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Research Article

Physical, Chemical, and Microbiological Characterization of a Flour with Antioxidant and Anti-Inflammatory Properties for Patients with Sickle Cell Anemia in Togo

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Abstract

Problem: The medical management of sickle cell disease remains a challenge since its discovery as a genetically inherited disease. Growing interest in the nutritional problems associated with the disease has led to the development of nutritional alternatives as a means of reducing its morbidity and mortality. *Objectives*: The aim of this study is to develop a flour with antioxidant and anti-inflammatory properties to reduce vaso-occlusive crises and improve the overall health of malnourished sickle cell patients. *Materials and methods*: The composition of the plant material used was designed based on a basic formulation from the nutrition division in collaboration with ITRA, consisting of corn, soybeans, and moringa. It was enriched with monkey bread, turmeric, and sesame, taking into account the recommendations of ANSES. This developed flour, flour C, consists of two subunits, flour A and flour B. The physicochemical and microbiological characteristics of this flour C were determined according to ISO standard methods. *Results*: The main results of the biochemical analyses gave the following values per 100 g of flour: 16.55% protein, 8.65% lipids, and 67.38% carbohydrates, with a theoretical caloric value of 413.57 kcal. The trace element content in mg/100g was as follows: iron 4.33; zinc 2.02; magnesium 2.51; sodium 5.53; potassium 9804.25; calcium 28.14; and phosphorus 315.18. Phytochemical compounds with antioxidant and anti-inflammatory properties were identified: polyphenols, flavonoids, saponoside alkaloids, and vitamin C. Microbiological analyses revealed no salmonella or staphylococcus aureus, and yeast and mold counts were below the threshold (10 CFU/g). *Conclusion*: The flour analyzed can meet the protein and energy needs of sickle cell patients, but also help them combat crises related to the inflammatory impact and oxidative stress associated with the disease.

Keywords

Sickle Cell Disease, Malnutrition, Flour, Antioxidant, Anti-inflammatory

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1. Introduction

Sickle cell disease refers to a group of hemoglobinopathies in which at least one gene encoding sickle beta globin (S) is inherited together with another type of abnormal hemoglobin. The most common hemoglobinopathies are hemoglobin SS (HbSS), hemoglobin SC (HbSC), and hemoglobin Sβ-thalassemia (HbSβ-thalassemia) minor and major [1]. It is the most widespread genetic disease in the world and constitutes a real public health scourge, with a high prevalence of the major SS forms [2]. Every year, sickle cell disease affects more than 300,000 newborns, and nearly 5% of the world's population carries a gene responsible for hemoglobin abnormality [3]. The majority of people with this disease live in sub-Saharan Africa, with prevalence rates varying between 10 and 40% [4]. In Togo, the prevalence of sickle cell disease is estimated at 16% and has even reached 25% among the Adelé, a rural ethnic group [5]. The condition usually begins at the age of 6 months and is characterized by vaso-occlusive crises, chronic hemolytic anemia, and severe infectious episodes. The occurrence of these vaso-occlusive crises is influenced by inflammatory syndromes linked to endogenous malnutrition [6]. This endogenous malnutrition, coupled with undernourishment, has been described in elderly people and cirrhotic patients who also suffer from chronic inflammatory syndrome [7, 8]. Patients with major forms of sickle cell disease are prone to chronic inflammatory processes that affect height and weight growth. This malnutrition also results from the absence or insufficiency of several essential nutrients. These include protein and micronutrient deficiencies [9]. Deficiencies in magnesium, zinc, vitamins A, B1, B6, B9, B12, and vitamin D have been described. These deficiencies can contribute to growth retardation, increased hemolysis, insufficient hemoglobin synthesis, and bone fragility. In addition, intra- and extracellular dehydration and oxidative stress have also been described, caused by insufficient water intake [10].

Since sickle cell disease was first described in 1910 by James Herrick, efforts have been made to develop clinical care to alleviate serious clinical problems, primarily frequent hospitalizations for recurrent painful episodes. However, it is only since the late 1980s that malnutrition has been recognized as a serious complication of the disease that should be treated as part of the required clinical care [11-13]. Given the chronic nature of HbSS symptoms, the best hope for patients would be low-cost, self-administered oral therapies. Self-administered therapeutic flours have been used in the past to combat protein-energy malnutrition [15]. In Togo, the management of the laboratories of the Togolese Institute of Agricultural Research and the National Nutrition Service have committed to implementing a strategy to combat malnutrition by improving food supplements through the enrichment of flours with local foods rich in vitamins and minerals [16]. The protein-energy and micronutrient nutritional status of children and adults with sickle cell disease should be assessed in order to provide them with fortified foods that help combat inflammatory syndrome and chronic oxidative stress. This assessment and nutritional monitoring are not yet integrated into the care of sickle cell patients treated at the National Center for Sickle Cell Research and Care (CNRSD). The aim of this study is to formulate a flour with antioxidant and anti-inflammatory effects from local products to reduce vaso-occlusive crises and correct malnutrition.

2. Materials and Methods

2.1. Type, Period, and Scope of the Study

This was an experimental characterization study conducted from April 1, 2022, to December 24, 2024. The study, initiated within the university research structure, the Laboratory of Biomedical, Agri-Food and Environmental Health Sciences (LASBASE), was carried out in the laboratories of the Togolese Institute of Agricultural Research (ITRA). The flour was produced in the ITRA's Nutrimix workshop. The physicochemical analyses were carried out at ITRA's Food Quality Control Laboratory (LCQA), specifically in the bromatology unit accredited International Organization for Standardization (ISO) 17025 No. ES 23005 by the West African Accreditation System (SOAC). Biochemical analyses were carried out at the biochemistry laboratory of the Faculty of Sciences of the University of Lomé (UL). Microbiological analyses were carried out at the Laboratory of Microbiology and Food Quality Control (LAMICODA) of the University of Lomé The taste test took place at the CNRSD.

