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Manufacturing of Cowpea (*Vigna unguiculata*) based infant flour to fight child malnutrition in the Boeny Region, Madagascar

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ABSTRACT :

Child malnutrition is a major health and socio-economic challenge in Madagascar, with a rate exceeding the emergency threshold in certain areas like the Boeny Region. Facing the necessity for local and sustainable nutritional solutions, this study objective to validate the feasibility and nutritional performance of infant flour formulated with cowpea flour and green banana flour. Following a literature review, a computer assisted formulation of infant flour combining these two flours was undertaken with Solver software. A familial technology for its manufacturing is then set up. Macroelements and microelements composition, hygienic as well as the sensory qualities of the flour were analyzed. Its nutritional performance and toxicity were evaluated through weight monitoring and 21-day subchronic preclinical study on 15 Wistar rats, divided into three groups of five rats each fed with habitual laboratory fed, plumpy nut and our infant flour. Anatomopathological examination was finally performed to confirm the safety. The results showed an average cowpea flour manufacturing yield of 65% and banana flour yield of 16%. Data processing leads to mix 50/50 proportion of cowpea and green banana flours, forming by carbohydrate (56,94%), protein (18,4%), fat (16,8%) and ash (7,86%). Its micronutrients include chiefly phosphorus (1291,67 mg/kg), sodium (3439,17mg/kg) , potassium (1850,78mg/kg) , calcium (387,49mg/kg) and iron (30,15mg/kg). In vivo comparison test confirmed good nutritional performance with non-significant weight gain (p=0.057). The histological examination of vital organs revealed no tissue or cellular lesions, attesting to the flour's non-toxicity alongside satisfactory the organoleptical acceptability. It is recommended to promote the production and valorization of this flour among households and nutritional centers in the Boeny Region. Future work will focus on the popularization and in vivo experimentation of this infant flour in children.

Keywords : Child malnutrition, Vigna unguiculata, mixed cowpea-banana infant flour, nutritional performance, preclinical study, Boeny, Madagascar.

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I. INTRODUCTION

Universal access to adequate and healthy food is a fundamental right and an essential prerequisite for human development, as defined by the Food and Agriculture Organization of the United Nations **[1]**. However, food insecurity remains a persistent scourge, complicated by a convergence of factors such as conflicts, climate shocks, economic crises, and structural poverty **[2]**.

The consequences of this food insecurity are particularly devastating for vulnerable populations, including children under five years old. According to the World Health Organization (WHO), approximately 150.8 million children under five years old suffered from stunting in 2018, with 50.5 million affected by wasting **[3]**. Madagascar faces a critical nutritional challenge, with chronic malnutrition (stunting) affecting 42 % of children under five **[4]**. A 2024 survey conducted by our team in the Boeny Region revealed

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alarming figures where the acute malnutrition rate was 6.5%, chronic malnutrition was 56.4%, and underweight was 6.8% **[5]**, exceeding the humanitarian emergency threshold set by the WHO.

Traditional complementary foods used during the weaning period often have low energy and protein density, failing to meet the increased nutritional needs of the growing child **[5, 6]**. Given this deficit, the search for local, accessible, and high-performing nutritional solutions is essential. In this topic, cowpea, a resilient legume widely cultivated in Africa and Madagascar, represents an exceptional source of vegetable protein and other micronutrients, positioning it as a choice ingredient for enriching weaning foods **[7]**.

Hence, this study aims to evaluate nutritional value and safety of infant flour formulated from cowpea and green banana. The specific objectives are (1) to determine the optimal formulation of an infant flour combining cowpea and green banana flours, (2) to evaluate the nutritional performance of this new formula through a preclinical study compared to an international therapeutic standard, (3) to confirm the non-toxicity of the product through biometric analysis and anatomopathological examination of organs, and (4) to evaluate its organoleptical acceptability among a panel of tasters.

II. MATERIALS AND METHODS

II.1. Materials

II.1.2. Study setting

The flour formulation was carried out in urban Commune of Mahajanga I, Region Boeny, Madagascar. It is located in the northwest of the island, bordered by the Indian Ocean to the west, and by the Regions of Sofia (north and east), Betsiboka (southeast) and Melaky (southwest). Its area is 31,046 km2, housing 1,169,782 inhabitants, distributed across 46 Communes and 522 Fokontany.

