Article de recherche

Rev. Cong. Sci. Technol., Vol. 02, No. 03, pp. 392-398 (2023)

OPEN ACCESS

Revue Congolaise des Sciences & Technologies

ISSN: 2959-202X (Online); 2960-2629 (Print)

http://www.csnrdc.net/

REVUE CONGOLAISE DES SCIENCES ET TECHNOLOGIES

Evaluation of the antioxidant capacity of a complementary food based on traditional resources: the case of Vamine®

[Evaluation de la capacité antioxydante d'un aliment de complément à base de ressources traditionnelles : le cas de la Vamine®]

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Résumé

L'évaluation des activités antiradicalaire et antioxydante de la Vamine® et de ses constituants nous a permis de constater que l'évaluation de l'activité antiradicalaire et antioxydante de la Vamine® et de ses constituants, nous a permis de constater que la capacité de piégeage de la radicaux de Vamine® est prononcée par rapport aux constituants évalués séparément. En effet, Vamine® a une valeur IC50 à ABTS de 96,16 ± 5,47 µg/ml par rapport à *Dioscorea bulbifera* (150,44 ± 9,64 µg/ml), Maïs (150,44 ± 9,64 µg/ml), Manioc (218,27 ± 12,4 µg/ml), Voandzou (150,31 ± 4,93 µg/ml) Arachides (338,06 ± 133,59 µg/ml) et témoin acide gallique (0,71 ± 0,08 µg/ml). Au vu de ces résultats, on peut supposer que la capacité de piégeage de la Vamine® provient de la synergie obtenue à partir du mélange de différents constituants. L'analyse effectuée avec le radical DPPH n'a pas montré de bonnes valeurs d'IC50 ceci pourrait s'expliquer par le fait que pour les réactions avec le DPPH, seules les molécules polaires réagissent alors que les réactions avec le radical ABTS concernent les molécules polaires et non polaires.

Mots clés: activité antiradicalaire, activité antioxydante, Vamine®, radical DPPH, radical ABTS.

Abstract

The evaluation of antiradicalaire and antioxidant activities of Vamine® and its constituants has allowed us de constate that the the evaluation of the anti-free radical and antioxidant activity of Vamine® and its constituents, allowed us to note that the capacity of trapping of the free radicals of Vamine® is pronounced compared to the constituents evaluated separately. Indeed, Vamine® has an IC50 value at ABTS of 96.16 ± 5.47 µg / ml compared to *Dioscorea bulbifera* (150.44 ± 9.64 µg / ml), Corn (150.44 ± 9.64 µg / ml), Cassava (218.27 ± 12.4 µg / ml), Voandzou (150.31 ± 4.93 µg / ml) Peanuts (338.06 ± 133.59 µg / ml) and control gallic acid (0.71 ± 0.08 µg / ml). In view of these results, we can assume that the trapping capacity of Vamine® comes from the synergy obtained from the mixture of different constituents. The analysis carried out with the radical DPPH did not show good values of IC50 this could be explained by the fact that for the reactions with DPPH, only the polar molecules react while the reactions with the radical ABTS relate to the polar molecules and nonpolar.

Key words: antiradicalaire activity, antioxidant activity, Vamine®, radical DPPH, radical ABTS.

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Reçu le 03/08/2023; Révisé le 19/09/2023 ; Accepté le 09/10/2023

https://doi.org/10.59228/rcst.023.v2.i3.43

1. Introduction

Widespread food shortages have led to the persistence of hunger and malnutrition, particularly among low-income population groups in developing countries (FAO, 2009). This problem is more important among children under five in developing countries and in those of Africa in particular because of the socio-economic conditions favorable to the onset of malnutrition (UNICEF, 2004).

