PHYSICO-CHEMICAL AND, NUTRITIONAL PROPERTIES OF UNFERMENTED AND FERMENTED ROSELLE (*HIBISCUS SABDARIFFA* LINN) SEEDS.

ABSTRACT

The aim of this study was to investigate the Physicochemical and nutritional properties of unfermented and fermented Roselle seeds. *Datou* is a product of the traditional alkaline fermentation of *Hibiscus sabdariffa* seeds consumed as food condiment in many African countries including Mali. However, no physicochemical and nutritional data has been reported on fermented Roselle seeds make in Mali. The study was conducted in the faculty of sciences and techniques between June 2021 and February 2022.

The unfermented and fermented Roselle seeds were used to determine their contents in moisture, ash, fat, crude proteins carbohydrate, fatty acids, amino acids and minerals.
The results showed that the studied unfermented seeds were composed 27.32% of crude proteins, 20.83% of crude lipids, 39.23% of carbohydrates and the fermented seeds showed 21.70% for proteins, 18.64% for crude lipids and 47.42% for carbohydrates.

The most predominant inorganic elements were found to be potassium, magnesium, calcium and sodium in all samples, but in different orders. Phosphorus was relatively low in all samples, however, the fermented compared to the unfermented seeds showed the highest content.

The fatty acids composition analysis showed the high differences in unsaturated compared to the saturated. The most predominant unsaturated fatty acid in both samples was found to be oleic acid.

The nutritional parameters were estimated based on the amino acids composition of our both samples. The results suggested that our samples have high nutritional quality. Based on the results obtained from this study we can concluded that unfermented and fermented Roselle seeds could be a good source for protein deficient consumers as well as a potential food ingredient

Keywords: unfermented-fermented-Roselle-seeds, physicochemical, minerals, fatty–acids-amino-acids-compositions, nutritional-parameters

1. INTRODUCTION

Hibiscus sabdariffa L. also known as Roselle, sorrel mesta belongs to the family of Malvaceae. The origin of *H. sabdariffa* is not fully known, but it is believed to be native of tropical Africa. The plant is widely distributed in the tropical regions, especially in the Middle Eastern countries [1], and “it is generally considered as a medicinal plant. The calyces or petals of the flower are extensively used to prepare herbal drinks, cold and warm beverages, as well as making jams and jellies” [2, 3]. Traditional fermented foods take a significant place in the African nutrition.

“They improve the population nutritional state for their nutritive values (proteins, minerals, vitamins). Datou is a food condiment obtained by a traditional uncontrolled fermentation of *H. sabdariffa* seeds in African countries, including Burkina Faso, Mali Niger, Nigeria, Cameroon and Sudan among others. It is also known as dawadawa botso (Niger), Datou (Mali), Furundu (Sudan), Mbuja (Cameroon)” [4, 5].

“Plant proteins are extensively recognized as an important source of affordable protein. In many African countries foods from animal sources are mainly consumed by households of higher socio-economic status and majority of the population does hardly access these foods due to poverty” [2].

Roselle is rich in protein and abundant in many countries (Africa and Asia). Besides that, in recognition of the worldwide need for cheaper protein sources for low-income groups in developing countries, there have been efforts to develop low-cost protein of plant origin. Previous studies showed that Roselle seeds could be used as a potential source of proteins and oil [6, 7].

“The physicochemical and nutritional proprieties of fermented Roselle seeds have not been documented as compared with the unfermented seeds. Literature indicated that Roselle whole seeds powder from other countries contained high amounts of protein, oil and carbohydrate” [4, 8]. However, no physicochemical and nutritional data has been reported on fermented Roselle seeds made in Mali. Therefore, the objectives of this study were to investigate the effects of the traditional uncontrolled alkaline fermentation on the physicochemical properties of Roselle seeds; that include proximate composition, minerals, fatty acids, and estimation of the nutritional parameters based on their amino acids composition.

2. MATERIAL AND METHODS

2.1 Materials

Fermented and unfermented Seeds of *Hibiscus Sabdariffa* were obtained from Bamako-Republic of Mali and the samples were transported to the laboratory for identification and analysis. All the chemicals were obtained from the commercial source and were of analytical grade quality.

2.2 Proximate chemical composition

The proximate analysis of unfermented Roselle seed (UFRS) and fermented Roselle seed (FRS) were determined according to AOAC [9]. The moisture content was determined by drying in an oven at 105 °C until a constant weight was obtained. Ash was determined by weighing the incinerated residue obtained at 550 °C for 8-12 hours. Total crude protein content was determined using the Kjeldahl method. The total lipid content in samples was determined by Soxhlet method. Available carbohydrates were calculated as 100% - [% (moisture +ash + fat + protein)].