Nutritional elements analysis were undertaken in the Laboratories of the Department of Zootechnical and Fishery Research of Rural Research Center (FOFIFA) and the National Institute for Nuclear Science and Technique (INSTN), while sensory and hygienic quality analysis in the laboratories of Food and Agricultural Industries of the High School of Agricultural Sciences and the Food Security and Quality Control Agency (ASCQDA) of the Ministry of Public Health. Performance and toxicity studies were conducted in the laboratory of the National Center for Pharmaceutical Research (CNARP) and the anatomopathological examination took place at the laboratory of the University Hospital Center of Joseph Ravoahangy Andrianavalona (CHU-JRA) Antananarivo.

II.1.2. Study samples

Cowpea grains, green banana and ingredients (salt, sodium bicarbonate and citric acid) were purchased at the local market. After its production, appropriated flour samples were provided to targeting laboratories. Habitual laboratory feed, Plumpy Nut (reference food for the management of child malnutrition) and formulated infant flour were used for performance and toxicity study.

II.1.3. Study population

Fifteen rats, divided into three lots of five rats each, were subjected to the flour's performance and toxicity study. Following weight monitoring and 21-day subchronic preclinical study, rats were sacrificed, autopsied and five vital organs (Liver, spleen, kidney, heart and stomach) were sampled for the histological examination.

Twenty-two Master degree students of Food and Agro-Industries Department performed the two stages sensory analysis. Firstly, local popular infant flour (Koba aina) was used to evaluate nature formulated infant flour in order to get panelists recommendation for its improvement. Then, optimized formulated infant flour submitted hedonic and descriptive tests.

II.1.4. Data processing tools

Computer equipped with various softwares such as Solver, Word, Excel and XLSTAT were used to formulate infant flour from cowpea and green banana alongside to process data and undergo statistical analysis.

II.2. Methods

II.2.1. Cowpea flour production

Cowpea flour process includes sorting, misting, shelling and winnowing soaking.

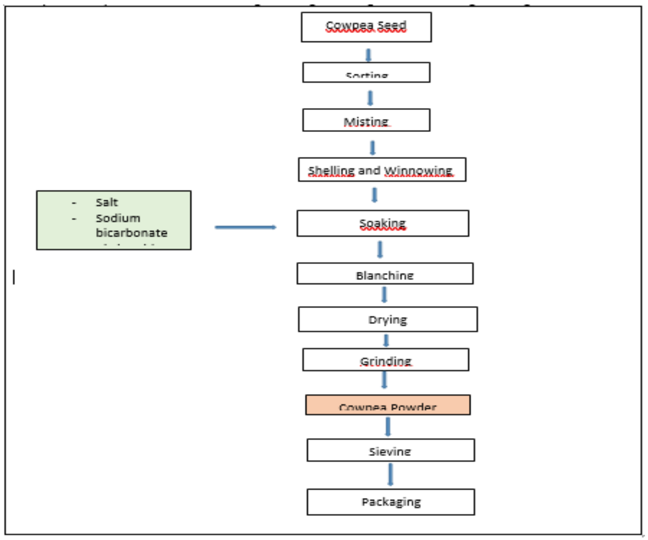

Figure 1. **Cowpea flour process production**

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II.2.2. Banana flour production

After selecting well-green and firm bananas, banana flour process included five steps: washing (to remove dirt), peeling, and cutting, sun drying, grinding and packaging.

II.2.3. Infant flour formulation

Infant flour formulation was undertaken with extended Excel SOLVER software by linear procreation. Using nutritional value of cowpea and green banana flour as raw materials to fulfill recommended daily infant nutritional needs, it leads to 50/50 proportion. Its process included easily three stages: weighting of each flour, mixing and packaging.

