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Development of the Process for a Drinkable Plant-Based Infant Food

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Abstract

Provision of nutritionally adequate foods is crucial for the prevention and management of malnutrition. In order to meet the nutrient requirements of infants and young children using locally available cereals and legumes, foods need to be of low viscosity, of high caloric content and nutrient density and easily digestible. This study developed a processing protocol for three plant food formulations to yield nutrient dense, easily digestible and safe therapeutic foods for malnourished children. Pre-digestion of the milled ingredients at 40°C for 4 hours with grain amaranth that was malted for 48 hours and 1% Bromelain achieved 70-84% starch hydrolysis and 55-61% protein hydrolysis. Sterilization of the therapeutic food in glass jars in an autoclave at 121°C, 15 PSI for 20 minutesgave non- detectable total plate and yeast and mold counts. The final products had viscosities of 2745-2913 Cps. These results show that pre-digestion can improve the nutrition quality of infant foods made from plant sources.

Keywords: Amaranth; Malt; Hydrolysis; Bromelain; Legumes; Therapeutic food

Introduction

Malnutrition is a direct cause of 40% of deaths among children under five years old in Uganda. Over two-thirds of these deaths are associated with inappropriate feeding practices during the first year of life [1]. Populations with a high prevalence of malnutrition tend to have diets that are typically bulky, of low nutrient density, and have low bioavailability of nutrients [2].

Plant foods provide over 65% of the world protein for humans with 45 to 50% of it from cereals and legumes [3]. In Uganda, children aged 6 to 23 months consume foods made from grains more often than any other food group [1]. Legumes are not only one of the cheapest and richest sources of dietary protein, but they also play an important role in the traditional diets [4]. Despite their moderately high content of proteins, calories, minerals and vitamins, the use of legumes is limited by long cooking time, low protein digestibility, low levels of sulphur-containing amino acids and presence of several anti-nutritional factors [5,6]. Different processing methods and traditional treatments such as de-hulling, soaking, germination, fermentation and cooking or a combination of these, have been used to reduce the anti-nutritional factors, thus improving the nutritional and cooking quality of legumes to various extents [7].

A number of traditional complementary foods in Africa are only a slight modification of adult foods, involving only mashing and dilution without taking into consideration the special nutritional requirements of young children [8]. Cereal porridges are usually diluted to attain a suitable consistency for feeding children under than 3 years [9]. A number of treatments such as use of industrial amylase treatment, pre-cooking, extrusion and malting, have successfully been used to decrease the viscosity of weaning foods thus reducing their bulk and increasing the energy density [10]. Malting increases the amylase activity and amount of free nitrogen which improves the digestibility and quality of starch and proteins in the grains, reduces viscosity of the food, and increases the energy and nutrient densities making malted foods suitable for infants [11,12].

Grain amaranth (*Amaranthus caudatus* L.) has 12.5 to 17.6% crude protein content, 5 to 17%, lipid content, with 50% linoleic acid and a variety of minerals, such as calcium, sodium, iron, magnesium, potassium and vitamins A, E, C [13-16]. With its high protein content, greater protein quality, high content of essential fatty acids and micronutrients, grain amaranth has the potential to contribute greatly to the nutritional needs of vulnerable people especially children under 5 years of age [16,17].

Computer formulation software was used to design low cost formulations of a therapeutic food meeting the nutrient composition of F100 using plant foods in a related study [18]. The purpose of this study was to develop a processing protocol for the formulations that would yield a nutrient dense, easily consumable, digestible and safe therapeutic food (TF) for malnourished children.

Methods

Ingredients and pre-processing: Three products (Table 1) were formulated using Concept-4, Least Cost Formulation computer software in a related study [18]. The peanuts, beans, sesame, and cowpeas were purchased from a market in Kampala while the golden amaranth grain was bought from farmers in Kamuli district in rural Uganda.

The peanuts and sesame were roasted in an infrared oven (GU-6 Orimas, Kuala Lumpur Malaysia) at 180°C for 20 minutes. The beans were soaked in tap water at a ratio of 1:2 (beans to water, w/v) for 12 hours at room temperature, drained and there after pressure cooked for 15 minutes. The cowpeas were soaked in tap water at a ratio of 1:2 (cowpeas to water, w/v) for 30 minutes at room temperature, dried in an infrared oven (GU-6 Orimas, Kuala Lumpur Malaysia) for 8 hours at 60°C followed by de-hulling [19]. The cowpeas were then soaked for 12 hours in tap water at a ratio of 1:2 (cowpeas to water, w/v), drained and pressure cooked for 20 minutes. All ingredients were blended together

using a blender (Black and Decker BX260, England) with water at a ratio of 1:3.5 (product to water, w/v) until a uniform consistency was achieved.