2.2. Flour Production Equipment

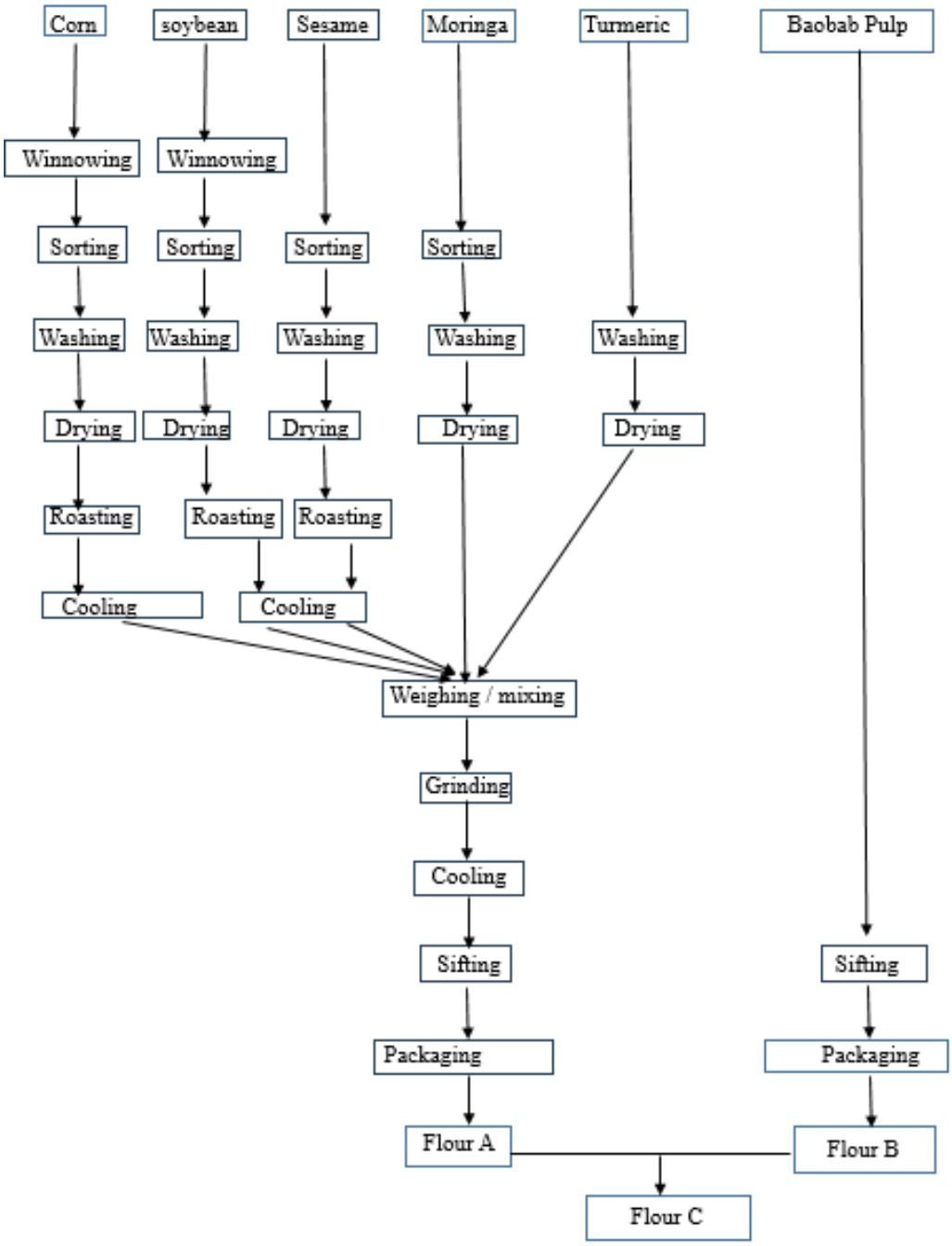
The equipment used consisted of an electric roaster (Juno, Germany), a mill, weighing scales, a gas dryer (Sam Logistics, Togo), sealing machines (Cynox, China), drying and sorting tables, and small kitchen equipment.

2.3. Origin of the Ingredients in the Formulation

The composition of the plant material used was based on a basic ITRA formulation developed in collaboration with the nutrition division of the Togolese Ministry of Health and Public Hygiene (MSHP). It consisted of yellow corn (Zea mays), soybeans (Glycine max), and moringa leaves (Moringa oleifera). It was enriched with baobab fruit (Adansonia digitata), turmeric rhizome (Curcuma longa) and sesame seeds (Sesamum indicum), taking into account the recommendations of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) [17]. The moringa powder was purchased from Kinomé, a social and solidarity economy company that aims to ensure food security [18]. An

agreement was signed with this company to ensure the quality of production, which must take into account traceability and hygiene criteria in accordance with the HACCP (Hazard Analysis Critical Control Point) standard. Compliance with this same standard was applied to the procurement of monkey bread from the company NI-RAJOUT, which was selected following a compliance check on vitamin C content and microbiological quality. The turmeric powder was supplied by the Sisters of Providence group based in Atchangbadé in the Kara region. The sesame seeds were purchased in the same region at the Lama market. The yellow corn was purchased in Sotouboua at the seed farm, and the soybeans were delivered by the FAMETAM project based in Sokodé. The purchase of all ingredients was financed by the Food and Agriculture Organization of the United Nations (FAO) and partly by KINOME and NI-RAJOUT.

Figure 1 below shows the flour production diagram.



NB: 100g kit of this flour consists of 78g of flour A and 22g of flour B

Figure 1. *Production diagram for flour with antioxidant and anti-inflammatory properties.*

Figure 2 below shows flour C with its two flours A and B.

Photo: Ouro-bere

Figure 2. *Farine C avec ses deux sous composantes A et B.*

2.4. Physicochemical Analyses

The physicochemical analysis of the samples consisted of determining the water, protein, lipid, carbohydrate, energy value, mineral composition, and vitamin C content.

The different contents were determined according to methods described in international ISO or AOAC (Association of official analytical collaboration) standards, in triplicate tests. The test samples of flours A, B and C were obtained with a balance (OHAUS, USA) with a capacity of 520g calibrated in accordance with the requirements of ISO 17025 version 2017 [19].

The water content was determined by oven drying in accordance with ISO 24557: 2009 [20]. This was carried out using a German-made ARGOLAB oven with a 5 g test sample.

The protein content was determined using the Kjeldahl method described in ISO 20483: 2013 [21], using a 0.5 g sample. This method involves determining the nitrogen content and calculating the crude protein content. The quantification of total nitrogen by distillation with the BUCHI distiller, of German origin, made it possible to determine the amount of protein according to the formula: Vegetable protein = nitrogen content x 6.25.

The lipid content was determined using hexane extraction in a Soxhlet extractor (NANBEI, China) using the Randall method described in ISO 11085: 2015 [22]. The test sample was 2 g.

