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# **Sweetpotato-based complementary food for infants in Ghana**

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A thesis presented in fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Human Nutrition



Institute of Food, Nutrition and Human Health

Massey University

Palmerston North, New Zealand.

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Title: Sweetpotato-based complementary food for infants in Ghana

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*“We are guilty of many errors and many faults, but our worst crime is abandoning the children, neglecting the foundation of life. Many of the things we need can wait. The child cannot. Right now is the time his bones are being formed, his blood is being made and his senses are being developed. To him we cannot answer “Tomorrow”. His name is “Today”.” Gabriela Mistral, 1948.*

To my

Prudent wife

Flora

And our angels

Mawutor

Mawuena

Makafui



## **Abstract**

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**Background:** In an effort to reduce the prevalence of protein-energy malnutrition among Ghanaian infants after the period of exclusive breastfeeding when complementary food (CF) is introduced, Weanimix, which is a blend of non-dehulled maize, groundnut and non-dehulled soyabean/cowpea, was introduced in Ghana, in 1987 through collaboration between the Nutrition Division, Ministry of Health, Ghana and the United Nations Children's Fund. Weanimix is an improvement over traditional maize-, millet-, or sorghum-only porridge in protein and energy densities, but it is high in phytate. Phytate limits the bioavailability of nutrients such as iron and zinc, and probably calcium and some proteins. Also, unless fortified, Weanimix is low in  $\beta$ -carotene (vitamin A precursor) as the white maize is the commonly consumed variety in Ghana. Additionally, cereal-based CF (example, Weanimix) forms a very thick porridge that requires dilution with water to get the desirable viscosity, leading to "energy and nutrient thinning" (that is, the reduction of energy and nutrient densities). Thus, the widely used unfortified cereal-based CF could be a major contributing factor to the persistently high occurrence of vitamin A, iron and zinc deficiencies among infants in sub-Saharan Africa. **Purpose:** The main focus of this study was to develop a CF using locally accessible ingredients in Ghana that will be low in phytate, contain measurable levels of  $\beta$ -carotene and forms a low viscous porridge, which could be produced at home or industrially. **Method:** A computer-assisted programme (Nutrition Calculator) obtained from Global Alliance for Improved Nutrition- Infant and Young Child Nutrition Programme was used to formulate composite flours containing sweetpotato, soyabean and fish powder from anchovies or skim milk powder to meet the protein, fat and energy specifications in the Codex standards (CAC/GL 8 and STAN 074-1981, Rev.1-2006) for CF. The household-level ComFa formulation with fish powder as an ingredient was toasted in an oven and denoted oven-toasted ComFa, while the industrial-level formulations (roller-dried ComFa and extrusion-cooked ComFa) had skim milk powder (a common ingredient in industrial-processed dry infant cereal) as a component and were produced using a roller drier or an extruder. The nutritional, functional and consumer acceptance analyses of sweetpotato-based CFs were carried out and compared with enriched

Weanimix. In this research, Weanimix was slightly modified by using dehulled maize and soyabean flours, and further addition of 17% fish powder and 0.50% sugar to the basic formulation, and referred to as enriched Weanimix. Additionally, the stability of  $\beta$ -carotene in the oven-toasted ComFa was evaluated under simulated average temperature of 32°C and 85% relative humidity, mimicking the ambient conditions of Ghana. **Results:** The ComFa formulations and the enriched Weanimix met the stipulated energy and fat values specified in the Codex standards. However, the protein content of the industrial ComFa formulations was lower by 17%, but the oven-toasted ComFa and enriched Weanimix met the protein specification. The sweetpotato-based formulations had total dietary fibre that was about twice the Codex specification of less than 5.0%, but was likely to be partly soluble fibre and, thus beneficial. The phytate content in all the ComFa formulations was approximately a quarter of the level of 0.80 g/100 g in the enriched Weanimix. Only the sweetpotato-based infant foods contained measurable levels of  $\beta$ -carotene, resulting in significantly higher vitamin A content of the oven-toasted ComFa compared with enriched Weanimix (28.80 vs. 1.20  $\mu$ g retinol equivalents/100 kcal). Most of the  $\beta$ -carotene in the oven-toasted ComFa was retained for up to eight weeks when stored in containers with a good moisture barrier under simulated ambient conditions of Ghana. Oven-toasted ComFa, roller-dried ComFa and enriched Weanimix, using an estimated daily ration of 40 g (dry weight), contained less than half of the World Health Organization recommended levels for calcium (400 mg/day), iron (9.3 mg/day) and zinc (4.1 mg/day) from CFs processed for 6 to 8 month-old breastfeeding infants, with the exception of the oven-toasted ComFa, which contained 60% of the recommended calcium level. The two selected ComFa formulations are likely to be less inhibitory regarding calcium, iron and zinc absorption by infants than the enriched Weanimix based on the phytate: calcium, iron and zinc molar ratios, and the level of  $\beta$ -carotene to predict relative availability of these essential minerals. All the CFs had phytate: calcium molar ratios lower than the maximum recommended ratio of 0.17, but their phytate: iron molar ratios exceeded the maximum recommended ratio of 1.0. However, the phytate: zinc molar ratio of the oven-toasted ComFa and roller-dried ComFa were approximately lower by 24% than the recommended

ratio of 15. In contrast, the phytate: zinc ratio of enriched Weanimix was higher by 53% compared to the recommended ratio. Also, the ComFa formulations and enriched Weanimix, had levels of total polyphenols (84.70 and 76.96 mg/meal, respectively), which may inhibit iron absorption. On average, sweetpotato-based formulations were higher in maltose (26 times), sucrose (5 times), free glucose (19 times) and fructose (7 times) than levels in enriched Weanimix, but the ComFa formulations contained significantly less starch (10-13 vs. 47 g/100 g). The high simple sugar and low starch levels in the ComFa formulations could explain the lower apparent viscosity (9-, 13- and 20-times, for peak, “consume” and final viscosities), higher water solubility index (7 times), and higher consumer acceptance compared with the maize-based formulation.

**Conclusion:** On the basis of the compositional, functional and sensory findings of this study, the sweetpotato-based formulations have significant advantages as complementary food compared with Weanimix due to the low level of phytate, the high levels of endogenous  $\beta$ -carotene and low viscosity. Importantly, the ingredients used to produce the household-level ComFa formulation could be easily accessed by caregivers in Ghana. However, there is a need to conduct field trial and consumer acceptance studies in Ghana before substantive recommendations on the use of the sweetpotato-based infant formulation could be made. **Limitation:** The conclusions made are based on compositional, functional and consumer acceptance studies but not on any in vivo experiment or randomised feeding trial among Ghanaian infants. However, because the comparisons were made between the ComFa formulations and Weanimix, the conclusions drawn in this thesis are relevant.

**Key words:** Carotene, Complementary/infant food, Ghana, Maize, Phytate Sweetpotato, Vitamin A

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## **List of papers**

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The thesis is based on papers either published, under review or yet to be submitted to a journal.

- Paper I. Development of sweet potato-soybean blend, an alternative to maize-legume mix as complementary food for infants in Ghana. (Conference proceedings of the Nutrition Society of New Zealand (Inc.), Vol 34, 2010).
- Paper II. Complementary food blends and malnutrition among infants in Ghana—a review and a proposed solution. (Scientific Research Essays, 7(9), 2012).
- Paper III. Sweetpotato-based complementary food for infants in low-income countries. (*Food and Nutrition Bulletin*, 33 (1), 2012).
- Paper IV. A household-level sweetpotato-based infant food to complement vitamin A supplementation initiatives. (*Maternal and Child Nutrition*, 8(4), 2012).
- Paper V.  $\beta$ -carotene retention in sweetpotato-based complementary food stored in different containers under simulated tropical temperature and humidity. To be submitted to the *Journal of Science of Food and Agriculture*.
- Paper VI. Sweetpotato-based complementary food would be less inhibitory on mineral absorption than a maize-based infant food assessed by compositional analysis. (*International Journal of Food Sciences and Nutrition*, DOI: 10.3109/09637486.2012.687368).
- Paper VII. Carbohydrate composition, viscosity, solubility and sensory acceptance of sweetpotato- and maize-based complementary foods. Under review in *Food and Nutrition Research*. (Manuscript ID: 18717).

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*International Journal of Food Sciences and Nutrition*. Copyright © 2012, Informa Healthcare (Paper V).

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## **Chapter 1: General Introduction**

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### **1.1 Thesis layout**

The chapters of this thesis are manuscripts under review or published in scientific journals.

This current chapter, chapter 1, describes the layout of thesis and introduces the problem (extensively considered in chapter 2) that needs to be addressed and ends with the study objectives. The first attempt of formulating and processing a household-level sweetpotato-based complementary food is included as Paper I in this chapter.

Chapter 2 is a literature review on the types of complementary foods available in Ghana. The focus is on cereal-based blends (example, maize-soyabean blend) and not on either maize, millet or sorghum only infant food. This chapter ends with a proposed alternative complementary food based on sweetpotato (*Ipomoea batatas* [L.] Lam).

Processing of household- and industrial-level sweetpotato-based complementary foods (oven-toasted ComFa, extrusion-cooked ComFa and roller-dried ComFa), their macronutrients (protein, fat and carbohydrate) composition, total dietary fibre and phytate in comparison with a cereal-legume blend referenced Weanimix (Agble, 1997; Lartey, Manu, Brown, Peerson, & Dewey, 1999) are presented in Chapter 3. In this work, Weanimix was slightly modified and denoted enriched Weanimix. The version of this cereal-legume blend considered in Paper I did not contain fish powder.

Chapter 4 considers the possible contribution of the oven-toasted ComFa to complement vitamin A supplementation initiatives, which is an effort to reduce the prevalence of vitamin A deficiency in low-income countries. The potential is in terms of the level of vitamin A and its stability under simulated temperature of 32°C and relative humidity of 85%.

The availability of calcium, iron and zinc from the ComFa formulations and enriched Weanimix, based on compositional analysis, is examined in chapter 5. Highlights of data on an *in vitro* digestion/Caco-2 model iron uptake study to

estimate iron availability from the complementary food formulations will be included at the end of this chapter.

In chapter 6, the focus is on other findings of nutritional significance: carbohydrate composition, functional properties such as viscosity and water solubility index, and consumer acceptability of the sweetpotato-based and the maize-based complementary food.

A general summary and conclusion based on all the findings of this thesis are outlined in chapter 7.

In chapter 8, the limitations, recommendations and future perspectives are highlighted.



## **1.2 Background**

Chronic micronutrient deficiencies such as vitamin A, zinc and iron deficiencies during early childhood may increase susceptibility to infections, stunted growth or blindness and, may have irreversible effects on cognitive development (Black et al., 2008; Grantham-McGregor et al., 2007). These nutritional deficiencies subsequently lead to growth faltering and/or death and limit an individual from achieving his/her full potential during adulthood; they have been associated with the intergenerational transmission of poverty in low-income countries (Atinmo, Mirmiran, Oyewole, Belahsen, & Serra-Majem, 2009; Grantham-McGregor et al., 2007; Victora et al., 2008). Black et al. (2008) identified vitamin A and zinc deficiencies as having the largest disease burdens among all of vitamins and minerals deficiencies.

Childhood malnutrition in sub-Saharan Africa is among the worst in the world (UNICEF, 1998). Vitamin A deficiency (VAD) among children <5 years in sub-Saharan Africa remains high (44%), second to South-East Asia (50%), compared with the worldwide occurrence of 33% [World Health Organization (WHO), 2009]. The reason could be the widely used cereal-legume blends as complementary foods in low-income countries (Gibbs et al., 2011), which are naturally low in provitamin A/ $\beta$ -carotene (Dewey & Brown, 2003; Lartey, Manu, Brown, Pearson, & Dewey, 1998), and high in phytate (Gibson, Bailey, Gibbs, & Ferguson, 2010). Phytate forms insoluble complexes with some micronutrients (Gibson & Hotz, 2001; Greiner & Konietzny, 2006; Kumar, Sinha, Makkar, & Becker, 2010) and, because the intestinal phytase activity in humans is low (Sandberg & Andersson, 1988; Weaver & Kannan, 2002), hydrolysis of such complexes to release the bound nutrients is limited.

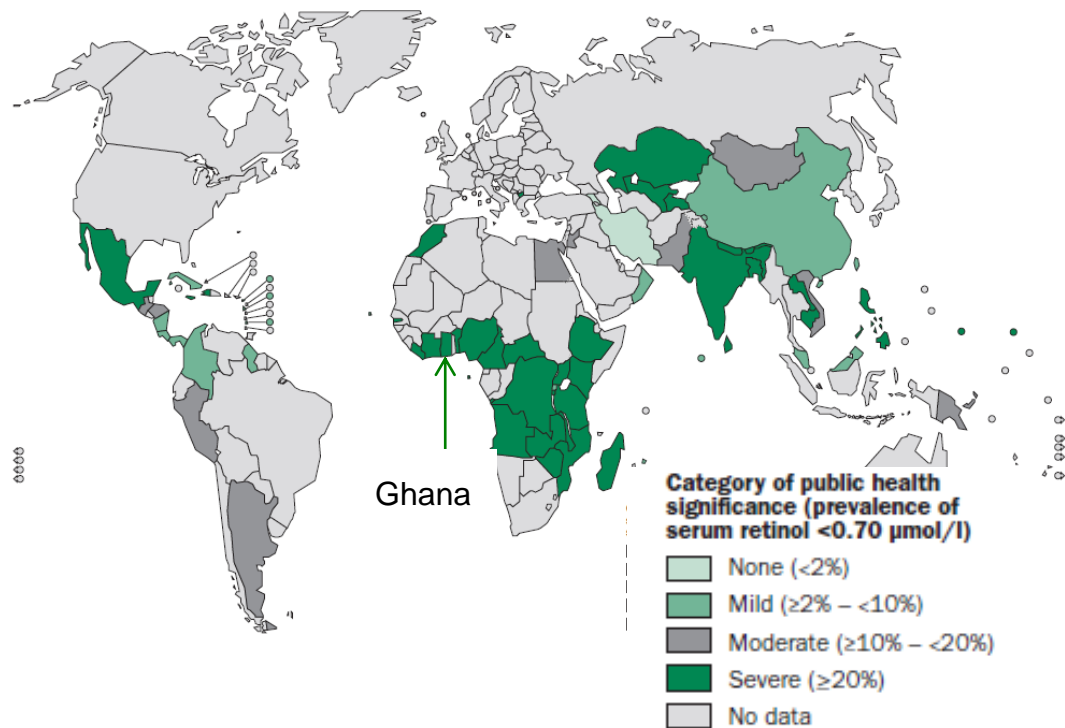
Ghanaian infants are particularly vulnerable to nutritional deficiencies when complementary foods are introduced as documented in Ghana Demographic Health Survey (GDHS) Reports [Ghana Statistical Service (GSS), Ghana Health Service (GHS), & ICF Macro, 2009; GSS, Noguchi Memorial Institute for Medical Research (NMIMR), & ORC Macro, 2004]. Complementary foods are mostly processed from white maize (*Zea mays*), pearl millet (*Pennisetum glaucum*) or sorghum (*Sorghum bicolor*), with or without the addition of a leguminous food crop, example, soyabean (*Glycine max*), groundnut (*Arachis*

*hypogaea*) or cowpea (*Vigna unguiculata*) based on the findings from the literature review presented in Chapter 2 (Paper II) of this thesis. White maize, the preferred choice of maize for human consumption in Africa (Nuss et al., 2012), is devoid of  $\beta$ -carotene (Nuss & Tanumihardjo, 2010). Therefore, the major cause of nutritional deficiencies among infants in Ghana could be the complementary foods given to them.

The GDHS reported that the occurrence of anaemia among 6-11 mo old infants ranged from 75-88% based on haemoglobin concentration of  $\leq 100$  g/L as the cut-off (GSS et al., 2004 & 2009). Similarly, the occurrence of vitamin A deficiency (VAD) (**Figure 1.1**), using serum retinol level of 0.70  $\mu\text{mol/L}$  as the cut-off, was 76% among children under five years (WHO, 2009).

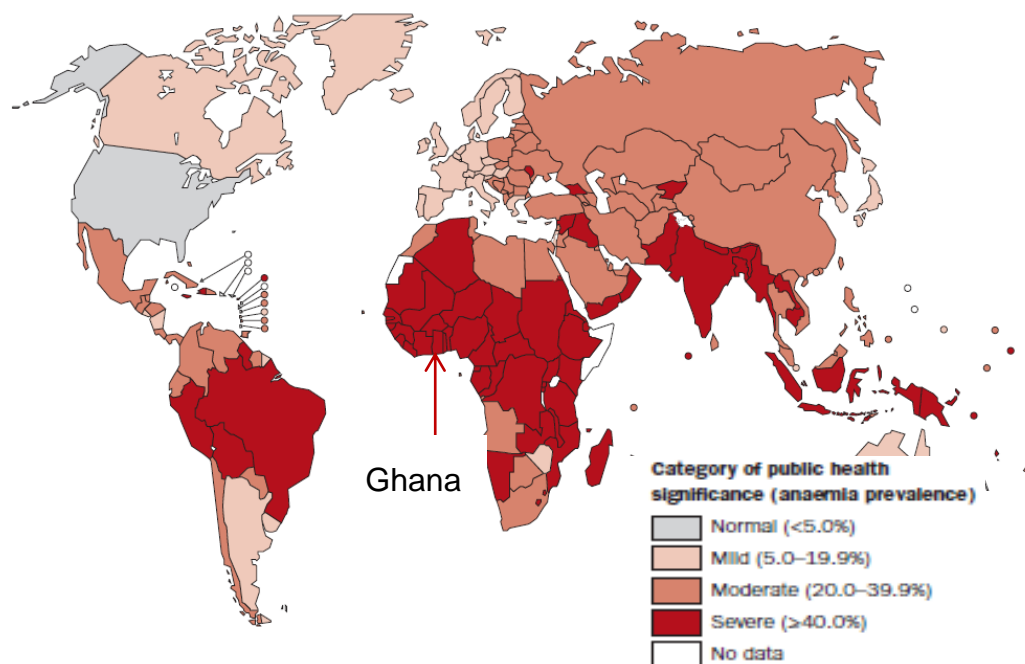
The low level of vitamin A intake (Dewey & Brown, 2003; Lartey et al., 1998; Nuss & Tanumihardjo, 2010) is the major contributing factor to the high occurrence of VAD among infants in sub-Saharan Africa (**Figure 1.1**). Also, the high levels of phytate in the cereals and legumes (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2002; Lukmanji et al., 2008) commonly used, coupled with the traditional lack of variety in complementary foods, can be associated with the prevalence of anaemia in Ghana (**Figure 1.2**). The limitations of some of the suggestions to reduce the level of phytate in complementary foods are considered in chapter 2. VAD and anaemia prevalence among infants and young children are both classified as being of “severe public health significance” (de Benoist, McLean, Egli, & Cogswell, 2008; WHO, 2009).

In addition to the low vitamin A and high phytate contents of household-level cereal-legume blends, another nutritional disadvantage is their high viscosity when not produced from malted cereals (Mosha & Svanberg, 1983; Mosha & Svanberg, 1990). Suitable viscosity of cereal-based porridge is obtained by dilution with water, consequently, leading to “*energy and nutrient thinning*”, that is, the reduction of energy and nutrient densities (Kikafunda, Walker, & Abeyasekera, 1997; Mosha & Svanberg, 1983; Mosha & Svanberg, 1990).



**Figure 1.1** Global prevalence of vitamin A deficiency (based on serum retinol <0.70 µmol/l) in populations at risk 1995–2005

Source: WHO (2009)



**Figure 1.2** Worldwide prevalence of anaemia (based on haemoglobin concentration of <110 g/l) 1993–2005

Source: de Benoist, B., McLean, E., Egli, I., & Cogswell, M. (Eds.). (2008)

Using micronutrient fortification/supplementation to improve infant nutriture in developing countries like Ghana is impractical, because micronutrient preparations cannot be produced at the household level from available local resources and there is a lack of commitment by policy makers to ensure their provision at a subsidised price. Hence, the availability of micronutrient supplements undesirably ends with the nutritional trials they are designed for. Vitamin and mineral supplements, if made affordable or available for resource-poor households, remain the most efficacious way of improving the nutritional status of infants in developing countries. Until vitamin and mineral supplements, lipid-based nutrient supplement or sprinkles are affordable in developing countries through government subsidies, dietary diversification (example, food-to-food fortification) is the key to improving nutritional status of infants in low-income countries (Chakravarty, 2000; Gibson et al., 2000) as this approach can easily be replicated at the household-level.

Therefore, complementary food made from coloured\* varieties of sweetpotato to process complementary food as an alternative infant food, will be naturally high in provitamin A carotene (Hagenimana et al., 2001; Ofori, Oduro, Ellis, & Dapaah, 2009; Ssebuliba, Nsubuga, & Muyonga, 2001) and low in phytate [Food and Agriculture Organization (FAO), 1990; Lung'aho & Glahn, 2009a; Phillippy, Bland, & Evens, 2003]. This alternative complementary food will meet the WHO guideline of daily consumption of vitamin A-rich food by infants [Pan American Health Organization (PAHO) & WHO, 2003]. Thus, an alternative complementary food, which would naturally be high in  $\beta$ -carotene, low in phytate and forms a low viscous porridge, could contribute significantly to improving the nutritional status of infants in sub-Saharan Africa countries such as Ghana; hence the relevance of this thesis.

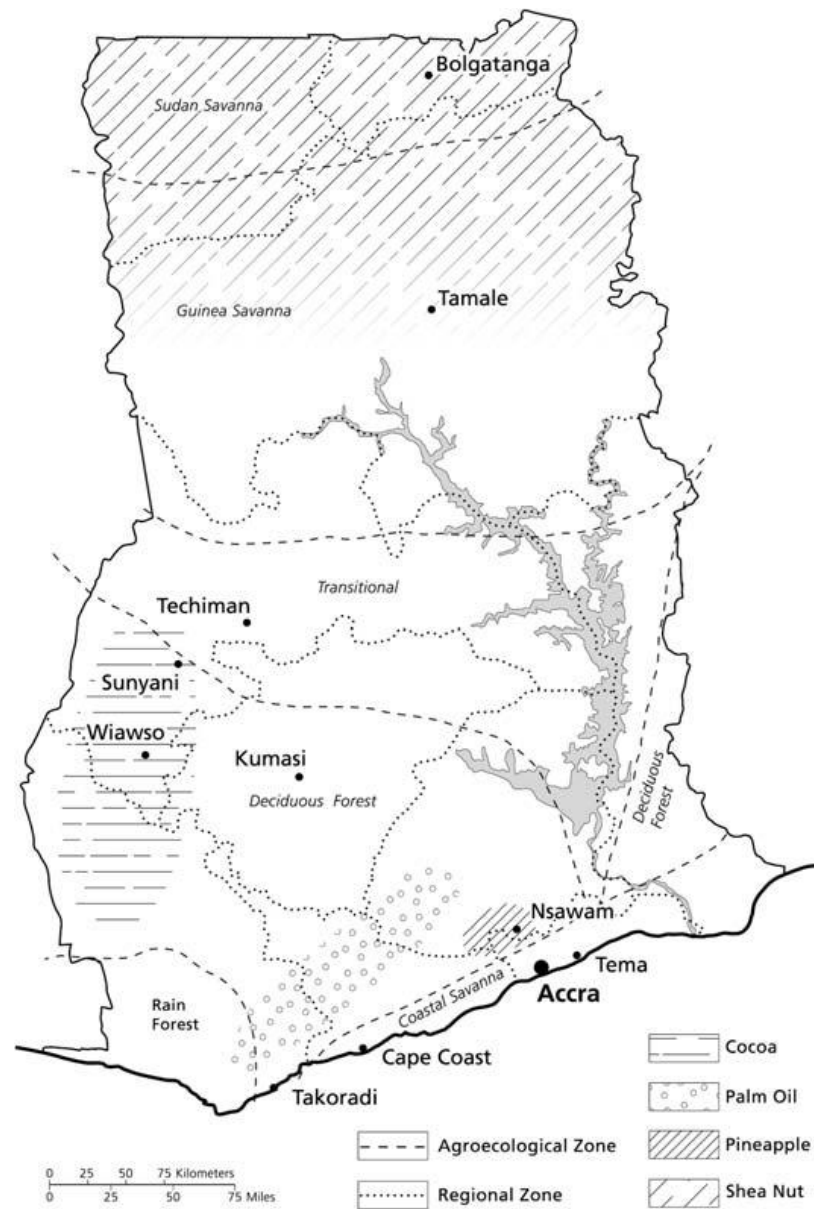
### **1.3 Crop production in Ghana**

Ghana is located on the southern coast of West Africa, between latitudes 05° 35'N and longitudes 00° 15'W with a land surface area of 238,535 km<sup>2</sup> (Wikipedia contributors, 2009). The economy is said to be driven by agriculture, but this sector's potential has not been fully harnessed, making most people in

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\*Referred to orange-, red- or cream-fleshed variety but not the white-fleshed variety.

the rural communities who are farmers, very poor. The country can be classified into 6 major agro-ecological zones on the basis of climatic conditions, namely, rain forest, coastal savannah, deciduous forest, transitional, Guinea savannah and Sudan savannah (Fold, 2008) (**Figure 1.3**). The transitional zone is the bridge between the forest and the savannah belts. Ghanaians cultivate varieties of crops and the types of crop cultivated depend on the location. The crops cultivated (**Table 1.1**) can broadly be divided into two major groups; namely food crops- for household consumption and local market, which comprise of cereals, roots, tubers, legumes, vegetables, fruits, spices, etc, and industrial or cash crops- cocoa, coffee, palms, fibres, sugar cane, rubber, etc mainly for export (Abbiw, 1990). Cocoa is the main export crop. In addition, fruits and vegetables like papaya, mango, chillies and pineapple are also cultivated for export. The eastern part of the Sudan savannah zone is noted for growing vegetable (tomato, garden eggs, lettuce, carrots, chillies, onions) (Fold, 2008) and sweetpotato (Dittoh, 2003; FAO, 2005).



**Figure 1.3** Agro-ecological zones in Ghana indicating major economic crops

Source: Fold, 2008

**Table 1.1** Major crops grown in the different agro-ecological zones of Ghana

| Zone                       | Cereals                      | Starchy crops              | Legumes                    | Vegetables                     | Tree crops                        |
|----------------------------|------------------------------|----------------------------|----------------------------|--------------------------------|-----------------------------------|
| Coastal savannah           | Maize, rice                  | Cassava                    | Cowpea                     | Tomato, shallot                | Coconut                           |
| Rain forest                | Maize, rice                  | Cassava, cocoyam, plantain |                            | Pepper, okra, eggplant         | Citrus, coconut, oil-palm, rubber |
| Semi-deciduous rain forest | Maize, rice                  | Cassava, cocoyam, plantain | Cowpea                     | Pepper, okra, eggplant, tomato | Citrus, oil-palm, coffee, cocoa   |
| Guinea savannah            | Maize, rice, sorghum, millet | Yam, cassava               | Cowpea, groundnut, bambara | Tomato, pepper                 | Shea, cashew                      |
| Sudan savannah             | Maize, rice, sorghum, millet | Sweetpotato                | Cowpea, groundnut, bambara | Tomato, onion                  |                                   |

Source: FAO (2005)

#### 1.4 Sweetpotato and soyabean cultivation and utilisation in Ghana

Sweetpotato, though not a major staple food crop, is not new to Ghanaians. The white-fleshed variety is common. The coloured varieties (cream-, yellow-, and orange-fleshed) are being promoted in Ghana through encouraging their cultivation and value addition to the roots (Amenyenu, David, Bonsi, & Zabawa, 1998; Bonsi, Zabawa, David, & Nti, 1998; Otoo et al., 1998). Depending on the variety, sweetpotato may contain high level of  $\beta$ -carotene (Hagenimana et al., 2001; Ofori et al., 2009; Ssebuliba et al., 2001). Assessment of  $\beta$ -carotene levels in new sweetpotato genotypes introduced in Ghana ranged from trace to 2100  $\mu\text{g}/100\text{ g}$  levels, on fresh weight basis (Ofori et al., 2009). Oduro, Ellis, & Owusu (2008) have reported on the potential of the leaves as a source of minerals. Importantly, sweetpotato has low phytate level (FAO, 1990; Lung'aho & Glahn, 2009a; Phillippy et al., 2003).

Sweetpotato (presumably, the coloured varieties) was mentioned in the GDHS Report as one of the food sources consumed by infants between 6-9 months of age that is rich in vitamin A (GSS, 2004). Extensive research in Ghana has resulted in the production of early maturing (3 to 3.5 months) of the coloured varieties known as *Okumkom*, *Sautie*, *Faara* and *Santom Pona* [Council for Scientific and Industrial Research (CSIR), 2006; Otoo, Missah, & Carson, 2001].

There is evidence suggesting that growing of sweetpotato in Ghana has increased producers' income, but this was not seen with poor farmers due to limited options for processing, marketing and consumption [International Fund for Agricultural Development (IFAD), 2004]. A FAO Report indicates that the crop is gaining popularity in Ghana particularly the Upper East Region, where it is grown and exported to neighbouring Burkina Faso (FAO, 2005). Opare-Obisaw, Danquah, Doku, Boakye, & Ansah-Kissiedu (2000) stated that climatic conditions in Ghana favours sweetpotato production.

Soyabean was introduced in Ghana in 1910 (Plahar, 2006); the northern sector of the country is its major producer. Plahar (2006) reported that flour, grits, paste, milk and maize-soyabean blend were products developed from it at the household level. The author indicated that, there are 18 companies in Ghana that process soyabean commercially into products such as oil, cake, flour, milk, curd, extruded meal and high protein food.

In addition, soyabean flour is widely promoted by public health workers in Ghana; and soyabean in combination with cereals as complementary food is especially encouraged (Annan & Plahar, 1995; Anonymous, 2012; Mensa-Wilmot, Phillips, & Hargrove, 2001).

The use of coloured varieties of sweetpotato, which is available in Ghana, but not exploited (based on review of literature on complementary food presented in Chapter 2), to replace cereal in cereal-legume mix would present a double-advantage over cereal by being a dietary source of vitamin A, and also the sweetpotato-based infant food matrix could enhance absorption of micronutrients such as iron and zinc. Besides, it is also a good source of carbohydrate (Padmaja, 2009).



Although some earlier researchers have developed sweetpotato-based infant foods (Adenuga, 2010; Akaninwor & Okechukwu, 2004; Akubor, 1997, 2005; Espinola, Creed-Kanashiro, Ugaz, van Hal, & Scott, 1997; Ijarotimi & Ashipa, 2006; Nandutu & Howell, 2009; Nnam, 2000; Omwamba, Nanua, & Shalo, 2007), only the following authors indicated the levels of phytate and tannins (Akaninwor & Okechukwu, 2004) and vitamin A (based on  $\beta$ -carotene) (Espinola et al., 1997; Omwamba et al., 2007) in addition to the macronutrient levels. Nandutu & Howell (2009) reported that their sweetpotato infant formulation had comparable viscoelastic properties to a proprietary Heinz baby food. Due to the little published information on phytate, vitamin A, polyphenols, carbohydrate composition and viscosity, this research focussed on making available this information of nutritional significance.

A feeding trial of the sweetpotato-based complementary food among Ghanaian infants would have appropriately ascertained the effect of the consumption on micronutrients status of infants; but it was not carried out due to financial constraint. Further, there was the need to prove that sweetpotato-based complementary food could have some nutritional advantages over cereal-based infant food. However, approval from Massey University Human Ethics Committee for conducting a feeding trial on infants was sought, summarised as a study protocol (**Appendix 1**) for conducting a randomised infant feeding trial in Ghana.

As the infant feeding study was not feasible, the phytate to calcium, iron and zinc molar ratios (Paper VI) as used by other researchers (Abebe et al., 2007; Chan et al., 2007; Gibbs et al., 2011; Tizazu, Urga, Belay, Abuye, & Retta, 2011) was employed to estimate availability of these micronutrients; while an *in vitro* digestion/Caco-2 model iron uptake for estimating iron availability (Frontela, Scarino, Ferruzza, Ros, & Martinez, 2009; Lung'aho & Glahn, 2009a, 2009b; Pynaert et al., 2006) was utilised to estimate the availability of iron from infant food formulations. The *in vitro* digestion/Caco-2 model iron uptake studies was a collaborative work with another PhD student, who is evaluating methods for estimating iron bioavailability from different food sources. Therefore, in this thesis, the data of one of the several uptake studies being carried out on complementary food formulations are briefly highlighted in Chapter 5. A detailed

description of these uptake studies would be presented in the thesis of the other student.

There is repetition of the description of the materials and the methods used in processing the sweetpotato- and maize-based complementary foods, and the justification of the choice of the ingredients in the various papers listed above, in order to provide sufficient information for readers. Also, in-text citations and list of references in the various papers are not uniform; they were presented using the journals' requirements. However, with the exception of Paper I, the APA 6<sup>th</sup> referencing style was used in Chapters 1, 7 and 8.

The limitation of this thesis is that the conclusions made are based on compositional, functional and sensory studies but not on either data from an *in vivo* experiment or randomised feeding trial conducted on the formulations. However, because the compositional, functional and sensory data collected were compared with enriched Weanimix, the maize-based formulation that was developed through collaboration between United Nations Children's Fund (UNICEF) and the Nutrition Unit of the Ministry of Health, Ghana, in 1987 (Agble, 1997; Lartey et al., 1999), and currently being promoted in Ghana (Anonymous, 2012), the conclusions drawn in this thesis are relevant.

## **1.5 Main objective**

The main objective was to develop sweetpotato-based products that are more suitable as complementary food than maize-based products such as Weanimix. Suitability was assessed based on levels of micro- and macro-nutrients, antinutrients, physical and consumer acceptance properties.

### **1.5.1 Specific objectives**

- To formulate complementary food based on sweetpotato using either household- or industrial-level processing technology to meet the protein, fat and energy specifications in the Codex standards (CAC/GL 8 and STAN 074-1981, Rev.1-2006).
- To compare the nutrient (macronutrient and vitamin A) and the antinutrient (phytate and polyphenol) composition of the sweetpotato- and maize-based complementary foods.
- To assess stability of  $\beta$ -carotene in the sweetpotato-based complementary food stored in three different containers under simulated tropical temperature of 32 °C and 85% relative humidity in an environmental chamber for 24 weeks.
- To assess the availability of calcium, iron and zinc from sweetpotato-based infant foods compared with a maize-based complementary food on the basis of compositional analysis.
- To compare physical properties (viscosity and water solubility index) and sensory attributes of the sweetpotato- and maize-based complementary foods.

## **1.6 Development of sweetpotato-soyabean blend, an alternative to maize-legume mix as complementary food for infants in Ghana**

Data in Paper I were orally presented at the 45<sup>th</sup> Annual Conference of the Nutrition Society of New Zealand (Inc.); subsequently, the data were published in Volume 34 of the Proceedings of the Society (Amagloh et al., 2010).

### **Abstract**

**Background:** *The composition of the foods given to infants and young children in Ghana significantly contributes to the prevalence of malnutrition. Currently, a better option may be Weanimix (maize-soyabean-groundnut blend) designed to be processed as either industrial- or household-level complementary food. Weanimix has adequate protein and energy densities, but is high in phytate, an antinutrient which inhibits nutrient bioavailability.* **Objective:** *To formulate a low-phytate complementary food from cream-fleshed sweetpotato to contain comparable levels of macronutrients as Weanimix.* **Design:** *A composite blend of sweetpotato, defatted soyabean and soyabean oil was cooked on a stove-top, oven-dried, milled and enriched with fishmeal (referred to as stove-top cooked ComFa).* **Outcomes:** *Stove-top cooked ComFa had a protein level that was higher than that of Weanimix ( $25.49 \pm 0.10$  vs.  $14.26 \pm 0.29$  g/100 g;  $p=0.001$ ). However, the energy content was low compared with Weanimix ( $370 \pm 1.70$  vs.  $431 \pm 0.71$  kcal/100 g;  $p=0.001$ ). Stove-top cooked ComFa had a 7.5% energy deficit compared to the recommended level of at least 400 kcal/100 g in Codex standard, but met the calcium (105 mg/100 kcal) and zinc (1.6 mg/100 kcal) densities as recommended by WHO for complementary foods.* **Conclusion:** *The sweetpotato-soyabean blend has the potential to serve as an alternative complementary food if the energy content could be improved.*

**Key words:** Complementary food, Ghana, Maize, Soyabean, Sweetpotato

### **1.6.1 Introduction**

In 1987, Weanimix, a complementary food, which can be processed at either the household-level or at the industrial-level, was introduced in Ghana through collaboration between the Nutrition Unit of the Ministry of Health, Ghana and UNICEF to improve the nutritional status of older infants (Lartey *et al.*, 1999). Weanimix is a blend of maize, soyabean and groundnut; it is higher in protein and energy densities compared with *koko* - a household-level complementary food prepared from fermented dough of maize, millet or sorghum only. Weanimix improved growth (height and weight indices) but not micronutrient status unless fortified with a vitamin and mineral premix (Lartey *et al.*, 1998, Lartey *et al.*, 1999). Weanimix is also high in phytate (480 mg/100 g), which will limit bioavailability of iron (Hurrell *et al.*, 2003, Hurrell and Egli, 2010), zinc (Gibson and Ferguson, 1998), probably calcium (Perlas and Gibson, 2005) and to some extent protein (Greiner *et al.*, 2006). Therefore, formulation of another complementary food with low phytate, and made from available local resources warrants attention.

The objective of this study was to formulate a low-phytate complementary food from cream-fleshed sweetpotato and compare the levels of macro- and micro-nutrients of the sweetpotato-based product and Weanimix. Both formulations were also compared to recommended standards (Codex Alimentarius Commission, 1991, Dewey and Brown, 2003).

### **1.6.2 Methods**

#### **1.6.2.1 Ingredients used for processing complementary foods**

All ingredients were sourced from New Zealand, unless otherwise stated. *Toka Toka Gold* (cream flesh with orange streaks) sweetpotato (*Ipomoea batatas*) was supplied by Delta Produce Co-op Ltd in Dargaville and processed into flour by peeling, drying and grinding. Defatted toasted soyabean (*Glycine max*) flour was purchased from Oppenheimer, Wellington. White maize meal (Springbok™, South Africa), soyabean seed, groundnut (*Arachis hypogea*) paste and soyabean oil (SIMPLY™) were obtained from local supermarkets in Palmerston North. Fishmeal prepared by milling smoke-dried anchovies (*Engraulis hepsetus*), with the heads removed was imported from Ghana.

#### **1.6.2.2 Formulation and processing of sweetpotato-soyabean complementary food**

The formulation was estimated using published nutrient composition of sweetpotato (FoodWorks, 2009) and the nutritional information on labels of the other ingredients to obtain a product that could have an energy level of 400 kcal/100g, fat content between 10–25 g/100 g and protein content of at least 15 g/100 g on dry matter basis to meet the specified guidelines for complementary foods for infants and young children (Codex Alimentarius Commission, 1991).

ComFa, a composite blend containing 66% sweetpotato flour, 23% defatted soyabean, 10.3% soyabean oil, 0.20% lecithin and 0.50% iodised salt, was cooked on a stove to replicate the usual household-level food preparation and then oven-dried to a moisture content of 9.31 g/100 g. The dried sweetpotato-soyabean formulation was broken into smaller chunks, milled and then enriched with 20% wt/wt fishmeal prepared from anchovies. This is a recommended practice in Ghana to improve the nutrient quality of household-level complementary food (Akor *et al.*, 2001). The complementary food obtained was referred to as stove-top cooked ComFa. Sweetpotato was chosen to replace the maize and groundnut of Weanimix because it is low in phytate (Lukmanji *et al.*, 2008, Gibson *et al.*, 2010), high in vitamin A precursor and available in Ghana (Ofori *et al.*, 2009). It has been confirmed in another study that the calcium content of household-level prepared complementary food increased when this fishmeal was added (Perlas and Gibson, 2005).

#### **1.6.2.3 Processing of maize-soyabean-groundnut blend (Weanimix)**

The method described by Lartey *et al.* (1999) was used with a slight modification. Refined (dehulled) maize flour was used instead of flour prepared from roasted white maize grain. This modification was necessary due to the unavailability of white maize grain for human consumption in New Zealand.

#### **1.6.2.4 Nutrient analysis**

The moisture, protein, fat and ash contents of the ComFa formulation and Weanimix were determined using the standard methods described by the AOAC International (AOAC, 2005). Carbohydrate and the energy contents were estimated (FAO, 2003). Calcium, iron and zinc in the samples were determined

by atomic absorption spectrophotometry. All the analyses were done on three independent replicates.

#### **1.6.2.5 Data analysis**

The data were analysed using two-sample t-test procedure in Minitab v15.1™ (Minitab Inc., US). Means were considered to be significantly different at  $p < 0.05$ . Results are reported as means of triplicate determinations  $\pm$  standard error of the mean (SEM).

### **1.6.3 Results**

#### **1.6.3.1 Nutrient composition**

The stove-top cooked ComFa met protein and fat specifications of the Codex Standard (Codex Alimentarius Commission, 1991) for complementary foods for infants and young children (**Table 1.2**). However, the ComFa formulation met approximately 70% of the estimated carbohydrate (60–75%) and 92% of the specified energy (400 kcal/100 g) in the Codex standard.

**Table 1.2** Macronutrient composition of stove-top cooked ComFa and Weanimix as complementary food formulations<sup>a</sup>

| Nutrient(/100 g)                   | Codex Standard <sup>b</sup> | Stove-top cooked ComFa | Weanimix          | p-value <sup>c</sup> |
|------------------------------------|-----------------------------|------------------------|-------------------|----------------------|
| Protein, g                         | 15                          | 25.49 $\pm$ 0.10       | 14.29 $\pm$ 0.29  | 0.001                |
| Fat, g                             | 10 - 25                     | 11.76 $\pm$ 0.11       | 12.02 $\pm$ 0.23  | 0.22                 |
| Ash, g                             |                             | 6.07 $\pm$ 0.01        | 1.84 $\pm$ 0.01   | <0.001               |
| Carbohydrate, g<br>(by difference) | 60 – 75 <sup>d</sup>        | 47.36 $\pm$ 0.27       | 66.52 $\pm$ 0.37  | <0.001               |
| Energy, kcal                       | 400                         | 370.06 $\pm$ 1.70      | 431.27 $\pm$ 0.71 | 0.001                |

<sup>a</sup> Values (mean  $\pm$  SEM,  $n=3$ ) reported on dry matter basis;

<sup>b</sup> Source: Codex Alimentarius Commission (1991);

<sup>c</sup> ComFa formulation and Weanimix are significantly different at  $p < 0.05$ ;

<sup>d</sup> Estimated from data given for protein and fat in the Codex Standard.

It is evident that the fat in the ComFa formulation and Weanimix was not significantly different. Although, the protein content of the stove-top cooked

ComFa was higher than Weanimix by about 56% ( $p=0.001$ ), the estimated energy value of the ComFa formulation was lower than that of Weanimix by 15% ( $p<0.001$ ). The estimated carbohydrate content of ComFa was lower than that of Weanimix by 34% ( $p<0.001$ ). The protein level of Weanimix was slightly lower (by 4.7%) than the protein level stipulated in the Codex standard but met the stipulated fat level. Weanimix met the minimum energy specification of complementary foods stated in the Codex standard.