In sub-Saharan African countries, the problem of food availability Ruel (2003) is compounded by deficiencies in proteins, vitamins A, iron, zinc and folic acid. Malnutrition leads to high infant mortality, diseases characterized by physical and intellectual growth retardation and permanent sequelae (FAO, 2009; Camara, 2009). However, food availability can lead to another form of child malnutrition, that due to an excessive supply of nutrients. In this case, children face obesity. Most studies show an increased risk of obesity during childhood and adolescence, especially among bottle-fed children. Overweight and obesity are a growing concern of children in developing countries, which could constitute a new public health problem in the future.

In the Democratic Republic of Congo, the results of the 2007 Demographic and Health Survey EDS, (2007), showed that 43% of children aged 0-59 months are suffering from chronic malnutrition, 23% of whom are under the severe form, 8% of acute malnutrition (3% in the severe form) while 23% are underweight (7% in the severe form). In addition to health care, the strategies adopted to eradicate child malnutrition consist of the search for new plant resources rich in proteins and micronutrients for the formulation of infant flours (FAO/OMS, 2008). During the weaning period, food given to the child in addition to breast milk should also provide proteins and micronutrients, but also photochemical compounds that are good for health. This could be beneficial in combating both malnutrition and nutritional diseases (cancer, obesity, type I diabetes) (Yang & Tsou, 2006).

To overcome this scourge we use the use of local products for the formulation of infant flours. The formulation of this type of infant flour covers the problem of using local plant resources. Several products such as soybeans, fruits and vegetables are known for their high protein, micronutrient or vitamin A, vitamin C and photochemical compounds (Abdullah et al., 2012; Sandberg, 2002; Yang & Tsou, 2006). Each year, the deaths of 2.7 million people are attributed to insufficient consumption of fruits and vegetables. This ranks among the top 10 risk factors for mortality (Ezzati, 2002). An increase in the use and consumption of fruits and vegetables would therefore be essential to reduce the incidence of nutritional deficiencies. This will promote better nutrition and family health (Yang & Tsou, 2006).

However, children, because of their fluid porridge foods, do not have easy access to fruits and vegetables. Can we use raw materials of plant origin for the formulation of infant flours of high nutritional quality and with functional food characteristics? The main objective of this study is to visualize the appearance of the starch grains of the various raw materials used in the composition of the complementary food before treatment and of Vamine®, our complementary food, and finally to assess their antioxidant capacities.

2. Materials et methods

The complementary food Vamine was prepared from sweet cassava tubers (*Manihot esculenta CRANTZ var. Dulcis*), Voandzou seeds (Vigna subterranea (L.) Verdc., Peanuts (*Arachis hypogea L.*), corn kernels (*Zea mays L.*) and yam tubers (*Dioscorea bulbifera L.*).

2.1. Production of complementary feed

2.1.1. Sweet cassava flour

It is obtained from the tubers of sweet yellow cassava (1 Kg). After washing, washed, peeled and cut into small slices using a knife; the latter were cooked in boiling water in a porcelain pot for 30 minutes. The cooked slices were dried in the Heraeus brand oven at 60 °C for 48 hours, pulverized in the hammer mill of the brand mentioned above and sieved using 0.25 - 0.5 mm mesh (Memina, 1994; Saskia & Annoek, 2005; Zannou et al., 2011).

2.1.2. Grilled corn flour

The flour was obtained from corn seeds (1 kg) which were sorted manually, washed with clean water, roasted in an Electrotech household oven at a moderate temperature (40 °C) for at least 45 minutes in the aim to destroy the antitrypsic factors, allow precooking of the food and obtain a partial degradation of the starch. A Mapo brand hammer mill bjt 3176223 was used for the preparation of very fine flour, after sieving with a 0.25 - 0.5 mm mesh (Memina, 1994; Saskia & Annoek, 2005; Zannou et al., 2011).

