# Nutritional and Microbiological Qualities and Storage Stability of Ready-to-Use Foods Based on Cocoa or Cashew


## Abstract

Child malnutrition in Côte d'Ivoire represents a major public health challenge, requiring effective nutritional solutions such as Ready-to-Use Foods (RUFs). However, their limited accessibility complicates the effective management of malnourished children. This study aims to develop Ready-to-Use Foods based on cocoa or cashew while evaluating their nutritional quality, microbiological safety, and storage stability. Four RUFs (LF1, LF2, LF3, and LF4) were formulated and produced using linear programming and domestic production processes. Their nutritional and microbiological composition was analyzed according to standard methods. Their stability over a one-month storage period was assessed in 100 mL polypropylene plastic containers using acid value (AV) and peroxide value (PV) indices, enabling the estimation of their shelf life. The results showed that the tested formulae (LF1 to LF4) met nutritional recommendations for malnourished children, with high protein content (15.02 to 15.40 g/100 g) and energy density (526.66 to 530.76 kcal/100 g). Microbiological analyses confirmed the absence of pathogens, and lipid stability indicated minimal degradation, with an estimated shelf life of at least four months.In conclusion, these locally developed RUFs offer a promising solution to combat child malnutrition, although further studies are necessary to validate their acceptability and clinical efficacy.



**Keywords**: Child malnutrition, Ready-to-Use Foods (RUFs), nutritional quality, microbiological quality, lipid stability.


## Introduction

Child malnutrition remains a major public health challenge in Côte d'Ivoire, where approximately 30% of children under five suffer from chronic malnutrition, and 6% experience severe acute malnutrition **[1]**. These alarming figures highlight the need for targeted nutritional interventions for this particularly vulnerable population. Ready-to-Use Foods (RUFs), known for their effectiveness in treating child malnutrition **[2, 3]**, remain largely inaccessible due to their high cost and limited availability, especially in peri-urban and rural areas.

In response to these challenges, the development of RUFs using local ingredients represents a sustainable and economically viable solution **[4, 5]**. Raw materials such as cocoa and cashew, which are abundantly available in Côte d'Ivoire, offer remarkable nutritional potential. Cocoa is a significant source of lipids, antioxidants, and minerals such as magnesium and iron **[6]**. Cashew, on the other hand, is rich in high-quality proteins, essential fatty acids, and vitamins, making it suitable to meet the specific nutritional needs of malnourished children **[7]**.

However, the formulation of such foods requires a rigorous assessment of their nutritional quality to ensure adequate supplies of macronutrients (proteins, lipids, and carbohydrates) and essential micronutrients (iron, zinc, vitamins) to meet the needs of malnourished children **[8, 9]**. In addition to their nutritional quality, the microbiological safety and storage stability of RUFs are critical parameters to consider. Foods must be free of microbial contaminants to avoid health risks, particularly in contexts where preservation and distribution infrastructures are limited **[10, 11]**.Lipid stability, often challenged by oxidation, poses a significant hurdle as this process can degrade the organoleptic and nutritional properties of products, while also presenting potential health risks.

This study aims to develop Ready-to-Use Foods based on cocoa or cashew, while evaluating their nutritional quality, microbiological safety, and storage stability.

## Materials and Methods

### Design of Local RUFs

In this pilot study, local Ready-to-Use Foods (RUFs) were designed to meet the nutritional needs of children aged 6 to 59 months suffering from moderate acute malnutrition **[12]**. The design of these foods considered the following factors: the nutritional value of each ingredient, their availability (quantity, regular supply, seasonality, and relative price stability), and cost. Linear programming was employed to achieve the theoretical formulation of the RUFs, following the method described by **[4]**. Consequently, four RUFs were formulated and named Local Formula (LF) 1, 2, 3, and 4 (**Table 1**).

**Table I. Formulations of Local RUFs**

| Ingredients (%) | LF1 | LF2 | LF3 | LF4 |
|---|---|---|---|---|
| **Cashew paste** | | 16 | | 16 |
| **Cocoa paste** | 07 | | 07 | |
| **Rice flour** | 26 | 22 | 26 | 22 |
| **Soy flour** | 28 | 27 | 26 | 25 |
| **Enrichedeggyolkpowder** | | | 03 | 03 |
| **Refined palm oil** | 22 | 21 | 21 | 20 |
| **Icingsugar** | 13 | 10 | 13 | 10 |
| **MVP (Mineral-VitaminPremix)** | 03.5 | 03.5 | 03.5 | 03.5 |
| **Emulsifier** | 0.5 | 0.5 | 0.5 | 0.5 |
| **Total** | **100** | **100** | **100** | **100** |

**Note**: MVP = Mineral-Vitamin Premix; LF = Local Formula

### Domestic Production Process of Local RUFs

The production of RUFs, including ingredient processing, domestic tools, and the actual preparation, was carried out according to the method described by **[5]**.