Product hydrolysis

Preparation of amaranth malt: To increase the digestibility and reduce viscosity of the TFs, grain amaranth was malted to provide amylases that would pre-digest the starch. To determine the germination time of the grain amaranths that produced the highest amylase activity: the grains were soaked for 12 hours in tap water at a ratio of 1:2 (amaranth to water, w/v) at room temperature; then rinsed in tap water three times; laid out on trays lined with wet cloth at a thickness not greater than half an inch and placed in a darkroom at 23-26°C. Samples were removed every 12 hours over 72 hours, placed in an infrared oven (GU-6 Orimas, Kuala Lumpur Malaysia) at 60°C for 4-5 hours until dry and milled into fine flours using a mill (Wonder mill grain mill, Pocatello Idaho, USA).

Amylase activity of amaranth malt: The amylase activity of the amaranth malt flour samples obtained was determined using the residual starch method [20]. 10 g of amaranth malt flour was dissolved in 100 ml of 0.1 N sodium acetate buffer (pH 5.9) and placed on ice for 40 minutes. The solution was centrifuged for 20 minutes at 4°C at 12,000 rpm. The supernatant (0.2 ml) was then added to 1 ml of starch (2 mg/ml) and incubated at 40°C for 60 minutes. At 10 minute intervals, 0.2 ml of the solution was removed and added to 5 ml of an iodine-HCl reagent, prepared by adding 1ml of stock solution (0.5% Iodine in 5% potassium iodide) and 5 ml, 5 M HCl to 500 ml distilled water. The absorbance of the reaction solution was read at 620 nm against a blank. A standard curve was obtained using 2 mg/ml starch solution and used to determine the amount of starch hydrolysed and degree of starch hydrolysis.

Hydrolysis of starch in the therapeutic food: A modification of the Dextrose Equivalents (DE) method of Dextrin Analysis D-52 was used to determine the optimum temperature for activity of the amylases in the amaranth malt [21]. After blending, the product was incubated at 40, 50 and 60°C for 4 hours. Two grams of the product was removed every hour, diluted to 100 ml with distilled water and centrifuged at 1100 rpm at 4 °C for 20 minutes. Thereafter, 2 ml of the supernatant was placed in boiling tubes to which 3 ml of water, 2 ml of Fehling's A solution and 2 ml of Fehling's B solution were added. The tubes were boiled in a 1000 ml beaker with water for 5 minutes, and rapidly cooled in an ice bath. To each tube, 2 ml of 30 % Potassium Iodide solution and 2 ml of 28% sulphuric acid were added. The reaction solution was titrated with 0.02 M sodium thiosulphate solution with continuous agitation until the solution turned white. A standard dextrose curve was prepared with 3 g of dry anhydrous dextrose. The amount of dextrose was expressed as grams of dextrose in 100 g of solids in the sample.

The amylase activity of the amaranth malt flour in the TF product was determined using the residual starch method [20]. The amaranth in the formulation (Table 1) was added to the rest of the ingredients in form of the amaranth malt flour. After pre-processing and blending, the product was incubated at 40°C for 240 minutes. Samples were removed at 30 minute intervals, centrifuged at 1200 rpm for 20 minutes and 8 ml of the resultant supernatant removed and diluted to 100 ml with distilled water. Using the diluted supernatant, 0.2 ml was added to test tubes with 5 ml of the Iodine-HCl reagent. The absorbance was read at 620 nm against a blank. The amount of starch hydrolysed and degree of hydrolysis was then calculated.

Hydrolysis of protein in the therapeutic food using commercial bromelain: Activity of the commercial bromelain (Herbal Extracts Plus, Croydon, Pennsylvania USA) in the TF was determined using modifications of the Biuret assay [22,23]. Varying amounts of commercial bromelain: 0.5, 1, 5, 10, and 15% (w/w) of the predicted protein content of the TF were added to the blended product. The product was incubated at 40°C for 4 hours. At 30 minute intervals, samples were obtained and centrifuged for 10 minutes at 1200 rpm; 10 ml of the supernatant was removed and diluted to 100 ml with distilled water. Biuret reagent (9 ml) was then added to 1 ml of the diluted supernatant. The solution was left to stand for 20 minutes and the absorbance read at 550 nm. A standard curve was prepared using BSA. The degree of hydrolysis reflected the percentage of the total number of peptide bonds in the proteins that were cleaved during hydrolysis [24].

