with boiling water prior to feeding. iii) the food products, which are produced centrally reduce the use of firewood for frequent cooking, thus saving the environment from further degradation. iv) the products minimize incidences of food-borne infections, because they are produced under high sanitary conditions and eliminate the need for mothers to keep cooked foods for later feeding during the day.

v) the products based on cereal-bean-sardine blends expand the utilization of beans and provide more market outlets for the smallholder bean farmers and women bean grower groups. vi) they serve as a source of income for private small scale entrepreneurs, NGOs, women groups and other interested groups.

This study was designed as part of the national/international efforts to address the problem of protein and energy undernutrition in Tanzania. It was planned in accordance with the WHO/UNICEF [37] recommendation of producing low-cost, fortified ready-to-feed supplementary foods from locally available materials using appropriate small-scale technology. Specifically, this study aimed at formulating, processing and evaluating the quality of low-cost, processed maize/sorghum/rice-bean-sardine composite products suitable for feeding pre-school age children in low-income communities in Tanzania. The products were formulated to maximize the amino acid score and energy density as recommended by the FAO/WHO Codex Alimentarius Standards [39] and the Tanzanian standard (*TZS 180:198)* [40] specifications for processed cereal-based weaning/supplementary foods for infants and young children.

Hypothesis

It is hypothesized that, processed, ready-to-feed composite supplementary foods formulated from locally produced food ingredients would be as good as the standard dried skim milk and corn-soy meals in supporting normal growth and rehabilitating of undernourished children.

Specific Objectives

1. Formulate high nutrient-dense products from locally produced ingredients such as corn, beans, sorghum, rice and sardines to meet the FAO/WHO/UNU

[41] amino acid recommendations for cereal-based weaning/supplementary foods.

2. 2. Determine optimal processing conditions for ready-to-feed "instant" products from the formulated products using extrusion and/or drum-processing methods.

3. Evaluate the nutritional quality of the processed products including:

   i) amino acid profile,

   ii) protein digestibility amino acid scores,

   iii) residual levels of phytohemagglutinins and

   iv) anti-nutritional factors (trypsin, chymotrypsin, and α-amylase inhibitors),

   v) evaluate cooking doneness (starch gelatinization rate and residual urease activity).

4. Evaluate the potential of the supplementary foods to support normal growth and rehabilitation of undernourished children using an animal model.

5. Evaluate the storage stability of the products under simulated tropical conditions.

1.1 Organization of the Dissertation

This dissertation is organized into six chapters. References are presented at the end of each chapter for easy referencing by the reader. Chapter one provides the general introduction of the problem and the rationale for the study. It points out the specific objectives for the study. Chapter two provides a review of literature on the broad concepts of food products formulation, processing methods and conditions, evaluation of nutritional and sensory quality and storage stability. The chapter also covers the most contemporary recommendations on supplementary foods development based on the WHO/UNICEF [37] guidelines. Chapter three contains

article number one titled "protein quality of drum-processed cereal-bean-sardine composite supplementary foods for pre-school age children". This study evaluated the protein quality and growth/rehabilitation potential of supplementary foods developed from locally produced materials in Tanzania. This article addressed objectives number one, two and four of the study. Chapter four contains article number two titled "nutritional quality of drum-processed and extruded composite supplementary foods". The study evaluated the suitability of two food processing methods – extrusion and drum-processing for producing well-cooked, ready-to-feed supplementary foods for pre-school age children. This article-addressed objectives number three (ii and iv) of the study.

Chapter five contains article number three titled "protein digestibility corrected-amino acid scores, acceptability and storage stability of ready-to-eat supplementary foods for pre-school age children". This study evaluated the amino acid profile of the processed cereal-bean-sardine composite products and determined the protein quality using the FAO/WHO [42] approved – protein digestibility-corrected amino acid scores and evaluated the storage stability of the processed products under simulated tropical conditions. This article addressed objectives three (ii and iii), and five of the study. Chapter six provides an overall summary of the three studies and presents some conclusions. Chapter seven presents recommendations for future research.

This report is intended primarily for nutrition and health professionals and others concerned with nutrition, health, and well-being of children in developing countries. The discussion and conclusions in this report focus on the particular needs of children in low-income settings, as well as the economic and environmental constraints, that are common in those areas. The level of presentation of this report

assumes that the reader has some familiarity with the basic concepts of food science, nutrition, protein quality and food safety.

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CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 Protein Energy Undernutrition

Protein energy under-nutrition is a nutrition related disorder associated with inadequate intake and/or poor utilization of protein, energy and micronutrients [1]. Protein energy undernutrition affects mostly children between the ages of 6 – 60 months and accounts for the high rates of childhood morbidity and mortality [2]. Sub-Saharan Africa contains the largest number of undernourished children in the world, experiencing various types of nutrient deficiencies, such as protein, iron, zinc, vitamin A and iodine deficiency [1]. In Tanzania, protein energy undernutrition affects many children under the age of 5 years. According to the Tanzania Bureau of Statistics [3] and Tanzania Food and Nutrition Center (TFNC) [4], more than 44% of all the children in the country are stunted (height-for-age less than 60% of the National Center for Health Statistics (NCHS) reference values for children of the same age and sex), 30% are underweight (weight-for-age less than 60% of the NCHS reference values for children of the age and sex), 66% are anemic, 24% are vitamin A deficient and 7.2% are wasted. Infant mortality rate is 81 per 1000 live births while under-five mortality is 117 per 1000 live births [4].

2.2 Causes of Protein Energy Undernutrition

The major causes of protein energy undernutrition are summarized in the UNICEF [5] conceptual framework (Fig. 1). The immediate causes of protein energy undernutrition are inadequate intake of nutrients due to unavailability, low daily feeding frequency, high dietary bulkiness, poor utilization of nutrients associated with low

digestibility and poor bio-availability, and high incidence of diseases. Underlying factors include insufficient household food security, inadequate childcare system, insufficient health services, poor sanitary, and environmental conditions; while the deep-rooted causes include insufficient and poor allocation of human, economic and institutional resources, political and ideological superstructure that lead to civil strife, poor ecological conditions and misuse and/or misallocation of the available basic resources [5]. Factors relating protein energy undernutrition with inadequate food intake have been highlighted in various reports to account for more than 60% of deaths of children under the age of five years [6, 7].

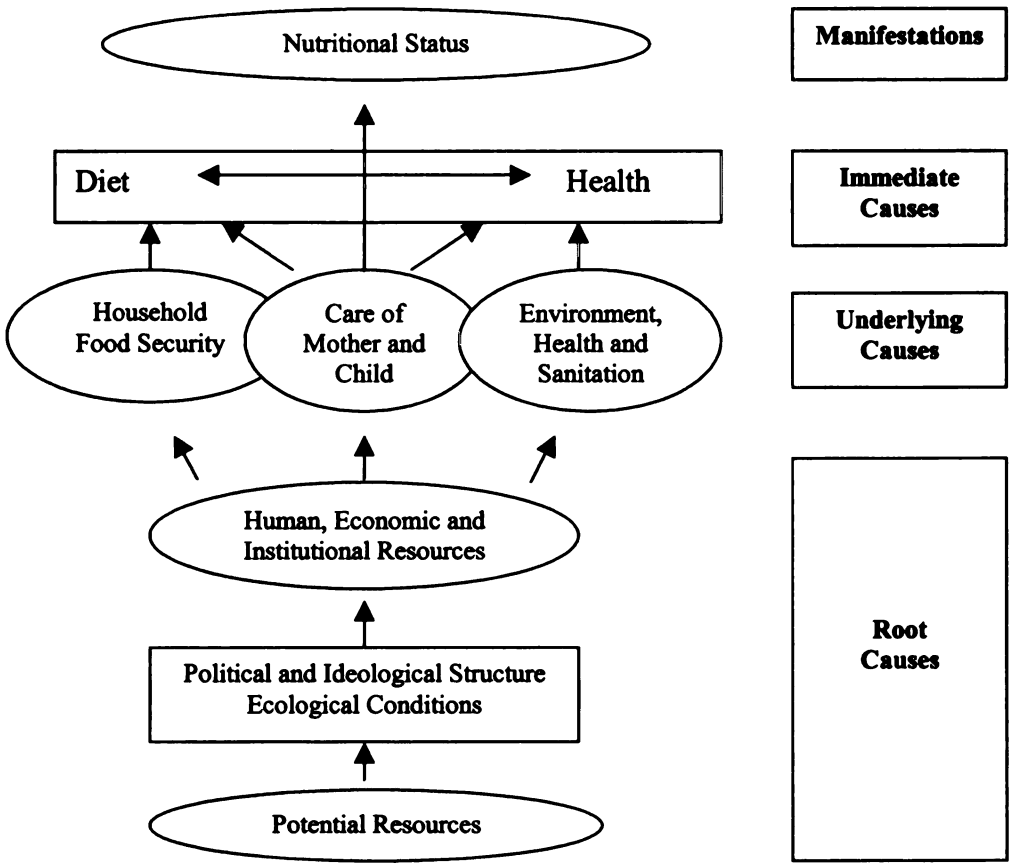

Fig. 1: UNICEF conceptual framework for determinants of nutritional status

Protein energy undernutrition has also been shown to delay motor development [8] and impaired cognitive function and school performance [9]. Inadequate nutrient intake and disease reinforce each other synergistically to cause protein energy undernutrition [10]. Protein energy undernutrition makes a child more susceptible to infection and decreases the immune defenses against invading pathogens. In turn, certain pathogens influence the nutritional status through reduced nutrient intake (anorexia), reduced nutrient absorption (malabsorption and intestinal damage), nutrient losses (diarrhea, intestinal damage) and increased nutrient requirement (increased metabolic rate, redistribution of nutrients in the body and activation of inflammatory and/or immune response). Diseases such as malaria, diarrhea, acute respiratory infections, measles and parasite infections by *Entamoeba*, *Ascaris*, *Schistosomes*, *Trichuris* and hookworm are most common and most severe in children [10]. Furthermore, about 200,000 children in Tanzania are infected with HIV/AIDS mainly through mother-to-child transmission. It is estimated that by the end of 2005 the number of children orphaned in the country will exceed 3 million and most of these will suffer from protein energy undernutrition due to lack of adequate food and parental care [11].

2.3 Weaning and Supplementation

The World Health Organization [12] recommends that infants should be exclusively breastfed for the first six months of their life. From the age of six months onwards, children enter a vulnerable period of weaning during which they make a gradual transition to eating ordinary family foods. To achieve a diet that is nutritionally adequate and safe for children at the age of 6 - 24 months is difficult, particularly from plant-based

diets. Many supplementary foods are mainly based on starchy cereals and root tubers. A diet prepared from these food sources usually result in a thick, gelatinous gruel with low nutrient density. In addition, mineral bioavailability is poor in many plant-based foods due in part to the high content of anti-nutritional factors such as tannins, lectins, trypsin, chymotrypsin and α-amylase inhibitors which impair the bioavailability of nutrients [13]. Microbial contamination of the weaning foods is common and contributes to the high rates of diarrheal diseases, growth faltering, and death. Particular problems exist in situations where the food is stored for some hours after preparation. Chemical toxins also occur in some foods e.g. afflatoxins in peanuts and cereals, cyanogenic glycosides in cassava and phytohemagglutinins in legumes [14]. The incidences of protein-energy undernutrition rise sharply during the period from 6 – 18 months of age in most countries, and the deficits acquired at this age are difficult to compensate for later in childhood [13]. Growth faltering is most evident in this age period and is often accompanied by micronutrient deficiencies and high rates of infection [15].

During the past decade, there have been considerable efforts to improve child-feeding practices. These efforts have culminated in a series of WHO recommendations and guidelines on complementary feeding of young children in developing countries [12, 16, 17]. Nutritional benefits of food supplementation on children have been widely documented in developing countries [18, 19]. A study to examine the impact of nutrition supplementation on annual growth rates in length and weight in children from birth to 7 y revealed that, during the first year of life, each 100 kcal/day supplemented resulted in ~ 9 mm in additional length/height gain and 350 g in additional weight gain. In the second year of life, the benefits decreased slightly to 5 mm in height/length gain and 250 g in

weight gain. Between 24 and 36 mo of age, food supplement had significant impact only on length but not on weight. Between 3 – 7 years of age, nutrition supplementation did not have any impact on either height or weight gain [18]. In a study to investigate the long-term effect of nutrition supplementation with high protein-energy food during early childhood on body size and composition at adolescence, Rivera et al. [19] observed that, the supplement improved significantly the dietary intake among 0 – 3 y children. Consequently, the children grew better (weight and height) compared to their peers who were receiving low protein-energy supplement. At adolescence, children who received supplements at 0 – 3 y of age were heavier, taller, and had greater fat free mass compared to their peers who received low protein-energy diet. A meta-analysis of 21 supplementary feeding programs in 14 countries for children 6 – 12 mo revealed that nutrition supplementation improved infants dietary intake by 65 - 302 kcal/day and infant growth by 0.10 – 0.50 standard deviations (SD) (mean 0.46 SD) of the z-scores. This range of improvement, in absolute terms, would reduce prevalence of protein energy undernutrition (z-scores < -2 SD) at 12 mo of age by 1 – 19% and would reduce deaths due to protein energy undernutrition by 2 – 13% [20]. Similar supplementation studies conducted in Bolivia [21], Thailand [22], Guatemala [18], Colombia [23], Jamaica [24], Congo (DR) [21], Senegal [21], and Indonesia [25] showed significant improvement in both weight and length growth of young children.

Nutritional supplementation at childhood may also have long-term effects at adult age. A 5 year longitudinal study of children who participated in a nutrition supplementation program of high protein-energy diet at age 0 – 3 y revealed that, at adolescence, children who received nutrition supplementation had a greater physical

work capacity (measured by oxygen consumption VO_{2max}) compared to their peers who did not receive the supplement [26]. The study also showed a positive relationship between amount of supplement consumed and the physical work capacity. Also, early nutrition supplementation has shown to increase biological/skeletal maturity at adolescence [27] as well as increased bone mineral content, bone width and bone mineral density [28]. Supplementation at early childhood therefore has long lasting effects in the skeletal development, bone mineralization and physical performance of the individuals.

In a study to investigate the effect of early nutrition supplementation on cognitive and intellectual development, Pollitt et al. [29] observed that supplemented children scored significantly higher at tests of knowledge, numeracy, reading and vocabulary compared to those who did not receive the supplemental feeding. Supplemented children also showed faster reaction time in information processing tasks. These studies give considerable evidence that; addressing protein energy undernutrition early in life has beneficial effects on human performance, health and survival. Continued reduction of protein energy undernutrition and mortality will thus require improved nutrition through interventions targeting marginal populations at the early stages of life [30]. The fact that malnutrition impairs human function and its effects persist to the adult life, any effort to address protein energy undernutrition at the early stages of growth is worthwhile.

2.4 Nutritional Rehabilitation

Children identified with mild and/or severe protein energy undernutrition are usually referred to hospital-based (residential) or community-based (non-residential) rehabilitation centers where they receive feeding supplements to restore their reference

body weights while continuing with medical treatment of the complications associated with protein energy undernutrition [31, 32]. The major criteria for admission into the residential and non-residential rehabilitation centers are -- weight-for-age < 60% of NCHS reference values for the age and sex or weight-for-height z-scores < -2 SD of the NCHS reference values for children of the same age and sex. The child must also meet the following criteria -- eating well, has improved mental state (e.g. can smile, respond to stimuli, interested in surroundings), can sit, crawl, stand or walk alone (depending on age), has normal body temperature (36.5 – 37.5^{0}C), has no vomiting or diarrhea, has no edema and has started gaining weight at a rate of > 5g/kg body weight/day (for at least 3 consecutive days) after the edema phase [31].

The common food used for rehabilitation of malnourished children is "energy food" made from dried skim milk and reconstituted with vegetable oil and sugar [31]. The average weight gain during rehabilitation for children on dried skim milk is 2 – 20 g/kg body weight/day [33]. A child in a residential facility is considered to be fully rehabilitated and ready for discharge when his/her weight-for-height z-scores has reached –1 SD of the median NCHS/WHO reference values for children of the age and sex. Another criterion based on weight-for-age ≥ 80% of the NCHS reference values has also been used for discharge [33]. Criteria for discharging children from non-residential care are – child attained weight-for-height z-scores of – 1 SD of the NCHS/WHO median reference values, child able to eat adequate amount of a nutritious diet that the mother can prepare at home, child gaining weight at a normal or increasing rate, all vitamin and mineral deficiencies have been corrected, all infections and other conditions have been

treated - including diarrhea, anemia, intestinal parasitic infestations, malaria, tuberculosis and otitis media, and full immunization program has been started [32].

The major limitation with nutrition rehabilitation is the failure to sustain the recovery attained at the centers once children return to their respective homes [34 - 36]. Reports by Roosmalen-Wienbenga [35] and WHO [31] showed that the proportion of relapses after discharge were 25 – 35% and in some hospitals exceeded 50%. When rehabilitated children return home from the hospital/ community-based rehabilitation centers, they no longer have access to the "energy food". Dried skim milk is imported, and is not readily available in rural areas, and whenever available it is too expensive for the low-income families to afford [31]. Return of the child to the unfavorable socioeconomic conditions and lack of access to the "energy food" after discharge make the children relapse to the same problem. Follow-up studies of some children discharged from such rehabilitation centers revealed that the rehabilitated children had higher mortality rates compared to their non-referred peers; most remained underweight and stunted until adulthood and only 30% attained satisfactory catch-up growth [37, 38]. The major reason for the relapse into protein energy undernutrition is inadequate access to well balanced nutritious foods.

In light of the foregoing, the report by the WHO [12] Global Consultation on supplementary feeding emphasized that, in order to insure sustainable healthy growth of normal and rehabilitated children, there must be adequate availability of low-cost nutritious supplementary foods prepared from locally available ingredients using small-scale production technologies in centralized or community settings. There is ample evidence showing that if supplementary foods are formulated solely from unmodified

locally produced plant ingredients, they cannot meet the recommended energy and nutrient needs for growing children [39 - 41]. Approaches to increase availability of nutritious supplementary foods at household level should be emphasized including the appropriate technologies to process them. Centrally processed and fortified foods products can also play a significant role in insuring adequate availability of low-cost supplementary foods for children.

2.5 Supplementary Foods

Supplementary foods may be processed at central strategic place where services are available or at a community level. Centrally processed foods are marketed through the existing commercial channels, with or without subsidization, or distributed through public institutions, such as health clinics or ration shops. The foods may target specific vulnerable subgroups of the population, such as low-income families or undernourished children, or they may be distributed universally to children within a particular age range. Community-based processing of foods involves special groups such as mother and child health (MCH) group, mothers' clubs, women groups or other similar community organizations. In this approach, food is processed by the group members and distributed for on-site consumption or as a take home ration [42]. In both central and community-based processing approaches, a nominal charge may be assessed on the food to cover the cost of the basic ingredients and labor (where applicable) and probably a little margin of profit for the non-service groups. Reports from various groups of the world indicate that high quality; low-cost fortified supplementary foods with good distribution system can be effective in ameliorating protein energy undernutrition and micronutrient deficiency in

target populations [43]. For examples, *Alli Alimentum* – a fortified supplementary food in Peru has shown to be effective in improving the energy intake and micronutrient status of children and has reduced significantly the prevalence of iron deficiency anemia and vitamin A deficiency among pre-school age children. Other foods that have shown considerable impact in ameliorating protein energy undernutrition among children under the age of five in developing countries include *Superamine* (Algeria), *Tsabana* (Botswana), *Ouando* (Bennin), *Musalac* (Burundi), *MICAF* (Cape Verde), *Vitafort* (Chad), *Faffa* (Ethiopia), *Weanimix* (Ghana), *Likuni Phala* (Malawi), *Actamine* (Morocco), *Bitamin* (Niger), *SOSMA* (Rwanda), *Lisha* (Tanzania), and *Nutrimix* (Togo) [42].

2.5.1 Products Formulation and Ingredients

The nutritional basis of supplementary food formulation is the principle of nutrient complementation described by Harper and Jansen [44]. Foods deficient in some specific nutrients are blended with others that are rich on the deficient nutrients to correct the deficiency. For example, cereal grains such as corn that are deficient in lysine but rich in sulfur amino acids (Cysteine and Methionine) may be blended with legumes (rich in Lysine, but low in sulfur amino acids) to correct the deficiency. A wide variety of cereals e.g. maize, millet, sorghum, rice and wheat; legumes e.g. kidney beans, cowpeas and chickpeas; oilseeds e.g. soybeans; and animal products e.g. dry milk, dry fish, beef and chicken may be combined in carefully determined proportions to achieve optimum nutrient content for promoting growth of the social group the product is formulated for. Some of the optimum nutrient levels used as quality markers for supplementary foods

include protein (≥ 14 g/100 g dry food), protein-energy (≥ 14.5%), energy (> 360 kcal/100 g dry food), net dietary protein-energy (NDpE) (6 – 10%), essential amino acid index (1.0), amino acids score (≥ 65%) and protein digestibility-corrected amino acid scores (≥ 60%) [45, 46, 47].

In the past, major emphasis was placed on food blends from cereals and legumes because the ingredients were readily available and inexpensive. Most blends from these components however, could not meet the requirements for protein, essential amino acids, energy, and micronutrients [42]. Recently, there have been deliberate efforts to make food products providing not only adequate energy and high quality protein, but also generous amounts of micronutrients such as iron, zinc and vitamin A. As a result, supplementary food mixtures are increasingly containing animal source foods such as dry milk and fish in combination with legumes and oil seeds [44]. Animal source foods provide high quality and readily digested protein and an array of readily available micronutrients that are often limiting in plant-based diets [48]. A study by Ferdinandez et al. [49] has shown that, the protein efficiency ratio of 30:70 mixtures of animal: vegetable protein was similar or higher than those of the animal foods alone. This implies that blending plant-based foods with animal source foods even in modest amounts can greatly improve the nutritional quality of the products and thus assist in mitigating multiple nutrient deficiencies [48]. The WHO [50] global strategy on infant and young children emphasizes that processed food products for infants and young children should, when sold or otherwise distributed be of high quality by meeting the compositional, nutritional, and safety standards recommended by the FAO/WHO Codex Alimentarius Commission [51] and the Codex Code of Hygienic Practice for Foods for Infants and Children [52].

2.5.2 Recommended Composition of Supplementary Foods

2.5.2.1 Energy Density

Dietary energy is important in maintaining the physiological functions of the body and in sparing the body protein [53]. For infants and young children adequate energy intake is important because they have higher rates of growth. Energy density also influences the feeding frequency required per day to attain the recommended amount of energy. For children in developing countries where the frequency of feeding per day is limited due to heavy maternal workload, energy density of their supplementary foods should be high enough to be able to supply the recommended daily energy from the few meals taken. The recommended energy density in supplementary foods is based on the WHO/UNICEF [42] recommendations for energy that were first presented by the International Dietary Energy Consultative Group in 1994. Table 1 shows the minimum dietary energy density required to attain the level of energy needed from supplementary foods in one, two, three or four meals per day by level of breast milk energy intake and age group, for well-nourished and undernourished children aged 6 – 23 months. As shown in Table 1, energy densities recommended for well-nourished, 6 – 8 mo children who are no longer receiving breast milk energy are 342 kcal/100 g (receiving only one meal per day), 171 kcal/100 g (receiving two meals per day), 114 kcal/100 g (receiving three meals per day), and 86 kcal/100 g (receiving four meals per day). For children of the same age group (6 – 8 mo) receiving low amount of breast milk energy, the recommended energy densities in their supplementary foods are 255 kcal/100 g (receiving only one meal per day), 128 kcal/100 g (receiving two meals per day), 85

kcal/100 g (receiving three meals per day), and 64 kcal/100 g (receiving four meals per day). For undernourished, 6 – 8 months old children who are no longer receiving breast milk energy, the recommended energy densities in their supplementary foods are 444-kcal/100 g (receiving only one meal per day), 222-kcal/100 g (receiving two meals per day), 148 kcal/100 g (receiving three meals per day), and 111 kcal/100 g (receiving four meals per day). For their peers of the same age (6 – 8 mo) receiving low amount of breast milk energy, the recommended energy densities in their supplementary foods are 331 kcal/100 g (receiving only one meal per day), 165 kcal/100 g (receiving two meals per day), 110 kcal/100 g (receiving three meals per day), and 83 kcal/100 g (receiving four meals per day). The variability in energy density requirement in supplementary foods for children of same age receiving and not receiving low breast milk energy is 25%. This underscores the importance of breast milk for older infants and young children. The energy densities recommended for supplementary foods for undernourished children are higher by 23% due to their low average body weights and gastric capacity. This would imply that diets for undernourished children require high energy density to meet the needs for compensatory or catch-up growth [54]. Estimation of the minimum appropriate energy density for supplementary foods is made by dividing the amount of energy required from supplementary foods by the number of meals providing these foods and by an assumed gastric capacity of 249 g/meal at 6 – 8 mo, 285 g/meal at 9 – 11 mo, and 345 g/meal at 12 – 23 mo (30 g/kg reference body weight). The energy requirements from supplementary foods are based on age-specific total energy requirements plus 2 SD (to meet the needs of almost all children) minus the amount of energy provided by the breast

Table 1: Minimum dietary energy density required to attain the level of energy needed from supplementary foods in one, two, three or four meals/day by level of breast milk energy (BME) intake and age group, for well- and under-nourished children[1]

Age Group	Minimum energy density (kcal/100g)				Gastric Capacity (g)	Total energy/ kcal required + 2 SD
Well-nourished children	One meal/d	Two meals/d	Three meals/d	Four meals/d		
6 – 8 mo						
BME - None	342	171	114	86	249	852
- Low	255	128	85	64	249	852
- Average	176	88	59	44	249	852
- High	98	49	33	24	249	852
9 – 11 mo						
BME - None	364	182	121	91	285	1038
- Low	309	155	103	77	285	1038
- Average	231	116	77	58	285	1038
- High	153	77	51	38	285	1038
12 – 23 mo						
BME - None	396	198	132	99	345	1365
- Low	370	185	123	92	345	1365
- Average	295	148	98	74	345	1365
- High	221	111	74	55	345	1365
Undernourished children						
6 – 8 mo						
BME - None	444	222	148	111	192	852
- Low	331	165	110	83	192	852
- Average	229	114	76	57	192	852
- High	127	63	42	32	192	852
9 – 11 mo						
BME - None	455	228	152	114	228	1038
- Low	386	193	129	97	228	1038
- Average	289	145	96	72	228	1038
- High	192	96	64	48	228	1038
12 – 23 mo						
BME - None	500	250	167	125	273	1365
- Low	467	234	156	117	273	1365
- Average	373	187	124	93	273	1365
- High	279	140	93	70	273	1365

[1] Assumes body weight (BW) of 8.3 kg (6 – 8 mo), 9.5 kg (9 – 11 mo), and 11.5 kg (12 – 23 mo) for well-nourished children and BW of 6.4 kg (6 – 8 mo), 7.6 kg (9 – 11 mo), and 9.1 kg (12 – 23 mo) for under-nourished children and gastric capacity of 30 g/kg BW. Energy needs from supplementary foods were set at total energy requirement + 2 SD (i.e. 25%) minus estimated energy intake from breast milk. Adopted from WHO/UNICEF [42].

milk [42, 54]. For general population feeding guidelines, data based on low-breast milk energy or no-breast milk energy are used instead of average- and high-breast milk energy because these data provide the most conservative assumptions regarding the minimum desirable energy density or number of meals [54].