The stove-top cooked ComFa met the requirement for calcium and zinc, and about 49% of the recommended level of iron from complementary food for breastfeeding infants (6–8 mo old) (Dewey and Brown, 2003) (**Table 1.3**). Weanimix met only approximately 3.0% of the calcium, 30% of the iron and 94% of the zinc recommended levels. The nutrient densities for calcium and iron, but not zinc, of the ComFa formulation were significantly higher than that of Weanimix.

**Table 1.3** Calcium, iron and zinc densities of stove-top cooked ComFa and Weanimix as complementary food formulations compared to average desired densities<sup>a</sup>

| Nutrient density (mg/100 kcal) | Recommended level (WHO 2002) <sup>b</sup> | Stove-top cooked ComFa | Weanimix    | p-value <sup>c</sup> |
|--------------------------------|---|------------------------|-------------|----------------------|
| Calcium                        | 105                                       | 131.50 ± 8.10          | 3.59 ± 1.10 | 0.004                |
| Iron                           | 4.5                                       | 2.19 ± 0.02            | 1.34 ± 0.14 | 0.03                 |
| Zinc                           | 1.6                                       | 2.13 ± 0.52            | 1.50 ± 0.17 | 0.37                 |

<sup>a</sup> Values (mean ± SEM,  $n=3$ ) reported on dry matter basis;

<sup>b</sup> Source: WHO recommended levels as cited by Dewey and Brown (2003);

<sup>c</sup> ComFa formulation and Weanimix are significantly different at  $p<0.05$ .



#### **1.6.4 Discussion**

These results show that it is possible to process a dried complementary food from sweetpotato as reported in another study (Nandutu and Howell, 2009). The low energy and carbohydrate content of the stove-top cooked ComFa could be increased by modifying the formulation. For instance, the use of full-fat soyabean flour instead of the defatted flour would increase the energy content. The fishmeal added to the ComFa formulation accounted for the high protein content compared with the level in Weanimix. A similar protein content of 28 g/100 g was obtained when Weanimix was enriched with anchovies-fishmeal at 20% (wt/wt) (Lartey *et al.*, 1999). The pronounced effect of the addition of the fishmeal on the calcium content in ComFa suggests that it could be used as a rich source of calcium in complementary feeding and should be encouraged in other localities where anchovy is available. Our findings support the observation that adding fishmeal prepared from anchovies improved calcium content of plant-based complementary food as reported in another study (Perlas and Gibson, 2005). A further advantage of the sweetpotato-based complementary food is that the overall micronutrient content is increased because of the fishmeal incorporated; and combined with its presumably low level of phytate (Gibson *et al.*, 2010), the bioavailability of micronutrient would be enhanced. This would make the sweetpotato-based product more useful for improving micronutrient status of infants than the maize-based complementary food.

### **1.6.5 Conclusion**

The preliminary results from this study suggest that the cream-fleshed sweetpotato has the potential for providing the basis of an alternative complementary food for infants in Ghana. However, the energy value of the formulation needs to be increased to make it suitable as complementary food. The addition of anchovies-fishmeal to sweetpotato-soyabean blend provided the requirement for calcium and zinc densities, and about half the recommended level for iron density.

Because the stove-top cooked ComFa in the preliminary study presented above was limited in energy content, the ingredient composition was modified to meet the energy specification of 400 kcal/100 g in the Codex Standard (Codex Alimentarius Commission, 1991) by using a computer-assisted programme (Nutrition Calculator) obtained from Global Alliance of Improved Nutrition-Infant and Young Child Nutrition Program. Instead of defatted soyabean flour, full-fat soyabean flour was used in the new formulations as suggested in the publication above. Household- and industrial-levels sweetpotato-based formulations were developed, with varying ingredients, but with sweetpotato as the basic ingredient in both formulations. The choice of ingredients and processing methods are extensively discussed in Chapter 2 (Paper II). Basically, both formulations were guided by practicality at the household- or industrial-level. The new sweetpotato-based complementary food formulations proposed in Chapter 2, were subsequently processed as described in Chapter 3 (Paper III), and were evaluated for suitability as complementary food in comparison with the maize-based infant food (Weanimix) being promoted in Ghana (Anonymous, 2012), in Chapters 4 to 6.

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## **Chapter 2: Literature Review**

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This chapter has been published in Scientific Research Essays, entitled, “*Complementary food blends and malnutrition among infants in Ghana—a review and a proposed solution*” (Amagloh, Weber, et al., 2012), and reprinted as Paper II. The rationale of this chapter was to review literature to identify the food-based approaches being promoted in Ghana aimed at improving the nutritional status of infants. Reports on unfortified cereal-only formulations based on maize, millet or sorghum, traditionally called *koko* (fermented cereal porridge), were not considered because *koko* has low energy and protein densities (Lartey, Manu, Brown, & Dewey, 1997), contributes to protein-energy malnutrition (Walker, 1990), and has been associated with the poor nutritional status of Ghanaian infants (Appoh & Krekling, 2005).

### **Highlights**

- Complementary food used in Ghana is cereal-based; example is Weanimix, the first of such formulations introduced in Ghana 1987.
- Cereal-legume blends have higher energy and protein densities compared with *koko*.
- Cereal-legume formulations, unless fortified, have low level of vitamin A and high phytate content, consequently, leading to the high occurrence of micronutrient deficiencies among Ghanaian infants.
- Suggested method (soaking of flour or addition of phytase) to reduce phytate in cereal-based formulations has limitation.
- Sweetpotato-based complementary food was proposed as a more appropriate alternative infant food because it will be lower in phytate and higher in provitamin A compared with Weanimix.
- Climatic conditions prevailing in Ghana is suitable for the growth of sweetpotato.

## **Abstract**

*Widespread malnutrition among Ghanaian infants could be attributed to unfortified plant-based complementary foods commonly used at the household level. This review summarises the publications on the development of complementary food blends and intervention trials aimed at improving the nutritional status of Ghanaian infants. The complementary food blends are cereal-based which are developed from maize (in higher proportion) together with soyabean, cowpea and/or groundnut- an effort to improve protein and energy levels. The cereal-legume blends affect growth more positively than cereal-only formulations but not micronutrient status unless fortified with micronutrients. The low level of micronutrients (including vitamin A) and the high phytate content of cereal-legume blends partly account for micronutrient deficiencies. Phytate limits the bioavailability of nutrients such as iron, calcium and zinc. We propose an alternative complementary food blend that is based on sweetpotato. This proposed formulation would be relatively high in endogenous  $\beta$ -carotene (vitamin A precursor) and low in phytate compared to household-level cereal-based complementary foods.*

**Key words:** Cereal-legume, Complementary food, Ghana, Malnutrition, Phytate, Sweetpotato

## **Abbreviations:**

|        |                                   |
|--------|-----------------------------------|
| FAO    | Food and Agriculture Organization |
| GDHS   | Ghana Demographic Health Survey   |
| GSS    | Ghana Statistical Service         |
| PAHO   | Pan American Health Organization  |
| UNICEF | United Nations Children's Fund    |
| VAD    | Vitamin A Deficiency              |
| WHO    | World Health Organization         |

## **2.1 Introduction**

Improving the diet of infants has been recommended as one of the solutions to tackle childhood malnutrition in developing countries (Save the Children, 2012). In their recent report, entitled, “*A life free from hunger: Tackling child malnutrition*” (Save the Children, 2012), food fortification (example, addition of iron to flour in mills) or plant breeding, which has resulted in the availability of  $\beta$ -carotene-rich sweetpotato, was identified among the strategies to reduce iron or vitamin A deficiency (VAD) during infancy.

Infants are more likely to become malnourished in low-income countries when complementary foods are introduced (Dewey et al., 1992; Dewey, 1998; Gibson et al., 1998; Lutter and Rivera, 2003). The inadequacy of infant nutrition and its negative influence on attainment of full potential in life is now well established [Pan American Health Organization (PAHO) and WHO, 2003; Engle et al., 2007; Adu-Afarwuah et al., 2008; Beard, 2008]. Grantham-McGregor et al. (2007) suggested that poor nutrition during infancy is likely to lead to poor academic achievement, low incomes in adulthood and inadequate care for the children of subsequent generations. This cycle has contributed to the inter-generational poverty bedevilling low-income countries in South Asia and sub-Saharan Africa (Walker et al., 2007).

The level of childhood malnutrition in sub-Saharan Africa, an area that includes Ghana, is among the worst in the world. The proportion of children under 5 y with chronic malnutrition ( $<-2$  SD from the reference median for height-for-age) in sub-Saharan Africa was 38%, against a worldwide prevalence of 28% (UNICEF, 2009). Globally, an estimated 33% of children under 5 y were vitamin A deficient; in Africa, the prevalence was higher (42%) (WHO, 2009). Although the prevalence of anaemia (haemoglobin threshold of  $\leq 110$  g/L) among children under 5 y was a worldwide problem (47%), the occurrence was markedly higher (68%) in Africa (de Benoist et al., 2008).

Therefore, a review of the types of complementary foods available/being promoted in low-income countries, such as Ghana, demands more attention. Ghana was chosen because of the vigorous efforts by local and international

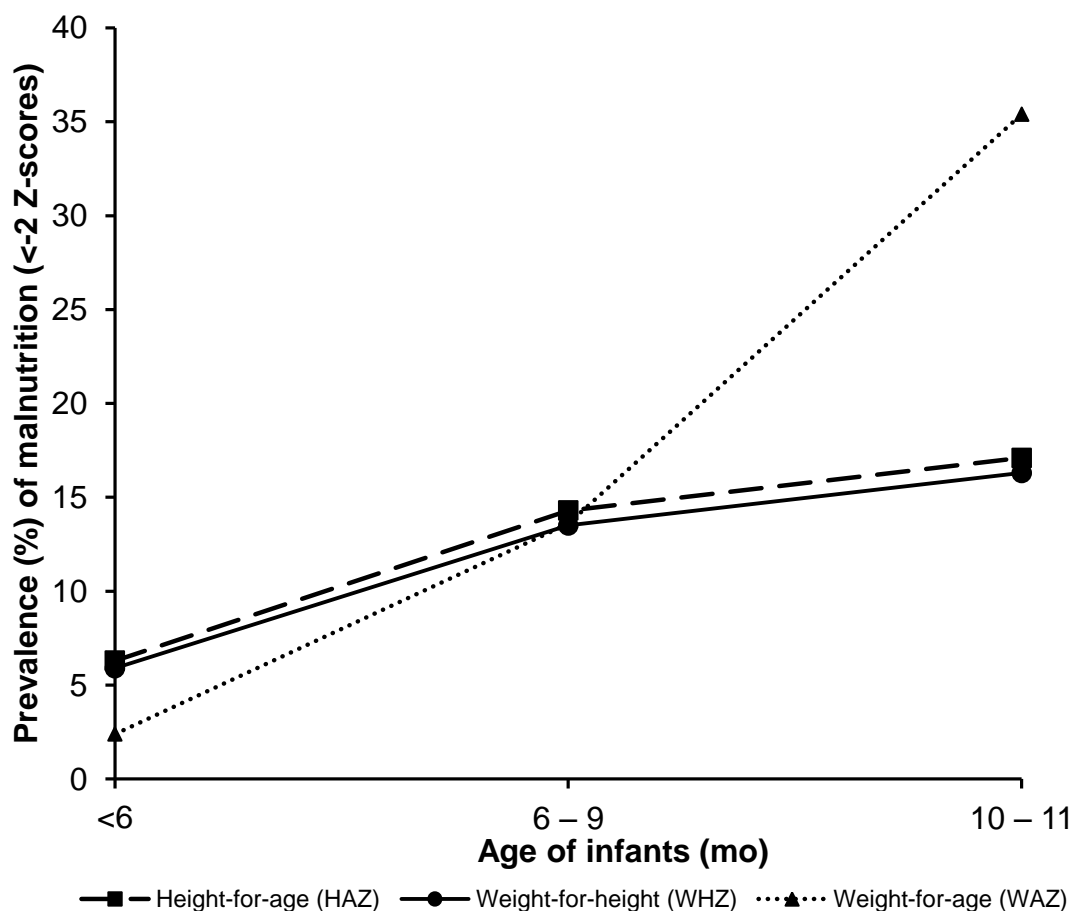
researchers to improve the nutritional status of infants in the country during the last two decades.

This review is limited to publications on the formulation of complementary foods based on cereal-legume blends and nutritional feeding trials conducted on complementary foods in Ghana. Reports on unfortified cereal-only formulations based on maize, millet or sorghum were not considered because such products have low energy and protein densities (Lartey et al., 1997) and contribute to protein-energy malnutrition (Walker, 1990). A negative association between consumption of cereal-only (fermented maize) porridge and child nutritional status among Ghanaian infants has been established (Appoh and Krekling, 2005). Also, nutritional interventions involving the use of vitamin and/or mineral supplements only, such as vitamin A supplementation, rather than added to complementary food prior to consumption do not fall in the scope of this review.

The database search for studies on the development of cereal-based complementary food blends and intervention trials involving complementary foods was done in Scopus, ISI Web of Knowledge<sup>SM</sup> and Google scholar. Two Ghana Demographic Health Survey (GDHS) Reports published in 2004 and 2009 [Ghana Statistical Service (GSS), 2004; GSS, 2009] were used to obtain information on feeding practices and nutritional status of Ghanaian infants.

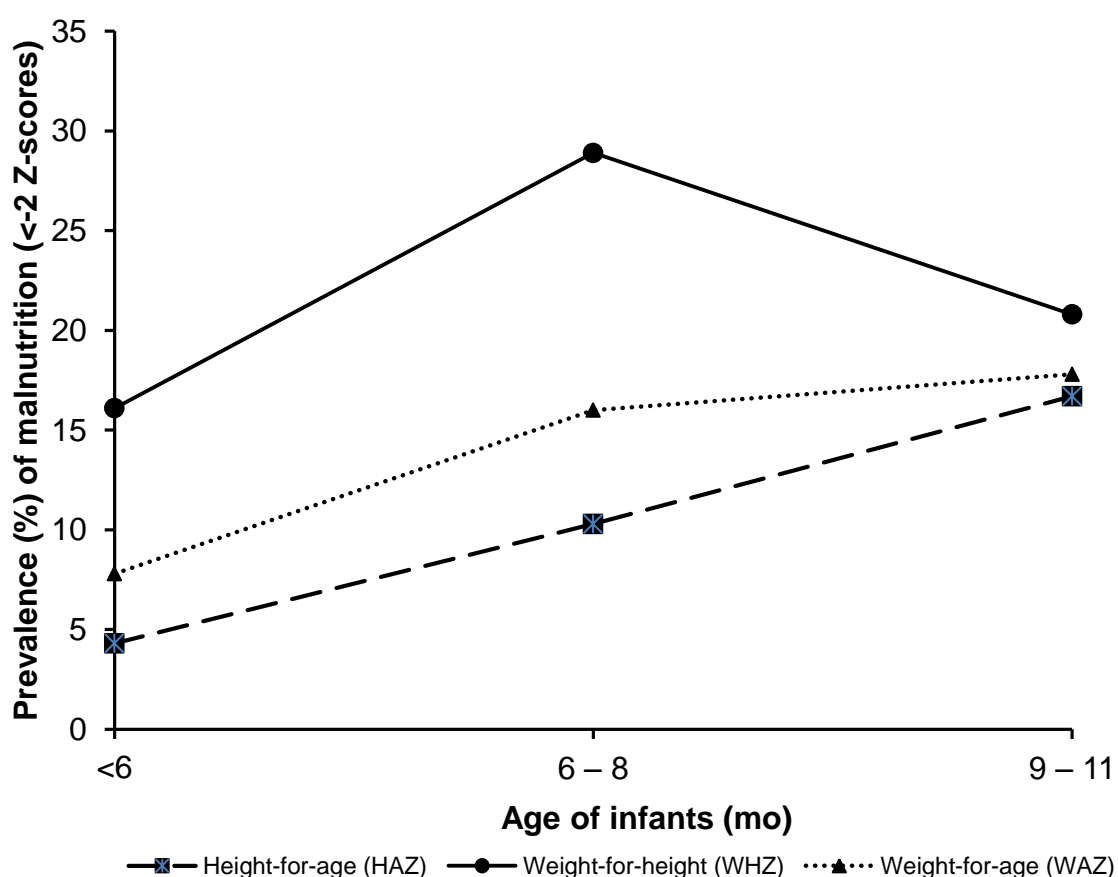
In Ghana, micronutrient deficiencies and growth faltering predicaments are worse in children under 5 y and particularly in infants after the transition from exclusive breastfeeding to complementary feeding. **Figures 2.1** and **2.2** show the prevalence of growth faltering among infants (<1 y) using the anthropometric indices: low height-for-age (stunting), low weight-for-height (wasting) and low weight-for-age (underweight). According to the 2004 GDHS report, the proportion of infants who were stunted (<-2 SD) was lower among infants <6 mo of age (6.3%) than among infants 10–11 mo old (17%) (**Figure 2.1**); in 2008 (**Figure 2.2**), the prevalence level followed a similar trend. It is not possible to compare the data in the two reports because different reference medians and age categorisations were used. However, both chronic and acute malnutrition indicators (stunting and underweight or wasting, respectively) had a similar trend in both reports: higher among infants 6 mo old onwards.





**Figure 2.1** Percentage of infants under 12 mo classified as malnourished using height-for-age (HAZ), weight-for-height (WHZ) and weight-for-age (WAZ) Z-scores

Source: GSS (2004). Each index was expressed in standard deviation units from the median of the NCHS/CDC/WHO International Reference population.



**Figure 2.2** Percentage of infants under 12 mo classified as malnourished using height-for-age (HAZ), weight-for-height (WHZ) and weight-for-age (WAZ) Z-scores

Source: GSS (2009). Each index was expressed in standard deviation (SD) units from the median of the WHO Child Growth Reference Standards published in 2006.

The national prevalence of anaemia ( $\leq 100.0$  g/L haemoglobin) according to the GDHS 2004 report was 75% among the 6–9 mo old infants, but in the 10–11 mo olds, the prevalence was 86%. Similar occurrence levels and patterns are reported in the GDHS 2009 report (76% for 6–8 mo olds and 88% for 10–11 mo olds). The causes of anaemia were attributed to inadequate dietary intake of iron, intestinal worm infestation and malaria. There were no data on VAD in either the GDHS 2004 or 2009 reports. However, a prevalence of 76% VAD among Ghanaian infants and young children <5 y old using blood serum retinol of  $<0.70$   $\mu\text{mol/L}$  as cut-off has been reported elsewhere (WHO, 2009). This high VAD prevalence is despite a vitamin A supplementation programme with 95%

coverage in which at least one of the required two doses offered was administered (UNICEF, 2009). This suggests that vitamin A supplementation alone will not bring the desired effect unless complemented by regular dietary intake of vitamin A.

The pattern of prevalence (lower around 6 mo of age and higher 8 mo of age and onwards) of anaemia and VAD among Ghanaian infants stated above, could undoubtedly be associated with food, specifically, complementary food as the major causal factor.

The mean duration of exclusive breastfeeding and predominant breastfeeding in Ghana was 3.8 and 6.9 mo, respectively from 1999–2002 (GSS, 2004). Predominant breastfeeding was defined as either exclusively breastfeeding or breastfeeding and consuming water only, and/or non-milk liquids only. From 2004 to 2007, exclusive breastfeeding or predominant breastfeeding duration was 4.4 or 6.2 mo, respectively (GSS, 2009). The percentage of infants between 12–15 mo who were breastfeeding was about 95% in both GDHS reports.

About 53% of infants from 1999–2002 and 63% from 2004–2007 were exclusively breastfed for the first 6 mo of infancy. Thus, about 40% of infants in Ghana are introduced to complementary food before the recommended period of exclusive breastfeeding of 6 mo (WHO, 2001); this is despite initiatives by the Ghana Health Service in establishing baby friendly centres in most health care facilities to encourage exclusive breastfeeding for the first 6 mo nationwide.

Comparison of the data from the GDHS reports indicates that there was a slight improvement in infant feeding practices in 2008 compared with 2003. From data in **Table 2.1**, the number of infants given complementary food from 6 mo, when breastmilk alone is likely to be insufficient (UNICEF, 1998; PAHO and WHO, 2003) to meet the nutrient demands of infants, was higher in 2008 than 2003 (75 vs. 62%). Likewise, the proportion of infants who were exclusively breastfed for the first 6 mo of infancy was higher in 2008 than 2003 as indicated above. The percentage of infants who were breastfed 6 mo onwards and consumed water alone was higher in 2003 (23%) than in 2008 (16%). Furthermore,

complementary foods given to infants contained more fruits and vegetables and included more animal derived foods in 2008 than in 2003 (**Table 2.2**).

In rural settings of Ghana, porridge prepared from cereals such as maize, millet or sorghum is usually the first complementary food. The data in **Table 2.2** show that about half of Ghanaian infants are given this cereal-only porridge as their normal complementary food, an unsuitable complementary food, which has been associated with poor nutritional status in infancy (Appoh and Krekling, 2005). The average energy content is about 100 kJ/100 g and the average protein level is 0.6 g/100 g (Lartey et al., 1997) compared with the desirable levels of at least 1670 kJ/100 g and 15 g/100 g, respectively (Codex Alimentarius Commission, 1991).

**Table 2.1** Breastfeeding practices\* (%) among Ghanaian infants

| Age of infants<br>(mo) | Breastfeeding and consuming |             |   |             |                     |             |                           |             |                    |             |
|------------------------|-----------------------------|-------------|---|-------------|---------------------|-------------|---------------------------|-------------|--------------------|-------------|
|                        | Exclusive breastfeeding     |             | Breastfeeding and consuming plain water |             | water-based liquids |             | Milk products (non-human) |             | complementary food |             |
|                        | <u>2003</u>                 | <u>2008</u> | <u>2003</u>                             | <u>2008</u> | <u>2003</u>         | <u>2008</u> | <u>2003</u>               | <u>2008</u> | <u>2003</u>        | <u>2008</u> |
| <6                     | 53.4                        | 62.8        | 24.0                                    | 17.1        | 1.2                 | 0.6         | 5.5                       | 2.6         | 15.6               | 16.9        |
| 6–9                    | 9.6                         | 4.1         | 22.8                                    | 15.9        | 2.4                 | 0.7         | 2.7                       | 1.1         | 62.2               | 75.3        |

\*Breastfeeding practice refers to a "24-hour" period (yesterday and last night) status. For 2003,  $n = 308$  for infants <6 mo old and  $n = 239$  for infants 6-9 mo old; 2008,  $n = 308$  for infants <6 mo old and  $n = 188$  for 6-9 mo old.

Source: GSS (2004; 2009)

**Table 2.2** Types of complementary foods (%) given to infants using a "24-hour" period (yesterday and last night) recall

| Age of infants (mo)* | Infant formula |             | Grains <sup>†</sup> |             | Legumes and nuts <sup>‡</sup> |             | Fruits and vegetables <sup>§</sup> |             | Root and tuber <sup>  </sup> |             | Animal source <sup>¶</sup> |             |
|----------------------|----------------|-------------|---------------------|-------------|-------------------------------|-------------|------------------------------------|-------------|------------------------------|-------------|----------------------------|-------------|
|                      | <u>2003</u>    | <u>2008</u> | <u>2003</u>         | <u>2008</u> | <u>2003</u>                   | <u>2008</u> | <u>2003</u>                        | <u>2008</u> | <u>2003</u>                  | <u>2008</u> | <u>2003</u>                | <u>2008</u> |
| 6–8/9                | 11.2           | 13.7        | 53.1                | 53.2        | 10                            | 9.7         | 28.9                               | 54.2        | 12.3                         | 16.6        | 20.9                       | 28.4        |

Source: GSS (2004; 2009);

\*2003, *n* = 238 for infants 6–9 mo old, 2008, *n* = 144 for infants 6–8 mo old;

<sup>†</sup>Porridge prepared from maize, millet or sorghum;

<sup>‡</sup>Beans (including soyabean) and groundnuts;

<sup>§</sup>Includes those rich in vitamin A: pumpkin, red or yellow yams or squash, carrots, red sweetpotatoes, green leafy vegetables, mangoes, papayas, and other locally grown fruits;

<sup>||</sup>White potatoes, white yams, cocoyam or cassava;

<sup>¶</sup>Meat, fish, shellfish (prawn or lobster), poultry or eggs.

## **2.2 Efforts to improve the nutritional status of Ghanaian infants**

Attempts have been made by international organisations and local researchers to improve the energy and protein content of complementary foods for infants in Ghana. In 1987, UNICEF collaborated with the Nutrition Unit of the Ministry of Health, Ghana, to formulate a cereal-legume complementary food called Weanimix which can either be processed at the household or industrial level (Agble, 1997; Lartey et al., 1999). It contains 75–80% maize, 10–15% soyabean/cowpea and 10% groundnut. A number of such improved complementary foods (with regards to energy and protein) have been developed using a range of ingredients, though these are generally driven by intent to incorporate a particular crop to conform to the objectives of funding agencies. Product composition and processing of complementary food blends are presented in **Table 2.3**. Key summary points of the formulations of complementary foods are:

- Most of the formulations include maize (predominantly) together with legumes such as soyabean, cowpea or groundnut; soyabean is the most popular legume added to maize. This, coupled with the data in **Table 2.1** (from GDHS reports), indicates that the complementary foods given to infants are mostly cereal-based.
- All the formulations involve the combination of flours from the grains/seeds prepared by roasting and milling. Extrusion cooking has been used to process some of the cereal–legume blends at industrial-level (Mensa-Wilmot et al., 2003; Plahar et al., 2003b).
- Complementary foods are formulated as dried products which need to be reconstituted with liquid to form porridge before being served to infants.
- Formulation involves the use of available local resources in line with the WHO (2001) recommendation of processing complementary food for infants.
- Most of the formulations satisfy the energy (1670 kJ/100 g) and protein (15 g/100 g) levels in the Codex guidelines for complementary food (Codex Alimentarius Commission, 1991).

The use of unfortified cereal-legume blends instead of unfortified cereal-only formulation as complementary food in Ghana has the potential to reduce the

incidence of protein-energy malnutrition among infants. Cereal-legume blends are relatively high in energy as well as protein (both quality and quantity); the legumes supply the lysine lacking in cereal and the cereals provide cysteine and methionine that are low in legumes (Annan and Plahar, 1995). Weanimix, which contained 75% maize, 15% soyabean and 10% groundnut, was reported to have an energy value of 1820 kJ/100 g and protein level of 15 g/100 g (Lartey et al., 1999) compared with 100 kJ/100 g and 0.6 g/100 g mentioned earlier for *koko*, a maize-only porridge prepared from fermented cereal dough (Lartey et al., 1997).

There is evidence that Weanimix adequately meets the growth demands of infants using weight gain as an index (Lartey et al., 1999); but is inadequate to meet the demand for vitamin A (Lartey et al., 1998), iron or zinc (Lartey et al., 1999; Lartey et al., 2000b) (**Table 2.4**). The poor vitamin A status of the infants may be attributed to the low levels (about 2.0 µg retinol equivalents/100 kJ) of vitamin A (Lartey et al., 1999) compared with the recommended range of 14–43 µg retinol equivalents/100 kJ (Codex Alimentarius Commission, 2006). Vitamin A has been identified as one of the “problem nutrients” in Weanimix (Dewey and Brown, 2003). Poor iron or zinc absorption leading to deficiency has been associated with cereal–legume based diets (Taylor et al., 1995; Hotz and Gibson, 2001; Hotz et al., 2001; Hurrell et al., 2003) because of the high phytate levels (Egli et al., 2002; Hurrell et al., 2003). The ingredients commonly used to formulate cereal-legume blends all have relatively high levels of phytate: maize (1.15 g/100 g), cowpea (0.66 g/100 g) and soyabean (1.40 g/100 g) (Egli et al., 2002) and groundnut (1.76 g/100 g) (Lukmanji et al., 2008). The phytate level of 0.48 g/100 g of Weanimix (Lartey et al., 1997) could inhibit iron and zinc absorption by infants. Therefore, for unfortified plant-based complementary foods, not only is the level of iron important, but the phytate content is crucial since it reduces the amount of non-haem iron that can be absorbed.

The inhibitory effect of phytate on non–haem iron and zinc bioavailability has been shown to be dose-dependent (Hallberg and Hulthen, 2000; Greiner et al., 2006); hence effort has been directed towards identifying methods for reducing phytate level in foods. Traditional processing methods such as fermentation or germination are ineffective in reducing the amount of phytate in foods



processed from maize, cowpea or soyabean as most of these crops have low levels of endogenous phytase (Egli et al., 2002). Using natural lactic acid fermentation of maize flour slurry, Hotz and Gibson (2001) found that phytate content was reduced by 12% after 86 h. Songré-Ouattara et al. (2010) reported that addition of lactic acid bacteria with high phytase activity did not reduce the phytate level in pregelatinised millet and soyabean slurry after 24 h of fermentation. This is in spite of findings that suggested that damaging cell membranes by thermal processing increases phytase activity (Cheryan, 1980). The traditional soaking of maize flour in water and decanting the excess water afterwards reduces the level of phytate by only 57% (Hotz et al., 2001). However, the soaking method, which could easily be adopted at the household level, leads to leaching and loss of water-soluble micronutrients (Hotz and Gibson, 2001); it is therefore not a suitable approach particularly in preparing household-level complementary food, which is seldom fortified with vitamin and mineral supplements (Gibson et al., 1998). Additionally, the soaking method has not been tested on composite flours of cereal and legume which have been shown to be superior nutritionally and more suitable as complementary food than cereal-only flour (**Tables 2.3 and 2.4**). The use of phytase-containing micronutrient powder led to improved iron and zinc status in humans consuming a phytate-rich meal prepared from maize (Troesch et al., 2009; Troesch et al., 2011). Although the phytase-containing micronutrient powder optimised iron bioavailability at low levels of iron (2.5–3.0 mg of per meal), Troesch et al. (2009) cautioned that the product is expensive and presents ethical issues because the phytase used was extracted from genetically modified *Aspergillus niger*.

Cooking usually leads to approximately 50% phytate degradation, whereas high temperature and short time thermal processing methods such as extrusion cooking results in approximately 30% degradation (Sathe and Venkatachalam, 2002). The ineffectiveness of thermal processing on phytate reduction and enhanced iron absorption has been demonstrated by Hurrell et al. (2002). Extrusion cooking of cereal flour (polished rice, degermed maize or wheat) at about 160°C or roller drying of precooked slurry with steam injection at about 135°C had no effect on phytate degradation and consequent iron absorption

compared with home-cooking of the respective cereals (Hurrell et al., 2002). The combination of fermentation for 2 h and baking at 220°C for 15 min was the only processing method which completely degraded phytate and significantly increased iron absorption in that study.

Other constituents of food, either exogenous or endogenous, such as ascorbic acid (Gillooly et al., 1983; Siegenberg et al., 1991) and  $\beta$ -carotene (provitamin A) (Garcia-Casal et al., 2000; Garcia-Casal, 2006), have been reported to promote iron absorption in the presence of phytate. However, roasting ingredients in the open-pans used in cereal-legume blends formulations (**Table 2.3**) and further cooking of the reconstituted blend as porridge would totally degrade the endogenous ascorbic acid (Teucher et al., 2004). The effect of heat on the endogenous  $\beta$ -carotene levels in orange-fleshed sweetpotato reduction is moderate (Low et al., 2009), ranging from 12 to 57%, depending on the cooking method (Wheatly and Loechl, 2008). Preparing cereal porridge with fruit juice could increase the ascorbic acid content, but this is not a common practice in Ghana because of the additional cost. Therefore, a plant-based complementary food which has appreciable amounts of endogenous  $\beta$ -carotene is a more feasible strategy to counteract the inhibitory effect of phytate on nutrient bioavailability.

An alternative approach of reducing the inhibitory effect of phytate on nutrient absorption is to add vitamin and mineral supplements to the complementary food as carried out by Larrey et al. (1999) in a trial on Ghanaian infants (**Table 2.4**). For example, fortification of complementary food with iron or zinc would decrease phytate: iron or zinc molar ratio, an index used to estimate iron or zinc availability (considered in detail in Chapter 5). The key summary points in the nutritional intervention studies conducted in Ghana on infants and young children presented in **Table 2.4** are:

- Addition of vitamin and mineral supplements to cereal-legume or cereal-only porridges had a positive effect on the micronutrient status of infants and young children.
- A lipid-based nutrient supplement (Nutributter) which met the recommended nutrient intake for 14 vitamins and minerals plus calcium, potassium, phosphorus, magnesium, manganese as well as energy (452 kJ/day) mainly

from fat (including 1.29 g linoleic acid/day and 0.29 g  $\alpha$ -linolenic acid/day), improved iron status as well as growth.

- Cereal-legume blends with/without fish powder as well as cereal-only plus fish powder complementary foods improved growth but not the iron and vitamin A status of infants. In fact, infants fed Weanimix for 6 mo did not maintain their vitamin A status at the end of the study (Lartey et al., 1998).

Using micronutrient fortification/supplementation to improve infant nutriture in developing countries like Ghana is impractical, because micronutrient preparations cannot be produced at the household level from available local resources and there is a lack of commitment by policy makers to ensure their provision at a subsidised price. Hence, the availability of micronutrient supplements undesirably ends with the nutritional trials they are designed for. Vitamin and mineral supplements, if made affordable or available for resource-poor households, remain the most efficacious way of improving the nutritional status of infants in developing countries. Until vitamin and mineral supplements, lipid-based nutrient supplement or sprinkles are affordable in developing countries through government subsidies, dietary diversification (example, food-to-food fortification) holds the key to improving nutritional status of infants in low-income countries (Chakravarty, 2000; Gibson et al., 2000) as this approach can easily be replicated at the household-level.

An easy-to-adopt strategy by most households in low-income countries would be to replace some of the ingredients in cereal-legume food mix with sweetpotato (*Ipomoea batatas*); a locally-available food crop, high in  $\beta$ -carotene (precursor of vitamin A), and low in phytate. The  $\beta$ -carotene content of the roots of coloured (red-, yellow-, cream- or orange-fleshed) sweetpotato varieties ranged from 500–8000  $\mu\text{g}/100\text{ g}$  (fresh weight basis) (Hagenimana et al., 2001; Ssebuliba et al., 2001; Ofori et al., 2009). Flour prepared from a cultivar of orange-fleshed sweetpotato [Pumpkin (CIP 420027)] with a dry matter of 89% was reported to have  $\beta$ -carotene as high as 13,900  $\mu\text{g}/100\text{ g}$  (Hagenimana et al., 2001). The level of phytate in sweetpotato is generally low ranging from non-detectable (Phillippy et al., 2003; Lung'aho and Glahn, 2009) to 6.0 mg/100 g (Gibson et al., 1998; Gibson et al., 2010) or 10.0 mg/ 100 g (Lukmanji et al., 2008).

**Table 2.3** Complementary food formulations developed for Ghanaian infants

| Authors                 | Composition and focus   | Major findings and conclusion  |
|-------------------------|---|--|
| Annan and Plahar (1995) | Maize (70%), soyabean (20%), groundnut (5%) and full-fat milk powder (5%). This combination was to meet the minimum protein requirement of 15% in the Codex Alimentarius Commission standards. This formulation was referred to as FRI Weaner   | <p>The FRI Weaner had protein (17.1 g/100 g), fat (10.56 g/100 g) and carbohydrate (67.80 g/100 g); these were similar to the levels in Nestlé® Cerelac®—a nutritionally adequate commercial cereal-based complementary food in Africa</p> <p>FRI Weaner was higher in fat and protein by a difference of 84% and 56%, respectively compared with traditional tom brown (TTB)—roasted maize only flour.</p> <p>FRI Weaner and Nestlé® Cerelac® supported growth of weanling albino rats better than TTB based on anthropometric and biochemical data.</p> <p>The FRI Weaner could be used as an ideal weaning food to improve nutritional status of infants and help to alleviate protein–energy malnutrition.</p> |
| Mensah et al. (1995)    | <p>Maize and soyabean in ratio of 4:1.</p> <p>The complementary food blends were either fermented (F) or non-fermented (NF). Focus was on acceptability and nutrient intake of porridge from the complementary food blends compared with traditional fermented maize-only (P) porridge among infants recovering from diarrhoea.</p> | <p>Mothers rated P more highly accepted than F and NF. However, using quantitative intake by infants as an index of acceptability, there were no differences among the three products.</p> <p>Daily protein and energy intakes for NF and F were higher by a difference of at least 78% (protein) and 65% (energy) than for P.</p> <p>The high nutrient density of NF and F porridges make them better suitable vehicle for macronutrients supply</p>  |

Overall literature review and proposal (Paper I)

|  |   |  |
|--|---|--|
|  |   | during the recovery diarrhoea than P.  |
| Nti and Plahar (1995)                    | <p>Maize and cowpea.</p> <p>To replace maize flour with cowpea flour in proportions: 0, 20, 30, 40 and 100% and assess the protein quality and chemical characteristics of such complementary food blends.</p> <p>Also, the effect of amino acid supplementation of the maize-cowpea blend on protein quality was assessed.</p> | <p>Protein level increased with increasing cowpea flour substitution at 20, 30 and 40% (13–15% for maize: cowpea blend vs. 10% for maize only flour) but fat (3.5–4.1% vs. 4.8% and carbohydrate (79–81% vs. 83%) levels decreased.</p> <p>Calcium and iron contents of the maize: cowpea blend were higher than the maize only flour: calcium (42.6–53.8 mg/100 g vs. 33.9 mg/100 g) and iron (5.5–6.3 mg/100 g vs. 4.0 mg/100 g).</p> <p>Increasing levels of cowpea flour in maize–cowpea blend led to increase in the levels of lysine (3.9–4.8 g/16 g N compared with 2.6 g/16 g N for maize–only flour) and tryptophan (0.8–1.0 g/16 g N against 0.7 g/16 g N for maize), but a decrease in cysteine and methionine (4.7 g/16 g N for maize flour compared with 3.4–3.9 g/16 g N for the maize–cowpea blend. The protein score for the maize–cowpea blend was higher (62–78%) than for maize flour (43%).</p> <p>Addition of tryptophan, lysine, tryptophan and methionine to maize: cowpea (70:30) blend improve the protein quality–biological value (82% compared with 67% without supplementation) and net protein utilisation (73% against 61% without supplementation) using Wister rats as model.</p> |
| Mensa-Wilmot et al. (2001a; 2001b; 2003) | <p>Maize (43%) cowpea (42%) and groundnut (15%); maize (50%), cowpea (35%), soyabean (10%), soyabean oil (5%).</p>  | <p>The levels of protein (17–19 g/100 g), fat (6–9 g/100 g) and energy (1720–1760 kJ/100g) of the extrudates indicated that the formulations could be used as</p>  |

Overall literature review and proposal (Paper I)

|                              |   |  |
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|                              | <p>These two blends were formulated to meet daily requirement for energy, two-thirds to three-quarters of daily protein requirement and at least a third of essential nutrients for 0.5–0.9 y old infant.</p> | <p>weaning supplements.</p> <p>The formulated blends were sufficient to support growth because of the protein quality indices: true protein digestibility of the blends ranged from 87–92% compared with 96% for casein, and the protein digestibility corrected amino acid score ranged from 0.72–0.82.</p> <p>Sensory data showed that the formulation were least preferred in colour, flavour, texture and general acceptability compared with two commercial products (Nestlé® Cerelac® and Nestlé® Frisocrema®, Nestle Ghana). However, mothers found the processing of weaning food from local staples attractive.</p> |
| Plahar et al. (2003a; 2003b) | <p>Maize (75%), groundnut (10%) and soyabean (either roasted or not roasted) (15%).</p>   | <p>The extruded non-roasted and extruded pre-roasted weaning formulations had higher protein (17 g/100 g) than roasted maize only flour (9.2 g/100 g).</p> <p>The energy content was similar for the extruded formulations and roasted maize product (1600 vs. 1580 kJ/100 g, respectively).</p> <p>The extruded formulations had excellent rat growth response than the roasted maize formulation, indicating high protein quality of the cereal-legume blends.</p> <p>The extruded cereal-legume blends were more accepted and had better consistency when prepared as porridge compared with roasted maize product.</p>   |
| Nelson-Quartey et al. (2007) | <p>Maize (malted), breadfruit pulp, breadnut</p>  | <p>Blends were higher in nutrient compared with Traditional</p>  |

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|                        | seed and/or groundnut in varying proportions. The blends were enriched with full-fat milk powder and carrot. Sugar was added as a sweetener.       | <p>tom brown (roasted maize flour).</p> <p>The protein level was between 13.9 and 16.0 g/100 g and fat was from 6.6 to 12.0 g/100 g.</p> <p>Malting of cereals reduced the viscosity and increased the solubility of the complementary food formulations.</p> <p>One formulation containing 50% breadfruit pulp, 40% malted maize and 10% groundnuts, had the most preferred sensory attributes.</p>                    |
| Amankwah et al. (2009) | Maize (fermented) (43.96%), rice (31.81%), soyabean (20.09%) and fishmeal (4.14%); Maize (fermented) (51.53%), soyabean (25.97%) and rice (22.50%) | <p>Protein contents of the formulation containing the fishmeal and without fishmeal were 19.13 and 17.18 g/100 g, respectively with corresponding 1690 and 1650 kJ/100 g as energy; both satisfying the Codex Standard specifications.</p> <p>The blends were equally accepted as complementary food based on sensory evaluation.</p> <p>Blends can be used as weaning foods to lessen protein-energy malnutrition.</p> |
| Amankwah et al. (2010) | Maize flour (fermented) (64%), soyabean (32%) and groundnut (4%)   | <p>The blend had excellent rat growth response: weight at end of a 10-week period (190–230 vs. 180–185 g, respectively, for blend and maize only diet) and haemoglobin [12.5–14.0 (blend) vs. 10.5–12.0 g/dL (maize only)].</p> <p>The blend was therefore recommended as complementary food to improve nutritional status of growing infants compared with the fermented maize only flour.</p>                         |