Effect of pH and heat treatment on the microbiological quality of the TFs: The possibility of lowering the pH of the product into the low acid food range by adding acetic acid and citric acid was considered. The effect of lowering the pH of the TF on the effectiveness of the heat treatment thus on the microbiological quality of the product was investigated. The blended product with 48 hour amaranth malt flour and 1% Bromelain was hydrolysed at 40°C for 4 hours. The mixture was filled into glass jars (250 g, wb.) and heated in a pressure cooker for 20 minutes. Varying amounts of acetic acid (0-0.1% w/w) and citric acid (0-0.7% w/w) were then added to the cooled jars and the pH determined.
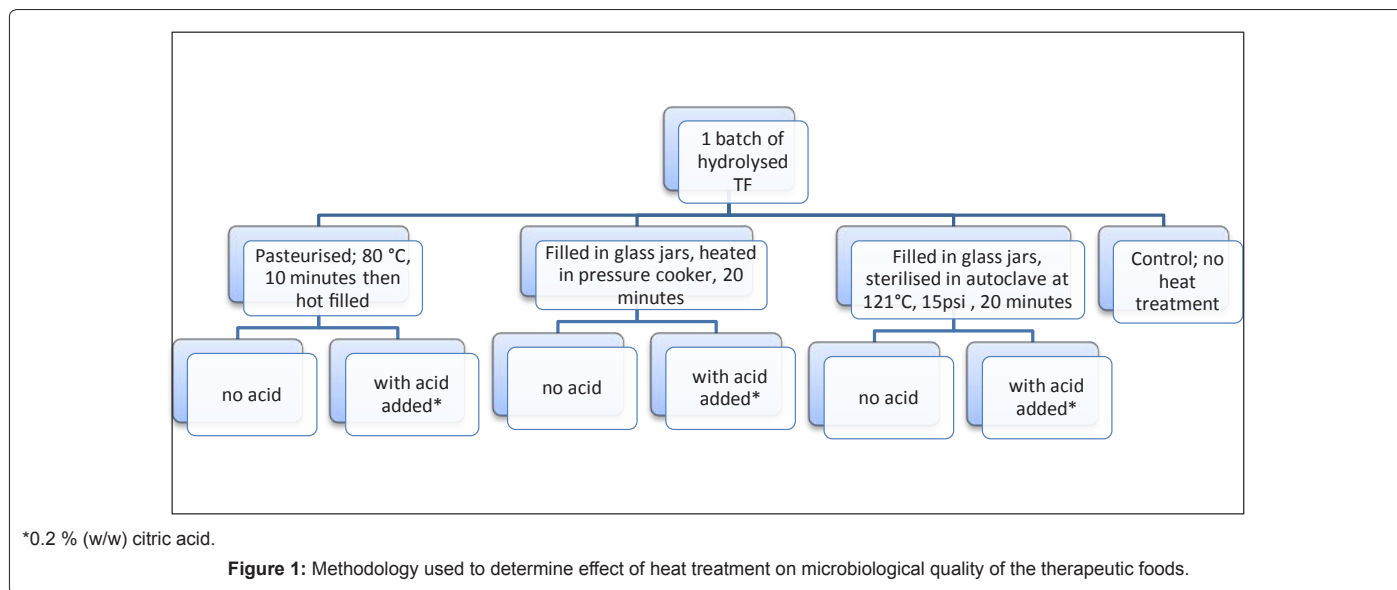
The effect of citric acid on the product was further analysed by adding 0, 0.2, 0.4, 0.6, and 0.8 % (w/w) of the acid to the cooled product in jars and the pH was determined. The acceptable level of acidity in products with varying amounts of citric acid was preliminarily evaluated by 12 untrained panellists, based on the taste, appearance and sensory acceptability.

The result of combining low pH and various heat treatments on the microbiological quality of the TF was then determined. After hydrolysis of the blended product, with 48 hour amaranth malt flour and 1% Bromelain at 40°C for 4 hours, the product was either filled in glass jars and sterilized in an autoclave, or pressure cooker, or pasteurized by heating it a saucepan followed by hot filling (Figure 1).