Table 2 shows the minimum daily number of meals required to attain the level of energy needed from supplementary foods with mean dietary energy density of 80, 100 and 120 kcal/100 g for children with low level of breast milk energy intake, according to age group. This implies that, when most households are able to prepare meals with energy density of 120-kcal/100 g, children in all age groups would be able to consume enough energy if they receive at least three meals per day. When most household are able to prepare foods with minimum energy density of only 100 kcal/100 g, children from 6 – 11 mo of age would be able to satisfy their energy needs from supplementary foods if they receive at least three meals/day, whereas those from 12 – 23 mo of age would need to consume at least four meals per day.

Table 2: Minimum daily number of meals required to attain the level of energy needed from supplementary foods with mean dietary energy density of 80, 100 and 120 kcal/100 g for children with low level of breast milk energy intake, according to age group[1]

Energy density (kcal/100 g)	Number of meals		
	6 – 8 mo	9 – 11 mo	12 – 23 mo
80	3.7	4.1	5.0
100	2.8	3.1	3.7
120	2.2	2.5	3.0

[1] Estimated energy requirement based on WHO/UNICEF [42] data averages plus 25% (2 SD). Assumed functional gastric capacity (30 g/kg reference BW) is 249 g/meal at 6 – 8 mo, 285 g/meal at 9 – 11 mo and 345 g/meal at 12 – 23 months.

2.5.2.2 Fat Content

Lipids have traditionally been considered as part of the dietary energy supply. Lipids are the main energy source in children foods, thus essential for normal and physical activity. Fats enhance the taste and acceptability of foods, and lipid components largely determine the texture, flavor and aroma of foods. In addition, they slow gastric emptying and intestinal motility, affecting satiety [55, 56]. Dietary lipids provide essential fatty acids and facilitate the absorption of fat-soluble vitamins [57 - 59]. Lipids are also structural components in all tissues and are indispensable for cell and plasma membrane synthesis. The brain, retina and other neural tissues are particularly rich in long-chain poly-unsaturated fatty acids [60]. Some long-chain poly-unsaturated fatty acids derived from the *n*-6 and *n*-3 essential fatty acids are precursors for eicosanoid production (prostaglandins, prostacyclins, thromboxanes and leukotrienes). These autocrine and paracrine mediators are powerful regulators of numerous cell and tissue functions e.g. thrombocyte aggregation, inflammatory reactions and leukocyte functions, vasocontriction and vasodilation, blood pressure, bronchial constriction and uterine contractility. Lipids, particularly essential fatty acids and long-chain poly-unsaturated fatty acids have also been shown to affect neural development and function [56, 57, 61, 62]. Evidence indicates that specific fatty acids exert their effect by modifying the physical properties or membranes, including membrane-related transport systems, ion channels, enzymatic activity, receptor function and various signal transduction pathways. More recently the role of specific fatty acids e.g. polyunsaturated fatty acids in determining levels of gene expression for key transcription factors, peroxisome

proliferator-activated receptors and retinoic acid receptors has renewed the interest in better defining the role fatty acids in the regulation of lipid metabolism, energy partitioning, insulin sensitivity, adipocyte development and neural functions across the lifespan [56, 57, 61, 62].

The *n*-6 and *n*-3 essential fatty acids can be synthesized from their respective precursors, linoleic and α-linolenic acid, but the synthesis capacity decreases progressively in older infants and young children as intakes of breast milk decrease and supplementary foods increase. FAO/WHO [57] recommends that linoleic acid provide at least 3% of total energy in supplementary foods. Thus, if seed oils such as maize oil, or soybean oil which contain more than 50% linoleic acid are used in the supplementary foods, no more than 6% of total energy would have to be supplied by these sources to meet the nutritional requirements for linoleic acid. Conversely, if fats such as palm oil or coconut oil which contain less than 10% linoleic acid are used as major dietary sources, as much as 30% of energy would be required from these fats to meet the nutritional needs.

Although fat is essential in increasing the overall energy density of diets, the dietary fat should not be too great as to dilute excessively the density of protein and micronutrients per 100 kcal. Low dietary energy usually co-exists with low nutrient density. Dietary modifications to increase energy density of supplementary foods such as adding fats or sugar have a potential adverse affect on the protein and micronutrient density since these nutrients are computed as proportions of total energy. Some concerns have also been raised that dietary lipids affect cholesterol metabolism at an early age and excessive intake of fat may pre-dispose children to the risk of future hyperlipidemia and

cardiovascular morbidity and mortality, though little direst evidence is available to support this [56, 62 - 65]. Expert consultative bodies have given varying recommendations on fat intake for infants and young children [56]. While the American Academy of Pediatrics Committee on Nutrition [66], American Academy of Pediatrics [67] and the Canadian Society of Pediatrics [68] recommend that fats intake should not be restricted for children under two years old, European Union [56] and European Society for Pediatric Gastroenterology, Hepatology ad Nutrition Committee on Nutrition [69] recommend fat intake of $\geq$ 40 – 58.5% of energy intake for 0 – 4/6 mo old children, $\geq$32 – 58.5% of energy intake for 6 – 12 mo old children, and 30 – 35% of energy intake for children over two years old. No restriction of fat intake was recommended for children aged 12 – 24 months. Conversely, FAO/WHO [57] recommends fat intake of 50 – 60% of energy intake for children aged 0 – 12 mo and 30 – 40% of energy intake for children aged 12 – 36 mo.

In view of the current knowledge concerning low fat intakes from mixed diets in developing countries and the potential difficulties in achieving adequate micronutrient densities with high fat diets, it is recommended that fat content in supplementary foods for older infants and young children be 30% of the total energy [57]. This would imply that supplementary foods should contain at least 3.33 g of fat per 100 kcal. For under-nourished 6 – 8 mo old children whose energy requirement is 852 kcal/day they would need a total of 28.4 g of fat per day and if this energy is to be obtained from three meals/day, each meal should contain at least 9.5 g of fat. For under-nourished older infants (9 – 11 mo) whose average energy requirement is 1036 kcal/day, they need a total of 34.6 g of fat per day and if this energy is to be obtained from three meals/day, each

meal should contain at least 11.5 g of fat. For under-nourished young children (12 – 23 mo) receiving an average of three meals/day, a greater proportion of fat – 15.2 g is required per meal to meet the energy requirement of 1365 kcal/day. The recommended fat contents of the supplementary foods are based on the assumptions that the under-nourished children receive very low or no breast milk. If the children are still receiving some amount of breast milk (Table 1), the recommended fat contents per meal of supplementary food would be considerably lower.

The amount of fat recommended for supplementary foods may however pose a significant quality problem during storage of dehydrated products. Auto-oxidation of lipids occurs largely via a self-propagating free radical mechanism, in the presence of light, metal catalysts, heat or other pro-oxidants to form hydroperoxides and a range of low molecular breakdown products, some of which impart the rancid off-flavor to the food [70]. Fat content, its molecular structure (i.e. the number and location of double bonds), type of oxidant, oxygen tension, temperature, surface area, pH, storage time, physical state and presence of pro- and anti-oxidants are the major factors that influence the stability of dehydrated supplementary foods [70]. Numerous anti-oxidants, including α-tocopherol, α-tocopherol acetate, ascorbyl palmitate, butylated hydroxytoluene, butylated hydroxyanisole, di-t-butylhydroquinone, green tea catechins, and flavanoids have been studies with mixed results [71]. It is therefore pertinent that in addition to using antioxidants in supplementary foods, control of the type and amount of fat added is critical in minimizing oxidative deterioration of the dehydrated products.

2.5.2.3 Protein Density

Protein is important for growth and well being of children because it is a major source of essential amino acids that act as substrates for synthesis of new proteins and maintenance of homeostatic balance of body fluids. Amino acids can also act as gluconeogenic substrate, regulators of protein turnover, neurotransmitters or precursors of signal transducers and as signaling molecules (Table 3) [72 - 76]. Since essential amino acids are not stored in the body, amino acid metabolism can be drastically altered in response to various forms of malnutrition or trauma (e.g. sepsis, fevers or thermal burns). Alterations of amino acid profiles have been reported as a result of a deficiency of any one of the essential amino acids, a dietary imbalance of amino acid or an insufficient intake of protein. To adapt to the amino acid limitation, humans have to adjust several of their physiological functions by regulating numerous genes. Example; an amino acid limitation that occurs when animals take low protein diet causes an increase in the level of insulin-like growth factor binding protein-1 mRNA, thereby participating in down regulation of growth [77]. Likewise, deficiency of amino acids reduces the abundance of C/EBP homologous protein (also called human gene for growth arrest and DNA damage protein 153), asparagines synthetase, and C/EBPβ (ubiquitin) mRNAs [78]. The C/EBP homologous protein encodes a transcription factor that regulates cellular response to stress while asparagines synthetase is gene-encoding enzyme responsible for the biosynthesis of asparagine from aspartate and glutamate.

The mechanisms involved in the amino acid control of gene expression are poorly understood, however, both transcription and translation are regulated by amino acid

availability. In this respect, essential amino acids serve as signaling molecules that facilitate signal transduction process that leads to changes in transcription rates for specific genes. As signaling molecules, essential amino acids cause changes in phosphorylation thereby altering functions of a number of proteins that regulate the

Table 3: The involvement of amino acids in physiological and metabolic functions

System	Function	Product	Precursor
Intestine	Energy generation	Adenosine tri-phosphate	Glutamine, Aspartate Glutamine
	Proliferation	Nucleic Acids	Glutamine, Glycine, Aspartate
	Protection	Glutathione Nitric oxide Mucins	Cysteine, Glutamine, Glycine Arginine Threonine, Cysteine, Serine, Proline
Skeletal Muscle	Energy generation	Creatine	Glycine, Arginine, Methionine
	Peroxidative protection	Taurine	Cysteine
Nervous system	Transmitter synthesis	Adrenergic Serotinergic Glutaminergic Glycine Nitric oxide	Phenylalanine Tyrosine Glutamine Glycine Arginine
	Peroxidative protection	Taurine	Cysteine
Immune system	Lymphocyte proliferation	Unknown	Glutamine, Arginine, Aspartate
	Peroxidative protection	Glutathione	Cysteine, Glutamine, Glycine
Cardiovascular	Blood pressure regulation	Nitric oxide	Arginine
	Peroxidative protection	Red cell Glutathione	Cysteine, Glutamine, Glycine

initiation of mRNA translation. The major mechanism by which amino acid regulate initiation of translation in mammalian cells include phosphorylation of eukaryotic initiation factor-2α (eIF2α) which becomes a competitive inhibition of eIF2B, thereby making eIF2B inactive [75]. Since translation of essentially all mRNAs involve eIF2B, phosphorylation of eIF2α (which renders eIF2B inactive) results in a decline in the synthesis of almost all proteins. The effect of deprivation of essential amino acids on translation inhibition is summarized on Fig. 2. As can be noted, deprivation of essential amino acids inhibits the initiation phase of mRNA translation at one or more steps, including those involving eukaryotic initiation factors – elF2α, elF2β, elF4E and S6 (rp S6). A study of a DNA micro-array global analysis of gene expression in the liver of rats

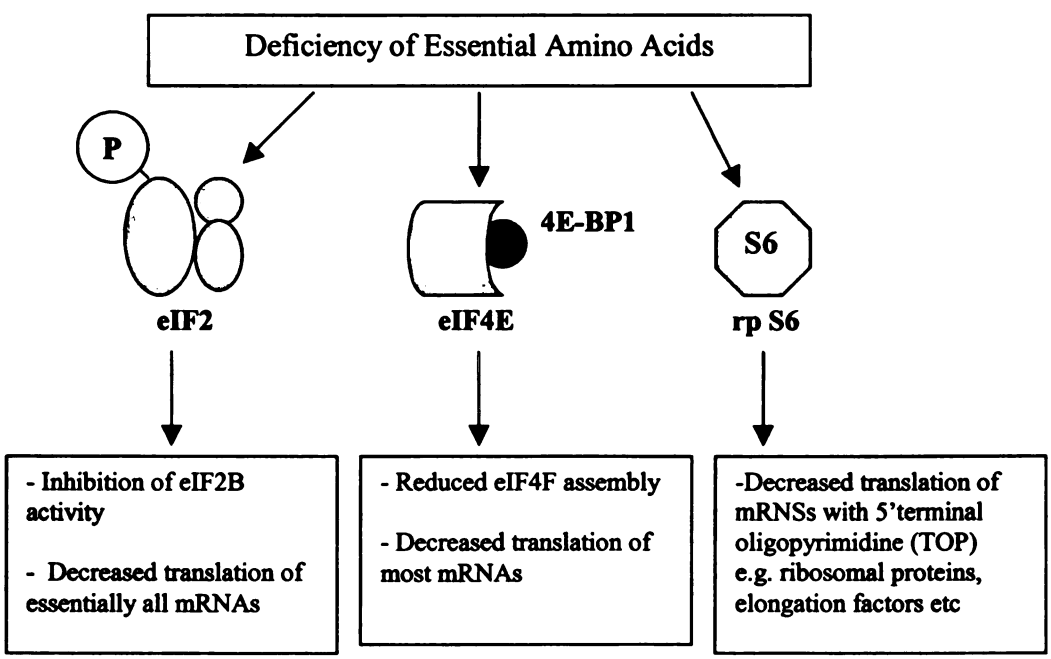

Fig. 2: Effect of deficiency of essential amino acids on gene translation.

fed inadequate and poor quality protein diet (wheat gluten) for one week revealed that, the deprivation of essential amino acids caused changes in the expression of 111 genes. When the animals were fed a protein-free diet for the same period of time, changes were observed in the expression of 281 genes.

Among the genes affected were the Id proteins (inhibitor of DNA binding), which are used in the regulation of multiple genes involved in various metabolic pathways [79]. Continuous supply of a complete complement of essential amino acids is thus a pre-requisite for maintenance of optimal rates of protein synthesis. Deficiency of even single essential amino acids causes a decrease in the synthesis of essentially all the cellular proteins through an inhibition of the initiation phase of the mRNA translation and impairs gene expressions for a number of key biochemical pathways. Deficiency of any essential amino acids also affects feeding behaviors [80]. Studies based on animal models have shown that animals rapidly reduce intake of diets deficient in any one of the essential amino acids [81- 88]. Low plasma concentration of essential amino acids is associated with decreases in the concentrations of limiting amino acids, norepinephrine and cyclic medroxyprogesterone acetate and with altered protein synthesis. These changes are sensed by the anterior pyriform cortex of the brain. In response, the brain induces release of serotonin that mediates development of taste aversion at the level of the vagus. As a result, animals modify their meal patterns by reducing both meal size and meal frequency [88]. This adaptive method has advantage to animals because it helps them to minimize tissue breakdown to maintain nitrogen balance since essential amino acids are not stored in the body [88]. The hypophagic behaviors shown in animals fed imbalanced protein or low protein diets are similar to those observed in malnourished children fed low-protein

starchy diets. Many studies in developing countries [53] have cited anorexia and apathy as sone of the major reasons for inadequate nutrient intake from traditional weaning foods.

According to WHO/UNICEF [42], the recommended protein densities in supplementary foods for children receiving low or no breast milk are 1.1-g/100 kcal (6 – 8 mo), 1.0 g/100 kcal (9 – 11 mo), and 0.9-g/100 kcal (12 – 23 mo). For children receiving an average amount of breast milk, the protein density recommended for their supplementary foods is 0.7-g/100 kcal for all ages from 6 to 23 mo. Protein in supplementary foods should be of high quality with high digestibility.

2.5.2.4 Micronutrient Density

Micronutrients are essential for the various physiological and metabolic processes of the body. The most common "problematic nutrients" (those for which there is most discrepancy between their content in supplementary foods and the amount required by infants and young children) are iron, zinc, vitamin A and to some extent, calcium. Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by the red blood cell hemoglobin, as a transport medium for electrons within cells and as an integrated part of important enzyme systems in various tissues. Several iron-containing enzymes (cytochromes) act as electron carriers within the cells by transferring energy within the cells, especially in the mitochondria. Other key functions of iron-containing enzymes (e.g. cytochrome P450) include the synthesis of steroid hormones and bile acids, detoxification of foreign substances in the liver, and signal controlling in some neurotransmitters e.g. dopamine and serotonin systems in the brain.

The role of iron in the growth of children has been shown in several studies [89, 90]. Iron supplementation of young children has shown a positive impact on both length and weight gains, although a greater improvement was noted on the weight gain [89]. In a study of Kenyan school children, iron supplementation improved appetite and doubled the rate of weight gain [90]. The major limitation with the iron supplementation studies is that the supplementation trials take only 2 – 3 months, which is long enough to treat anemia, however this period is too short too detect improvement in the linear growth [91].

Like iron, zinc is an essential component of a large number (> 300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids as well as in the metabolism of other micronutrients [92]. Zinc stabilizes the molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all forms of life [92]. Zinc also plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity [92].

Investigations of the impact of zinc supplementation on the growth of infants and young children in developing countries have shown positive impact on improving children's linear growth. Example, providing a zinc supplement of 10 mg/day to 3 – 5 year old children resulted in significant increases in length within 6 months [93]. Several studies have also demonstrated that zinc supplementation increases the rates of growth

during recovery from severe malnutrition and reduces the rates of morbidity among children [94, 95].

Vitamin A (retinol) is needed in small quantities by humans for the normal functioning of the visual system, growth and development, and maintenance of epithelial cellular integrity, immune function and reproduction. Vitamin A cellular functions are mediated through specific nuclear receptors. These receptors are activated when they bind with specific retinoic acid isomers (i.e. *all-trans* and *9-cis* retinoic acid). The activated nuclear receptors bind to DNA response elements to regulate the level of expression of those genes [96]. The synthesis of a large number of proteins, vital to the maintenance of normal physiological functions of the body, is regulated by these retinoid-activated genes [96]. Supplementation of vitamin A to infants and young children has shown a significant impact on reducing night blindness and xerophthalmia, improving weight gain and reducing the risk of morbidity and mortality [89].

Meeting the micronutrient requirements of infants and young children from supplementary foods posses the greatest nutritional challenge. In many cases, plant-based supplementary foods cannot provide adequate amount of iron, zinc, vitamin A and sometimes calcium without addition of animal products or fortifying with micronutrients. The recommended [42] densities for the "problematic" and other micronutrients in the supplementary foods for children receiving low or no breast milk are summarized in Table 4. Recommended iron concentrations for the moderately bioavailable heme-iron range from 2.3 mg/100 kcal for children aged 6 – 8 mo to 0.6 mg/100 kcal for those in age 12 – 23 mo. For children receiving average amount of breast milk, the recommended iron densities in their supplementary foods are 4.0-mg/100 kcal (6 – 8 mo), 2.4-mg/100

kcal (9 – 11 mo), and 0.8-mg/100 kcal (12 – 23 mo). For iron with low bioavailability e.g. non-heme iron, higher densities of iron per 100 kcal would be needed to meet the nutritional needs of the children (see Table 4). The recommended densities of zinc for children receiving low or no breast milk range from 1.0 mg/100 kcal for children age 6 – 8 mo to 0.6-mg/100 kcal for children aged 12 – 23 mo. For children receiving average amount of breast milk, the recommended zinc densities in the supplementary foods range from 1.6-mg/100 kcal for children aged 6 – 8 mo to 0.8-mg/100 kcal for those in age 12 – 23 mo [42].

For vitamin A, the recommended densities for children receiving low or no breast milk are – 35-μg RE (Retinol Equivalents)/100 kcal (6 – 8 mo), 32-μg RE /100 kcal (9 – 11 mo), and 31-μg RE /100 kcal (12 – 23 mo) while those receiving average amount of breast milk, the recommended vitamin A densities in the supplementary foods range from 5-μgRE /100 kcal for 6 – 8 mo old children to 17-μgRE /100 kcal for those aged 12 – 23 mo (Table 4). The recommended densities of calcium for children receiving low or no breast milk range from 91-mg/100 kcal (for 6 – 8 mo old children) to 30-mg/100 kcal (12 – 23 mo old children) while those receiving average amount of breast milk, the recommended calcium densities in the supplementary foods range from 125-mg/100 kcal (for 6 – 8 mo old children) to 26-mg/100 kcal (for 12 – 23 mo old children) (see Table 4). The recommended densities for the other micronutrients in the supplementary foods for children of various age groups are summarized in Table 4.

Based on the recommended micronutrient densities, it is practically very difficult to supply enough iron from unmodified supplementary foods based on cereal-legume mixtures to meet the required needs of infants at 6 – 11 months of age. To meet

Table 4: Desired nutrient density of supplementary foods (per 100 kcal) by level of breast milk intake[1]

Nutrient	6 - 8 months			9 - 11 months			12 - 23 months		
	Breast milk intake			Breast milk intake			Breast milk intake		
	Low	Avg	High	Low	Avg	High	Low	Avg	High
Protein (g)	1.1	0.7	0	1	0.7	0	0.9	0.7	0.2
Vitamin A (µgRE)	35	5	0	32	9	0	31	17	0
Folate (µg)	0	0	0	1	0	0	3	0	0
Niacin (mg)	0.6	1.1	4.1	0.7	0.9	1.7	0.8	0.9	1.4
Pantothenic acid (mg)	0.2	0.2	0	0.2	0.1	0	0.1	0.1	0
Riboflavin (mg)	0.06	0.07	0.14	0.04	0.04	0.04	0.05	0.05	0.06
Thiamin (mg)	0.02	0.04	0	0.03	0.04	0.04	0.05	0.05	0.06
Vitamin B_6 (mg)	0	0	0	0	0	0	0	0	0
Vitamin B_{12} (µg)	0	0	0	0.01	0	0	0.03	0	0
Vitamin C (mg)	2.2	0	0	2.1	0	0	2.3	1.1	0
Vitamin D (µg)	1.5	2.5	8.9	1	1.5	2.8	0.7	0.9	1.3
Vitamin K (µg)	2	3.3	11	1.4	2	3.5	1	1.2	1.6
Calcium (mg)	91	125	345	67	78	112	30	26	19
Chloride (mg)	74	81	123	57	53	42	73	76	84
Copper (µg)	0.04	0.04	0.14	0.03	0.02	0.04	0.04	0.04	0.04
Fluoride (µg)	0	0	0	0	0	0	0	0	0
Iodine (µg)	4	0	0	4	0	0	5	1	0
Iron (Med bioav) (mg)	2.3	4	14.7	1.6	2.4	4.7	0.6	0.8	1.2
Magnesium (mg)	13	19	56	10	13	20	8	9	11
Manganese (µg)	3	4	14	2	3	4	1	2	2
Phosphorus (mg)	75	114	360	54	70	116	25	26	29
Potassium (mg)	109	129	258	83	84	86	71	69	64
Selenium (µg)	0.6	0	0	0.7	0	0	1.1	0.5	0
Sodium (mg)	54	74	197	45	53	77	47	54	68
Zinc (mg)	1	1.6	5.2	0.7	1	1.7	0.6	0.8	1.1

[1]The categories Low, Average and High correspond to breast milk intake being mean -2SD, mean and mean +2SD. Adapted from WHO/UNICEF [42].

children's needs for iron, they need to take large amounts of iron-rich animal foods such as liver, fish and/or beef. Due to large quantities of these foods that would need to be consumed to meet the iron requirements, supplementary foods designed for children < 23 months require to be fortified with iron [42]. Likewise, it is very difficult to meet zinc requirements from unmodified supplementary foods at 6 – 8 mo of age unless there is a high intake (totaling about 50 – 70 g/day) of liver, dried fish, milk powder, eggs, chicken and/or beef [42]. At 9 – 12 mo of age, zinc needs can be met by relatively high intake (50 – 200 g/day) of zinc-rich foods. Sandstead [97] reported that zinc deficiency is more prevalent in areas of the developing world where diets are low in animal products and high in phytates e.g. where diets are based on maize, legume, whole wheat or unpolished rice. In many parts of developing countries therefore animal food sources are scarce and expensive and the practicality of such high intakes of animal foods for children is questionable. The only practical alternative to supply adequate zinc in the supplementary foods is thus to fortify with multi-minerals containing zinc [42].

Vitamin A requirements can be met from supplementary foods if good sources of vitamin A such as liver, leafy greens, milk, eggs, cheese and some orange or red fruits are incorporated. For infants aged 6 – 11 mo, the amounts of these foods required to supply adequate amount of vitamin A or pro-vitamin A are not large (generally 1 – 50 g/day) in part because breast milk is a rich source of the vitamin. In situations where the red-palm oil or other local food sources of easily absorbable precursor carotenoids are available and can be incorporated into the supplementary foods, vitamin A requirements can be met readily [42]. Likewise, calcium needs can be met if sufficient amounts of milk products or fish (including the soft bones) are consumed. About 20 – 35 g/day of milk

powder or dried ground fish would provide adequate calcium for infants 6 – 11 mo of age while 12 – 20 g/day of the same would meet the calcium needs for children at 12 – 23 mo of age [42].