**Table 2.4** Nutritional intervention trials in Ghana using complementary food for infants and young children

| Authors                           | Study description  | Main results and conclusion/recommendation   |
|-----------------------------------|--|--|
| Lartey et al. (1998; 1999; 2000a) | <p>Design: Longitudinal, randomised feeding trial.</p> <p>Group: Four groups based on food allocations:</p> <ul style="list-style-type: none"> <li>• Weanimix (W)</li> <li>• Weanimix fortified with vitamins and minerals premix (WM)</li> <li>• Weanimix plus fish powder prepared from smoke-dried anchovies (WF)</li> <li>• <i>Koko</i> (fermented maize only) plus fish powder (KF).</li> </ul> <p>Subject: Breastfeeding infants (<math>n=216</math>).</p> <p>Non-intervention group (<math>n=464</math>). Data on non-intervention group was collected before and after the intervention trial.</p> <p>Approach: Infants were recruited when &lt;1 mo. Trial started when 6 mo old until infants were 12 mo of age.</p> <p>Mothers were supplied 500 g of their allocated foods weekly.</p> | <p>Plasma retinol increased in the WM group (<math>0.17\pm0.4</math> <math>\mu\text{mol/L}</math>) from baseline (6 mo) to end of study (12 mo).</p> <p>Plasma retinol decreased in W, WF and KF (<math>-0.08\pm0.3</math>, <math>-0.01\pm0.3</math>, <math>-0.02\pm0.3</math> <math>\mu\text{mol/L}</math>, respectively).</p> <p>The group given WM had better vitamin A status than infants fed non-fortified improved complementary foods.</p> <p>Haemoglobin, hematocrit or zinc level was not significantly different among the intervention groups.</p> <p>The proportion of infants with serum ferritin level &lt;12 <math>\mu\text{g/L}</math> (index of poor iron store) increased in the W, WF and KF groups but not in WM group from baseline to the end of the study.</p> <p>Fortification of locally processed complementary food with vitamin and mineral supplements led to no depletion of iron stores.</p> <p>The improved complementary foods formulated were associated with less growth faltering compared with the non-intervention group.</p> |
| Zlotkin (2001)                    | <p>Design: Longitudinal, randomised controlled trial.</p> <p>Group: Sprinkles group (<math>n=245</math>) and Ferrous sulphate drops group (<math>n=247</math>).</p> <p>Subjects: Infants (6 -18 mo of age) with haemoglobin</p>  | <p>No significant difference at baseline or at the end of the study period in terms of haemoglobin and ferritin concentrations between the groups.</p> <p>There was significant increase in haemoglobin and ferritin</p>   |



|                            |  |  |
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|                            | <p>concentration from 70 to 99 g/L.</p> <p>Approach: Ferrous drops to be given 3 times a day (a total of 40 mg elemental iron) and microencapsulated ferrous fumarate plus ascorbic acid to be added to cooked complementary food before consumption (a total of 80 mg of elemental iron + 50 mg ascorbic acid). Treatment was for a period of 2 mo.</p> <p>This was the first initial field testing of Sprinkles</p>  | <p>concentrations from baseline to end of study.</p> <p>No difference in compliance on the use of the drops or the sprinkles.</p> <p>Microencapsulated ferrous fumarate plus ascorbic acid sprinkled once daily on complementary foods had similar rate of treatment effect on anaemia among infants as ferrous drops (administered as three drops per day).</p>   |
| Christofides et al. (2006) | <p>Design: Longitudinal, randomised, clustered (housing as clustering unit).</p> <p>Group: Four Sprinkles groups based on varying doses of iron and one iron drops group.</p> <p>Sprinkles</p> <ul style="list-style-type: none"> <li>○ 12.5 mg iron as ferrous fumarate (<math>n=26</math>)</li> <li>○ 20 mg iron as ferrous fumarate (<math>n=28</math>)</li> <li>○ 30 mg iron as ferrous fumarate (<math>n=27</math>)</li> <li>○ 20 mg iron as ferric pyrophosphate (<math>n=27</math>).</li> </ul> <p>Each sachet of the Sprinkles also contained 30 mg ascorbic acid, 300 retinol equivalents of vitamin A, 5 mg zinc, 7.5 µg vitamin D and 160 µg folic acid.</p> <p>Ferrous sulphate (FS)</p> <ul style="list-style-type: none"> <li>○ 12.5 mg iron as ferrous sulphate drops (<math>n=25</math>).</li> </ul> <p>Subjects: Anaemic infants (6–18 mo of age) with haemoglobin concentration from 70 to 99 g/L.</p> | <p>Haemoglobin and serum ferritin improved from baseline to the end of the treatment period in all group, and were not different among the groups.</p> <p>Iron deficiency anaemia (defined as haemoglobin &lt;100 g/L and soluble transferrin receptor &gt;8.5 mg/L) prevalence was not different across the groups and decreased significantly from baseline to end of study, except the FS group.</p> <p>Sprinkles were easier to use than the drops. Also, greater teeth staining occurred in the drops group than the sprinkles.</p> <p>The 12.5 mg Sprinkles containing 30 mg ascorbic acid, 300 retinol equivalents of vitamin A, 5 mg zinc, 7.5 µg vitamin D and 160 µg folic acid was recommended as effective for treating anaemia by sprinkling it on complementary food before consumption.</p> |

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|                                  |  | Approach: Sprinkles were added to food after cooking once daily while 1 ml of FS was to be given once daily between meals for an 8 week period.  |  |
| Zlotkin et al. (2006)            | Design: Longitudinal, randomised, controlled.<br>Group: Three Sprinkles groups<br>5 mg elemental zinc as <sup>67</sup> Zn-labelled zinc gluconate combined with 50 mg ascorbic acid (LoZn group) ( <i>n</i> = 21)<br>10 mg elemental zinc as <sup>67</sup> Zn-labelled zinc gluconate combined with 50 mg ascorbic acid (HiZn group) ( <i>n</i> = 21)<br>5 mg elemental zinc as zinc gluconate, no ascorbic acid ( <i>n</i> = 18).<br>Each also contained 30 mg of elemental iron as <sup>57</sup> Fe-labelled microencapsulated ferrous fumarate.<br>Subjects: Anaemic and non-anaemic infants (12–24 mo of age).<br>Approach: Sprinkles were added to smaller portion of cooked food to ensure that all that Sprinkles were fully ingested before feeding the entire dish. | The percentage zinc absorbed did not differ at 5 or 10 mg intakes.<br>The zinc absorption in the form of Sprinkles was low.<br>The amount of zinc absorbed from the HiZn group was higher and significantly different from the LoZn group (0.82 vs. 0.31 mg).<br>Across the three groups, ascorbic acid was not associated with increased iron absorption.<br>There was no effect of the levels of zinc or the level of ascorbic acid on iron absorption from sprinkles added to maize-based complementary food. |  |
| Adu-Afarwuah et al. (2007; 2008) | Design: Longitudinal, randomised feeding trial and cross-sectional for non-intervention group.<br>Group: Three groups based on supplement allocation as intervention group:<br>Sprinkles (SP) ( <i>n</i> =105)   | Plasma ferritin levels were not significantly different across the interventions (SP, NT and NB), but were significantly higher than the NI.<br>SP, NT and NB effect on haemoglobin concentration were not significantly different. However, NT and NB but not SP effect   |  |

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|-------------|--------|--|--|
|             |        | <p>Nutritabs (NT) (<math>n=105</math>)</p> <p>Nutributter (NB) (<math>n=103</math>).</p> <p>Non-intervention (NI) group (<math>n=96</math>).</p> <p>SP met the Recommended Nutrient Intake (RNI) for 6 vitamins and minerals; NT met the RNI for 14 vitamins and minerals plus some calcium and potassium; NB met RNI for 14 vitamins and minerals plus calcium, potassium, phosphorus, magnesium, manganese as well as energy (108 kcal/day) mainly from fat (including 1.29 g linoleic acid/day and 0.29 g <math>\alpha</math>-linolenic acid/day).</p> <p>Subject: Infants.</p> <p>Approach: Infants were recruited when 5 mo old but longitudinal trial started when 6 mo old until 12 mo of age. Infants for NI were selected from the eligible subjects but who were not randomly assigned to the intervention groups at the end of study (12 mo).</p> <p>Mothers were told to mix supplement with 1 to 2 tablespoon of the child's food to ensure that the infants took the entire dose.</p> <p>Each supplement was given once daily.</p> | <p>on haemoglobin was significantly higher than NI.</p> <p>There was no significant effect on plasma zinc concentration among the intervention groups as well as the NI group.</p> <p>Head circumference and length-for-age Z-score were not significantly different among the four groups.</p> <p>NB group had significantly higher weight-for-age and weight-for-length z scores than NT but not SP or NI groups.</p> <p>The percentage of children at 12 mo who were able to stand without support was not significantly different across the 4 groups. However, the children who were able to walk independently were significantly higher in intervention groups than the NI.</p> <p>NB supplement improved weight and length indices better than Sprinkles and NT.</p> <p>All 3 supplements had positive impact on the ability of the children to walk independently at 12 mo compared to NI.</p> <p>All supplements were well accepted.</p> |
| Adom (2010) | et al. | <p>Design: Longitudinal, randomised controlled feeding trial.</p> <p>Group: Two groups based on food allocations:</p> <ul style="list-style-type: none"> <li>• Test group (plus iron fortification) (<math>n=31</math>)</li> </ul>   | <p>Anaemia prevalence increased in the control group, but decreased in the test group.</p> <p>Haemoglobin concentration was significantly different between the groups but serum iron and total-iron binding capacity were not significantly affected.</p>   |

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- Control group (without iron fortification) ( $n=29$ )  
The intervention food was made from 80% maize and 20% cowpea.  
Subject: Infants and young children (6–18 mo old); haemoglobin  $\geq 9$  g/dL.  
Approach: Dry rations of fermented blend (with or without iron fortification) were given to mothers to take home and encouraged to prepare and give thrice daily.  
The intervention period was 6 mo.
- 

The percentage of children who were slightly underweight decreased from baseline to the end of the study, but this difference was not significant between the groups.

It was concluded that maize-cowpea blend could be a vehicle for fortification because children liked the blend and also did not present additional burden for mothers when preparing the porridge from it.

Findings from two randomised control feeding trials using orange-fleshed sweetpotato among children in South Africa (van Jaarsveld et al., 2005) and Mozambique, South Eastern Africa (Low et al., 2007) showed that the coloured sweetpotato had positive impact on vitamin A status of infants and young children. Other researchers, van Jaarsveld et al. (2005) found greater improvement of liver vitamin A stores among 5–10 y old school children fed boiled mashed orange-fleshed sweetpotato ( $n=90$ ; an increase from 78% to 87% of children with normal vitamin status) compared with those consuming an equal amount of white-fleshed sweetpotato [ $n=90$ ; a slight decrease from 86% to 82% (normal vitamin A status)] for 53 school days. About 48% (232 of 490) of children (mean age at baseline was 13 mo) in the intervention group, where farmers had increased access to orange-fleshed sweetpotato vines and nutritional education, had low serum retinol concentration ( $<0.70 \mu\text{mol/L}$ ) compared with 58% (141 of 243) children in the control group (Low et al., 2007). In another study, among 3–6 y old children, conducted in Indonesia, in which red-fleshed sweetpotato contributed about 80% of  $\beta$ -carotene from test meals (children did not like spinach and swamp cabbage as the other sources of  $\beta$ -carotene), serum retinol level improved five times more in the group whose basic test meal was enriched with sweetpotato compared with those on the basic test meal (Jalal et al., 1998). These findings suggest that  $\beta$ -carotene from sweetpotato is bioavailable in humans.

The coloured varieties of sweetpotato are gaining attention in Ghana since their introduction in the mid 1990s. An extensive research programme in Ghana has resulted in the production of early maturing (3 to 3.5 months) of the coloured varieties of sweetpotato, locally known as *Okumkom*, *Sautie*, *Faara* and *Santom Pona* (Otoo et al., 2001; Council for Scientific and Industrial Research (CSIR), 2006). A recent report also indicates that several varieties of the coloured sweetpotato are being introduced in Ghana (Ofori et al., 2009). In the Upper East region of Ghana, it has been reported that sweetpotato is exported to the neighbouring country, Burkina Faso (FAO, 2005). Sweetpotato (presumably, the coloured varieties) was mentioned in the GDHS Reports as one of the food sources rich in vitamin A consumed by infants between 6–9 mo (GSS, 2004; GSS, 2009). The leaves and/or cooked

roots of these new varieties available in Ghana have been recommended as sources of minerals and vitamin A (Oduro et al., 2008; Ofori et al., 2009). These findings, coupled with the fact that sweetpotato is less laborious to cultivate than cereals (Woolfe, 1991; Padmaja, 2009), presents sweetpotato as a suitable choice for processing a dried complementary food.

However, there is no report in the output of the database search conducted on complementary food formulation from coloured varieties of sweetpotato for Ghanaian infants. The processing of the coloured varieties of sweetpotato as complementary food is likely to have a double advantage over cereal-legume blends by both improving vitamin A status and by enhancing iron absorption due to the relatively high  $\beta$ -carotene and low phytate content of sweetpotato.

### **2.3 Complementary foods processed using sweetpotato**

The use of sweetpotato in complementary foods using either household-level processing technologies (fermentation, germination, drying, milling or blending) or industrial-level methods (drum drying or freeze drying) have been reported in the literature and summarised as follows.

Cereal (maize or sorghum) (65%)-legume (soyabean or cowpea) (30%)-sweetpotato (5%) blends have been processed as complementary foods by either malting or cooking or fermenting the raw ingredients, followed by drying and milling into separate fine flours which were then blended (Nnam, 2000). Although the blends formulated satisfy the protein (15 g/100 g) and energy (1670 kJ/100 g) specifications of the Codex Standard for infant foods (Codex Alimentarius Commission, 1991), the sweetpotato was not the main ingredient. Sweetpotato flours (fermented or non-fermented) were combined with flours processed from the seeds of African breadfruit (*Treculia africana*) (with/without prior fermentation) in the ratio of 1:4 (Akubor, 2005). These formulations also meet the protein and energy specifications stated above. A combination of sweetpotato and soyabean in proportion of 70% and 30%, respectively has been suggested by Ijarotimi and Ashipa (2006) as an ideal combination to meet the protein and energy levels desired of foods for infants. However, using soyabean at 30% in combination with sweetpotato

would also result in a formulation which would be high in phytate. A sweetpotato-cowpea-groundnut household-level complementary food formulated with the legume incorporated at a level between 30–40% (Adenuga, 2010) would likely be high in phytate also. Therefore, if the proportion of soyabean incorporated in complementary food formulations could be reduced, the phytate level in the formulation would be low; consequently increasing nutrient bioavailability. Hence, we aimed to use a lower proportion of soyabean in our proposed sweetpotato-based formulations presented later in this report. Khan et al (2011) processed complementary food formulations by drum drying composite flours of sweetpotato (either 21.73% or 22.31%), rice (11.11%), wheat (12.78%), maize (5.56%) and whole milk powder (24.28%), rice bran protein isolate (either 6.16% or 6.29%), sugar (8.89%), vegetable oil (8.33%), antioxidant (0.01%) and vitamin-mineral mixture (0.10%) which met the protein, fat and energy specifications in the Codex Standard. However, these formulations would be difficult to replicate and also expensive to process because of the number of ingredients used. In all the sweetpotato-based formulations mentioned above, the variety of sweetpotato, the vitamin A level and the phytate level were not reported. It is worth stating that the focus of the research by Khan et al. (2011) was value addition to agro-industrial waste (rice bran) and not the promotion of sweetpotato as an ingredient in complementary food formulation. Only the work of Nandutu and Howell (2009) described below stated the variety of sweetpotato used in their complementary food formulations.

Nandutu and Howell (2009) used freeze-dried slices of orange-fleshed sweetpotato, to which fish (Tilapia skinned fillets), sunflower oil and either skim milk or soyabean flour was added to process their sweetpotato-based complementary food. The ingredients were mixed and blended into a soft paste. Antioxidants (ascorbic acid and  $\alpha$ -tocopherol) were added to the paste prior to cooking. The soup obtained was freeze-dried and milled into flour as a complementary food. The protein (24 g/100g), fat (3.3 g/100 g), calculated carbohydrate (62 g/100 g) and *in vitro* starch digestibility (68%), as maltose equivalent per total starch, compared well with the levels determined for a

nutritionally adequate commercial cereal-based complementary food in Africa (Nestlé® Cerelac®). However, the level of fat in this orange-fleshed sweetpotato-based complementary food is far below the specification (10–25 g/100 g) in the Codex Standard (Codex Alimentarius Commission, 1991). Additionally, the vitamin A and phytate levels were not reported.

With reference to the information presented for sweetpotato-based complementary foods above, we have proposed a new formulation which could meet the protein, energy and fat requirements of complementary foods using the Codex Standard (Codex Alimentarius Commission, 1991).

## **2.4 Proposed formulation of sweetpotato-based complementary food**

It is noteworthy that food-to-food fortification without addition of vitamins and minerals supplements seldom meets the nutrient recommendations for infants and young children (Lutter and Rivera, 2003). However, a careful choice of ingredients, as described below, is likely to have some positive impact. The primary target for this formulation was to meet energy content of at least 1670 kJ/100 g, protein level of 15 g/100 g and fat content in the range of 10–25 g/100 g as specified in Codex Alimentarius Commission guidelines on formulated foods for older infants and young children (Codex Alimentarius Commission, 1991). The ingredients to be used in the proposed formulations are listed in **Table 2.5**, and include cream-fleshed sweetpotato, soyabean flour, soyabean oil and either skim milk powder or fish powder prepared from anchovies.

A computer programme (Nutrition Calculator) developed by Global Alliance for Improved Nutrition (GAIN) (Jonathan Siekmann, personal communication, 15/07/2010) was used to obtain the proportions of ingredients needed to get the energy, protein and fat values stated above. To improve both the content and quality of protein, fish powder prepared from anchovies was included as an ingredient for the household-level formulation and skim milk powder was included for the industrial-level prototypes. Anchovies are available, inexpensive and culturally acceptable additions to porridge for Ghanaian infants (Lartey et al., 1999; Akor et al., 2001). The inclusion of anchovy



powder would also serve as sustainable source of calcium, iron and omega 3 fatty acids in complementary foods produced at the household-level. Skim milk powder was chosen because of the suggested positive association with linear growth (de Pee and Bloem, 2009). Further, milk is an ingredient in most industrial level dried complementary food used worldwide, example, Nestlé® cereal-based products. To increase fat content, and consequently the energy, soyabean oil was included in the proposed sweetpotato-based formulations.

Two formulations (**Table 2.5**) were developed, one to be processed at household-level and the other, industrial-level. The household-level formulation containing the fish powder could be processed by toasting in an open-pan or in an oven. The industrial formulation with the skim milk powder could be processed using either roller drying or extrusion cooking as possible industrial-level methods. Roller drying is proposed because it is the oldest commercial drying technique in the food industry used to produce powdered products including complementary food (Bhandari and Hartel, 2005). On the other hand, extrusion cooking is a more recent, versatile, high temperature short time (residence time of 1 to 2 min) and widely used technique in cereal processing such as snack foods and breakfast cereals (Cheftel, 1986). The additional advantage of extruders is that several ingredients (pre-processed into grits or flour) can be combined in one run (Guy and Benjamin, 2003) into a single product (extrudates). With infant food, the extrudates are subsequently dried and milled.

The proposed complementary food formulations based on sweetpotato will be referred to as ComFa. The data in **Table 2.5** show that the estimated energy, protein and fat would meet the requirements in the Codex Alimentarius Commission guideline. ComFa Industrial and ComFa Home have estimated vitamin A content of 780 and 870 µg RE/100 g, respectively. Thus, both ComFa formulations are likely to contribute positively to initiatives designed to reduce VAD in Ghana compared with Weanimix, which has vitamin A content of 36 µg RE/100 g. The estimated levels of phytate in the ComFa Industrial and ComFa Home would be 154 mg and 45 mg/100 g, respectively after correcting for 30% loss due to heat processing (Sathe and

Venkatachalam, 2002). The estimated phytate levels are based on the composition of the formulations and the phytate levels in sweetpotato and soyabean indicated earlier. The phytate content of Weanimix is 480 mg/100 g (Lartey et al., 1997); thus sweetpotato-based formulations would have about a third of the phytate content of Weanimix.

**Table 2.5** Ingredients and estimated levels of macronutrients of proposed sweetpotato-based complementary food

| Ingredient (g/100 g) *          |  | Household-level | Industrial-level | Estimated nutrient composition (/100 g dry weight basis) assuming final moisture of 5% |                 |                  |
|---------------------------------|--|-----------------|------------------|--|-----------------|------------------|
| Cream-fleshed sweetpotato flour |  | 66              | 72               |  |                 |                  |
|                                 |  |                 |                  | Nutrient   | Household-level | Industrial-level |
| Full fat soyabean flour         |  | 10              | 15               | Energy, <i>kJ</i>  | 1858            | 1787             |
| Soyabean oil                    |  | 6               | 6                | Protein, <i>g</i>  | 22              | 20               |
| Iodised salt                    |  | 0.5             | 0.5              | Fat, <i>g</i>  | 24              | 21               |
| Sugar                           |  | 0.5             | 0.5              | Carbohydrate, <i>g</i>   | 36              | 43               |
| Skim milk powder                |  | -               | 6                | Vitamin A (µg RE <sup>†</sup> )  | 870             | 780              |
| Fish powder (anchovies)         |  | 17              | -                |  |                 |                  |

\*Nutrient composition of the ingredients was available in the GAIN Nutrition Calculator except for sweetpotato and fishmeal. Nutrient composition of sweetpotato FoodWorks version 6 (FoodWorks, 2009). Data on fish (*Engraulis hepsetus*) to be used was not available; therefore the data on *Engraulis encrasicolus* from USDA (<http://www.nal.usda.gov/fnic/foodcomp/search/>) was used.

<sup>†</sup>Retinol equivalents

## **2.5 Conclusion**

Complementary food processed from maize, soyabean and groundnut with/without fish powder has been shown to be positively associated with growth over cereal-only porridge. Cereal-only or cereal-legume porridge without fortification with vitamin and mineral supplements will lead to deficiency of micronutrients such as iron and vitamin A among infants because such foods are both low in the micronutrients (example, vitamin A) and high in phytate, which compromises nutrient bioavailability. Vitamins and minerals supplements are not available for use by families in households in Ghana due to cost.

Sweetpotato-based complementary food blends, which could be processed industrially (using either extrusion cooking or roller drying) or at the household-level (using toasting in an oven or open-pan) are proposed. These formulations would satisfy the requirements for energy, protein and fat specified in the Codex Alimentarius Commission guidelines and also be a better source of  $\beta$ -carotene. In this regard, we suggest that the ComFa-home formulation should be used as household-level complementary food as it would be a better source of endogenous vitamin A than unfortified cereal-legume blends commonly used in Ghana. Also, the relatively low estimated level of phytate and high level of  $\beta$ -carotene in the sweetpotato-based complementary food would likely make iron, calcium and zinc more bioavailable than from the cereal-based products.

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### **Chapter 3: Processing, Macronutrient and Phytate Levels**

*“...while developing better quality complementary food supplements clearly has an important role to play in addressing the malnutrition crisis and saving lives now, particularly in emergency situations, donor-supported food programs are not enough as a long-term strategy.”* (The PLoS Medicine Editors, 2008)\*

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\*The PLoS Medicine Editors. (2008). Scaling up international food aid: Food delivery alone cannot solve the malnutrition crisis. *PLoS Med*, 5(11), e235.

This chapter, Paper III, has been published in Food and Nutrition Bulletin, entitled, “*Sweetpotato-based complementary food for infants in low-income countries*” (Amagloh, Hardacre, et al., 2012). The sweetpotato-based formulations (ComFa) proposed in the previous chapter, were processed using both household- or industrial-level methods into products similar to a dried infant cereal. The macronutrient, fructose and phytate levels of the ComFa formulations were compared with enriched Weanimix, the maize-based complementary food being promoted in Ghana.

### **Highlights**

- Sweetpotato can be processed into products similar to a dried infant cereal.
- The sweetpotato-based infant food could be prepared either at the household- or industrial-level. The household-level method employed was oven toasting, while extrusion cooking and roller drying were used as industrial processing methods.
- Sweetpotato-based complementary foods are relatively low in phytate compared with maize-based infant food. Thus, the sweetpotato-based food matrix may not compromise nutrient bioavailability as much as that of a non-dephytinised cereal-legume blend.
- The phytate content was not significantly affected by the processing methods used for the ComFa formulations.
- The fructose level in the sweetpotato-based complementary foods was more than five times that in enriched Weanimix.

## **Abstract**

*In low-income countries, most infants are given cereal-based complementary foods prepared at the household level. Such foods are high in phytate, which limits the bioavailability of nutrients, including iron, calcium, zinc, and in some cases proteins, which are crucial to the development of infants. In this study, the levels of macronutrients (protein, fat, and carbohydrate), gross energy, and fructose, as well as phytate in sweetpotato-based (denoted ComFa) formulations and enriched Weanimix (dehulled maize–dehulled soyabean–groundnut blend with fish powder and sugar incorporated) were compared. A composite flour of sweetpotato and soyabean containing fish powder was processed by oven toasting as a home-based complementary food. Another blend containing skim milk powder was processed by extrusion cooking or roller drying as industrial-based prototypes. The macronutrient composition and the levels of fructose and phytate were determined in the ComFa formulations and enriched Weanimix. The ComFa formulations and the enriched Weanimix met the stipulated values in the Codex Alimentarius Commission standard for energy (400 kcal/100 g), protein (15 g/100 g), and fat (10 to 25 g/100 g) for complementary food, with the exception of the industrial-based ComFa formulations, which satisfied 83% of the protein requirement. The ComFa formulations had a quarter of the phytate level of enriched Weanimix. The fructose level in the sweetpotato-based complementary foods was more than five times that in enriched Weanimix. The sweetpotato-based formulations were superior to enriched Weanimix as complementary foods for infants in low-income countries, based on the fructose, which makes the porridge naturally sweet, and phytate levels.*

**Key words:** Complementary food, fructose, phytate, sweetpotato

## **Abbreviation:**

UNICEF      United Nations Children's Fund



### **3.1 Introduction**

Malnutrition among infants in low-income countries can be related to the composition of the complementary foods introduced after the period of exclusive breastfeeding. The high incidence of anaemia among children in sub-Saharan Africa is partly due to poor iron bioavailability from their diet, which tends to be high in the antinutrient, phytate [1]. Phytate is the storage form of phosphorus in plants [2] and limits the bioavailability of essential nutrients such as iron [3, 4], zinc [5], probably calcium [6], and protein [7]. Improved starch and protein digestibility has also been observed in foods when the level of phytate was reduced [8, 9].

Cereals and legumes processed into dry products and reconstituted as porridge are widely promoted as complementary foods in sub-Saharan Africa by researchers and health organisations. Such food mixes have improved protein and energy contents compared with cereal-only gruel [10]. In Ghana, for example, collaboration in 1987 between UNICEF and the Ghanaian Ministry of Health led to the development of Weanimix, a complementary food blend of maize (75%), soyabean/cowpea (15%), and groundnuts (10%) [11]. The composition of Weanimix formulations has been the prototype of other cereal-based complementary foods developed in sub-Saharan Africa [10, 12, 13]. Although Weanimix has adequate protein (15 g/100 g) and energy (435 kcal/100 g) contents [11], it is a combination of high phytate-containing food crops, hence the high phytate level in the product. The phytate contents of maize, soyabean, and groundnut are 0.80, 1.5, and 1.8 g/100 g, respectively [14]. Larrey et al. [15] reported the level of phytate in Weanimix processed from unrefined maize (75%), unrefined soyabean (15%), and groundnut (10%) as 480 mg/100 g.

Complementary foods based on either root or tuber crops have been shown to be significantly lower in phytate (by 3% to 20%) than cereal and legume-based foods [16, 17]. A level of 0.01 g/100 g of phytate has been found in sweetpotato [14]. The inhibitory effect of phytate has been shown to be dose-dependent [18]; hence the use of sweetpotato as the main ingredient to process complementary food is likely to result in a product that would be low in phytate and would be less inhibitory on nutrient absorption.

The red-, orange-, or cream-fleshed sweetpotato (herein referred to as the coloured variety) is ideal because it is naturally sweet, liked by young children, well suited to the tropical climate in Africa, and high in  $\beta$ -carotene, the precursor of vitamin A [19-21]. Sweetpotato contains inulin in addition to starch as a carbohydrate reserve [22]. Inulin is a soluble, fermentable, non-starch carbohydrate containing fructose as monomers [22]. When inulin was added to bread or liquid food in a human feeding trial, it was associated with increased calcium bioavailability [23]. Fructose, as well as imparting sweetness to food [24], could possibly improve iron bioavailability [25]. Human infants were found to consume fructose and sucrose (table sugar) in similar quantities, which indicates that the infants liked the sweetness of fructose [26]; hence the presence of fructose in food would increase food intake without the need to add sweeteners. Daily fructose intake of 50 g or less, or approximately 10% of total food energy, has been recommended as not deleterious [27].

Despite the above-mentioned nutritional benefits to be derived from processing the coloured variety of sweetpotato into dried products that could be used as complementary foods for infants in Africa, only a few such formulations have been reported in the literature [28, 29]. In these studies, defatted or full-fat soyabean flour was added to orange-fleshed sweetpotato at various ratios, and the mixture was processed in an extruder. The extrudates were dried and milled into flour that could be used as complementary food. In another study, freeze-dried slices of orange-fleshed sweetpotato, fish (skinned *Tilapia* fillets), and sunflower oil with either skim milk or soyabean flour [30] were blended into a soft paste and cooked as soup. The soup was then freeze-dried and milled into flour as a complementary food. The nutritional composition and *in vitro* starch digestibility of the freeze-dried processed complementary food compared favourably with that of Nestlé® CERELAC®, a popular commercial cereal-based complementary food used in Africa. However, Nandutu and Howell [30] did not indicate the levels of phytate in their product. Therefore, in this study we developed complementary food formulations containing cream-fleshed sweetpotato and determined their phytate contents.

Extrusion cooking and roller drying were used as possible industrial-based processing methods because of their popularity in processing dried food products [31, 32]. Oven toasting was used as a possible home-based processing method. It is noteworthy that heat processes, such as toasting (or other home cooking methods), extrusion cooking, or roller drying, have been shown to be less effective at counteracting the negative effect of phytate on iron absorption [33]. Cooking usually reduces phytate levels by 50%, whereas high-temperature and short-term heat processing, such as extrusion cooking, results in degradation of this antinutrient by about 30% [34].

The purpose of this study was to assess the macronutrient composition and the fructose and phytate contents of cream-fleshed sweetpotato-based complementary foods (ComFa formulations) and enriched Weanimix (dehulled maize–dehulled soyabean–groundnut blend containing fish powder and sugar).

We hypothesised that the ComFa formulations would be lower in phytate, and that both ComFa formulations and enriched Weanimix would satisfy the recommended macronutrient levels of complementary food according to the Codex Alimentarius Commission standard [35].

## **3.2 Materials and methods**

### **3.2.1 Description of ingredients used in the formulations**

Sweetpotato (*Ipomoea batatas*), marketed as “gold kumara” in New Zealand and “O’Henry” in the United States, was sourced from Delta Produce Co-op Ltd, Dargaville, New Zealand. The root has a cream flesh with orange streaks and is a variant of Beauregard (an orange-fleshed variety). Fish powder prepared from smoke-dried anchovies (*Engraulis hepsetus*) without the heads was imported from Ghana. Full-fat dehulled soyabean (*Glycine max*) flour (Floursoy 25, The Three Mac Company), soyabean oil (AMCO Soya, Goodman Fielder Ltd.), and skim milk powder (NZMP, Fonterra Ltd.) were sourced from companies in New Zealand. Iodised salt and white sugar were purchased from local supermarkets in Palmerston North, New Zealand. The ingredients of enriched Weanimix were refined (dehulled) maize (*Zea mays*) meal (Springbok, South Africa), soyabean seed, and groundnut (*Arachis hypogea*) paste. These were also obtained from local supermarkets in Palmerston North.

### **3.2.2 Preparation of cream-fleshed sweetpotato flour**

The roots of sweetpotato were washed, peeled with a kitchen knife, and immersed into 0.5 g/100 g sodium acid pyrophosphate (SAPP) solution to prevent discolouration of the roots. The peeled roots were diced into chips approximately 1.4 cm thick with a Dito-Sama TR 21slicer (Model G59979, Aubasson, France) and re-immersed in the SAPP solution. The chips were then blanched at 90°C for 1 minute in a steam-jacketed pan and dried in a forced-air oven (Whitlock Speedy Smoke “N” Cooker, Progressive Machinery Design Ltd., Auckland, New Zealand) at 60°C to constant weight (final moisture content, 2.5 to 3.3 g/100 g). The dried chips were milled into flour with a grain mill fitted with a 1-mm screen fabricated in China for Plant and Food Research, Palmerston North, New Zealand.

### **3.2.3 Formulation of sweetpotato-based complementary foods (ComFa)**

The proportions of the ingredients used for each of the processing methods are shown in **Table 2.5** (Chapter 2, Page 52). A computer programme (Nutrition Calculator) developed by Global Alliance for Improved Nutrition

(GAIN) (Jonathan Siekmann, personal communication, 15 July 2010) was used to calculate the proportions of ingredients needed for an energy content of 400 kcal/100 g, a protein level of 15 g/100 g, and a fat content in the range of 10 to 25 g/100 g, as specified in the Codex Alimentarius Commission guidelines for complementary foods for older infants and young children [35]. The composite flour of the entire ingredients (approximately 2.0 kg) was homogenised in a food mixer (NSF, ARM-02, Thunderbird, Canada) with mixing speed set at position 2 for 10 minutes.

In this paper, the ComFa home-based formulation refers to sweetpotato–soyabean blend enriched with fish powder prepared from smoke-dried anchovies and toasted in an oven, and ComFa industrial-based formulations denote sweetpotato–soyabean blend enriched with skim milk powder either extrusion cooked or roller dried. Fish powder was incorporated in the ComFa home-based formulation as a sustainable source of animal protein, calcium, iron, and omega-3 fatty acids. Skim milk powder was incorporated in the industrial prototypes because of the suggested positive association of milk with linear growth [36]; it is also an ingredient in Nestlé® CERELAC®.

#### **3.2.4 Extrusion-cooked ComFa**

A twin-screw extruder (Clextral BC21, Firminy Cedex, France) with a barrel length of 700 mm and seven temperature-controlled barrel sections was used. The temperatures were set from the first barrel (from the feeder end) as 30, 30, 80, 120, 120, 80, and 80°C. The screw speed was 250 rpm, with a feed rate of 165 g/min and a water feed rate of 6.0 g/min. A die with 3.4-mm diameter aperture was used. The moist, noodle-like extrudates were dried in the forced-air oven at 60°C for 48 hours and milled with the grain mill described above to obtain the extrusion-cooked ComFa sample.

#### **3.2.5 Roller-dried ComFa**

A laboratory-level roller drier (Richard Simon & Sons Ltd., Nottingham, England) with a revolving drum 200 mm wide and 170 mm in diameter was used. A slurry was prepared by mixing 2.0 kg of homogenized composite flour with 3.5 L of potable water and partially cooked in a steam-jacketed pan for 10 minutes at temperatures between 80° and 83°C to partially gelatinise

the starch. A tablespoonful of the precooked blend was transferred to the rotating drums of the roller drier to obtain dry flakes. The pressure of the steam was set at 100 kPa and the temperature at 106.7°C. The rollers rotated at 1.2 rpm. The flakes obtained were oven-dried at 60°C for 48 hours and passed through 800-µm mesh to obtain the roller-dried ComFa sample.

### **3.2.6 Oven-toasted ComFa**

The homogenous composite flour was toasted in a commercial oven (AR 85, Electrolux, Steelfort Engineering Company Ltd., Palmerston North, New Zealand) pre-heated to 120°C for 30 minutes with intermittent stirring at 5-minute intervals. The oven-toasted ComFa (also referred to as ComFa home-based) was further dried in the forced-air oven at 60°C for 48 hours, as was done for the other ComFa formulations.

### **3.2.7 Enriched Weanimix**

Whole soyabean seeds were toasted in an electric frying pan (Kambrook 9708, Hong Kong), dehulled by hand, and milled into flour with the grain mill described previously. The maize meal and groundnut paste were added to the dehulled soyabean flour. The composite flour was toasted in the electric frying pan with continuous stirring until the blend turned golden brown. Typically, a blend containing 75%, 15%, and 10% of maize, soyabean, and groundnut, respectively, is referred to as Weanimix [11]. Fish powder and white sugar were added to Weanimix at 17% and 0.5% by weight, and the complementary food sample obtained was referred to as enriched Weanimix because it contained refined maize and soyabean flours and added sugar, which were not used in the earlier formulation.

## **3.3 Laboratory analyses**

Three samples of the above formulations were taken and stored separately in airtight plastic containers at -1.0°C prior to analysis. The laboratory analyses were performed on each of the samples.

### **3.3.1 Nutritional analyses**

Proximate composition was determined by the method described by the AOAC International [37]: moisture (AOAC 925.10), crude protein (AOAC

960.52), crude fat (AOAC 922.06), and ash (AOAC 969.32). Moisture analysis was modified as follows. The samples were dried at 108°C overnight (approximately 16 hours). An assay kit (K-ACHDF 11/08; Megazyme Int., Wicklow, Ireland) was used to analyse total dietary fibre and D-fructose contents. Available carbohydrate was estimated by difference as  $100 - (\text{moisture} + \text{protein} + \text{fat} + \text{ash} + \text{total dietary fibre})$  [38]. The gross energy content of the formulations was analysed by the Nutrition Laboratory (Massey University, Palmerston North, New Zealand) using bomb calorimetry.

### **3.3.2 Phytate analysis**

The phytate content of the formulations was determined with an assay kit (K-PHYT 05/07; Megazyme Int., Wicklow, Ireland). Defatted samples were used to reduce the interference of fat during the assay. Briefly, the phytate assay involved an extraction stage using hydrochloric acid followed by an enzymatic dephosphorylation step with phytase and alkaline phosphatase and a precipitation stage using a colour reagent prepared from ascorbic acid in sulphuric and ammonium molybdate. The absorbance of phosphorus standards and samples was measured with a UV/Visible spectrophotometer (Pharmacia LKB Ultrospec II, England) at 655 nm.

### **3.4 Statistical analysis**

The univariate and ANOVA procedures in SAS, version 9.1, were used to perform descriptive analysis and compare the means of triplicate measurements of macronutrient, fructose, and phytate levels. Means were considered to be significantly different when  $p < .05$ . The least significant difference test was used to separate the means when the difference was significant.

### **3.5 Results**

#### **3.5.1 Nutrient composition of the complementary foods**

The levels of macronutrients (protein, fat and carbohydrate) and gross energy in the ComFa formulations and enriched Weanimix are presented in **Table 3.1**. ComFa home-based (oven-toasted ComFa) and ComFa industrial-based (extrusion-cooked ComFa and roller-dried ComFa) formulations satisfied the requirements for energy (400 kcal/100 g) and fat (10 to 25 g/100 g), as stipulated in the Codex Alimentarius Commission standard [35].

However, the protein content of the ComFa industrial-based prototypes met 83% of the stipulated minimum level of 15 g/100 g in the Codex standard, and was lower than the protein content of the ComFa home-based product, which satisfied the stipulated value. The total fat level was not markedly different between the ComFa home-based and industrial-based formulations. The ash content was 66% greater in the ComFa home-based than the ComFa industrial-based formulations. The total dietary fibre level was not significantly different among the ComFa formulations, but the level was approximately twice the minimum level of 5 g/100 g for dietary fibre specified in the Codex standard [35]. The industrial-based ComFa formulations were approximately 8.0% higher in total available carbohydrate than the home-based product. There was a difference of approximately 1.0% in gross energy content between the ComFa home-based and industrial-based formulations.

Enriched Weanimix met the protein, fat, and energy values stipulated in the Codex standards [35], and contained about 22% more dietary fibre than the level recommended in the Codex standard. It was also significantly higher in protein, fat, and energy than all the ComFa formulations but was 40% lower in total dietary fibre than the ComFa formulations.



**Table 3.1** Macronutrient composition of sweetpotato- and maize-based complementary foods

| Complementary food <sup>b</sup> | Nutrient composition [g/100 g, except gross energy (kcal/100 g)] dry matter basis <sup>a</sup> |                         |                            |                         |                           |                          |                          |
|---------------------------------|--|-------------------------|----------------------------|-------------------------|---------------------------|--------------------------|--------------------------|
|                                 | Moisture   | Protein                 | Fat                        | Ash                     | Total dietary fibre       | Available carbohydrate   | Gross energy             |
| Extrusion-cooked ComFa          | 7.33±0.07 <sup>w</sup>   | 12.39±0.09 <sup>y</sup> | 10.07± 0.23 <sup>y</sup>   | 5.45±0.04 <sup>x</sup>  | 10.25±1.32 <sup>w</sup>   | 56. 07±1.14 <sup>x</sup> | 464.24±0.15 <sup>y</sup> |
| Roller-dried ComFa              | 3.52±0.06 <sup>y</sup>   | 12.48±0.09 <sup>y</sup> | 10.39±0.20 <sup>x, y</sup> | 5.34±0.04 <sup>x</sup>  | 10.57±0.70 <sup>w</sup>   | 58.92±0.48 <sup>w</sup>  | 463.42±0.30 <sup>y</sup> |
| Oven-toasted ComFa              | 2.36±0.04 <sup>z</sup>   | 20.14±0.15 <sup>x</sup> | 10.67±0.04 <sup>x</sup>    | 10.69±0.08 <sup>w</sup> | 8.18±0.44 <sup>w, x</sup> | 53.28±0.54 <sup>y</sup>  | 469.66±0.08 <sup>x</sup> |
| Enriched Weanimix               | 6.05±0.01 <sup>x</sup>   | 22.22±0.11 <sup>w</sup> | 11.25±0.15 <sup>w</sup>    | 11.25±0.10 <sup>w</sup> | 6.08±0.26 <sup>x</sup>    | 50.25±0.43 <sup>z</sup>  | 491.34±0.56 <sup>w</sup> |
| <i>p</i> -value                 | <0.0001  | <0.0001                 | 0.01                       | <0.0001                 | 0.01                      | 0.0001                   | <0.0001                  |

a. Values are means of triplicate determinations ± standard error of the mean; means with different superscript letters in a column are significantly different ( $p < .05$ ).

b. Extrusion-cooked ComFa and roller-dried ComFa contained 72% cream-fleshed sweetpotato flour, 15% full-fat soyabean dehulled flour, and 6.0% skim milk powder; oven-toasted ComFa contained 66% cream-fleshed sweetpotato flour, 10% full-fat soyabean dehulled flour, and 17% fish powder prepared from smoke-dried anchovies; enriched Weanimix contained 75% refined (dehulled) maize flour, 15% full-fat soyabean dehulled flour, and 10% groundnut, plus 17% (wt/wt) fish powder and 0.5% (wt/wt) sugar.

The ComFa formulations and enriched Weanimix satisfied the recommended minimum energy density requirement of 0.8 kcal/g [39] for complementary food (**Table 3.2**). The estimated daily protein and fat intakes from the ComFa formulations and the enriched Weanimix were higher than the suggested intakes from complementary foods (**Table 3.2**), but neither met the suggested daily energy intake of 200 kcal/day [39]. The average daily energy intakes from the ComFa formulations and the enriched Weanimix were about 77% and 81% of the recommended intake, respectively.