When all the jars were sufficiently cool, the total plate and the yeast and mould counts were determined [25]. Plate count agar and potato dextrose agar were used for total plate count and yeasts and moulds respectively. The most effective heat treatment method for the TF was determined based on the microbiological and sensory evaluation. For sensory evaluation, products sterilized in the pressure cooker and autoclave had their appearance, taste, and overall acceptability evaluated by 20 untrained panellists using a 5-point hedonic scale where 1 was dislike extremely and 5 was like extremely [26].

Change in viscosity: The TF had been hydrolysed with 48 hour amaranth malt flour and 1% Bromelain at 40°C for 4 hours, was filled in glass jars and sterilized in an autoclave at 121°C, 15 PSI for 20 minutes. The change in viscosity of the product was determined using Brookfield viscometer (LVDV-II + Pro, MA, USA) at 30 ± 2°C, 60 rpm, 50 ± 5% torque with the No. 62 spindle. The viscosity was determined before hydrolysis, after hydrolysis and after heat treatment.

All the results of each treatment were subjected to Analysis of variance. The means were also compared using Tukey's range tests using SPSS 16.0 statistical software to allow for all possible pair-wise treatment comparisons. Significance was accepted at $p<0.05$.

*0.2 % (w/w) citric acid.

Figure 1: Methodology used to determine effect of heat treatment on microbiological quality of the therapeutic foods.

Results

Activity of amaranth malt

After malting amaranth for 0 to 72 hours, the 48 hour malt hydrolysed the highest amount of starch from 2 mg/ml to 0.93 mg/ml in 15 minutes. This yielded a degree of hydrolysis of 46.35 ± 4.31%. There was however no significant difference between the amounts of residual starch, the amount of enzyme acting on 0.1 mg of starch, nor the degree of hydrolysis of the different malts ($p<0.05$). The 48 hour malt was used for hydrolysis of the TF.

After 60 minutes, the 48 hour amaranth malt was able to hydrolyse 1.422 mg of the 2 mg/ml starch solution at 40°C, achieving a 71 ± 6.48% degree of starch hydrolysis (Figure 2). There was a significant decrease in the starch concentration at the start of hydrolysis and that after 60 minutes of hydrolysis ($p<0.05$). The amylase enzyme activity of the amaranth malt was 3.532 mg of malt flour acting on 0.1 mg of starch in 60 minutes at 40°C.

When the amaranth malt was added to the rest of the TF ingredients, maximum starch hydrolysis was achieved at 40°C (Table 2). After four hours of hydrolysis, there was a significant increase in the DE of the products at 40°C, 50°C, and 60°C. There was no significant difference in the DE of the product in the first three hours of hydrolysis at any of the temperatures. However, after four hours of hydrolysis, there was a significant difference between the DE at 40°C and that at 50°C and 60°C ($p<0.05$).

After four hours of hydrolysis at 40°C, the degree of starch hydrolysis achieved in TF products A, B, and C was 70.2 ± 5.3, 83.6 ± 7.3, and 69.9 ± 5.5% respectively. At the end of hydrolysis, the change in starch concentration observed in products A, B, and C was 13.3, 24.0, and 26.3 mg of starch per ml respectively. Though a reduction in starch concentration with time was observed for all three products (Figure 3), only product A had a statistically significant reduction. There was no significant difference between the final starch concentrations of the three products ($p<0.05$).

Hydrolysis of protein in the therapeutic food

Using commercial Bromelain, the degree of protein hydrolysis obtained in the TFs at 40°C, 50°C, and 60°C was 51.1 ± 0.17, 49.6 ± 0.04, and 51.6 ± 0.06% respectively. There was no significant difference observed between the activity of commercial Bromelain on BSA obtained at the above temperatures ($p<0.05$).

When the amount of Bromelain added to the products was increased from 0.5, 1, 5, 10, and 15%, the degree of protein hydrolysis recorded after two hours was 39.4, 43.1, 30.3, 52.7, and 34.4% respectively. Increase in the amount of Bromelain added to the products did not yield significantly higher levels of hydrolysis ($p<0.05$).

When 1% Bromelain was added to each of the 3 products, a significant reduction in the BSA concentration in the products was observed after hydrolysis at 40°C for 4 hours. The degree of protein hydrolysis achieved by the Bromelain in products A, B, and C was 55.3 ± 7.5, 60.7 ± 0.9, and 60.9 ± 3.5% (Figure 4). There was no significant difference between the BSA concentrations and degree of hydrolysis of the three products ($p<0.05$).

