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RESEARCH ARTICLE

DETERMINATION OF VITAMINS IN FN, FW, FK FLOURS OF NÉRÉ (PARKIA BIGLOBOSA) FROM KORHOGO IN NORTHERN CÔTE D'IVOIRE

Niamke Arthur Michel[1]*, Diarrassouba Gnoh Minata[1] and Djaman Allico Joseph[2]

[1]Department of Biochemistry- Genetics, Biological Sciences Training and Research Unit, Peleforo Gon Coulibaly University, PO Box 1328, Korhogo, Côte d'Ivoire; [2]Department of Biochemistry, Biosciences Training and Research Unit, Félix Houphouët-Boigny University, 22 PO Box 582, Abidjan, Côte d'Ivoire

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**Corresponding Author:*
Niamke Arthur Michel

ABSTRACT

The néré fruit is known in the north of Côte d'Ivoire for its seeds, which are used to prepare soumbara, and for its pulp, which is also consumed. Unfortunately, the nutritional value of the floury pulp is not known by the population and it is not processed by local industries. The flour is largely discarded by the "washing" system, which only preserves the seed. This study contributes to the valorisation of néré fruits. After hulling the néré fruit, the pulp is dried in the sun, crushed and sieved to produce three types of flour, depending on the source. Flours from Niofoin, Waragniéré and Kanoroba are coded FN, FW and FK respectively. The study of these flours by AOAC method and HPLC revealed, depending on the source, the presence of numerous vitamins such as vitamin C (178.94 to 198.53mg/100g), vitamin B1 (154 to 407µg/100g), vitamin B2 (59.7 to 91.6µg/100g), vitamin B3 (89 to 140µg/100g) vitamin B5 (53.67µg/100g on average), vitamin B6 (25µg/100g on average), vitamin B9 (3555 to 4718µg/100g), vitamin A (820µg/100g on average), vitamin E (8700µg/100g on average) and vitamin K1 (66.67µg/100g on average). The three (3) flours FN, FW, FK of néré contain vitamins that are beneficial to the health of local populations and can be used by the food industry.

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INTRODUCTION

The néré tree is a typical tree of the semi-arid and sub-humid zones of West Africa. While the cowpea tree grows naturally in the bush, it is a 'useful' tree. The néré tree represents an important source of harvested products. Its name 'néré' in Bambara is the most commonly used to designate the tree, whose scientific name is Parkia biglobosa (Touré, 2018). According to Traoré (2007), it is a large tree of 15 to 20 m in height, with a broad umbrella-like crown and dark green biparipenous leaves. The fruits are long pods of about 45 cm, 2 cm wide, slightly arched, hanging in clusters from the receptacle on the club-shaped flower. The pods open at maturity and contain numerous flattened black seeds embedded in a yellow pulp rich in sucrose. About twenty countries in West Africa (Benin, Burkina Faso, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea Bissau, Mali, Niger, Nigeria, Senegal, Sierra Leone, Togo), Central Africa (Cameroon, Central African Republic, Chad, Democratic Republic of Congo) and East Africa (Sudan, Uganda) are home to the species (Shao, 2000). The socio-economic importance of P. biglobosa is explained by its multiple food, agroforestry, medicinal and cultural functions. Considered in several societies as a symbol of peace, harmony of social life and well-being of communities, it is a species whose products are used in all rituals marking the different stages of life, i.e. birth, baptism, excision, marriage, funerals, etc. (Ouédraogo, 1995).In terms of food, its pulp is very rich in carbohydrates and is consumed fresh or after processing into fermented drinks or fritters (Ki, 1994).

The seeds are rich in protein and lipids especially when fermented (Adedayo et al., 2010). The product obtained from the fermentation of the seeds is widely used and is known as soumbala (in Bambara) or dawadawa (in Hausa in Nigeria). It is a product of high nutritional value and is used as a condiment in the preparation of sauces (Ouédraogo, 1995). The Néré seed trade is flourishing. For example, in Burkina Faso, more precisely in the Hauts-Bassins region, 4,149,783 kg of seeds were traded during 2012 (APFNL, 2013). It is a source of income and employment especially for women (Babalola, 2012).