The carbohydrate content was determined by calculation using the following CAC (Continuous Assisted Coding) difference method: % Carbohydrates = 100% - (% Moisture + % Protein + % Fat + % Ash). The total ash obtained by incinerating the dry matter in an oven (Nabertherm, Germany) was quantified according to the ISO 2171: 2023 [23] method. The ash content per 100 grams of dry matter (%C) was calculated using the following formula: $\%C = ((P3- P1)/(P2- P1)) x100$. [24]

P1: weight of the empty crucible, P2: weight of the crucible containing the dry test sample, P3: weight of the crucible containing the dry test sample after incineration in the furnace and cooling.

The minerals contained in the ashes obtained were measured by atomic absorption spectrometry (Agilent Technology, Malaysia) [25]. Iron and zinc were measured after microwave digestion; calcium, magnesium, sodium, and potassium were measured in accordance with the requirements of method NF EN 14084 version 2003. Phosphorus was measured using the DABIN 1965 method. The results were expressed in mg/100 g DM.

The theoretical energy value of flours A, B, and C was calculated based on the calorific values of carbohydrates, proteins, and lipids [26]. Thus, for a sample whose analysis gives P% protein, G% carbohydrates, and L% lipids, the energy value was obtained using the formula: Kcal/g or Kcal/100g = (P x 4 Kcal) + (G x 4 Kcal) + (L x 9 Kcal).

The vitamin C content of the samples was determined by titration using 20 g of flour samples soaked for 10 min in 40 mL of a metaphosphoric acid-acetic acid solution (2%, w/v) [27]. The mixture was centrifuged CYAN (Cyan, Germany) at 0.15G for 20 min and the supernatant obtained was diluted and adjusted with 50 mL of distilled water. Ten (10) mL of this mixture was titrated to the color change with dichlorophenol-indophenol (DCPIP) 0.5 g/L.

2.5. Biochemical Analyses

Quantitative and qualitative phytochemical analyses were performed on aqueous extracts of the flours produced in order to detect the presence or quantify secondary metabolites of pharmacological interest. The metabolites with anti-inflammatory and antioxidant effects sought were: flavonoids (antioxidant, anti-inflammatory), saponins, triterpenes, alkaloids (anti-inflammatory), and total polyphenols (antioxidant). The presence of these phytochemical constituents was determined by colorimetric reactions. Total polyphenols, flavonoids, and anti-radical activity were measured on a spectrophotometer (Metash, China) at specific wavelengths.

Flavonoid testing: A dry sample of 1 g of each flour was dissolved in 10 mL of distilled water and divided into two tubes. Three drops of a 1% sodium hydroxide (NaOH) solution were added to one tube. The formation of an intense yellow color, which became colorless upon addition of 35%

hydrochloric acid diluted to one-third, detected the presence of flavonoids. The second tube served as a negative control [28].

Saponin testing: This was done using the foam test. The experimental protocol used consisted of two tubes in which 0.5 g of each flour extract was diluted in 10 mL of distilled water. After shaking the solution in one of the tubes for 1 minute, the solution was left to stand for 15 minutes. The formation of a persistent foam layer at least 1 cm thick, measured with a graduated ruler, confirmed the presence of saponins in the samples studied. The second tube, containing only distilled water, did not produce any foam and therefore served as a negative control [28].

Triterpene testing: Each dry extract of the mixed flour was dissolved in distilled water and divided into two tubes. A few drops of sulfuric acid (1 M) were added to one tube, then the tubes were shaken and left to rest. The appearance of a golden yellow color indicated the presence of triterpenes. The second tube served as a control [28].

Alkaloid testing: Alkaloids in the extracts were detected using Dragendorff's test [28]. The dry extract of each flour was dissolved in distilled water and divided into two tubes. A few drops of Dragendorff's reagent, a solution of potassium iodide and bismuth, were added to one tube. The formation of a red precipitate indicates the presence of alkaloids. The second tube, which did not receive Dragendorff's reagent, served as a negative control.

Total polyphenol assay: Total phenols were assayed according to the method described by Singleton et al. in 1999 [29]. This method is based on an oxidation-reduction reaction in which the hydroxyl group (-OH) of phenols is oxidized while Folin Ciocalteu Reagent (FCR) is reduced, resulting in a decrease in optical density that was read at 760 nm using a spectrophotometer, against a blank obtained from a mixture consisting of: 500 µL of FCR, 500 µL of sodium carbonate, and 200 µL of distilled water.

Determination of anti-radical activity: The principle used is based on the reduction of the molybdate ion Mo^{6+} to the molybdate ion Mo^{5+} by the antioxidant compounds contained in the flour extracts, resulting in the formation of a green phosphomolybdate complex ($PMo_{12}O_{40}^{3-}$) which showed maximum absorption at 695 nm [30].

2.6. Microbiological Analyses

The microbial flora associated with the flour batches was counted using recognized methods described in ISO standards. These analyses consisted of counting yeasts and molds [31], total mesophilic aerobic flora [32], thermotolerant coliforms [33], Salmonella spp [34], and Staphylococcus aureus [35].

2.7. Method for Preparing the Porridge

This method was developed to preserve the antioxidant properties of vitamin C in monkey bread [36].

Pour 200 ml of water into a saucepan, add 78 g of flour A (yellow corn, soy, moringa, turmeric, sesame) and mix until completely dissolved. Gradually add 500 ml of water while continuing to stir. Bring to a boil over medium heat and simmer for 10 minutes, stirring regularly. Remove from the heat and leave to cool to below 60 ℃. Add 22 g of flour B (baobab fruit pulp powder) and commercial sugar, then mix until smooth. Serve and enjoy the porridge according to preference.

A group of patients or parents of children with sickle cell disease were trained in this preparation method. They then took part in a tasting test.

2.8. Organoleptic and Acceptability Test

Samples of porridge prepared from flour produced according to the instructions described above were presented simultaneously to each panelist selected at the CNRSD. Each porridge was evaluated based on its appearance (color), taste (flavor), smell (aroma), consistency, and overall acceptability using a nine-point hedonic scale [37]. A score of 9 corresponds to an extremely pleasant product, while 8 is assigned to a very pleasant product. The number 7 corresponds to a pleasant product, and 6 to a fairly pleasant product. The number 5 is assigned to a product that is neither pleasant nor unpleasant (neither good nor bad), and 4 corresponds to a fairly unpleasant product. The number 3 corresponds to an unpleasant product, 2 is assigned to a very unpleasant product, and 1 to an extremely unpleasant product.