**Table 3.2** Estimated daily nutrient intakes from sweetpotato- and maize-based complementary foods against recommended daily requirements for 6 to 8 month-old breastfeeding infants in developing countries<sup>a</sup>

| Nutrient composition    | ComFa                       |                              |                             | Enriched Weanimix           | p-value | Reference value <sup>b</sup> |
|-------------------------|-----------------------------|------------------------------|-----------------------------|-----------------------------|---------|------------------------------|
|                         | Extrusion-cooked            | Roller-dried                 | Oven-toasted                |                             |         |                              |
| Energy density (kcal/g) | 4.64<br>±0.00 <sup>y</sup>  | 4.63<br>±0.00 <sup>y</sup>   | 4.70<br>±0.00 <sup>x</sup>  | 4.91<br>±0.01 <sup>w</sup>  | < .0001 | ≥ 0.8                        |
| Energy (kcal/day)       | 153.20<br>±0.0 <sup>y</sup> | 152.93<br>±0.1 <sup>y</sup>  | 154.98<br>±0.0 <sup>x</sup> | 162.14<br>±0.1 <sup>w</sup> | < .0001 | 200                          |
| Protein (g/day)         | 4.09<br>±0.03 <sup>y</sup>  | 4.12<br>±0.03 <sup>y</sup>   | 6.64<br>±0.05 <sup>x</sup>  | 7.33<br>±0.04 <sup>w</sup>  | < .0001 | 2                            |
| Fat (g/day)             | 3.32<br>±0.07 <sup>y</sup>  | 3.43<br>±0.06 <sup>x,y</sup> | 3.52<br>±0.02 <sup>x</sup>  | 3.71<br>±0.05 <sup>w</sup>  | .01     | 0                            |

a. Values are means of triplicate estimations ± standard error of the mean for ComFa formulations and Weanimix based on estimated average daily intake of 33 g (dry weight) of complementary food foods from findings by Lartey et al. [11]; means in the same row with different superscript letters are significantly different ( $p < .05$ ).

b. Source: Dewey and Adu-Afarwuah [39] for 6- to 8-month-old infants on average daily breastmilk intake (600 to 650 mL/day).

The fructose level in the sweetpotato-based complementary foods was more than five times that in enriched Weanimix (**Table 3.3**).

**Table 3.3** Fructose and phytate levels of sweetpotato- and maize-based complementary foods<sup>a</sup>

| Complementary food     | Fructose (g/100 g)       | Phytate (g/100 g)        |
|------------------------|--------------------------|--------------------------|
| Extrusion-cooked ComFa | 8.44 ± 0.15 <sup>w</sup> | 0.19 ± 0.03 <sup>w</sup> |
| Roller-dried ComFa     | 8.48 ± 0.32 <sup>w</sup> | 0.20 ± 0.02 <sup>w</sup> |
| Oven-toasted ComFa     | 7.92 ± 0.14 <sup>w</sup> | 0.23 ± 0.03 <sup>w</sup> |
| Enriched Weanimix      | 1.45 ± 0.05 <sup>x</sup> | 0.80 ± 0.03 <sup>x</sup> |
| <i>p</i> -value        | < .0001                  | < .0001                  |

a. Values are means of triplicate determinations ± standard error of the mean; means with different superscript letters in a column are significantly different ( $p < .05$ ).

### 3.5.2 Phytate content of the complementary foods

There were no differences in phytate content between the extrusion-cooked, roller-dried and oven-toasted ComFa formulations (**Table 3.4**). The phytate content of the ComFa formulations was one-quarter of that in the enriched Weanimix.

### 3.6 Discussion

The high protein and ash (an indication of the mineral content) levels in the ComFa home-based formulation compared with ComFa industrial-based prototypes may be attributed to the proportion of fish powder used, which was 17% (wt/wt), compared with 6.0% (wt/wt) of skim milk for the ComFa industrial-based formulations. The amount of skim milk used could be increased in the ComFa industrial-based formulations to increase the protein content to that of the ComFa home-based product. The absence of a significant difference in fat content between the ComFa home-based and industrial-based formulations suggests that the fish powder did not significantly contribute to the total fat content in the ComFa home-based formulation.

The higher total dietary fibre in the ComFa formulation compared with the requirement for complementary food specified in the Codex standard could be a concern for the use of these sweetpotato-based formulations as complementary foods as it was suggested in the Codex standard that fibre may affect the efficiency of the bioavailability of nutrients present in marginal levels. However, a significant proportion of the fibre in the ComFa formulations is likely to be soluble fibre, as it has been reported that about 25% to 50% of the total dietary fibre in sweetpotato is soluble [40, 41]. The benefits of soluble fibre as a fermentable substrate for *lactobacilli* and *bifidobacteria* (health-promoting bacteria) [42, 43] and in improving calcium bioavailability [23] suggest that the high level of total dietary fibre in the ComFa formulations could be beneficial. The difference in gross energy content between the home-based and the industrial-based ComFa formulations was due to the relatively high protein content, since the ComFa industrial-based formulations were higher in total available carbohydrates.

The estimated intake of fructose, on a daily intake of 33 g dry weight, [11] from the ComFa formulations is about 2.6 g/day, which is much less than the recommended daily maximum intake of 50 g/day [27]. This suggests that the benefits of fructose in imparting sweetness [24], subsequently leading to increased food intake [26, 44], could be achieved better from ComFa formulations than from enriched Weanimix. Therefore, the higher level of

fructose in the ComFa complementary foods could be a nutritional advantage.

The recommendation of Dewey and Adu-Afarwuah [39] for 200 kcal/day of energy from complementary food could be satisfied if the daily portion is increased, which is likely for the sweetpotato-based formulations due to the level of fructose. A daily portion intake of 43 g dry weight of ComFa formulations instead of 33 g would meet the suggested requirement.

The phytate levels of the home-based and industrial-based ComFa formulations were not different, which confirms that the effect of thermal processing on phytate degradation is minimal, as observed in another study [33]. In that study, the use of home cooking, roller drying, and extrusion cooking as thermal processing methods did not affect iron absorption. Since the inhibitory effect of phytate on micronutrient bioavailability is dose-dependent [18, 45], it is expected that micronutrient bioavailability from ComFa formulations would be higher than that from the enriched Weanimix. This finding also suggests that formulating complementary foods using ingredients such as sweetpotato, which is low in phytate, is a viable option to reduce the intake of this antinutrient.

Although the research focused on complementary food intended for infants (6 to 8 months of age) in sub-Saharan Africa, the findings can be translated into processing of similar products for other regions. The use of coloured-fleshed sweetpotato varieties (not white-fleshed variety) in complementary foods (such as ComFa) will help meet the recommended daily intake of vitamin A–rich fruits and vegetables by infants and young children [46]. In comparison with maize (corn)–soyabean blends used by international organisations in food aid programmes, ComFa has lower phytate and higher fructose levels, both of which are beneficial to the health of infants.

In conclusion, sweetpotato can be combined with soyabean, soyabean oil, and either fish powder or skim milk powder, and processed into a complementary food suitable for 6- to 8-month-old infants using home-based methods such as oven toasting and by industrial-based processes such as extrusion cooking or roller drying. The levels of energy, protein, and fat in

ComFa formulations and enriched Weanimix compared favourably with those stipulated in the Codex Alimentarius Commission guideline for complementary foods for infants and young children. Although the ComFa industrial formulations contained slightly less protein than specified in the Codex standard, the formulations met the suggested daily intake of protein from a complementary food for breastfeeding infants 6 to 8 months of age. The levels of phytate and fructose in the sweetpotato-based formulations make them superior to enriched Weanimix as complementary foods.

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## **Chapter 4: Sweetpotato-based formulation as a dietary source of vitamin A (Papers IV & V)**

*“Promoting provitamin A sources alongside of supplementation and fortification efforts is safe and desirable to achieve the goal of eradicating hidden hunger among those at risk for vitamin A deficiency.” (Tanumihardjo, Palacios, & Pixley, 2010)\**

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\*Tanumihardjo, S. A., Palacios, N., & Pixley, K. V. (2010). Provitamin A carotenoid bioavailability: What really matters? *International Journal for Vitamin and Nutrition Research*, 80(4-5), 336-350. doi: 10.1024/0300-9831/a000042

This chapter is based on Papers IV and V. Paper IV was been published in Maternal and Child Nutrition, entitled, “*A household-level sweetpotato-based infant food to complement vitamin A supplementation initiatives*” (Amagloh et al., 2012). Paper V, entitled, “*β-carotene retention in sweetpotato-based complementary food stored in different containers under simulated tropical temperature and humidity*” is presented after Paper IV. The overall rationale of this chapter was to investigate the contribution of the oven-toasted ComFa, the household-level sweetpotato-based complementary food, to reducing VAD based on the level of vitamin A and the stability of β-carotene under simulated tropical ambient conditions; partly addressing one of the objectives raised in Chapter 1. In Chapter 2, the choice of the fishmeal for the household-level ComFa formulation was to increase the mineral and protein (both in quantity and quality) contents.

### **Highlights (Paper IV)**

- The sweetpotato-based formulation and enriched Weanimix nearly met the amino acid score of at least 80% as specified in the Codex Standard.
- Addition of fishmeal prepared from small fish with bones intact improves the calcium content of the complementary food prepared at the household level comparing the level of calcium in Weanimix without fishmeal (3.59 mg/100 kcal) (Chapter 1, Paper I) and Weanimix with fishmeal (75.46 mg/100 kcal) reported in this Chapter.
- The vitamin A content of cream-fleshed sweetpotato-soyabean blend is far higher than the vitamin A content in maize-soyabean-groundnut processed as a household-level complementary food.

## **Abstract**

*Vitamin A deficiency prevalence in sub-Saharan Africa is high in spite of vitamin A supplementation programmes among children in most countries. Plant-based complementary foods remain the key source of nutrients in addition to breastmilk for infants in lower income countries. Cereal-legume blends are superior in protein and energy densities compared with maize, millet or sorghum-only porridge. However, unfortified cereal-legume and cereal-only porridges are low in vitamin A. A household-level sweetpotato-based infant food, rich in vitamin A, has been developed to complement vitamin A supplementation initiatives in sub-Saharan Africa. Composite flour containing sweetpotato, soyabean, soyabean oil and fishmeal was processed as complementary food by oven toasting (denoted oven-toasted ComFa). The oven-toasted ComFa and enriched Weanimix (processed from dehulled maize, dehulled soyabean, groundnut and fishmeal) were assessed for suitability as complementary food based on the nutrient composition using specifications in the Codex Standard (CS) (CODEX STAN 074 – 1981) as a reference. The sweetpotato-based formulation and enriched Weanimix met the energy, protein, fructose and fat specifications but barely met the amino acid score as indicated in the CS. However, only the oven-toasted ComFa met the calcium and almost half the vitamin A levels as specified in the CS. Oven-toasted ComFa was slightly lower in energy, protein and fat by a difference not greater than 4.0% but was higher by a difference greater than 100% in terms of fructose and vitamin A levels. Therefore, the sweetpotato-based complementary food is likely to support vitamin A supplementation initiatives in low-income countries better than the cereal-based formulation.*

**Key words:** Codex Standard, Fructose, Household-level, Soyabean, sub-Saharan Africa, Sweetpotato

## **Abbreviations:**

|        |                                   |
|--------|-----------------------------------|
| CS     | Codex Standard                    |
| FAO    | Food and Agriculture Organization |
| UNICEF | United Nations Children's Fund    |

|     |                           |
|-----|---------------------------|
| VAD | Vitamin A Deficiency      |
| WHO | World Health Organization |

## 4.1 Introduction

Vitamin A deficiency (VAD) is a worldwide health problem responsible for growth failure and increased susceptibility to infection, blindness and it contributes to anaemia by impairing iron transport and utilisation for haemoglobin synthesis (Ross, 1998; Administrative Committee on Coordination & Sub-Committee on Nutrition, 2001). An estimated 33% of children under 5 years worldwide have VAD using serum retinol level of  $<0.70 \mu\text{mol/L}$  as a cut-off (WHO, 2009). In Africa, the prevalence is higher (44%). In sub-Saharan Africa, all the countries are in the “severe” degree of VAD severity (defined as prevalence  $\geq 20\%$  among children under 5 years) with the exception of Gabon, Namibia and South Africa (WHO, 2009). This is in spite of on-going vitamin A supplementation programmes with a coverage rate of 67% (full supplementation; child receives 2 doses per year) to 77% (part supplementation; child receives at least 1 dose) for children under 5 years in sub-Saharan Africa (UNICEF, 2009). Poor diet and infection rates were identified as the main causes of VAD (WHO, 2009). The prohibitive cost of nutritionally adequate complementary food available in low-income countries makes it unaffordable to most resource-poor households. Cereal-based complementary foods are low in most vitamins and minerals (Lutter & Rivera, 2003) and have low bioavailability but are the key source of nutrients in addition to those in breastmilk for most infants in lower income countries (Gibson *et al.*, 2010). The primary ingredient for such foods can either be maize (corn), millet or sorghum which is low in lysine and energy (Annan & Plahar, 1995).

Cereal-legume blends, processed at the household-level, are not fortified with mineral or vitamin premix (Gibson *et al.*, 2010); hence, their micronutrient (particularly vitamin A) content is likely to be low and thus contribute to relatively high VAD in low-income countries where infants are fed such complementary food. In Ghana, Weanimix [a composite blend of maize (75%), soyabean (15%) and groundnut (peanut) (10%)] is considered to be an adequate household-level complementary food because it has relatively high energy and protein densities compared with weaning foods

from maize, millet or sorghum only (Lartey *et al.*, 1999). However, Weanimix alone is low in many micronutrients particularly vitamin A [8.3 µg retinol equivalents (RE)/100 kcal] (Lartey *et al.*, 1999) compared with 60 RE/100 kcal, which is the lowest vitamin A level specified in the Codex Standard (CS) (CODEX STAN 074–1981) for foods for infants and young children (Codex Alimentarius Commission, 2006). The effect of consumption of this improved household-level complementary food on vitamin A status among breast-fed infants using serum retinol level as an index had been investigated (Lartey *et al.*, 1998). Infants were randomly assigned to groups: Weanimix, Weanimix fortified with vitamin and mineral premix, Weanimix plus fish powder prepared from anchovies (*Engraulis hepsetus*) or *koko* (fermented maize only porridge) plus fish powder. The intervention foods were supplied for a 6-month period, starting when infants were 6 months of age. At the end of the study, there was a decrease in the serum retinol levels compared with the baseline serum retinol status in all the groups with the exception of those infants assigned to the Weanimix fortified with vitamin and mineral premix. The changes in serum retinol levels were: Weanimix =  $-0.08 \pm 0.3$  µmol/L, Weanimix fortified with vitamin and mineral premix =  $0.17 \pm 0.34$  µmol/L, Weanimix plus fish powder =  $-0.01 \pm 0.3$  µmol/L and *koko* plus fish powder =  $-0.02 \pm 0.3$  µmol/L). These findings suggest appropriate serum retinol levels that would support the growth demands for vitamin A were not met by giving these improved foods unless fortified with vitamin and mineral premix. Despite this, dried composite flours of cereals and legumes served as porridge to infants are currently promoted as complementary food in sub-Saharan Africa (Elemo *et al.*, 2011, Ayoya *et al.*, 2010, Mensa-Wilmot *et al.*, 2001).

Because household-level complementary foods in sub-Saharan Africa are not usually fortified with vitamin and mineral premix due to the cost of such products, there is a need to find alternative food crops that are higher in β-carotene (precursor for vitamin A) and highly bioavailable. Kapinga *et al.* (2010) reported that the coloured variety (the orange-, red-, or cream-fleshed but not the white-fleshed) of sweetpotato could be a significant dietary source



of vitamin A in sub-Saharan Africa. Two studies conducted in sub-Saharan Africa have demonstrated that consumption of boiled orange-fleshed sweetpotato improved vitamin A status of children and can significantly complement vitamin A supplementation initiatives to minimize VAD (Low *et al.*, 2007, van Jaarsveld *et al.*, 2005). Thus, the coloured sweetpotato has the potential to serve as a more sustainable food-based strategy for controlling VAD in children (van Jaarsveld *et al.*, 2005).  $\beta$ -carotene from sweetpotato is substantially better (in terms of bioavailability) than that from leafy vegetables or other vegetables (Administrative Committee on Coordination & Sub-Committee on Nutrition, 2001). However, the potential of the coloured-fleshed sweetpotato has not been fully exploited in terms of processing it into a dried complementary food like Weanimix to be prepared as porridge before serving to infants.

Few studies have used sweetpotato to prepare a cereal-like complementary food (Nandutu & Howell, 2009, Akubor, 1997, Ijarotimi & Ashipa, 2006, Ameny *et al.*, 1994, Akubor, 2005). In these product formulation studies, only Nandutu and Howell (2009) used the orange-fleshed sweetpotato variety; the others used the white-fleshed sweetpotato, which is devoid of  $\beta$ -carotene (van Jaarsveld *et al.*, 2005). We investigated the possibility of processing a non-cereal based complementary food using cream-fleshed sweetpotato (a variant of the orange-fleshed sweetpotato) using household-level food processing technologies as a measure to improve the vitamin A content of such foods.

The objective of this study was to assess the suitability of cream-fleshed sweetpotato-based complementary food and enriched Weanimix (processed from dehulled maize, dehulled soyabean, groundnut and fishmeal) using the nutrient composition including vitamin A specifications in the CS (Codex Alimentarius Commission, 2006) as a reference. This standard has been created by Food and Agriculture Organization (FAO) and WHO to serve as food standards and guidelines to ensure quality and safety of food worldwide (Zlotkin *et al.*, 2010). The current CS (CODEX STAN 074 – 1981) covers four categories of cereal-based foods for infants and young children listed below:

*Potential of ComFa formulation as dietary source of vitamin A (Paper IV)*

- cereals for infants (6 mo old onwards) to be prepared with milk or other nutritious drinks before consumption;
- cereals for infants to be prepared with water only or non-protein liquids before consumption;
- pasta;
- rusks and biscuits.

Each category has different nutritional specifications and should have a cereal such as wheat, rice, barley, oats, rye, maize, millet, sorghum or buckwheat as the base ingredient. Legumes, starch stems, oil seeds or starchy roots such as arrow root, yam or cassava may be added in smaller proportions.

## **4.2 Materials and Methods**

### **4.2.1 Ingredients for processing the complementary food**

All materials used were obtained in New Zealand, except where otherwise stated. Cream-fleshed sweetpotato (*Ipomoea batatas*) was sourced from Delta Produce Co-op Ltd. and processed into flour using household-level processing techniques comprising peeling, dicing, drying and grinding. Full-fat soyabean (*Glycine max*) flour (Floursoy 25) and soyabean oil (AMCO Soya) were purchased from local distributors. Iodised salt and white sugar were purchased from local supermarkets in Palmerston North. Fishmeal prepared from smoked-dried anchovies (*Engraulis hepsetus*) was imported from Ghana, West Africa.

The ingredients for Weanimix were refined maize meal (Springbok™, South Africa), soyabean seed and groundnut paste. These were obtained from local supermarkets in Palmerston North. The soyabean seeds were roasted in an electric frying pan (Kambrook 9708, Hong Kong) until golden brown, dehulled manually and milled into flour.

### **4.2.2 Processing of complementary foods**

Although the vitamin A content of complementary food was part of the basis of this work, we also targeted our formulation to have a protein density between 2.0–5.5 g/100 kcal and energy density of  $\geq 0.8$  kcal/g as indicated in CS for processed cereal-based foods for infants and young children (Codex Alimentarius Commission, 2006). We employed a computer programme (Nutrition Calculator) developed by Global Alliance for Improved Nutrition (GAIN) (Jonathan Siekmann, personal communication, 15/07/2010) to obtain the proportions of ingredients needed to get our target for protein and energy values stated above. Based on the GAIN Nutrition Calculator, we developed a formulation with the following proportions of ingredients: sweetpotato flour (66%), full-fat soyabean flour (10%), fishmeal (17%), soyabean oil (6%), iodised salt (0.5%) and granulated white sugar (0.5%). Fishmeal was included in the formulation as a household-level strategy to improve protein quality as well as the mineral contents. This composite food blend was mixed

in a food mixer (NSF<sup>®</sup>, ARM-02, Thunderbird<sup>®</sup>, Canada). The homogenous composite flour (approximately 2 kg) was toasted in an oven (AR 85, Electrolux, Steelfort Engineering Company Ltd., Palmerston North, New Zealand) at 120°C for 30 min with intermittent stirring (manually) every 5 min. The sample was further dried in a forced-air oven (Whitlock Speedy Smoke “N” Cooker, Progressive Machinery Design Ltd., Auckland, New Zealand) at 60°C for 48 h. The product obtained was referred to as the oven-toasted ComFa.

The composition of the maize-based complementary food was the same (75% maize, 15% soyabean and 10% groundnut) as reported by Lartey *et al.* (1999) with slight modifications. Refined (dehulled) maize flour was used instead of roasted unrefined maize flour because white maize grain for human consumption was not available in New Zealand. Dehulled flour instead of non-dehulled soyabean flour was used in the formulation. The composite flour was toasted until golden brown (approximately 20 min) in an electric frying pan (Kambrook 9708, Hong Kong). Fishmeal and granulated white sugar were added in similar proportion as in the oven toasted ComFa, which were 17% (w/w) and 0.5% (w/w), respectively. This formulation was referred to as enriched Weanimix.

#### **4.3 Nutrient composition analyses**

Three samples were taken from each formulation after processing and stored separately in air-tight plastic containers at -1.0°C in a chiller prior to analyses. The acid hydrolysis method for fat [Association of Official Analytical Chemists (AOAC) 922.06] described by the AOAC International (AOAC, 2005) was used for crude fat determination, and the AOAC 925.10 was used for moisture determination. An assay kit (K-ACHDF 11/08) (Megazyme Int., Wicklow, Ireland) was used to determine the levels of D-glucose and D-fructose contents. Carbohydrate was computed as the sum of D-glucose and D-fructose. The gross energy content (using bomb calorimetry), retinol and  $\beta$ -carotene [using HPLC, Carr-Price method, AOAC 974.29 (4)] and amino acid profile (hydrochloric acid hydrolysis followed by HPLC separation, AOAC

994.12) were performed by the Nutrition Laboratory (Massey University, Palmerston North, New Zealand). The protein content was determined as the sum of the individual amino acids residue (FAO, 2003). A conversion factor of 1 retinol equivalent (RE):12  $\beta$ -carotene was used in calculating the vitamin A content of oven toasted ComFa as has been used for boiled sweetpotato roots (Hagenimana *et al.*, 2001). The amino acid score (chemical score) was calculated using casein as reference protein. Calcium was determined using quadrupole inductively coupled plasma mass spectrometry (ICP-MS) after sample digestion by Campbell Micro Analytical Laboratory, Department of Chemistry, Otago University, New Zealand.

The three samples were all separately analysed for the nutrients mentioned above with the exception of the retinol,  $\beta$ -carotene and amino acid profile which were done on two samples because of logistical constraint. The energy and the other nutrient composition data were converted to energy density (kcal/g) and nutrient density (/100 kcal), respectively, to enable comparison with specifications in the CS (Codex Alimentarius Commission, 2006).

#### **4.4 Data analysis**

The mean and standard deviation were calculate using Minitab v15.1™ (Minitab Inc., US) and were plotted as bars using SigmaPlot v11.0 (Systat Software, Inc., GmbH, Germany).

## **4.5 Results and discussion**

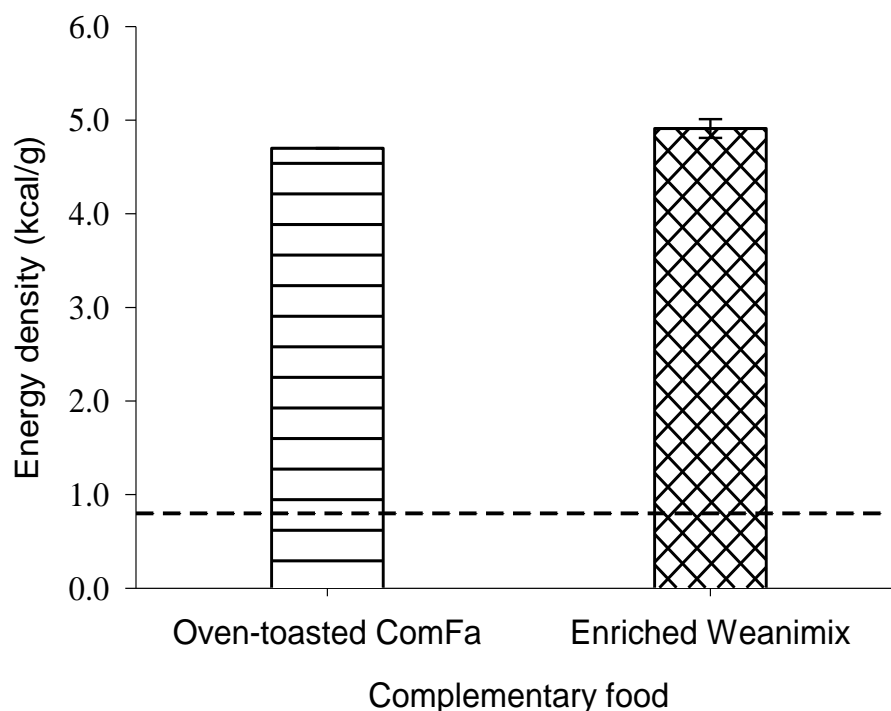
### **4.5.1 Product description for cereal-based infant food in the Codex Commission Standard**

The oven-toasted ComFa and enriched Weanimix were designed to be prepared as porridge with water only before consumption. The protein contents of the oven toasted ComFa and enriched Weanimix were  $4.01 \pm 0.12$  (mean  $\pm$  standard deviation) and  $4.19 \pm 0.12$  g/100 kcal, respectively. This protein level places both the oven-toasted ComFa and enriched Weanimix into the second category CS product definition. Such cereal-based infant food should have a protein content ranging from (2.0 to 5.5 g/100 kcal) and should be processed to be prepared with water only or non-protein liquids before consumption. For clarity, use of the CS henceforth refers to the standards for cereal-based complementary food for infants to be prepared with water only or non-protein liquids before consumption. Based on the proportion of the ingredients used to formulate the oven-toasted ComFa (see materials and methods section), this sweetpotato-based infant food did not contain cereal as the base ingredient. Thus, the oven-toasted ComFa should not be compared to the CS as it was established for cereal-based infant foods (Codex Alimentarius Commission, 2006). But in the absence of a suitable standard to assess the suitability of the oven-toasted ComFa as a complementary food, the CS was used. In contrast, the enriched Weanimix, which contained three times as much maize (cereal) as the combined proportion of soyabean and groundnut (legumes), qualifies as cereal-based complementary food for infants because maize is the base ingredient. The use of the CS also enabled us to compare the nutrient composition including vitamin A in oven-toasted ComFa and enriched Weanimix as household-level complementary foods.

### **4.5.2 Energy density**

The energy density of cereal-based complementary food should be  $\geq 0.8$  kcal/g (Codex Alimentarius Commission, 2006). The oven-toasted ComFa and enriched Weanimix met the minimum specified energy density in the Codex Standards as shown in **Figure 4.1**. Therefore, both formulations will

meet the energy required from complementary foods for infants. However, the oven-toasted ComFa ( $4.70 \pm 0.00$  kcal/g) was lower by a difference of 4.0% compared with the level of the enriched Weanimix ( $4.91 \pm 0.01$  kcal/g). The energy level of enriched Weanimix was similar with that indicated for



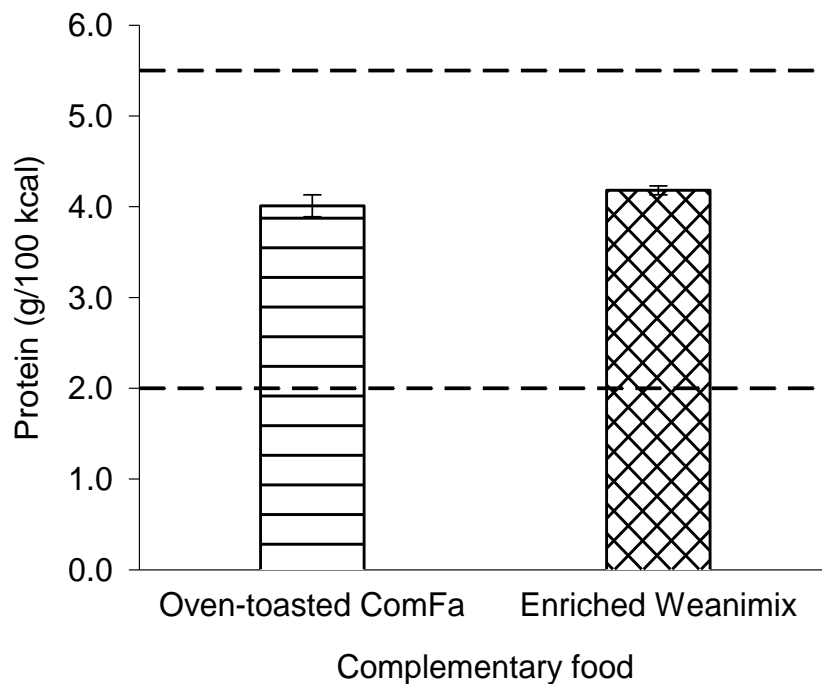
**Figure 4.1** Energy density of household-level processed complementary foods

Line (- - -) represents the minimum level for complementary foods for infants in the Codex Standard. Bar values (means  $\pm$  standard deviation,  $n = 3$ ).

#### 4.5.3 Protein

The protein content of the oven-toasted ComFa was  $4.01 \pm 0.12$  g/100 kcal and that of enriched Weanimix was  $4.19 \pm 0.12$  g/100 kcal; both were near the upper limit of protein specification (from 2.0 to 5.5 g/100 kcal) in the CS as shown in **Figure 4.2**. However, as shown in **Figure 4.3**, both products barely met the minimum amino acid score (index for protein quality) of at least 80% using casein as the reference protein as specified in the CS (Codex Alimentarius Commission, 2006). The amino acid scores of the oven-toasted

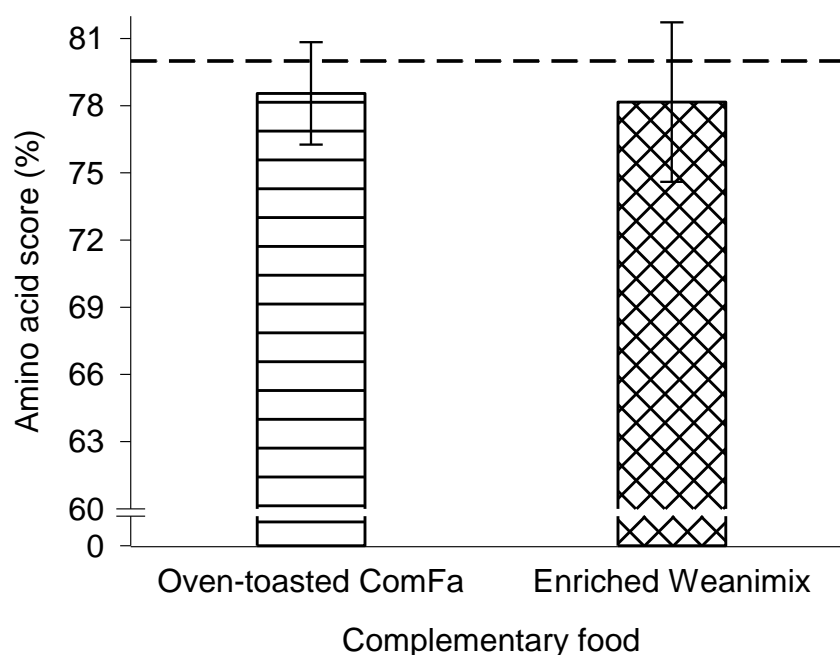
ComFa and enriched Weanimix were approximately 79% and 78%, respectively. The oven-toasted ComFa was lower in protein by a difference of 4% than the enriched Weanimix, but was slightly higher in amino acid score (1.0%). These data suggest that high protein quantity does not necessarily indicate high protein quality. Because the fishmeal used contributed significantly to the total protein level, we suggest that the proportion could be increased to at least 20% as used by Lartey *et al.* (1999) to increase the amino acid score.



**Figure 4.2** Protein density of household-level processed complementary foods

Lines (- - -) represent the minimum and maximum levels for complementary foods for infants in the Codex Standard. Bar values (means  $\pm$  standard deviation,  $n = 3$ ).





**Figure 4.3** Amino acid score of household-level processed complementary foods

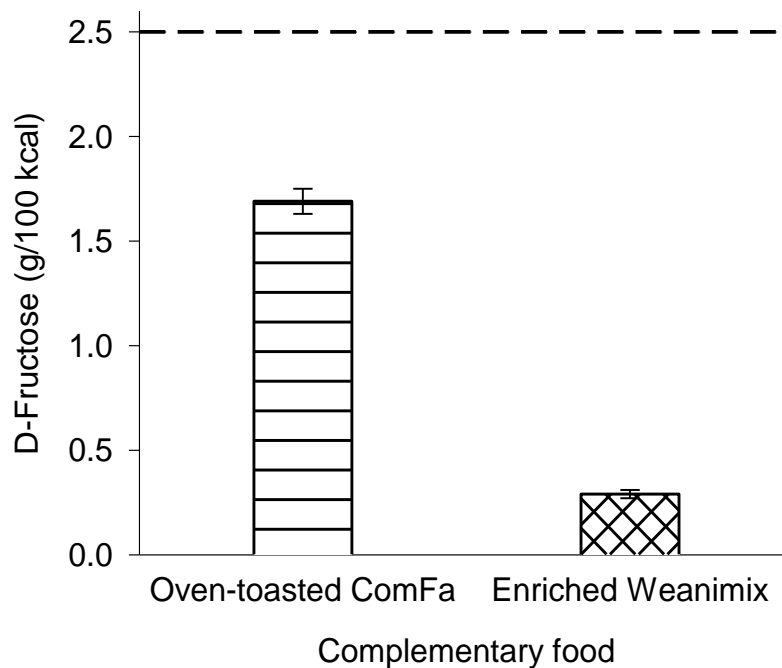
Line (- - -) represents the minimum level for complementary foods for infants in the Codex Standard. Bar values (means  $\pm$  standard deviation,  $n = 2$ ).

However, addition of more fishmeal to this level will accentuate the fishy smell and likely have sensory implications, particularly in the case of the oven toasted ComFa.

#### 4.5.4 Carbohydrate

There is no specification for carbohydrate in the CS for the main ingredients used to prepare cereal-based complementary food. According to the CS, when sucrose, fructose, glucose, glucose syrup or honey is added to infant food, the amount of carbohydrates from these sources should not exceed 5.0 g/100 kcal (Codex Alimentarius Commission, 2006). As shown in **Figure 4.4**, the fructose level of the oven-toasted ComFa was  $1.69 \pm 0.06$  g/100 kcal, and lower than the value of fructose stipulated in the CS (2.5 g/100 kcal). The fructose content of the oven-toasted ComFa was higher by a difference greater than 100% compared with enriched Weanimix ( $0.29 \pm 0.02$  g/100 kcal). The sweetpotato used in our formulation contributed significantly to the

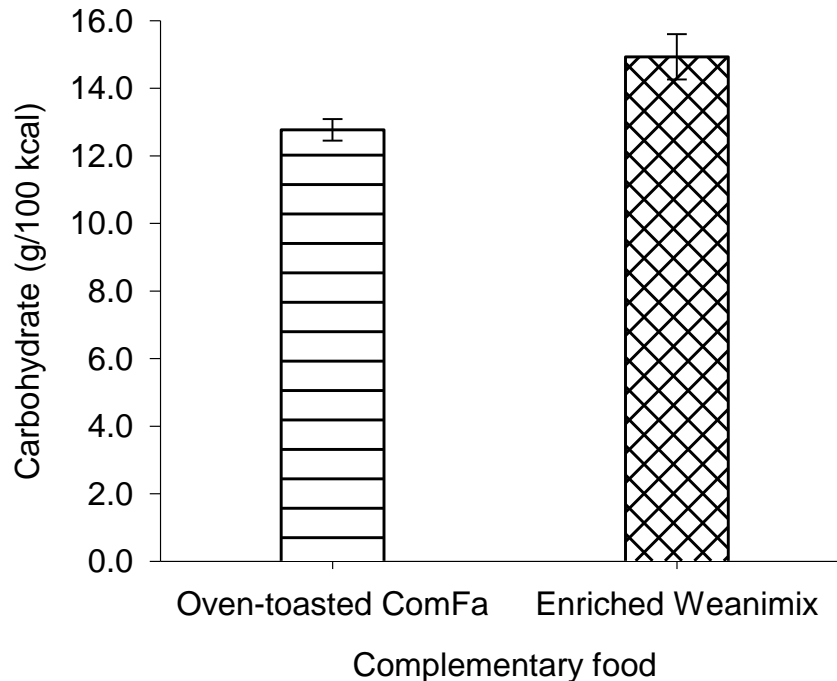
fructose content and could be responsible for the sweetness of sweetpotato-based food products.



**Figure 4.4** Fructose density of household-level processed complementary foods

Line (- - -) represents the maximum level if fructose is added as an ingredient to complementary foods for infants in the Codex Standard. Bar values (means  $\pm$  standard deviation,  $n = 3$ ).

The high fructose level in sweetpotato-based formulation compared with maize-based product is likely to impart sweetness when prepared for consumption reducing the need for additional sugar for sweetening. Hence, the cost of sugar is likely to be reduced when our proposed formulation is promoted and accepted by mothers as household-level complementary food in low-income countries. Furthermore, the higher fructose content in the oven-toasted ComFa can be seen as a nutritional advantage over enriched Weanimix because it would result in higher food intake by infants as it has been reported that the level of sweetness of complementary food increased the quantity of product consumed (Dewey & Brown, 2003).



**Figure 4.5** Carbohydrate (glucose + fructose contents) density of household-level processed complementary foods

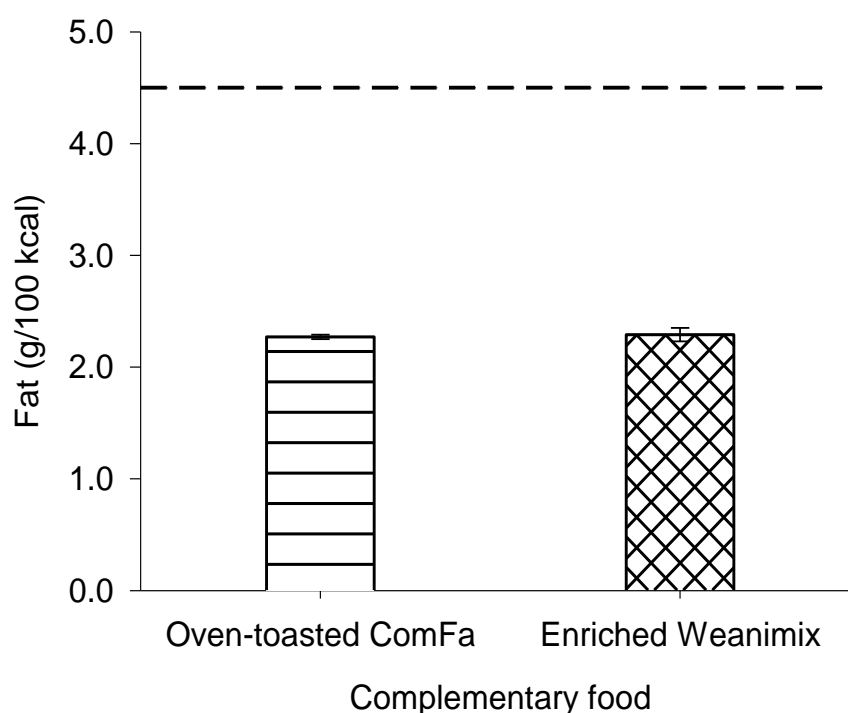
Bar values (means  $\pm$  standard deviation,  $n = 3$ ).

As shown in **Figure 4.5** above, the carbohydrate (as the sum of the measured glucose and fructose contents) in the oven-toasted ComFa was  $12.77 \pm 0.32$  g/100 kcal, and lower by a difference of 7.7% compared with the level of enriched Weanimix ( $14.93 \pm 0.67$  g/100 kcal). The lower glucose content of the oven-toasted ComFa by a difference of 2.8% compared with the level of enriched Weanimix is an indication of less starch content in the oven-toasted ComFa. Starch granules are polymers of D-glucose units and thicken when mixed with water and heat is applied (BeMiller & Huber, 2008). Enriched Weanimix is therefore likely to form a more viscous porridge than the oven-toasted ComFa because of its high starch content when it is prepared as porridge for consumption and will require dilution with water to reduce the viscosity (Mensah & Tomkins, 2003). Reducing the viscosity to a consistency that an infant will be able to consume will lead to dilution of nutrients; therefore the quantity of nutrient intake will be reduced. The high glucose content in enriched Weanimix contributing to the higher carbohydrate

than the oven-toasted ComFa is not of any nutritional significance as both complementary food formulations did not widely vary in energy as previously discussed.

#### 4.5.5 Lipids

Both the oven-toasted ComFa and enriched Weanimix had fat at levels not exceeding the maximum level stipulated in the CS (4.5 g/100 kcal) (Codex Alimentarius Commission, 2006). The difference between the oven-toasted ComFa ( $2.27 \pm 0.02$  g/100 kcal) and enriched Weanimix ( $2.29 \pm 0.06$  g/100 kcal) was below 1.0% (**Figure 4.6**).

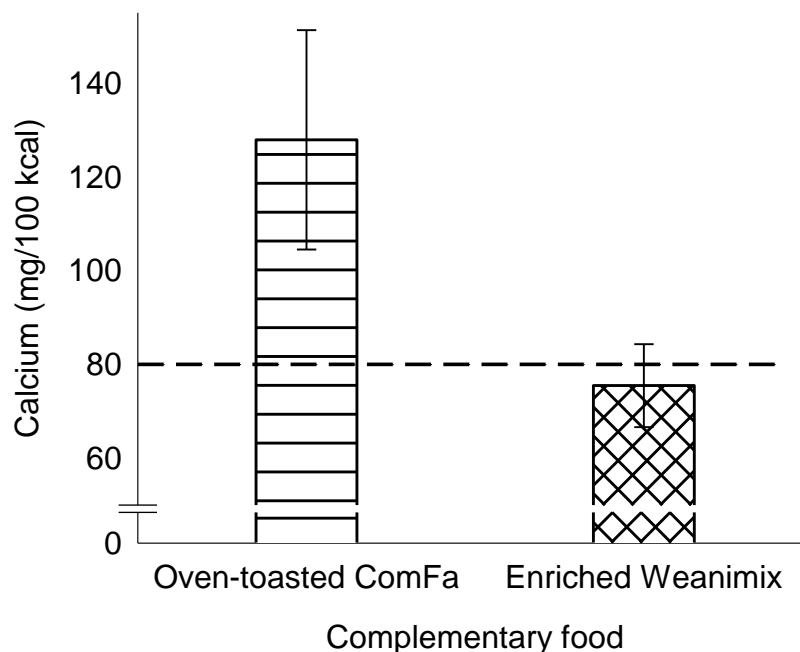


**Figure 4.6** Fat density of household-level processed complementary foods  
Line (- - -) represents the maximum level for complementary foods for infants in the Codex Standard. Bar values (means  $\pm$  standard deviation,  $n = 3$ ).