2.6 Processing of Supplementary Foods

The aims of food thermo-processing are to make the food edible by developing appealing texture, color, taste and flavor; pasteurizing/sterilizing the food and making it microbiologically safe, destroying natural toxins and anti-nutrients such as phytohemagglutinins, enzyme inhibitors and cyanogenic glycosides; improving nutrient digestibility and bioavailability, improving the shelf-life and making foods more convenient for use [98]. There are a variety of thermo-processing methods that are commonly used in food preparation such as baking, roasting, frying, conventional cooking, drum processing and extrusion.

Selection of any one of these processing methods is influenced by a number of factors including the nature and type of ingredients to be processed and the product desired e.g. ready-to-eat products, availability of equipment and the cost of production. WHO/UNICEF [42] recommends the use of low-cost extrusion and drum processing for making supplementary foods in developing countries because these methods are low-cost and they give products of high nutritional and sanitary quality.

2.6.1 Extrusion

Food extrusion is a process in which a food material is forced to flow, under one or more varieties of conditions of mixing, heating and shear, through a die that is

designed to form and/or puff-dry the ingredients. The use of extruders for food cooking has been expanding rapidly in the food industry over the past few years due to their versatility, high productivity, efficiency, hygiene conditions and low operation cost [99]. Extrusion involves high-temperature-short-time processing which is able to denature enzymes, inactivate antinutritional factors such as trypsin and chymotrypsin inhibitors, and kill microorganisms present in food materials to render the food sterile. High-temperature-short-time treatment also improves digestibility of the food ingredients by gelatinizing starches or heat treating proteins while minimizing detrimental reactions such as the loss of lysine through reactions with reducing sugars in browning reactions, or reduction in the activity of enzymes [100, 101]. Other advantages of extruders include ability to provide texture and shape to food constituents e.g. ready-to-eat cereals and snacks, texturization of vegetable proteins to form fiber-like structures used as meat analogs.

There are four types of extruders commonly used in food processing. These include single screw, twin screw, dry extruders and interrupted flight screw extruders. Single screw extruders are available in a number of sizes and shapes and their screw, barrel and die configurations can usually be varied to suit a particular product's specifications [100]. Single screw extruders are classified into cold forming extruders (low-shear machines with smooth barrels, deep flights and low screw speed), high pressure forming extruders (low-shear machines with grooved barrels and compression screws), low-shear cooking extruders (moderate–shear machines with high compression screws and grooved barrels to enhance mixing but no puffing), collet extruders (high-shear machines with grooved barrels and screws with multiple shallow flights to enhance

heating and puffing) and high-shear cooking extruders (high-shear machines, with screws for changing flight depth and/or screw pitch to increase compression, pressure, heat and puffing). These extruders may be adiabatic (autogenous) - generate all the heat by friction in the barrel, isothermic – operate at constant product temperature throughout the entire length of the barrel or polytropic extruders - with provision to alternatively add or remove heat depending on the specific process [99].

Collet extruders are the most commonly used machine for the production of various low-cost puffed snacks and instant foods. They are designed to create extremely high-shear environment within the flights of the screws and grooves in the barrel. No external heat is usually applied to the barrel and the entire heat input comes from the viscous dissipation of mechanical shaft energy applied to the extruder. The short-barrel, short flighted screws and high shear of the typical collet extruder limits its operations to low-moisture ingredients that are used to produce highly expanded products e.g. instant/convenience foods.

Collet extruders vary in sizes and shapes and their screws, barrel and die configuration can be varied to suit specific product's cooking and shaping requirements. Jacketed barrels, direct steam injection, and hollow screws fitted for cooling water or steam help to regulate and control the extrusion operations. The wide range of moisture contents (12 – 40%), wide variety of feed ingredients, cooking temperatures (80 – 200^{0}C) and controllable residence times, make the collet extruders greatly versatile. Table 5 shows the major classes of cooking extruders with typical operating data. As requirements move from a low-temperature- and-shear machine to a high-temperature-and-shear extruder, several significant features change in the extruder design and

operation. The low-temperature-and-shear extruder is characterized by the use of high moisture foods, relatively deep flighted screws and low screw speed. Under these conditions, little mechanical energy input is dissipated as heat and the net energy per unit mass of extrudate is low.

Conversely, high temperature-and-shear extruders operate on relatively low moisture feeds with shallow flighted screws that result in high-shear rates and a significant amount of heat energy entering the food product from viscous dissipation. Example; a high-temperature-high-shear extrusion profile and screw configuration for wheat flakes is as follows: - material – wheat flour, moisture content – 12.17%, feed rate – 3.6 kg/h, moisture injection – 9%, screw speed – 300 rpm, barrel temperatures – 70^0C (zone 1), 100^0C (zone 2), 122^0C (zone 3), 143^0C (zone 4), and 131^0C (zone 5); product temperature – 148^0C, die pressure – 410 psi, die size – 3 mm, and torgue – 55.1%. Screw configuration: - 8D (8 twin lead (TL) screws), $7x30^0$ (7 forward kneading paddles (FKP) at an angle of 30^0), 4D (4 TL screws), $4x60^0$ (4 FKP at an angle of 60^0), $4x30^0$ (4 reverse kneading peddles (RKP) at an angle of 30^0), 2D (2 TL screws), $6x60^0$ (6 FKP at an angle of 60^0), $4x30^0$ (4 RKP at an angle of 30^0), 1D (1 single lead (SL) screws), $7x90^0$ (7 FKP at an angle of 90^0) and 2D (2 SL screws).

For a low-temperature-and-shear wheat product the screw configuration is as follows:- 8D - (8 TL screws), $7x30^0$ (7 FKP, at an angle of 30^0), 8D (8 TL screws), $3x60^0$ (3 FKP, at an angle of 60^0), $3x30^0$ (3 RKP, at an angle of 30^0), 2D (2 SL screws), $4x60^0$ (4 FKP, at an angle of 60^0), $3x30^0$ (3 RKP at an angle of 30^0), and 2D (2 SL screws). Screw diameter equals 19.0 mm (1 D) and one kneading paddle = ¼ D [102].

Table 5: Major classes of cooking extruders with typical operating data[1]

Measurement	Extruders – typical operating data				
	Low-shear Forming	High-pressure forming	Low-shear cooking	Collet	High-shear cooking
Food moisture (%)	22	25	28	11	15
Product moisture (%)	22	25	25	2	4
Max product temperature (^{0}C)	52	79	149	199	149
Screw diameter/flight height ratio (D/H)	6	4.5	7 – 15	9	7
No. of parallel screw flights, p	1 – 2	1	1	2 – 4	1 – 3
Screw speed (rpm)	30	40	60	300	450
Shear rate in screw (sec^{-1})	9.5	9.5	22 – 47	140	165
Mechanical energy input (kw-hr/kg)	0.05	0.11	0.11	0.20	0.36
Part of mechanical energy dissipated as heat (kw-hr/kg)	0.02	0.03	0.02	0.10	0.11
Heat transfer from barrel jackets, q, (kw-hr/kg)	0	0	0.05	0	-0.04
Net energy input to product (kw-hr/kg)	0.02	0.03	0.07	0.10	0.07
Product type	Pasta	RTE Cereals	Soups bases, soft moist products	Puffed "instant" products	Textured vegetable protein

[1] Adapted from Harper [100].

The screws are the central components of an extruder and their configuration greatly influences the quality of the extruded product. Large extrusion screws (length/diameter > 10) tend to have greater operating flexibility and allow greater

precision of control of the extrusion process. Usually, screws in the feed section of the barrel contain deep flights, which easily accept food materials and begin to convey them down the barrel. During conveying process, the food materials are worked into a continuous mass and the air and voids are expelled such that the flights of the screws are completely filled.

The compression or transition section of the barrel (middle) contains screws with small flight heights, which restrict the cross-sectional area of the screw for flow. This restriction or compression increases the shear rate and mechanical energy input to the food, resulting in temperature rise. In some cases, screws are configured to reverse the flow of the materials, which increases the shear, pressure, heat, and the residence time of the food. Screws in the metering section of the barrel (end) have very shallow flights or flights with decreasing pitch. In this section, the shear rate is very high, internal mixing is increased dramatically and the dissipation of mechanical energy is at a maximum. Correspondingly, the temperature rise in the metering section is very rapid and reaches maximum just before the product emerges from the die. The puffed extrudates are usually chopped or molded to desired shapes [99, 100].

2.6.2 Drum Processing

Drum processing involves use of twin-counter rotating, smooth surfaced drums which are heated by steam or electric coils. A raw or pre-cooked slurry (~ 80 – 90% water) of cereal, legume, food mixture or other suspended ingredients is spread in a thin layer on the surface of the slowly rotating drums where the food gets cooked and dried very quickly. The cooked, dry product is scraped from the drums surfaces before they are

recoated with slurry. The speed of the rotating drums is adjusted to increase/reduce the product residence time on the drums. Likewise, the amount of heat in the drums can be adjusted to regulate the cooking/drying process [44, 102]. Drum cooking is commonly used for processing cereal and tuber flours and mixes (e.g. corn, sorghum, rice, wheat and oats) and vegetable proteins (e.g. defatted soy flour). In some developing countries, drum processing has been used to produce low-cost nutritious supplementary foods for children [103]. The thin, dry, well-cooked flakes produced by drum processing are highly soluble in water. This physical-chemical property makes drum processed products particularly suitable for older infants and young children [103].

2.7 Evaluating the Quality of Processed Foods

The quality of a supplementary food is influenced by both the quantity and the relative proportions of the essential nutrients. A high quality product must contain adequate amount of all the essential nutrients in the right proportions for supporting optimal growth. It must also be - free of toxins and anti-nutrients, well cooked, organoleptically acceptable and shelf-stable for a reasonable length of time. For ready-to-eat supplementary foods, the key quality parameters include, protein digestibility-corrected amino acid score, starch gelatinization and dextrinization rate, sensory quality and acceptance, and the shelf-life.

2.7.1 Protein Quality

The importance of protein to overall diet and health has been recognized for many years; however, the quality of protein sources has been a subject of debate. Food proteins

vary greatly in their content of amino acids. Both plant and animal proteins are made up of about 20 common amino acids, but only nine are essential. The concept of essentiality of amino acids underlies the whole concept of protein quality. According to Mercer et al. [104], proteins are divided into three categories based on their absolute or relative importance in protein synthesis in vivo: 1) indispensable amino acids – histidine, isoleucinele, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; 2) conditionally indispensable amino acids – arginine, cysteine, and tyrosine; and 3) dispensable amino acids – alanine, aspartate, aspartic acid, glutamine, glutamic acid, glycine, proline, and serine. Other amino acids have also been classed as indispensable due to their specific physiological roles in promoting growth in children, preventing diseases and maintaining a positive nitrogen balance in children and adults. These amino acids are – taurine (for infants and feline), cysteine (for pre-term infants, older children with metabolic disorders and malnourished patients suffering from compromised liver function such as cirrhosis), Tyrosine (for pre-term infants, malnourished children and the elderly) and basic amino acids – arginine, citrulline, ornithine (for urea cycle) [72, 105, 106].

Protein quality is influenced by the concentration of both essential and non-essential amino acids making up the protein. The greater the proportion of the essential amino acids, the greater is the biological value or quality. Proteins that are deficient in one or more of the essential amino acids are of poor quality and this is usually reflected in their amino acid scores. Example, tryptophan and lysine are limiting in corn, lysine in wheat and other cereals, and methionine in beans and other legumes.

2.7.1.1 Effect of Digestibility on Protein Quality

The effect of digestibility on protein quality has been widely reported [107 - 109]. Digestibility involves breakdown of large molecules e.g. protein to simple constituent components e.g. amino acids that can be absorbed into the body. Digestibility of protein is facilitated by a number of hydrolytic enzymes such as trypsin, chymotrypsin, pepsin, rennin (infants), carboxypepsidase and aminopepsidase. When a protein molecule cannot be hydrolyzed into simple, absorbable amino acids, the protein is excreted undigested through the feces. Likewise, if a protein is poorly digestible (cannot be hydrolyzed completely), it limits the amino acid availability since only part of the protein is utilized by the body. The indigested protein fraction is lost through the feces. Digestibility therefore influences the proportion of the ingested protein that would ultimately be utilized by the body. When digestibility of a protein is low, so also the amount of its constituent amino acids that would be absorbed into the body. Example, egg protein has a digestibility rate of 99% and thus most of the protein (99%) is digestible and can be utilized by the body. Its biological value is therefore very high. Conversely, bean protein whose digestibility rate is 80% has only 80% of its protein digestible and utilizable while 20% of the bean protein is excreted in the feces.

The effect of digestibility on protein quality affects also availability of its constituent amino acids. For example, isolated soy protein has 26 mg of histidine per gram of protein. The digestibility rate for soy protein is 97%. This means that, out of 26 mg of histidine reaching the small intestine, only 25.2 mg would be absorbed while about 0.78 mg would be lost in feces. In the light of the foregoing, digestibility is an important determinant of protein quality. Food proteins with high digestibility rate are of high

quality while those with low digestibility are often of poor quality. Since digestibility affects the amount of amino acids that are absorbed by the body, it thus affects growth performance of the subjects fed on those proteins. Highly digestible proteins may thus induce greater growth performance relative to poorly digestible proteins. A food with high protein density may fail to elicit good growth if it is poorly digestible. Improving the protein digestibility of a food may thus improve its protein quality.

Protein digestibility is determined by measuring nitrogen in food and in feces. Apparent crude protein digestibility is given by the relationship:

Apparent crude protein digestibility (%) = {(total N consumed – total fecal N) x 100}/ total nitrogen consumed.

True crude protein digestibility (%) = [{total N consumed – (total fecal N – metabolic fecal N)} x 100]/ total nitrogen consumed.

The fecal nitrogen output on a nitrogen-free (non- or low-protein) diet provides the estimate of metabolic fecal nitrogen i.e. nitrogen not derived from undigested residue of foods such as microbial proteins, unabsorbed residue of gastrointestinal secretions and mucosal cells of the intestinal lining [107]. Since true protein digestibility measurements take into account the metabolic fecal nitrogen that is not of dietary origin, true protein digestibility of a food is always higher that the apparent digestibility. Apparent protein digestibility values increase with increase in food intakes whereas the true protein digestibility values are not independent of protein intake [107]. Therefore, true rather than apparent digestibility of protein and amino acids would provide more accurate value for comparing different diets or protein sources and for diet formulations. Digestibility of crude proteins varies widely among foods. While proteins in animal foods are often

highly digestible, proteins in plant foods e.g. cereals and grain legumes are often poorly digestible [39].

There are several factors that influence protein digestibility. These include: Structure of protein -- the protein configuration and amino acid bonding. Fibrous protein such as collagen, keratins, and elastin tend to be insoluble, tough and resistant to digestion. Conversely, globular protein such as albumins, globulins and histines tend to be fairly soluble with high solubility [108]. Example, inaccessibility of native phaseolin protein by enzymes has been attributed to its structural constraints and its compact structure [109]. Interaction of the phaseolin protein with carbohydrates to form stable glycoproteins and the stearic hindrances of the proteases created by the saccharide chain contributes to the difficulty of proteases to cleave the hydrogen and sulfur-sulfur bonds near the glycosylation sites.

Presence of natural protease inhibitors also affects protein digestibility. During processing and cooking, most of these inhibitors are inactivated; however, residual levels of active inhibitors may remain, particularly in improperly cooked foods. Presence of trypsin and chymotrypsin inhibitors in foods such as legumes has been reported to reduce protein digestibility [110, 111]. Also, phytates have been implicated in protease inhibition and therefore may affect protein digestibility. Production of adequate amounts of digestive enzymes is a major determinant of protein digestibility. Trypsin acts t break-up peptide linkages in the carboxyl sides of arginine and lysine. It also activates conversion of chymotrypsinogen and procarboxydases to active forms chymotrypsin A, B, and C and carboxydase A ad B. Chymotrypsin A, B and C all act to break peptide linkages on the carboxyl side, but chymotrypsin A also acts on linkages next to phenylalanine,

tryptophan and tyrosine, chymotrypsin B acts on linkages next to leucine, while chymotrypsin C acts on linkages next to methionine and glutamine [108]. Carboxypeptidase A breaks the peptide linkage on terminal amino acid that have a free carboxyl group, but also aromatic and aliphatic side chains while carboxypeptidase B slits off only the basic amino acids. Aminopeptidase on the other hand splits off the terminal amino acids to form free amine groups. Any antinutritional factor e.g. lectins or disease conditions e.g. Celiac's sprue that causes absolute or apparent lack of these digestive enzymes may cause significant decrease in the digestibility of food proteins and hence lowering their biological quality [108, 109].

Formation of complexes between proteins and starch, hemicellulose, polyphenolic compounds, fiber, and minerals greatly limits their digestibility. The decrease in protein digestibility could be due to physical entrapment, premature release of food particles from the stomach and/or direct inhibition of digestive enzymes [110]. Example, conjugated proteins formed when protein interacts with lipids, metals, nucleic acids and/or carbohydrates have different digestibilities depending on the nature of chemical bonding formed [108]. Polyphenols on the other hand have been shown to form insoluble complexes with protein, making the complexed proteins less susceptible to proteolysis [112]. Polyphenolic compounds have been related to reduced digestibility of chickpeas [113], dry beans [114] and the phaseolin protein of dry beans [109, 115]. Newman et al. [116] reported also that the true digestibility of chickpeas was inversely associated with their fiber content while Hughes [117] reported from a rat study that soluble dietary fiber reduced significantly the digestibility of dry beans protein and increased the fecal nitrogen.

Thermo-processing greatly improves digestibility of most protein fractions however, heat treatment may reduce digestibility of other protein fractions. Example, an in vivo study by Antunes and Sgarbieri [118] and an in vitro study by Sathe et al. [119] indicated that heat treatment improved the digestibility of both albumin and globulin fractions from beans (*Phaseolus vulgaris*). However, in vitro studies by Marguez and Lajolo [120] and Deshpande and Nielesen [121] suggested that the digestibility of albumin fraction might be reduced upon thermo-processing. Protein-protein interactions in the albumin fraction apparently lead to the formation of high-molecular weight aggregates that are not readily degraded by the digestive enzymes. Other processing conditions such as treatment with oxidizing agents (such as hydrogen peroxide which is used widely in the food industry), organic solvents, alkalis and acids may result in the formation of Maillard compounds (reaction of sugars with free NH_2 group of lysine and other amino acids), oxidized forms of sulfur amino acids (such as methionine sulfoxide, methionine sulfone and cysteic acid), in racemization of optically active amino acids and formation of cross-links in the protein (such as lysinoalanine, ornithionalanine and lanthionnine), all of which tend to make amino acids less available, and in general the protein less digestible [107].

2.7.1.2 Methods for Evaluating Protein Quality

Evaluating the adequacy of the protein component of a human diet is essential in estimating the amount of the protein that is biologically available and utilizable. Such estimates depend on the total amount of protein consumed, its quality and digestibility.

The common methods that are used to evaluate the quality of protein are *in vivo* (using e.g. rats, pigs, chicks or young children's growth) and *in vitro* [47, 122].

2.7.1.2.1 *In Vivo* Methods

Rat growth assays are widely used for predicting protein quality in foods [123]. The most common rat growth models include:

(i) *Protein efficiency ratio (PER)* = weight gain of test group/protein consumed by the test group. The most serious criticism of the PER assay is its inability to properly credit protein used for maintenance purposes. A protein source may not support growth and have a PER near zero, yet may be adequate for maintenance purposes. Due to the error introduced by not making allowance for maintenance, the PER values of proteins of differing quality are not proportional in protein quality to each other i.e. a PER of 2.0 cannot be assumed to be twice as good as a PER of 1.0. The lack of proportionality to protein quality makes the PER method unsuitable for the calculation of utilizable protein such as in protein rating (protein in a reasonable daily intake, g x PER), which is the official method of evaluating protein claims in some countries e.g. Canada [123]. The PER, which measures the ability of a protein to support growth in young rats, also severely overestimates the value of some animal proteins for human growth while underestimating the value of some vegetable proteins for that purpose [124]. The more rapid growth rate of rats, which increases their needs for certain essential amino acids compared to humans and the differences between the amino acid requirements of rats against humans are the reasons for this discrepancy.

(ii) *Net protein ratio (NPR)* = (weight gain of test group + weight loss of non-protein group)/protein consumed by test group. NPR method credits protein used for both growth

and maintenance. The method assumes, however, that the protein required to prevent weight loss of the rats fed the non-protein diet is equivalent to the amount required for maintenance purposes [123, 124]. As normally carried out, NPR values are uncorrected.

(iii) Biological Value = (retained nitrogen/absorbed nitrogen x 100).

(iv) *Net protein utilization (NPU)* = (body nitrogen of test group – body nitrogen of group fed non-protein diet) x 100/nitrogen consumed by test group. Both BV and NPU rate proteins on a scale of 1 to 100.

(v) *Relative NPR (RNPR)* = NPR of test protein expressed relative to a value of 100 for NPR of reference protein. RNPR is a corrected NPR and expressed in a scale of 1 to 100. It is a modified NPU method (based on weight) that is shorter (2 wk) than the standard PER method (4 wk). It is thus less expensive. Unlike PER values, RNPR values are proportional to each other in protein quality within reasonable limits, are more accurate, and more reproducible. RNPR is a better predictor of protein quality for human infants than the other methods based on animal growth. RNPR method has a number of weaknesses. RNPR method overestimates the quality of lysine-deficient proteins for the rats [123]. Also, in the standard PER and NPR methods, foods are tested at 10% dietary protein, and unsupplemented casein is used as the reference protein. However, diets containing 8 – 10% protein from unsupplemented casein do not meet rat growth requirements for sulfur amino acids [125]. Therefore, methionine supplemented casein was used as the reference protein in the determination of RNPR. Since it is now well known that the requirements of rats for methionine + cysteine are much higher than those of humans, then any rat growth assay (especially those which do not credit protein used for maintenance such as PER) will underestimate the protein quality for humans

especially for products limiting in sulfur amino acids such as soybean protein products, peanuts, and legumes or pulses. Modifications for higher essential amino acids requirements of rats compared with humans have been suggested for accurate prediction of protein quality for humans by rat bioassays [123].

(vi) *Corrected RNPR (CRNPR)* = RNPR x Factor (1.5). Studies comparing the essential amino acid requirements of rats and humans [126] showed that, rat requirements for sulfur amino acids was about 50% higher than that of humans, whereas the difference between the requirements for other essential amino acids were relatively small. Based on these comparisons, a factor of 1.5 was selected to correct the RNPR values (casein + methionine = 100) of food products deficient in sulfur amino acids for rat growth. The major limitation with CRNPR is that, it cannot be used to predict appropriately the protein quality for infants because of higher essential amino acids requirements of infants compared to adults and children [127]. Also, the CRNPR method cannot discriminate against proteins deficient in sulfur amino acids because only the proteins deficient in sulfur deficient amino acids are supposed to use the factor of 1.5. The use of the constant factor 1.5 regardless of the degree of deficiency in sulfur amino acids can introduce a significant variation in the total amount of sulfur amino acids in the diets. FAO [128] suggested the use of the correction factor basing on the total sulfur amino acids content of the test proteins, however, this approach made the CRNPR method very inefficient because its calculation would also require determination of total sulfur amino acids. Moreover, the accuracy of the sulfur amino acid requirements of growing rats that would be used in the CRNPR method would be a source of disagreement.

2.7.1.2.2 In Vitro Methods

In theory, the most logical approach for evaluating protein quality is to compare amino acid content of food with human amino acid requirements. Resulting amino acid scores may be calculated from the content of the single most limiting amino acid or from 2, 3, 4 or 5 key essential amino acids (which are likely to be deficient in mixed human diets) such as lysine, methionine, cysteine, threonine and tryptophan. The validity of amino acid scoring procedures has been limited by lack of standardized and reproducible procedures for determining tryptophan, methionine and cysteine. Insufficient data on digestibility of protein and bioavailability of amino acids in foods and the uncertainty about human amino acid requirements to be used for the scoring pattern have added to the invalidity [128]. In the recent years however, significant advancements have been made in standardizing amino acid methodology, in reaching a consensus about human amino acids requirements and in obtaining information about digestibility of protein and bioavailability of amino acids in a number of protein sources [129]. These developments have facilitated the use of amino acid scoring procedure that is a better predictor of protein quality for humans than *in vivo* methods [129].

The protein digestibility – corrected amino acid score method, recommended initially by the FAO/WHO Joint Expert Consultation on Protein Quality Evaluation [130] is based on scoring the amino acids against reference values. The methodology takes into account three critical parameters of protein quality: i) the food protein's essential amino acid profile ii) its digestibility and iii) its ability to supply the essential amino acids in the amount required by humans. Amino acid requirements of humans vary depending on the

ratio of new protein synthesized versus protein that is maintained. Table 6 summarizes the FAO/WHO/UNU [127] amino acid requirement patterns for various age groups, including the mean and range recommended for infants and young children.

The protein digestibility – corrected amino acid score of a food protein is established by comparing the essential amino acid profile of the food, corrected for digestibility to the FAO/WHO/UNU [127] 2 – 5 year old essential amino acid requirement pattern. The 2 – 5 year old requirement pattern is used because it is the most demanding pattern of any age group other than the infants. To determine the protein digestibility – corrected amino acid score of a food protein, the following procedure is used: (i) analyze the proximate nitrogen (N) composition of the food protein; (ii) calculate the protein content (N x 6.25 or use a specific AOAC [131]-conversion factor). For food products consisting of mixed protein sources, the factor 6.25 is used in calculating protein content and a weighed average procedure based on the proportional contribution of each protein component in the product is followed [130]; (iii) analyze for essential amino acid profile; (iv) determine the amino acid score (uncorrected) using the relationship: Amino acid score (uncorrected) = (mg of essential amino acid in 1 g of test protein)/mg of essential amino acids in 1 g of reference protein. Reference protein essential amino acids profile = FAO/WHO/UNU [127] requirement for 2 – 5 year old children; (v) analyze for the protein true digestibility using animal assay; (vi) calculate the protein digestibility – corrected amino acid score from the relationship: Protein digestibility – corrected amino acid score = Lowest uncorrected essential amino acid score x protein digestibility.