II.2.4. Infant flour quality analysis

Infant flour quality analysis included nutritional value analysis by Near Infrared Spectrophotometry and utilization of Energy-Dispersive X-ray Fluorescence and gravimetry, habitual flour microbiology quality control of the Food Security and Quality Control Agency (ASCQDA) Laboratory (numeration of total mesophilic flora, total coliform, *E. coli*, *Staphylococcus aureus*, yeast and mold and Bacillus cereus) and sensorial analysis by trained panelists (hedonic, discriminative and descriptive tests).

II.2.5. Nutritional performance and toxicity tests

The experiment followed standardized protocol conducted in the laboratory of the National Center for Pharmaceutical Research (CNARP) in Antananarivo laboratory. Fifteen rats have been divided into three lots of five rats each: lot 1 (control) served with usual feed, lot 2 (reference food) served with Plumpy Nut (reference food for the management of child malnutrition) and lot 3 (formulated Infant Flour) served with the infant flour.

Daily fed ration was served according to the recommendation of the National Center for Pharmaceutical Research laboratory. Residual feed was weighed daily, and body weight of the rats was taken every three days. They were monitored for 21 days daily weighting before being sacrificed for the toxicity study. In this topic, organs such as liver, spleen, kidneys, heart, and stomach were collected, stored in 10% formol and send for anatomopathological examination.

II.2.6. Data processing

The data were processed using WORD, EXCEL, and XLSTAT softwares. Analysis was performed using the descriptive statistic and ANOVA test. Specific responses of sensorial analysis were scored (/5 and or /20) and plotted.

III. RESULTATS

III.1. Production yield and nutritional values of the infant flour

The production yield for cowpea flour was 65% and 16% for banana flour. Table 1 presents the nutritional values of the formulated infant flour.

Table 1: Nutritional values of the formulated infant flour

Macronutrients	Values (%)	Micronutrients	Values (mg/kg)
Moisture	7.27	Sodium	3,439.17
Dry matter	92.73	Phosphorus	1,291.67
		Potassium	1,850.78
Carbohydrate	56.94	Magnesium	193.00
Protein	18.40	Calcium	387.49
Fat	16.80	Zinc	16.33
Ash	0.59	Iron	30.15

Crude energy (Kcal/100g) : 452.56

Table 1 shows that the formulated infant flour is not only highly energetic but also nutritive.

III.2. Production yield and nutritional values of the infant flour

Following Table 2 presents the microbiological quality of the formulated infant flour.

Table 2 presents the microbiological quality of the formulated infant flour.

Microorganism	Criteria (UFC/g)	Résults	Assessment
TAM at 30°C	<3. 105	<3.106	Satisfaying
Total coliforms	$1,10.10^2$	<1	Satisfactory
Coagulase-positive Staphylococci	<1	<1	Satisfactory
Escherichia coli	10	<1	Satisfactory
Bacillus cereus	1,0.	<1	Satisfactory
Yeasts and molds	$1,0.\ 10^3$	<1	Satisfactory
ASR à 46 ° C	$1,0.\ 10^2$	<1	Satisfactory
	Conclusion		Satisfactory

Table 2 confirms that its microbiological quality is satisfactory.

III.3. Nutritional performance

Table 3 presents the nutritional performance of the formulated infant flour

Table 3 Evolution of rat weight according to the different feeds (g)

Jour	LOT 1	LOT 2	LOT 3	*P value*	Conc lusio

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J3	105,060±1,729^{a} 103,000-107,100	105,340±1,919^{a} 102,400-107,500	103,660±4,824^{a} 95,200-106,900	0,675	NS
J6	105,620±3,183^{a} 101,000-109,000	104,180±1,384^{a} 102,300-106,100	103,300±2,678^{a} 100,000-106,200	0,373	NS
J9	108,680±3,520^{a} 104,000-113,300	105,760±1,885^{a} 103,800-108,100	109,040±5,086^{a} 102,400-115,000	0,346	NS
J12	112,120±3,899^{a} 108,300-118,600	106,480±2,487^{a} 103,500-109,100	105,680±7,306^{a} 97,600-115,000	0,126	NS
J15	114,020±3,931^{a} 110,400-120,400	108,520±2,690^{ab} 105,800-112,900	105,120±3,018^{b} 102,100-110,000	**0,003**	**S**
J18	115,220±3,946^{a} 111,100-120,400	106,120±6,977^{b} 93,800-110,600	109,650±3,034^{ab} 106,000-112,900	**0,048**	**S**
J21	115,960±3,808^{a} 110,000-119,469	110,244±2,616^{a} 106,977-113,131	111,811±3,749^{a} 107,143-115,714	0,057	NS

Mean ± standard deviation, Min – Max (minimum - maximum) S (significance) NS (non significance).