Change in viscosity of the therapeutic food

Hydrolysis of the TF resulted in significant reduction in viscosity of 28.6, 25.6, and 21.6% for products A, B, and C at 60 rpm and 30 ± 2°C (Table 3). After heat treatment of the hydrolysed products, there was an insignificant increase in the viscosities of the three products.

Adjustment of pH of the therapeutic food

The effects of citric and acetic acid on the processed TF were compared and citric acid lowered the pH more effectively than acetic acid. The pH of the product was lowered by 0.7% (w/w) citric acid 6.20 to 4.27 by, while 0.097% (w/w) acetic acid lowered the pH of the product from 6.03 to 5.04. Of the 8 untrained panellists that screened the taste and appearance of the products, all accepted the products with no acid and rejected products with the lowest pH based on the taste and appearance. Overall, products with citric acid were preferred to those with acetic acid as the former were rated higher by 75% of the panellists.

When the effect of citric acid was further analysed, 0.8% (w/w) of citric acid was observed to significantly lower the pH of the product from 6.38 ± 0.05 to 4.49 ± 0.02 (Table 4). According to the 12 untrained panellists that screened the taste, appearance and general acceptability

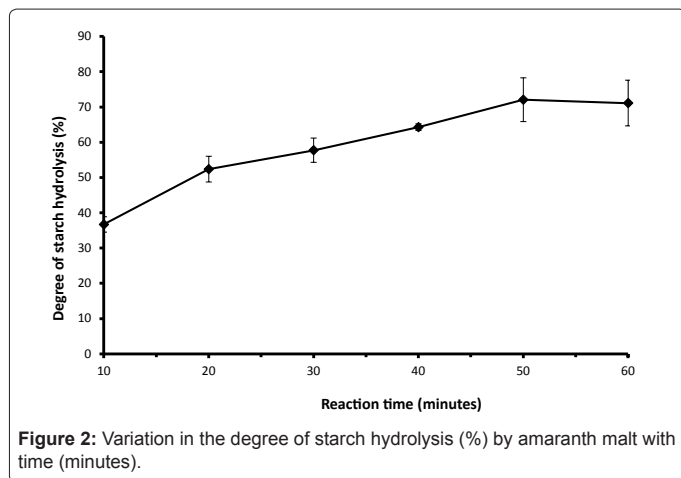**Figure 2:** Variation in the degree of starch hydrolysis (%) by amaranth malt with time (minutes).

	Therapeutic food formulations		
Ingredients	**A**	**B**	**C**
Peanuts	50	50	40
Beans	25.5	25	35
Cowpeas		10	
Sesame	10		11
Grain amaranth*	7	7	7
Sugar	7.5	7.5	7

*Added as Amaranth malt flour

Db.: Dry Basis

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Table 1: Percentage weight composition of selected low cost therapeutic food formulations (db.).

Hydrolysis time (hours)	Average DE (g) per 100 g solids		
	40°C	**50°C**	**60°C**
1	1.3 ± 0.7[a,f]	2.2 ± 0.6[a,f]	2.3 ± 0.7[a,f]
2	3.7 ± 2.2[b,f]	2.4 ± 0.9[a,f]	2.6 ± 1.2[a,f]
3	8.4 ± 1.0[c,f]	8.1 ± 1.9[b,f]	7.6 ± 0.7[b,f]
4	9.7 ± 0.7[c,f]	8.2 ± 0.9[b,g]	8.4 ± 0.3[b,g]

Values are mean ± Standard Deviation

[a, b, c]Means in the same column followed by the same letter are not significantly different from each other at *P<0.05*.

[f, g]Means in the same row followed by the same letter are not significantly different from each other at *P<0.05*.

Table 2: Effect of temperature on activity of amaranth malt in TF product A.

of the products, 92% accepted the product without acid while all (100%) rejected with 0.8% citric acid. When the ratings for the products with citric acid were compared, 0% accepted the jars with 0.6%, 0.7%, and 0.8% citric acid. On the other hand, 42% accepted the product with 0.2% citric acid, while 25% accepted that with 0.4% citric acid.

Whereas the ratings for taste and acceptability of the products reduced with decrease in pH, the ratings for appearance increased as pH decreased. As the pH lowered, the pink/peanut colour of the product intensified. Using a Lovibond tintometer, a change in colour was observed from orange + 1.4 to orange 2.1 as the amount of citric acid added increased (Table 4). There was a statistically significant increase in red and yellow colours between the product with 0, 0.2% and that with 0.8% citric acid. There was no statistical difference between the values for blue as the amount of citric increased ($p<0.05$).