2.3 Minerals composition

Samples were digested in 100 mL micro-Kjeldahl flask with HNO_3/HClO4 until the solution became colourless. The samples were cooled and diluted to volume in a 25 mL volumetric flask with 0.1 [M] HCl. Sodium, potassium, calcium, magnesium, iron and zinc, were measured by atomic absorption spectrophotometry, [10], modified by Tounkara et al.[2];

using a Varian spectra atomic absorption spectrophotometer (Varian SpectrAA220, Varian, Palo Alto, CA).

2.4 Fatty acid analysis

Fatty acids for the UFRS and FRS were determined according to the method of Ceirwyn [11]. Fat was extracted with methyl ether that was prepared directly with the treatment of the fat with sodium methoxide. Gas chromatography/mass spectra (GC/MS) system was used to identify and quantify the fatty acids of the product developed on a FINNIGAN TRACE MS gas chromatograph/mass spectra equipped with a 30 m x 0.25 mm Ov-1701 column. Column flow rate was 0.8 mL min^{-1} with helium as the carrier gas, split was 64 mL min^{-1} and the source temperature was 270 °C. The fatty acid methyl esters were identified by comparison with the retention times of NU CHECK Inc. standards (Elysian, 1L) and quantified by internal normalization.

2.5 Amino acids analysis

The dried samples were digested with HCl (6 mol L^{-1}) at 110 °C for 24 hours under nitrogen atmosphere. Reversed phase high performance liquid chromatography (RP-HPLC) analysis was carried out in Agilant 1100 (Agilent Technologies, PaloAlto, CA, U.S.A.) assembly system after precolumn derivatization with o-phthaldialdehyde (OPA) [12]. Each sample (1 μL) was injected on a Zorbax 80 A C18 column (i.d. 4.6x180 mm, Agilent Technologies, Palo Alto, CA, U.S.A.) at 40 °C with detection at 338 nm. Mobile phase A was 7.35 mmol L^{-1} sodium acetate/triethylamine/tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mmol L^{-1} sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g of amino acid per 100 g of protein.

2.6 Protein nutritional parameters

The nutritional parameters of FRS and UFRS were calculated using their amino acid composition including: (1) Proportion of essential amino acids (E) to the total amino acids (T) of the proteins. (2) Amino acid score (AAS) = (mg of amino acid per g of test protein/mg of amino acid per g of standard protein) ×100. The FAO/WHO/UNU reference pattern of essential amino acid requirements (g $100g^{-1}$ of protein) was used as the standard. (3) Predicted Protein Efficiency Ratio (PER) values. The predicted PER values of FRS and UFRS were estimated by three regression equations developed by [13].

I. PER = - 0.684 +0.456(Leu) - 0.047(Pro)

II. PER = -0.468 + 0.454(Leu) - 0.105(Tyr)

III. PER = -1.816 + 0.435(Met) + 0.780(Leu) + 0.211(His) - 0.944(Tyr).

2.7 Statistical analysis

All experiments were conducted at least in triplicate with SPSS software (version 16.0, the predictive analytics company, Chicago, U.S.A.). The data were subjected to a one way analysis of variance (ANOVA), followed by Duncan's multiple range test**.**

3. RESULTS AND DISCUSSION

3.1 Proximate chemical composition

Preliminary studies were conducted to assess the major chemical composition of the studied samples. The unfermented Roselle seed (UFRS) and fermented Roselle seed (FRS) flours were analyzed for moisture, crude protein, crude fat and ash using AOAC[9] and carbohydrate, which was determined as the remaining fraction. The results are shown in Table 1. The moisture content of all the tested samples was low; but the highest value was observed in FRS which can be attributed to the processing method. The URSF contained 27.32% and 39.23%of protein and carbohydrates respectively. The fat content was 20.83% for UFRS. Moreover, the fermented RSF contained 21.70% and 47.42% of protein and carbohydrates respectively. The fat content was 18.64% for FRS. The results showed that the carbohydrate content increased, moreover the crude protein decreased in FRS. "The results of our study were within the range reported for other samples studied" [4, 14]. "Other researchers found that the carbohydrates were mainly composed of dietary fibers" [15].