### Nutritional Composition of Local RUFs

The locally produced RUFs and the reference RUF (Plumpy'Sup) were analyzed to determine total dry matter, moisture, crude lipids, proteins, fiber, and ash content, using standard methods described by

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**[13]**, in triplicate. Carbohydrate content was estimated through differential calculation **[14]**, while the energy value was calculated using the metabolic energy conversion factor, also known as Atwater's general factors **[15]**.

### Microbiological Analysis of the Formulae

Samples of the food formulae were subjected to a series of microbiological tests following the recommendations of **[16]**. The analyses targeted Total Aerobic Mesophilic Germs (TAMG), lactic acid bacteria, yeasts and molds, Salmonella, coliforms (total and fecal), enterobacteria, Escherichia coli, and staphylococci, in accordance with appropriate ISO or NF standards. Ten grams of each formulation were homogenized in 90 mL of Buffered Peptone Water (BPW), followed by serial decimal dilutions up to $10^{-5}$. The inoculations, performed in duplicate, were incubated on suitable media, and colony counts were performed as per the **[17]** standard. For Salmonella, 25 g of sample were analyzed using specific procedures. Results are expressed in Colony Forming Units (CFU/g), calculated according to the **[18]** standard. Samples were analyzed immediately after production in 100 mL polypropylene plastic containers stored at room temperature.

### Lipid Stability Analysis

The lipid stability of the formulae was assessed by monitoring the evolution of acid value (AV) and peroxide value (PV) during storage in 100 mL polypropylene (PP) plastic containers. The AV, corresponding to the amount of potassium hydroxide (KOH) needed to neutralize free fatty acids, was determined using the method of **[19]** after extracting fats through the Soxhlet method. The PV, indicating peroxide content, was measured according to the **[20]** standard. Analyses involved controlled titrations, and AV and PV were calculated using standard formulae based on the volume of solutions used and the mass of the samples. These analyses were performed at the start of storage and weekly for one month to assess lipid quality over time. For each indicator, an estimation of the formulae' shelf life was proposed based on standard limits of 3 mg KOH/g/day **[21]** for AV and 10 meq $O_2$/kg **[22]** for PV.

### Statistical Analysis

Collected data were entered into Excel and statistically analyzed using R (version 3.5.2), with graphical representations created using GraphPad Prism (version 7.00). Results are presented as means ± standard error (m ± SEM). A one-way analysis of variance (ANOVA) followed by Tukey's test was applied for all studies except for chemical stability, where a two-way ANOVA followed by the Least Significant Difference (LSD) test was conducted. The significance level was set at 5%.

## Results and Discussion

The comparative analysis of the local formulae (LF-1, LF-2, LF-3, LF-4) and the reference formula (Plumpy'Sup®) revealed significant differences (**Table II**). The cashew-based formulae (LF-2 and LF-4) exhibited higher moisture content, though still within recommended limits, resulting in lower dry matter compared to the cocoa-based formulae and Plumpy'Sup® (Reference). These lower moisture levels could be attributed to drying processes and the addition of sugar, which favor extended shelf life **[10, 23]**.

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The protein content in the local formulae, primarily derived from soy flour, ranged from 15.02 to 15.40 g/100 g. These values were higher than those of Plumpy'Sup® and met recommendations for malnourished children **[24, 25]**. The LF-3 and LF-4 formulae, enriched with egg powder, showed increased protein levels.Lipids were more abundant in the cocoa-based formulae, while remaining within the limits set by the World Food Programme. Fiber content was significantly higher in the local formulae, particularly in those without egg powder, providing additional physiological benefits.

Furthermore, carbohydrates in the local formulae, mainly sourced from rice and sugar, ranged from 37.01 to 38.34 g/100 g. However, these values were lower than those of Plumpy'Sup®. The energy values of the local formulae and the reference formula (526.66 to 539.11 kcal/100 g) were in line with recommendations, despite variations depending on the ingredients, and are suitable for nutritional rehabilitation **[25]**.