2.9. Ethical Considerations

This study was authorized by the Bioethics Committee for Health Research of the Ministry of Health and Public Hygiene under number 013/2022/CBRS/ of May 24, 2022. Informed consent was obtained from each participant in the organoleptic tests. The anonymity of the subjects included in this study was maintained. The results obtained in this study were used exclusively for scientific purposes.

2.10. Statistical Analyses

All measurements were performed in triplicate. The data were collected in an Excel file after verification of the transcripts. The macronutrient, mineral, and vitamin C content of Flour C was compared to the FAO/WHO nutritional recommendations to ensure that children's needs were met [3]. Means and standard deviations were determined using GraphPad Prism version 9.0 software (Graph Pad Software Inc, USA). The results were exported to Stata 16 IC Software from Stata Corp LLC to compare the means with the reference values using the T-test. The same test was used to compare the means of photochemical compound content and anti-radical activity for different flours. Statistical significance was set at $P<0.05$.

3. Results

3.1. Nutritional Composition of Flours

This refers to the composition in terms of moisture content, macronutrients, micronutrients, and vitamin C.

3.1.1. Macronutrient and Vitamin C Content of Different Flours

Macronutrients provide the calories needed for energy metabolism, and vitamin C helps combat oxidative stress. Table 1 shows their content in 100 g of flour.

Table 1. *Macronutrient and vitamin C content in 100g of flour.*

Parameters	Flour A M ±ET	Flour B M ±ET	Flour C M ±ET	Recommendations FAO/WHO*	p value
Moisture (%)	2,77 ±0,02	8,47 ±0,23	4,16 ±0,07	≤5,00	0,0012
Protéin (%)	19,73 ±0,55	1,72 ±0,02	16,55 ±0,09	15,00	0,0006
Fat (%)	9,12 ±0,09[e]	0,997 ±0,01	8,65 ±0,25	8,00	
Carbohydrate (%)	34,5 ±0,69	83,66 ±0,33	67,38 ±0,37	68,00	0,0505
Valeur calorique (kcal)	350,62 ±2,76	299 ±2,290	413,57 ±2,48	400,00	0,0055
Vitamin C (mg)	1,96 ± 1,42	297,67 ± 2,31	70,99 ± 2,56	>25,00	0,0005

M: mean, SD: standard deviation *corresponding unit

The protein and fat content is significantly in line with or higher than FAO recommendations, and the carbohydrate content is at the limit of the reference value.

The vitamin C content is more than twice the recommended value.

3.1.2. Micronutrient Content of Different Flours

Minerals are essential for antioxidant and anti-inflammatory metabolic mechanisms. The mineral composition of different flours is shown in Table 2.

Table 2. *Mineral content (mg) per 100 g of flour.*

Parameters	Flour A M ±SD	Flour B M ±SD	Flour C M ±SD	Recommendations FAO/OMS (mg)	P value
Iron (mg)	5,06 ±0,02	3,27 ±0,07	4,33 ± 0,02	>4,00	0,0006
Zinc (mg)	1,80 ±0,02	0,71 ±0,02	2,02 ±0,09	3,20	0,0010
Magnésium (mg)	2,50 ±0,03	2,54 ±0,03	2,51 ±0,02	>19,00	<0,0001
Sodium (mg)	5,37 ±0,14	2,59 ±0,01	5,53 ±0,02	>74,00	<0,0001
Potassium (mg)	952,38 ±21,73	1968,85 ±1,15	1968,60 ±40,85	>129,00	<0,0001
Calcium (mg)	47,78 ±0,24	64,1 ±0,21	28,14 ±0,08	>125,00	<0,0001
Phosphorus (mg)	354,64 ±1,28	167,67 ±0,36	315,18 ±0,43	>114,00	<0,0001

M: mean, SD: standard deviation.

The results show that requirements for magnesium, sodium, calcium, and, to a lesser extent, zinc are not being met, unlike requirements for iron, potassium, and phosphorus.

3.2. Assessment of the Impact of Porridge Preparation Methods on Macronutrient and Vitamin C Content Cooking Methods

Cooking method, especially heat, can have an impact on vitamin C. The different macronutrient and vitamin C contents in porridges depending on the preparation method are shown in Table 3.

Table 3. *Macronutrient and vitamin C content in different porridges.*

Paramètres	FCB	P*	FAB	P§	FAB/FB	P+	Recommandation FAO/OMS
Moisture %	84,78±0,31	0,0000	86,79 ±0,14	0,0000	83,75 ±0,18	<0,0001	≤5,00
Protein %	2,46 ±0,2	0,0000	3,175 ±0,15	0,0000	3,46 ±0,02	0,0000	15,00
Fat %	0,25 ±0,01	0,0000	0,635 ±0,02	0,0000	0,3 ±0,01	0,0000	8,00
Carbohydrate %	11,88 ±0,12	0,0000	8,75 ±0,08	0,0000	11,585 ±0,14	0,0000	68,00
Energy density (kcal)	59,61 ±1,23	0,0000	53,415 ±0,06	0,0000	62,88 ±0,57	0,0000	400,00
Vitamin C (mg)	8,03 ±0,32	0,0001	0,070 ±0,014	0,0000	61,18 ±2,94	0,1639	>25,00

P*, P§, P+: significance thresholds for comparisons between FCB, FAB, and FAB/FB and the recommended levels of the FAO/WHO, respectively. FCB: mixture of flour A and B boiled, FAB: flour A boiled, FAB/FB: Mixed flour A porridge with flour B added after cooling to below 60 °C.

Heat has a significant impact on vitamin C content. Only the FAB+FA preparation method preserves the vitamin C content, which is a good antioxidant.

3.3. Research into Phytochemical Compounds

Phytochemical compounds have anti-inflammatory and antioxidant properties. Saponins, alkaloids, and triterpenes were measured qualitatively, unlike total polyphenols, flavonoids, and radical activity, which were measured quantitatively. Table 4 presents the results of qualitative research carried out on each test sample of flour, and Table 5 shows the total polyphenol and flavonoid content and anti-radical activity of dry test samples of different flours.

Table 4. *Identification of phytochemical compounds in different flours.*

Paramètres	Farine A	Farine B	Farine C
Saponins	+	+	+
Alkaloids	+	-	+
Triterpènes	-	-	-

Tri terpenes were not detected in flours A, B, and C.