Using the CS specifications for protein, energy and lipid, the oven-toasted ComFa and enriched Weanimix met the requirements of infant foods to be prepared with water only or non-protein liquids before consumption.

#### 4.5.6 Calcium

As shown in **Figure 4.7**, the oven-toasted ComFa met the minimum calcium specification of 80 mg/100 kcal in the CS (Codex Alimentarius Commission, 2006). The calcium content of the enriched Weanimix was 6.0% lower than the minimum stipulated value in the CS. The oven-toasted ComFa was higher in calcium by a difference of 52% than the enriched Weanimix, despite the two formulations having similar amounts of fishmeal incorporated. In Ghana, West Africa, anchovies are eaten with the bones intact (Lartey *et al.*, 1999), which provides a source of calcium. The fishmeal prepared from smoked-dried anchovies is cheaper and a more readily available source of calcium for resource-poor households in low-income countries. The calcium in small fish eaten with bones has been reported to have similar bioavailability as calcium from milk (Hansen *et al.*, 1998). Therefore, localities with access to anchovies should be encouraged to use its fishmeal as it is a good source of calcium needed by the growing infant.

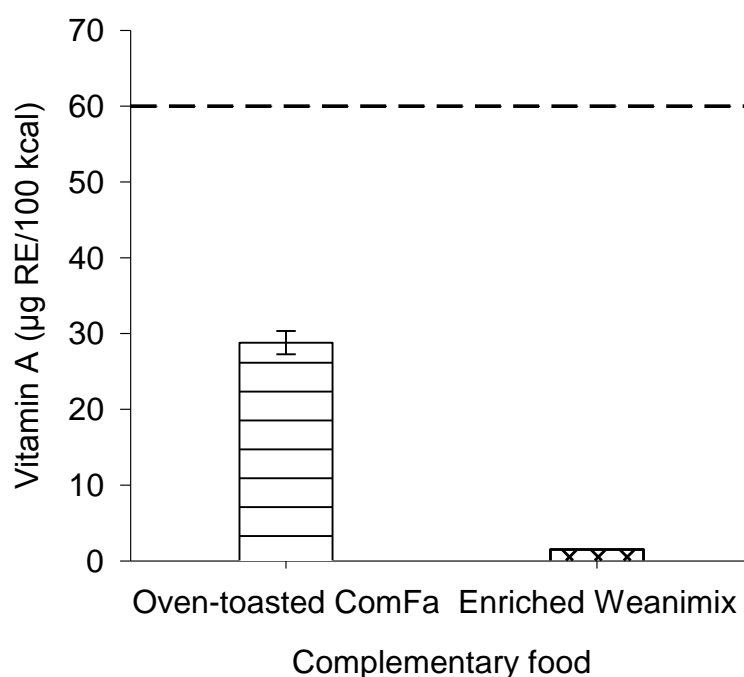


**Figure 4.7** Calcium density of household-level processed complementary foods

Line (- - -) represents the minimum level for complementary foods for infants in the Codex Standard. Bar values (means  $\pm$  standard deviation,  $n = 3$ ).

#### 4.5.7 Vitamin A

According to the CS, vitamin A added to cereal-based complementary foods for infants should be between 60 - 180 µg retinol equivalents (RE)/100 kcal. A typical unfortified maize-based complementary food such as enriched Weanimix had a vitamin A density of 1.52 µg RE/100 kcal (**Figure 4.8**), which is far below the minimum stipulated value in the CS. Conversely, the sweetpotato-based infant food had a vitamin A density of 28.04±1.08 RE/100 kcal; about 50% the minimum vitamin A specification in the CS for cereal-based complementary food, which is fortified with vitamin A premix. The vitamin A level (not added as a fortifier) in oven-toasted ComFa was higher than enriched Weanimix by a difference greater than 100%. Therefore, the level in the oven-toasted ComFa is likely to support on-going initiatives to reduce VAD better than unfortified cereal-based complementary food.



**Figure 4.8** Vitamin A density of household-level processed complementary foods

Line (- - -) represents the minimum level for complementary foods for infants in the Codex Standard. Bar values (means ± standard deviation,  $n = 2$ ). RE, retinol equivalents.

In addition, it has been shown in previous intervention trials that consumption of orange-fleshed sweetpotato improves vitamin A status of infants (van Jaarsveld *et al.*, 2005, Low *et al.*, 2007). Also, it has been reported that the  $\beta$ -carotene from sweetpotato is substantially bioavailable (Administrative Committee on Coordination & Sub-Committee on Nutrition, 2001).

To the best of our knowledge, our work is the first to report on the vitamin A content of sweetpotato-based cereal-like complementary food. Our findings support earlier suggestions that coloured-fleshed varieties of sweetpotato will be a long-term sustainable food-based approach to reduce VAD in low income countries (van Jaarsveld *et al.*, 2005, Low *et al.*, 2007).

## **4.6 Conclusion**

The energy, protein and fat densities in oven-toasted ComFa and enriched Weanimix met the specification in the CS for infant foods to be prepared with water only or non-protein liquids before consumption. However, the level of amino acid scores in both formulations was slightly lower than the stipulated level in the CS. The oven-toasted ComFa had significant levels of fructose, calcium and vitamin A using CS specifications compared with enriched Weanimix. The high fructose, calcium and vitamin A contents in the oven-toasted ComFa make the sweetpotato-based complementary food superior as a household-level food for infant feeding.



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Paper V, entitled, “*β-carotene retention in sweetpotato-based complementary food stored in different containers under simulated tropical temperature and humidity*” will be submitted to the Journal of Science of Food and Agriculture.

### **Highlight (Paper V)**

- Vitamin A precursor, β-carotene, in the household-level ComFa formulation is stable when stored in containers with good water barrier under simulated tropical conditions of temperature at 32 °C and relative humidity of 85%.
- Oven-toasted ComFa stored in a transparent low-density polyethylene bag, a likely food storage container at the household-level in Ghana, with poor water barrier had only 37% of the initial β-carotene remaining at week 24.

## **Abstract**

*Vitamin A deficiency among infants in sub-Saharan Africa can be associated with the naturally low  $\beta$ -carotene content of cereal-based formulations. We investigated the stability of  $\beta$ -carotene in sweetpotato-based complementary food stored in three different containers under simulated tropical temperature of 32 °C and 85% relative humidity in an environmental chamber for 24 weeks. The formulation stored in a metallised polyester film in a walk-in refrigerator (approximately 3.0 °C and 95% relative humidity), as the control sample, retained about 81% of  $\beta$ -carotene at week 24, with no significant change in the moisture content. The formulation stored under the simulated tropical conditions in either a metallised polyester film or a translucent polypropylene container, retained approximately half of the  $\beta$ -carotene at week 24, with an increase in moisture content. However, the formulation stored in a transparent low-density polyethylene bag had the lowest  $\beta$ -carotene retention (37%) and the highest moisture content at week 24. Within the limits of this study, which include the fixed and drastic simulated tropical conditions, it has been demonstrated that  $\beta$ -carotene in sweetpotato-based complementary food is well retained when stored in containers with good moisture barrier under tropical conditions. Thus, the formulation could be a dietary source of vitamin A for infants in sub-Saharan Africa.*

**Key words:**  $\beta$ -carotene, complementary food, storage, sweetpotato, vitamin A.

## **Abbreviations:**

LDPE      Transparent low-density polyethylene bag containing formulation stored at 32 °C and 85% relative humidity;

MPF      Metallised polyester film containing formulation stored at 32 °C and 85% relative humidity;

MPF(control) Metallised polyester film containing formulation stored at 3 °C and 95% relative humidity;

*Potential of ComFa formulation as dietary source of vitamin A (Paper V)*

|     |  |
|-----|--|
| PP  | Translucent polypropylene container with low-density polyethylene snap-on lid <i>containing formulation stored at 32 °C and 85% relative humidity;</i> |
| VAD | Vitamin A Deficiency;  |
| WHO | World Health Organization.   |

#### **4.7 Introduction**

The vitamin A deficiency (VAD) among children <5 years in sub-Saharan Africa remains high (44%), second to South-East Asia (50%), compared with the worldwide occurrence of 33%.<sup>1</sup> The reason could be the widely used cereal-based complementary foods in low-income countries,<sup>2</sup> which are naturally low in provitamin A carotene/ $\beta$ -carotene.<sup>3-5</sup>

However, there is increasing evidence that the coloured varieties (including orange-, red- and cream-fleshed) of sweetpotato could reduce the occurrence of VAD among children.<sup>6-9</sup> The orange-fleshed sweetpotato can contribute about half <sup>10</sup> of the Adequate Intake of 500  $\mu$ g retinol activity equivalents per day for 7 to 12 month old infants.<sup>11</sup>

In spite of the increasing potential of sweetpotato to contribute to vitamin A status of infants in lower income countries, only a few studies incorporating sweetpotato as an ingredient are available;<sup>5, 12-18</sup> and few publications <sup>5, 17, 18</sup> specified the vitamin A level. To our knowledge, no study has investigated the stability of provitamin A carotene in sweetpotato-based complementary food. However, the stability of carotenoid in the roots,<sup>19, 20</sup> chips<sup>21</sup> and flour<sup>22</sup> of sweetpotato has been investigated.

Storage of the roots of orange- and cream-fleshed sweetpotato varieties in earthenware pots and exposed to ambient temperatures ranging from about 22 to 33 °C, with relative humidity ranging from 65 to 80%, resulted in an increase in  $\beta$ -carotene contents.<sup>19</sup> This observation was likely due to loss of moisture (that is, increase in total solids) during storage rather than synthesis of  $\beta$ -carotene. Bechoff and others<sup>21</sup>, reported losses of provitamin A carotene ranging between 63% and 78% when sweetpotato chips were stored for 125 days (4 months) in black or clear low-density polyethylene bag under ambient room temperature ranging from about 19 to 28 °C with 43 to 87% relative humidity. The total carotenoids of flour stored in low-density polyethylene bag and wrapped with aluminium foil was stable for only two weeks, after which there was a significant degradation.<sup>22</sup>

The stability of  $\beta$ -carotene in sweetpotato-based complementary food is a major nutritional factor to consider if such formulation could be recommended as a dietary source of vitamin A to support on-going vitamin A supplementation programmes in low-income countries.<sup>5, 15, 18</sup> Storage studies are needed to provide information on the stability of provitamin A carotene in processed foods based on the coloured varieties of sweetpotato.

This study was conducted to determine the stability of  $\beta$ -carotene in a household-level sweetpotato-based complementary food in likely storage containers under a simulated tropical temperature of 32 °C and 85% relative humidity.



## **4.8 Materials and Methods**

### **4.8.1 Preparation of the sweetpotato-based complementary food**

The ingredients and the methods used to prepare the household-level sweetpotato-based complementary food (referred to as oven-toasted ComFa) have been previously described in detail <sup>23</sup>. In brief, a composite flour of 66% cream-fleshed sweetpotato, 10% full-fat soyabean, 6.0% soyabean oil, 0.50% iodised salt, 0.50% sugar and 17% fish powder from anchovy, was toasted at 120 °C for 30 minutes in a pre-heated oven (AR 85 Electrolux, Steelfort Engineering Company Ltd., Palmerston North, New Zealand). For this storage study, three batches of the household-level sweetpotato-based complementary formulation were prepared from the same stock of ingredients for the oven-toasted ComFa described in an earlier report.<sup>5</sup> About 500 g from each batch were sampled and put in three different containers.

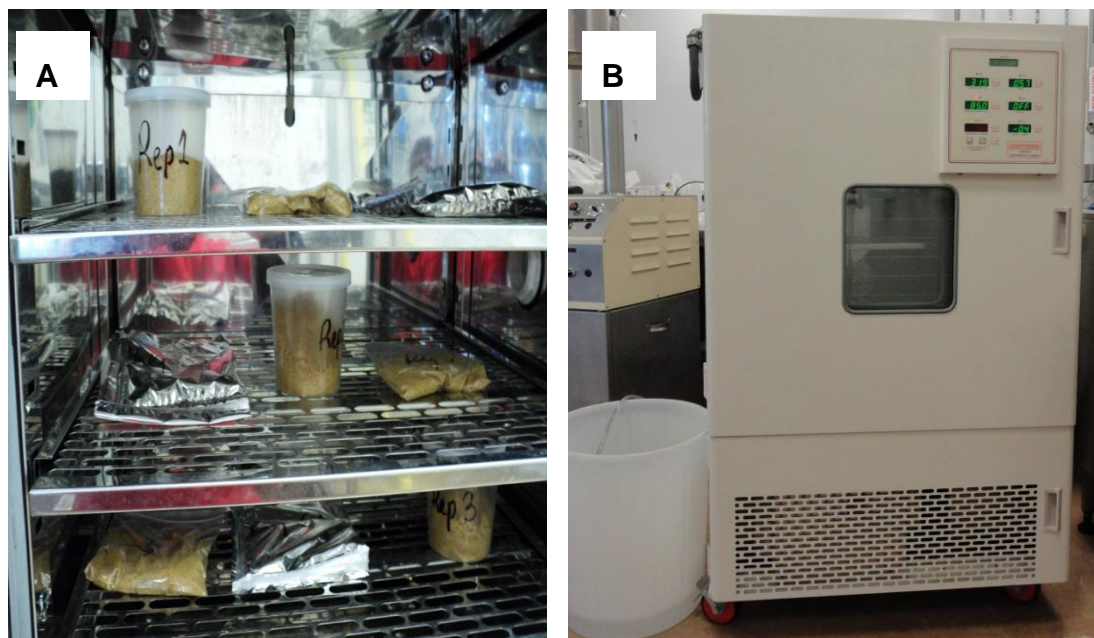
### **4.8.2 Storage container**

The three different storage containers used were (**Figure 4.9A**): a transparent low-density polyethylene bag (LDPE) (UBL<sup>®</sup>, China), a translucent polypropylene container with LDPE snap-on lid (PP) (Huhtamaki Australia, New South Wales, Australia) and a bag made from a roll of metallised polyester film (MPF). A control sample was kept in the metallised polyester film bag and stored in a walk-in refrigerator at 3.0±1.0 °C with a relative humidity of 95% [MPF (control)]. The LDPE and the PP containers are probable household-level storage containers in Ghana (personal observation).

### **4.8.3 Storage study under simulated temperature and humidity**

An environmental chamber (**Figure 4.9B**) (Contherm Precision Environmental Chamber, Contherm Scientific Ltd., Lower Hutt, New Zealand) was used in this study. The storage of the sweetpotato-based complementary food was conducted for 24 weeks under a simulated average temperature of 32 °C and relative humidity of 85%, mimicking ambient conditions in Ghana, West Africa.<sup>24</sup> Ghana was chosen because it was the

representative country for Africa in the World Health Organization Multicentre Growth Reference Study,<sup>25</sup> which led to the development of the international growth reference for monitoring growth and development of infants and young children worldwide.



**Figure 4.9** The three storage containers (A) and Contherm precision environmental chamber (B)

#### **4.8.4 Sampling**

The sampling points were weeks: 0 (beginning of storage study), 8, 16 and 24. At each sampling point, the contents of the sample in each storage container including the control were mixed by inverting the containers four times and further mixing with the sampling spoon before aliquots were taken. About 50 g from each of the three batches (as replicates) of the formulation stored in the MPF, PP, and LDPE as well as MPF (control) were taken and put into different sample containers. Each sample container was coded with a random 3-digit figure for moisture and provitamin A analyses. The remaining samples were returned to the environmental chamber or to the walk-in refrigerator, and the sampling described above was repeated at the selected weeks until the end of the study.

In addition, previously reported  $\beta$ -carotene level in oven-toasted ComFa prepared in October 2010,<sup>26</sup> and was still in storage at approximately -1.0 °C in an opaque polypropylene container with snap-on lid was also determined, in duplicate, in October 2011, and the data were included in this manuscript.

#### **4.8.5 Moisture analysis**

About two grams of each of the aliquots sampled were weighed and dried in a forced-air oven (Contherm Oven 240, Contherm Scientific Company Ltd., Lower Hutt, New Zealand) at 108 °C for approximately 16 hours as previously described.<sup>23</sup>

#### **4.8.6 $\beta$ -carotene analysis**

The level of  $\beta$ -carotene in the blind aliquots (as 3-figure coded samples) of the MPF, PP, LDPE and MPF (control) was determined using the Carr-Price method, AOAC 974.29 (4) by the Nutrition Laboratory, Massey University, New Zealand, using a high performance liquid chromatography (HPLC) (Shimadzu HPLC, Japan). The aliquots were stored in a dark room at about 20 °C, and analysed within two weeks of sampling.

For the purpose of quality control, the coded samples were further randomised into four batches by the analyst at the laboratory, and the first sample of each batch was determined in duplicate. The coefficient of variation for the duplicate ranged from 0.47 to 2.3%. Also, a sample void of carotenoids was spiked with  $\beta$ -carotene (C4582, synthetic crystalline  $\geq 95\%$  HPLC grade, Sigma-Aldrich) and analysed with each of the other three batches. The percentage recovery ranged from 83% to approximately 100%.

The standard curve generated from  $\beta$ -carotene (C4582, synthetic crystalline  $\geq 95\%$  HPLC grade, Sigma-Aldrich), which was used to determine the provitamin A levels, had coefficients of determination ranging from 0.9997 to 0.9999, indicating an almost perfect linearity.

The  $\beta$ -carotene values of the samples were corrected for their respective moisture contents.

#### **4.9 Statistical analysis**

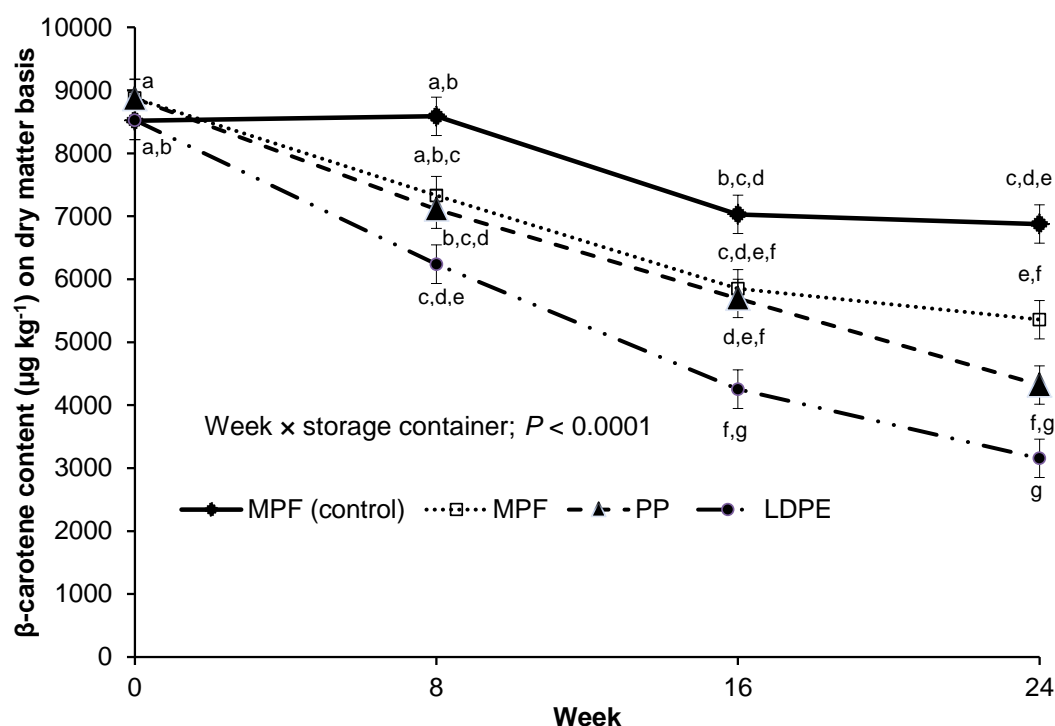
The data were analysed using the general linear model for repeated measures design in Minitab v15.1™ (Minitab Inc., State College, PA, USA).<sup>27</sup> The between-group effect was the storage containers and the within-group effect was the weeks of sampling. The  $\beta$ -carotene and moisture values were the response variables. All the predictor variables: storage containers, weeks of sampling, and storage containers  $\times$  weeks of sampling, were considered as fixed factors. Statistical significance was set at  $P < 0.05$ . Tukey's method for pairwise comparison of the least square means<sup>28</sup> was employed for post hoc test when required.

Data were expressed as least square means  $\pm$  standard error of means of three independent samples, unless otherwise specified.

## 4.10 Results and Discussion

### 4.10.1 Provitamin A precursor retention

Data on the loss of  $\beta$ -carotene in the oven-toasted ComFa, a household-level complementary food, stored in the different containers for 24 weeks are shown in **Figure 4.10**.



**Figure 4.10** Loss of  $\beta$ -carotene content in oven-toasted ComFa in different storage containers under simulated tropical temperature and humidity<sup>#</sup>

<sup>#</sup>Values are least square means  $\pm$  standard error of means of three independent sample;

Values with the same letter are not significantly different ( $P > 0.05$ );

All treatment levels were stored in an environmental chamber set at 32.0°C and 85% relative humidity, except MPF (control) that was stored at approximately 3.0 °C and 95% relative humidity;

MPF: Metallised polyester film;

PP: Translucent polypropylene container with low-density polyethylene snap-on lid;

LDPE: Transparent low-density polyethylene bag.

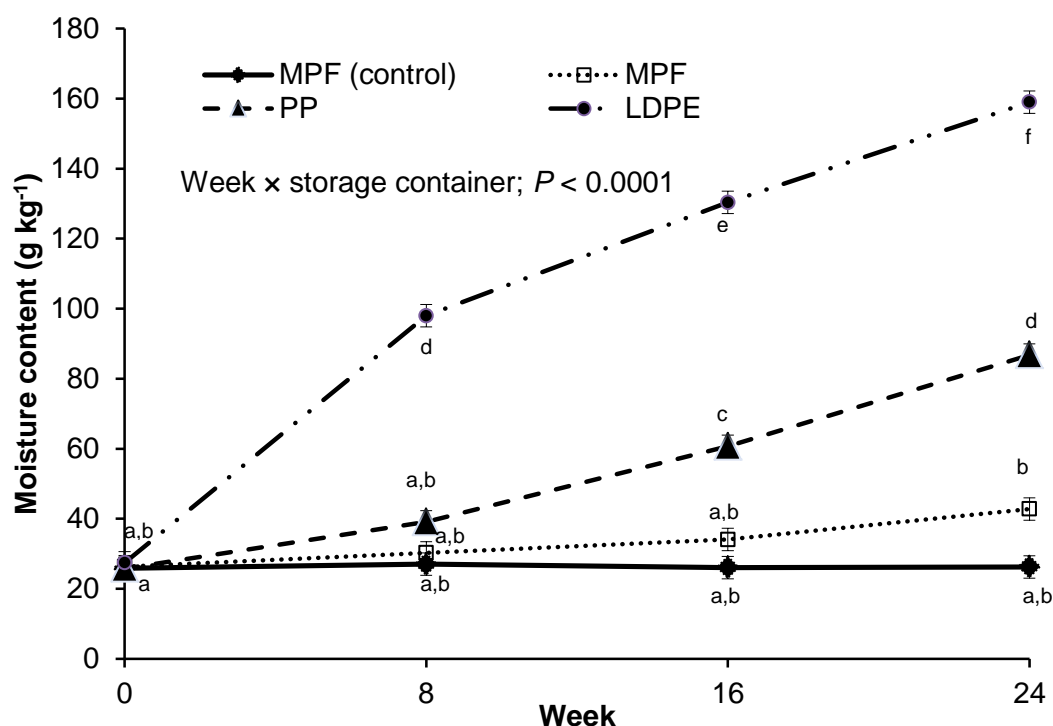
The MPF (control), the sample in the metallised polyester film stored at  $3.0 \pm 1.0$  °C with a relative humidity of 95%, had the highest retention of provitamin A carotene and was significantly ( $P < 0.05$ ) higher from weeks 8 to 24 compared with the sample stored LDPE in the environmental chamber (**Figure 4.9**) at simulated tropical temperature of 32 °C with 85% relative humidity. Approximately 81% of the initial  $\beta$ -carotene content was retained at the 24th week in the MPF (control) treatment. The MPF and the PP stored in the environmental chamber had similar ( $P > 0.05$ ) retention of  $\beta$ -carotene. The levels of  $\beta$ -carotene retention for the formulation in the MPF and the PP containers were respectively: 83 vs. 80% (week 8), 66 vs. 64% (week 16) and 60 vs. 49% (week 24). At week 16, the LDPE treatment level had the highest loss of provitamin A carotene, approximately 50% the initial content.

#### **4.10.2 Moisture uptake by the oven-toasted ComFa from the different containers**

There was no significant change in the moisture content (**Figure 4.11**) of the MPF (control) treatment level during the entire storage period of 24 weeks. However, there were approximately 2-, 3- and 6-fold increases in the moisture content of the formulation in the MPF, PP and LDPE containers, respectively. The moisture content was two times higher for the formulation stored in the MPF than the sample stored in the MPF (control) suggesting that the constant high temperature of the environmental chamber increased the permeability of the metallised polyester film to moisture.

An expected trend was observed between moisture uptake by the formulations in the different containers and provitamin A carotene retention. A bivariate correlation between moisture and  $\beta$ -carotene (expressed on dry matter basis) showed a significant inverse correlation ( $P < 0.0001$ , Pearson correlation coefficient = -0.80). Thus, about 64% of the variability in the provitamin A carotene levels during storage could be explained by the variation in the moisture content. Therefore, when moisture uptake by sweetpotato-based complementary food is controlled during storage, for example, by keeping the formulation in high-density polyethylene container instead of low-density polyethylene bag; the loss of  $\beta$ -carotene would be

minimal in formulations stored for 24 weeks. However, we do not expect such a long storage period for a household-level processed complementary food.



**Figure 4.11** Uptake of moisture by the oven-toasted ComFa in the different storage containers under simulated tropical temperature and humidity<sup>#</sup>

<sup>#</sup>Values are least square means  $\pm$  standard error of means of three independent sample;

Values with the same letter are not significantly different ( $P > 0.05$ );

All treatment levels were stored in an environmental chamber set at 32.0°C and 85% relative humidity, except MPF (control) that was stored at approximately 3.0 °C and 95% relative humidity;

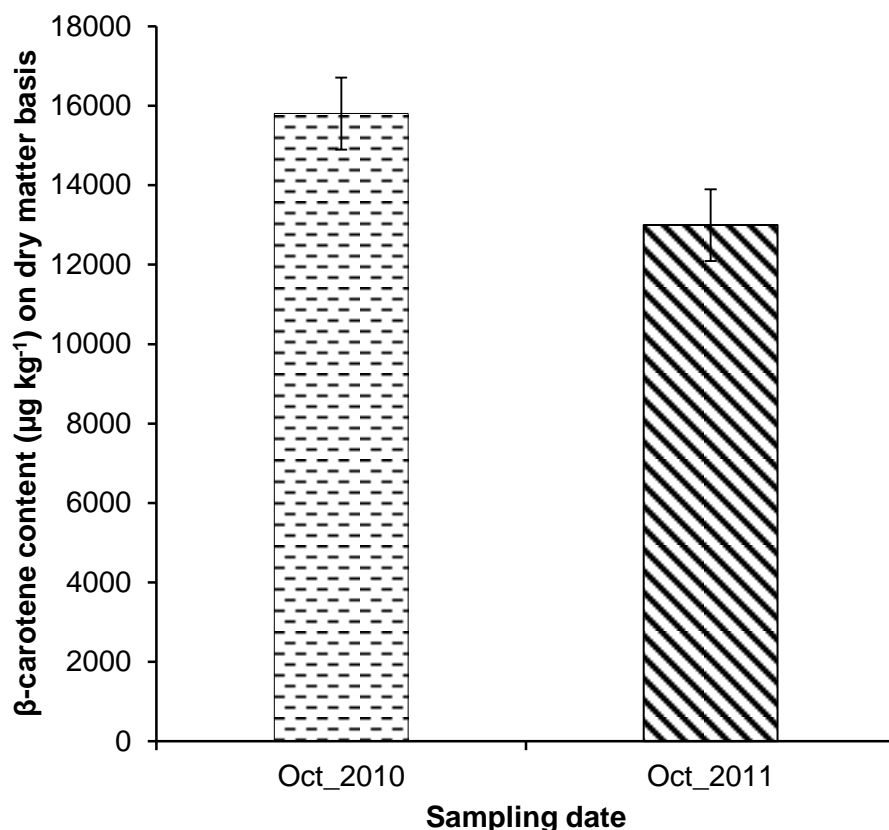
MPF: Metallised polyester film;

PP: Translucent polypropylene container with low-density polyethylene snap-on lid;

LDPE: Transparent low-density polyethylene bag.

The data suggest that high ambient temperature (32 °C) with high relative humidity (85%) could be detrimental to provitamin A carotene retention during storage. However, a higher relative humidity of 95% and low temperature ranging between 2-5 °C do not significantly degrade  $\beta$ -carotene

when stored in a container with good moisture barrier as observed in our control sample [MPF (control)].



**Figure 4.12**  $\beta$ -carotene content in oven-toasted ComFa over a year in storage<sup>#</sup>

<sup>#</sup>Sample was stored at approximately  $-1.0^{\circ}\text{C}$  in an opaque polypropylene container with snap-on lid;

Values are means  $\pm$  standard deviations of two and three independent samples for 2010 and 2011, respectively.

An interesting finding of nutritional significance is the level of  $\beta$ -carotene in the oven-toasted ComFa measured when it was processed in October 2010,<sup>26</sup> and then after a year in storage (**Figure 4.12**). About 82% of  $\beta$ -carotene was retained after a year of storage. The  $\beta$ -carotene content, on dry matter basis, of the 2010 oven-toasted ComFa was  $15802.90 \mu\text{g kg}^{-1}$



compared with 8697.11  $\mu\text{g kg}^{-1}$  for the 2011 oven-toasted ComFa used for the storage studies. Both formulations were prepared from the same stock of ingredients and same equipment. The moisture contents of the products were comparable, 23.60  $\text{g kg}^{-1}$  (2010 formulation) vs. 26.35  $\text{g kg}^{-1}$  (2011 formulation). This finding suggests that there was a significant loss of  $\beta$ -carotene in the sweetpotato flour, about 45%, during storage. The sweetpotato flour and the 2010 oven-toasted ComFa were stored under similar conditions as stated previously (plastic containers with good moisture barrier at approximately  $-1.0\text{ }^{\circ}\text{C}$ ). This finding suggests that other components in the food matrix of the oven-toasted ComFa tend to limit the degradation of provitamin A carotene. This suggested “protective effect” of the oven-toasted ComFa food matrix on  $\beta$ -carotene warrants further investigation to elucidate the mechanism as it would be of nutritional significance for reducing VAD.

The storage of sweetpotato flour for more than two weeks should not be encouraged as there is significant loss of  $\beta$ -carotene after this period.<sup>22</sup> It is therefore suggested that sweetpotato roots should be stored in earthenware pots<sup>19</sup> or in pits<sup>20</sup> but not processed into flour and stored unless it is intended to be used within a short time, such as two weeks or less.<sup>22</sup>

Due to the lower level of phytate (an antinutrient that inhibits iron, zinc and calcium absorption) and higher vitamin A content of the oven-toasted ComFa compared with a maize-based complementary food, we previously suggested that the sweetpotato-based infant food is likely to contribute positively to the nutritional status of infants in lower income countries such as Ghana,<sup>5, 23</sup> where cereal-only or cereal-legume blends are widely used for complementary feeding.<sup>29</sup> The results of this current study support our suggestion that sweetpotato-based complementary food can be a dietary source of vitamin A (when stored appropriately) to support on-going vitamin A supplementation initiatives in low-income countries<sup>6-9</sup> to reduce VAD occurrence.

The limitations of our storage study include the fixed and drastic simulated tropical conditions (temperature of  $32\text{ }^{\circ}\text{C}$  and relative humidity of 85%) in

which the storage containers were held, which may not be the ideal situation. Also, the airflow in the environmental chamber was not controlled as it was not equipped with such a function. However, within the limits of this study, it has been demonstrated that  $\beta$ -carotene in sweetpotato-based complementary food is well retained when was stored in containers with good moisture barrier at a relatively high temperature and relative humidity.

#### **4.11 Conclusion**

The  $\beta$ -carotene in the sweetpotato-based formulation is fairly stable when stored in containers with good moisture barrier under tropical temperature (32°C) and relative humidity (85%). Thus, the sweetpotato-based formulation could be a dietary source of vitamin A when stored appropriately.

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## **Chapter 5: Estimation of Calcium, Iron and Zinc Availability**

As part of assessing the suitability of the formulated sweetpotato-based complementary food (main objective, Chapter 1), the availability of calcium, iron and zinc were estimated using phytate: mineral molar ratio and the levels of  $\beta$ -carotene (possible enhancer of iron absorption) and polyphenols, an inhibitor of iron absorption. This chapter, based on Paper VI, entitled “*Sweetpotato-based complementary food would be less inhibitory on mineral absorption than a maize-based infant food assessed by compositional analysis*”, has been published in the International Journal of Food Sciences and Nutrition (Amagloh, Brough, et al., 2012).

### **Highlights**

- The sweetpotato-based complementary foods had at least half the phytate: calcium, iron and zinc molar ratios of maize-based infant food.
- Both the sweetpotato- and maize-based infant foods phytate: iron ratio predicts low iron bioavailability.
- All the infant foods contained similar polyphenols levels that may limit the absorption of iron.
- Only the sweetpotato-based complementary foods contained measurable levels of  $\beta$ -carotene, a possible enhancer of iron absorption.

It is worth mentioning that the lower phytate: iron molar ratio of the ComFa formulations compared with enriched Weanimix may not improve the iron status of infants. In a randomised control trial conducted in Tanzania, East Africa, in which processed and unprocessed complementary food samples with phytate: iron molar ratios being 4:1 and 17.5:1, there was no significant difference in iron status parameters (Mamiro et al., 2004). The ingredients in their formulation were 65.2% finger millet, 19.1% kidney beans, 8.0% peanuts and 7.7% mango puree. Detailed description of the processing methods in the processed and unprocessed formulations has been published (Mbithi-Mwikya et al., 2002). Clearly, the formulation investigated in that study was cereal-based, different from ComFa formulation, which is

sweetpotato-based. Therefore, it is difficult to suggest that the lower phytate: iron molar ratio of the ComFa formulations and the level of  $\beta$ -carotene would not enhance iron absorption. Findings from an *in vitro* digestion/Caco-2 uptake model study reported by Pynaert et al. (2006), investigating the effect of the processed or unprocessed millet-based formulation described above (Mbithi-Mwikya et al., 2002) on iron bioavailability, were similar to the outcome of field trial mentioned earlier (Mamiro et al., 2004). An *in vitro* digestion/Caco-2 uptake model study was carried to evaluate the bioavailability of iron from oven-toasted ComFa. Data from that study will be presented after Paper VI.



## **Abstract**

*The availability of micronutrients from sweetpotato-based complementary foods (CFs): oven-toasted and roller-dried ComFa and a maize-based infant food (enriched Weanimix) was compared using phytate: mineral molar ratios, polyphenols and  $\beta$ -carotene levels. The phytate: calcium, iron and zinc molar ratios of approximately 0.17, 1 and 15 predict better absorption of calcium, iron and zinc, respectively. Generally, the sweetpotato-based CFs had at least half the phytate: mineral ratios of enriched Weanimix. The phytate: iron ratio in both the sweetpotato- and the maize-based CFs was greater than 1. Only the ComFa formulations had phytate: zinc ratio lower than 15. The level of polyphenol (iron inhibitor) in the formulations was similar. Only the sweetpotato-based CFs contained measurable levels of  $\beta$ -carotene, a possible iron enhancer. The lower phytate: mineral ratios and the  $\beta$ -carotene level of the sweetpotato-based CFs suggest that calcium, iron and zinc absorption could be better from them than from the maize-based infant food.*

**Key words:** availability; complementary/infant food; maize; mineral; phytate; sweetpotato

## **Abbreviation:**

CFs: Complementary foods

## **5.1 Introduction**

The high phytate levels of complementary foods (CFs) prepared from unrefined/non-dehulled cereals such as maize, millet, sorghum and legumes (soyabean, cowpea or groundnut), commonly used in low-income countries of sub-Saharan Africa (Greiner et al. 2006, Gibson et al. 2010), partly contributes to the high iron and zinc deficiencies among infants (Hotz et al. 2001, Hurrell et al. 2003, Hurrell and Egli 2010). Phytate forms insoluble complexes with some micronutrients (Gibson and Hotz 2001, Greiner et al. 2006, Kumar et al. 2010) and, because the intestinal phytase activity in humans is low (Sandberg and Andersson 1988, Weaver and Kannan 2002), hydrolysis of such complexes to release the bound nutrients is limited. The inhibitory effect of phytate on nutrients is dose-dependent (Hallberg and Hulthen 2000, Greiner et al. 2006). Therefore, reduction of the phytate level in plant-based infant foods is likely to improve the absorption of essential micronutrients including iron and zinc.

Strategies suggested to reduce the inhibitory effect of phytate on nutrient absorption include soaking of cereal flour in water and discarding the excess water (Hotz et al. 2001) or the addition of phytase-containing micronutrient powder to the cereal porridge prior to consumption (Troesch et al. 2009). Generally, thermal processing is not an effective method for reducing the phytate levels because phytate is relatively heat stable (Sathe and Venkatachalam 2002), and heating also denatures endogenous phytase (Sandberg et al. 1987). Importantly, lower derivatives of phytate (inositol tri- and tetra-phosphates) alone do not limit nutrient bioavailability (Sandberg et al. 1987), but do so in the presence of penta- and hexa-phosphates (Sandberg et al. 1999).

Other constituents of food such as polyphenols (Gillooly et al. 1983, Teucher et al. 2004, Petry et al. 2010) and  $\beta$ -carotene (Garcia-Casal et al. 1998, Garcia-Casal et al. 2000, Layrisse et al. 2000, Garcia-Casal 2006) have been reported to affect iron absorption from plant-based foods. Levels of total polyphenols, determined by the Folin-Ciocalteu method, of greater than 20 mg/meal Gallic acid equivalents have been shown to inhibit iron absorption

(Petry et al. 2010). Polyphenols are located in the seed coat of legumes and outer layer of cereals (Gillooly et al. 1984), so dehulling of legumes and grains could reduce the levels in foods.

A human study in which  $\beta$ -carotene was added to test diets prepared from rice, corn or wheat led to an increase in iron absorption that was 2-3 times greater compared with meals given without  $\beta$ -carotene supplementation (Garcia-Casal et al. 1998). Other findings that suggest that  $\beta$ -carotene could limit the inhibitory effect of phytate and/or polyphenols on iron absorption have been published (Garcia-Casal et al. 2000, Layrisse et al. 2000, Garcia-Casal 2006). Further, an algorithm available for predicting iron bioavailability from a vegetarian diet, which has been validated *in vitro* (coefficient of determination of 0.81) and *in vivo* (coefficient of determination of 0.79), includes  $\beta$ -carotene as a positive predictive factor (Chiplonkar and Agte 2006). It is noteworthy that there are no contradictory findings on the effect of  $\beta$ -carotene on iron absorption as have been observed for vitamin A (Walczyk et al. 2003, Hurrell and Egli 2010).

Sweetpotato is relatively low in phytate (Phillippy et al. 2003, Lukmanji et al. 2008, Lung'aho and Glahn 2009) and, depending on the variety, may contain high  $\beta$ -carotene levels (Hagenimana et al. 2001, Ssebuliba et al. 2001, Ofori et al. 2009) compared with cereals. For these reasons, it has been used to develop CFs (Amagloh et al. 2012a) as an alternative to cereal-based infant foods. Some previous researchers formulated similar sweetpotato-based infant foods (Espinola et al. 1997; Nnam, Akaninwor and Okechukwu 2004; Akubor, Ijarotimi and Ashipa 2006; Omwamba et al. 2007; Nandutu and Howell 2009; Adenuga 2010). However, there is little published information about the bioavailability of mineral nutrients such as calcium, iron and zinc from the sweetpotato-based food matrix (Gibson et al. 2010).

Before conducting micronutrient bioavailability trials, which are expensive and time-consuming to undertake, the phytate: calcium, iron or zinc molar ratio can be a useful index to assess the potential availability of these essential micronutrients from plant-based CFs (Gibson et al. 2010). These ratios have been used to assess the availability of calcium, iron and zinc in

other CFs (Abebe et al. 2007, Chan et al. 2007, Gibbs et al. 2011, Tizazu et al. 2011). Dephytinised commercial infant cereals with lower phytate to iron and zinc ratios resulted in higher bioavailability of iron and zinc in Caco-2 cells model compared with the non-dephytinised infant cereals (Frontela et al. 2009). Furthermore, in absorption studies conducted in either humans (Adams et al. 2002, Hambidge et al. 2004) or weanling rat model (Lönnerdal et al. 2011), test meals with lower phytate: zinc molar ratios resulted in higher fractional zinc absorption compared with those with higher phytate: zinc molar ratios.

The availability of calcium, iron and zinc from sweetpotato-based CFs and a maize-based infant food was compared using phytate: calcium, iron and zinc molar ratios; and polyphenols and  $\beta$ -carotene levels.

## **5.2 Materials and methods**

Detailed description of the two prototype formulations of sweetpotato-based CFs: oven-toasted ComFa (household-level formulation) and roller-dried ComFa (industrial-level formulation) has been documented in an earlier publication (Amagloh et al. 2012a).

Briefly, a composite flour of 66% sweetpotato (cream-fleshed), 10% soyabean, 6.0% soyabean oil, 0.50% iodised salt, 0.50% sugar and 17% fish powder from anchovy (*Engraulis hepsetus*) was toasted for 30 min in a preheated oven at 120°C, and referred to as *oven-toasted ComFa*. Another composite flour of cream-fleshed sweetpotato (72%), soyabean (15%), soyabean oil (6.0%), iodised salt (0.50%), sugar (0.50%) and skim milk powder (6.0%) was pre-cooked in a steam-jacketed pan for 10 min (80-83°C) before roller drying with steam at 107°C under 100 kPa, and referred to as *roller-dried ComFa*.

The proportion of the ingredients was computed as described by Amagloh et al. (2012b) using Nutrition Calculator for formulating complementary food blends developed by Global Alliance for Improved Nutrition. The primary target for the ComFa formulations was to meet energy level of at least 1670 kJ/100 g, protein content of 15 g/100 g and fat ranging from 10–25 g/100 g specified in the Codex standard for CFs (Codex Alimentarius Commission 1991).

*Enriched Weanimix* is a maize-based complementary food and was processed as previously described (Agle 1997, Lartey et al. 1999) with some modifications (Amagloh et al. 2011). The modifications included the use of dehulled maize and soyabean flours and further addition of 17% fish powder (wt/wt) and 0.50% sugar to the basic Weanimix formulation.

Three samples of each formulation were prepared and stored at approximately -1.0°C in an air-tight plastic container before analysis.