**Table II. Proximate composition of the local formulae compare to reference**

| Parameters (/100g) | WFP recommendations | | *Plumpy'Sup®* | LF1 | LF2 | LF3 | LF4 |
|---|---|---|---|---|---|---|---|
| | Min | Max | | | | | |
| **Moisture (%)** | _ | 5* | $2.05 \pm 0.07^{b}$ | $2.20 \pm 0.02^{b}$ | $2.40 \pm 0.18^{a}$ | $2.23 \pm 0.03^{ab}$ | $2.42 \pm 0.04^{a}$ |
| **Dry matter (%)** | _ | _ | $97.95 \pm 0.07^{a}$ | $97.80 \pm 0.02^{a}$ | $97.60 \pm 0.18^{b}$ | $97.77 \pm 0.03^{b}$ | $97.58 \pm 0.04^{c}$ |
| **Protein (g)** | 11 | 16 | $13.86 \pm 0.07^{c}$ | $15.02 \pm 0.32^{b}$ | $15.22 \pm 0.07^{b}$ | $15.40 \pm 0.07^{a}$ | $15.34 \pm 0.02^{a}$ |
| **Fat (g)** | 26 | 36 | $34.97 \pm 0.25^{b}$ | $35.28 \pm 0.05^{ab}$ | $35.64 \pm 0.02^{a}$ | $35.44 \pm 0.02^{ab}$ | $34.66 \pm 0.01^{c}$ |
| **Ash (g)** | _ | _ | $4.67 \pm 0.01^{a}$ | $4.35 \pm 0.01^{b}$ | $4.48 \pm 0.05^{b}$ | $4.63 \pm 0.05^{a}$ | $4.65 \pm 0.03^{a}$ |
| **Crude fiber (g)** | _ | _ | $2.18 \pm 0.04^{d}$ | $5.59 \pm 0.02^{a}$ | $5.25 \pm 0.05^{b}$ | $4.75 \pm 0.05^{c}$ | $4.59 \pm 0.0^{c}$ |
| **AC (%)** | _ | _ | $42.34 \pm 0.28^{a}$ | $37.56 \pm 0.08^{c}$ | $37.01 \pm 0.19^{d}$ | $37.55 \pm 0.19^{c}$ | $38.34 \pm 0.06^{b}$ |
| **Energy (kcal)** | 510 | 560 | $539.11 \pm 1.11^{a}$ | $527.84 \pm 0.18^{c}$ | $529.68 \pm 0.98^{b}$ | $530.76 \pm 0.98^{b}$ | $526.66 \pm 0.12^{c}$ |

Source: * **[10]**; AC: Available Carbohydrate. Results are expressed as the mean value ± standard error of three replicates. For assessing statistical significance, a one-way analysis of variance (ANOVA) followed by the Turkey test at the threshold of 5% was used. On the same column, the means followed by different superscript letters are significantly different (*p <0.05*).

Regarding microbiological quality (**Table III**), the local formulae exhibit aerobic mesophilic germ counts below the microbiological thresholds (<10□ CFU/g) established for managing acute malnutrition **[16, 25, 26]**. These low counts are likely due to the cooking, drying, and roasting processes applied to the ingredients (cocoa, soy, rice, eggs), which reduce moisture and water activity, as observed by **[27]** and **[28]**. The absence of hygiene indicator organisms (fecal coliforms) and pathogens (S. aureus, Salmonella) confirms the effectiveness of thermal treatments and adherence to good hygiene and manufacturing practices (GHP/GMP), ensuring satisfactory microbiological quality.

**Table III: Microbiological Quality of Produced Local Formulae**

| Microorganisms | Standards* | LF-1 | LF-2 | LF-3 | LF-4 |
|---|---|---|---|---|---|
| **TAMG** | < 10□ CFU/g | $2.54\times10^{2}$ | $2.37\times10^{2}$ | $2.75\times10^{2}$ | $2.10\times10^{2}$ |
| **Lacticacidbacteria** | | $5.9\times10^{1}$ | $3.6\times10^{1}$ | $5.5\times10^{1}$ | $3.4\times10^{1}$ |

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| | | | | | |
|---|---|---|---|---|---|
| **Total coliforms** | < 10 CFU/g | 1 | 1 | 1 | 1 |
| **Fecalcoliforms** | Abs | Abs | Abs | Abs | Abs |
| **Yeasts** | < 10 CFU/g | 2 | 2 | 2 | 2 |
| **Molds** | < 50 CFU/g | Abs | Abs | Abs | Abs |
| ***Salmonella*** | Abs/25 g | Abs | Abs | Abs | Abs |
| **Enterobacteria** | < 10 CFU/g | 1 | 1 | 1 | 1 |
| ***Escherichia coli*** | Abs/g | Abs | Abs | Abs | Abs |
| **Staphylococci** | Abs/g | Abs | Abs | Abs | Abs |

**Source**: ***** *[16]*; **TAMG**: Total Aerobic Mesophilic Germs; **Abs**: Absence; **CFU**: Colony Forming Units; **LF**: Local Formula

The stability of RUFs based on local ingredients was assessed over four weeks of storage at room temperature in 100 mL polypropylene containers. Acid values (AV), reflecting triglyceride hydrolysis (**Figure 1**), and peroxide values (PV), indicating primary lipid oxidation (**Figure 2**), showed moderate linear increases during storage.