Microbiological quality of the therapeutic food

While, there were no yeasts and moulds detected in all products after heat treatment, only the autoclaved product had non-detectable total plate counts. There was no significant difference in the total plate counts of products that had a lower pH due to addition of citric acid (0.2% w/w) and those without citric acid ($p<0.05$). There was a significant difference between the total plate counts of the control (1.4×10^7) and the pasteurised product (4.4×10^4), the pressure cooked product (2.3×10^3) and the autoclaved product (non-detectable counts). The microbiological quality of the TF according to Gilbert et al. and WHO [27,28] rated the control and the pasteurised product as unsatisfactory, the pressure cooked product as acceptable and the autoclaved product having satisfactory quality.

When the effect of autoclaving and pressure cooking on the sensory attributes of the TF were determined, the general acceptability of the autoclaved product was rated as moderately liked by 65% of the panellists. The pressure cooked product was neither liked nor disliked by 55% and liked moderately by 30%, while 35% moderately liked the pressure cooked product with citric acid added. There was a statistically significant preference of the autoclaved product to that which had citric acid added. There was however no significant difference between the acceptability of the autoclaved product and that which was pressure cooked ($p<0.05$).

Discussion

The peak of the amylase activity of amaranth malt was 48 hours which concurs with previous findings that used 48 hour amaranth malt to hydrolyse weaning foods [16,29]. The amylase activity of malt increases with germination time up to a point after which it starts to decline [12]. The amylase activity of sorghum has been observed to increase with germination time to an optimum of 55-60 hours before decreasing [30].

The optimum temperature of 40°C for amaranth amylases was lower than the optimum temperature of 60°C and 65°C reported for α-amylases from *Bacillus spp.* acting on wheat and rice starch [20,31]. The difference in optimum temperatures could be related to variation in stability of the amylases with enzyme source. Adewale et al. [32] found differences in the relative activity of α-amylases from sorghum, millet and maize malts with temperature. Sorghum and millet amylases were more stable to heat inactivation than maize amylases. At 80°C,
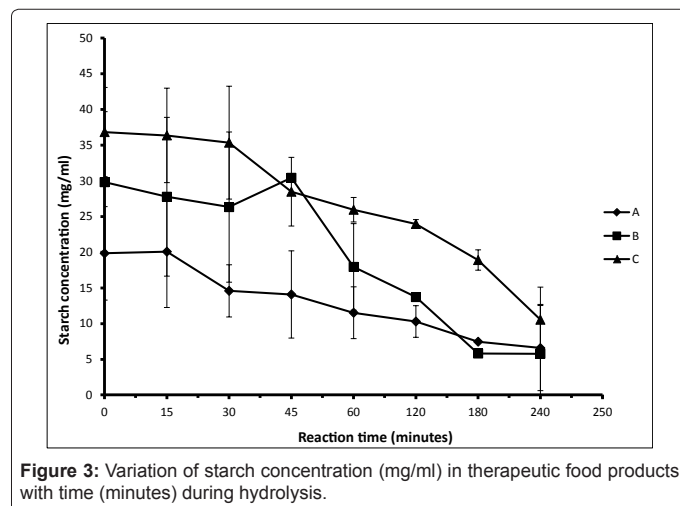

Figure 3: Variation of starch concentration (mg/ml) in therapeutic food products with time (minutes) during hydrolysis.

	Viscosity (Cps) at 60 rpm		
	A	**B**	**C**
Before hydrolysis	3839[e]	3752[e]	3562[e]
After hydrolysis	2732[f]	2782[f]	2783[f]
After heat treatment	2745[f]	2794[f]	2913[f]

[e] [f]Means in the same column followed by the same letter are not significantly different from each other at *P<0.05.*

Table 3: Effect of processing on viscosity of the therapeutic food.
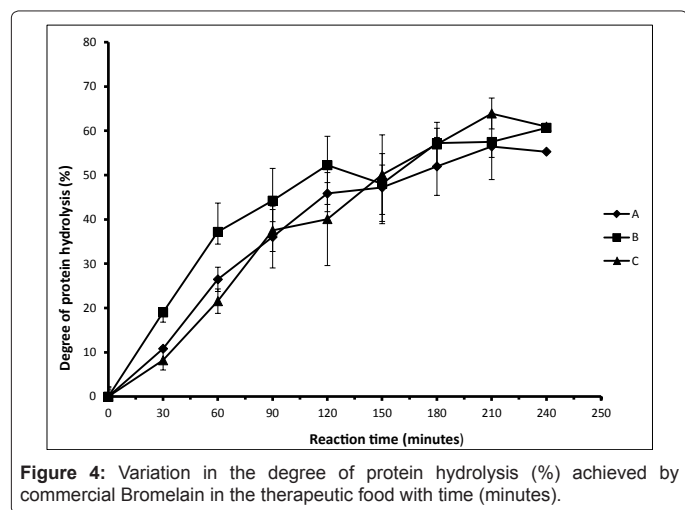
Figure 4: Variation in the degree of protein hydrolysis (%) achieved by commercial Bromelain in the therapeutic food with time (minutes).