The monitoring of rat growth shows that the effect of the diet was not statistically significant (NS) until Day 12, indicating similar initial growth performance between the lots. A significant difference (S) appeared at Day 15 (P=0.003) and Day 18 (P=0.048). On these days, Lot 1 (Control) showed superior performance compared to Lot 3 (Infant Flour) But, this difference tends to fade at Day 21 (P=0.057). Formulated infant flour has then similar nutritional performance as the laboratory usual feed and Plumpy Nut which is the reference food for the management of child malnutrition. This result is very encouraging.

III.4. Toxicity

Table 4 of the next page presents the effects of the consumption of formulated infant flour on vital organs.

The table 4 shows significant weight variations in the direction of atrophy for Lot 1 (Control) compared to Lot 2 (Plumpy nut) and Lot 3 (formulated infant flour) for the liver, spleen, kidneys, and heart. Only the stomach weight variation was non-significant (NS).

Table 4: Weight of rat vital organs according to the different feeds (g)

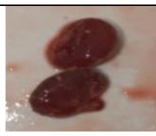
Organs	**LOT 1**	**LOT 2**	**LOT 3**	***P value***	**Conclusion**
Liver	7,625±0,171^{a} 7,400-7,800	6,425±0,619^{b} 5,900-7,100	4,700±0,717^{c} 4,000-5,500	**<0,001**	**S**
Spleen	0,900±0,216^{a} 0,600-1,100	0,650±0,578^{a} 0,600-0,700	0,525±0,126^{b} 0,400-0,700	**0,017**	**S**
Kidney	1,500±0,141^{a} 1,400-1,700	1,125±0,09^{b} 1,000-1,200	1,000±0,141^{b} 0,900-1,200	**0,001**	**S**
Heart	0,825±0,050^{a} 0,800-0,900	0,625±0,958^{b} 0,500-0,700	0,600±0,115^{b} 0,500-0,700	**0,013**	**S**
Stomach	4,000±2,392^{a} 2,100-7,500	1,600±0,216^{a} 1,400-1,900	3,975±2,640^{a} 1,500-6,900	0,222	NS

Mean ± standard deviation, Min – Max (minimum - maximum) S (significance) NS (non significance).

The table 3 shows significant weight variations in the direction of atrophy for Lot 1 (Control) compared to Lot 2 (Plumpy nut) and Lot 3 (formulated infant flour) for the liver, spleen, kidneys, and heart. Only the stomach weight variation was non-significant (NS).

III.5. Anatomopathological examination of organs

Photo 1 presents the histological examination of the organs of each rat of the 3 lots.

Photos	**Results**	**Photos**	**Results**
	Hearts were of normal structure, composed of non-atypical myocardial tissue		Spleens were also normal with non-atrophic white pulp and moderately congested red pulp.
Heart		Spleen	

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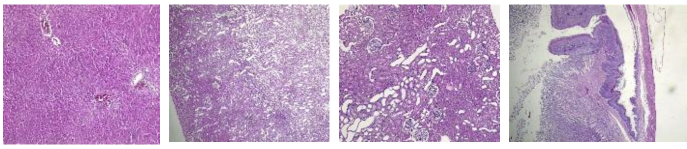
	Kidneys were also normal, without necrosis, fibrosis, or inflammatory infiltrate.		Livers were also normal, devoid of atypia, inflammation, vacuolization, or overload pathology
Kidney		Liver	

	Stomachs were well-differentiated, keratinized malpighian and glandular, devoid of atypia, metaplasia, or inflammation.
Sacrificed rat	Stomach

Photo 1 Histological examination of the organs of sacrificed rat

Despite the significant variations in the relative weight of some organs, the anatomopathological examination concluded that all vital organs were of normal structure, without lesions, necrosis, or atypia.