When calculating protein digestibility – corrected amino acid score of a food protein, any score above 100% is rounded to 100% for further calculations. There is no nutritional advantage to consuming proteins with scores greater than 100% since the body does not utilize the excess amino acids. All proteins with a protein digestibility – corrected amino acid score of 100% complete proteins that meet the essential amino acid requirements of humans. Food proteins with a protein digestibility – corrected amino acid score of lower than 100% are of low quality and may fall short of meeting the 2 – 5 year old amino acid requirements for growth. Since the protein digestibility – corrected amino acid score is based on human amino acids requirements and thus is inherently more appropriate for evaluating protein quality of foods intended for human consumptions, FDA [132] adopted it as an official method of evaluating protein quality to replace the PER. Protein digestibility – corrected amino acid score is now being used for nutritional labeling purposes for all food products intended for young children above one year old and adults.

Table 6: Essential amino acid requirement patterns for people of various age groups[1]

	Pattern of requirement in mg/g crude protein				
Essential amino acid	Infants (mean)	Infants (range)[2]	2 – 5 y	10 – 12 y	Adults
Histidine	26	18 – 36	19	19	16
Isoleucine	46	41 – 53	28	28	13
Leucine	93	83 – 107	66	44	19
Lysine	66	53 – 76	58	44	16
Methionine + Cystine	42	29 – 60	25	22	17
Phenylalanine + Tyrosine	72	63 – 118	63	22	19
Threonine	43	40 – 45	34	28	9
Tryptophan	17	16 – 17	11	9	5
Valine	55	44 – 77	35	25	13

[1] Adapted from FAO/WHO/UNU [127]

[2] Amino acid composition of human milk

2.7.2. PDCAAS and Protein Requirement

For children and adults, protein is required for two major roles -- first, for maintenance of the normal physiological functions e.g. production of enzymes, antibodies and hormones, and second, for normal growth e.g. production of new cells and tissues. Protein requirement for maintenance for children aged 0 – 1 year is estimated at 0.75-g/kg-body weight/day. Maintenance requirement decreases gradually with age, reaching 0.64 g/kg/day at age 18 years. Likewise, the protein component that is required to support growth is highest in infants and decreases gradually with age. According to FAO/WHO/UNU [127], the safe protein intakes for people of various age groups are 1.47 g/kg/day (0 – 5 mo), 1.15 g/kg/day (7 – 12 mo), 1.09 g/kg/day (3 – 4 years), 0.99 g/kg/day (9 – 10 years), 0.94 g/kg/day (girls 13 – 14 years), 0.97 g/kg/day (boys 13 – 14 years), 0.75 g/kg/day (young adults, 19+ years) ad 0.75 g/kg/day for elderly women. These protein requirements however have been revised extensively by the International Dietary Energy Consultative Group [133], Millward [134] and Young and Bergonha [135] and lower levels of safe protein intakes have been suggested.

Meeting these protein requirements is strongly influenced by the quantity consumed and the ability of the protein to provide sufficient amounts of the essential amino acids after digestion. When the quantity of protein ingested is adequate for the age group and the PDCAAS is 100%, the protein would provide adequate amount of essential and non-essential amino acids needed for maintenance and growth. Nevertheless, as the PDCAAS value decreases, the ability of the protein to meet the needs for maintenance and growth decreases accordingly. For example, when the quantity of a dietary protein is adequate but the PDCAAS value is low e.g. 80%, the protein would supply essential

amino acids that would be enough to support the maintenance component and probably a limited growth. When the PDCAAS value decreases further, e.g. to 65%, the amount of essential amino acids supplied from the protein would be adequate to support the maintenance only and no growth will occur. If the food protein has a PDCAAS value lower than 65%, the body would not be able to obtain from the diet the necessary essential amino acids to maintain it. As a result, the body will start breaking down body cells and tissues to obtain the essential amino acids needed for maintenance. To avoid tissue breakdown therefore, the protein supplied in diet must have a PDCAAS value of at least 65%. FAO/WHO/UNU [127] recommends the PDCAAS value of 65% as the minimum cut-off score for growing children.

It may not be possible to provide adequate amount of essential amino acids by increasing intake of low quality protein. This is because increased intake of low quality protein would lower the plasma concentration of essential amino acids, which in turn would suppress appetite [83 – 88]. Also, intake of large amount of incomplete protein would provide excess non-essential amino acids. The excess non-essential amino acids cannot be utilized to synthesize more proteins (because they do not alter the rate of protein catabolism) and only a limited amount of it can be used to produce energy. Since the non-essential amino acids cannot be stored in the body, any excess amount would be catabolized into urea through the urea cycle (Fig. 3) [136]. In this pathway, both essential and non- essential amino acids are indiscriminately deaminated to form urea, causing more deficiency of the essential amino acids. In light of the foregoing, a child who is receiving a low or poor quality protein may not compensate for the deficiency of essential

amino acids by eating larger amount of the food. Doing so would put the child into a more negative nitrogen balance.

2.7.3 Starch Gelatinization and Dextrinization

Starch is an important dietary source of energy in developing countries. Any changes in starch during extrusion or drum processing may have important nutritional effects. Gelatinization is the breaking of hydrogen bonds among and within starch molecules that opens the granules to hydration and enzymic hydrolysis.

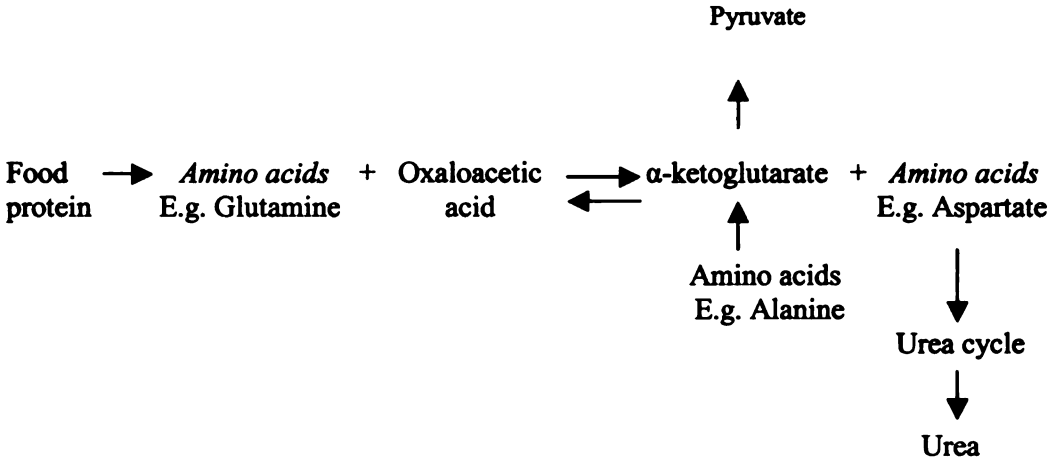

Fig. 3: Metabolic fate of excess amino acids.

The crystalline structure of starch must be disrupted for complete enzymatic hydrolysis of the starch to occur, especially when eaten. Starch gelatinization starts at 60 – 70^{0}C and is typically accomplished at 90 – 100^{0}C [137]. Starch gelatinization is the major effect that occurs on starch during drum and/or extrusion cooking and is often used as a measure of the extent of cooking (cooking doneness) for the ready-to-eat "instant" foods [138]. The rate of gelatinization is greatly influenced by the extrusion/ processing conditions, and the processing conditions must therefore be carefully controlled to achieve a complete

gelatinization. Gelatinization rate is also affected by the content of other food components including lipids, proteins, sugars, salt and dietary fiber [137]. Several studies have investigated the effects of extrusion cooking on gelatinization rate of cereal starches. According to Mercier and Feillet [139], extrusion cooking resulted in gelatinization rates ranging from 80 to 100%. In modified versions of low-cost extruders however, results on gelatinization rates have been inconsistent. In a model of low-cost extruder developed for the developing countries, Harper and Jansen [44] reported starch gelatinization rates above 90% while a similar model of a "very low-cost extruder" showed a wide starch gelatinization rates ranging from 52 to 98% [140]. Starch gelatinization rates above 80% have been associated with good sensory and eating qualities and were thus accepted as ideal for processed ready-to-eat human foods [137]. Starch digestibility on the other hand is largely dependent upon gelatinization rate. Incomplete gelatinization leads to low digestibility. *In vitro* starch gelatinization studies have shown that starch in extruded foods was highly digestible compared to starch in foods cooked by other methods [137, 140 - 142]. High starch digestibility is an essential quality parameter for foods designed for older infants and young children.

Apart from gelatinization, extrusion cooking also induces dextrinization of starch in foods. The high temperature, pressure and shear generated during extrusion process cause degradation of the native starches in the food to form dextrins [137]. Starch dextrinization in supplementary foods is desired because it improves the taste and digestibility of the food and reduces bulkiness during meal preparation allowing more dry matter to be consumed per meal [137].

2.7.3 Anti-nutrients and Toxins in Processed Foods

In many developing countries, plant foods such as cereals, root and stem tubers and legumes are used as a basis for developing supplementary foods. These plant-based foods contain a wide range of antinutritional factors and naturally occurring toxins. Antinutritional factors are food components that interfere with the digestion, absorption or with some other aspect of metabolism of a nutrient or nutrients contained in that food or other foods [42]. Of the antinutritional factors, enzyme inhibitors, phytates, lectins, polyphenols and allergens are the most common. Some antinutritional and toxic factors are specific to just a few plant species such as cyanogens in cassava and some cruciferous vegetables, gossypol in cottonseed and favism in fava beans [143].

The nutritional significance of antinutritional factors has been widely reviewed [42, 143, 144]. Protease inhibitors block the activity of pancreatic proteolytic enzymes particularly trypsin and chymotrypsin, leading to enlargement of the pancreas (hypertrophy) and hyperplasia. There is also an increase in the secretion of digestive enzymes including trypsin, chymotrypsin and elastase, the action modulated by cholecytokinin through a monitor peptide [143]. This effect suggests that, the growth depression caused by the trypsin/chymotrypsin inhibitors is a result of poor digestibility of proteins and endogenous loss of amino acids in the form of enzymes being secreted by a hyperactive pancreas. Since pancreatic enzymes are rich in sulfur containing amino acids, the effect of a hyperactive pancreas is to divert the amino acids from the synthesis of body tissue proteins to the synthesis of these enzymes that are subsequently lost in the feces [143, 144].

Alpha-amylase inhibitors, just like the other protease inhibitors, inhibit the activity of α-amylase enzyme. Alpha-amylase inhibitors have been reported in several studies involving normal and diabetic rats to reduce starch digestion [145], reduce serum glucose and insulin concentrations and increase metabolism of non-esterified fatty acids from adipose tissues [146].

Phytohemagglutinins are natural, high molecular weight glycoproteins that are potent toxins to animals and humans. They are widely distributed in plants consumed as part of human diet especially in legume seeds. Phytohemagglutinins have high affinity for binding carbohydrate -containing molecules, with high specificity towards the sugar component. They have ability to agglutinate red blood cells and other cells from various animals because phytohemagglutinins have multiple binding sites on their molecules that interact with specific glyco-conjugate receptors on the surface of the cell membrane. The lethal effects of phytohemagglutinins are therefore due to their adherence to glycoproteins of the intestinal mucosal membrane surface, causing disruption on the brush-border, atrophy of the microvilli, and reducing the viability of the epithelial cells. In this case, they also adversely affect absorption of all nutrients. Due to interactions of the phytohemagglutinins with epithelial surface of the small intestine, there is an increase in weight of the small intestine because of the hyperplasia of the crypt cells. Consumption of phytohemagglutinins also stimulates hyperplasia and hypertrophy of the pancreas due in part to a mechanism similar to that in protease inhibitors reviewed [50, 143, 144]. Other nutritional and physiological effects of phytohemagglutinins are lowering insulin levels in blood, inhibition of disaccharidases and proteases in the

intestines, degenerative changes in the liver and kidneys, and interference with absorption of non-heme iron and lipid from diet [146].

Because of their protein nature, enzyme inhibitors and phytohemagglutinins are inactivated under the conditions leading to irreversible protein denaturation. Thermo-processing of foods denatures the inhibitors, however, because of the necessity of achieving a balance between the amount of heat necessary to destroy the trypsin /chymotrypsin inhibitors and that which may result in damage to the nutritional or functional properties of the protein, most commercially available edible grade products e.g. soybean products, retain some amount of trypsin and chymotrypsin inhibitor activity originally present in the raw food [143]. The most common food processing methods such as conventional cooking, steaming, microwave cooking, drum processing and extrusion are efficient in inactivating most or even all of the enzyme inhibitors [147]. Inactivation is dependent on cooking time, temperature, particle size, and moisture conditions. Some reducing agents e.g. thiol-containing compounds such as cysteine, N-acetyl-cysteine and glutathione help to effect inactivation of the inhibitors. Use of these agents therefore allows processing of the foods at relatively lower temperatures, which preserves other thermo-labile nutrients [148]. Ellenreider et al. [148] observed that in natural food milieu, trypsin inhibitors are more resistant to thermo-denaturation than the chymotrypsin inhibitors. In a dietary surveys in England and USA, Doell et al. [149] and Billings et al. [150] observed that many of the products consumed in these countries contained some amounts of protease inhibitors.

Like protease inhibitors, phytohemagglutinins were inactivated by thermo-processing when products were subjected to moist heat for 5 – 15 minutes. However,

phytohemagglutinins in ground flours appear to be more resistant to inactivation than in whole grains and also to dry heat treatment than moist heat. Armour et al. [151] and Coffey et al. [152] observed that extrusion cooking was not effective in reducing phytohemagglutinins in ground and split beans but was very effective in inactivating protease inhibitors. Alpha-amylase inhibitors are effectively inactivated even by modest thermo-processing conditions [153].

The physiological and nutritional significance of chronic ingestion of low-levels of residual enzyme inhibitors and phytohemagglutinins is not clearly known. It has been reported that, soy products retain 5 – 20% of the trypsin inhibitory activity due to inadequate thermal treatment in the effort to minimize damage of functional and nutritional properties of protein [143]. Burns [154] also observed that, processed soy products contained ~ 3% of trypsin inhibitory activity, which was equivalent to an inhibition of 2 – 5 μg of trypsin/mg protein, the amounts that were insignificant nutritionally or physiologically. In conventionally cooked beans, about 2 – 5% of trypsin inhibitor activity was detected [155], while the same level of inhibitory activity was detected in soy-based infant formulas. On the basis of these findings, Liener [143] concluded that the residual trypsin inhibitor activities in most foods are safe and are unlikely to cause any health risk to humans. However, the effect of long-term consumption of low concentrations of trypsin and/or chymotrypsin inhibitors on human health is not known. Long-term feeding of rats with concentrated trypsin inhibitors or raw soy flour resulted in development of adenomatous nodules in the pancreas and significantly increased the probability of pancreatic adenoma formation [143, 144, 156]. Likewise, consumption of raw soy flour and/or concentrated trypsin inhibitors has been

shown to potentiate the carcinogenic effect of azaserine and di(2-hydroxyproxyl) nitrosamine on the pancreas of rats [144]. For phytohemagglutinins and α-amylase inhibitors, there is usually no residual activity left if the foods are properly processed. phytohemagglutinins however, are resistant to dry heat and hence flour-based dry products such as cookies may contain some residual phytohemagglutinins activity [143].

2.7.4 Sensory Quality and Consumer Acceptability

The characteristics of a food product (i.e. appearance, flavor, and texture), the conditions under which it is consumed, and the appeal that it has for a specific consumer, determine its acceptance. Sensory evaluation is the science of judging and evaluating the quality of food by the use of the five senses i.e. the taste, smell, sight, touch, and hearing. Sensory measurement of liking is used during product development to predict consumer response before investments are made in equipment, production and distribution [157]. Preference and liking are generally thought to be almost the same, and techniques used for their measurement are often similar [158]. However, preference implies a choice between products, without considering how well liked each one is. Therefore, measurement of the degree of liking or "hedonic value" is a means of determining not only whether one food is preferred to another, but how acceptable or well liked it is. The relevance and reliability of the hedonic scale method, based on known rating scale methods used in psychology, were established through extensive field-testing of army rations of many types [158]. Since the development of the technique, the nine-point and five-point hedonic scales have been used extensively and validated by numerous studies

of food products. Hedonic rating is very popular because it may be used with trained and untrained panelists with minimum literacy and communication skills [156, 159, 160].

In hedonic rating, testers are presented with a continuous or discrete scale with five or nine points where 1 is "dislike extremely" and 3 or 5 is "neither like nor dislike" and 5 or 9 is "like extremely" [158]. Other points are like, or dislike "very much", "moderately" or "slightly". Samples are resented in succession and the testers are asked to test the food product and express their honest opinion of liking accordingly on the scales. They are reassured that there is no correct answer. The data are then interpreted numerically and analyzed statistically. Interpretation of the hedonic testing results is based on the food product; some novel products such as candy and ice cream usually achieve higher hedonic ratings while staple foods usually receive slightly lower hedonic ratings.

The weakest part of hedonic rating is its inability to predict consistently long-term food acceptability [161]. A food with long-term acceptability is a food that can be repeatedly (daily, weekly) eaten without the consumer becoming "tired" of eating it. Few studies of repeated consumption or repeated taste tests suggest that initial hedonic ratings of foods sometime, but not always, predict the long-term acceptability. In a repeated consumption test of breakfast cereals, Goldman [162] found that the hedonic scores were consistent over the five test days. Likewise, studies by Siegel and Pilgrim [163], Schutz and Pilgrim [164], Kamen and Peyam [165] and Tuorila-Ollikainen et al. [166] showed that initial hedonic ratings of some foods were maintained over repeated consumption while the hedonic ratings of others decreased significantly. Foods with higher initial hedonic ratings tended to maintain their ratings better than foods with initially lower

hedonic ratings. On the basis of these observations, Koster [167] concluded the rapid preference test procedures are not valid for the prediction of long-term food acceptance because the methods did not necessarily incorporate the effects of adaptation, habituation or post ingestional effects. Both rapid and long-term acceptability is required for foods that are to be consumed frequently e.g. supplementary foods [161].

Food product testing environment can strongly influerence consumer's liking and acceptance. Cardello et al. [168] reported that, determining consumer preference using the standard, controlled or simulated testing situations e.g. in laboratories, might not be a reliable method to predict consumer behavior towards a food product. They suggested that, the best approach is to test the products in the real life situations using a test panel of people who would eventually consume the foods. This approach however has been shown to be intricate and costly. These limitations notwithstanding, hedonic scales remain the most useful tools for determining consumer acceptance and an integral part of food product development and marketing.

2.7.5 Product Storage Stability

The length of time that a food product is acceptable and meets the consumer expectations of its quality is considered to be its shelf life [169]. Procedures for determining shelf life include microbiological, physical-chemical, and sensory evaluation. These measurements give objective point for stating that the food product meets or do not meet the expected quality. Other food parameter including moisture content, water activity (a_w), lipid oxidation and vitamin loses can be correlated with sensory changes and serve as indices of shelf-stability. Water activity, which is defined as

the ratio of the equilibrium vapor pressure of food system (p) to the vapor pressure of pure water (p_0) ($a_w = p/p_0 = ERH/100$) at the same temperature, affects the shelf life, food safety, texture, flavor and smell of foods. While temperature, pH and several other factors can influence if and how fast organisms will grow in a food product, water activity may be the most important factor in controlling spoilage. Most bacteria, for example, do not grow at water activities below 0.91, while most molds and yeasts cease to grow at water activities below 0.80 and 0.88, respectively [170]. By measuring water activity, it is possible to predict which microorganisms will and will not be potential sources of spoilage. Water activity--not water content--determines the lower limit of available water for microbial growth. In addition to influencing microbial spoilage, water activity can play a significant role in determining the activity of enzymes and vitamins in foods and can have a major impact on their color, taste, and aroma.

Although reduction of moisture has beneficial effects of discouraging or inhibiting growth of microorganisms in foods during storage, residual moisture when too low may promote some chemical reactions such as non-enzymatic browning and enzymic reactions that are detrimental to the food quality. In general, food systems have optimum water activity-moisture range above and below which the food deteriorate at a more rapid rate. Depending on the storage system, deteriorative chemical reactions are normally high at elevated water activity ($a_w \sim 1.0$) and decreases as the water activity decreases. Example, at high water activity (0.91) (moisture > 14%) the rates of deterioration are usually very high in most foods, but at low water activity (moisture 4 – 5%), the rates of deterioration are very slow if the food products are properly formulated, processed, and packaged and can thus remain in good condition for an extended period of time. There is

however an exception with respect to oxidative deterioration of lipids and fat-soluble nutrients. It has been shown in various studies that lipid oxidation decreases as the water activity decreases from the equilibrium (a_w = 0.75) to the water activity 0.3. Further reduction in water activity below this level (a_w = 0.3) resulted in an increase in lipid oxidation [171]. The water activity of 0.3 is therefore a critical value below which oxidative deterioration increased at a rapid rate. This critical water activity level therefore serves as a minimum demarcation for lowering water activities in dehydrated food products that contain lipids. According to Nelson and Labuza [170], the water activity range 0.15 – 0.3 enhances lipid oxidation whereas the range 0.3 – 0.6 minimizes the lipid oxidation and other deteriorative reactions. There are a number of proposed explanations for this effect that implicate the state of hydration of catalysts (e.g. metals), and hydroperoxides, phase transition, mobilization of pro- and anti-oxidants, and diffusion-related phenomena [172].

For dehydrated supplementary foods, oxidative changes in the lipid phase would be of much concern especially when unsaturated vegetable and fish lipids and minerals are present in significant amounts, for not only they will cause adverse changes in flavor and acceptability, but also may produce non-desirable by-products and destruction of fat soluble vitamins [173]. Conversely, lowering the water content of the product to a very dry state (e.g. < 5% moisture) may promote lipid oxidation. A solution to this dilemma would be to use antioxidants and packaging the product in air- and moisture-proof polyethylene materials to minimize lipid oxidation. For products to be stored for a long time (at least a year), such as cereal-based supplementary foods, oxidative rancidity, sensory quality, and other physical-chemical changes (e.g. total acids and pH) must be

measured analytically and used to establish the storage stability. Data from such parameters whether determined in real-time study or accelerated shelf-life testing conditions [174], can be used to build prediction models for storage of similar products [175].

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CHAPTER THREE

3.0 EVALUATION OF PROTEIN QUALITY AND GROWTH/ REHABILITATION POTENTIAL OF DRUM-PROCESSED, READY-TO-EAT CEREAL-BEAN-SARDINE COMPOSITE SUPPLEMENTARY FOODS FOR PRE-SCHOOL CHILDREN.

3.1 Abstract

Acute, severe undernutrition during childhood remains a common health problem in many parts of the world and makes significant contribution to childhood mortality. This study was conducted to evaluate protein quality and growth/rehabilitation potential of supplementary foods developed from locally produced materials in Tanzania.

Six diets namely rice-meal (RM), bean-meal (BM), rice-bean composite meal (RBM), rice-bean-sardines composite meal (RBSM), corn-bean-sardines composite meal (CBSM) and corn-bean meal (CBM) were formulated to maximize amino acid score as recommended by FAO/WHO/UNU (1985) for preschool children. Biological qualities of the diets including apparent and true protein digestibility, net protein retention ratio, food efficiency ratio, protein digestibility corrected amino acid scores (PDCAAS%) and rehabilitation potential were evaluated using Sprague Dawley weanling rats.

Our results showed that, net protein retention ratios varied significantly ($p < 0.05$) among the products. The net protein retention ratios were; control diet (0.93), RBSM (0.92), CBSM (0.86), RM (0.66), RBM (0.44), CBM (0.28), BM (0.12) and CM diet (-0.40). True protein digestibility ranged between 82 and 99% with BM showing the lowest digestibility. The protein digestibility corrected amino acid scores (PDCAAS%) were

100% (control diet), 77% (CBSM), 89% (RBSM), 58% (RM), 90% (RBM), 47% (CBM), 85% (BM) and 48% (CM). Two test diets, CBSM and RBSM showed greatest potential to support growth and rehabilitation of undernourished rats, while CBM, RBM, BM and CM did not display acceptable growths. These results suggest that, cereal-bean-sardine composite were of high quality and have a potential for use as supplementary/ rehabilitation foods for pre-school and school-age children and adults.

3.2 Introduction

Acute, severe malnutrition during childhood remains a common health problem in many parts of the world and makes significant contribution to childhood mortality [1]. In Tanzania, more than 200,000 children die before reaching their fifth birthday due to inadequate intake of protein, energy and/or micronutrients [2]. The situation is worsened by the current scourge of HIV/AIDS, which renders many children orphans and leaves them with no adults to take care of them [3 – 4]. The causes of malnutrition are multi-factorial, however, the immediate cause of this condition is inadequate intake of nutrients due to inadequate food or poor quality foods [5 – 7]. Lack of nutrient-dense foods especially during and after weaning and during illness has been associated with more than 75% of the infant and young children mortalities [5]. Usually, children identified with mild or severe protein energy malnutrition (PEM) are referred to hospital-based or community-based rehabilitation centers where they receive feeding supplements while continuing with medical treatment of the complications associated with PEM [8 – 10].

The common food used for nutritional rehabilitation is dried skim milk (DSM), which is reconstituted with vegetable oil and sugar to increase the energy density "energy food" [11]. The DSM is imported by the government as part of its public social support service. The rapid increase in the number of children needing nutritional rehabilitation has left the government unable to import adequate amount of DSM for all children. When the rehabilitated children return home from the treatment centers, they no longer have access to the DSM. Dried skim milk is not readily available in rural areas, and where available, it is too expensive for the majority of the low-income families to afford. As a result, children who were discharged from the nutritional rehabilitation centers often

become malnourished again as they return to the same dietary regime that caused PEM originally. Such children often become malnourished again and are readmitted to the same centers [8 – 10].