sorghum and millet α-amylases retained 18% and 20% of their activity respectively, while maize α-amylases had no activity after 10 minutes of incubation.

The reduction is starch concentration in the therapeutic food during hydrolysis can be attributed to the release of alpha-amylases during the malting of the amaranth [33]. A large proportion of the carbohydrate content of legumes occurs as starch, providing a substrate for the amylase from the malt. Peas and beans are about 55 - 68 % carbohydrates of which 32-57% is starch [34]. Grain amaranth has 48-62% starch depending on the variety [13]. The higher degree of starch hydrolysis for Product B could thus be attributed to the higher starch content from cowpeas which were not included in products A and C. During germination of grain amaranth, there is a decrease in storage carbohydrates and an increase in total soluble and reducing sugars. The raffinose and stacchyose content of amaranth also decrease rapidly during the first 24 hours of germination and almost disappear after 48 hours [35]. Malting not only increases the amylase activity and amount of free nitrogen, it also improves the digestibility and quality of protein in the grains, making them suitable for infants [12]. This coupled with the high degree of starch hydrolysis (70-84%) achieved during pre-digestion yields a product with increased starch digestibility.

Malting is an effective method of reducing bulk and improving nutrient density of weaning foods [10]. The reduction in viscosity of malt-based blends like the formulated TFs is attributed to the starch degradation caused by the action of alpha and beta amylase that are released during the malting process [36]. The rate of reduction in viscosity observed was however lower than that achieved by Delgado and Saldivar [11] using sorghum malt. This could be due to differences in the enzyme source and the nature of the foods in the formulations. Starch in legumes is more difficult to digest than starch in cereals or tubers because of the high amylose to amylopectin ratio and the physical insulation of starch by thick walled cells [37,38]. However, the

effect of thick walled cells does not apply as the TF ingredients were pressure cooked and blended, processes that disrupt the cell structures thus increasing the rate of digestion of starch [38]. Legumes contain 30 to 40% amylose and 60 to 70% amylopectin in their starch granules, whereas other carbohydrate foods have 25 to 30% amylose and 70 to 75% amylopectin. Starches high in amylopectin are digested more quickly than those high in amylose [39].

In addition, legumes contain twice the protein of cereals and the interaction of protein with starch has an effect on starch hydrolysis. Proteins located at the surface of starch granules may act as an obstacle to the access of amylolytic enzymes or interact with them. The nature of the starch present also affects this interaction. There is limited interaction between amylopectins and proteins compared to amylose [40]. The high amylopectin content of legumes implies that this interaction may not be a major factor affecting starch hydrolysis and viscosity in the products. Nevertheless, preliminary protein hydrolysis of the product with the Bromelain prior to addition of the malt (for starch hydrolysis) may have a profound effect on the starch hydrolysis and viscosity [40].

The final viscosities of the three products (2745-2913 Cps) were all within the recommended range of 1000 to 3000 Cps that is appropriate for feeding children less than three years of age. This range indicates a semi-liquid consistency that is needed to prevent the child from chocking [9,41]. Increasing the concentration of the malt and the hydrolysis time decreases the viscosity of the food blends [11]. Further increase in hydrolysis time beyond four hours resulted in a change in the aroma and taste of the TF that was undesirable. Though the amount of malt in the TF in this study was fixed by the amount of amaranth in the formulations generated by the software, an increase in the quantity of malt may not only lead to a further reduction of viscosity but the nutrient density as well.