Liver (x40) Kidney (x40) Kidney (x100) Stomach (x40)

Photo 2: Tissue microphotography

These microphotographies confirm the non-toxicity of the formulated infant flour.

III.5. Sensory quality

Table 5 presents sensorial quality of the Banana flour and nature Infant flour.

Table 5: Sensorial quality of the Banana flour and nature Infant flour

	General appreciation	Color	Odor	Taste	Texture in mouth	Consistency
Banana flour	2,682 a	3,000 a	2,182 a	2,273 a	3,455 a	3,500 a
Infant flour	2,409 a	2,455 b	2,045 a	1,955 a	2,864 b	3,682 a
Pr> F	0,219	0,025	0,584	0,181	0,007	0,33

						8
Signification	No	**Yes**	No	No	**Yes**	No

The results of the hedonic test show a significant difference concerning Color and Texture in the mouth, but a non-significant difference for General appreciation, Odor, Taste and Consistency. Improvement has been then undertaken in order to improve its acceptability. Its concerns sugar dose, color and taste. Sensorial score of hedonic, discriminative and descriptive tests of the improved infant flour are presented on figures in the following page.

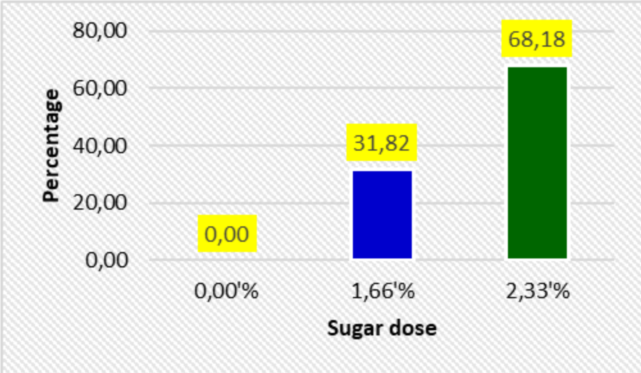


Figure 1: Sugar dose recommended

Figure 2: Product detection

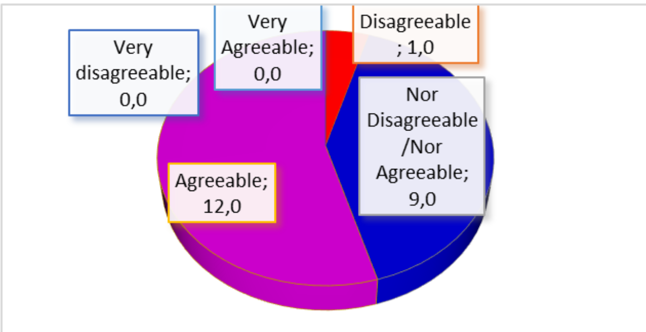


Figure 3: Overall acceptability of the improved formulated infant flour

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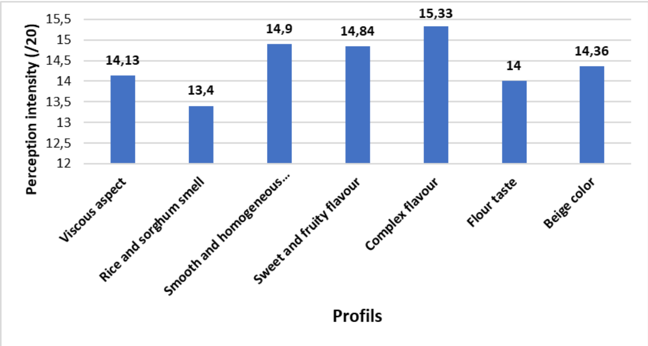

Figure 4: Sensory score of the improved formulated infant flour

Panelists recommended sugar dose of 2,33% equal to 1 coffee spoon for 20g of formulated infant flour and It is easy to detect among various infant flour presented. Hedonic attests to a positive overall product appreciation; it is generally agreeable. Our infant flour has a complex flavor, particularly sweet and fruity, with wrongly percept of rice and sorghum smell but a smooth and homogeneous texture. Flour taste has been recalled and its color is beige.