### **5.2.1 Calcium, iron and zinc analysis**

Mineral analysis was conducted by the Campbell Micro-analytical Laboratory, Department of Chemistry, University of Otago, New Zealand. The samples

were digested in triplicate using quartz distilled concentrated HNO<sub>3</sub> heated to 104°C. The acid strength was reduced by evaporation to near dryness at 90°C. After suitable dilution in 2.0% HNO<sub>3</sub> and the addition of reference elements, the calcium, iron and zinc levels were determined by quadrupole inductively coupled mass spectrometry (Agilent 7500ce, Agilent Technologies Inc., CA, USA). The instrument was tuned in accordance with the manufacturer's recommendations, and calibration used certified multi-element solutions. Helium collision cell gas was used to eliminate interferences. Spiked samples and blanks were run with mean recoveries 103%, 103% and 98% for calcium, iron and zinc respectively. There were no measureable minerals present in the blanks. Results were reported on a dry weight basis after correction for recovery.

### **5.2.2 Phytate analysis**

The phytate content of defatted samples of the CFs has been reported (Amagloh et al. 2012a). A *myo*-inositol phosphate specific phytase was used to quantify together the total di-, tri-, tetra-, penta- and hexa-phosphates as phytate content in the samples on a UV/Visible spectrophotometer (Pharmacia LKB Ultrospec II, England) using a commercial phytate assay kit (K-PHYT 05/07) from Megazyme International Ireland Ltd.

### **5.2.3 Total polyphenols analysis**

The total polyphenols in the samples, quantified as Gallic acid equivalents, were determined using the Folin-Ciocalteu method according to the procedure described by Akond et al. (2010) following extraction of total polyphenols in the samples using the method described by Zimmermann et al. (2008). Total polyphenols were extracted with acetone and Milli-Q water (acetone:water, 4:1 vol/vol). The suspension obtained was centrifuged (Heraeus multifuge 1S-R, ThermoFisher SCIENTIFIC, Osterode, Germany), and the extraction solvent was removed using a rotary evaporator (Rotavapor R-215, Büchi Labortechnik AG., Fawil, Switzerland). The absorbance of the total polyphenols in the sample was read at 760 nm on a UV/Visible spectrophotometer (Pharmacia LKB Ultrospec II, England). The coefficient of determination of the standard curve was 0.994, indicating high linearity.

#### **5.2.4 $\beta$ -carotene analysis**

The level of  $\beta$ -carotene in the sweetpotato- and maize-based infant foods was determined in duplicate using the Carr-Price method, AOAC 974.29 (4) by a commercial laboratory (Nutrition Laboratory, Massey University, New Zealand) using high performance liquid chromatography (Shimadzu HPLC, Japan). The coefficient of determination of the standard curve was 0.999, indicating almost a perfect linearity. The percentage recovery of an internal standard spiked with  $\beta$ -carotene and run in parallel to the samples was 85%.

#### **5.2.5 Calculation of phytate: mineral molar ratio**

The phytate: calcium, iron or zinc ratio was calculated by dividing the moles of phytate by moles of calcium, iron or zinc per sample. The moles of phytate in the samples were calculated by dividing the amount of phytate by the molecular mass of phytate (660.40 g/mol). The moles of calcium, iron and zinc were calculated by dividing the levels in the samples by their respective molecular masses (40.07, 55.85 and 65.38 g/mol).

### **5.3 Statistical analysis**

Univariate and One-way ANOVA analyses were used for the descriptive and ANOVA respectively using Statistical Analysis Systems software package version 9.1 (SAS Institute, Cary, NC, USA). Tukey's studentized range test was used to compare differences between means when significant ( $p < 0.05$ ). Student's T-test was used to compare the means of  $\beta$ -carotene content of the two sweetpotato-based infant foods only.

## 5.4 Results

The phytate, calcium, iron and zinc levels in the sweetpotato-based CFs (oven-toasted ComFa and roller-dried ComFa) and the maize-based infant food, enriched Weanimix, are shown in **Table 5.1**.

**Table 5.1** Levels of phytate, calcium, iron and zinc (mg/kg) in sweetpotato- and maize-based CFs\*

| Infant food        | Phytate <sup>†</sup> |        | Calcium                                    |        | Iron                        |      | Zinc                        |      |
|--------------------|----------------------|--------|--|--------|-----------------------------|------|-----------------------------|------|
|                    | Mean                 | SEM    | Mean                                       | SEM    | Mean                        | SEM  | Mean                        | SEM  |
| Sweetpotato-based  |                      |        |  |        |                             |      |                             |      |
| Oven-toasted ComFa | 2263.70 <sup>b</sup> | 320.01 | 6008.40 <sup>a</sup><br>(60%) <sup>‡</sup> | 633.34 | 69.74 <sup>a</sup><br>(30%) | 0.37 | 21.17 <sup>b</sup><br>(21%) | 0.75 |
| Roller-dried ComFa | 1949.70 <sup>b</sup> | 208.40 | 2017.70 <sup>b</sup><br>(20%)              | 18.28  | 27.88 <sup>b</sup><br>(12%) | 1.26 | 15.79 <sup>c</sup><br>(15%) | 0.25 |
| Maize-based        |                      |        |  |        |                             |      |                             |      |
| Enriched Weanimix  | 8032.70 <sup>a</sup> | 356.81 | 3707.80 <sup>b</sup><br>(37%)              | 251.17 | 81.22 <sup>a</sup><br>(35%) | 4.49 | 30.69 <sup>a</sup><br>(30%) | 0.21 |
| P-value            | <0.0001              |        | 0.001                                      |        | <0.0001                     |      | <0.0001                     |      |

\*Mean value ( $n=3$ ) with respective standard error of mean (SEM) reported on dry matter basis; mean value within a column with unlike superscript letters<sup>a,b,c</sup> is significantly different ( $p<0.05$ ).

<sup>†</sup>Data extracted from Amagloh et al. (2012a).

<sup>‡</sup>Value in parenthesis is percentages of the WHO desirable levels (Dewey and Brown 2003) met by the infant foods based on daily ration size of 40 g (dry weight) for 6- to 11-month-old breastfeeding infants (Dewey 2003).



### Calcium, iron and zinc availability based on compositional analysis (Paper VI)

The sweetpotato-based CFs contained less phytate, approximately 26%, compared with enriched Weanimix (Amagloh et al. 2012a). The calcium content of the oven-toasted ComFa was approximately three and two times significantly higher ( $p=0.001$ ) than the levels in the roller-dried ComFa and enriched Weanimix respectively. The level of calcium in the roller-dried ComFa and the enriched Weanimix was not significantly different ( $p>0.05$ ). Also, the iron in the oven-toasted ComFa and enriched Weanimix was similar and both levels were significantly higher than the content of the roller-dried ComFa ( $p<0.0001$ ). The estimated daily intakes of calcium, iron and zinc from the sweetpotato- and the maize-based CFs based on 40 g (dry weight) (Dewey 2003) for 6- to 11-month-old infants were less than half of the World Health Organization recommended levels for calcium (400 mg/day), iron (9.3 mg/day) and zinc (4.1 mg/day) from CFs processed for 6- to 8-month-old breastfeeding infants (Dewey and Brown 2003). The only exception was the oven-toasted ComFa, which contained 60% of the recommended calcium level.

There were significant differences between the sweetpotato-based CFs and the maize-based infant food regarding the phytate: calcium ( $p=0.001$ ) and zinc ( $p=0.001$ ) molar ratios (**Table 5.2**). All the CFs differed in the phytate: iron molar ratio ( $p<0.0001$ ). The oven-toasted ComFa, containing 17 g/100 g fish powder, had the lowest phytate: mineral molar ratios, followed by roller-dried ComFa, which contained 6.0% milk powder. The enriched Weanimix, containing 17% fish powder as in the oven-toasted ComFa (both could be replicated at the household level), had the highest ratios. All the CFs had lower phytate: calcium ratios than the maximum recommended ratio of 0.17 (Gibson et al. 2010). The oven-toasted ComFa and the roller-dried ComFa were, respectively, about nine and three times below the maximum recommended ratio for the phytate: calcium ratio. Enriched Weanimix was slightly lower (1.3 times) than the recommended phytate: calcium molar ratio. The phytate: iron molar ratios for the sweetpotato-based infant foods as well as the enriched Weanimix were significantly different ( $p<0.0001$ ) and exceeded the maximum recommended ratio of 1 (Hurrell 2004) by more than

100%. However, the phytate: iron molar ratios for the oven-toasted ComFa and roller-dried ComFa were lower by approximately 67% and 30% respectively compared with the enriched Weanimix. The phytate: zinc molar ratio of the oven-toasted ComFa and roller-dried ComFa were approximately two times ( $p=0.001$ ) lower than the ratio for enriched Weanimix. The sweetpotato-based CFs were averagely 24% lower compared with the maximum recommended phytate: zinc molar ratio of 15 (World Health Organization 1996). In contrast, the phytate: zinc ratio of enriched Weanimix was higher by 53% compared with the maximum recommended ratio.

**Table 5.2** Phytate: calcium, iron and zinc molar ratios of sweetpotato- and maize-based CFs\*

| Infant food               | Phytate:calcium   |      | Phytate:iron      |      | Phytate:zinc       |      |
|---------------------------|-------------------|------|-------------------|------|--------------------|------|
|                           | Mean              | SEM  | Mean              | SEM  | Mean               | SEM  |
| Sweetpotato-based         |                   |      |                   |      |                    |      |
| Oven-toasted ComFa        | 0.02 <sup>b</sup> | 0.01 | 2.74 <sup>c</sup> | 0.38 | 10.72 <sup>b</sup> | 1.81 |
| Roller-dried ComFa        | 0.06 <sup>b</sup> | 0.01 | 5.89 <sup>b</sup> | 0.43 | 12.28 <sup>b</sup> | 1.49 |
| Maize-based               |                   |      |                   |      |                    |      |
| Enriched Weanimix         | 0.13 <sup>a</sup> | 0.01 | 8.38 <sup>a</sup> | 0.24 | 25.94 <sup>a</sup> | 1.31 |
| <i>P</i> -value           | 0.001             |      | <0.0001           |      | 0.001              |      |
| Maximum recommended ratio | 0.17 <sup>†</sup> |      | 1 <sup>‡</sup>    |      | 15 <sup>¶</sup>    |      |

\*Mean value ( $n=3$ ) with respective standard error of mean (SEM); mean value within a column with unlike superscript letters<sup>a,b,c</sup> is significantly different ( $p<0.05$ ).

<sup>†</sup>Source: Gibson et al.(2010)

<sup>‡</sup>Source: Hurrell (2004)

<sup>¶</sup>Source: World Health Organization (1996)

The data in **Table 5.3** indicate the amounts of total polyphenols, an inhibitor, and  $\beta$ -carotene, as a possible enhancer, that could affect iron absorption from the formulations.

**Table 5.3** Levels of total polyphenols (an inhibitor of iron absorption) and  $\beta$ -carotene (a possible enhancer of iron absorption) in sweetpotato- and maize-based CFs

| Infant food        | Total polyphenols<br>(mg/kg) <sup>*</sup> |        | $\beta$ -carotene<br>( $\mu$ mol/kg) <sup>†</sup> |      |
|--------------------|---|--------|---|------|
|                    | Mean                                      | SEM    | Mean  | SD   |
| Sweetpotato-based  |   |        |   |      |
| Oven-toasted ComFa | 2195.09                                   | 162.35 | 28.73   | 1.11 |
| Roller-dried ComFa | 2039.85                                   | 85.61  | 24.84   | 1.24 |
| Maize-based        |   |        |   |      |
| Enriched Weanimix  | 1923.95                                   | 152.25 | ND <sup>‡</sup>                                   |      |
| <i>P</i> -value    | 0.43                                      |        | 0.19  |      |

<sup>\*</sup>Mean value ( $n=3$ ) with respective standard error of mean (SEM) on dry matter basis;

<sup>†</sup>Mean value ( $n=2$ ) with respective standard deviation (SD) on dry matter basis;

<sup>‡</sup>Not detected at the minimum detection limit 0.09  $\mu$ mol/kg for  $\beta$ -carotene (1  $\mu$ mol  $\beta$ -carotene=537  $\mu$ g  $\beta$ -carotene).

The level of the total polyphenols, quantified as Gallic acid equivalents, in both the sweetpotato- and maize-based CFs was not significantly different ( $p=0.43$ ). Using an estimated daily ration of 40 g (dry weight) for 6- to 11-month-old infants (Dewey 2003), the estimated total polyphenols would be 84.70 mg/meal and 76.96 mg/meal from the sweetpotato- and the maize-based CFs respectively. The oven-toasted ComFa and roller-dried ComFa contained measurable amounts of  $\beta$ -carotene content, and the levels were

not significantly different ( $p=0.19$ ). The  $\beta$ -carotene level in the enriched Weanimix was below detection limit of  $0.09 \mu\text{mol/kg}$ .

## **5.5 Discussion**

The rationale and the choice of ingredients have been discussed elsewhere (Amagloh et al. 2012b). In summary, the sweetpotato-based CFs (oven-toasted ComFa and roller-dried ComFa) were formulated as alternatives to maize-based CFs, which are generally low in vitamin A (Lartey et al. 1998, Dewey and Brown 2003) unless fortified, and high in phytate (Greiner et al. 2006, Gibson et al. 2010). A typical example of such maize-based complementary food is Weanimix, developed through collaboration between UNICEF and the Nutrition Unit of the Ministry of Health, Ghana, in 1987 (Agble 1997, Lartey et al. 1999), and was formulated to be processed at either the household-level or by small-scale food manufacturers in Ghana. Lartey et al. (1999) added fish powder from anchovies to the basic Weanimix to enhance the nutrient composition of infant porridge prepared at the household-level. The oven-toasted ComFa was formulated for possible replication at the household-level by caregivers, while the roller-dried ComFa was formulated as an industrial-level product. The roller-dried ComFa was formulated to be fortified with vitamins and minerals as done with proprietary cereal-based CFs, but the sample in this study was not fortified. The energy, macronutrients and phytate levels of both the sweetpotato- and maize-based CFs have been discussed elsewhere (Amagloh et al. 2012a). All the formulations met the stipulated energy (1670 kJ/100 g), and fat (10–25 g/100 g) contents recommended by the Codex Alimentarius Commission (1991). However, the roller dried-ComFa contained protein that was lower by 17%, but both the oven-toasted ComFa and enriched Weanimix met the requirement of 15 g/100 g.

The results of this study show that the fish powder prepared from anchovy (milled with bones) used in the oven-toasted ComFa and enriched Weanimix resulted in higher levels of calcium compared with the roller-dried ComFa. A similar observation was reported in another study where fish powder was added to cereal-based household level infant foods (Perlas and Gibson 2005). Although both the oven-toasted ComFa and enriched Weanimix had the same levels of fish powder incorporated, which served as the main

source of calcium, the higher level of calcium in this sweetpotato-based infant food indicates that the sweetpotato contributed about 40% of the total calcium in the formulation. The calcium content of Weanimix without added fish powder was 155 mg/kg, which was only 4.2% of the calcium of the enriched Weanimix. Therefore, the fish powder contributed approximately 95% (3552 mg/kg) of the total calcium content of the enriched Weanimix. For this reason, the incorporation of fish powder prepared from anchovies into infant foods should be encouraged especially in cultures where this practice is acceptable such as in Ghana, as suggested in an earlier report (Amagloh et al. 2011). Hence, the sweetpotato used to formulate the oven-toasted ComFa accounted for about 2400 mg/kg of the total calcium of this product.

Without considering the inhibitory effect of phytate, the enriched Weanimix might be thought to be superior to the sweetpotato-based CFs as it was slightly higher in iron and zinc. However, when the phytate: calcium, iron and zinc molar ratios are taken into account, these minerals are likely to be more available from the sweetpotato-based CFs than from the maize-based infant food. The low phytate: calcium ratios of the CFs compared with the maximum recommended ratio suggest that the phytate in the foods would be less inhibitory on calcium absorption. On the basis of the phytate: iron ratio, the iron absorption would be limited from both the sweetpotato-based CFs and enriched Weanimix, but the inhibition is likely to be higher for the enriched Weanimix than for the ComFa formulations.

The assay method used to assess the phytate content in this study may slightly overestimate the levels as it also measured inositol di-, tri- and tetra-phosphates, in addition to penta- and hexa-phosphates, which are the usually reported phytate level in CFs (Hotz et al. 2001, Frontela et al. 2009, Gibbs et al. 2011). Tri- and tetra-phosphates, in the presence of penta- and hexa-phosphates, a likely occurrence in thermally processed foods, limit iron absorption (Sandberg et al. 1999), but this effect is ignored when only penta- and hexa-phosphates are determined. Therefore, the Megazyme assay procedure is appropriate in assessing the phytate level for processed foods.

All the CFs had a higher total polyphenols content per serving than 20 mg/meal that has been shown to reduce iron absorption (Petry et al. 2010) and is expected that the inhibitory effect of polyphenols would be similar in all the CFs.

The oven-toasted ComFa and roller-dried ComFa contained measurable amounts of  $\beta$ -carotene (26  $\mu\text{mol/kg}$ ) and is likely to enhance iron absorption by off-setting the inhibitory effect of phytate and/or polyphenols as reported in other studies (Garcia-Casal et al. 1998; Garcia-Casal et al. 2000; Layrisse et al. 2000; Chiplonkar and Agte 2006; Garcia-Casal 2006).

On the basis of the compositional analyses carried out in this study, the sweetpotato-based formulations have the potential to be a valuable complementary food after the period of exclusive breastfeeding, particularly for infants in low-income countries, who are especially vulnerable to micronutrient deficiencies.

## **5.6 Conclusion**

The phytate: mineral molar ratio for calcium, iron or zinc and possibly, the  $\beta$ -carotene level indicate that the sweetpotato-based infant foods would have less inhibitory effect on calcium, iron and zinc absorption than the maize-based product. Feeding trial among human infants is required to evaluate the impact the sweetpotato-based CFs will have on their micronutrients status.

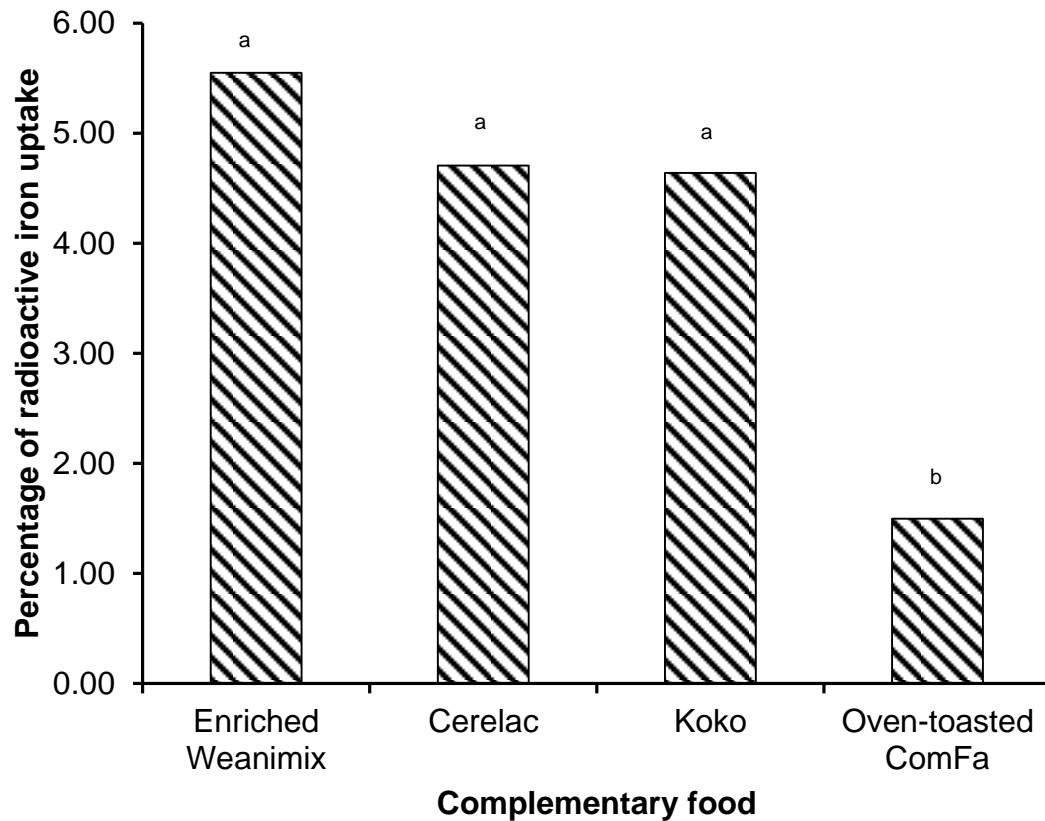


## **5.7 Data from an *in vitro* digestion/Caco-2 model iron uptake study**

An *in vitro* digestion/Caco-2 model uptake study was conducted to estimate iron availability from oven-toasted ComFa, enriched Weanimix, *koko* (fermented maize porridge) and Nestlé® Cerelac® maize. As stated in Chapter 1 (thesis overview, page 3), this work will be submitted in detail by another PhD student who is investigating models for measuring iron absorption. Briefly, the iron contents in the formulations above were determined and made approximately the same by adjusting with FeCl<sub>3</sub>. Before the *in vitro* digestion, radioactive iron (<sup>55</sup>Fe) (Perkin Elmer, Ma, USA) was added so that the final ratio of radioactive to food iron (native + exogenous as FeCl<sub>3</sub>) was 1:10. The *in vitro* digestion method was based on the protocols previously described (Glahn et al. 1998, Swain et al. 2002). The digests were further prepared until suitable to be transferred to the Caco-2 cell monolayers for the iron uptake study. Scintillation counting (Wallac Trilux 1450 Microbeta PerkinElmer, Waltham, Massachusetts, USA) of radioactive iron was used to estimate iron uptake from the infant formulations.

**Figure 5.1** is the percentage mean radioactive iron uptake from wells to which the oven-toasted ComFa, enriched Weanimix, *koko* and Nestlé® Cerelac® maize were added. Unexpectedly, oven-toasted ComFa with lower phytate: iron molar ratio (2.74 vs. 8.38) and phytate (2264 vs. 8033 mg/kg) compared with enriched Weanimix had iron uptake that was only 27% of that of enriched Weanimix. Data is from Paper VI. This is contrary to the generally accepted view in nutritional science of the inhibitory effect of phytate on iron absorption (Hurrell and Egli 2010). Secondly, the suggestion of enhancing effect of β-carotene on iron absorption (Garcia-Casal et al. 2000, Layrisse et al. 2000, Garcia-Casal 2006) was also not supported in this uptake study. Moreover, it has been shown that counting efficiency of <sup>14</sup>C-carotenoids in a solution increased from 1.0% to 79% when a solution containing 1 mg of β-carotene was decolourised using benzoyl peroxide (Walter and Purcell 1966). Thus, it could be that the β-carotene in the oven-toasted ComFa quenched the radioactive iron, hence, the low count, and consequently, the

low iron uptake of that sample. As reported in Paper VI, the  $\beta$ -carotene of enriched Weanimix was below the detection limit of 0.09  $\mu\text{mol/kg}$  compared to 29  $\mu\text{mol/kg}$  of the ComFa formulation.



**Figure 5.1** Uptake of radioactive iron (in percentages) across cells per total radioactive iron added from different complementary food

Further, both enriched Weanimix and koko, which will be far lower in vitamin C than the Cerelac formulation, had comparable effect on iron uptake. These findings refute the enhancing effect of vitamin C on iron absorption. Ascorbic acid in foods (exogenous or endogenous) is generally accepted to counteract the inhibitory effect of phytate on non-haem iron absorption owing to its reducing properties and ability to chelate and form a soluble complex with non-haem iron, making it unavailable to form insoluble complexes with phytate or polyphenols (Hallberg 1981, Teucher et al. 2004). The vitamin C content of a similar product by Nestlé® Cerelac® maize is 35 mg/100 g compared with <0.10 mg/100 g for enriched Weanimix. Vitamin C level was

not determined in *koko* as it has been previously reported to be zero (Lartey et al. 1999).

This measure of iron uptake from Caco-2 cells may not be appropriate for complementary foods such as the oven-toasted ComFa, which contain measurable amounts of  $\beta$ -carotene. This uptake data should therefore be interpreted with caution.

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## **Chapter 6: Nutritional Implications of Functional and Sensory Properties**

Among the concerns raised in Chapter 1 was the consequence of high viscose porridge, which requires dilution, usually with water, leading to decrease in energy and nutrient densities. Cereal-legume blend forms a viscous porridge that requires dilution, normally done with water, at the household-level, to get a suitable consistency of porridge for feeding infants (Kikafunda et al., 1997; Mosha & Svanberg, 1983; Mosha & Svanberg, 1990). Therefore, in this chapter, Paper VII (submitted to a journal), entitled *“Carbohydrate composition, viscosity, solubility and sensory acceptance of sweetpotato- and maize-based complementary foods”* compares the carbohydrate composition (particularly, simple sugars), which has been reported to influence viscosity when starch-containing slurry is heated (Ryu, Neumann, & Walker, 1993; Sharma, Oberoi, Sogi, & Gill, 2009) between the ComFa formulations and enriched Weanimix. Also, consumer acceptability involving infant caregivers was evaluated.

### **Highlights**

- Simple sugars naturally present in the sweetpotato-based complementary foods can be associated with the decreased viscosity during porridge preparation. Hence, less water is needed to thin the consistency, and consequently, less *“energy and nutrient thinning”*, that is, higher energy and nutrient densities than enriched Weanimix.
- The relatively high composition of carbohydrate as simple sugars makes the sweetpotato-based infant foods to have higher water solubility.
- Sweetpotato-based complementary foods are more acceptable to infant caregivers than the maize-based formulation.

## **Abstract**

*Cereal-based complementary foods from non-malted ingredients form a relatively high viscous porridge. Therefore, excessive dilution, usually with water, is required to reduce the viscosity to be appropriate for infant feeding. The dilution invariably leads to “energy and nutrient thinning”, that is, the reduction of energy and nutrient densities. Carbohydrate is the major constituent of food that significantly influences viscosity when heated in water. The objectives were to compare the sweetpotato-based complementary foods (extrusion-cooked ComFa, roller-dried ComFa and oven-toasted ComFa) and enriched Weanimix (maize-based formulation) regarding their: (a) carbohydrate composition; (b) viscosity and water solubility index (WSI); and (c) sensory acceptance evaluated by sub-Saharan African women as model caregivers. The level of simple sugars/carbohydrates was analysed by spectrophotometry, total dietary fibre by enzymatic-gravimetric method, and total carbohydrate and starch levels estimated by calculation. A Rapid Visco™ Analyser was used to measure viscosity. WSI was determined gravimetrically. A consumer sensory evaluation was used to evaluate the product acceptance of the roller-dried ComFa, oven-toasted ComFa and enriched Weanimix. The sweetpotato-based complementary foods were, on average, significantly higher in maltose, sucrose, free glucose and fructose, and total dietary fibre, but were markedly lower in starch content compared with the levels in the enriched Weanimix. Consequently, the sweetpotato-based complementary foods had relatively low apparent viscosity, and high WSI than that of enriched Weanimix. The scores of sensory liking given by the caregivers were highest for the roller-dried ComFa, followed by the oven-toasted ComFa, and lastly, the enriched Weanimix. The sweetpotato-based formulations have significant advantages as complementary food due to the high level of endogenous sugars and low starch content that reduced the viscosity, increased the solubility and imparted desirable sensory characteristics, and potentially avoiding excessive “energy and nutrient thinning”.*

**Key words:** *carbohydrate; complementary/infant food; sensory; simple sugars; sweetpotato; viscosity*

## **6.1 Introduction**

The physical and sensory properties of plant-based complementary foods for infants are indirectly of nutritional significance as they impact on the quantity of food eaten and, invariably, nutrient intake. The estimated carbohydrate content of complementary food is between 60-75 g/100 g (1), and thus, is the major factor that influences the viscosity of cooked products (2). When starch slurry is heated, the starch granules swell increasing the viscosity, which directly relates to the quantity of starch (2, 3). Complementary foods that form a very viscous paste during cooking will require excessive dilution with water before they become suitable for feeding infants (3, 4). However, diluting the paste to reduce the viscosity leads to “*energy and nutrient thinning*” (that is, the reduction of energy and nutrient densities). Therefore, a household-level sweetpotato-based complementary food has been suggested as a more suitable complementary food as it would be lower in starch resulting in a less viscous porridge compared with a maize-based infant product (5).

Apart from viscosity, water solubility index (WSI), which is a measure of the soluble constituents in foods, is another physical property that relates to the carbohydrate composition of foods (6). High WSI can be used to predict the ease of digestion of complementary foods by infants (7, 8), although the actual digestibility in infants was not investigated. Starch is efficiently digested by infants when is present in complementary food in small quantities (9, 10); therefore, higher level of carbohydrate as sugars in complementary foods could be used as an indicator for food digestibility for infants (1, 11).

The level of simple carbohydrates/sugars can affect not only the physical properties (viscosity or solubility), but also the sensory properties and, consequently, food intake. Other researchers reported that infants consumed more cereal-based porridge when sweetened than unsweetened (12).

As an effort to improve the nutritional status of infants in lower income countries of sub-Saharan Africa, sweetpotato-based complementary foods (extrusion-cooked ComFa, roller-dried ComFa and oven-toasted ComFa) were proposed as alternatives to commonly used cereal-legume blends, such

as Weanimix (13). Weanimix was developed in 1987 in Ghana through collaboration between UNICEF and the Nutrition Unit of the Ministry of Health, Ghana; it contains maize, groundnut and soyabean or cowpea (14, 15), and is an improved complementary food compared with traditional cereal-only porridge in terms of energy and protein (15). In our studies, Weanimix was slightly modified by using dehulled maize and soyabean instead of using non-dehulled ingredients, and denoted as enriched Weanimix (5, 16, 17). The extrusion-cooked ComFa and roller-dried ComFa contained 83% of the protein content of 15 g/100 g recommended by the Codex Alimentarius Commission (1), but the oven-toasted ComFa and enriched Weanimix met the specification. The ComFa formulations as well as enriched Weanimix met the energy (1670 kJ/100 g) and fat (10–25 g/100 g) stipulated levels in the Codex standard (1). However, the sweetpotato-based infant foods have nutritional advantages over enriched Weanimix because of their relatively low phytate, approximately a quarter of the 0.80 g/100 g in enriched Weanimix (16), and high vitamin A (28 vs. 2 retinol equivalents/100 kcal) (5). In addition, using the phytate: mineral ratio for predicting calcium, iron and zinc availability from food, the roller-dried ComFa and oven-toasted ComFa were predicted not to adversely affect the absorption of these essential nutrients as much as the enriched Weanimix (17).

Sucrose level in sweetpotato roots increases during storage (18-20), and maltose increases when the roots are heated at 65°C or above (18, 20-22). Therefore, it is expected that sweetpotato-based complementary foods would contain higher amounts of simple sugars and lower starch as previously suggested (5). Consequently, the sweetpotato-based infant foods would be expected to have lower viscosity and be more soluble in water and thus, require less dilution to reach an acceptable viscosity for infant feeding, that is, less “*energy and nutrient thinning*”.

The objectives of this study were to compare the sweetpotato-based complementary foods (extrusion-cooked ComFa, roller-dried ComFa and oven-toasted ComFa) and enriched Weanimix (maize-based formulation) regarding their: (a) carbohydrate composition; (b) viscosity and water

solubility index (WSI); and (c) sensory acceptance evaluated by sub-Saharan African women as model caregivers.

## **6.2 Material and methods**

### **6.2.1 Processing of complementary food formulations**

The choice and the proportion of the ingredients and the processing of the sweetpotato-based infant foods (ComFa formulations) have been previously reported (13, 16). The complementary foods were formulated to meet the recommended macronutrients specification of the Codex standard (1), and for easy replication at both the household- and industrial-level as previously discussed (13). Briefly, the sweetpotato-based formulations were processed as follows:

### **6.2.2 Extrusion-cooked ComFa and roller-dried ComFa**

Cream-fleshed sweetpotato flour (72%), soyabean flour (15%), soyabean oil (6.0%), iodised salt (0.50%), sugar (0.50%) and skim milk powder (6.0%) were mixed and the composite flour was either extrusion cooked or roller dried. Extrusion cooking of the composite flour was done at 120°C. For the roller-dried ComFa, slurry was prepared, cooked in a steam-jacketed pan for 10 min at 80-83°C before roller drying with steam at 107°C under 100 kPa.

These are the industrial-level ComFa formulations, which could be reconstituted in the same way as Nestlé Cerelac®, a popular proprietary dry infant cereal-based food in Africa.

### **6.2.3 Oven-toasted ComFa**

Composite flour of sweetpotato (66%), soyabean (10%), soyabean oil (6.0%), iodised salt (0.50%) sugar (0.50%) and fish powder from anchovy without the heads (17%) was toasted in a pre-heated oven at 120°C for 30 min. This is the household-level sweetpotato-based formulation.

### **6.2.4 Enriched Weanimix**

A maize-based complementary food, described as Weanimix, was processed as previously described (14, 15), with slight modification (5) by further enrichment with 17% (wt/wt) anchovy powder and 0.50% (wt/wt) sugar.

## **6.3 Chemical and physical properties analyses**

### **6.3.1 Carbohydrate composition**



Three samples of each complementary food were separately defatted using the Soxhlet method (23), and then oven-dried at about 108°C for 16 h to a constant weight. An assay kit (K-MASUG 10/04, Megazyme Int., Wicklow, Ireland) was used to determine the levels of simple carbohydrates/sugars: maltose, sucrose and free glucose (the assay enzymes do not hydrolyse glucose from glucose polymers) in the defatted samples using a UV/Visible spectrophotometer (Pharmacia LKB Ultrospec II, England) at 340 nm. Approximately 0.5 g of each defatted sample was weighed and transferred into 50-mL beakers and deproteinised as described in the kit to reduce possible interference by other substances.

Total available carbohydrate was calculated as 100% minus the sum of moisture, protein, fat, ash and total dietary fibre obtained using proximate analysis as previously reported (16). Levels of free fructose and starch contents in the samples were estimated by calculation, while total dietary fibre in the samples was analysed by enzymatic-gravimetric method using the K-ACHDF 11/08 kit (Megazyme Int., Wicklow, Ireland). Free fructose was calculated by adjusting for the fructose from the sugar used as an ingredient after determining the total fructose levels in the formulations using the K-ACHDF 11/08 assay kit by spectrophotometry at 340 nm. The starch content was calculated as the difference between total available carbohydrate and the sum of maltose, sucrose, free glucose and free fructose; corrected for approximately 3 g/100 g of lactose from skim milk powder (24) used as an ingredient in processing the extrusion-cooked ComFa and roller-dried ComFa.

### **6.3.2 Viscosity measurement**

A Rapid Visco™ Analyser [Super 4 (RVA-S4), Newport Scientific Pty Ltd., NSW, Australia] interfaced with a personal computer equipped with the software Thermocline for Windows v3.0 was used to measure the pasting profile using the Newport Scientific Method Version 13. In summary, about 3 g (14% moisture basis) of non-defatted complementary foods (in triplicate) were weighed and transferred to an aluminium canister containing approximately 25 mL of distilled water (corrected to compensate for 14% moisture used for the sample). A plastic paddle was used to mix the slurry to

break up any lumps before inserting the canister with the paddle into the instrument to measure the pasting profile for 20 min. The initial equilibration of the slurry was set for 2 min at 25°C, which was followed by heating to a maximum of 95°C for 5 min, and then holding at this temperature for a further 3 min. The paste was cooled to 25°C for 5 min, and held at this temperature for 3 min.

The maximum apparent viscosity during heating, or the heating/holding phase of the test, was reported as the peak viscosity and the final viscosity was the viscosity recorded at the end of the test (25). Using the recommended viscosity measurement at 45°C for infant porridge (12, 26), the average of seven apparent viscosity readings between 13.60 and 14.00 min (RVA time) corresponding to 45°C was taken as “consume viscosity” for the non-defatted complementary foods.

#### **6.3.3 Water solubility index**

The gravimetric method described by Jin et al. (27) was used to determine the WSI of the non-defatted infant formulations in triplicate.

#### **6.4 Consumer sensory evaluation**

The sensory acceptance of the products: roller-dried ComFa, oven-toasted ComFa and enriched Weanimix, were measured using the method of Stone & Sidel (28). The extrusion-cooked ComFa was not included in the sensory acceptance test to reduce evaluation fatigue. Twenty women from nine sub-Saharan Africa countries (Congo, Gabon, Ghana, Kenya, Malawi, Nigeria, Rwanda, Zambia and Zimbabwe), resident in Palmerston North (PN), New Zealand (NZ), who had experience of feeding a child with cereal-based porridge prepared at the household level and responded to the advertisement to participate in the consumer sensory evaluation were invited to the Institute of Food, Nutrition and Human Health, Massey University, PN, NZ. Mothers have been employed to assess the consumer acceptance of cassava-soyabean complementary foods (29). The sensory attributes evaluated (**Appendix 6**) for the selected complementary foods were overall acceptability, colour, smell, texture and taste using a 9-point hedonic scale with one as least acceptable/dislike extremely and nine as highly

acceptable/like extremely. A just-about-right scale (5 being just right) was used to evaluate the level of sweetness and saltiness. The women were also asked whether they would be willing to feed their babies with each of the complementary food formulations using a 9-point scale (1=Not likely; 5=Neutral; 9=Very likely).

Ethical approval was obtained from the Massey University Human Ethics Committee (HEC: Southern A Application-09/58), PN, NZ. Before the sensory test began, written consent to take part in the study was obtained from each participant after explaining the objectives to them.

Two of the participants who were feeding complementary foods to their babies at the time of the sensory evaluation testing were selected to prepare suitable food for babies from the roller-dried ComFa, oven-toasted ComFa and enriched Weanimix, as they would do at their homes in sub-Saharan Africa. One hundred and fifty grams of each of the three formulations were given to the selected women. These two women were informed that oven-toasted ComFa and enriched Weanimix were to be prepared as traditional household-level infant porridge while the roller-dried ComFa was to be reconstituted in the same way as Nestlé Cerelac®.

About 20 g of each of the prepared samples were randomly served together to all of the participants (including the two caregivers who prepared the porridge) by the researchers in three-figure coded plastic cups at room temperature for the product liking evaluation in a sensory booth.

## **6.5 Statistical analysis**

The composition of carbohydrate, viscosity and the WSI data were subjected to Univariate Analysis of Variance (One-Way ANOVA). The Tukey's studentized range test was used to compare differences between means when the ANOVA was significant ( $P < 0.05$ ). The relationship between apparent viscosity and WSI and either the sum of simple sugars, starch or total dietary fibre was evaluated using Pearson's correlation. Minitab v15.1™ (Minitab Inc., State College, PA, USA) was used for the statistical analyses.

The sensory acceptance test data were statistically analysed using both the Kruskal-Wallis nonparametric test (not assuming normality of data) for three or more independent samples and the usual One-Way ANOVA, assuming normality of data using the PASW Statistics 18, Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, USA).

For the sweetness and saltiness intensities scoring using the just-about-right scale, the responses were categorised as, “much too low” ( $\leq 2$ ), “too low” (3-4), just-about-right (5), “too high” (6-7) and “much too high” ( $\geq 8$ ). As recommended by Stone & Sidel (28), if 70% of the responses were in the just-about-right category, the intensity was taken as acceptable by the caregivers.

## 6.6 Results

The levels of sugars and starch in all the infant foods are presented in **Table 6.1**. As expected, the sum of simple sugars as percentage of total carbohydrate (that is, total dietary fibre and total available carbohydrate) was approximately 64% for the ComFa formulation while the maize based formulation was 6.0% only. There were significant differences in the levels of simple carbohydrates between the sweetpotato-based formulations compared with those of the maize-based product. On average, sweetpotato-based formulations were higher in maltose (26 times,  $P < 0.0001$ ), sucrose (5 times,  $P < 0.0001$ ), free glucose (19 times,  $P < 0.0001$ ) and fructose (7 times,  $P = 0.002$ ). The roller-dried ComFa was slightly higher in maltose than the extrusion-cooked ComFa (1.12 times) and oven-toasted ComFa (1.21 times). All the other carbohydrate fractions, with the exception of free glucose, were not significantly different between the ComFa formulations ( $P > 0.05$ ). The sum of maltose, sucrose and glucose in the sweetpotato-based complementary foods is comparable to the level of 50 g/100 g (dry matter basis) in sweetpotato roots reported by other researchers (18). The sweetpotato-based complementary foods contained significantly less starch (10-13 vs. 47 g/100 g,  $P < 0.0001$ ), and were higher in total dietary fibre (8-11 vs. 6 g/100 g) than the levels in enriched Weanimix. The levels of the calculated total available carbohydrate and total dietary fibre together in the ComFa formulations indicated that the sweetpotato-based formulations satisfied the estimated carbohydrate content of 60-75 g/100 g in the Codex standard (1), but the enriched Weanimix was slightly lower by about 6.0%.