Table 1. Proximate chemical composition (%) of fermented and unfermented Roselle Seeds

Samples	**Parameters (%)**				
	Protein(%N x 6.25)	**Moisture**	**Fat**	**Ash**	**Carbohydrate**
FRS	21.70±0.2	6.96±0.1	18.64±0.32	5.25±0.1	47.42±0.5
UFRS	27.32±0.39	8.15±0.1	20.83±0.5	4.47±0.11	39.23±0.60

All values are Means and standard deviations of three replicates. FRS: Fermented Roselle seeds, UFRS: Unfermented Roselle seeds. %N: Nitrogen Percentage

3.2 Fatty acid analysis

"Fatty acid composition of the oil extracted from Roselle seeds is given in Table 2. The oil of FRS showed considerable fat content even when compared with oil seeds; with around 70% of unsaturated fatty acids. Oleic and linoleic acids were the highest fatty acids, and accounted for 40.29 and 22.57% in FRS; 36.9 and 35.02¨% in UFRS. However, arachidic acid had the lowest levels among the unsaturated fatty acids (0.47 and 0.67%) respectively for FRS and UFRS. Whereas, palmitic acid (19.21% UFRS; 12.34 FRS) was the highest among the saturated fatty acids content. The results of fatty acids composition confirmed the high shear in unsaturated fatty acids, indicating the nutritional benefit of our studied samples. Linoleic acid had beneficial effect on blood lipids, lowering blood pressure and serum cholesterol" [16, 17]. These results are in good agreement with the findings reported by [18, 19].

Table 2. Comparative Fatty acid profiles of Fermented and unfermented Roselle seeds (%).

Fatty acid	FRS	UFRS
	%	
Saturated		
Palmitic acid	12.34	19.21
Staric acid	16.35	5.13
Arachidic acid	0.47	0.67
Total	29.16	25.01
Unsaturated		
Oleic acid	40.29	36.90
Linoleic	22.57	35.02
Alpha-linolenic acid	2.33	1.85
Total	69.19	73.77

FRS: Fermented Roselle seeds, UFRS: Unfermented Roselle seeds

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3.3 Minerals

The mineral composition of FRS and UFRS seeds is presented in Table 3. The mineral compositions of Roselle seeds fermented and unfermented were found to contain respectively between 2182.83 and 1470.69 µg g-1 calcium and 128.83 and 114.72 µg g-1 zinc. In general, the results showed that fermenting decreased the concentration of almost all the mineral elements investigated in this study, except iron and phosphorus. However, the predominant elements in the studied samples, where potassium, magnesium, calcium and sodium but; in different orders for our both samples. The FRS showed calcium as the major predominant element followed by potassium, Magnesium and sodium. Therefore, the UFRS showed potassium as the major predominant element followed by magnesium, calcium and sodium. Phosphorus was relatively low in all the samples studied. Mineral elements were reported to be significantly influenced by variety, location and environmental conditions [8, 18]. These factors may be responsible for different variations exhibited by the current and previous values of our samples. [19]. reported K, Mg, Na and Ca to be the major predominant elements in Roselle seeds. Similar work was also reported by Hainida et al (2008) [8]. In their study on nutritional and amino acid contents of differently treated Roselle seeds.

Table 3. Minerals composition of the FRS and UFRS powders (µg g-1).

Elements	FRS	UFRS
	µg g-1	
Calcium (Ca)	1470.69±32.13	2182.83±65.34
Phosphorus (P)	9.97±0.12±0.10	8.81±0.10
Potassium (K)	1114.54±1.02	20341.67±1.04
Iron (Fe)	105.45±5.10	93.78±6.50
Zinc (Zn)	114.72±1.05	128.83±1.04
Magnesium (Mg)	1009.55±27.13	5433.33±131.2
Sodium (Na)	256.95±2.14	489.33±3.92

All values are Means and standard deviations of three replicates. FRS: Fermented Roselle seeds, UFRS: Unfermented Roselle seeds.

3.4 Amino acids composition

The nutritional value of food products is based on their amino acid composition. In order to determine the nutritional values of the samples, amino acid compositional analysis was carried out. Apparently, the fermented and the unfermented Roselle seed were observed to have similar amino acid composition. The results of the amino acids tests were shown in Table 4. Glutamic acid was the major amino acid in nearly all the both samples. In general, arginine and aspartic acid were predominant in all the samples. Roselle is considered to be related to okra and results from this study on amino acid composition of Roselle seed proteins were in agreement with the finding of [14] for okra seeds. According to [20] high levels of albumin will elevate sulfur-containing amino-acids. In the Roselle seeds, the albumin content is lower when compared to globulin [2, 7, 21]. This might explain the low values of cysteine and methionine found in our studied samples (Table 4).

Table 4. Comparative Amino acids profiles of Fermented and unfermented Roselle seeds (g 100g-1 of protein).