IV. DISCUSSION

Malnutrition is a worldwide public health problem, chiefly in developing country like Madagascar **[1, 2, 3, 5]** but our results demonstrate that fabrication of infant flour based on Malagasy local raw material is feasible and benefic to cope with. These support other African work **[8, 9, 10]**.

Processing production is closed to all dray beans treatment **[11, 12]**. The production yield of the finished product compared to the raw material is 65%. This is significantly lower than the 88% found by Ramiadamanana (2022) **[12]**. The difference in yield may be due to the traditional grinding technique used, such as the mortar and pestle. Although optimization is possible, such as it is done by Ihediohanma et al. (2018) **[13]**, Jarrard (2007) **[14]** and Christ (2018) **[11]**.

Many studies have shown the benefit of combining cereals or tubers with legumes to improve the nutritional value of weaning foods **[15]**. Cowpea is valued for its richness in protein (about 22.4g per 100g) **[8]** and its agronomic resilience. The mineral analysis of the raw material revealed naturally high contents of essential elements, confirming the potential of cowpea to naturally enrich the flour and directly combat childhood deficiencies. The presence of Iron and Zinc is particularly relevant, as these micronutrients are vital for the prevention of anemia and the improvement of immunity and growth in children, which are endemic problems in Madagascar. The use of green banana, which is rich in starch, also improves the energy density of the porridge, a major challenge for traditional African flours which are often too diluted and hypocaloric **[6]**.

The absence of a significant difference in weight gain between the infant flour lot (Lot 3) and the Plumpy Nut lot (Lot 2) at Day 21 (p=0.057) indicates that the infant flour has a comparable feeding efficacy to a reference product used to treat child malnutrition. This capacity to compete with a high-energy and high-protein product like Plumpy Nut reinforces the argument that cowpea is a viable and local alternative for the treatment and prevention of malnutrition.
In addition, the anatomopathological examination concluded that all vital organs were of normal structure, without lesions, necrosis, or atypia. This confirms the non-toxicity of the formulated infant flour base on cowpea, as it reported by Dadie et *al*. (2003) **[16]**.
In this study, the general appreciation of the new formula is not significantly different from that of the local reference product ($p > 0.181$). This is a major success, because even if variations were noted in color and texture, the taste and overall acceptability are maintained. This suggests that the cowpea processing (soaking and blanching) successfully mitigated unwanted flavors or texture factors. This formulated infant flour is as good as it is produced somewhere else **[6, 8, 9, 16]**.

V. CONCLUSION

The objectives of this research, focused on the valorization of cowpea for the production of an effective infant flour to cope with infant malnutrition in the Region of Boeny have been achieved. In fact, the set of analysis and experimentation results validated our hypothesis. Hence, it provided a solid scientific basis for promoting this local flour, thereby contributing to strategies for improving the nutritional status of children in the Boeny Region.

The optimal formulation lead to 50/50 cowpea/banana and yielded a product that demonstrated high nutritional value and good hygienic quality. Furthermore, the subchronic preclinical study showed that the weight gain of rats receiving the cowpea-based flour is comparable to that obtained with Plumpy Nut, a standard therapeutic reference and habitual laboratory fed. The anatomopathological examination of organs concluded the total absence of toxicity signs after 21 days of consumption, guaranteeing the product's safety. Finally, the formulated flour was judged comparable to the reference flour by the panel with satisfactory acceptability, which facilitates its potential integration into dietary habits.

The infant flour developed from cowpea and green banana constitutes an effective, local nutritional products alternative for complementary foods. This work paves the way for

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popularization and in vivo trials in children, significantly contributing to the strengthening of food security and offering decision-makers and field actors a sustainable solution to improve the health and development of Malagasy children in the Boeny Region. It is recommended to promote the production and valorization of this flour among households and nutritional centers in the Boeny Region. Future work will focus on the popularization and in vivo experimentation of this infant flour in children.

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