Table 5. *Photochemical compound content and anti-radical activity measurement of extracts from different flours.*

Paramètres	Farine A M±SD	P+	Farine B M±SD	P*	Farine C M±SD
Polyphenols totaux (mg EAG/g)	91,47 ±1,80	0.0336	90,26 ±1,70	0,0157	96,53 ±2,09
Flavonoids (mg EQ/g)	36,13 ±1,71	0,0009	34,13 ±1,70	0,004	45,42 ±0,65
radical activity (mg EAA/g)	3,65 ±0,09	0,0132	2,05 ±0,08	0.0004	3,20 ±0,16

P+: comparison between flour A and C, P*: comparison between flour B and C, M±SD (mean ±standard deviation), GAE: gallic acid equivalent, QE: quercetin equivalent, AAE: ascorbic acid equivalent

Flour C is significantly richer in polyphenols and flavonoids than flours A and B. The anti-radical activity is higher in flours A and C.

3.4. Microbiological Analyses

The safety of the flours prevents contamination. The results of the microbiological analysis are presented in Table 6 below.

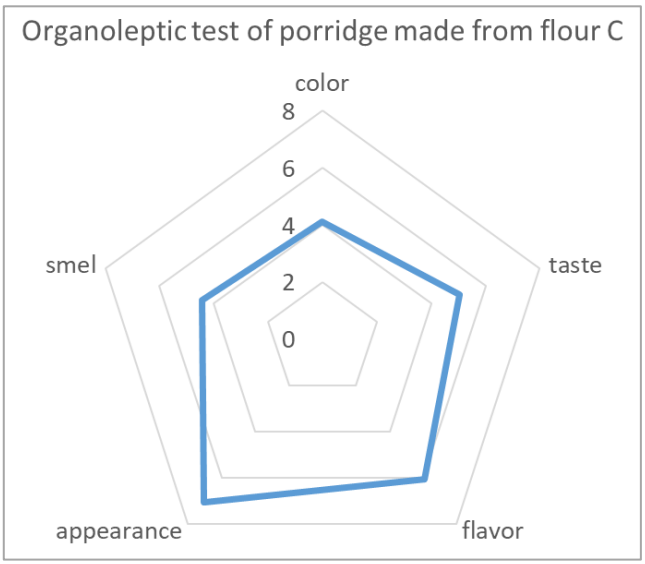
Table 6. Study of the microbiological quality of flours A and B from the kit.

N °	Germes Recherches	Unit	Criteria*	Germes nomber	
				Flour A	Flour B
1	Total mesophilic flora (30 ℃)	UFC/g	10^6/g	6600	3700
2	Total coliforms (30 ℃)	UFC/g	10 ⁋g	<10	<10
3	Sulfite-reducing anaerobes (44 ℃)	UFC/g	<10/g	<10	<10
4	Yeasts and molds (30 ℃)	UFC/g	<10/g	<10	<10
5	Staphylococcus aureus	UFC/g	<10 ⁋g	<100	<100

The microbiological quality research data clearly show that the components of flour C tested are of satisfactory hygienic quality.

3.5. Taste test

Sensory evaluation tests are designed to detect deviations or defects in the product with a view to improving it. The organoleptic characteristics scores are illustrated in the diagram in Figure 3.



Author: ouro-bere

Figure 3. Diagram illustrating the organoleptic characteristics ratings.

According to the diagram, the color and smell were rated lower for the porridge prepared using the flour provided (Flour C).

4. Discussions

The aim of this study was to offer malnourished sickle cell patients flours with antioxidant and anti-inflammatory effects derived from local products. The appropriate carbohydrate, fat, calorie, and protein content of enriched flour C helps combat the protein-energy malnutrition described in sickle cell patients, as chronic inflammation leads to endogenous malnutrition coupled with anorexia nervosa induced by the action of cytokines [7]. Indeed, the study by Umeakunne et al. showed that protein, fat, and carbohydrate requirements are increased in sickle cell disease [38]. Energy content is also crucial for children's growth, and C flour meets their daily requirements. The average energy value of 413.74 kcal per 100 g of dry matter in enriched C flour was lower than that reported in the work of Loba et al., who produced infant cereals and legume flours prepared using wet and dry methods [39, 40].

The protein content of flour C is beneficial for child growth and adult maintenance, as protein has a positive impact on tissue repair and muscle building [41]. Reports on the administration of protein-rich supplements containing L-arginine to mice in which sickle cell production was induced showed significant reductions in inflammation, oxidative stress, and painful episodes, as well as improved microvascular function [42, 43].

Significantly low circulating levels of antioxidant vitamins (A, C, and E) have been measured in patients with sickle cell

disease [44]. Vitamin C levels higher than the recommended intake were found in porridge made from C flour. In 2000, Ohnishi et al. reported that adding vitamin C to human sickle red blood cells in vitro inhibited the formation of dense cells that cause occlusive crises [45].

Magnesium, iron, and zinc requirements are increased in sickle cell disease, but the mineral composition of C flour in terms of magnesium, sodium, calcium, and, to a lesser extent, zinc was below the minimum recommended values [3]. The subjects in this study will not be fed exclusively on flour; they will receive nutritional recommendations to compensate for any deficiencies that flour C porridge cannot meet. They will be advised to eat foods rich in iron, zinc, and magnesium (offal, seafood, green vegetables, legumes, nuts, seeds, dark chocolate, seeds, nuts, pulses, dried fruit, oilseeds, whole grains, soy milk, and oily fish) [38]. Numerous health consequences have been reported in sickle cell patients as a result of zinc deficiency, including immune dysfunction, abnormal or delayed sexual maturation, abnormal growth patterns, poor wound healing, and decreased levels and activity of zinc metalloproteins. This highlights the need to monitor their consumption of local foods rich in zinc, such as oysters, red meat, fish, legumes, seeds, and dark chocolate [46]. Low total magnesium levels in sickle cell erythrocytes have been associated with increased sickling due to the propensity of red blood cells to dehydrate and, consequently, increased HbS polymerization [11]. Dehydration in sickle cell patients has been shown to be due to abnormally high red blood cell permeability and potassium (K+) loss via at least three weakly connected pathways, in which the relative contribution of each is not yet known. One of these pathways, K-Cl cotransport, is abnormally activated by low Mg^{2+} levels in cells [11]. This causes rapid and irreversible loss of K+ and Cl– ions, followed by water through osmosis. The phosphorus and potassium levels in flour are higher than the recommended values of 281.2 mg and 408.7 mg/100g of dry matter, respectively [3, 47]. In 2003, Lutter et al. reported that phosphorus and potassium are beneficial for child development and can limit the onset of nutritional deficiencies [48]. Although the flour meets many nutritional needs, the results show that levels of magnesium, sodium, calcium, and zinc are below recommended values. While these deficiencies would be addressed through personalized nutritional recommendations, it would be worth considering potential fortification of these specific minerals within the flour itself to improve its overall nutritional profile.