Follow-up studies of children discharged from nutritional rehabilitation centers indicated higher mortality rates compared to their non-referred peers. Most remained underweight until adulthood and only 30% attained satisfactory catch-up growth [12 – 13]. An inexpensive follow-up diet is therefore critical for the rehabilitated children to survive and attain normal growth. Shortage of DSM-based diet to feed the large number of malnourished children in the households, rehabilitation centers, orphanages, street children homes, and refugee camps has been a major problem limiting nutritional improvement of children in Tanzania. As part of the efforts to address this problem, this study was designed to evaluate the nutritional quality and growth/ rehabilitation potential of formulated supplementary foods from locally produced food ingredients. It is hypothesized that, developing high quality supplementary foods based on locally produced ingredients would enhance availability, affordability and will strongly augment the nutritional care and nutritional rehabilitation efforts, thus, improving the survival and well-being of vulnerable populations in the country.

3.3 Materials and Methods

3.3.1 Product Formulation

Six products namely rice meal (RM), rice-bean meal (RBM), rice-bean-sardine meal (RBSM); corn-bean meal (CBM), corn-bean-sardine meal (CBSM) and bean meal (BM) were formulated in the laboratories of the Department of Food Science and Human

Nutrition, Michigan State University. Products were optimized to deliver greatest amino acid score and the desired amount of energy and fat according to the FAO/WHO Codex Alimentarious guidelines (CAC/GL 08-1991) for supplementary foods for older infants and young children [14]. The products were designed for enhancing growth and for nutrition rehabilitation interventions. All the ingredients used i.e. rice (*Oryza sativa*), corn/maize (*Zea mays)*, beans (*Phaseoulus vulgaris*), sardines (*Sardinops melanosticta*) and red palm-oil (derived from the mesocarp of the oil palm (*Elaeis guineensis*) are commodities that are inexpensive and readily available in Tanzanian markets.

3.3.2 Product Processing

The raw materials were processed into pre-cooked flour that could be reconstituted into porridge for child feeding as recommended by FAO/WHO Codex standard [14] for processed cereal-based foods for infants and children (CODEX STAN 74-1991). The beans, corn, and rice were sorted to remove extraneous materials, washed in double distilled water, dried and milled into fine flours (mesh size 0.8 mm). The sun-dried small fish (sardines - *Sardinops melanosticta*) were sorted to remove pebbles and other extraneous materials and washed in double distilled water. The fish were thereafter cooked in boiling water for 30 min, then dried and ground into a fine powder. The basic ingredients – rice, rice-bean, rice-bean-sardine, corn, corn-bean, corn-bean-sardine and beans - were separately mixed with distilled water in a 25:75 solid: water ratio to form thin slurry. The slurry was thereafter cooked in a steam heated drum drier set at 100.6^0C. Residual moisture in the products was removed by additional drying in an oven.

3.3.3 Diet Preparation

Six test diets were formulated to meet the FAO/WHO [14] Codex Alimentarius (CAC/GL 08-1991) guidelines (Table 1). A cornmeal was prepared according to the traditional processing practices in Tanzania [15]. A modified AIN 93G diet [16] was used as a control. A low protein diet was also prepared in which the casein in the control diet was replaced by cornstarch and 2 g of lactalbumin per 100 g of diet. The low protein diet was used to estimate the endogenous nitrogen excretion of the rats. Lipid content of the control, the test and the low protein diets was adjusted to 13% using a mixture of red-palm oil (10%) and corn oil (3%).

3.3.4 In vivo study

Growth Study: This study was conducted according to the AOAC [17] procedure 45.3.06. Male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), 21days old and weighing 35 – 50 g were housed individually in suspended stainless steel cages with wire bottoms. The temperature of the animal room was set at 22^0C and 40 – 60% relative humidity with alternating 12-hour periods of light and darkness throughout the study. The weanling rats were fed a stock diet for an acclimatization period of three days. Subsequently, the rats were weighed and those rats at the extremes of the distribution curve were excluded from the study. The remaining animals were randomly assigned into seven groups of six animals each. The differences in mean weight between any two groups did not exceed 2 g. Each group was randomly assigned to a diet and food and water were provided *ad libitum*. The animals were allowed to acclimatize to the test diets for three days before data collection started. Feed intake and weight changes of the

animals were monitored and recorded daily for the study period of 21 days. Feces were collected from individual rats during the last three days of experiment.

Table 1: Composition (g kg^{-1}) of the cereal-bean based supplementary foods used for the *in vivo* rat feeding study[1]

Ingredients/Diet[2]	CTRL	RM	RBM	CBSM	RBSM	CBM	BM	CM
Bean	00.0	00.0	453.4	102.0	153.0	434.4	566.8	00.0
Corn	00.0	00.0	00.0	570.9	00.0	312.6	00.0	770.0
Rice	00.0	740.0	314.6	00.0	519.1	00.0	00.0	00.0
Sardines	00.0	00.0	00.0	73.8	73.8	00.0	00.0	00.0
Sucrose	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Cornstarch	535.8	03.3	41.4	50.0	29.5	72.2	244.3	13.7
Cystine	02.3	00.0	00.0	00.0	00.0	00.0	00.0	00.0
Casein	152.2	00.0	00.0	00.0	00.0	00.0	00.0	00.0
Red Palm Oil	100.0	100.0	100.0	100.0	95.4	100.0	100.0	99.2
Corn oil	30.0	05.9	12.9	12.9	00.0	10.9	21.4	00.0
Fiber	112.2	83.3	10.2	10.2	61.7	00.4	00.0	49.6
Multi-mix[3]	47.514	47.514	47.514	47.514	47.514	47.514	47.514	47.514
TOTAL	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

[1] FAO/WHO Codex standard for processed cereal-based foods for older infants and young children (CODEX STAN 74-1991)[14].

[2] Diets – CTRL = control, RM = rice meal, RBM = rice-bean meal, CBSM = corn-bean-sardine meal, RBSM = rice-bean-sardine meal, CBM = corn-bean meal, BM = bean meal and CM = corn meal

[3] Multi-mix = (mineral mix – 3.5 g + vitamin mix – 1.0 g, + choline bitartate – 0.25 g + butylated hydroxytoluene - 0.0014 g).

3.3.5 Rehabilitation Study

Animals on low protein diet, CM, BM and CBM, which did not show satisfactory growth, were rehabilitated by feeding them the diets that showed the greatest potential to support growth, i.e. control diet, RBSM and CBSM. The animals were rehabilitated for 14 days and their feed intake and weight changes monitored daily.

3.3.6 Computations

The following protein quality indices were calculated from the data collected:

$$\text{Net Protein Retention Ratio} = \frac{\text{(g of weight gain + g of weight loss in protein free diet)}}{\text{g of protein consumed}}$$

Food Efficiency Ratio = g of weight gain/g of food consumed

$$\text{Apparent Protein Digestibility} = \frac{I - F}{I}$$

$$\text{True Protein Digestibility} = \frac{I - (F - F_0)}{I}$$

Where I = Nitrogen intake of the test diet

F = Fecal nitrogen of the test diet

F_0 = Fecal nitrogen of the 2% lactalbumin diet

3.4 Chemical Assays

Feces collected in the balance period (last three days of the experiment) were separated from the spilled food, dried to a constant weight at 100^0C and ground. Total nitrogen in the feces and diets was determined by Kjeldahl method [17] and the crude protein content was calculated using the factor of 6.25. The moisture content of food and fecal matter was determined by AOAC [17] procedure 925.09.

3.4.1 Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The PDCAAS was determined using the FAO/WHO [18] and Gertjan [19] procedure. The essential amino acid profile of the test diets was compared with the FAO/WHO/UNU [20] essential amino acid requirement pattern for a pre-school (2 – 5 year old) child to compute the amino acid scores. The PDCAAS index was computed for

all experimental diets using their essential amino acid profile compiled from the USDA database [http://www.nal.usda.gov] and mean true digestibility value for each diet.

PDCAAS = True Digestibility x Lowest Amino Acid ratio

3.5 Statistical Analysis

Results are presented as mean values and standard deviations. Data were subjected to one-way analysis of variance (ANOVA) where applicable, using the SAS system for Windows® Version 8E, 2000 and a difference was considered to be significant at $p < 0.05$. *Post hoc* analysis of means was done by the Duncan's Multiple Range Comparison Test.

3.6 Results and Discussion

3.6.1 Amino Acid Profile

Proportionality pattern of amino acids in foods is the most important determinant of protein quality [18]. Table 2 data show the proportions of the various essential amino acids in the control and test food products relative to the FAO/WHO/UNU [20] reference patterns for children 0 – 1 year and 2 – 5 years and for NRC [21] amino acid reference pattern for growing rats. All the products except RM, CBM and CM had amino acid patterns that are considered acceptable for pre-school children aged 2 – 5 years i.e. His - 19, Ile - 28, Leu - 66, Lys - 58, SAA (Met + Cys) - 25, AAA (Tyr + Phe) - 63, Thr - 34, Trp - 11 and Val - 35 gkg^{-1}. The PDCAAS index reflects the estimated ability of the test protein to meet the protein needs of an individual. The FAO/WHO/UNU [20] recommended the use of the amino acid requirement pattern for the 2 – 5 year old child

as the reference for foods meant for preschool children and the amino acid composition of human milk for foods intended for infants. PDCAAS values ranged from 47 – 91% when the amino acid profile of 2 – 5 yr old children was used as reference. Rice-bean meal, RBSM and CBSM had the highest PDCAAS values ($p < 0.05$) while RM, CBM and CM had the lowest scores. The most limiting amino acids were Lys in RM and CM and Trp in CBM. Lys and Trp are inherently limiting in rice and maize/corn-based products, respectively [22]. The CBSM was limiting in Trp, however, the PDCAAS value for the food product was higher than the minimum score (70%) recommended by the FAO/WHO [14] Codex Alimentarius. This could be attributed to the presence of fish protein (5.30 gkg^{-1} of dried product), which improved its amino acid profile.

When the FAO/WHO/UNU [20] amino acid profile for breast milk was used as a reference pattern, all products except the control diet had serious deficiencies in essential amino acids. Trp was the most deficient amino acid (in control diet, CBSM, RBSM, CBM and CM meals), followed by Sulfur containing Amino Acid (in RBM and BM). Lys was limiting in RM. The PDCAAS values (range 30 – 94%) for all food products except the control were lower than the minimum level (85%) recommended by FAO/WHO [14] Codex Alimentarius for follow-up infant foods (CODEX STAN 156-1989). This would imply that, although these food products can adequately meet the nutritional needs of pre-school children (2 – 5 years), school children (10 - 12 years) and adults [18, 20], they would be suitable only as supplements for infants and should not be used to substitute the mother's breast milk.

The NRC [21] requirements of Sulfur containing Amino Acid for rats are much greater than for pre-school children. This explains the low PDCAAS values (range 33 – 70%) for rats. Most test diets (RBSM, CBSM, RBM, CBM and BM) were limiting in sulfur containing amino acids for rats while CM and RM were limiting in Lys. The control diet contained the minimum PDCAAS (70%) required for growing rats. Since the amino acid requirements for growing rats are much higher than that of pre-school age children, the growth patterns observed in the growing rats are lower than what would have been if the test diets were fed to children. For this reason, diets with protein that supports only modest growth in rats would be adequate to support optimal growth in pre-school age children.

3.6.2 Protein Quality

Table 3 shows the apparent and true protein digestibility, net protein retention and food efficiency ratios for the various cereal-bean formulations. The casein in the control diet was significantly ($p<0.05$) more digestible than the protein in the other products, while BM, RBM and CBM were the least ($p < 0.05$) digestible. The apparent and true protein digestibility levels observed in the BM, RBM and CBM diets were similar to those reported in other studies [22, 24 – 25]. A study by Sarwar [24] on protein and amino acids digestibility in various foods revealed that, products containing beans usually have low digestibility ranging from 70 to 85% resulting in lower overall utilization of the protein in the foods.

Table 2: Comparison of the amino acid composition (g kg^{-1} of protein) of the various food products with the FAO/WHO/UNU [20] recommended requirement patterns for pre-school (2 – 5 years) children and infants and with the NRC [21] recommended pattern for rats

Amino acid/Diet[1]	CTRL	RM	RBM	CBSM	RBSM	CBM	BM	CM	FAO/WHO[2]	FAO/WHO[3]	NRC(Rat)[4]
Trp	16	12	12	9	11	6	12	7	11	17	13
Thr	47	35	41	42	42	41	42	38	34	43	41
Ile	54	41	44	42	44	43	44	36	28	46	41
Leu	95	82	80	100	82	88	80	123	66	93	71
Lys	81	35	64	56	64	61	69	28	58	66	61
SAA	46	42	28	35	35	28	26	39	25	42	65
AAA	110	106	86	87	91	84	82	90	63	72	68
Val	67	58	58	53	56	52	52	51	35	55	49
His	29	25	27	33	32	28	28	30	19	26	19
TOTAL	**545**	**436**	**440**	**457**	**457**	**431**	**435**	**442**	**339**	**457**	**430**
PDCAAS[5]	100	58	83	77	90	47	82	46			
Limiting AA[5]	NIL	Lys	NIL	Trp	NIL	Trp	NIL	Lys			
PDCAAS[6]	94	51	56	50	59	30	51	39			
Limiting AA[6]	Trp	Lys	SAA	Trp	Trp	Trp	SAA	Trp			
PDCAAS[7]	70	55	36	51	48	37	33	44			
Limiting AA[7]	SAA	Lys	SAA	SAA	SAA	SAA	SAA	Lys			

[1] Diets – CTRL = control, RM = rice meal, RBM = rice-bean meal, CBSM = corn-bean-sardine meal, RBSM = rice-bean-sardine meal, CBM = corn-bean meal, BM = bean meal and CM = corn meal.

[2] FAO/WHO/UNU [20] amino acid reference pattern for children aged 2 – 5 years.

[3] FAO/WHO/UNU [20] amino acid reference pattern for children aged 0 – 1 year.

[4] National Research Council (NRC) 21 amino acid reference pattern for growing rats.

[5] Protein digestibility corrected amino acid scores with respective limiting amino acids based on FAO/WHO/UNU [20] amino acid reference pattern for children aged 2 – 5 years.

[6] Protein digestibility corrected amino acid scores with respective limiting amino acids based on FAO/WHO/UNU [20] amino acid reference pattern for children aged 0 – 1 year.

[7] Protein digestibility corrected amino acid scores with respective limiting amino acids based on NRC[21] amino acid reference pattern for growing rat.

In a feeding study of young children, Graham et al. [23] observed low protein digestibility in diets containing beans and inferred that, poor protein digestibility was the major limiting factor for efficient utilization of bean-based proteins by small children. Protein digestibility levels in these trial products influenced the ratio of net protein retained. Net protein retention was significantly higher ($p < 0.05$) in the control (93%), RBSM (92%), and CBSM (86%) meals compared to the CM (- 40%), BM (12%), CBM (28%) and RBM (44%). Inclusion of sardines in the RBSM and CBSM significantly improved net protein retention. The ratio of net protein retained reflects the

Table 3: *In vivo* true and apparent protein digestibility, net protein and food efficiency ratios of the various cereal-bean based food products[1]

Diet[2]	True Protein Digestibility (%)	Apparent Protein Digestibility (%)	Net Protein Ratio[3]	Food Efficiency Ratio[4]
CTRL	99 ± 1.2^{a}	95 ± 1.23^{a}	0.93 ± 0.26^{a}	0.15 ± 0.04^{a}
RM	96 ± 2.1^{b}	85 ± 1.97^{c}	0.66 ± 0.22^{bc}	0.05 ± 0.02^{c}
RBM	83 ± 1.5^{f}	75 ± 2.24^{f}	0.44 ± 0.19^{cd}	0.06 ± 0.03^{c}
CBSM	94 ± 2.0^{c}	86 ± 1.62^{c}	0.86 ± 0.25^{ab}	0.10 ± 0.03^{b}
RBSM	90 ± 1.7^{d}	83 ± 0.97^{d}	0.92 ± 0.17^{a}	0.12 ± 0.02^{b}
CBM	86 ± 1.3^{e}	78 ± 0.96^{e}	0.28 ± 0.01^{de}	0.03 ± 0.01^{cd}
BM	82 ± 1.5^{f}	72 ± 1.56^{g}	0.12 ± 0.02^{e}	0.01 ± 0.00^{d}
CM	95 ± 1.7^{b}	88 ± 1.68^{b}	-0.40 ± 0.67^{f}	-0.03 ± 0.03^{e}

[1] Means (n = 6) within a column with different superscripts are significantly different at $p < 0.05$.
[2] Diets – CTRL = control, RM = rice meal, RBM = rice-bean meal, CBSM = corn-bean-sardine meal, RBSM = rice-bean-sardine meal, CBM = corn-bean meal, BM = bean meal and CM = corn meal
[3] (g of weight gain + g weight loss in low-protein diet)/g of protein consumed
[4] g of weight gain/g of food consumed.

ability of the animal to grow. FAO/WHO [14] recommends addition of edible fishmeal and fish protein concentrate to cereal-based supplementary foods to increase their protein quality. Fish protein has high content of Lys and is highly digestible [24].

Amount and type of protein in these food products greatly influenced their intake. As reported in previous studies [26 – 28], foods containing low and/or imbalanced protein suppress food intake. Likewise, foods containing too high protein content (> 40g/100g) suppress food intake [29]. In this study, the highest levels of food intake ($p < 0.05$) were observed in RM (15.54 g/day) followed by RBSM, control and CBSM (12.22, 11.55, and 11.43 g/day, respectively; $p > 0.05$). Cornmeal was the least consumed (5.21 g/day). Food intake is a critical factor for growth as it influences both energy and protein intake. Low food intake in animals receiving CM, BM, RBM and CBM diets could have adversely affected their growth. Both sensory and metabolic effects have been described to influence the food intake. Sensory attributes influence the food taste, physical characteristics, and overall acceptability (immediate effect) while metabolic factors influence plasma amino acid homeostasis and satiety (long-term effect) [28 – 29].

Food efficiency ratio was significantly higher ($p < 0.05$) in the control diet than in the other food products. The food efficiency ratio ranged from 15% in the control diet to -3% in CM. Food efficiency ratio was significantly lower ($p > 0.05$) in the products that contained beans, which could be attributed to poor digestibility of bean protein [23].

The low protein digestibility of food products containing beans could be associated with presence of strong, covalent disulfide linkages between Cys residues in some of the bean protein fractions e.g. globulins GII, glutellins and protease inhibitor proteins that form compact, dense polymers which prevent proteolytic enzymes – pepsin,

trypsin and chymotrypsin from reaching the internal catalytic sites. Other factors such as high dietary fiber, residual activity of lectins after cooking, steric hindrance of proteolysis by the carbohydrate moieties of the glycoproteins (especially in globullin GII and protease inhibitor protein fractions), and presence of carbohydrate-protein and protein-protein bands that are not susceptible to mammalian protease hydrolysis have been reported to reduce the overall protein digestibility [30]. High food efficiency ratio is an important quality attribute for supplementary foods, especially the cereal-based foods that have a high dietary bulk which limits the amount of food children can take in a day [31].

3.6.3 Assessment of the cereal-bean products as supplementary foods

Table 4 shows protein quality attributes and energy content of the test products compared to the recommendations of FAO/WHO [14] Codex Alimentarius for pre-school age children diets. FAO/WHO [14] recommends that, supplementary foods for children have PDCAAS value $\geq$ 70%, $\geq$ 400 kcal per 100 g of dry product, $\geq$7.0% of the energy obtained from protein, net dietary protein energy value $\geq$ 7.0% and protein quality index of 100% [32]. All products except CBM and CM met the recommended requirements of protein and energy for young children. These products therefore are suitable for maintenance and growth of pre-school, school age children and adults [20].

3.6.4 Evaluation of rehabilitation potential

Fig. 1 shows the effect of protein quality on growth. Weight gain was significantly greater ($p < 0.05$) (2.69 g/day) for rats eating the control diet while rats receiving the CM diet lost weight (-0.17 g/day). Rates of weight gain for animals receiving the other food

products were 1.65 g/day (RBSM), 1.4 g/day (CBSM), 0.57 g/day (RM), 0.37 g/day (CBM), 0.45 g/day (RBM), and 0.21 g/day (BM).

Table 4: Protein quality and energy content of selected test diets compared with the recommendations of FAO/WHO [14] Codex Alimentarius for pre-school age children[1]

	Quality Criteria				
Diet[2]	Total Energy (kcal)	Protein-energy (%)	PDCAAS (%)	NDpE%[3]	PQI (%)[4]
CTRL	400	15.2	100	9.6	159
RM	405	4.94	58	3.1	52
RBM	405	14.1	83	8.8	97
CBSM	400	11.9	77	7.2	121
RBSM	400	11.8	90	7.9	121
CBM	401	16.4	47	6.2	97
BM	400	17.6	82	9.6	90
CM	412	6.8	46	3.4	42
FAO/WHO[1]	≥ 400	≥7.0	≥ 70	≥7.0	≥100

[1] FAO/WHO [14] Codex Alimentarius quality criteria for supplementary foods for older infants and young children.

[2] Products – CTRL = control, RM = rice meal, RBM = rice-bean meal, CBSM = corn-bean-sardine meal, RBSM = rice-bean-sardine meal, CBM = corn-bean meal, BM = bean meal and CM = corn meal

[3] Net dietary protein energy % = (1.25 x Protein energy % x Amino acid score)/(100 + 0.064 x Protein energy % x Amino acid score) [32].

[4] Protein quality index = requirement of protein for 2 – 5 y child (1.09 g/kg/day)/Amount of test protein to satisfy requirement of the most limiting amino acid for a 2-5 y old child

Both food intake and protein adequacy strongly affect growth. As discussed above, low protein quality results in reduced food intake and therefore reduced growth. Two trial products (RBSM and CBSM) and the control diet were used to rehabilitate the undernourished rats. As shown in Fig. 2, the rate of weight gain during rehabilitation was significantly higher ($p < 0.05$) than during the normal growth phase. During nutritional rehabilitation, the rate of weight gains for the animals receiving the test diets RBSM and CBSM did not differ significantly ($p > 0.05$) from the weight gained by the rats receiving the control diet. During nutritional rehabilitation the rates of weight gain were 5.61, 5.47

and 5.29 g/day for the animals receiving the control, RBSM and CBSM diets, respectively.

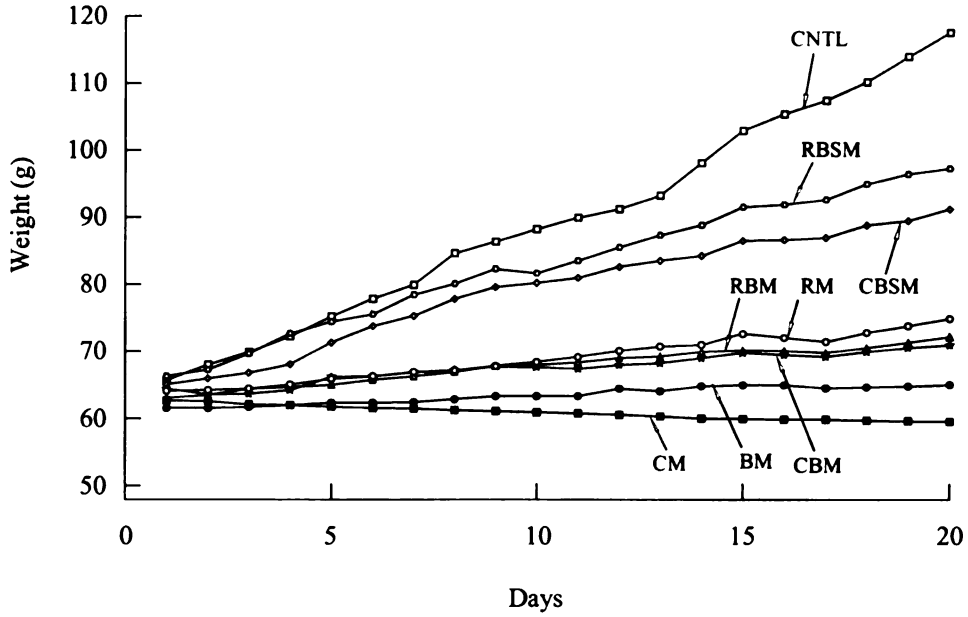

Fig. 1: Growth patterns for rats fed varying sources of protein

The catch-up growth phenomenon observed in these animals was similar to that observed in malnourished children during nutritional rehabilitation. Fjeld et al. [33] and Rowland et al. [34] reported a rapid increase in weight gain ranging between 3 – 8 times the average daily growth rates when undernourished children were rehabilitated using high energy, protein diets. In this study, the rates of catch-up growth were 2 – 3 times the average daily rate of growth. Hastening catch-up growth would be advantageous because it reduces the residence time in a hospital or community-based rehabilitation centers, thus reducing the cost and enabling more children to be served.

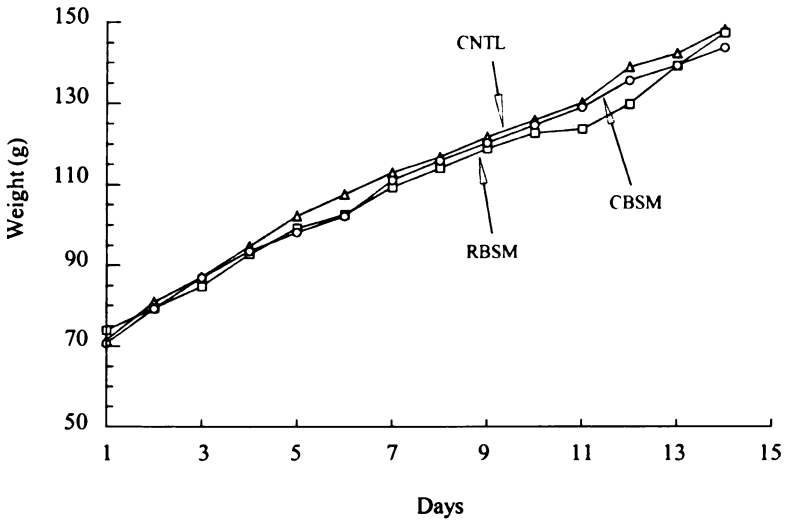

Fig. 2: Growth pattern during rehabilitation phase

3.7 Conclusion

This study showed that, the drum-processed RBSM and CBSM products developed from locally produced ingredients were of high quality and displayed a great potential to support normal and catch-up growth during nutritional rehabilitation of undernourished animals. Values of true protein digestibility and growth response based on rat feeding trials correspond closely with human measurements [18]. These results therefore, may be extrapolated to pre-school and school-age children.