The AVs of the LF-1 to LF-4 formulae (0.014 to 0.016 mg KOH/g/day) remained below the threshold of 3 mg KOH/g/day **[22]**, indicating minimal degradation and the preservation of nutritional value over the four weeks. The estimated shelf life ranged from 6.4 to 7.1 months, depending on the formula.

The PVs (0.024 to 0.04 meq $O_2$/kg/day) were also below the standard limit of 10 mEq$O_2$/kg **[23]**, confirming limited oxidation. The estimated shelf life ranged from 3.9 to 4.9 months. These findings are consistent with those of Pokhrel **[29]** and Niraula **[30]**, who reported similar increases using high-density polyethylene containers.

Finally, variations in PVs, influenced by the plastic packaging **[31, 32]** and the richness in unsaturated fatty acids (UFAs) in the egg yolk powders of LF-3 and LF-4, explained the slightly higher oxidation observed in these formulae.

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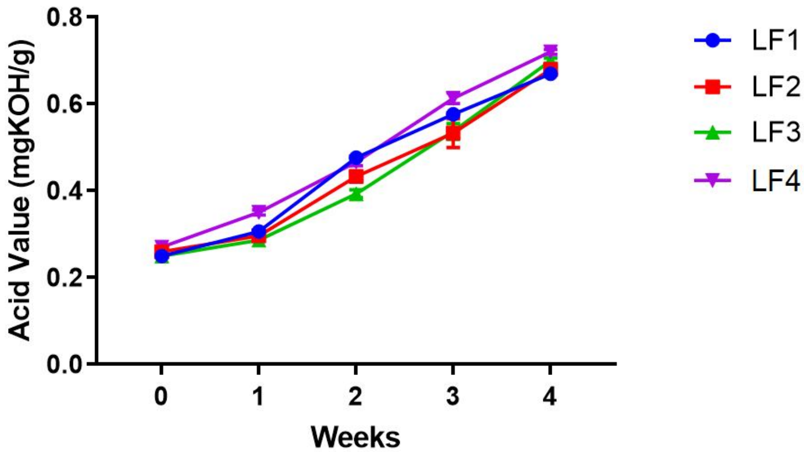


**Figure 1: Evolution of Acid Value During Storage of Local Formulae**
Each value represents the mean ± standard error (n = 3 repetitions). Following a two-factor analysis of variance (ANOVA), mean comparisons were performed using the Least Significant Difference (LSD) test at a 5% significance level.

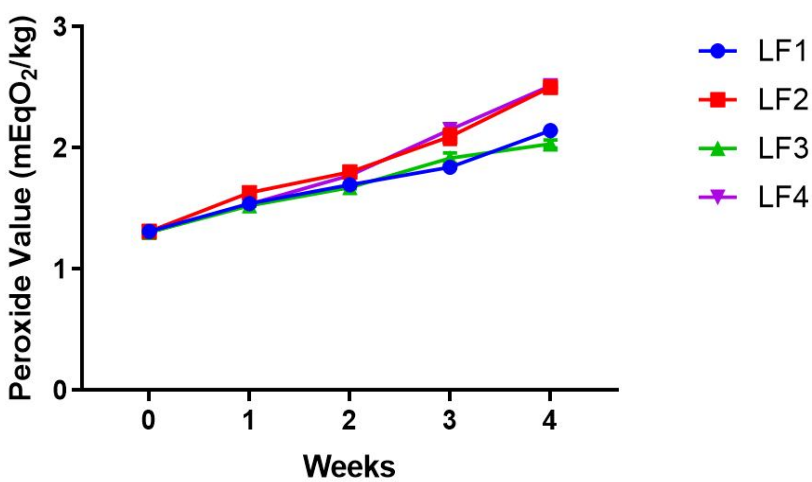


**Figure 2: Evolution of Peroxide Value During Storage of Local Formulae**
Each value represents the mean ± standard error (n = 3 repetitions). Following a two-factor analysis of variance (ANOVA), mean comparisons were performed using the Least Significant Difference (LSD) test at a 5% significance level.

## Conclusion

Local Ready-to-Use Foods (RUFs) based on cocoa and cashew offer a sustainable alternative for combating child malnutrition in Côte d'Ivoire. Their good nutritional quality, microbiological safety, and storage stability make them promising candidates for large-scale deployment. However, further studies on their sensory acceptability and clinical efficacy are needed to optimize their formulation and ensure their impact.

## Data Availability Statement

The data are available from the corresponding author upon reasonable request.

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