In West Africa, the different organs of the tree are widely and diversely used in traditional medicine and pharmacopoeia either internally and/or externally, or in combination with other plants for the treatment of several ailments such as parasitic diseases, circulatory system diseases, respiratory system diseases, skin diseases and most digestive system diseases (Sina, 2006). Nevertheless, the nutritional value of the floury pulp of néré is not known by the population in the department of Korhogo, in localities such as Niofoin, Waragniéré, Kanoroba, despite the presence of many trees, and it is little used in industry. The floury pulp is discarded by the "washing" process, which allows only the seed to be recovered. This study is therefore a valorization of the néré by determining the vitamins of the floury pulp.

MATERIAL ET METHODS

Biological material: The biological material used was essentially made up of néré (*Parkia biglobosa*) pods collected in three (3) large areas (Kanoroba, Niofoin and Waragnéré), in the department of Korhogo, in the north of Côte d'Ivoire, which have a significant néré stand.

Methods

Sampling: For each zone (Kanoroba, Niofoin and Waragnéré), 20 kg of néré pod samples were collected from three (3) different locations, making 60 kg of pods per zone. For each zone, the samples were put together in 100kg bags and transported to Korhogo for flour production.

Production of néré flour

The néré flour was obtained through different steps: The néré pods were sorted and dehulled by area. After hulling, the yellow pulp containing seeds was obtained. It was sorted and then stored in a bag before drying. For each zone, the yellow pulp obtained, containing the seeds, was dried for two (2) days in the sun. The dried yellow pulp was crushed on the third day, by zone, in a mortar with a hand pestle to obtain the seed and the yellow néré flour separately. The sieving was done with a 2mm diameter sieve, by zone, to separate the flour from the seed. Thus three flours were obtained according to their origin. Flour from Niofoin, Waragnéré and Kanoroba were coded FN, FW and FK respectively. The flour obtained was then weighed with a scale to determine the number of kilograms obtained for each zone and finally the flour was stored in plastic buckets.

Vitamin C content: The vitamin C was evaluated from the three néré flours using 2,6- dichlorophenol-indophenol (DCPIP) reagent **(AOAC, 1984).** Ten (10) grams of ground dried néré flours sample were dissolved into 40 mL of metaphosphoric acid-acetic acid solution (2%, w/v). The resulted mixture was centrifuged at 3,000 rpm for 20 min. Thus, the supernatant was recovered, added with boiled distilled water for 50 mL, and titrated with 2, 6- DCPIP solution (0.5 g/L) previously calibrated with a pure vitamin C solution

Determination of water-soluble vitamins: The method for the determination of water-soluble vitamins is based on reverse phase HPLC assay, using a C18 column and the detection is done by UV at 272 nm at a flow rate of 1.1 ml/min. For this purpose, the preparation of the standard solution was done by dissolving respectively 15; 17; 13; 10; 8; 12 and 21 mg of (B1; B2; B3; B5; B6; B9 and B12 in the dark in 100 ml of a mixture containing water (94v/v), acetonitrile (5v/v) and acetic acid (1v/v) and heated in a water bath at 65°C with stirring until complete dissolution. The above solutions were diluted 1:10 with the mobile phase (the mobile phase is a methanol/phosphate buffer mixture (50/50 v/v)). For the test solutions, 1g of each néré flour was dissolved in the dark in the mixture containing water (94v/v), acetonitrile (5v/v) and acetic acid (1v/v) and heated in a water bath at 65°C with stirring until complete dissolution. The different solutions were then filtered on whatman filter paper and diluted 1:10 in the mobile phase. 20 µl of each solution was injected at a flow rate of 1.1ml/min and detection was done by UV at 272nm.

Determination of fat-soluble vitamins: The extraction of fat-soluble vitamins was performed according to the method described by **(Jedlicka and Klimes, 2005).** To 1.0 g of the néré flour sample, 10 mL of a 10% KOH solution in methanol-water (1:1, v/v) was added. To avoid the oxidation process during saponification, 0.025 g of ascorbic acid was added. The mixture was then refluxed in a water bath at 70 °C for 30 min. The mixture was then cooled and extracted with 5mL of hexane (the operation was repeated three times). The hexane phases were combined and dried over anhydrous sodium sulphate and evaporated to dryness. The residue obtained (about 0.3 g) was taken up in methanol (10 ml) for analysis.