2.1.3. Voandzou flour

It is obtained from voandzou grains which have been sorted manually, washed in water to remove sand and other debris. We boiled water in a porcelain pot and the seeds were immersed in the pot until boiling for 15 minutes, then drained and then roasted in an oven at 60 °C for 24 hours. After drying the voandzou seeds were skinned by crushing with a mortar, winnowed then ground in a porcelain mortar and sieved (mesh of 0.25 - 0.5 mm). The flour obtained was placed in polyethylene bags by an ElectrothermalMx Spi LlproutMantle heat sealer and stored in an oven at 40 °C (Memina, 1994; Saskia & Annoek, 2005).

2.1.4. Peanut paste

It is obtained from peanuts. These were sorted manually before grilling them in the brand domestic oven at a moderate temperature of 40 ° C for 45 minutes. Then, they were peeled by hand rubbing, winnowed and then ground and pulped using a Miller aluminum mill to obtain a paste (Memina, 1994; Saskia & Annoek, 2005; Zannou et al., 2011).

2.1.5. Yam flour

This one is obtained from tubers of yams, sources of α-amylases, coming from Bandundu were put in germination in the experimental Garden of the Department of Biology of the Faculty of Sciences of the University of Kinshasa where they have watered regularly to keep the soil moist before harvesting them according to the protocol defined by Mayamba (1996). The different pieces taken were washed, peeled, cut into thin slices and dried in an oven (HERAEUS type T5050 EK) for 48 hours at 40 ° C before grinding and sifting (mesh 0.15 - 0.5 mm) to obtain a flour and then stored in polyethylene bags at 4 ° C (Memina, 1994; Saskia & Annoek, 2005; Zannou et al., 2011).

2.2. Theoretical formulation of the complementary feed

Despite the fact that there are software programs that allow the formulation of the complementary food taking into account the composition tables, we have to manually use the different food composition tables. There are :

- the FAO Food Composition Table (FAO, 1970);
- levels of amino acids in food from FAO and the Catholic University of Louvain (FAO, 1970);
- the Table of typical compositions of Essential Amino Acids (AAE) of FAO / WHO (FAO / WHO, 1973);
- the chemical composition table of traditional foods from Kwango-Kwilu (Mbemba & Remacle, 1992);
- data on food composition. Production, management and use (FAO, 2007)

2.3. Formulation of the complementary feed

Our complementary food has been prepared from mixtures of flours, sources of protein voandzou and peanuts and energy sources of cassava and corn. These flours were mixed using an Mx type 676 mixer, while adding using a spatula, yam flour as a source of enzymes, cane sugar, vanilla sugar and 1% mineral supplement (0.5% iodized salt and 0.5% baking soda). The complementary feed was called ‟Vamine®.

2.4. Preparation of the instant form of Vamine®

The preparation of Instant Vamine® consisted of mixing an equivalent amount of Vamine® flour with hot water until elastic dough was obtained. The resulting dough was spread evenly on metal trays and then placed at 50 ° C for 30 minutes in the WTB Binder incubator, before baking it in the Electrotech OVEN household oven at 130 ° C for at least 45 minutes. To the dough thus cooked and dried were added 10 g of sugar, 1 g of flavoring and 1 g of mineral supplement; this mixture was sprayed with a porcelain mortar and pestle to obtain Instant Vamine®.

3. Results et Discussion

3.1. Micrograph of flour

Starches are plant-based polysaccharides found primarily in cereal grains and tubers. Some fruits can also be rich in starches which differ from each other by the shape and size of their granules, and by the respective proportions of the amylose and amylopectin chains which condition their physicochemical properties (Delpeuch et al., 1978). The nutritional role of starches is particularly important since they constitute, after digestive hydrolysis into glucose, the main source of calories in human food (Atrous, 2011).

Starch granules vary in shape and size depending on their botanical origin. Their size varies from 1 to 100 μm (Hooper & Aedin, 2006). Most granules are oval, but you can find rounded, spherical or irregularly shaped granules.

The micrograph of the flour powders of food resources used gave us the characteristic elements below shown in figure 1.