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CHAPTER FOUR

4.0 NUTRITIONAL QUALITY OF DRUM-PROCESSED AND EXTRUDED READY-TO-EAT COMPOSITE SUPPLEMENTARY FOODS

4.1 Abstract

This study was conducted to evaluate the nutritional quality of ready-to-eat composite foods cooked by extrusion, conventional and drum-processing methods. Four supplementary foods, namely corn-bean-sardine meal (CBSM), bean meal (BM), sorghum-bean-sardine meal (SBSM), and rice-bean-sardine meal (RBSM) were formulated according to the FAO/WHO/UNU guidelines. The food mixtures were extruded, drum-processed, and cooked conventionally in the traditional way. Cooking doneness was evaluated by percent starch gelatinization and residual urease activity while biological qualities -- true protein digestibility and growth performance -- were evaluated using Sprague Dawley weanling rats. Efficiency in destroying phytohemagglutinins and the anti-nutritional factors – trypsin, chymotrypsin and α-amylase inhibitors were also evaluated. Results of the study showed that starch gelatinization and residual urease activity were not significantly different ($p > 0.05$) between the extruded and drum-processed diets. Relative to conventional cooking, starch gelatinization was 95 - 100% in extruded and 90 - 100% in drum-processed products. Inactivation of urease activity ranged from 93 to 100% in extruded and 83 to 100% in drum-processed diets. The true protein digestibilities were significantly ($p \leq 0.05$) higher when extruded foods compared to drum-processed foods were fed to experimental animals. Animals fed extruded products showed slightly greater but insignificant growth rates relative to those fed drum-

processed foods. Destruction of phytohemagglutinins was ranged between 91– 97% in extruded and between 90 – 95% in the conventionally cooked and drum processed foods. Extrusion, drum-processing and extrusion cooking also resulted in a significant destruction of the anti-nutritional factors - trypsin, chymotrypsin and α-amylase inhibitors. These results suggest that extrusion and drum-processing of cereal-bean-sardine composite foods resulted in products meeting the required nutritional quality.

4.2 Introduction

Protein energy under-nutrition during childhood remains a common health problem in many parts of sub-Saharan Africa and contributes significantly to childhood morbidity and mortality [1]. In Tanzania for instance, more than 30% of young children suffer from protein, energy and micronutrient under-nutrition whereas more than 0.2 million children are estimated to die before reaching their fifth birthday [2, 3]. The first year of life is usually the vulnerable period for developing under-nutrition, which usually coincides with the introduction of weaning foods. Various studies have shown that children in Tanzania, as is elsewhere in developing countries, start their life in a sound health and their growth rate during the first 4 – 6 months is comparable to that of children in developed countries [4 – 6]. Growth however, starts to falter during weaning and/or thereafter [6].

Inadequate intake of nutrients due to low nutrient density and infrequent feeding of children due to heavy maternal workload have been identified as some of the underlying causes of acute and severe undernutrition. Due to mothers' competing roles such as working in farms, collecting firewood, fetching water, washing clothes, cleaning house and caring for the other members of the family, they do not have time to prepare meals and feed the child in a frequency (4 – 6 times/day) that would provide enough nutrients [7, 8]. This period also is accompanied with high incidences of childhood diseases due to increased infections caused by contaminated weaning foods and decline in the immune-related factors acquired from the mother [9]. According to WHO [10], WHO/UNICEF [11], and Dewey and Brown [12], preparing low-cost, fortified supplementary foods from locally available ingredients using suitable small-to-medium

scale production technologies in community settings, can help to meet the nutritional needs of young children hence reducing the prevalence of under-nutrition drastically. Likewise, centrally processed, fortified and ready-to-eat products would provide a reliable option for families who have the means to buy them and can thus contribute immensely in mitigating the problem of protein energy under-nutrition among older infants and young children in developing countries. It is thus hypothesized that developing nutrient-dense, fully cooked, ready-to-eat, inexpensive and culturally acceptable supplementary foods from locally grown ingredients would help mitigate child under-nutrition by increasing nutrient intake through increased feeding frequency and reducing food-borne diseases caused by the intake of contaminated and/or improperly cooked foods. Identifying an appropriate processing method is therefore critical because it determines the food quality, digestibility, palatability, overall acceptability, market price and sustainability of production. This study was designed to evaluate the nutritional quality of processed, ready-to-eat composite products cooked by extrusion, drum-processing and conventional cooking methods. Specifically, this study was aimed at identifying an appropriate method(s) of choice for processing ready-to-eat composite foods for undernourished children in developing countries.

4.3 Materials and Methods

4.3.1 Products formulation and composition

Four supplementary products, namely corn-bean-sardine meal (CBSM), bean meal (BM), sorghum-bean-sardine meal (SBSM) and rice-bean-sardine meal (RBSM), were formulated in the laboratories of the Department of Food Science and Human Nutrition, Michigan State University. Products were optimized to deliver greatest amino

acid score and the desired amount of energy and fat according to the FAO/WHO Codex Alimentarius guidelines (CAC/GL 08-1991) for supplementary foods for older infants and young children [13]. The products were designed for enhancing growth and for nutrition rehabilitation interventions. All the ingredients used, i.e., rice (*Oryza sativa*), corn/maize (*Zea mays)*, dry beans (*Phaseoulus vulgaris*), sardines (*Sardinops melanosticta*), and red palm-oil [derived from the mesocarp of the oil palm (*Elaeis guineensis*)] are commodities that are inexpensive and readily available in Tanzanian markets.

4.3.2 Product Processing

The raw materials were processed into pre-cooked flour that could be reconstituted into porridge for child feeding as recommended by FAO/WHO Codex standard [13] for processed cereal-based foods for infants and children (CODEX STAN 74-1991). The dry beans, corn, sorghum, and rice were sorted to remove extraneous materials, washed in distilled water, dried and milled into fine flours (mesh size 0.8 mm). The sun-dried small fish (sardines - *Sardinops melanosticta*) were sorted to remove pebbles and other extraneous materials and washed in distilled water. The fish was thereafter cooked in boiling water for 30 min, then dried and ground into a fine powder. The basic ingredients were formulated into diets: corn-bean-sardine, bean flour, sorghum-bean-sardine, and rice-bean-sardine. Each diet was divided into four portions. The first, second and third portions of each diet were processed by drum-processing, extrusion and conventional cooking, respectively into pre-cooked, ready-to-feed flour that could be reconstituted into porridge for child feeding as recommended by FAO/WHO [13] Codex

standard for processed cereal-based foods for infants and children (CODEX STAN 74-1991). The fourth portion was analyzed unprocessed.

Extrusion of the formulated foods was carried out in a small laboratory twin-screw extruder type APV-MP19T2-28 (APV Baker Ltd, Staffs, UK) with a barrel length/diameter ratio of 25. The following extrusion conditions were adopted: feed moisture content - 12 – 14.4%, moisture injection – 9 – 15%, feed rate – 3.61 kg/h, screw-speed – 300 rpm, barrel temperatures – 70^0C (zone 1), 100^0C (zone 2), 127^0C (zone 3), 141^0C (zone 4), 131^0C (zone 5 - die), product temperature – 147^0C, die pressure – 350 psi, die diameter – 3 mm, motor load – 52.4% and mechanical energy input – 0.174 kw-hr/kg. Drum-processing was done by mixing the food ingredients with distilled water in a 25:75 solid: water ratio to form a thin slurry. The slurry was slowly poured between the two counter-rotating drums, which were heated by steam. The slurry was spread on the surface of the slowly rotating drums to form a thin layer. High temperatures on the surface of the drums cooked and dehydrated the food swiftly. The cooked, dehydrated food product was scraped out of the drum surface before it was recoated with fresh food slurry. The speed of the rotating drums was adjusted to increase/reduce product residence time on the drums. Likewise, the amount of steam in the drums was adjusted to regulate the cooking and drying process.

Conventional cooking of the food products followed the traditional Tanzanian cooking practices adapted to the laboratory conditions as described by Mosha and Svanberg [14] and Kikafunda et al. [15]. It involved preparing a smooth slurry of the foods containing 10% solid matter and pouring the slurry into boiling water. The mixture was stirred constantly until boiling. The heat was thereafter reduced from high to medium

and the porridge was allowed to simmer while maintaining gentle constant stirring until the desired cooking time was completed. The CBSM, SBSM and RBSM were cooked for 25 min each while the BM was cooked for 35 min. After cooking, the conventionally cooked foods were freeze-dried while the extruded and drum-processed foods were dried further in an air oven (set at 60 ^{0}C) for 3 h.

4.3.3 Diet Preparation

Four test diets were formulated to meet the FAO/WHO [13] Codex Alimentarius (CAC/GL 08-1991) guidelines (Table 1). A modified AIN 93G diet [16] was used as a control. A low protein diet (METDIET) was also prepared in which the casein in the control diet was replaced by cornstarch and 2 g of lactalbumin per 100 g of diet. The low protein diet was used to estimate the endogenous nitrogen excretion of the rats. Lipid contents of the control, the test and the low protein diets were adjusted to 13% using a mixture of red-palm oil (10%) and corn oil (3%).

4.3.4 In vivo Study

Growth Study: This study was conducted according to the AOAC [17] procedure 45.3.06. Male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), 21days old and weighing 35 – 50 g were housed individually in suspended stainless steel cages with wire bottoms. The temperature of the animal room was set at 22^{0}C and 40 – 60% relative humidity with alternating 12-hour periods of light and darkness throughout the study. The weanling rats were fed a stock diet for an acclimatization period of three days. Subsequently, the rats were weighed and those rats at the extremes of the distribution

Table 1: Composition (g/100 g) of the cereal-bean-based supplementary foods used for the *in vivo* rat feeding study[1]

Ingredients	Diet[2]			
	CBSM	BM	SBSM	RBSM
Bean	10.20	56.68	11.28	15.30
Corn	57.09	0.00	0.00	0.00
Rice	0.00	0.00	0.00	51.91
Sorghum	0.00	0.00	49.68	0.00
Sardines	7.38	0.00	7.38	7.38
Sucrose	2.00	2.00	2.00	2.00
Cornstarch	5.00	24.43	10.56	2.95
Red Palm Oil	9.02	10.00	9.65	9.54
Corn Oil	0.00	2.14	0.00	0.00
Fiber	4.56	0.00	4.70	6.17
Mineral Mix	3.50	3.50	3.50	3.50
Vitamins Mix	1.00	1.00	1.00	1.00
Choline Cl	0.25	0.25	0.25	0.25
BHT	0.0014	0.0014	0.0014	0.0014

[1] FAO/WHO [13] Codex standard for processed cereal-based foods for older infants and young children (CODEX STAN 74-1991).

[2] BM - bean meal; CBSM - corn-bean-sardine meal; SBSM - sorghum-bean-sardine meal; RBSM - rice-bean-sardine meal

curve were excluded from the study. The remaining animals were randomly assigned into 13 groups of six animals each. The differences in mean weight between any two groups did not exceed 2 g. The animals were divided into 12 groups in a 4 X 3 factorial design of four different diets (CBSM, BM, SBSM, and RBSM) each processed by three different methods (drum-processing, extrusion and conventional cooking). The 13th group of rats was fed the modified AIN-93 control. Food and water were provided *ad libitum*. The animals were allowed to acclimatize to the test diets for three days before data collection started. Feed intake and animal weight were recorded daily for 21 days. Feces were collected from each rat during the last three days of experiment. On day 22 one rat was

randomly selected from each group to form a 14th group. These rats were fed a stock diet for three days after which they received a low-protein - metabolic diet. As for the other groups, feed intake and animal weights were recorded daily for 3 days and feces were also collected from each rat during the last three days. Fecal protein and metabolic fecal protein were determined using the protein intake and fecal output data.

4.3.4.1 Computations

The following protein quality index was calculated from the data collected: True protein digestibility = PI – (FP – MFP)/ PI x 100%; where PI = protein intake, FP = fecal protein, MFP = metabolic fecal protein

4.4 Chemical Assays

4.4.1 True Protein Digestibility

Feces collected in the balance period (last three days of the experiment and the during metabolic study) were separated from the spilled food, dried to a constant weight at 100^0C and ground. Total nitrogen in the feces and diets was determined by Kjeldahl method [17] and the crude protein content was calculated using the factor of 6.25. The moisture content of food and fecal matter was determined by AOAC [17] procedure 925.09.

4.4.2 Starch gelatinization

The degree of starch gelatinization in the cooked and raw products was determined by using an enzyme hydrolysis method [18]. A dry sample ca 0.8 g was

hydrolyzed with 0.2 mL invertase (3000 IU/mL) at 37^0C for 30 min. The free glucose (FG) formed was condensed with *o*-Dianisidine (colorless) in presence of glucose oxidase and peroxidase to form oxidized *o*-Dianisidine (brown color). Absorbance was determined at 510 nm with a spectrophotometer. Percent free glucose was computed from the relationship:

$FG\% = (A_t \times V_t \times C \times D \times 100\%)/A_{std} \times Wt$ (mg) where A_t = absorbance of the test food, V_t = total volume of test food solution (25.2 mL), C = concentration of glucose standard (0.394 mg/mL), A_{std} = absorbance of glucose standard solution, D = dilution factor (18) and W_t = weight of dry food sample used in mg.

The sample residue was subsequently digested with 0.1 mL amyloglucosidase to determine total glucose (TG). The glucose liberated was analyzed as above and calculated from the relationship:

$TG\% = (A_t \times V_t \times C \times D \times 100\%)/A_{std} \times Wt$ (mg) where A_t = absorbance of the test food, V_t = total volume of taste food solution (35.7 mL), C = concentration of glucose standard (17.55 mg/mL), A_{std} = absorbance of glucose standard solution, D = dilution factor (1) and W_t = weight in mg of dry food sample used.

Total starch (TS) was thereafter calculated from the FG% and TG% values using the relationship: TS = (TG% - FG%) x 0.9 [18]. The total starch served as an index of the rate of starch gelatinization because it measured the amount of glucose released by enzymic hydrolysis of the food samples after gelatinization. Total starch was corrected for the free glucose.

4.4.3 Urease Activity Test

Residual urease activity in the cooked and raw foods was determined by the AOCS [19] method Ba 9-58.

4.4.4 Tryspin Inhibitor Assay

Trypsin inhibitor activity (TIA) was determined by the method of Smith et al. [20] as modified by Liu and Markakis [21]. One g of ground sample was extracted in 50 mL of 10 mM NaOH and the pH adjusted to 9.4. The extract was then incubated with trypsin enzyme at 37^0C. Tyrpsin inhibitor activity (TIA) was determined by the reduction in hydrolysis of a substrate – benzoyl-DL-arginine-*p*-nitroanilidine hydrochloride (BAPNA) by the enzyme trypsin. TIA was defined as mg of pure trypsin inhibited per g of food sample at 410 nm.

4.4.5 Chymotrypsin Inhibitor Assay

Chymotrypsin inhibitor activity (CIA) was assayed by the procedure of Kakade et al. [22]. CIA was defined as the number of chymotrypsin units inhibited per mg of the food sample at 275 nm under the conditions of the experiment.

4.4.6 Alpha-amylase Inhibitor Assay

Alpha-amylase inhibitor activity was determined by the method of Bernfend [23] as modified by Deshpande et al. [24]. Alpha-amylase inhibitor activity was defined as mg maltose equivalent inhibited from α-amylase hydrolysis under the conditions of the experiment.

4.4.7 Phytohemagglutinins Assay

Active phytohemagglutinins in the food samples were determined by enzyme-linked immunosorbent assay (ELISA) described by Boniglia et al. [25]. One g of finely ground food sample was extracted in 20 mL of PBS (phosphate buffer solution, 10 mM, pH 7.2 containing 150 mM NaCl) by stirring overnight at room temperature. A 96-well microtier plate (NUNC, USA) was coated with 0.1 mL/well of porcine thyroglobulin and incubated overnight at 4 ^{0}C. The treated microtiter wells were treated with 0.1 mL/well of PBS supplemented with 0.5% bovine serum albumin (BSA) and the plate incubated for 60 min at 37^{0}C. Aliquots of 0.1 mL of sample extracts were loaded into each well of the microtiter plate and the plate incubated at 37^{0}C for 60 min. The plate was thereafter washed twice with PBT (phosphate buffer 10 mM, pH 7.2 containing 0.05% Tween 20) and once with PBS. The microtiter plate was subsequently treated with: 1) 0.1 mL aliquots of BSA (0.05% BSA in PBS); 2) rabbit anti-phytohemagglutinin IgG (diluted 1:5,000 in PBS containing 0.25% BSA); 3) alkaline phosphate-conjugate monoclonal anti-rabbit IgG (diluted 1:10,000 in PBS containing 0.25% BSA); and 4) color development solution – p-nitrophenyl phosphate (Sigma Cat. N7653). Between each treatment, the microtiter plate was incubated for 60 min at 37^{0}C and rinsed twice with PBT and once with PBS. Finally, 50 uL of 3M NaOH was added to each well to stop the reaction. Absorbance of the resulting solution was measured at 405 nm using a Synergy HT Multi-detection Microplate reader (Bio-Tek® Instruments Inc., Winooski, VT 05404-0998, U.S.A.). Samples and phytohemagglutinin standard solutions were loaded in the microtiter plate in triplicate.

4.5 Statistical Analysis

Results are presented as mean and standard values. Data were subjected to one- and two-way analysis of variance (ANOVA) where applicable, using the ProStat [Version 3.01 (Poly Software International, Pearl River, N.Y. 10965)] and a difference was considered to be significant at $p \leq 0.05$. Since the TPD values were in percent, the data were subjected to arcsine transformation [26] prior to analysis by ANOVA. *Post hoc* analysis of means was done by the Fisher's LSD test.

4.6 Results ad Discussion

The percent starch gelatinization and residual urease activity for foods processed by extrusion, drum-processing and conventional cooking are summarized in Table 2. Starch gelatinization rates were highest in the extruded and conventionally cooked foods (94.8 – 100.0%) but were slightly lower ($p > 0.05$) in the drum-processed products. Starch gelatinization rates in the drum-processed foods were 90.4% in BM, 93.1% in RBSM, and 100.0% in CBSM and SBSM. Starch gelatinization rate is an important food quality index for processed, ready-to-eat foods because it measures the cooking doneness and also influences food palatability, digestibility, and consumer acceptance [27]. According to Camire et al. [27] and Camire [28], levels of starch gelatinization that are palatable and acceptable to consumers range from 80 to 100%. All our experimental foods had starch gelatinization rates within the acceptable range.

Urease activity was reduced significantly ($p \leq 0.05$) by all the processing methods. The thermo-processing methods reduced the urease activity by 97 – 99%. Urease activity is a measure of cooking doneness for foods that contain legumes. This

Table 2: Percent starch gelatinization and residual urease activity of foods processed by various methods[1].

Food Product[2]	Food Processing Method						
	Extrusion		Drum-processing		Conventional cooking		Uncooked food
	% SG[3]	UA[4]	% SG[3]	UA[4]	% SG[3]	UA[4]	UA[4]
CBSM	100.0[a]	0.020[bc]	100.0[a]	0.030[b]	100.0[a]	0.015[c]	1.460[a]
BM	94.8[a]	0.030[bc]	90.4[b]	0.020[c]	100.0[a]	0.040[b]	2.166[a]
SBSM	100.0[a]	0.030[b]	100.0[a]	0.015[c]	100.0[a]	0.025[bc]	1.330[a]
RBSM	99.6[a]	0.050[b]	93.1[b]	0.010[c]	100.0[a]	0.016[c]	1.460[a]

[1] Residual urease activity (UA) and starch gelatinization (SG) values in a row with similar superscripts are not significantly different at $p \leq 0.05$.
[2] CBSM - corn-bean-sardine meal, BM - bean meal, SBSM - sorghum-bean-sardine meal, RBSM - rice-bean-sardine meal
[3] Starch gelatinization
[4] Residual urease activity

quality indicator is particularly important for processed ready-to-eat foods that do not involve further cooking prior to feeding. A residual urease activity level of 0.8 units has been recommended for all infants'/young children's supplementary foods that contain legumes [29]. High levels of residual urease enzyme in foods containing legumes have been associated with gastrointestinal illness and diarrhea in children [29]. In the drum-processed foods, residual levels of urease activity ranged between 0.01 and 0.03 units while in the extruded and conventionally cooked products the residual activities ranged between 0.02 and 0.05 and between 0.015 and 0.04 units, respectively. Although the reduction in the urease activity was significant ($p \leq 0.05$) for all the cooking methods, the residual enzyme activities were in fact about 10-fold lower than the recommended maximum allowable residual activity (0.8) units [29]. Based on the residual urease

activity levels therefore, all the processing methods were effective in inactivating the enzyme and the foods were thus suitable for children consumption.

Table 3 data show the residual activity/units of different anti-nutritional factors in the foods processed by extrusion, drum-processing and conventional cooking. Trypsin inhibitors (TIU/g) were significantly reduced ($p \leq 0.05$) by all the heat-processing methods. Residual trypsin inhibitors in the drum-processed foods ranged from 1.04 – 7.94 TIU/g while in the extruded and conventionally cooked foods residual levels of trypsin inhibitors were 0.29 – 0.78 and 0.43 – 0.72 TIU/g of food, respectively. These results suggest that, extrusion and conventional cooking were more effective in inactivating the trypsin inhibitors compared to the drum-processing. The thermo-processing methods also resulted in a significant reduction ($p \leq 0.05$) in the amount of chymotrypsin inhibitors in the foods. The residual levels of chymotrypsin inhibitors in the drum-processed, extruded and conventionally cooked foods were 0.00 – 9.01, 0.00 – 8.08 and 0.00 – 1.05 CIU/g of food, respectively. In this case, conventional cooking was superior in inactivating the chymotrypsin inhibitors while extrusion and drum-processing were equally effective in inactivating the inhibitors.

The extent of the trypsin and chymotrypsin inhibitor inactivation by the various cooking methods was comparable to those reported in other studies [30, 31]. Ellenreider et al. [32] and Armour et al. [33] observed that in natural food milieux, like the ones used in this study, trypsin inhibitors were more resistant to heat-inactivation than chymotrypsin inhibitors. Because of the necessity of achieving a balance between the amount of heat necessary to destroy the trypsin/chymotrypsin inhibitors and that which may result in damage to the nutritional or functional properties of the protein, most foods,

Table 3: Residual activity/levels of various anti-nutritional factors in the composite foods processed by the various methods[1, 2]

Anti-Nutritional Factor	Food product	Processing Method			
		Drum-processing	Extrusion	Conventional cooking	Control
Trypsin inhibitors (TIU/g food)	CBSM	2.3539[b]	0.2873[c]	0.4339[c]	2.4338[a]
	BM	7.9435[b]	0.7800[c]	0.5219[c]	11.1064[a]
	SBSM	1.0436[b]	0.6400[b]	0.7213[b]	1.9702[a]
	RBSM	2.1445[b]	0.3126[c]	0.5498[c]	3.7241[a]
Chymotrypsin inhibitors (CIU/g food)	CBSM	0.0000[b]	0.0000[b]	0.0000[b]	3.3708[a]
	BM	9.0051[b]	8.0859[b]	0.0000[c]	19.3538[a]
	SBSM	7.0758[b]	2.1286[c]	1.0482[d]	23.1758[a]
	RBSM	0.5281[b]	0.0000[c]	0.0000[c]	25.8577[a]
Alpha-amylase inhibitors (AAI/g food)	CBSM	0.0000[b]	0.0000[b]	0.0000[b]	258.5497[a]
	BM	0.0000[b]	0.0000[b]	0.0000[b]	357.4456[a]
	SBSM	0.0000[b]	0.0000[b]	0.0000[b]	202.5027[a]
	RBSM	0.0000[b]	0.0000[b]	0.0000[b]	324.0213[a]
Phytohemagglutinins (μg/ g food)	CBSM	0.1781[b]	0.1690[b]	0.1894[b]	2.1992[a]
	BM	0.3131[b]	0.2044[c]	0.2091[c]	6.5854[a]
	SBSM	0.1984[b]	0.1797[b]	0.1818[b]	2.0053[a]
	RBSM	0.1767[a]	0.1849[a]	0.1699[a]	3.5124[a]

[1] CBSM - corn-bean-sardine meal, BM - bean meal, SBSM - sorghum-bean-sardine meal, RBSM - rice-bean-sardine meal.

[2] Values in a row with similar superscripts are not significantly different at $p \leq 0.05$.

even the commercially available edible grade products, e.g., soybean products, retain some amount of trypsin and chymotrypsin inhibitor activities originally present in the raw food [34]. In dietary surveys in England and U.S.A., Doell et al. [35] and Billings et al. [36] observed that many of the products consumed in these countries contained residual amounts of protease inhibitors. In a study of ready-to-feed forms of commercial soybean infant formulas, Churella et al. [37] reported residual TIU ranging from 0.24 – 1.21 TIU per gram of the foods while Rackis and Gumbmann [38] reported higher trypsin inhibitor residues in commercial soy protein products ranging from 3.2 – 13.7 TIU per g of the products. Trypsin inhibitor residues in our test products ranged from 0.3 to 7.9 TIU per g of cooked food. These residual typsin inhibitor units were comparable to those reported in commercial soybean products and infant formulas [37 – 39]. The residual trypsin inhibitor levels found in these products were considerably low and may not present a health risk to consumers. Liener [34] concluded in this regard that the residual trypsin and chymotrypsin inhibitor activities in most foods are safe and are unlikely to cause any health risk to humans. For the α-amylase inhibitors, extrusion, drum-processing and conventional cooking resulted in 100% inactivation of the enzyme inhibitors. Grant et al. [40] have stated that α-amylase inhibitors are readily inactivated even by modest thermo-processing conditions.