The evaluation of the fat-soluble vitamin content was carried out by HPLC coupled with a fluorimetric detector. The analysis was performed in isocratic mode on a Hypersil ODS RP18 column (stationary phase). The mobile phase was a mixture of acetonitrile/methanol (80:20, v/v) with a flow rate of 1 mL/min. Detection of vitamin A was performed at λ=455nm, vitamin K1 at λ=245 nm and vitamin E at λ=295 nm. The standards for the establishment of the regression lines were prepared by series of dilutions (1/10th then 1/2) from: α-tocopherol (E): 3.4µg/100 mL; Retinol (A): 11.3µg/100 mL; Phylloquinonel (K1): 8.6µg/100 mL

Statistical studies: All analyses were performed in triplicate and the data processed using the Statistical Program for Social Sciences (SPSS version 20.0, SPSS for Windows, USA). For each characteristic, the results were expressed as means followed by their standard deviations as data dispersion parameters. A one-way analysis of variance (ANOVA 1) was also performed to test the effect of flour on the characteristics evaluated, at the 5% statistical significance level. For statistically different means, classification was performed with the Student-Newman-Keuls test.

RESULTS

Water-soluble vitamins: For vitamins C, B1, B2, B3, B9, the statistical studies showed a significant difference between the contents of the three flours FN, FW, FK at the 5% threshold. Thus, for vitamin C, FN flour had the highest content (198.53 mg/100g) and FW flour the lowest (178.94 mg/100g). As for vitamin B1, FN flour still has the highest value (407 µg/100g) while FK flour has the lowest (154 µg/100g). For vitamin B2, FK flour had the highest content (91.6 µg/100g) while FN flour had the lowest (59.7 µg/100g). For vitamin B3, the highest content was observed in the FN flour (140 µg/100g); conversely, the FK flour had the lowest content (89 µg/100g). For vitamin B9, the FN flour had the lowest content (3555 µg/100g) and the highest content was observed in the FW flour (4718 µg/100g). The three (3) flours did not show any significant difference at the 5% threshold between the contents of vitamins B5 and B6. The overall averages obtained are: 53.67 µg/100g for vitamin B5; 25 µg/100g for vitamin B6 (Table 1).

liposoluble vitamins : The three flours did not show any significant difference at the 5% level between their contents for vitamins A, E and K1. The overall averages obtained are: 820 µg/100g for vitamin A; 8700 µg/100g for vitamin E; 66.67 µg/100g for vitamin K1 **(Table 2)**

DISCUSSION

The vitamin A content of néré flour (820 µg/100g on average) is higher than that of the pulp of the fruit of Nauclea latifolia (63µg/100g) as shown by (Kini et al., 2008). Vitamin A plays a major role in vision, growth and development, epithelial cell integrity, immunity, cell differentiation and reproduction (West, 2002). The vitamin E content of néré flour (8700 µg/100g on average) is higher than that of the pulp of the fruit of Vitex doniana Sweet (Verbenaceae) (32µg/100g) highlighted by (Kini et al.,2008). Many biological processes involve vitamin E, such as the stabilisation of cell membranes or platelet aggregation. It is also involved as a cofactor in many enzymatic activities as well as in gene regulation, particularly in relation to the inflammatory response (CSS, 2016). The vitamin K1 content of néré flour (66.67 µg/100g) is higher than that of Ziziphus mauritiana Lam. (Rhamnaceae) (48µg/100g) reported by (Kini et al., 2008). Vitamin K plays a key role in blood coagulation and bone metabolism. An association between dietary intake of vitamin K1 and K2 and a reduced risk of type 2 diabetes has been suggested by some studies as well as a reduction in cardiovascular risk (Ibarolla-Jurado et al., 2012). The vitamin C content of néré flour (178.94 to 198.53 mg/100g) is higher than that of EF01 flour (24.7 mg/100g), which is composed of young shoot of roan flour, moringa leaves and cowpea powder, described by (Mahan et al., 2017).

Table 1. Water-soluble vitamins

Parameters	FN	FW	FK	General average	P-value
Vitamin C (mg/100g)	198.53±0.57c	178.94±0.32a	189.53±0.6b		0.000
Vitamin B1 (µg/100g)	407±8c	245±13b	154±8a		0.000
Vitamin B2 (µg/100g)	59.7±0.4a	74±1b	91.6±0.3c		0.000
Vitamin B3 (µg/100g)	140±6c	101±3b	89±13a		0.001
Vitamin B5 (µg/100g)	44±8a	65±13a	52±3a	53.67	0.075
Vitamin B6 (µg/100g)	24±10a	31±8a	20±3a	25	0.290
Vitamin B9 (µg/100g)	3555±5a	4164±26b	4718±8c		0.000

Per line, values followed by different letters are statistically different at 5%. P-value: value of the statistical probability test. With a < b<c; P value < 0.05 (5%) so the difference is significant.