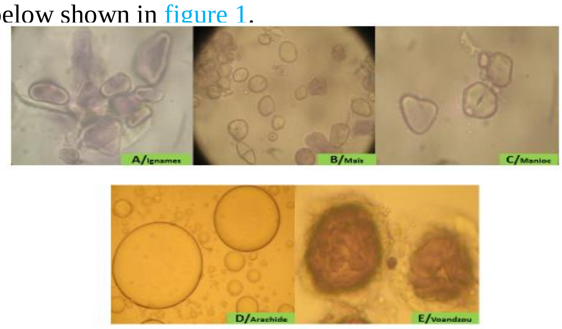

Figure 1. A. Oval, polyhedral starch seeds of Dioscorea Bulbifera (40X); B. Small grain cluster of Polyhedral corn starches (10X), C. Ovoid starch grains (40X) of cassava, D. Oil droplets of peanuts and spherical starches (40X) of peanuts, E. Cauliflower-shaped cluster of starches from Voandzou powder (40X)

According to the description of (Fréderich, 2011; Shailenra, & Nilambari, 2014; FAO, 1970), starches from potatoes, legumes, tubers and chestnuts are large in size and generally have oval, hemispherical, pear-shaped or ellipsoidal shapes (Fréderich, 2011; Shailenra, & Nilambari, 2014).

As shown in figure 1, *D. bulbifera* exhibits starches having either an oval shape or an ellipsoidal shape. Spiral-shaped vessel fragments have also been identified in the powder of *D. bulbifera* tubers. Corn, rice, buckwheat, millet and oats have starches that are more or less regular in blocky form. This is also observed for the starches studied in figure 1.

The starch grains in the sweet cassava flour under analysis have an ovoid appearance and others are ellipsoidal. (Sioibe et al., 2007) studied the appearance of starches from five varieties of cassava (*Manihot esculenta crantz*) [attiéké Mossi 1, attiéké Mossi2, agbablé 1, kétévie and TA and found that their starch grains are mostly spherical and only a small minority had shown an ovoid appearance. Hélène Angellier (2005) also obtained similar results. The literature reports that peanut seed starches have a spherical shape and are found near oil droplets (Fréderich, 2011).

Microscopic examination of the peanut paste used also shows oil droplets alongside the spherical starches. The fragment of spiral vessels was also found on microscopic examination. Voandzou seeds have many hairs and oil droplets (Fréderich, 2011). This observation was also made for the voandzou seeds used, and whose clusters of starches are cauliflower-shaped.

3.2. Micrograph of Vamine®

The micrograph of Vamine® gives the various constituents of which have undergone treatments liable to modify the rheology of their starches. Microscopic examination of figure 1 shows the presence of different starch grains of polyhedral, oval, ovoid, spherical shapes next to the fragments of fibers and oil droplets originating respectively from cassava and voandzou.

From this microscopic analysis we retain that the appearance of voandzou starch could be considered in the future as the key element during the quality control of Vamine® and will make it possible to detect and protect the latter from possible falsifications (Fréderich, 2011).

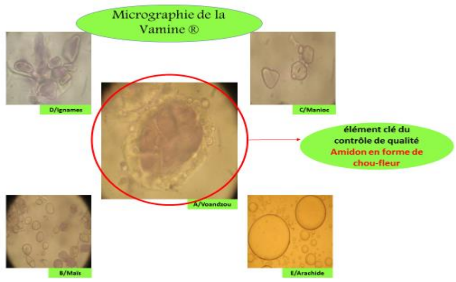

Figure 2. A. Cauliflower-shaped cluster of starch from Voandzou powder (40X), B. Polyhedral, oval, ovoid starch grains from corn powder, (40X), C. Polyhedral, oval starch grains from cassava powder,… (40X), D. Oval and ellipsoidal starch grains. Of yams, E. Spherical peanut starches.