**Table 6.1** Carbohydrate composition<sup>1</sup> (g/100 g dry matter basis) of sweetpotato- and maize-based complementary foods

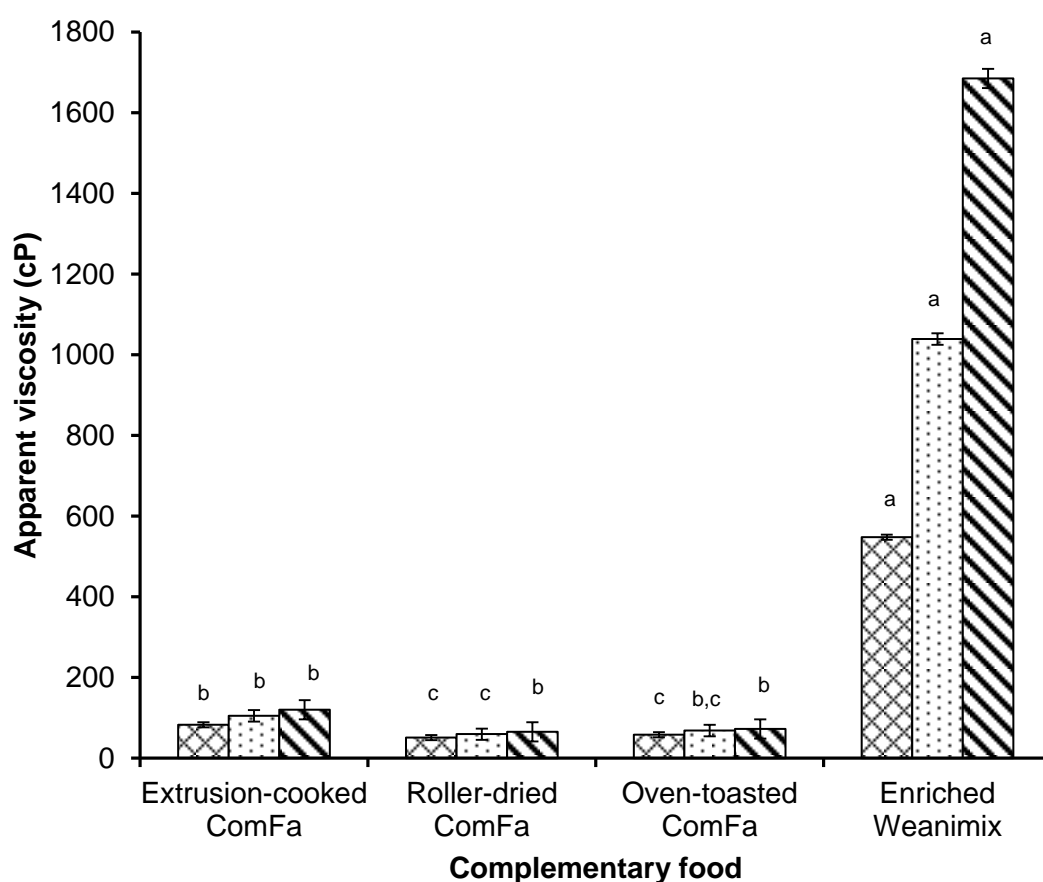
| Complementary food     | Maltose                   | Sucrose                   | Free glucose             | Free fructose            | Starch <sup>2</sup>       | Total dietary fibre <sup>3</sup> | Total available carbohydrate <sup>4</sup> |
|------------------------|---------------------------|---------------------------|--------------------------|--------------------------|---------------------------|----------------------------------|---|
| Sweetpotato-based      |                           |                           |                          |                          |                           |                                  |   |
| Extrusion-cooked ComFa | 27.50 (2.45) <sup>a</sup> | 10.20 (0.31) <sup>a</sup> | 1.24 (0.03) <sup>b</sup> | 3.07 (0.37) <sup>a</sup> | 11.32 (1.87) <sup>b</sup> | 10.25 (2.29) <sup>a</sup>        | 56.07 (1.97) <sup>a</sup>                 |
| Roller-dried ComFa     | 30.85 (3.84) <sup>a</sup> | 10.53 (1.06) <sup>a</sup> | 1.34 (0.03) <sup>a</sup> | 2.94 (1.04) <sup>a</sup> | 10.53 (3.70) <sup>b</sup> | 10.57 (1.21) <sup>a</sup>        | 58.92 (0.83) <sup>a</sup>                 |
| Oven-toasted ComFa     | 25.43 (1.17) <sup>a</sup> | 10.08 (0.43) <sup>a</sup> | 1.40 (0.05) <sup>a</sup> | 2.61 (0.46) <sup>a</sup> | 13.75 (0.72) <sup>b</sup> | 8.16 (0.77) <sup>a,b</sup>       | 53.28 (0.94) <sup>a,b</sup>               |
| Maize-based            |                           |                           |                          |                          |                           |                                  |   |
| Enriched Weanimix      | 1.06 (0.18) <sup>b</sup>  | 2.01 (0.12) <sup>b</sup>  | 0.07 (0.02) <sup>c</sup> | 0.39 (0.13) <sup>b</sup> | 46.72 (0.64) <sup>a</sup> | 6.08 (0.44) <sup>b</sup>         | 50.25 (0.75) <sup>b</sup>                 |
| <i>P</i> -value        | <0.0001                   | <0.0001                   | <0.0001                  | 0.002                    | <0.0001                   | 0.01                             | <0.0001                                   |

<sup>1</sup>Value is mean (standard deviation) of triplicate; values with the same superscript letter in a column are not significantly different ( $P > 0.05$ );

<sup>2</sup>Starch=total available carbohydrate minus sum of maltose, sucrose, free glucose and free fructose; the starch content for extrusion-cooked ComFa and roller-dried ComFa was corrected for approximately 3 g/100 g of lactose from skim milk powder (24) used as an ingredient;

<sup>3,4</sup>Data previously published (16); Tukey's studentized range test was used for the Post Hoc mean analysis instead of least significant difference in published manuscript.

The average apparent viscosity of the sweetpotato-based infant foods was 9-, 13- and 20-times lower at the peak, “consume” and final viscosities compared with enriched Weanimix (**Figure. 6.1**). All the complementary foods showed an increase in viscosity from high temperature (peak viscosity) to low temperature (final viscosity). The increase in viscosity was less pronounced (1.35-fold) in the sweetpotato-based products but substantially high (3.07-fold) for the maize-based formulation.



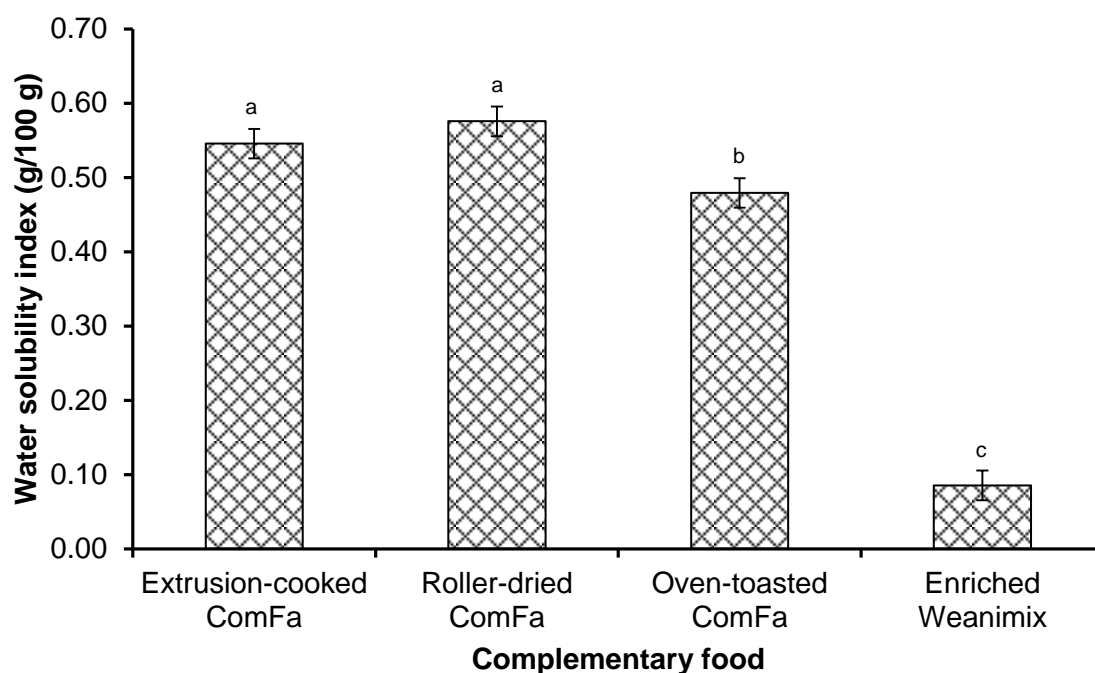
**Figure 6.1** Viscosity during cooking (peak viscosity), at serving (“consume viscosity”) and during storage (final viscosity) of porridge from the complementary foods

▨ Peak viscosity    ▤ “Consume viscosity”    ■ Final viscosity

Bar value (mean ± standard deviation,  $n=3$ );

Bar with different letter per each variable for the complementary foods was significantly different ( $P < 0.0001$ ).

The ComFa formulations were significantly higher in WSI (6.63 times,  $P < 0.001$ ) compared with the maize-based complementary food (**Figure 6. 2**).



**Figure 6.2** The water solubility index of the complementary foods

Bar value (mean  $\pm$  standard deviation,  $n=3$ ).

Bar with a different letter for a variable was significantly different ( $P < 0.0001$ ).

**Table 6.2** Correlation coefficient<sup>1</sup> between carbohydrate composition, apparent viscosity and water solubility index (WSI)

| Carbohydrate fraction      | Physical property           |                                  |                              | WSI               |
|----------------------------|-----------------------------|----------------------------------|------------------------------|-------------------|
|                            | Peak viscosity <sup>2</sup> | “Consume viscosity” <sup>3</sup> | Final viscosity <sup>4</sup> |                   |
| Simple sugars <sup>5</sup> | -0.992<br>(0.008)           | -0.992<br>(0.008)                | -0.992<br>(0.008)            | 0.998<br>(0.002)  |
| Total dietary fibre        | -0.852<br>(0.148)           | -0.854<br>(0.146)                | -0.854<br>(0.146)            | 0.935<br>(0.065)  |
| Starch                     | 0.995<br>(0.005)            | 0.996<br>(0.004)                 | 0.996<br>(0.004)             | -0.995<br>(0.005) |

<sup>1</sup>Cell content: Pearson correlation coefficient ( $P$ -value);

<sup>2</sup>Maximum apparent viscosity during heating or the heating/holding phase during RVA (Rapid Visco Analyser) test;

<sup>3</sup>Viscosity corresponding to approximately 45°C;

<sup>4</sup>Viscosity during storage, recorded at 25°C;

<sup>5</sup>Sum of maltose, sucrose, free glucose and fructose.

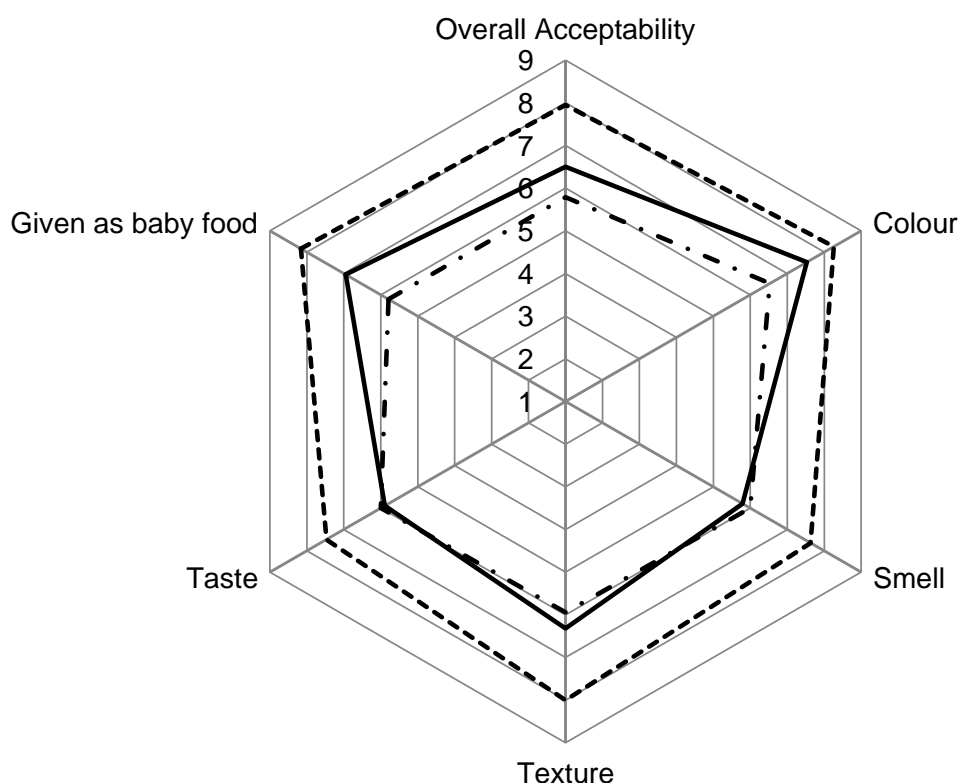


The relationships between the simple carbohydrates, total dietary fibre as well as starch and the physical properties (viscosity and solubility) are shown in **Table 6.2**. As expected, the level of simple sugars correlated negatively with the viscosities and positively with WSI.

For the sensory testings, slurries of oven-toasted ComFa and enriched Weanimix were prepared with potable water before cooking on a hot-plate for about 10 min. The roller-dried ComFa was reconstituted as ready-to-eat complementary food using potable water that was boiled and allowed to cool to 40°C. The average age of the participants who took part in the consumer sensory evaluation was 37±8 yr (mean±standard deviation). All the women also indicated that sweetpotato was readily available in their respective countries. The oven-toasted ComFa was mixed with approximately 750 mL of potable water before cooking but for the enriched Weanimix, initially about 600 mL of water, and further addition of about 300 mL and 150 mL of water during cooking to reduce the viscosity. Approximately 450 mL of the warm water was used for reconstitution of the roller-dried ComFa. The amount of water used for the reconstitution was discussed and accepted by the two selected women, and the final viscosity was unanimously accepted by the other participants.

Both the Kruskal-Wallis nonparametric test and the usual ANOVA gave similar results regarding the rejection of the null hypothesis (**Figure 6.3**). Therefore ANOVA was chosen, as recommended by Montgomery (30), to compare the means of the scores of the sensory attributes evaluated by the participants. The order of liking among the products was roller-dried ComFa, followed by oven-toasted ComFa, and lastly, enriched Weanimix for colour, texture, overall acceptability and willingness to use the formulations in their households as complementary food (**Figure 6.3**). All the three formulations were judged to be acceptable on all the indicators. The mean scores of sensory liking for colour, texture, overall acceptability and willingness to give product as baby food on the 9-point scale ranged from 6.3 to 8.3 for the two ComFa formulations selected for the sensory study. In contrast to the ComFa formulations, the equivalent range for the above sensory attributes was 5.8 to

6.5 for the enriched Weanimix.



**Figure 6.3** Diagrammatic presentation of product liking for sensory attributes and choice to give the formulations as complementary food to infants by female sensory participants

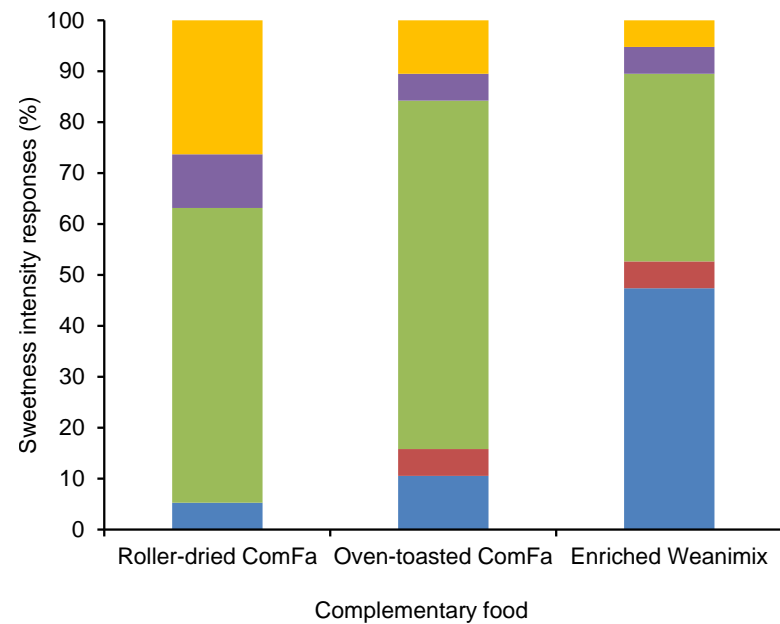
----Roller-dried ComFa —Oven-toasted ComFa - · -Enriched Weanimix

Sensory attribute with  $P < 0.05$  indicates that significant difference among the complementary foods; italicised value in parenthesis is asymptotic significance from Kruskal-Wallis test.

A 9-point hedonic scale was used (1=least acceptable/dislike extremely; 5=Neutral; 9=highly acceptable/like extremely) for all attributes except for willingness to give product to babies (1=Not likely; 5=Neutral; 9=Very likely).

Using the just-about-right scale, with the responses collected on a 9-point scale categorised as, “much too low” ( $\leq 2$ ), “too low” (3-4), just-about-right (5), “too high” (6-7) and “much too high” ( $\geq 8$ ); about 58% and 68% of the caregivers liked the sweetness intensity of the roller-dried ComFa and oven-toasted ComFa respectively compared with about 37% for enriched Weanimix compared with about 37% for enriched Weanimix (**Figure 6.4**). About 53% of the caregivers

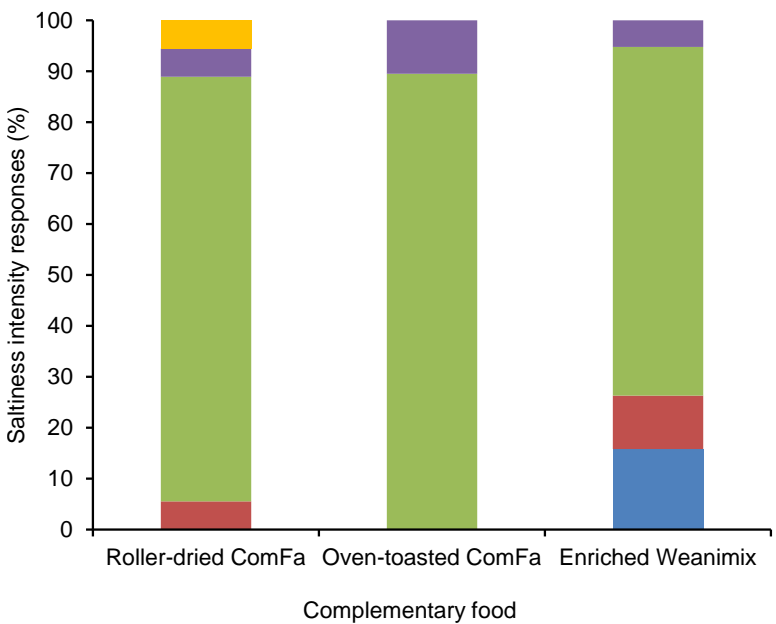
scored the sweetness intensity of enriched Weanimix as being at least too low.



**Figure 6.4** Diagrammatic presentation scoring for sweetness intensity of the complementary food formulations on just-about-right scale

■ Much too low ■ Too low ■ Just-about-right ■ Too high ■ Much too high

Scoring was done on a 9-point scale and responses were categorised as, “much too low” ( $\leq 2$ ), “too low” (3-4), just-about-right (5), “too high” (6-7) and “much too high” ( $\geq 8$ )



**Figure 6.5** Diagrammatic presentation scoring for saltiness intensity of the complementary food formulations on just-about-right scale

■ Much too low ■ Too low ■ Just-about-right ■ Too high ■ Much too high

Scoring was done on a 9-point scale and responses were categorised as, “much too low” ( $\leq 2$ ), “too low” (3-4), just-about-right (5), “too high” (6-7) and “much too high” ( $\geq 8$ )

However, the percentage of the score in the just-about-right category for all the formulations did not meet the established agreed-on acceptability of 70%.

The saltiness intensity (**Figure 6.5**) the roller-dried ComFa and oven-toasted ComFa was higher than the established agreed-on acceptability of 70% by 13%, while the saltiness intensity of the enriched Weanimix was only 2.0% lower than the agreed-on acceptability score.

## **6.7 Discussion**

The higher level of sugars in sweetpotato-based complementary foods will make them naturally sweeter than the maize-based product. Therefore, addition of sugar would not be necessary when preparing porridge. The natural sweetness of the sweetpotato-based infant foods could offer a nutritional benefit over the maize-based complementary food as it may lead to higher intake of food (12).

The slightly higher level of maltose in the roller-dried ComFa than the extrusion-cooked ComFa and oven-toasted ComFa is likely due to gradual heating of the slurry that allows the endogenous  $\beta$ -amylase in sweetpotato (22) a longer interaction time with gelatinised starch before being denatured. However, for the extrusion-cooked and oven-toasted ComFa, the composite flour was introduced into the extruder or the oven, both pre-heated to 120°C, which could lead to early denaturation of  $\beta$ -amylase. A similar trend was observed when the roots of cream-fleshed sweetpotato, the same variety used in this study, were mashed and heated slowly from 75-100°C for 40 min compared with those cooked rapidly for 10 min at either 95-100°C or 105°C (20).

The total dietary fibre in the ComFa formulations exceeded the maximum specified content of 5 g/100 g in the Codex standard (1). As discussed in an earlier publication (16), the high levels of fibre in the sweetpotato-based formulation may be beneficial rather than a nutritional limitation (10). It has been reported that about 25-50% of the fibre in sweetpotato is soluble (31, 32), which may serve as fermentable substrate for health-promoting colonic bacteria.

The peak viscosity measured during heating, or the heating/holding phase on the RVA represents the maximum viscosity likely to occur during porridge preparation on a kitchen stove. For example, in Ghana, a slurry is made from cereal or cereal-based dough/flour in a cooking pot, which is then cooked on a stove with frequent stirring. The porridge is usually left to cool to between 40-45°C before being fed to infants. The apparent viscosity at approximately 45°C (the temperature of porridge served to infants), thus represents the

likely consistency of the porridge given to infants (4, 12, 26). The final viscosity, measured at 25°C, could correspond to the viscosity of the porridge when left to cool at room temperature. The increase in apparent viscosity upon cooling observed in this study compares well with similar observation in other studies (4, 33), and may be due to the partial association or realignment of starch granules (particularly, the linear molecules, amylose, and linear parts of amylopectin molecules) to form a precipitate or a gel, a phenomenon referred to as retrogradation (2). The substantial increase in the viscosity during cooling in the enriched Weanimix indicates higher retrogradation, and can be related to the corresponding higher starch content than the ComFa formulations. The suggestion in our earlier publication that the oven-toasted ComFa will be lower in starch and consequently, have a lower viscosity compared with the enriched Weanimix, both as household-level complementary food (5) is confirmed in this study.

The higher level of sugars in the ComFa formulations largely contributed to the lower apparent viscosities of the sweetpotato-based products as reported in other studies (34, 35). Because of the inherently low viscosity of the sweetpotato-based formulations, less water will be added during porridge preparation than would be needed for the maize-based formulation so there will be less “*energy and nutrient thinning*” for the ComFa formulation.

The high WSI of the sweetpotato-based complementary food in this study can be explained by the quantity of soluble molecules (example, sugars and possibly soluble fibre). The relatively high WSI and simple carbohydrates to starch ratio may suggest easier digestibility of the sweetpotato-based formulations than enriched Weanimix as proposed by other researchers (7-9). The lower solubility of the oven-toasted ComFa compared with the other ComFa formulation could be due to the slightly higher starch content.

The participants in the sensory evaluation test were purposely recruited because the ComFa formulations were developed for infants in sub-Saharan Africa. The women were chosen instead of infants because of the common practice (example, in Ghana), that caregivers taste food before serving to their babies, hence they could serve as suitable consumer sensory panellists for complementary foods as done in another study (29). Our sampling criteria

limited us from achieving the recommended 25-50 responses per product for reliable and credible laboratory-based sensory liking test (28). In any case, the reasons for the number of responses per product include improving the statistical power for detecting minimal differences in the hedonic scale ratings given for products (28). Notwithstanding this limitation, there was a clear significant trend ( $P < 0.05$ ) that indicated that the sweetpotato-based complementary foods were preferred by the participants than the maize-based product for all the eight attributes evaluated, including the willingness to give the products as baby food. The just-about-right sweetness intensity score for the ComFa formulations supports our earlier suggestion that mothers would probably not sweeten the porridge for infants with sucrose. The higher consumer rating for the roller-dried ComFa compared with the oven-toasted ComFa was probably because the fish powder included in the oven-toasted ComFa imparted a flavour that was disliked by some of the participants. We have previously discussed elsewhere that the fish powder was incorporated in the household-level formulation to improve the protein quality and calcium (5).

Another important observation of nutritional significance relates to the amount of water used to prepare the same quantities of complementary food flour. Less water was used for the oven-toasted ComFa porridge compared with the enriched-Weanimix [1:5 (wt/wt) and 1:7 respectively of flour: water]. Therefore, it is the desired viscosity of porridge that guides mothers/caregivers on the suitable proportions of complementary food flour and water to be used during preparation. The lower amount of water used for the oven-toasted ComFa porridge indicates less “*energy and nutrient thinning*” compared with the maize-based formulation porridge. Also, the amount of water used for the maize-based porridge supports the relatively high peak viscosity of this product.

The sweetness score and the amount of water used for the porridge correspond to the data on the composition of carbohydrate and apparent viscosity, respectively. This therefore validates the sensory data.



## **6.8 Conclusion**

The sweetpotato-based formulations had a higher sugar to starch ratio than the maize-based complementary food. The levels of the total sugars and starch are the likely reasons for the lower apparent viscosity and higher WSI of the ComFa formulations compared with the enriched Weanimix resulting in requirement for less water when preparing the porridge from the ComFa formulations. The scores of the consumer preference given by the infant caregivers indicated highest liking for roller-dried ComFa, followed by the oven-toasted ComFa, and lastly, the enriched Weanimix. These findings, combined with the relatively low phytate and phytate: mineral ratio, and high vitamin A levels (5, 16, 17), suggest that the use of sweetpotato in complementary food should be encouraged as it has potential to have significant nutritional benefit in low-income countries.

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## **Chapter 7: General Summary and Conclusion**

*“Good nutrition in early life helps adults to become taller, stronger, and more intelligent, thus improving school achievement, economic productivity, and earnings.” Cesar G. Victora, 2009, Lancet (374)\**

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\* Victora, C. G. (2009). Nutrition in early life: a global priority. *Lancet*, 374(9696), 1123-1125. doi: 10.1016/s0140-6736(09)61725-6

The studies conducted in this thesis were to investigate the possibility of formulating a suitable dry infant cereal-like complementary food blend from cream-fleshed sweetpotato (Paper I: Amagloh, Weber, et al., 2012). Weanimix was used for comparison since it is being promoted by health workers in Ghana (Anonymous, 2012), and was formulated in 1987 through collaboration between UNICEF and the Nutrition Unit of the Ministry of Health, Ghana (Lartey et al., 1999). This maize-soyabean-groundnut blend can be processed at both the household- or industrial-level.

Cereal-legume food blends prepared at the household-level from unrefined/non-dehulled cereals such as maize, millet, sorghum and legumes (soyabean, cowpea or groundnut) have high levels of phytate, an antinutrient that limits the bioavailability of nutrients including iron and zinc (Gibson et al., 2010; Greiner, Konietzny, & Jany, 2006) and, partly contributes to the high iron and zinc deficiencies among infants (Hotz, Gibson, & Temple, 2001; Hurrell & Egli, 2010; Hurrell, Reddy, Juillerat, & Cook, 2003). Additionally, cereal-based complementary foods are naturally low in vitamin A (Dewey & Brown, 2003; Lartey et al., 1998).

Also, non-malted cereal-legume blends form viscous porridges that require dilution with water to reduce the consistency to make them suitable for infant feeding; reducing energy and nutrient densities (Kikafunda et al., 1997; Mosha & Svanberg, 1983; Mosha & Svanberg, 1990). The dilution with water, thus, leads to “*energy and nutrient thinning*” of already nutrient-low infant formulations (Dewey & Brown, 2003).

The sweetpotato-based complementary foods, ComFa formulations, were formulated to be replicated at the household-level (oven-toasted ComFa) or the industrial-level (roller-dried ComFa and extrusion-cooked ComFa).

The ComFa formulations and the enriched Weanimix met the stipulated energy and fat values (Papers II & III: Amagloh et al., 2011; 2012) as specified in the Codex standards (CAC/GL 8 and STAN 074-1981, Rev.1-2006) (Codex Alimentarius Commission, 1991, 2006). However, the protein content of the industrial-based ComFa formulations (roller dried- and extrusion-cooked ComFa), was lower by 17%, but both the oven-toasted

ComFa and enriched Weanimix met the protein specification (Paper II: Amagloh, Hardacre, et al., 2012). The protein content of roller-dried ComFa and extrusion-cooked ComFa could be increased by adjusting the proportions of the soyabean and the skim milk powder.

The sweetpotato-based formulations had total dietary fibre that was more (about twice) than the maximum specification of 5 g/100 g by the Codex Alimentarius Commission (1991). This may be a disadvantage for using the ComFa formulations as complementary food, but it is likely that significant proportion of the fibre would be soluble; about 25-50% of the total dietary fibre in sweetpotato is soluble (Huang, Tanudjaja, & Lum, 1999; Mullin, Rosa, & Reynolds, 1994). Soluble fibre is not digested, but serves as a fermentable substrate, and thus stimulates the growth of beneficial colonic microflora such as lactobacilli and bifidobacteria (Anderson et al., 2009; Anderson, Smith, & Gustafson, 1994). In healthy young men, soluble fibres have been shown to improve calcium bioavailability (Coudray et al., 1997). However, it is worth-mentioning that the ability to ferment complex carbohydrate during infancy is not fully developed until around 8 mo old (Parrett, Edwards, & Lokerse, 1997), thus improvement in the calcium bioavailability may not be realised in infants as in adults. Fructo-oligosaccharides, which exhibit soluble dietary fibre-like properties, are added to infant formula to promote the growth of non-pathogenic microflora (Sabater-Molina, Larque, Torrella, & Zamora, 2009). Therefore, the high level of total dietary fibre in the ComFa formulations could be beneficial.

The sweetpotato-based infant foods had a quarter of the level of phytate of enriched Weanimix (Paper II: Amagloh, Hardacre, et al., 2012), which was prepared using dehulled maize and soyabean flours instead of non-dehulled flour. The dehulling was to done to reduce the fibre and polyphenol contents in the enriched Weanimix formulation.

As expected, vitamin A content of the oven-toasted ComFa was markedly higher than enriched Weanimix because of the cream-fleshed sweetpotato used in the formulation (Paper III: Amagloh et al., 2011). Most of the  $\beta$ -carotene in the oven-toasted ComFa was retained for up to eight weeks when stored in containers with good moisture barrier under simulated

average temperature of 32°C and relative humidity of 85%, as prevails in Ghana (Paper IV).

The ComFa formulations may inhibit calcium, iron and zinc absorption less than the enriched Weanimix using the phytate: calcium, iron and zinc molar ratios, and the level of  $\beta$ -carotene to predict relative availability of these essential minerals (Paper V: Amagloh, Brough, et al., 2012).

All the ComFa formulations contained more simple sugars (mono- and disaccharides) than complex carbohydrate, starch; this is contrary to the carbohydrate composition in enriched Weanimix (Papers VI). The higher simple sugar and lower starch contents of the ComFa formulation was likely responsible for the lower viscosity compared with enriched Weanimix when the pasting profile of slurry prepared from samples was evaluated using the Rapid Visco™ Analyser. The low viscosity of the sweetpotato-based complementary food could be a nutritional benefit because during porridge preparation, less dilution with water would be required, thus reducing “*energy and nutrient thinning*” as will be the case for Weanimix, unless amylase-rich flour is prepared by malting the maize as shown in other studies (Afoakwa, Adjonu, & Asomaning, 2010; Mosha & Svanberg, 1990). Therefore, the use of sweetpotato instead of maize will eliminate the treatment with amylase-rich flour to reduce porridge viscosity. The level of sugar also contributed to the higher consumer preference by caregivers for the sweetpotato-based formulation than the maize-based formulation.

Within the limits of this research, that is, the compositional, functional and sensory data presented in this thesis, the sweetpotato-based formulations are more suitable than maize-based product as complementary food because the sweetpotato-based food matrix:

- contains less phytate, thus less inhibition of iron and zinc absorption;
- contains more carotenoids, thus a sustainable source of dietary vitamin A to complement vitamin A supplementation initiative in Ghana.
- contains more simple sugars than starch that resulted in porridge with lower viscosity; therefore, there is no need for either excessive dilution with water or preparation of amylase- rich flour, to thin the viscosity and;



Importantly, the climatic condition in Ghana is suitable for cultivation of sweetpotato as documented elsewhere (FAO, 2005; IFAD, 2004; Opare-Obisaw et al., 2000).

## **Chapter 8: Limitations, Recommendations and Future Perspectives**

In the course of this research, it was realised that maturity of the sweetpotato roots may affect the levels of phytate and  $\beta$ -carotene. Phytate rapidly accumulates during maturation of legumes and grains (Reddy, 2002), but it is not known if it also occurs in sweetpotato roots.  $\beta$ -carotene concentration is affected by farming and storage practices, as well as environmental conditions (Burri, 2011). Like other root and tuber crops (example, cassava, yam), sweetpotato can be stored in underground in Ghana as investigated in Tanzania (Van Oirschot et al., 2007). Also, the storage of the roots increased the sucrose levels (Deobald, Hasling, Catalano, & McLemore, 1969; Takahata, Noda, & Sato, 1995). However, these factors were not considered in this work. This could affect the phytate and carotenoid contents of the sweetpotato-based complementary food processed described in this thesis. Generally, because root and tuber crops are lower in phytate than cereals or legumes (Gibson et al., 2010), a similar trend would be found when the study is replicated. For this thesis, market-ready processing grade sweetpotato roots were used for preparing the ComFa formulations.

In a review on sweetpotato and its impact on vitamin A deficiency by Burri (2011), it was stated that the more orange the colour of the flesh of sweetpotato, the higher the concentration of  $\beta$ -carotene. Since one of the objectives of formulating the sweetpotato-based complementary food was to have a product that will be high in vitamin A compared with an unfortified cereal-legume blend such as Weanimix used at the household-level in Ghana, it is recommended that if an orange-fleshed sweetpotato variety with high dry matter is available, it should be used for preparing the complementary food as described in this thesis. The orange-fleshed sweetpotato variety (Beauregard) available in New Zealand, contained high moisture, and the flour prepared from it was hygroscopic; hence, it was not suitable for processing the ComFa formulations described in this work.

Another possible household-level formulation is to eliminate the processing of flour from sweetpotato roots. The roots can be cooked and mashed, and

mixed with the broth of the other ingredients as described in Paper I (Chapter 1) (Amagloh et al., 2010). The difference between this proposed formulation and the one described in Chapter 1 is that the chipped roots will directly be cooked with the other ingredients instead of being dried and milled into flour as described in the stove-top cooked ComFa in Chapter 1. This processing method would make it possible to use high moisture varieties of sweetpotato. The use of palm oil instead of soyabean oil and leafy vegetables (example, moringa, sweetpotato and cocoyam leaves, etc) could be added to the proposed formulation to improve the nutritional contents of vitamin A in the ComFa formulations. These could be considered in future studies. The proposed formulations look more attractive as there will be cost saving (no milling cost for dried sweetpotato chips), less time consuming (no need to dry chipped roots) and possibly resulting in a formulation with higher levels of essential micronutrients such as vitamin C and  $\beta$ -carotene.

The compositional, functional and sensory data in this thesis suggest that the consumption of sweetpotato-based complementary foods could improve the nutritional status of infants than Weanimix. Therefore, a randomised feeding trial as described in **Appendix 1** to investigate this potential is warranted.

Although the household-level ComFa formulation did not meet all the requirements for vitamin A, iron and zinc from complementary food, it would positively contribute, particularly, to vitamin A supplementation initiatives in Ghana to reduce VAD when regularly consumed better than enriched Weanimix. However, effort by health workers, nutrition researchers, food technologists and policy makers will be required for nutritional education before the potential of the ComFa formulation could be realised.

Could the high fibre and simple sugars contents in the ComFa formulations be detrimental or beneficial? These issues need to be investigated by organising well-designed nutritional studies before the sweetpotato-based complementary foods could be recommended to institutions involved in infants and young children feeding programme.

## **References: Chapters 1, 7 & 8**

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## **Appendices**

**Appendix 1:** Summarised information of the human ethics application as a study protocol for conducting feeding trial on infants in Ghana.



## Study protocol

This trial will be registered with Clinical trials and the protocol will be published in BMC Public Health when funding application is approved.

**Title: Randomised feeding trial for evaluating the effect of the consumption of sweetpotato- and maize-based complementary foods on the nutritional status of infants from 6 months and followed up to 9 months in the Kassena Nankana District, Ghana**

### Abstract

**Background:** The commonly used cereal-based complementary foods in developing countries such as Ghana have been implicated in the high prevalence of micronutrient deficiencies, particularly iron and zinc because of the phytate levels, and vitamin A deficiency due to the low levels of vitamin A in unfortified cereal-based infant foods. This project seeks to evaluate the consumption of sweetpotato-based infant foods (oven-toasted ComFa and roller-dried ComFa) and maize-based formulations (enriched Weanimix and Nestlé® Cerelac®) on the nutritional status of Ghanaian infants, who are particularly nutritionally vulnerable with a high incidence of growth faltering, anaemia and vitamin A deficiency. **Methods/Design:** Two hundred and sixty mother-infant pairs will be recruited from Kassena-Nankana District, in the Upper East Region, Ghana, and equally randomised into four groups. Feeding trial will begin when the infant is six months old and followed up until the child is nine months old. Supply of project food allocations (800 g) and collection of dietary and health information will be conducted weekly. Weekly and monthly measurements of weight and length, respectively, will also be carried out. Venous blood sample will be taken at 6 (baseline), 7.5 and 9 months for biochemical analyses such as retinol, haemoglobin, ferritin, iron, total iron-binding capacity, transferring saturation, zinc, retinol-binding protein and C-reactive protein. Occult blood in faeces and malaria parasite count will also be assessed at 6, 7.5 and 9 months. **Discussion:** This feeding will be the first to evaluation the consumption of sweetpotato-based infant formulation, as an alternative to cereal-based complementary food on the nutritional status of infants. If the sweetpotato-based formulation is

*successful in improving the vitamin A or iron status of infants in the study, recommendation will be made to organisations focussed on improving the nutritional status of infants, particularly in low-income countries, to promote its use instead of cereal-based formulation for complementary feeding.*

## **Background**

The occurrence of childhood malnutrition in sub-Saharan Africa is among the worst in the world [1-3]. Data from a Ghana Demographic and Health Survey (2003) revealed that about 30% of children were stunted countrywide, and in Upper East Region about 80% of children aged 6 to 59 months have anaemia [4]. The recent finding published in 2009, has prevalence of anaemia among children less than five years in this region being approximately 89% [5]. Infants are particularly vulnerable when complementary foods are introduced, although adequate nutrition for the first two years of life is critical. Iron deficiency may have irreversible effects on cognitive development [6]. Vitamin A and zinc deficiencies increase susceptibility to infections and other childhood diseases, which subsequently lead to growth faltering and/or death [7]. The commonly used cereal-based complementary foods in developing countries such as Ghana have been implicated in the high prevalence of micronutrient deficiencies, particularly iron and zinc because of the phytate levels [8, 9]. Phytate forms insoluble complexes with some micronutrients [10-12] and, because the intestinal phytase activity in humans is low [13, 14], hydrolysis of such complexes to release the bound nutrients is limited. However, the inhibitory effect of phytate on nutrients is dose-dependent [10, 15]. Therefore, reduction of the phytate level in plant-based infant foods is likely to improve the absorption of essential micronutrients including iron and zinc. Hence, there is the need to have an alternative low-phytate complementary food, which could be prepared at the household-level from available food crops in low-income countries such as Ghana.

An alternative complementary food based on sweetpotato referred to as ComFa was proposed [16], developed and has been assessed for suitability as complementary food in comparison with a maize-based complementary food, enriched Weanimix [17-19]. The ComFa formulations (oven-toasted ComFa, roller-dried ComFa and extrusion-cooked ComFa) and the enriched Weanimix met the stipulated energy and fat values specified in the Codex standards (CAC/GL 8 and STAN 074-1981, Rev.1-2006) [17]. However, the protein content of roller-dried ComFa and extrusion-cooked ComFa was

lower by 17%, but the oven-toasted ComFa and enriched Weanimix met the protein specification. The sweetpotato-based formulations had total dietary fibre that was about twice the Codex specification of less than 5.0%. The fibre contents of the ComFa formulations may be beneficial as it is likely to be partly soluble fibre. The phytate content in all the ComFa formulations was approximately a quarter of the level of 0.80 g/100g in the enriched Weanimix [17]. Only the sweetpotato-based infant foods contained measurable levels of  $\beta$ -carotene, resulting in significantly higher vitamin A content of the oven-toasted ComFa compared with enriched Weanimix (28.80 vs. 1.20  $\mu$ g retinol equivalents/100 kcal), when both formulations were evaluated as household-level complementary foods [18]. Most of the  $\beta$ -carotene in the oven-toasted ComFa was retained for up to eight weeks when stored in containers with good moisture barrier under simulated average temperature of 32°C and 85% relative humidity, mimicking ambient conditions of Ghana (*unpublished data*). The ComFa formulations may be less inhibitory on calcium, iron and zinc absorption by infants than the enriched Weanimix using the phytate: calcium, iron and zinc molar ratios, and the level of  $\beta$ -carotene to predict relative availability of these essential minerals [19]. On average, sweetpotato-based formulations were higher in maltose (26 times), sucrose (5 times), free glucose (19 times) and fructose (7 times) than levels in enriched Weanimix, but the ComFa formulations contained significantly less starch (10-13 vs. 47 g/100 g) (*unpublished data, manuscript under review*). The high simple sugar levels in the ComFa formulations resulted in lower apparent viscosity (9-, 13- and 20-times, for peak, “consume” and final viscosities), higher water solubility index (7 times), and higher consumer acceptance compared with the maize-based formulation.

On the basis of the compositional, functional and consumer liking stated above, the sweetpotato-based formulations have the potential to improve the nutritional status of infants, and thus, a randomised feeding trial among Ghanaian infants is warranted to evaluate the effect of the consumption of these alternative complementary foods (that is, the ComFa formulations) on the nutritional status of infants.

The aims of this study will be:

The assessment of the consumption of the oven-toasted ComFa, roller-dried ComFa, enriched Weanimix and Nestlé® Cerelac® on the micronutrients (vitamin A, iron and zinc) status of infants (6-9 months).

The assessment of the impact of the oven-toasted ComFa, roller-dried ComFa, enriched Weanimix and Nestlé® Cerelac® on growth of infants (6-9 months) by measuring weight and length.