The porridge prepared with flour C has the highest energy density of the three porridges (62.88 ± 0.50 kcal) and complies with the Codex standard values for infant formula, which are between 60 and 70 kcal/100 ml of porridge [49]. For better coverage, it would be beneficial to double the number of doses for children per day or triple the number for adults per day, or to increase the energy density of the porridge by adding sugar [50].

Interest in natural products is attracting increasing attention as an integrative approach to sickle cell disease management. Many of these tropical plants are native to countries with high rates of sickle cell disease. Plant derivatives have been shown to contain antioxidant properties derived from bioactive components such as phytochemicals [51]. Ethanolic extracts of Moringa oleifera have shown antioxidant values ranging from 77 to 4,458 µg/ml [52]. The traditional use of curcumin as an anti-inflammatory and antioxidant in treatments is limited due to its low absorption in the gastrointestinal tract, low solubility, stability, low bioavailability, and rapid systemic elimination [53]. To this end, sesame, which is rich in lipids, could promote the permeability of curcumin in order to increase its bioavailability. This is what justified its use in the present study.

The moisture content of the flour was satisfactory, as it was below the 5.00% recommended by the FAO for infant flours. Above this value, the environment becomes favorable for the development of microorganisms. The enriched flour in this study can therefore be stored well. This result concerning water content was also found in Burkina Faso in the work of Alozie et al., who reported a moisture content of 5% in bambara infant flour [54].

The mesophilic aerobic germ loads of the two sub-kits are below the microbiological criteria for mesophilic aerobic germs of 105 CFU/g in infant flours [47]. These low loads could be explained by the low moisture content in the various compound flours. This moisture content is thought to be due to the combination of drying and roasting of the seeds, which significantly reduced the mesophilic aerobic bacteria and fungi counts in the flours [55]. The counts of total coliforms (30 ℃), sulfite-reducing anaerobes (44 ℃), yeasts and molds (30 ℃), and Staphylococcus aureus were below the limits set by the French Standardization Association (AFNOR [56]).

The porridge made from the flour provided was rated differently by the panel of participants recruited at the CNRSD. The average score was 6.22 ± 1.55, corresponding to a fairly pleasant porridge. According to the organoleptic criteria, color and smell greatly influenced the panelists' acceptance of the porridge. The same assessments were made when moringa powder was added to couscous in a study conducted by Hama-Ba in 2016 [57]. Although the addition of 10% moringa improved the nutritional quality of the porridge in a study by Bouka et al [58], the flavor and green color were not appreciated by the panelists in a study by Paka et al. [59]. This assessment remains unchanged in this study, despite the incorporation of 7% moringa. Due to the color of the porridge, extraction of chlorophyll from moringa leaves without denaturing the nutrients prior to incorporation could be considered in the outlook. Baking soda is very effective at eliminating odors. Its use with lemon juice or vinegar will be considered for this purpose.

5. Conclusion

This study has led to the development of a flour enriched with local food resources that meets WHO recommendations

in terms of proximate, microbiological, and organoleptic composition for complementary feeding. This flour, made from a combination of five of the seven recommended food groups, contributes in terms of quality to the minimum dietary diversity recommended for children and adults based on the number of kits to be consumed per day. It contains good levels of protein, lipids, carbohydrates, minerals, and photochemical compounds with antioxidant and anti-inflammatory properties. Any mineral deficiencies will need to be addressed through personalized nutritional recommendations. Looking ahead, this enriched C flour will be administered in the form of porridge to malnourished sickle cell volunteers for 6 to 8 months, and the impact of this re-nutrition will be assessed through anthropometric, biochemical, and impedance measurements.

Abreviation

ANSES	French Agency for Food, Environmental and Occupational Health & Safety
AOAC	Association of Official Analytical Chemists
CNRSD	National Center for Sickle Cell Research and Care
FAO	Food and Agriculture Organization
HACCP	Hazard Analysis Critical Control Point
ISO	International organization for standardisation
ITRA	Togolese Institute of Agricultural Research
MSHP	Togolese Ministry of Health and Public Hygiene
LAMICODA	Laboratory of Microbiology and Food Quality Control
LASBASE	Laboratory of Biomedical, Agri-Food and Environmental Health Sciences
LCQA	Food Quality Control Laboratory
M	Mean
NaOH	Sodium Hydroxide
SD	Standard deviation
SOAC	West African Accreditation System
UL	University of Lomé
WHO	World Health Organization

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Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Mehta AB and Hoffbrand AV. Haematology at a Glance. John Wiley & Sons, 2009.

[2] Shongo MYP, Mukuku O, Lubala TK, Mutombon AM, Kanteng GW, Umumbu WS, Lukamba RM, Wembonyama SO, Luboya ON. "Sickle Cell Disease in Stationary Phase Children from 6 to 59 Months of Age in Lubumbashi: Epidemiology and Clinical Practice," Pan Afr. Med. J., no. 19-71, 2014, https://doi.org/10.11604/pamj.2014.19.71.3684

[3] FAO/WHO., "Joint FAO/WHO Food Standards Programme. Report of the Twenty-seventh Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses," ALINOM, pp. 1-105, 2006.

[4] Thiam L, Dramé A, Zokèbé CI, Diouf FN, Seck N, Boiro D, Ndongo AA, Basse I, Niang B, Deme/Ly I, Sylla A, Diagne I, Ndiaye O, "Epidemiological, clinical and hematological profiles of homozygous SS sickle cell disease in the inter-critical phase in children in Ziguinchor, Senegal," Pan Afr. Med. J., vol. 28, 2017, https://doi.org/10.11604/pamj.2017.28.208.14006

[5] Tété-Bénissan A, Duriez P, and Parra HJ. "Serum nutritional markers in the Adélé of Togo," ResearchGate, 2000, https://doi.org/10.4314/jrsul.v4i1.17000

[6] Belala A, Marc I, Hajji A, Belghyti D, and K. El Kharrim A. "Sickle Cell Disease in Children Hospitalized in the Pediatric Department (CHR El Idriss de Kénitra, Morocco): About 53 Cases," Eur. Sci. J., Jan. 2016, https://doi.org/10.19044/ESJ.2016.V12N12P201