Phytohemagglutinins were reduced significantly ($p \leq 0.05$) by all the thermo-processing methods (Table 3). Reduction in the concentration of phytohemagglutinins for the drum-processed products ranged between 90% (in SBSM) and 95% (in BM) while for the extruded products the reduction ranged between 91% (in SBSM) and 97% (in BM). For the conventionally cooked products, reduction in phytohemagglutinins ranged

between 91% (in SBSM) and 97% (in BM). The residual levels of phytohemagglutinins in our food products were lower than those reported in other studies [25, 41]. In a study of two commercial dietary supplements containing kidney bean protein, Boniglia et al. [25] reported residual phytohemagglutinins concentrations of 1.7 and 7.74 mg/g. Rizzi et al. [41] on the other hand reported residual phytohemagglutinins concentrations of 2.5 and 4.7 μg/g g in soy hamburger and soymilk, respectively. Since the residual phytohemagglutinins in our food products were considerably low (range 0.1690 – 0.3131 μg/g), it suggested that the foods were safe for human consumption. Ingestion of active phytohemagglutinins interferes with the intestinal mucosal membrane brush-border function by causing atrophy of the microvilli, thus, disrupting the absorption of all nutrients [34, 42].

Data in Table 4 show the average food consumption for the experimental animals receiving foods processed by the various methods. Diet influenced food intake significantly ($p \leq 0.05$). Variations in amino acid balance affect the acceptance of foods. According to Koehnle et al. [43], a deficiency of any essential amino acid alters feeding behavior. Studies based on animal models indicate that animals rapidly reduce intake of food if there is deficiency in any one of the essential amino acids [44 – 47].

The average food consumption for animal groups receiving extruded, drum-processed and conventionally cooked foods were not significantly ($p > 0.05$) different. The average 21-day food intakes were 287.63, 258.34 and 277.12 g for animal groups receiving extruded, drum-processed, and conventionally cooked foods, respectively. Although the consumption of the foods processed by the various methods were not statistically different ($p > 0.05$), there was however a significant interaction between the

Table 4: Average food consumption (g/21 days) for experimental rats receiving composite foods processed by the various methods[1]

Food Product[2]	Food Processing Method		
	Extrusion	Drum-processing	Conventional cooking
CBSM	317.50 ± 31.07[a]	274.53 ± 52.43[b]	324.30 ± 34.72[a]
BM	178.92 ± 28.92[a]	132.13 ± 24.02[b]	183.14 ± 24.02[a]
SBSM	325.32 ± 20.14[a]	325.10 ± 21.52[a]	305.43 ± 17.75[a]
RBSM	323.80 ± 12.52[a]	319.93 ± 36.00[a]	303.42 ± 24.27[a]
Mean group intake	287.63 ± 66.56[a]	258.34 ± 87.14[a]	277.12 ± 63.74[a]

[1] Mean ± SD based on duplicate analyses. Food consumption values in a row with different superscripts are significantly different at $p \leq 0.05$.
[2] CBSM - corn-bean-sardine meal, BM - bean meal, SBSM - sorghum-bean-sardine meal, RBSM - rice-bean-sardine meal

food products and the processing methods that influenced the amount of food consumed. For each of the composite foods, the extruded and the conventionally cooked products were the most consumed while the drum-processed foods were the least consumed. Extrusion and conventional cooking therefore produced products that were slightly more acceptable by the animals than the drum-processing. Thermo-processing plays a significant role in improving the physical-chemical and sensory characteristics of foods that lead to development of appealing taste, flavor, and aroma. Well-cooked foods are more palatable, appealing and more acceptable [27].

Data in Table 5 show the true protein digestibility for the composite foods processed by the various methods. Extrusion cooking resulted in products with a significantly higher ($p \leq 0.05$) protein digestibility compared to the drum-processing and conventional cooking. The true protein digestibilities of the foods processed by drum-processing and conventional coking were not significantly different ($p \leq 0.05$). Extruded

Table 5: Percent true protein digestibility of foods processed by various methods[1]

Food Product[2]	Food Processing Method		
	Extrusion	Drum-processing	Conventional cooking
	TPD[3]	TPD[3]	TPD[3]
CBSM	93.7 ± 0.6^{a}	88.4 ± 1.4^{b}	90.5 ± 0.9^{b}
BM	91.1 ± 3.0^{a}	86.4 ± 4.3^{b}	82.4 ± 4.8^{c}
SBSM	91.4 ± 1.1^{a}	88.2 ± 1.0^{b}	86.4 ± 1.9^{b}
RBSM	91.3 ± 0.4^{a}	89.2 ± 1.2^{a}	88.9 ± 1.2^{a}
Mean group TPD	91.8 ± 1.8^{a}	88.0 ± 2.6^{b}	87.0 ± 4.1^{b}

[1] Mean $\pm$ SD based on duplicate analyses. Values in a row with different superscripts are significantly different at $p \leq 0.05$.
[2] CBSM - corn-bean-sardine meal, BM - bean meal, SBSM - sorghum-bean-sardine meal, RBSM - rice-bean-sardine meal
[3] TPD – True protein digestibility

CBSM had the highest true protein digestibility (93.7%) while the conventionally cooked BM showed the lowest digestibility (82.4%). There were also significant ($p \leq 0.05$) interactions between the types of foods and the processing methods that influenced protein digestibility. In this case, extrusion cooking resulted in products whose proteins were significantly ($p \leq 0.05$) more digestible compared to the other processing methods. True protein digestibility is a key food quality index because it indicates whether the amino acids present in a food protein would be digested and become available for utilization by the body [48]. Low protein digestibility limits the amount of amino acids in a food that can be absorbed into the body, and can therefore adversely affect growth [48]. The high levels of food consumption and high protein digestibility of the extruded products were echoed in the average weight gain of the experimental animals. Data in

Table 6 show the average weight gain (g/21 days) for the animals receiving the food products processed by the various methods. As for the food consumption and true protein digestibility, there were significant ($p \leq 0.05$) interactions between the types of food products and the processing methods that influenced the average weight gain. Animals receiving the extruded products tended to gain more weight, even though their average weight gain did not differ significantly ($p > 0.05$) from those receiving the drum-processed and conventionally cooked products. This suggests that foods processed by extrusion, drum-processing and/or conventional cooking were equally good in supporting growth of the experimental animals.

Table 6: Average weight gain (g/21 days) for experimental rats receiving composite foods processed by the various methods[1]

Food Product[2]	Food Processing Method		
	Extrusion	Drum-processing	Conventional cooking
CBSM	105.44 ± 14.07^{b}	87.01 ± 13.46^{c}	117.01 ± 7.41^{a}
BM	26.00 ± 3.76^{ab}	16.56 ± 3.63^{b}	27.27 ± 6.36^{a}
SBSM	113.27 ± 7.16^{a}	111.72 ± 8.56^{ab}	100.57 ± 5.77^{b}
RBSM	122.00 ± 5.13^{a}	117.08 ± 18.07^{a}	103.70 ± 11.71^{b}
Group weight gain	92.23 ± 39.55^{a}	80.68 ± 42.88^{a}	85.98 ± 37.66^{a}

[1] Mean $\pm$ SD based on duplicate analyses. Weight values in a row with different superscripts are significantly different at $p \leq 0.05$.

[2] CBSM - corn-bean-sardine meal, BM - bean meal, SBSM - sorghum-bean-sardine meal, RBSM - rice-bean-sardine meal

4.7 Conclusion

High rate of starch gelatinization in the foods and inactivation of urease enzyme affirmed that the foods were fully cooked. Residual levels of anti-nutritional factors –

trypsin, chymotrypsin and α-amylase inhibitors and the phytohemagglutinins were negligibly small. The composite food products had high true protein digestibility and supported normal weight gain in the experimental animals. Both extrusion cooking and drum-processing produced foods that met the required nutritional and consumption quality characteristics. Availability of equipment and energy costs will thus dictate the ultimate method of choice for processing these foods in developing countries.

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CHAPTER FIVE

5.0 PROTEIN DIGESTIBILITY-CORRECTED AMINO ACID SCORES AND STORAGE STABILITY OF READY-TO-EAT SUPPLEMENTARY FOODS FOR PRE-SCHOOL AGE CHILDREN

5.1 Abstract

Severe under-nutrition during childhood remains a common health problem in many parts of the world and contributes immensely to childhood morbidity and mortality. Foods formulated to ameliorate this condition must provide adequate protein of high quality and also must have reasonable shelf-life. This study was conducted to evaluate the protein quality (as measured by the FAO approved true protein digestibility and protein digestibility-corrected amino acid scores), acceptability and storage stability of processed cereal-bean-sardine composite supplementary foods for pre-school age children in Tanzania. Four composite products namely corn-bean-sardine meal (CBSM), bean-meal (BM), sorghum-bean-sardine meal (SBSM) and rice-bean-sardine meal (RBSM) (processed by extrusion, drum-processing and conventional cooking) were formulated to maximize the amino acid score as recommended by FAO/WHO/UNU for pre-school age children. The products were evaluated for true protein digestibility (TPD) and true protein digestibility-corrected amino acid score (PDCAAS). Extruded and drum-processed CBSM and SBSM were evaluated for storage stability. Results showed that TPD and PDCAAS were highest in the extruded products. The TPD values for the products ranged from 82 – 93% and the PDCAAS values were 51 – 86%. There were no significant ($p > 0.05$) variations in the amino acid contents for foods processed by either

extrusion, drum-processing or conventional cooking. Threonine was most limiting in the CBSM, SBSM and RBSM while cysteine + methionine (SAA) was most limiting in the BM. Storage of the products up to 16 weeks at 38^0C resulted in a significant increase ($p \leq 0.05$) in the malondialdehyde concentrations; nevertheless, the levels remained within the acceptable range found in processed commercial supplements. Total acids and pH did not change significantly ($p > 0.05$) during storage.

5.2 Introduction

Childhood undernutrition remains a common problem in much of the developing world. A recent comprehensive report prepared by World Health Organization using data collected from 1980 to1992 indicates that, more than one third of children less than five years of age in developed countries have a weight/height-for-age less than 2 standard deviations (SD) with respect to international reference data [1]. The WHO report shows that the proportion of children who are undernourished has changed very little during the past 20 years [2 – 5] and because of the considerable global population growth during this period, the number of undernourished children continues to escalate, especially in sub-Saharan Africa [6]. In Tanzania, about 40% of young children suffer from protein-energy undernutrition (PEU) [7, 8]. Apart from PEU, other deficiencies, particularly of micronutrients, are widespread among infants and young children and account for the high rates of child morbidity and mortality[8, 9]. The most prevalent nutrient deficiencies in Tanzania are protein, iron, vitamin A and iodine. Other macro- and micronutrient deficiencies (e.g. zinc, selenium, copper, manganese, calcium, folate, and B-vitamins) are prevalent and have adverse effect on the nutritional status of children; however, their deficiency disorders are not clinically pronounced, thus, masking their public health significance[8, 11].

The causes of undernutrition are multifaceted by nature. The immediate cause of undernutrition is inadequate intake and poor utilization of nutrients due to the high incidence of disease. Underlying factors are insufficient household food security, an inadequate childcare system and insufficient health services while the deeper-rooted basic factors relate to socioeconomic conditions [7]. Factors directly linked to food components

have been highlighted in many reports and have been reported to account for more than 75% of the deaths of infants and young children in Tanzania [12]. Also, childhood diseases such as malaria, diarrhea, acute respiratory infections, and measles aggravate the nutritional problems by impairing intake and utilization of nutrients, lowering the body's defense system thus predisposing the body to other infections and increasing requirements for other nutrients [12].

PEU and micronutrient deficiencies begin during weaning and/or immediately thereafter as most food used for weaning do not provide adequate amounts of energy, protein and micronutrients. The traditional weaning foods of Tanzania are based on starchy staples such as maize (*Zea mays*), sorghum (*Sorghum bicolor* L.), finger millet (*Eleucine coracona*), and rice (*Oryza sativa*) or non-cereals such as cassava (*Manihot esculenta*), round potato (*Solanum tuberosum*), sweet potato (*Ipomea batatas*), yams (*Dioscorea spp*) and plantains (*Musa paradisiaca sapientum*) and these foods have been widely associated with nutrient deficiencies among pre-school age children [13, 14]. Developing nutrient-dense, fully cooked, ready-to-eat, inexpensive weaning/ supplementary foods from the locally grown food ingredients using suitable household-level or small-to-medium scale production technologies in community settings has been strongly recommended as a viable and sustainable approach to address the problem of undernutrition in developing countries [15 – 22]. According to WHO [17], WHO/UNICEF [15], and Dewey and Brown [18] centrally processed, fortified and ready-to-eat low-cost foods would provide a reliable option for many families and can thus contribute immensely in ameliorating the problem of protein-energy and micronutrient undernutrition among older infants and young children in developing

countries. The food however, must be nutritionally sound to provide the essential amino acids needed for growth and the processing methods used should insure maximum retention of the essential nutrients in the food. Furthermore, the processing methods must result in products that are organoleptically acceptable to consumers. The products must also be stable during storage so as to allow ample time for transportation, storage, and marketing while still maintaining their nutritional and sensory wholesomeness [23. This study was conducted to determine the protein digestibility corrected amino acid scores of supplementary foods formulated for older infants and pre-school age children and the effect of various processing methods – extrusion, drum-processing and conventional cooking on the retention of essential amino acids. The study also evaluates the storage stability of the products processed by extrusion.

5.3 Materials and Methods

5.3.1 Product Formulation

Four supplementary products namely corn-bean-sardine meal (CBSM), bean meal (BM), sorghum-bean-sardine meal (SBSM) and rice-bean-sardine meal (RBSM) were formulated in the laboratories of the Department of Food Science and Human Nutrition, Michigan State University. Products were optimized to deliver greatest amino acid score and the desired amount of energy and fat according to the FAO/WHO Codex Alimentarius guidelines (CAC/GL 08-1991) for supplementary foods for older infants and young children [24]. The products were designed for enhancing growth and for nutrition rehabilitation interventions of pre-school age children. All the ingredients used i.e. rice (*Oryza sativa*), corn/maize (*Zea mays)*, dry beans (*Phaseoulus vulgaris*), sardines

(*Sardinops melanosticta*) and red palm-oil (derived from the mesocarp of the oil palm (*Elaeis guineensis*) are commodities that are inexpensive and readily available in Tanzanian markets.

5.3.2 Product Processing

The raw materials were processed into pre-cooked flour that could be reconstituted into porridge for child feeding as recommended by FAO/WHO Codex standard [24] for processed cereal-based foods for infants and children (CODEX STAN 74-1991). The dry beans, corn, sorghum and rice were sorted to remove extraneous materials, washed in distilled water, dried and milled into fine flours (mesh size 0.8 mm). The sun-dried small fish (sardines) were sorted to remove pebbles and other extraneous materials and washed in distilled water. The fish were thereafter cooked in boiling water for 30 min, then dried and ground into a fine powder. The basic ingredients were formulated into diets – corn-bean-sardine, bean flour, sorghum-bean-sardine and rice-bean-sardine. Each formulation was divided into four portions. The first, second and third portions of each product were processed by drum-processing, extrusion and conventional cooking, dried and ground into pre-cooked, ready-to-feed flour that could be reconstituted into porridge for child feeding as recommended by FAO/WHO [24] Codex standard for processed cereal-based foods for infants and children (CODEX STAN 74-1991). The fourth portion was analyzed unprocessed.

Extrusion of the composite foods was carried out in a small laboratory twin-screw extruder type APV-MPF-19 (APV Backer Ltd, Staffs, UK) with a barrel length/diameter ratio of 25. The following extrusion conditions were adopted: feed moisture content 12 –

14.4%, moisture injection – 9 – 15%, feed rate – 3.61 kg h^{-1}, screw-speed – 300 rpm, barrel temperatures – 70^{0}C (zone 1), 100^{0}C (zone 2), 127^{0}C (zone 3), 141^{0}C (zone 4), 131^{0}C (zone 5 - die), product temperature – 147^{0}C, die pressure – 350 psi, die diameter – 3 mm, motor load – 52.4% and mechanical energy input – 0.174 kw-hr kg^{-1}. Drum-processing was done by mixing the food ingredients with distilled water in a 25:75 solid: water ratio to form a thin slurry. The slurry was slowly poured between the two counter-rotating drums, which were heated by steam. The slurry spread on the surface of the slowly rotating drums to form a thin layer. High temperatures on the surface of the drums cooked and dehydrated the food swiftly. The cooked, dry food product was scraped off from the drum surface before they were recoated with fresh food slurry. The speed of the rotating drums was adjusted to increase/reduce product residence time on the drums. Likewise, the amount of steam in the drums was adjusted to regulate the cooking and drying process.

Conventional cooking of the food products followed the traditional Tanzanian cooking practices adapted to the laboratory conditions as described by Mosha and Svanberg [9] and Kikafunda et al. [25]. It involved preparing smooth slurry of the foods containing 10% solid matter and pouring the slurry into boiling water. The mixture was stirred constantly until boiling. The heat was thereafter reduced from high to medium and the porridge was allowed to simmer while maintaining gentle constant stirring until the desired cooking time was completed. The CBSM, SBSM and RBSM were cooked for 25 min each while the BM was cooked for 35 min. After cooking, the conventionally cooked foods were freeze-dried while the extruded and drum-processed foods were dried further in an air oven (set at 60^{0}C) for 3 h.

5.3.3 Diet Preparation for Protein Quality Evaluation

Four test diets were formulated to meet the FAO/WHO [24] Codex Alimentarius (CAC/GL 08-1991) guidelines (Table 1). A modified AIN 93G diet [26] was used as a control. A low protein diet (METDIET) was also prepared in which the casein in the control diet was replaced by cornstarch and 2 g of lactalbumin per 100 g of diet. The low protein diet was used to estimate the endogenous nitrogen excretion of the rats. Lipid content of the control, the test and the low protein diets was adjusted to 13% using a mixture of red-palm oil (10%) and corn oil (3%).

5.3.4 In vivo study

Growth Study: This study was conducted according to the AOAC [27] procedure 45.3.06. Male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), 21days old and weighing 35 – 50 g were housed individually in suspended stainless steel cages with wire bottoms. The temperature of the animal room was set at 22^0C and 40 – 60% relative humidity with alternating 12-hour periods of light and darkness throughout the study. The weanling rats were fed a stock diet for an acclimatization period of three days. Subsequently, the rats were weighed and those rats at the extremes of the distribution curve were excluded from the study. The remaining animals were randomly assigned into 13 groups of six animals each. The differences in mean weight between any two groups did not exceed 2 g. Twelve groups were assigned to experimental diets (four diets with each diet processed by either extrusion, drum-processing or conventional cooking). The 13^{th} group of rats was fed the modified AIN-93 control. Food and water were provided *ad*

Table 1: Composition (g kg^{-1}) of the cereal-bean based supplementary foods used for the *in vivo* rat feeding study[a]

	Diet[b]					
Ingredients	CTRL	METDIET	BM	CBSM	SBSM	RBSM
Beans	00.0	00.0	566.8	102.0	112.8	153.0
Corn	00.0	00.0	00.0	570.9	00.0	00.0
Rice	00.0	00.0	00.0	00.0	00.0	519.1
Sorghum	00.0	00.0	00.0	00.0	496.8	00.0
Sardines	00.0	00.0	00.0	73.8	73.8	73.8
Sucrose	20.0	20.0	20.0	20.0	20.0	20.0
Cornstarch	535.8	535.8	244.3	50.0	105.6	29.5
Cystine	02.3	02.3	00.0	00.0	00.0	00.0
Casein	152.2	00.0	00.0	00.0	00.0	00.0
Lactalbumin	00.0	20.0	00.0	00.0	00.0	00.0
Red Palm Oil	100.0	100.0	100.0	90.2	96.5	95.4
Corn oil	30.0	30.0	21.4	00.0	00.0	00.0
Fiber	112.2	112.2	00.0	45.6	47.0	61.7
Mineral mix	35.0	35.0	35.0	35.0	35.0	35.0
Vitamins mix	10.0	10.0	10.0	10.0	10.0	10.0
Choline Cl	02.5	02.5	02.5	02.5	02.5	02.5
BHT	00.014	00.014	00.014	00.014	00.014	00.014

[a] FAO/WHO [24] Codex standard for processed cereal-based foods for older infants and young children (CODEX STAN 74-1991).
[b] CTRL = modified AIN-93 diet; METDIET = Metabolic diet; BM = Bean meal; CBSM = Corn-bean-sardine meal; SBSM = Sorghum-bean-sardine meal; RBSM = Rice-bean-sardine meal

libitum. The animals were allowed to acclimatize to the test diets for three days before data collection started. Feed intake and animal weight were recorded daily for 21 days. Feces were collected from each rat on day 19 – 21. On day 22 one rat was randomly selected from each group to form a 14^{th} group. These rats were fed a standard AIN 93G diet for three days after which they received the low-protein – metabolic diet (Table 1) for seven days. Feces were collected on days 5 – 7 to estimate the metabolic fecal nitrogen. Fecal protein and metabolic fecal protein were determined using the protein

intake and fecal output data. The following protein quality index was calculated from the data collected: True protein digestibility = PI – (FP – MFP)/ PI x 100%;

where PI = protein intake, FP = fecal protein, MFP = metabolic fecal protein.

5.3.5 Preparation of Products for Storage Stability Study

Extruded CBSM and SBSM were formulated as shown in Table 2. The extruded and drum-processed CBSM and SBSM were used in the storage stability study. Uncooked CBSM and SBSM were used as controls.

Table 2: Composition (g kg^{-1}) of the extruded products used for evaluation of storage stability

	Diet[a]	
Ingredients	CBSM	SBSM
Bean	250.0	250.0
Corn	510.0	00.0
Rice	50.0	50.0
Sorghum	00.0	510.0
Sardines	65.0	65.0
Sucrose	55.0	55.0
Red Palm Oil	50.0	50.0
Vegetable oil	00.0	00.0
Mineral/Vitamin mix	10.0	10.0
Baking soda	05.0	05.0
Table salt	05.0	05.0
BHT+ BHA[b]	00.15	00.15

[a] CBSM = Corn-bean-sardine meal; SBSM = Sorghum-bean-sardine meal
[b] BHT = Butylated hydroxytoluene, BHA = butylated hydroxyanisole

5.3.6 Evaluation of Storage Stability

The extruded, drum-processed and uncooked CBSM and SBSM were packed and sealed in airtight polyethylene bags, with 500 g per bag. The food packages were stored in a

thermo-regulated room (simulating tropical temperature) with temperature set at 38^0C. The foods were stored at this temperature for up to 16 weeks. Sensory evaluation, total titratable acidity (TTA), pH and malondialdehyde (MDA) concentrations were determined in the food samples at baseline. Thereafter, a packet of each food sample and a control were removed from the stored lot at intervals of every two weeks and analyzed for TTA, pH and MDA concentration as in baseline.

5.4 Chemical Assays

5.4.1 Crude Protein and Amino Acid Profile

Feces collected in the balance period (last three days of the experiment) were separated from spilled food, dried to a constant weight at 100^0C and ground. Total nitrogen in the feces and diets was determined by Kjeldahl method [27] and the crude protein content was calculated using the factor of 6.25. The moisture content of food and fecal matter was determined by AOAC [27] procedure 925.09.

Amino acid concentrations (except tryptophan) were determined by *reverse phase* HPLC using the Pico Tag method [28]. For all amino acids except methionine, cystine/cysteine and tryptophan, food samples were hydrolyzed in 6 N HCl. The methionine and cystine/cysteine in foods were oxidized by performic acid to methionine sulfone and cysteic acid prior to hydrolysis by 6 N HCl. All amino acids (except tryptophan) were derivatized by phenylisothiocayanate (PITC) and detected at 254 nm. Tryptophan was analyzed by the ion exchange chromatographic method as described in the AOAC [27] method 988.15. Protein in the food was hydrolyzed under vacuum with 4.2 N NaOH. After pH adjustment and clarification, tryptophan was separated by ion

exchange chromatography (DC5A cation exchange resin) with measurement of the ninhydrin chromophore.

The essential amino acid profile of the test products was compared with the FAO/WHO/UNU [29] essential amino acid requirement pattern for pre-school age (2 – 5 year) children to compute the amino acid scores. The amino acid scores were used to compute the protein digestibility-corrected amino acid score (PDCAAS) values as follows: PDCAAS = True Digestibility x Lowest Amino Acid ratio [30, 31].

5.4.2 Thiobarbituric acid – malondialdehyde test

Lipid oxidation products in the foods were determined by the thiobarbituric acid – C_{18} solid-phase acid extraction method [32].

5.4.3 Total titratable acidity and pH

Ten g of the dry foods were reconstituted with 90 mL of ultrapure water into porridge. The TTA of the resulting porridge was determined by potentiometric titration using 0.1 M NaOH to pH 8.5 while stirring [33, 34]. Acidity was calculated as lactic acid equivalent as follows:

1 mL 0.1 M NaOH = 0.0090 g lactic acid

% Lactic acid = Titer x 0.0090 x 100/weight of sample.

The pH of the reconstituted foods was determined by the standard AOAC [27] procedure.

5.5 Statistical Analysis

Results are presented as mean and standard values. Data were subjected to one- and two-way analysis of variance (ANOVA) where applicable, using the ProStat

(Version 3.01; Poly Software International, Pearl River, N.Y. 10965) and a difference was considered to be significant at $p \leq 0.05$. Since the TPD values were in percent, the data were subjected to arc sine transformation [35] prior to analysis by ANOVA. *Post hoc* analysis of means was done by the Fisher's LSD test.