Tableau 2. Liposoluble vitamins

Parameters	FN	FW	FK	General average	P-value
Vitamin A (µg/100g)	750±50a	810±300a	900±100a	820	0.594
Vitamin E (µg/100g)	7900±1000a	8800±300a	9400±1800a	8700	0.389
Vitamin K1 (µg/100g)	50±30a	70±10a	80±30a	66.67	0.317

Per line, values followed by different letters are statistically different at 5%. P-value: value of the statistical probability test. With a < b<c; P value < 0.05 (5%) so the difference is significant.

Vitamin C is involved in collagen, catecholamine and carnitine synthesis, cholesterol and steroid metabolism and iron and nitrite reduction. The antioxidant effect of vitamin C plays a major role in the direct protection of cellular structures against the toxic effect of highly reactive free radicals. Vitamin C contributes to the immune system. Finally, vitamin C is a potent activator of intestinal absorption of non-heme iron in martial deficient subjects (EFSA, 2014). The vitamin B1 content of néré flour ranges from 154 to 407µg/100g. Vitamin B1 (also called thiamine or aneurine) acts as a cofactor for various enzymes involved in energy production, especially in the brain and heart (CSS, 2016). The vitamin B2 content of néré flour ranges from 59.7 to 91.6 µg/100g. It is lower than that of EF01 flour (410 µg/100g) (Mahan et al., in 2017). Vitamin B2 or riboflavin is involved in the catabolism of fatty acids and amino acids, as well as in mitochondrial energy production and in red blood cell metabolism (Buijssen et al., 2014). The vitamin B3 content of néré flour ranges from 89 to 140 µg/100g. Vitamin B3 (Niacin or vitamin PP) acts as a precursor of two essential cofactors for energy metabolism, namely NAD (Nicotinamide adenine dinucleotide) and NADP (Nicotinamide adenine dinucleotide phosphate).

In addition, it has an inhibitory effect on lipolysis, reducing circulating levels of triglycerides and to a lesser extent cholesterol (EFSA, 2014). Vitamin B5 (53.67 µg/100g on average), or pantothenic acid, plays a major role in the energy utilisation of carbohydrates, fats and several amino acids, as it is part of the structure of coenzyme A (CoA) and Acyl Carrier Protein (ACP). These two molecules (CoA and ACP) are also involved in fatty acid synthesis (CSS, 2016). The vitamin B6 in néré flour (25 µg/100g on average), or pyridoxine, has an essential influence on the metabolism of several amino acids.

Its deficiency results in growth retardation, alopecia, delayed bone maturation, anaemia and numerous neurological disorders linked to insufficient formation of neurotransmitters (dopamine, noradrenaline). Vitamin B6 also determines the activity of many enzymes involved in transamination, deamination, decarboxylation or desulphurisation reactions. Recent evidence suggests that adequate intake of vitamin B6 may have a protective effect against the development of certain types of cancer, cardiovascular disease, and Parkinson's disease (Pallas health research and consultancy, 2012). The vitamin B9 content of néré flour (3555-4718 µg/100g) is higher than that of EF01 flour (240 µg/100g) described by (Mahan et al., 2017). This content is also higher than that of bread (20µg/100g dry weight) (AFSSA, 2003). Vitamin B9 (folic acid) is an essential nutrient involved in protein metabolism and DNA and RNA synthesis. As such, folic acid plays a major role in cell growth, particularly neuronal growth in the brain and spinal cord (CSS, 2011).

Conclusion and Perspectives

The three (3) flours FN, FW, FK of néré do not show any significant difference at the 5% threshold, for vitamins B5, B6, A, E, K1. All other vitamins are significantly different at the 5% level. FN, FW, FK flours contain numerous vitamins (C, B1, B2, B3, B5, B6, B9, A, E, K) which help fight against diseases. They are beneficial for children (growth, immune defence, bone metabolism, energy production, against anaemia, etc.) but also for adults (reproduction, blood coagulation, reduction of type 2 diabetes, reduction of cardiovascular risk, against cancer, against Parkinson's disease, etc.). In the future, it would be interesting to propose a food formulation based on néré flour for children.

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