[7] Kouassi K, Bagny A, Kaaga L, Bouglouga O, Lawson ALS, Lamboni C, Redah D. "Prevalence of Protein-Energy Undernutrition by Tricipital Skinfold and Brachial Muscle Circumference Measurement in 103 Adult Cirrhotics Hospitalized in Hepato-Gastroenterology at the Lome University Hospital Campus (Togo)," John Libbey Eurotext, vol. 24, no. 2, p. 208-13, 2014, https://doi.org/10.1684/mst.2014.0334

[8] Kouassi K and Lamboni C, "Protein-energy nutritional status of elderly people hospitalized at the Lomé University Hospital Campus (Togo): pilot study of 33 cases versus 30 controls collected in three care units.", Médecine Santé Trop., vol. 23, no. 1, pp. 39-48, 2013, https://doi.org/10.1684/mst.2013.015

[9] Graham R. Serjeant. "The emerging understanding of sickle cell disease," Br. J. Haematol., vol. 112, no. 1, pp. 3-18, Jan. 2001, https://doi.org/10.1046/j.1365-2141.2001.02557.x

[10] Aboudoulaye D. "Evaluation of the nutritional status of children with sickle cell disease monitored in the pediatrics department of the Gabriel Toure University Hospital in Bamako." FMOS Medicine Thesis, 2021.

[11] De Franceschi, Bachir, Galacteros, tchernia, Cynober, Neuberg, Beuzard, Brugnara, "Oral magnesium pidolate: effects of long-term administration in patients with sickle cell disease," Br. J. Haematol., vol. 108, no. 2, pp. 284-289, Feb. 2000, https://doi.org/10.1046/j.1365-2141.2000.01861.x.

[12] Mitchell MJ, Carpenter OJG, Crosby LE, Bishop CT, Hines J, and Noll J. "growth status in children and adolescents with sickle cell disease," Pediatr. Hematol. Oncol., vol. 26, no. 4, p. 202-215, Jan. 2009, https://doi.org/10.1080/08880010902896882

[13] Tomer A, Kasey S, ConnorW, Clark S, Harker L, and Eckman J. "Reduction of Pain Episodes and Prothrombotic Activity in Sickle Cell Disease by Dietary n-3 Fatty Acids," Thromb. Haemost. vol. 85, no. 06, p. 966-974, 2001, https://doi.org/10.1055/s-0037-1615948

[14] M. J. Mitchell, G. J. O. Carpenter, and L. E. Crosby, "Growth status in children and adolescents with sickle cell disease" Pediatr Hematol Oncol, p. 202-15, 2009.

[15] Lachant NA and Tanaka KR. "Antioxidants in Sickle Cell Disease: The In Vitro Effects of Ascorbic Acid," Am. J. Med. Sci., vol. 292, no. 1, p. 3-10, July 1986, https://doi.org/10.1097/00000441-198607000-00001

[16] Bouka G, Bangana CE and Sidi-Touré S. "Fortification of baby with local foodstuffs, in: Grains for Feeding the World. Presented at the EXPO MILANO 2015", Univ. Expo 2015 Com. Sci. Comune Milano Milan Ital., p. 29-34, 2015.

[17] ANSES, "Opinion of the French National Agency for Food, Environmental and Occupational Health Safety on the adverse effects associated with the consumption of food supplements containing turmeric," Maisons-Alfort, 2022.

[18] Kinomé, "Projet Moringa PROMOSCO Togo," 2022. Accessed: July 1, 2025. Available at: https://kinome.fr/projet-moringa-promosco-togo/

[19] ISO 17025, ISO. General requirements for the competence of testing and calibration laboratories. ISO/IEC 17043: 2017 (fr) online available at: https://www.iso.org/fr/standard/66912.html 2017.

[20] ISO 24557, ISO. Legumes — Determination of moisture content — Oven-drying method. ISO/CD 24557 2024(en) available at: https://www.iso.org/en/search.html?PROD_isoorg_en%5Bquery%5D=iso24577%3A2025, 2024.

[21] ISO 20483, ISO Cereals and pulses — Determination of nitrogen content and calculation of crude protein content — Kjeldahl method. ISO 20483: 2013 (en) available at: https://www.iso.org/search.html?PROD_isoorg_en%5Bquery%5D=20483, 2013.

[22] ISO 11085, Cereals, cereal products and animal feeding stuffs — Determination of crude fat and total fat content by the Randall extraction method. ISO 11085: 2015 (en) available at: https://www.iso.org/search.html?PROD_isoorg_en%5Bquery%5D=11085, 2015.

[23] ISO 2171, Cereals, pulses and derived products — Determination of ash content by ashing. ISO 2171: 2 (en) available at: https://www.iso.org/en/search.html?PROD_isoorg_en%5Bquery%5D=2171%3A2, 2023.

[24] AOAC, "Association of Official Analytical Chemists, Official Methods of Analysis". vol. 1. Official Methods of Analysis, pp. 808, 831-835, 1113, 1990.

[25] Pinta M. "Reference Methods for the Determination of Mineral Elements in Plants. Determination of the Elements Ca, Mg, Fe, Mn, Zn and Cu by Atomic Absorption. Oilseeds," vol. 2, pp. 87-92, 1973.

[26] Merrill AL and Watt BK, "Energy Value of Foods: Basis and Derivation." Agriculture Handbook No. 74," ARS U.S. Dep. Agric. Wash. DC, no. 74, 1973.

[27] Pongracz G, Weiser H, and Matzinger D, "Tocphenol-Antioxidant. », Fact Sci-Technol, vol. 97, p. 90-104, 1971.

[28] Prashant T, Bimlesh K, Mandeep K, Gurpreet K, and Harleen K, "Phytochemical screening and extraction: A Review," 2011.

[29] Singleton VL, Orthofer R, and Lamuela-Raventós RM. "[14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent," in Methods in Enzymology, vol. 299, Elsevier, 1999, pp. 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

[30] Prieto P, Pineda M, and Aguilar M, "Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E," Anal. Biochem., vol. 269, no. 2, pp. 337-341, May 1999, https://doi.org/10.1006/abio.1999.4019

[31] ISO 21527-2, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and molds. Part 2: Colony count technique in products with a water activity of 0.95 or less. ISO 21527-2: 2008 (en) available at: https://www.iso.org/en/standard/38276.html, 2008.

[32] ISO 4833, ISO Microbiology of the food chain — Horizontal method for the enumeration of microorganisms Part 1: Colony count at 30 ℃ by the depth plating technique. ISO 4833: 2013 (en) available at: https://www.iso.org/en/standard/53728.html 2013.