5.6 Results and Discussion

Table 3 presents the amino acid profile (g kg^{-1} crude protein) of the supplementary foods processed by extrusion, drum-processing and conventional cooking. Lysine concentrations in CBSM, BM, SBSM and RBSM were 90 – 98, 93 – 95, 83 – 96 and 91 – 95 percent, respectively, of the FAO/WHO/UNU [29] recommended amount for pre-school age children (58 g kg^{-1} crude protein). The concentrations of sulfur-amino acids (SAA = cysteine + methionine) in the CBSM, BM, SBSM and RBSM were 112 – 132, 60 – 76, 160 - 176 and 104 – 124 percent, respectively, of the FAO/WHO/UNU [29] recommendation (25 g kg^{-1} crude protein) while for tryptophan the concentrations in the various foods as a proportion of the FAO/WHO/UNU [29] recommendation (11 g kg^{-1} crude protein) were CBSM (91 – 100%), BM (118 – 136%), SBSM (109%) and RBSM (90 – 136%). The concentrations of threonine, (the most limiting amino acid) in the various foods as a proportion of the FAO/WHO/UNU [29] recommendation for pre-school age children (34 g kg^{-1} crude protein) were CBSM (76 – 91%), BM (76%), SBSM (71 - 82%) and RBSM (74 – 82%). These data suggest that all our composite supplementary foods were good sources of the essential amino acids as they contained close to or more than the recommended amino acid concentrations. Lysine, SAA, tryptophan and threonine are the most common limiting amino acids in plant-based

Table 3: Amino acid profile of the supplementary foods processed by various methods[1]

Food product[2] and Processing method	Amino Acids (g kg^{-1} crude protein)																	
	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Ile	Leu	Trp	Lys	Phe	SAA[3]	AAA[4]
CBSM																		
Extrusion	92	156	38	37	26	54	31	53	50	23	48	43	91	11	57	40	32	64
Drum-processing	84	154	38	36	28	52	26	53	50	25	47	42	92	10	52	40	28	65
Conventional	86	149	38	35	25	51	28	51	49	25	45	40	89	10	52	40	33	64
BM																		
Extrusion	119	144	47	34	28	57	26	39	35	27	51	46	78	13	55	50	17	77
Drum-processing	120	144	47	33	26	57	26	39	35	32	50	44	76	15	54	48	19	80
Conventional	116	141	47	33	27	55	26	38	34	25	50	44	75	13	54	48	15	73
SBSM																		
Extrusion	87	156	36	33	20	47	24	57	47	22	47	42	92	12	48	40	40	62
Drum-processing	103	173	41	37	25	54	28	63	51	28	53	47	101	12	56	45	44	73
Conventional	98	168	39	35	22	51	25	61	49	25	50	44	97	12	51	43	41	68
RBSM																		
Extrusion	93	151	39	40	27	65	28	50	37	25	53	44	79	15	55	42	31	67
Drum-processing	99	141	37	38	28	60	25	45	34	22	48	40	73	10	53	39	29	61
Conventional	94	151	40	37	24	64	27	49	37	24	51	43	78	13	55	41	26	65
FAO/WHO/UNU Reference[5]					19		34				35	28	66	11	58		25	63

[1] Amino acid values are means of duplicate analyzes. No significant variations ($p > 0.05$) in amino acid concentrations were observed among CBSM, BM, SBSM and RBSM processed by the various methods

[2] BM – bean meal, CBSM - corn-bean-sardine meal, SBSM – sorghum-bean-sardine meal, RBSM – rice-bean-sardine meal.

[3] SAA – sulfur containing amino acids – methionine + cysteine

[4] AAA - aromatic amino acids – phenylalanine + tyrosine

[5] FAO/WHO/UNU [29] essential amino acid reference pattern for pre-school age (2 – 5 years) children.

weaning/ supplementary foods in developing countries [36, 37]. Lysine, tryptophan and threonine are usually limiting in cereal grains while SAA are limiting in legumes. The combination of corn/sorghum (with relatively good concentration of SAA) with beans and sardines (rich in lysine) helped to increase the protein quality of the products through nutrient complementation. The concentrations of the essential and non-essential amino acids also were not significantly ($p > 0.05$) different among the foods processed by either extrusion, drum-processing or conventional cooking. This would suggest that none of the processing methods caused a significant loss of the total amino acids.

Table 4 presents the TPD and PDCAAS (expressed as percent) for the food products processed by the various methods. All the food products had relatively high TPD values ranging from 88 to 94%, 82 to90%, 86 – 91% and 89 to 91% in CBSM, BM, SBSM and RBSM, respectively. There were significant differences ($p \leq 0.05$) in the digestibility of the foods, with the casein diet being the most digestible (TPD = 95%). For all the foods, the highest TPD were observed in the extruded products. High TPD values in extruded products could be due to the high mechanical shear occurring during extrusion which in turn lead to molecular fragmentation and high protein digestibility. As a result of high TPD, extruded products also have better PDCAAS [38]. A two-way analysis of variance for the TPD values (Table 4) showed significant ($p \leq 0.05$) interactions between the food products and the processing methods that influenced the protein digestibility. In this case, extruded foods had the highest TPD values while the drum-processed foods, with an exception of CBSM, had the next highest TPD values. Food cooked by conventional method had the lowest TPD values, except in CBSM where TPD value was lowest in the drum-processed product. True protein digestibility is a key

Table 4. True protein digestibility (TPD) and protein digestibility-corrected amino-acid scores (PDCAAS) for the food products processed by the various methods[1]

Food Product[2]	Cooking Method	TPD%	PDCAAS%	LAA[3]
CBSM	Conventional	90.5[b]	74.6	Threonine
	Extrusion	93.7[a]	86.4	Threonine
	Drum-processed	88.4[b]	67.6	Threonine
BM	Conventional	82.4[b]	51.3	SAA[4]
	Extrusion	90.1[b]	60.4	SAA[4]
	Drum-processed	86.4[b]	65.1	SAA[4]
SBSM	Conventional	86.4[b]	63.7	Threonine
	Extrusion	91.4[ab]	64.0	Threonine
	Drum-processed	88.2[b]	72.9	Threonine
RBSM	Conventional	88.9[b]	67.1	Threonine
	Extrusion	91.4[ab]	75.8	Threonine
	Drum-processed	89.2[b]	64.3	Threonine
CONTROL	-	95.1[a]	84.3	Threonine

Source of Variation[5]	Mean TPD%	P-Value
Food Products		0.01
CBSM	90.9	
BM	86.3	
SBSM	88.7	
RBSM	89.8	
Cooking Methods		0.01
Extrusion	91.8	
Drum-processing	88.0	
Conventional	87.0	
Products x Cooking Interaction		0.05

[1] Values in a column with different superscripts are significantly different at $p \leq 0.05$.

[2] CBSM - corn-bean-sardine meal, BM – bean meal, SBSM – sorghum-bean-sardine meal, RBSM – rice-bean-sardine meal.

[3] LAA – limiting amino acid

[4] SAA– sulfur containing amino acids – methionine + cysteine

[5] A two-way analysis of variance for the TPD of the food products cooked by the various methods

food quality index because it indicates whether the amino acids present in a food protein would be digested and become available for utilization by the body [39]. Low protein digestibility limits the amount of amino acids in a food that can be absorbed into the body and can therefore adversely affect growth [39].

The TPD values observed in this study were higher than those reported for bean-based products by Sarwar et al. [40] and by Kannan et al. [41] In a study to evaluate protein and amino acid digestibility in food mixtures, Sarwar et al. [40] reported that, products containing beans usually have low TPD ranging from 70 – 85% which adversely affected the PDCAAS and overall utilization of nitrogen in the foods. The high TPD values observed in our food products containing beans could be due to the effect of grinding the raw beans into flour prior to cooking. In the studies by Sarwar et al. [40] and by Kannan et al. [41], the dry beans were cooked whole and ground to flour after cooking. Grinding of the beans prior to cooking might have disrupted the protein structure, thus increasing the degree of protein denaturation during cooking. This in turn could have rendered the protein fractions in the cooked foods more susceptible to hydrolytic enzymes. Low TPD of food products containing beans has been associated with the presence of strong covalent disulfide linkages between cysteine residues in some of the bean protein fractions e.g. globulins GII, glutelins and protease inhibitors, that form compact, dense, polymers that hinder proteolytic enzymes from reaching the internal catalytic sites [42]. Low TPD can also be associated with high dietary fiber, presence of phytohemagglutinins in foods, stearic hindrance of proteolysis by the carbohydrate moieties of the glycoproteins (especially in globulin GII, and protease inhibitor protein fractions) and presence of carbohydrate-protein and protein-protein

bonds that are not susceptible to mammalian protease hydrolysis [42]. The data reported here indicate that grinding raw beans and then cooking the flour overcomes many of the factors reported to limit protein digestion when whole beans are cooked.

The amino acid scores and PDCAAS for the food products were computed according to FAO/WHO [30] using the FAO/WHO/UNU [29] amino acid reference pattern for pre-school age (2 – 5 y) children (Table 3), and are presented in Table 4. The PDCAAS values were generally high, ranging from 68 – 86% (CBSM), 51 – 65% (BM), 60 – 73% (SBSM) and 64 – 75% (RBSM). Extruded CBSM (86%) and RBSM (75%) showed the highest PDCAAS while the conventionally cooked BM (51%) and extruded BM (60%) and SBSM (60%) displayed the lowest PDCAAS. The product that showed the highest average PDCAAS was CBSM (76%) followed by RBSM (69%), SBSM (66%) and lastly by the BM (59%). The PDCAAS index reflects the ability of the test protein to meet the protein needs of an individual. The FAO/WHO/UNU [29] recommends that the PDCAAS be > 60% to meet the amino acid needs of pre-school age children (2 – 5 years). Threonine was the most limiting amino acid in all the foods except in the extruded, drum-processed and conventionally cooked BM in which the sulfur amino acids – cysteine and methionine - were most limiting.

Table 5 data show the changes in TTA (g lactic acid per kg food) of the cooked and uncooked foods during the 16-week storage at elevated temperatures (38^0C). For both cooked and uncooked CBSM and SBSM, storage at elevated temperature did not result in a significant increase ($p > 0.05$) in TTA. For extruded products, TTA increased by 0.95-g kg^{-1} (5.4%) in CBSM and 4.47- g kg^{-1} (19.2%) in SBSM. For the drum-

processed products, TTA increased by 1.60- g kg^{-1} (5.9%) in CBSM and 1.38 g kg^{-1} (3.9%) in SBSM. The TTA in the uncooked foods during storage increased by 4.59 g kg^{-1}

Table 5. Changes in total titratable acids (as g lactic acid kg^{-1} food) of the cooked and uncooked foods during storage at 38^0C[1]

Period of storage (Weeks)	Extruded products		Drum-processed products		Uncooked	
	CBSM	SBSM	CBSM	SBSM	CBSM	SBSM
0	17.50	23.33	25.44	35.07	44.66	34.95
2	17.54	23.39	25.64	35.52	44.91	35.76
4	17.54	23.39	25.65	35.52	44.91	35.77
6	17.67	23.93	26.99	35.53	45.91	36.36
8	17.79	24.18	26.78	35.57	47.02	36.33
10	17.90	24.49	26.99	35.59	47.23	37.39
12	18.28	25.98	26.84	35.84	47.60	38.03
14	18.23	26.06	26.94	36.09	48.79	39.80
16	18.45	27.80	27.04	36.45	49.25	39.88

[1] No significant differences ($p > 0.05$) in total titratable acids were observed during storage at 38^0C. CBSM = corn-bean-sardine meal; SBSM = sorghum-bean-sardine meal.

(10.3%) in CBSM and 4.93- g kg^{-1} (14.1%) in SBSM. The insignificant changes in the TTA during storage were also reflected in stable pHs of the products (Table 6). For both cooked and uncooked products, there was no significant decrease ($p > 0.05$) in the pH during storage. The pH of the foods decreased by only 1.5 – 2.0% in extruded, 0.5 – 1.6% in drum-processed and by 0.6 – 1.0% in uncooked products. Since the TTA and pH values in the cooked and uncooked foods were similar ($p > 0.05$), it suggested that thermo-processing did not have an influence on the rate of food deterioration by acidification. The method and materials used to package the foods played a significant role in protecting the foods from deterioration. The moisture proof, airtight polyethylene bags used to package the foods excluded air and moisture from the foods, thus

minimizing lipid oxidation and fermentation. Oxygen and moisture are the major factors that catalyze lipid oxidation in food systems [43, 44]. An increase in TTA and a decrease in pH of foods could be a result of low-grade fermentation of the starch in the stored

Table 6. The pH in cooked and uncooked foods during storage at 38^0C[1]

Period of storage (Weeks)	Extruded products		Drum-processed products		Uncooked	
	CBSM	SBSM	CBSM	SBSM	CBSM	SBSM
0	6.63	6.61	6.46	6.37	6.25	6.35
2	6.60	6.53	6.44	6.30	6.24	6.30
4	6.55	6.55	6.45	6.32	6.23	6.32
6	6.53	6.53	6.42	6.32	6.22	6.32
8	6.51	6.52	6.41	6.30	6.22	6.30
10	6.50	6.54	6.42	6.29	6.20	6.31
12	6.51	6.52	6.42	6.28	6.21	6.31
14	6.51	6.51	6.41	6.26	6.20	6.30
16	6.50	6.51	6.43	6.27	6.21	6.29

[1] No significant decrease ($p > 0.05$) in pH was observed during storage at 38^0C. CBSM = corn-bean-sardine meal; SBSM = sorghum-bean-sardine meal

foods (due to moisture) to produce organic acids, which in turn lower the pH [33, 34]. Alternatively, a decrease in pH could be a result of lipid oxidation, which might have led to formation of complex secondary lipid oxidation products such as organic acids, alcohols, and hydrocarbons that increase food acidity and lower pH [43]. Increase in food TTA and/or decrease in food pH are food quality indices for deterioration. They adversely affect the food taste and overall acceptability [44].

Table 7 data show the changes in MDA concentrations ($\mu g\ kg^{-1}$) in the cooked and uncooked foods during the 16 wk elevated temperature storage. There was a significant ($p \leq 0.05$) increase in the concentrations of MDA for both the cooked and

uncooked foods during storage. The SBSM had highest MDA concentrations at both the baseline (Week 0) and during storage. For the extruded CBSM, MDA concentration increased by 178-μg kg^{-1} over the 16 wk period, while for the SBSM the MDA concentration increased by 306-μg kg^{-1} over the same period. For drum-processed

Table 7. Changes in malondialdehyde concentrations (μg kg^{-1}) in cooked and uncooked foods during storage at 38^0C[1]

Period of storage (Weeks)	Extruded products		Drum-processed products		Uncooked	
	CBSM	SBSM	CBSM	SBSM	CBSM	SBSM
0	208^{c}	595^{f}	615^{c}	1232^{e}	201^{e}	515^{e}
2	212^{bc}	609^{f}	630^{c}	1239^{e}	238^{de}	533^{e}
4	245^{b}	657^{e}	632^{c}	1246^{e}	267^{d}	650^{d}
6	260^{b}	692^{c}	626^{c}	1249^{e}	332^{c}	721^{c}
8	260^{b}	782^{b}	625^{c}	1281^{e}	388^{b}	796^{b}
10	268^{b}	792^{b}	627^{c}	1325^{d}	391^{b}	853^{a}
12	380^{a}	818^{b}	650^{b}	1609^{c}	393^{b}	863^{a}
14	383^{a}	843^{a}	665^{b}	1715^{b}	413^{b}	869^{a}
16	385^{a}	901^{a}	718^{a}	1822^{a}	520^{a}	872^{a}

[1] Malondialdehyde values in a column with similar superscripts are not significantly different at $p > 0.05$. CBSM = corn-bean-sardine meal; SBSM = sorghum-bean-sardine meal

CBSM, the MDA concentrations were stable for the first 6 weeks but increased significantly ($p \leq 0.05$) thereafter. In CBSM, the MDA concentration increased by 103-μg kg^{-1} and by 590-μg kg^{-1} in SBSM. For the uncooked CBSM, the MDA concentration increased by 319-μg kg^{-1} while the increase for SBSM was 357-μg kg^{-1}. These results suggest that both cooking and length of storage had a significant ($p \leq 0.05$) influence on the MDA concentrations.

MDA is a secondary product of lipid autoxidation and is often used as an indicator for deterioration of food quality. The concentrations of MDA observed in these test products are similar with those reported in others studies [45 – 47]. In a study of commercial infant milk formulas and infant formula powders, Cesa [45] reported MDA levels ranging between 163 – 362 μg kg^{-1} (milk formula) and 278 – 1094 μg kg^{-1} in infant formula powders. In a similar study involving milk powder samples, Botsoglou et al. [47] reported MDA concentrations ranging between 845 and 1572 μg kg^{-1} while unsweetened condensed milk and infant milk formula contained 782 and 2633 μg of MDA per kg, respectively. Draper et al. [48] reported that MDA in commercial foods ranged from < 100 μg kg^{-1} to about 10,000 μg kg^{-1} depending upon their fatty acid composition and conditions of storage. Although the concentrations of the MDA in our test products increased significantly during storage, the concentrations were lower than or within the normal ranges reported in commercial infants and young children foods. Therefore the small amounts of MDA even after 16-week storage at 38 ^{0}C would not be expected to pose health risks. The effect of long-term consumption of low concentrations of MDA by humans and/or animals is still unknown. However, long – term consumption by experimental animals of large amounts of MDA (~ 10,000 μg kg^{-1} per day) was found to cause hepatic nucleotoxicity, liver lesions, liver cancer and increased mortality [48, 49].

Significant correlation exists between TBA-MDA concentrations and off-flavor formation in cooked foods [50 – 52]. In a study by Poste et al. [50], a significant relationship was found between MDA concentrations and sensory aroma scores whereby sensory scores corresponding to MDA concentrations of 7,830 – 10,000 μg kg^{-1} were associated with weak off-flavor while the sensory scores corresponding to MDA

concentrations > 10,600 μg kg^{-1} were associated with strong rancid flavor. In our study, the concentration of MDA in test products ranged between 208 - 718 μg kg^{-1} (CBSM) and between 595 - 1,822 μg kg^{-1} (SBSM). These MDA concentrations were lower than the levels (> 10,600 μg kg^{-1}) that can cause detectable rancidity.

Conclusions

These data show that both extrusion and drum-processing can be used to produce highly nutritious products suitable for supplementation of pre-school age children. The protein in the cereal-bean-sardine mixtures was highly digestible and the PDCAAS exceeded the minimum value set by the FAO/WHO. Extruded CBSM and SBSM were shelf-stable for at least 16 weeks when stored at 38^0C in airtight polyethylene bags. Development and consumption of such nutritious composite foods would be expected to help ameliorate the problem of undernutrition among pre-school age children in developing countries.

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CHAPTER SIX

6.0 SUMMARY AND CONCLUSIONS

This study focused on the problem of undernutrition among pre-school age children in Tanzania and the role that supplementary foods could play in ameliorating these conditions and thus increase quality of life and child survival. The study was conducted based on the WHO/UNICEF [1] recommendation that production of ready-to-feed fortified supplementary foods using locally produced ingredients at community settings is the most practical and sustainable approach to mitigate the problem of undernutrition in developing countries. The objectives of the study were to:

1. Formulate nutrient-dense products from locally produced ingredients such as corn, beans, sorghum, rice and sardines to meet the FAO/WHO/UNU [2] amino acid recommendations for cereal-based weaning/supplementary foods.
2. Determine suitable processing conditions for preparation of ready-to-feed "instant" products using extrusion and drum-processing methods.
3. Evaluate the nutritional quality of the processed products including:
    i) amino acid profile.
    ii) true protein digestibility.
    iii) protein digestibility-corrected amino acid scores (PDCAAS).
    iv) residual levels of phytohemagglutinins; anti-nutritional factors (trypsin, chymotrypsin, and α-amylase inhibitors); and evaluate cooking doneness (starch gelatinization rate and residual urease activity).
4. Evaluate the potential of the supplementary foods to support optimal growth and rehabilitation of undernourished children using an animal model.

5. Evaluate the storage stability of the products under simulated tropical conditions.

The first study was conducted to evaluate the protein quality and growth/rehabilitation potential of supplementary foods developed from locally produced materials in Tanzania. Six trial diets namely rice meal (RM), rice-bean meal (RBM), rice-bean-sardine meal (RBSM), bean meal (BM), corn-bean meal (CBM) and corn-bean-sardine meal CBSM) were processed by a drum-drier. The diets were formulated to maximize the amino acid scores as recommended by the FAO/WHO/UNU [2] for pre-school age children and evaluated for protein quality – true protein digestibility, PDCAAS and net protein retention ratio. They were also evaluated for their potential to support growth and for rehabilitating undernourished animals. The study showed that composite diets containing cereal-bean-sardine had high net-protein retention ratio, true protein digestibility, and PDCAAS. The composite products also showed potential to support growth during normal and rehabilitation of undernourished animals. Overall, the cereal-bean-sardine composite products were of highest quality and displayed good potential for use as supplementary and rehabilitation foods for pre-school age children (2 – 5 y) as well as adults.

Study two evaluated the safety and nutritional quality of the composite foods processed by extrusion and drum-processing. The study was based on the concept that the cereal-bean-sardine composite foods contain 20 – 30% beans, which contain high amounts of phytohemagglutinins toxins and anti-nutritional factors – trypsin, chymotrypsin and a-amylase inhibitors. Consumption of active phytohemagglutinins in foods interferes with the function of the gastrointestinal tract by binding to the brush-border cells of the intestinal mucosa and thus disrupting absorption of all nutrients.

Active phytohemagglutinins also cause hyperplasia and hypertrophy of the pancreas [3, 4, 5] thus disrupting secretion of other digestive enzymes. Likewise, consumption of active inhibitors of trypsin and chymotrypsin interferes with the activity of the pancreatic proteolytic enzymes particularly trypsin, and chymotrypsin leading to poor digestion of protein and enlargement of the pancreas. They also cause an increase in the secretion of digestive enzymes including trypsin, chymotrypsin and elastase, which in turn increase the loss of endogenous amino acids in the form of the secreted enzymes. These effects on the other hand depress growth and interfere with other metabolic processes of the body [6]. Alpha-amylase inhibitors reduce digestibility of starch, limiting its utilization by the body. Raw starch is also poorly digested and its presence in processed ready-to-feed foods may cause gastrointestinal illness. For these reasons, the food processing methods used must produce products that are fully cooked, free of active phytohemagglutinins and anti-nutritional factors.

Four composite food products, namely CBSM, BM, RBSM and sorghum-bean-sorghum meal (SBSM) were formulated according to the FAO/WHO/UNU [2] guidelines. The food mixtures were processed by extrusion, drum-processing and also cooked by conventional method. Cooking doneness of the processed foods was evaluated by the rates of starch gelatinization, and urease enzyme inactivation. Destruction of phytohemagglutinins and anti-nutritional factors – trypsin, chymotrypsin and α-amylase inhibitors were also determined. The final assessment of safety was done by using animal model to evaluate true protein digestibility and potential to support growth. The study showed that, foods were well-cooked for both extruded and drum-processed foods. Starch gelatinization was $\geq$ 90% while the residual urease activity was $\leq$ 0.05 units per 100 g of

processed food. Extruded products had higher ($p \leq 0.05$) true protein digestibility and produced greater growth when fed to experimental animals compared to the drum-processed foods. Both extrusion and drum-processing were equally effective in destroying the phytohemagglutinins and the anti-nutritional factors. The study suggested that, extrusion of cereal-bean-sardine composite foods result in products of nutritional quality similar to that of conventionally cooked foods but slightly higher than that of drum-processed foods. Both processing methods however, produced foods meeting the required nutritional quality. Availability of processing equipment and energy costs would therefore be the major factors dictating the ultimate method of choice for processing of these foods in developing countries.

Study three was based on the concept that foods produced to ameliorate undernutrition must provide protein of high quality, and must have reasonable shelf-life. This study 1) evaluated the amino acid profile of the processed cereal-bean-sardine composite products, 2) determined protein quality by using the FAO/WHO [7] approved – PDCAAS, and 3) evaluated the storage stability of the processed products at simulated tropical conditions. Results showed that extruded products had higher ($p \leq 0.05$) PDCAAS than the drum-processed foods. No significant variations in the amino acids concentrations were found among foods processed by either extrusion or drum-processing. Threonine was the most limiting amino acid in the CBSM, RBSM and SBSB while sulfur amino acids – cysteine and methionine - were limiting in the BM. The processed cereal-bean-sardine composite products were shelf-stable for at least 16 weeks at 38^0C.

Overall, the following conclusions could be drawn.

1. Cereal-bean-sardine composite products formulated from locally produced ingredients are of high nutritional quality, with potential for use as supplementary and rehabilitation foods for undernourished pre-school age children.
2. High quality, fully cooked, ready-to-eat foods may be produced by either extrusion or drum-processing. Both processing methods produced food products meeting the required nutritional quality.
3. Composite cereal-bean-sardine products have protein that is highly digestible, with PDCAAS values equal or higher than the FAO/WHO/UNU [2] recommended score of $\geq$ 60% for pre-school age children.
4. The processed cereal-bean-sardine composite products are shelf-stable for at least 16 weeks when stored at elevated temperature – 38^0C.

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CHAPTER SEVEN

7.0 RECOMMENDATIONS FOR FUTURE RESEARCH

1. Evaluation of the efficacy of the processed composite foods to support normal growth and rehabilitation of undernourished children was done by using an animal model. It is recommended that a clinical study using human subjects be conducted to re-evaluate the food products. This will help to corroborate the results from the current study before recommending the products for large-scale use.

2. This study successfully developed the processing conditions for the cereal-bean-sardine composite products. It is recommended that similar studies be conducted for other staple foods such as pearl-, finger- and bulrush-millet, cassava, yams, sweet potatoes, peanuts, lentils and other legumes which are widely available but underutilized for child feeding in Tanzania. The study should also investigate on the feasibility of incorporating sun-dried vegetables and fruits in the composite products to increase the mineral and vitamin density.

3. There is currently a new generation of *very low-cost extruders* [1] and simple food texturizers that are very appropriate for rural settings. Such simple food processors have the advantage that they are manual or semi-automated and can use conventional and/or non-conventional sources of energy such as charcoal, firewood, electricity and/or kerosene. Most of the low-cost extruders and texturizers, however, are still on the trial stages and the quality of the foods processed by these equipments is not clearly known. It is recommended that, studies be conducted to determine if the quality of foods produced

in these small scale equipments meet the required nutritional and safety quality for ready-to-feed products for young children.

4. For sustainable production of these products there should be a good market structure that allows efficient distribution of the foods. It is recommended that, a marketing study be conducted to establish the consumer preferences, market prices and the appropriate marketing channel to reach our target populations in the rural communities. The study should also try to do some cost-benefit analysis of the products, recommend appropriate market prices for the products and identify the potential players for the production and distribution of the foods.

7.1 Reference

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