Amino acids	FRS	UFRS	FAO /WHO (Child (Adult)
	g 100g-1		
Essential amino acids			
Lysine	4.66	4.91	4.8 (4.5)
Valine	5.26	5.56	2.9 (3.9)
Phenylalanine	5.52	5.25	
Histidine	2.45	2.40	1.6 (1.5)
Leucine	7.86	8.06	6(5.9)
Isoleucine	4.36	4.19	3(3)
Tryptophan	0.07	0.085	0.66 (0.6)
Méthionine	2.35	2.40	
Thréonine	3.83	3.53	2.5 (2.3)
Non-essential amino acids			
Arginine	12.05	11.85	
Alanine	4.46	4.36	
Aspartic acid	9.78	9.74	
Cysteine	0.85	0.83	
Glycine	3.74	3.68	
Glutamic acid	19.87	19.93	
Prolyne	4.06	4.12	
Serine	4.68	4.70	
Tyrosine	3.03	2.97	

FRS: Fermented Roselle seeds, UFRS: Unfermented Roselle seeds, The FAO/WHO/UNU (Child (Adult) reference pattern of essential amino acid requirements (g $100g^{-1}$ of protein) was used to compare the protein quality of our studied samples.

3.5 Nutritional parameters

"Protein is one of essential nutrients in the human diet. Both the amount and quality of protein provided by a food are important. The protein quality, also known as the nutritional or nutritive value, depends on the level at which essential amino acids needed for overall body health and growth" [22]. Since a direct assessment of protein nutritional value in human subjects is impractical for regulatory purposes, methods based on *in vitro* and *in vivo* bioassays for assessment of protein quality have been developed. In our study, amino acid composition has been used as a basis for estimating the nutritional quality of the fermented and unfermented Roselle seeds. Results of the ratio of essential to total amino acids (E/T), amino acid score (AAS) and protein efficiency ratio (PER) of the fermented and unfermented

Roselle seeds are shown in Table 5. In all samples the ratio of essential to total amino acids (E/T) was higher than the pattern recommended by FAO/WHO/UNU (at least 36%)[23], and fermented Roselle seeds had the highest ratio with 36.58%. In general, the protein efficiency ratio below 1.5 implies a protein of low or poor quality, while PER between 1.5 and 2.0 indicates an intermediate protein quality and then PER above 2.0 means protein of high quality [24]. The predicted PER values of all the samples are in range of high quality. The PER values of fermented and its unfermented Roselle seeds were quite satisfactory compared with a standard casein PER of 2.5 [24] and were higher than the findings reported by [1, 25] PER of 2.0 and 2.06 for Roselle seed protein concentrate (RSPC) and Roselle seed protein isolates (RSPI) respectively. Bryant et al. (1988) [26] reported PER of 2.14 and 2.17 for soybean and okra proteins respectively. However, the total essential amino acid scores for all samples reached the FAO/WHO requirement [23] for the essential amino acids for children except tryptophan.

Table 5. Nutritional parameters of Fermented and unfermented Roselle (*Hibuscus Sabdariffa L.*) seeds.

Parameters	FRS	UFRS
E/T %	36.58	36.18
Estimated PER		
I	2.74	2.82
II	2.79	2.80
III	2.99	3.21
Amino acid scores		
Leucine	133.22	136.60
Histidine	163.33	160.00
Threonine	166.52	153.48
Valine	134.87	142.56
Isoleucine	145.33	139.67
Lysine	103.55	109.51
Tryptophan	11.67	14.17

E/T: the ratio of essential to total amino acids, FRS: Fermented Roselle seeds, UFRS: Unfermented Roselle seeds, Amino acid scores(AAS) = (mg of amino acid per g of test protein/mg of amino acid per g of standard protein) ×100.

4. CONCLUSION

From the results obtained in this study it is evident that traditional uncontrolled alkaline fermentation has no negative effects on the physicochemical properties of Roselle seeds, apart the mineral composition, which be corrected by addition. Moreover, the fermented and unfermented Roselle seeds were found to have a high nutritional quality. The amino acid pattern of both samples was higher than those of the FAO/WHO requirement. All the estimated nutritional parameters based on amino acids composition showed that the fermented and unfermented Roselle seeds have a good nutritional quality and suggests their possible use as a supplementary Protein source.

The fermented and unfermented Roselle seeds could have excellent applications for future product development by virtue of their physicochemical and nutritional properties. This would add some economic value to the existing uses of the plant and expand to cultivation.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

AUTHORS' CONTRIBUTIONS

Author FT' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author MMF managed the analysis of the study and managed the literature searches. All authors read and approved the final manuscript

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