Protein hydrolysis of the TF was achieved not only by the added commercial Bromelain but also by the proteases from the amaranth malt. In addition to amylases, malted grains have other enzymes such as proteases and phytases that serve to increase protein digestibility and mineral bioavailability in the malt [11]. Various physical, chemical and enzymatic treatments have been used to modify the functional and nutritional properties of plant proteins. Enzymatic modification is preferable due to the milder processing conditions, easier control of the reaction, and minimal formation of by-products [42]. Having a pre-digested product with a degree of protein hydrolysis of 55 to 61% consequently improves the protein digestibility of an all plant protein therapeutic food. The *in vitro* protein digestibility of the three TF formulations A, B and C ranged from 81 to 85% [18]. Trials using Bromelain from fresh pineapples were carried out but due to inconclusive results, commercially available Bromelain was used.

The change in colour of the product that was observed as the amount of acid added was increased can be attributed to the destruction of anthocyanin as the pH lowered. Anthocyanins are a group of reddish water-soluble pigments common in plants. They exist primarily in the red flavylium form at a pH below 2 [43,44]. Destruction of anthocyanins is pH dependent. An increase in pH causes a greater destruction of anthocyanin. At higher pH, the flavylium salts lose their proton making the purple quinoidal base-bond with water to give a colourless compound and thus unstable [45].

According to the microbiological recommendations by Gilbert et al. and WHO [27,28] the autoclaved product had satisfactory counts that is, was of good microbiological quality. The counts for products

	Amount of citric acid					
	0	**0.2%**	**0.4%**	**0.6%**	**0.7%**	**0.8%**
pH Colour	6.42 ± 0.05^a	5.76 ± 0.22^b	5.16 ± 0.11^c	4.79 ± 0.05^d	4.73 ± 0.05^d	4.49 ± 0.02^d
Red	$1.41+0.11^a$	$1.87+0.22^b$	$2.05+0.05^b$	$2.03+0.08^b$	$2.00+0.00^b$	$2.05+0.05^c$
Yellow	$1.43+0.09^a$	$1.85+0.15^b$	$2.07+0.16^c$	$2.12+0.08^c$	$2.07+0.05^c$	$2.08+0.07^c$
Blue	$0.24+0.09^a$	$0.32+0.08^{a,b}$	$0.25+0.10^a$	$0.30+0.09^{a,b}$	$0.30+0.12^a$	$0.15+0.08^{a,b}$
Colour of Sample	orange+1.4	orange+1.9	orange+1.9	yellow/orange+0.1	yellow/orange+0.1	orange+2.1

Values are mean ± Standard Deviation

Means in the same row followed by the same letter are not significantly different from each other at *P<0.05*.

Table 4: Effect of different levels of citric acid on the pH and colour of the therapeutic food.

sterilised in a pressure cooker were acceptable that is of boarder line microbiological quality, while those for the pasteurised product and the control were above the recommended levels and thus unsatisfactory. Since the pressure cooker in use did not have any gauges, it was not possible to determine and control the exact pressure and temperature that was achieved. On its own, the contribution of pasteurization to the extension of shelf life is small, especially when the food lacks other preservative factors such as low pH or low water activity [46].

The acidity of a product has important implications for its microbial ecology and the rate and character of its spoilage. Bacteria grow fastest at a pH of 6.0 - 8.0 and yeasts at 4.5-6.0 [46]. A pH of less than 4.6 considerably reduces bacterial growth and mould and yeast spoilage [47]. The acid added to the TF (0.2% w/w) was only able to reduce the pH of the product to 5.76, a value not low enough to facilitate a significant improvement in microbiological quality. This explains the lack of a significant difference between microbial results for the products with acid added and those without. The high protein content of the TFs may also have increased the buffer capacity of the food thus requiring a larger amount of acid to substantially change the pH.

The therapeutic foods used in the management of malnutrition not only contain adequate amounts of all the nutrients required by malnourished individuals, but are also nutrient dense with a high digestibility [48]. The TF products A, B and C are nutrient dense with a 100 g portion providing 101-111 Kcal, 5 g protein and 5.3-6.5 g fat [18]. With the pre-digestion of the products (70-84% starch hydrolysis and 55-61% protein hydrolysis), the nutrients are thus more readily available. This suggests that the formulated TF products have the potential to be used in the management of malnourished children once mineral-vitamin mix is added and efficacy trials are concluded.

Conclusion

The study was able to produce a therapeutic food that is easily digestible and safe for consumption. Malting of the amaranth for 48 hours generated amylases that hydrolysed the starch in the therapeutic food. This protocol can be used to produce infant foods from plant sources that are of higher quality. However, there is a need to conduct further studies on the shelf life of the therapeutic food. It is recommended that a mineral-vitamin mix is added and the efficacy of the developed therapeutic food is determined using clinical trials before the formulated TFs can be used in management of malnourished children.

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