[33] ISO 4832, ISO Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony count method. ISO 4832: 2006 (en) available at: https://www.iso.org/en/standard/38282.html 2006.

[34] ISO 6579-1, ISO Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella, Part 1: Detection of Salmonella spp. ISO 6579-1: 2017 (en) available at: https://www.iso.org/en/standard/56712.html, 2017.

[35] ISO 6888-1, Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species), Part 1: Method using Baird-Parker agar medium. ISO 6888-1: 2021(fr) online available at: https://www.iso.org/fr/standard/76672.html 2021.

[36] Paka E, Bouka EC, Melila M, and Amouzou K, "Effect of temperature on the degradation of ascorbic acid (VitamiC) contained in infantil supplement flours during the preparation of porringes," int J. Adv. Res, pp. 116-121, 2020.

[37] AFNOR, Norm; food product; microbiology; analytical technique; microbiological analysis. in Agro-food Compendium. 2000.

[38] Umeakunne K and Hibbert JM, "Nutrition in sickle cell disease: recent insights," Nutr. Diet. Suppl., vol. Volume 11, pp. 9-17, May 2019, https://doi.org/10.2147/NDS.S168257

[39] Loba S, gbakayoro J, Kouame K, Gridji G, and Brou K, "Formulations of mixed flours, one based on rice (oryza sativa) and the other on corn (Zea mays) for children of weaning age." », European Scientific Journal, 2019.

[40] Mouquet C, Icard V, Guyot J, Tou E, Rochette I, and Tr èche S, "Consumption pattern, biochemical composition and nutritional value of fermented pearl millet gruels in Burkina Faso. », International Journal of Food Sciences and Nutrition, p. 716-729, 2008.

[41] Soro S, Konan G, Elleingand E, N'guessan D, and Koffi E, "Formulation of infant foods based on yam flours enriched with soy." African Journal of Food agriculture nutrition and development », p. 8313-8339, 2013.

[42] Archer DR, Stiles JK, and Newman GW. "C-reactive protein and interleukin-6 are decreased in transgenic sickle cell mice fed a high protein. », diet. J Nutr., p. 1148-52., 2008.

[43] Kaul DK, Zang X, and Dasgupta T, "Arginine therapy of transgenic-knockout sickle mice improves microvascular function by reducing non-nitric oxide vasodilators, hemolysis, and oxidative stress." », Am J Pediatr-Heart Circul Physiol., p. 39-47., 2002.

[44] Natta C, Tatum VL, and Chow CK, "Antioxidant status and free-radical induced oxidative damage of sickle erythrocytes. », Ann NY Acad Sci., p. 365-7, 1992.

[45] Ohnishi ST, Ohnishi T, and Ogunmola G. B, "Sickle cell anemia: A potential nutritional approach for a molecular disease. », Nutr., pp. 330-8., 2000.

[46] Prasad AS, « Clinical, immunological, anti-inflammatory and antioxidant roles of zinc », Exp. Gerontol., vol. 43, no. 5, pp. 370-377, May 2008, https://doi.org/10.1016/j.exger.2007.10.013

[47] CAC, « Guidelines for the development of formulated complementary foods for older infants and young children, pp. 1-223. », pp. 1-223, 2013.

[48] Lutter C and Dewey K. « Proposed nutrient composition for fortified complementary foods. », Journal Nutr., p. 3011S-20S., 2003.

[49] CODEX STAN 72 – 1981, "Standard for infant formula and formulas for special medical purposes intended for infants," Codex Alimentarius, pp. 388-397, 2007.

[50] WHO/UNICEF, "Complementary feeding of young children in developing countries: a review of current scientific knowledge WHO/NUT/98.1Geneva: World Health Organization," Journal of the Clinical Biology Society of Benin, pp. 12-18, 1998.

[51] Khan SA, Damanhouri G, Ali A, Sarah A. Khan, Khan A, Bakillah A, Marouf S, Ghazi Al H, Halawani SH and Makki A., "Precipitating factors and targeted therapies in combating the perils of sickle cell disease---A special nutritional consideration," Nutr. Metab., vol. 13, no. 1, p. 50, Dec. 2016, https://doi.org/10.1186/s12986-016-0109-7

[52] Wright R, Lee K, and Hyacinth H, "An investigation of the antioxidant capacity in extracts from moringa oleifera plants grown in jamaica. Plants. 2017; 6(48): 1–8. https://doi.org/10.3390/plants6040048 p. 1-8, 2017.

[53] Essa D, Kondiah P, Choonara Y, and Pillay V, "The Design of Poly (lactide-co-glycolide) Nanocarriers for Medical Applications." Front Bioeng Biotechnol. 2020; 8: 48", p. 48, 2020.

[54] Alozie Y, Iyam M, Lawalo O, Udofia U, and Ani I, "Use of Bambara flour as a complementary food.", Journal of Food Technology, pp. 111-114, 2009.

[55] Tarhouni A, Djendoubi N, Amri F, Elbour M, Sadok S, and Mihoubi BN, "Development of an integrated process for the valorization of sardinella: effect of temperature and blanching on the nutritional value and microbiological quality of finished products.", Bulletin of the National Institute of Marine Sciences and Technologies Salammb ô, p. 69-71, 2015.

[56] Sanou A, Tapsoba F, Zongo C, Savadogo A, and Traore Y, "Study of the nutritional and microbiological quality of infant flours from four production units: CMA Saint Camille in Nanoro, CSPS Saint Louis in Temnaor é, CM Saint Camille in Ouagadougou and CHR in Koudougou.", Nature & Technology Journal., pp. 25-39, 2017.

[57] Hama-ba F, Silga P and Diawara B, "Evaluation of the quality and acceptability of couscous based on three composite flour formulations enriched with soy (Glycine max) and moringa (Moringa oleifera)," in J. chem sci, p. 2497-2510, 2016.

[58] Bouka Goto C, Bangana A, and Sidi-Touré S, "Fortification of babies with local foodstuffs," in: Grains for Feeding the World. Presented at the EXPO MILANO 2015, Le Universit àper expo 2015, Comitato Scientifico Del Comune Di Milano, Milan, Italy," pp. 29-34, 2015.

[59] Paka E, Boyode P, Melila M, Houndji SVB, Bilabina I, and Amouzou K, "Physicochemical characteristics of infant supplement flours produced by the Togolese Institute of Agronomic Research (ITRA)," Afrique Science, pp. 71-82, 2019.