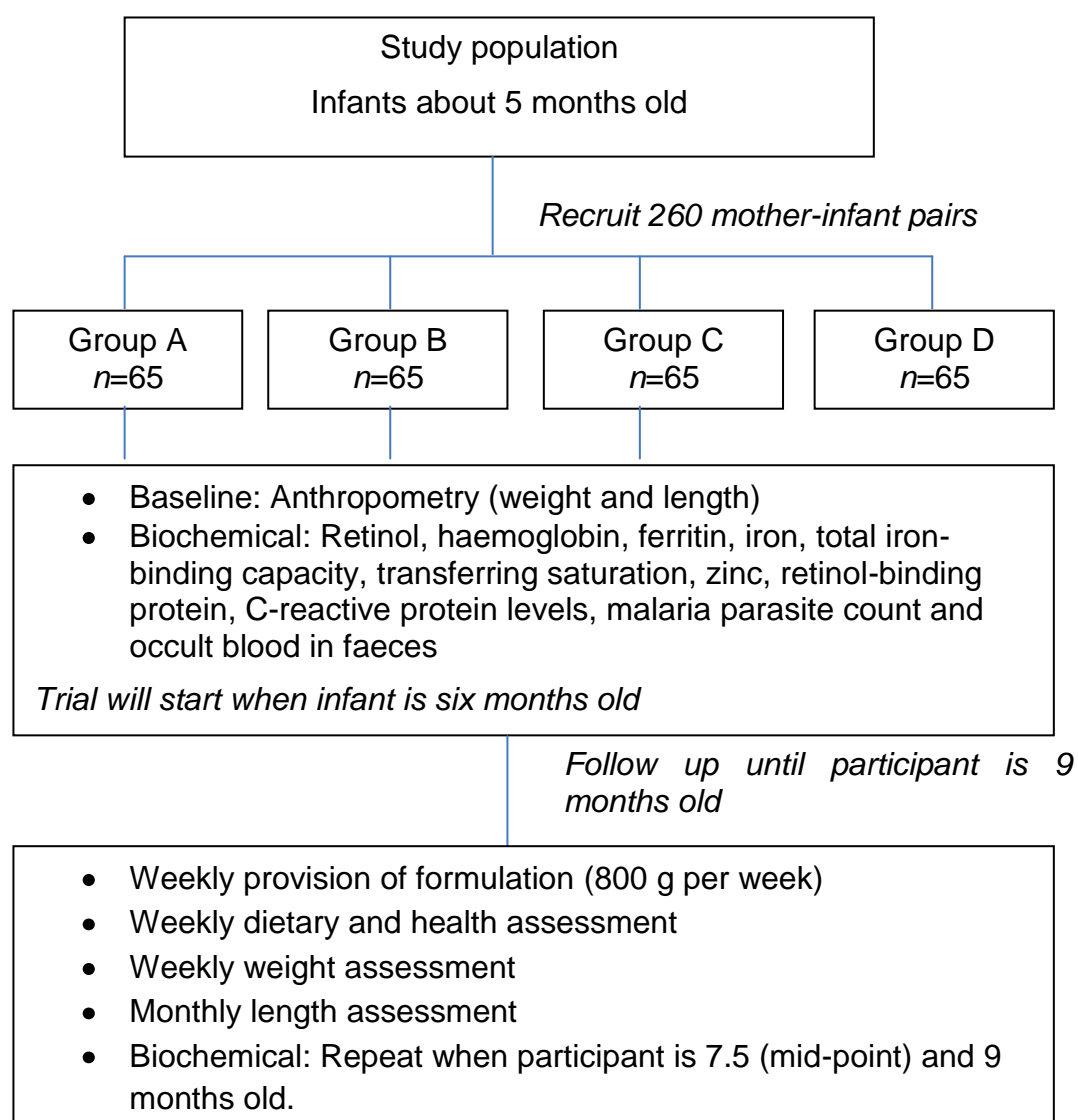
## Methods/Design

The study design is illustrated in **Figure A-1**. The first stage will involve publicising the study on a community radio station (Nabina FM) and also at postnatal clinic fora in the study area (Kassena-Nankana District). This will be carried out with the District Director of Nursing Services.

## Formulations

The oven-toasted ComFa, roller-dried ComFa and enriched Weanimix will be processed as previously described [17]. To ensure that mothers are not able to distinguish among the test formulations, the oven-toasted ComFa and enriched Weanimix will further be cooked and freeze-dried so they can be given to infants by reconstituting with warm water only as the roller-dried ComFa and Nestlé® Cerelac®. The reconstituted meals will be spoon-fed. The feeding frequency of the test meals will be twice daily in accordance to recommendation of feeding frequency of 2 or 3 per day of complementary food by the World Health Organization [7]. Also, the roller-dried ComFa, the industrial-level processed version of the sweetpotato-based complementary foods, will be fortified with micronutrient powder that will be sourced from DSM Nutritional Products (Isando, South Africa).

The micronutrient powder will have low level of iron of 500 mg/kg as NaFeEDTA as described in the study by Troesch and co-workers [20] conducted on school children in South Africa. The reason for using this low level of iron, as discussed elsewhere by Hurrell [21], was based on the result of a study conducted in Pemba, Tanzania, a high malaria endemic area [22]. In that Pemba study, the authors concluded that routine supplementation with iron and folic acid in preschool children in malaria endemic areas can result in an increased risk of severe illness and death. It was the basis for the use of phytase-containing micronutrient powder with low level of iron in the study by Troesch and co-workers [20]. However, the micronutrient powder to be added to the roller-dried ComFa will not contain the retinyl palmitate and phytase as roller-dried ComFa has measurable level of  $\beta$ -carotene (25  $\mu$ mol/kg) and is low in phytate (1950 mg/kg) [19].



**Figure A-1** Study design. Evaluating the effect of the consumption of sweetpotato- and maize-based complementary foods on the nutritional status of infants from 6 months and followed up to 9 months in the Kassena Nankana District, Ghana

### **Description of study area**

The Kassena-Nankana District is located in the Upper East Region of Ghana. The district capital is Navrongo. To the north of this district, is a neighbouring country, Burkina Faso. The area has a sub-Saharan climate, very hot (minimum 18°C and maximum 44°C) and dry, with three distinct seasons yearly: cold and dry (November to early February), hot and dry (mid February to early May) and rainy (late May to October) (data for 1999 to 2008, Joseph Achana, District Meteorologist, Navrongo, Ghana, personal communication, 2009). Most of the inhabitants live in a cluster of houses (a compound) with

their farmlands surrounding them. They mainly subsist from farming. The staple foods cultivated are millet, sorghum and groundnut in addition to small-scale livestock rearing (guinea fowl, goat and sheep). Crop farming is done in the rainy season but livestock is kept all year round. The study area is under continuous demographic surveillance by Navrongo Health Research Centre. The average hospital deliveries was 250 per month for 2008 (*Afua A. Williams, District Director of Nursing Services, War Memorial Hospital, Navrongo, Ghana, personal communication, 2009*).

### **Sample size calculation**

The sample size for the infant feeding study was calculated using Minitab statistical software [23], at a power of 80%. This would allow for a detection of a difference of haemoglobin concentration of 8.8 g/L among the four groups with standard deviation of 14 based on results of an earlier intervention study conducted in Ghana [6]. A calculated sample size of 57 per group was obtained and adjusted by a 15% non-response value, to give the working sample size of 65 per group.

### **Recruitment**

Sampling will be from mothers with infants attending postnatal clinic in Navrongo. The clinics usually end with a forum on infant feeding practices and family planning which is led by community health nurses. It is at this forum that this research will be mentioned to the mothers by the community health nurses who would have been briefed prior to the postnatal clinics by the District Director of Nursing Services and the researcher. Mothers with infants who are between 5 and 5.5 months old would be encouraged to stay at the end of the meeting to find out more about the study if they are willing to participate for detailed description of the project using the information sheet (**Appendix 2**). Those who opt to stay after they have been given detailed information on the project will be screened for eligibility using the subject screening form (**Appendix 3**). Eligible subjects would be contacted by researcher through phone calls to request for a home visit to discuss the project in detail with the parents and others family members. Parents who consent would be given 2 of the participant consent forms (**Appendix 4**) to



endorse; one will be kept by the family, and the other by the researcher. The period for them to endorse the form will be 2 days. When a participant is selected, the mother would be asked to pick a card from a non-transparent bag which she would keep. Initially, there would be a total of 260 tagged cards, consisting of 65 labels each for GP A, GP B, GP C and GP D representing the treatment (formulation) groups to be provided. This would continue until the sample size of 260 subjects is obtained. Assignment of the formulation to the groups would be done by one of the supervisors in New Zealand and not the researcher on the field, in order to minimize bias. All the intervention foods will be packaged similarly, using packaging materials that would be designed by Contour Packaging, New Zealand.

***Criteria for selecting participants from the pool of potential participants***

These are the criteria for selecting study participants.

- The baby should be singleton and should have a health card with his/her data on birth recorded. Birth weight should be  $\geq 2500$  grams (which will exclude most premature infants).
- Haemoglobin concentration should be  $>70.0$  g/L at baseline/during the study period.
- No confirmed congenital abnormalities.
- No confirmed blood borne diseases in mothers or infants.
- Infants who are breastfeeding and would continue partial breastfeeding after 6 months for the study period are the potential participants.
- Mother indicating that she would be staying in the study area the next 6 months after recruitment.
- Mother and/or father of the infant should understand English.
- Parents who will sign the written consent form to participate would be included in the study.

Infants participating in the study whose haemoglobin concentration would drop to below 70.0 g/L or consistently lose weight would be referred to Dr. John E. Williams, [MA. Dip (GUM & Ven), MB CHB]. Dr. Williams is currently the Director of Navrongo Health Research Centre, located in the study area.

***Data collection***

The questionnaires (**Appendix 5**) will be administered by the researcher and field assistants during home and hospital visits to obtain dietary and health

information, and anthropometric measurements using the appropriate equipment. Weight and length will be measured in duplicate. The interview will mostly be conducted at participant's home with prior knowledge given to them through phone calls and by word of mouth. When this is not possible, a convenient location suggested by the participant will be used. If it happens to be at a worksite, approval will be sought from the in-charge. The researcher, field assistants and phlebotomist employed by the Ghana Health Service who is in charge of the clinical laboratory of War Memorial Hospital, Navrongo will be responsible for collecting data. The use of the facilities and personnel at the hospital will be paid for by the project. The researcher will also double as the quality control officer for data collection. Payment would be provided to cover transportation costs for participants to attend hospital for blood sample collection by a phlebotomist.

Venous blood will be taken from the side within the fold of the elbow (median antecubital area) by a phlebotomist working at War Memorial Hospital, Navrongo employed by Ghana Health Service. About 5 ml of blood would be collected for 3 times when infants are 6 -, 7.5 - and 9 months old into lithium heparin tubes fitted with caps. Disposable syringes, latex gloves and antiseptic will be provided by the project to be used by the phlebotomist. A new syringe and a pair of glove will be used for each subject. About 4 ml of the whole blood collected would be centrifuged to separate the erythrocytes from the plasma. The plasma portion will be decanted into a tube. About 0.5 ml of the 1 ml whole blood will be put into separate tubes for reticulocyte counts. All the tubes will be placed in tube holders, which will be wrapped in aluminium foil and stored at - 40°C. The frozen samples would then be transported on dry ice to Noguchi Memorial Institute of Medical Research for biochemical analyses. Blood samples will be collated and transported fortnightly. The remaining 0.5 ml of whole blood will be used for haemoglobin and malaria parasites film counts.

Biochemical tests to be carried out on the blood samples by the Biochemistry Unit of the Noguchi Memorial Research Institute, Ghana, are as follows: plasma concentrations of ferritin, iron, total iron-binding capacity, transferring saturation, zinc, retinol-binding protein, C-reactive protein levels; erythrocyte

riboflavin on the erythrocyte; haemoglobin, malaria screening and reticulocyte count on whole blood.

The data obtained will be entered on the researcher's computer with a backup on an external disk that would be kept by the researcher during the field study. Weekly submission of information via email to Associate Professor Jane Coad (main supervisor of project) for back up would be done by the researcher. Photocopies of the signed consent form will be kept by the researcher during the field study, but the original would be sent to Associate Professor Jane Coad.

### ***Data handling and analysis***

The data and the consent forms will be stored in a locked filing cabinet in a locked office. The electronic data will be stored on computers and server, which will be protected with password. Data will be transferred to an official secure archive (Crown Records Management) after 5 years and destroyed after the 10 year period. Associate Professor Jane Coad will be responsible for this. Data collected would be entered directly into a data sheet that will be prepared in PASW Statistics 18, Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, USA). Data entry will be cross-checked by the researcher and the supervisors prior to data analysis. Descriptive statistics and One-Way Analysis of Mean with covariates will be performed. When needed, logarithmic transformation will be done to transformed data to achieve normality. Sample t-test will also be carried to compare Nestlé® Cerelac® and roller-dried ComFa, as industrial-level formulations; Same analysis will be used to compared oven-toasted ComFa and enriched Weanimix as household-level products. A paired-sample t-test will also be used to assess the effect of consumption of each of the formulations using the data biochemical and anthropometric measurements on collected at baseline and at the end of study.

### ***Funding and ethics***

Funding applications submitted to funding agencies including Thrasher Research Fund, Bill & Melinda Gates Foundation and Global Alliance for Improved Nutrition were not successful. At the time of the application, data on the compositional, functional and sensory presented in this thesis was not available to support the applications. This could partly have accounted for the rejection of request for funding to conduct the feeding trial. Responses from these organisations were encouraging with regards to the concept of developing an alternative complementary food based on sweetpotato.

Ethical approval was obtained from the Massey University Human Ethics Committee: Southern A, Application 09/58 (**Appendix 7**).

## Discussion

It is well established that the levels of phytate in cereals and legumes is associated with the occurrence of micronutrients such iron and zinc deficiencies [8, 9]. The study by Troesch [20], in which low level of iron was used and resulted in reduction of iron deficiency, could be partly be associated with the phytase that was added to the micronutrient powder. As commented elsewhere, Troesch cautioned that the product is expensive and presents ethical issues because the phytase used was extracted from genetically modified *Aspergillus niger* [16]. Because the phytate levels in the sweetpotato-based formulations are naturally low, there is no need to add phytase as suggested above under the formulation section. Coupled with its low phytate levels, it is naturally high level of  $\beta$ -carotene [18, 24].

As explicitly stated by Tanumihardjo [25], “Promoting provitamin A sources alongside of supplementation and fortification efforts is safe and desirable to achieve the goal of eradicating hidden hunger among those at risk for vitamin A deficiency.” Although the household-level ComFa formulation cannot meet all the required micronutrients (vitamin A, iron and zinc) from complementary food, it would positively contribute, particularly, to vitamin A supplementation initiatives in Ghana to reduce vitamin A deficiency when consumed regularly. This hypothesis will be tested in this proposed study.

Although, there is increasing evidence that orange- and red-fleshed varieties of sweetpotato could reduce the occurrence of vitamin A deficiency among children [26-29], it is incomprehensible why a food-based approach, such as complementary food processed from sweetpotato, has not been vigorously investigated by researchers. The ComFa formulation has the potential to improve the nutritional status of infants in low-income countries when used as complementary food, but this could only be evaluated in a randomised feeding trial as described in this protocol. This feeding will be the first to consider the effect of consuming sweetpotato-based infant formulation on the nutritional status of infants.

If the sweetpotato-based formulation is successful in improving the vitamin A or iron status of infants in the study, recommendation will be made to

organisations focussed on improving the nutritional status of infants, particularly in low-income countries, to promote its use instead of cereal-based formulation for complementary feeding.

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## **Appendix 2: Information sheet**

### **Introduction**

I am Francis Kweku Amagloh, a Ghanaian, a lecturer at the University for Development Studies, Tamale, Ghana. This study is collaborative study with the Institute of Food, Nutrition and Human Health, Massey University, New Zealand. The principal investigator at the Institute is Associate Professor Jane Coad.

### **Project Description**

This project is aimed at minimising malnutrition among infants (aged 6 to 9 months) by using baby food developed from sweetpotato and soyabean. We have developed this baby food because many of the cereals and legumes we use in Ghana for preparing food for infants contain substances that reduce the absorption of micronutrients, for example, iron and zinc, which are very important for brain development and growth in children. The recent Ghana Demography Health Survey conducted in 2008 revealed that in the Upper East Region, where Kassena Nankana District is located, the prevalence of anaemia was about 89%. This means that 9 out of 10 children below 5 years old in this region may not reach their full potential during adulthood if they survived the detrimental effects of nutritional deficiencies during childhood.

In order to minimise the prevalence of anaemia and overall malnutrition in Ghana, we developed baby food based on cream-fleshed sweetpotato variety and soyabean together with added minerals and vitamins or smoked-dried fish powder. We have also tried to remove the substances which block absorption of nutrients. This research study will investigate if iron and zinc absorption increase when this baby food is eaten. In addition, the type of sweetpotato we have used is rich in carotenes which improve the vitamin A levels in humans.

Four baby foods will be used in the study, two were manufactured by us under hygienic conditions in the Food Pilot Plant of the Institute of Food, Nutrition and Human Health in Palmerston North, New Zealand; the other two are Nestle Cerelac and cereal-soy mix baby foods purchased from the Ghanaian market.

We therefore invite you to participate in this study.

### **Participant Identification, Recruitment, and Project Procedures**

Two hundred and sixty (260) babies, who are breastfeeding and would continue with partial breastfeeding, would be randomly selected for the study. They would be assigned to 4 different groups to receive intervention food for free. We will visit you every week and bring food for your baby to eat every day. When we come, we will ask questions of your baby's health and eating habits. Each group will receive only one type of intervention food during the period of the study. All the babies will be allowed to eat other foods, but mothers would be encouraged to give them the intervention food. Mothers would be requested to go to War Memorial Hospital between 8:00 am and 11:00 am for 5 ml of venous blood from the side within the fold of the elbow of participant to be taken by a phlebotomist at War Memorial Hospital, Navrongo from your child when your baby is 6-, 7 and half, and 9-months old. About 5.0 g stool samples will be requested each time you are asked to come to the hospital. Plastic sample containers will be provided for this purpose. We will pay for your travel to the hospital. Length (using infantometer- a calibrated mat on which your child will lie with his/her back) measurements would be done at home when the child is 6-, 7-, 8-, and 9-months by the researcher. The weight of your baby will be measured using a weighing scale each week when we bring the food for your baby. The study period would begin when the child is 6 months old and end when your baby is 9 months old. Parents' weight and height may be taken once during the study period at their convenience. During the study, some activities may be filmed and pictures taken for the purpose of academic presentation. You should continue to breastfeed your child during the study period. World Health Organization recommends that all infants should receive breastmilk until they are at least 12 months old. Infants who become sick will be referred to the local doctor on the project (Dr. John E. Williams) for treatment.

### **Data Management**

Yours baby's weight, length and haemoglobin level will be given to you each time measurements are done. Your baby's data will be coded, ensuring the

confidentiality of participants. No material which could personally identify your family would be used in any reports on this study. When the study is completed, all material will be destroyed. We anticipate that the unspecific results will be part of articles that will be submitted to international journals for publication.

### **Participant's Rights**

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Not answer any particular question;
- Withdraw from the study (any time you think the health of your child is taken for granted or being compromised);
- Ask any questions about the study at any time during participation;
- Provide information with the understanding it would not be used to identify the family by any other person apart from the researcher; and
- Be given access to a summary of the project findings when it is concluded upon request.

### **Project Contacts**

Below are the contact details for the main supervisor and researcher. You can contact the main supervisor without the consent of the researcher for clarification or complaints.

#### **Main supervisor**

Associate Professor Jane Coad  
Institute of Food, Health & Human Health  
Riddet Building (PN452)  
Massey University, Private Bag 11 222, Palmerston North, 4442 New Zealand  
Phone: +0064 – 6 – 350 – 5962 Ext 81465  
Fax: +0064 – 6 – 350 – 5446  
Email: [j.coad@massey.ac.nz](mailto:j.coad@massey.ac.nz)

#### **Researcher**

Francis Kweku Amagloh  
Ghana Contact: University for Development Studies (UDS),  
Faculty of Applied Sciences  
Navrongo Campus  
Tel No: .....  
Email: [fkamagloh@uds.edu.gh](mailto:fkamagloh@uds.edu.gh), [francisamagloh@yahoo.com](mailto:francisamagloh@yahoo.com)

### Appendix 3: Participant screening form

Thank you for your interest in our research.

To ensure that you fill the criteria for the study, we need you to answer the questions in the form below.

#### Participant Details

Name of your child:.....

Name you would like your child to be called by:.....

Mother's name:.....

Father's name:.....

Suburb:.....

Nearest landmark:.....

Name I could use to identify your house:.....

Phone number(s):.....

Which is the best way to contact you during the day?.....

#### Infant's Birth Details

Was the birth singleton? Yes [ ] No [ ]

Was your child delivered at hospital/health centre? Yes [ ] No [ ]

Was your child assigned a Child Health Card? Yes [ ] No [ ]

Was child's birth weight recorded in the card? Yes [ ] No [ ]

Gestational age (in weeks) .....

#### Infant's Medical History

Has your child been diagnosed as having any of the following?

- Blood borne disease Yes [ ] No [ ]
- Any congenital abnormalities Yes [ ] No [ ]

Is your child scheduled for any operation within 1 year from now?

Yes [ ] No [ ]

Are you planning to leave where you are staying within the next 6 months?

Yes [ ] No [ ]

If yes, where would you be moving to?.....

***Thank you for your interest, we will be contacting you soon.***

#### Appendix 4: Participant consent form

**This consent form would be held for a period of five (5) years**

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I understand that the information about my family will be used for academic purposes only and that data about my family will be treated in such a way that my family will not be identified.

I agree to participate in this study and to do the following:

1. To prepare and feed my child with the study meal provided, and to breastfeed as he/she demands.
2. To allow the researcher into my home to measure my baby's weight each week.
3. To allow the researcher into my home to measure my baby's length when he/she is 6-, 7-, 8-, and 9-months old.
4. To attend War Memorial Hospital, Navrongo, around 10:00 am when my child is 6-, 7.5-, and 9-months of age for 5 ml blood samples to be taken from the side within the fold of the elbow of my child at each time by a phlebotomist as will be requested by the researcher.
5. To send my baby to see a doctor suggested by the researcher in Navrongo if he/she becomes sick and to share all health information of my child with the researcher.

In addition,

6. I request that the data of my child should be given to me at the end of the study. Yes [ ☐ ] No [ ☐ ]

7. I give permission for photographs or filming of my family during the study so it can be used for presentations about the study results.

Yes [ ☐ ] No [ ☐ ]

Signature/Thumbprint:.....

Date:.....

Full Name

(PRINTED):.....

## Appendix 5: Questionnaire (baseline & weekly) for dietary and health information for infant feeding trial

### **Baseline questionnaire**

| <p><b>Q1. Basic information</b></p> <p>Name of researcher:.....</p> <p>Name of infant:.....</p> <p>Name of father:.....</p> <p>Name of mother:.....</p> <p>Interview date:                     /.../.../.../</p> <p style="text-align: center;">/D/M/Y/</p> <hr/> <p><b>Q2. Infant birth details</b></p> <p>(i) Date of birth:                     /.../.../.../</p> <p style="text-align: center;">/D/M/Y/</p> <p>(ii) Birth weight:                   [    ].[    ] kg</p> <p>(iii) Length:                        [    ].[    ] cm</p> <p>(iv) Mode of birth delivery:</p> <p style="padding-left: 40px;">Spontaneous vaginal delivery [    ]</p> <p style="padding-left: 40px;">Induced vaginal delivery     [    ]</p> <p style="padding-left: 40px;">Caesarean birth                 [    ]</p> <p style="padding-left: 40px;">(Q 2i – iv) confirm from health card</p> <p>(v) Age(<b>completed months</b>)(calculate):   [    ]</p> <p>(vi) How many other children have you given birth to?   [    ]</p> <p style="padding-left: 40px;">If none, skip to (Q3)</p> <p>(vii) How old is the child born before study infant (<b>completed months</b>)?   [    ]</p> | <p><b>Q3. Postnatal medical information</b></p> <p>(i) Had your child had any of the following illness after birth?</p> <p style="text-align: right; padding-right: 20px;">Malaria [    ]</p> <p style="text-align: right; padding-right: 20px;">Shade choice(s)     Fever [    ]</p> <p style="text-align: right; padding-right: 20px;">Cough [    ]</p> <p style="text-align: right; padding-right: 20px;">Diarrhoea[    ]</p> <p style="text-align: right; padding-right: 20px;">Other, state:.....</p> <p style="text-align: center;">If none, skip to Q(v) below</p> <p>(ii) Did you send him/her to the hospital?</p> <p style="padding-left: 40px;">Yes [    ] No [    ] If No, skip to Q(v) below</p> <p>(iii) Was he/she admitted for any of the illnesses indicated above for more than 1 day in hospital?</p> <p style="text-align: right; padding-right: 20px;">Shade choice        Yes [    ]</p> <p style="text-align: right; padding-right: 20px;">No [    ]</p> <p>(iv) For which of those indicated above was he/she admitted? .....</p> <p>(v) How many times have you attended postnatal clinic (weighing) with baby? [    ]</p> <p>(vi) Request weighing card and record</p> <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 10px;"> <thead> <tr> <th style="width: 25%;">Date</th> <th style="width: 25%;">Wt(kg)</th> <th style="width: 50%;">Treatment</th> </tr> </thead> <tbody> <tr> <td style="height: 100px;"></td> <td></td> <td></td> </tr> </tbody> </table> | Date      | Wt(kg) | Treatment |  |  |  | <p><b>Q4. Infant dietary information</b></p> <p>(i) How old (in months) was your child when you introduced him/her to water? [    ]</p> <p>(ii) How old (in months) was your child when you introduced him/her to drinks other than water?   [    ]</p> <p style="padding-left: 40px;">Ask for the name of the drink(s)</p> <p>.....</p> <p>(iii) How old (in months) was your child when you introduced him/her to infant formula?   [    ]</p> <p style="padding-left: 40px;">Ask for the name of the formula</p> <p>.....</p> <p>(iv) How old (in months) was your child when you introduced him/her to solid food (example, koko, Tom Brown, etc)</p> <p style="padding-left: 40px;">Ask for description or the package containing the solid food</p> <p>.....</p> <p>(iv) During yesterday, how many times have you given him/her :</p> <p style="text-align: right; padding-right: 20px;">Water:   [    ]</p> <p style="text-align: right; padding-right: 20px;">Infant formula:                               [    ]</p> <p style="text-align: right; padding-right: 20px;">Solid food, state:.....                     [    ]</p> <p style="text-align: right; padding-right: 20px;">Breastmilk during the day:               [    ]</p> <p style="text-align: right; padding-right: 20px;">Breastmilk during the night:             [    ]</p> <p style="text-align: right; padding-right: 20px;">Vitamin or mineral supplement [    ]</p> <p style="padding-left: 40px;">Name of supplement:.....</p> |
|--|--|-----------|--------|-----------|--|--|--|---|
| Date   | Wt(kg)   | Treatment |        |           |  |  |  |   |
|  |  |           |        |           |  |  |  |   |

| Q5. Anthropometric data   |  |  |   |
|---|--|--|---|
| Person  | Weight(kg)                                 | Height (cm)  | Results(codes)<br>1. Measured<br>2. Not present<br>3. Refused |
| (i) Father  | [ ][ ][ ].[ ]<br>[ ][ ][ ].[ ]             | [ ][ ][ ][ ].[ ]<br>[ ][ ][ ][ ][ ].[ ]                            | [ ]   |
| (ii) Mother   | [ ][ ][ ][ ].[ ]<br>[ ][ ][ ][ ][ ].[ ]    | [ ][ ][ ][ ][ ][ ].[ ]<br>[ ][ ][ ][ ][ ][ ][ ].[ ]                | [ ]   |
| (iii) Child   | [ ][ ][ ][ ].[ ]<br>[ ][ ][ ][ ][ ][ ].[ ] | [ ][ ][ ][ ][ ][ ][ ][ ].[ ]<br>[ ][ ][ ][ ][ ][ ][ ][ ][ ][ ].[ ] | [ ]<br>Oedema Y[ ] N[ ]                                       |
| <b>Q6.</b> Have you given any vitamin supplement to your child after birth? Yes [ ]<br>No [ ] <b>Yes to Q6,</b><br>ask for name or the bottle and record the name:.....<br>How often do you give him/her per day? .....<br>Ask for the amount given per day (confirm with dosage on bottle) .....<br><b>Q7.</b> Does household have the following? <b>Observe, if not available, enquire.</b><br><div style="display: flex; justify-content: space-between;"> <div> <b>Shade choice(s)</b><br/> Electricity [ ]<br/> A refrigerator [ ]<br/> A mobile phone [ ]<br/> A television set [ ]<br/> A radio/tape/CD player [ ]<br/> A video deck/DVD/CD player [ ]<br/> Car/truck [ ]<br/> Motorbike [ ]<br/> Donkey plus cart [ ] </div> <div> <b>Q8.</b> Main material of the floor for room. <b>Observe, if not sure, enquire.</b><br/> Natural flour (earth/sand/mud) [ ]<br/> Mud mixed with dung [ ]<br/> <b>Shade choice</b> Finished flour (carpet, tiles, terrazzo, cemented) [ ] </div> </div> |  |  |   |
| <b>Q9.</b> What type(s) of fuel do you use for cooking?<br><b>Shade choice(s)</b><br>Electricity [ ]<br>Gas(LPG) [ ]<br>Kerosene [ ]<br>Charcoal [ ]<br>Firewood/Straw [ ]<br>Dung [ ]  |  |  |   |
| <b>Q10.</b> Source of drinking water at home.<br>Piped water [ ]<br>Open well [ ]<br>Covered well or borehole [ ]<br>Surface water (stream, river) [ ]<br>Bottled water [ ]<br>Satchel water [ ]  |  |  |   |
| <b>Q11.</b> Father's educational level.<br>No formal education [ ]<br>Up to Junior High School [ ]<br>Up to Senior High School [ ]<br>Above Senior High School [ ]  |  |  |   |
| <b>Q12.</b> Mother's educational level.<br>No formal education [ ]<br>Up to Junior High School [ ]<br>Up to Senior High School [ ]<br>Above Senior High School [ ]  |  |  |   |
| <b>Q13.</b> Father's average net income in Ghana Cedis per month.<br><50 [ ]<br>50-100 [ ]<br>101-200 [ ]<br>>200 [ ]   |  |  |   |
| <b>Q14.</b> Mother's average net income in Ghana Cedis per month.<br><50 [ ]<br>50-100 [ ]<br>101-200 [ ]<br>>200 [ ]   |  |  |   |



Weekly questionnaire

|   |            |                   |
|---|------------|-------------------|
| <b>Q1. Morbidity</b>  |            |                   |
| <b>(i)</b> In the last week, has your child had any illness? <i>Tick choice</i> |            |                   |
| <b>Illness</b>  | <b>Yes</b> | <b>No</b>         |
| (a) Malaria   |            |                   |
| (b) Fever   |            |                   |
| (c) Cough   |            |                   |
| (d) Diarrhoea   |            |                   |
| (e) Other, state:.....  |            |                   |
| If No to all above, skip to Q2  |            |                   |
| <b>(ii)</b> Did you take him/her to the hospital because of the illness?        |            |                   |
|   | Yes [    ] |                   |
|   | No [    ]  |                   |
| <b>(iii)</b> Was he/she admitted for the illness for more than 1 day?           |            |                   |
|   | Yes [    ] |                   |
|   | No [    ]  |                   |
| <b>Q2. (i)</b> Is your child taking any vitamin supplement?                     |            |                   |
|   | Yes [    ] |                   |
|   | No [    ]  | <b>Skip to Q3</b> |
| <b>(ii)</b> After my last visit, when did you start giving it to him/her?       |            |                   |
| .....   |            |                   |
| <b>(iii)</b> How many times did you give it to him/her yesterday? [    ]        |            |                   |
| <b>(iv)</b> Ask for the amount given (confirm with dosage on bottle)            |            |                   |
| .....   |            |                   |
| <b>(v)</b> Who advised you to give the supplement?                              |            |                   |
| .....   |            |                   |

|   |
|---|
| <b>Q3. (i)</b> After my last visit, did you attend postnatal clinic?  |
| Yes [    ]  |
| No [    ] <b>Skip to Q4</b>   |
| <b>(ii)</b> Request weighing card and record  |
| (a) Date :..... (b) Weight (kg): [    ] (c) Treatment:.....   |
| <b>Q4. Dietary information</b>  |
| Probe to find if yesterday was a typical day the child ate well; if not, take a typical day suggested by mother during the week to represent yesterday. |
| (i) During yesterday, were you with your baby all the time during the day?  |
| Yes [    ] No [    ]  |
| (ii) During yesterday before you slept in the night, how many times did you breastfeed your baby?   |
| [    ] times  |
| (iii) Do you sleep on the same bed with your baby during the night?   |
| Yes [    ] No [    ]  |
| (iv) For yesterday, how many times did you give your child the study food?  |
| [    ] times  |
| Estimated quantity consumed: ..... g  |
| (v) During the night, how many times did you give him/her the intervention food?  |
| [    ] times  |
| (vi) For yesterday, how many times did you give your child other solid food(s) during the day and night?  |
| [    ] times  |
| Probe and record the type of solid food:.....   |
| Estimated quantity consumed: .....g   |

| Dietary information Contd.   | Notes  |
|--|--|
| <p>(vii) During the last week, has your child refused to take the project food you prepared for him/her?</p> <p>Yes [ ]<br/>No [ ] <b>Skip to ix</b></p> <p>(viii) How many times during the week did he/she refuse to eat?</p> <p>.....</p> <p>(ix) Apart from water, did you add other things to the project food you prepared for your baby?</p> <p>Yes [ ]<br/>No [ ] <b>Skip to Q5</b></p> <p>(x) What did you add? .....</p> <p>(xi) Why did you add it/them? .....</p> <p>.....</p> <p>.....</p> <p><b>Q5. Anthropometric data</b></p> <p><u>Weekly basis</u></p> <p>Weight (kg): [ ] [ ] . [ ]<br/>[ ] [ ] . [ ]</p> <p>Oedema: Yes [ ] No [ ]</p> <p><u>Include only at monthly visit</u></p> <p>Length (cm): [ ] [ ] . [ ]<br/>[ ] [ ] . [ ]</p> | <p>Please request for the package containing the food supplied earlier for weighing.</p> <p>Booked next appointment day:.....</p> <p>Inform mother of hospital if it is due. Remind her to take stool sample in the container provided.</p> <p>General comments:</p> |

**Appendix 6: Questionnaire for consumer preference test**

**WELCOME TO OUR BABY FOOD RESEARCH PROJECT**

**Subject Code (Number on the card given to you): .....**

**Introduction**

I am Francis, a postgraduate student in Massey, working on baby food formulation from sweetpotato and soybean as an alternative to maize-soybean-groundnut blend for infants in Ghana. I have used household-level and industrial-level processing methods to develop such complementary food which is now ready for tasting to assess its acceptability as baby food. The food you will taste may contain one or more of the following ingredients: maize, groundnut/peanut, soyabean, fish (anchovies) and sweetpotato (kumara).

*All information provided will be used for academic purpose only.*

**Consent**

I agree to participate in this study based on the information given.

Name:.....

Signature:.....

Date:.....

**Instruction:**

**You will taste three different baby food samples and evaluate each of them one after the other.**

1. Please rinse your mouth with water provided before starting and rinse again before tasting any different sample.
2. Place the sample in your mouth and rate how you like it.
3. Please evaluate one after the other, starting with the one nearest to you.

**If at any time you have a question about the test or instructions, please feel free to ask us.**

Kindly complete the following questions:

1. Country of origin: .....

Questionnaire for sensory evaluation

2. Age: .....

3. Have you had experience feeding a child?

Yes [    ]      No [    ]

4. Age of your youngest child?

..... Months **or** ..... Years      No child [    ]

5. Do you have sweetpotato (kumara) in your home country?

Yes [    ]      No [    ]

Product Code: .....

1. Please indicate by placing a mark in the box your **overall acceptability** of this product?

|                          |                          |                          |                          |                          |                          |                          |                          |                          |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| <i>Least Acceptable</i>  |                          |                          | <i>Neutral</i>           |                          |                          | <i>Highly Acceptable</i> |                          |                          |

2. Please indicate by placing a mark in the box how you **like** the **colour** of this product?

|                          |                          |                          |                          |                          |                          |                          |                          |                          |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| <i>Dislike extremely</i> |                          |                          | <i>Neutral</i>           |                          |                          | <i>Like extremely</i>    |                          |                          |

3. Please indicate by placing a mark in the box how you **like** the **smell** of this product?

|                          |                          |                          |                          |                          |                          |                          |                          |                          |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| <i>Dislike extremely</i> |                          |                          | <i>Neutral</i>           |                          |                          | <i>Like extremely</i>    |                          |                          |

4. Please indicate by placing a mark in the box how you **like** the product's **texture** in your mouth?

|                          |                          |                          |                          |                          |                          |                          |                          |                          |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| <i>Dislike extremely</i> |                          |                          | <i>Neutral</i>           |                          |                          | <i>Like extremely</i>    |                          |                          |

5. Please indicate by placing a mark in the box how you **like** the **taste** of this product?

|                          |                          |                          |                          |                          |                          |                          |                          |                          |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| <i>Dislike extremely</i> |                          |                          | <i>Neutral</i>           |                          |                          | <i>Like extremely</i>    |                          |                          |

6. Please indicate by placing a mark in the box your **evaluation** on the **sweetness** of this product?

|                          |                          |                          |                          |                          |                          |                          |                          |                          |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| <i>Too low</i>           |                          |                          | <i>Just right</i>        |                          |                          | <i>Too sweet</i>         |                          |                          |

## Questionnaire for sensory evaluation

7. Please indicate by placing a mark in the box your **evaluation** on the **saltiness** of this product?

☐☐☐☐☐☐☐☐☐

*Too low*

*Just right*

*Too salty*

8. Please indicate by placing a mark in the box if you would **give** this product to a baby?

☐☐☐☐☐☐☐☐☐

*Not likely*

*Neutral*

*Very likely*

9. Overall comment(s) of this product as baby food?

.....

.....

.....

*Thank you very much for participating.*

**Appendix 7: Approval of human ethics application (Southern A Application-09/58)**



**Massey University**

2 December 2009

Francis Amagloh  
IFNHH  
PN452

OFFICE OF THE ASSISTANT  
TO THE VICE-CHANCELLOR  
(RESEARCH ETHICS)  
Private Bag 11 222  
Palmerston North 4442  
New Zealand  
T 64 6 350 5573  
64 6 350 5575  
F 64 6 350 5622  
humanethics@massey.ac.nz  
animalethics@massey.ac.nz  
gtc@massey.ac.nz  
www.massey.ac.nz

Dear Francis

**Re: HEC: Southern A Application – 09/58**  
**Development of a complementary food from sweet potato and soybean to improve the nutritional status of infants (aged 6-9 months) in Kassena Nankana District, Ghana**

Thank you for your letter dated 2 December 2009.

On behalf of the Massey University Human Ethics Committee: Southern A, I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

Please note that travel undertaken by students must be approved by the supervisor and the relevant Pro Vice-Chancellor and be in accordance with the Policy and Procedures for Course-Related Student Travel Overseas. In addition, the supervisor must advise the University's Insurance Officer.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

A handwritten signature in black ink, appearing to read 'J. Boddy'.

Professor Julie Boddy, Chair  
**Massey University Human Ethics Committee: Southern A**

cc A/Prof Jane Coad  
IFNHH  
PN452

Prof Richard Archer, HoI  
IFNHH  
PN452

---

Massey University Human Ethics Committee  
Accredited by the Health Research Council



To Kūmanga  
ki Pūrehuroa

**Appendix 8:** Statement of contribution to doctoral thesis containing publications





**MASSEY UNIVERSITY**  
GRADUATE RESEARCH SCHOOL

**STATEMENT OF CONTRIBUTION  
TO DOCTORAL THESIS CONTAINING PUBLICATIONS**

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: **Francis Kweku Amagloh**

Name/Title of Principal Supervisor: **Associate Professor Jane Coad**

Name of Published Research Output and full reference:

Amagloh, F. K., Hardacre, A., Weber, J. L., Brough, L., Mutukumira, A. N., & Coad, J. (2010, 6-8 December 2010). Development of sweet potato-soybean blend, an alternative to maize-legume mix as complementary food for infants in Ghana. Paper presented at the "Integrated nutrition: From cells to sales", Wellington, New Zealand.

In which Chapter is the Published Work: **Chapter 1 (Paper I)**

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: **70%**  
and / or

- Describe the contribution that the candidate has made to the Published Work:

Francis Kweku Amagloh designed the research, collected and analysed the data, and orally presented it the 45th Conference of the Nutrition Society of New Zealand (Inc.) held in Wellington, 2010. Afterwards, Francis wrote the first draft for publication as conference proceedings.

Francis Kweku Amagloh

Candidate's Signature

**June 08, 2012**

Date

Jane Coad

Principal Supervisor's signature

**June 08, 2012**

Date



**MASSEY UNIVERSITY**  
GRADUATE RESEARCH SCHOOL

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Name of Candidate: **Francis Kweku Amagloh**

Name/Title of Principal Supervisor: **Associate Professor Jane Coad**

Name of Published Research Output and full reference:

Amagloh, F. K., Weber, J. L., Brough, L., Hardacre, A., Mutukumira, A. N., & Coad, J. (2012). Complementary food blends and malnutrition among infants in Ghana—A review and a proposed solution. *Scientific Research and Essays*, 7(9), 972-988. doi: 10.5897/SRE11.1362

In which Chapter is the Published Work: **Chapter 2 (Paper II)**

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: **70%**  
and / or

- Describe the contribution that the candidate has made to the Published Work:

Francis Kweku Amagloh searched for the articles and other relevant publications used in this review, and wrote the first draft.

**Francis Kweku  
Amagloh**

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**June 08, 2012**

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**MASSEY UNIVERSITY**  
GRADUATE RESEARCH SCHOOL

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Name of Candidate: **Francis Kweku Amagloh**

Name/Title of Principal Supervisor: **Associate Professor Jane Coad**

Name of Published Research Output and full reference:

Amagloh, F. K., Hardacre, A., Mutukumira, A. N., Weber, J. L., Brough, L., & Coad, J. (2012). Sweet potato-based complementary food for infants in low-income countries. *Food and Nutrition Bulletin*, 33(1), 3-10.

In which Chapter is the Published Work: **Chapter 3 (Paper III)**

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: **70%**  
and / or

- Describe the contribution that the candidate has made to the Published Work:

Francis Kweku Amagloh designed the research, collected and analysed data, and wrote the first draft.

Francis Kweku  
Amagloh

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**MASSEY UNIVERSITY**  
GRADUATE RESEARCH SCHOOL

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Name of Candidate: **Francis Kweku Amagloh**

Name/Title of Principal Supervisor: **Associate Professor Jane Coad**

Name of Published Research Output and full reference:

Amagloh, F. K., Hardacre, A., Mutukumira, A. N., Weber, J. L., Brough, L., & Coad, J. (2011). A household-level sweet potato-based infant food to complement vitamin A supplementation initiatives. *Maternal and Child Nutrition*, Epub ahead of print. doi: 10.1111/j.1740-8709.2011.00343.x

In which Chapter is the Published Work: **Chapter 4 (Paper IV)**

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: **70%**  
and / or

- Describe the contribution that the candidate has made to the Published Work:

Francis Kweku Amagloh designed the research, collected and analysed data, and wrote the first draft.

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Amagloh

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Jane Coad

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Principal Supervisor's signature

**June 08, 2012**

Date





**MASSEY UNIVERSITY**  
GRADUATE RESEARCH SCHOOL

**STATEMENT OF CONTRIBUTION  
TO DOCTORAL THESIS CONTAINING PUBLICATIONS**

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We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: **Francis Kweku Amagloh**

Name/Title of Principal Supervisor: **Associate Professor Jane Coad**

Name of Published Research Output and full reference:

Amagloh, F. K., Brough, L., Weber, J. L., Mutukumira, A. N., Hardacre, A., & Coad, J. (2012). Sweetpotato-based complementary food would be less inhibitory on mineral absorption than a maize-based infant food assessed by compositional analysis. *International Journal of Food Sciences and Nutrition*, Epub ahead of print. doi: 10.3109/09637486.2012.687368

In which Chapter is the Published Work: **Chapter 5 (Paper VI)**

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: **70%**  
and / or

- Describe the contribution that the candidate has made to the Published Work:

Francis Kweku Amagloh designed the research, collected and analysed data, and wrote the first draft.

**Francis Kweku  
Amagloh**

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Principal Supervisor's signature

**June 08, 2012**

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