3.3. Secondary metabolites of Vamine®

In view of the protective role that antioxidants currently play, we were interested in identifying and evaluating some secondary metabolites, in this case total polyphenols and anthocyanins in Vamine® and its constituents.

The results of the contents of different samples, shown in Table 1 represent in each case the mean ± standard deviation of 3 measurements. These data show that *D. Bulbifera* has a high content of total polyphenols, followed by Voandzou, peanuts, maize and cassava. We did not detect the presence of tannins and flavonoids in all of the samples analyzed. Previous studies carried out on *Dioscorea* species have shown that *Dioscorea Bulbifera* is very rich in total polyphenols (Bhandhari et al. 2004, Bukatuka F, 2016). We have also detected anthocyanins, but in small amounts in Vamine®, peanuts and corn.

Table 1. Contents of secondary metabolites of Vamine® and its constituents

Samples	Total polyphenol content mg/g (EAG)	Flavonoid content mg/g (EQ)	Tannins content mg/g (EC)	Anthocyanin content mg/g (EC)
Dioscorea bulbifera (ignames) (1)	37.88 ± 5.97	Nd	Nd	0,057 ± 5,97
Maïs (2)	8.03 ± 0.23	Nd	Nd	0,147± 0,002
Manioc (3)	6.08 ± 0.02	Nd	Nd	0,064 ± 0,002
Voandzou (4)	18.77 ± 1.78	Nd	Nd	-
Arachides (5)	15.26 ± 1.42	Nd	Nd	0,175± 0,001
Vamine® (6)	17.05 ± 1.20	Nd	Nd	0,321± 0,007

Nd: Very low contents whose values cannot be calculated from the calibration line seen the negative absorbance values

Kayodé et al. (2012), found in infant flours composed of sorghum, soybeans, fry a total polyphenol content (6.29 ± 0.62 mg/g), lower than those of Vamine®, *Dioscorea bulbufera*, voandzou and peanuts while the anthocyanin content (0.039 ± 0.01) is lower than that of Vamine®.

[Songré et al. (2016)](), analyzing eight infant flours, found higher polyphenol contents (51.84 ± 17.92 - 492.86 ± 17.92 mg/100 g) compared to that of Vamine®.

The six flours composed of Bété Bété and Kponam yams have contents of polyphenols (62.79 ± 5.88 mg/g - 118.41 ± 2.19 mg/g), tannins (33.62 ± 2.12 mg/g - 56.28 ± 1.83 mg/g), in flavonoids (11.61 ± 1.29 mg/g - 76.58 ± 4.87 mg/g) higher compared to that of Vamine® (Soro et al., 2014). Tannins and flavonoids were not detected in all of the samples analyzed.

The content of total polyphenols in the extracts is in most cases correlated with their anti-free radical and antioxidant activities (Puttaraju et al., 2006; Abdullah et al., 2012). Their affinity for free radicals inhibits the oxidation of low density lipoproteins, thus playing a positive role in the prevention of cardiovascular disease. This antioxidant property is beneficial and helps prevent carcinogenesis (Hooper & Aedin, 2006) Polyphenols, particularly condensed forms such as tannins, also have the ability to complex cations (Fe^{2+} and Zn^{2+}), thus reducing their bioavailability (Kayodé et al., 2012). Their presence is therefore not desired in infant flours. The content of total polyphenols (16.71 mg/g) of infant flour made from rice, banana powder formulated by (Malvika & Rajinder, 2015) is lower than our formulated complementary food.

Considering the low content of anthocyanins and the absence of flavonoids and tannins in Vamine® and its constituents, we assume that the observed content of total polyphenols is due to the presence of another group of polyphenols in our samples. This was confirmed by the chemical screening carried out on all samples, which revealed the presence of phenol acids, a group of polyphenols with marked anti-free radical and antioxidant activity (Wagner et al., 2013). It would be desirable to also measure the total phenol acids in our various samples in order to establish a better correlation with the anti-free radical and antioxidant capacity.

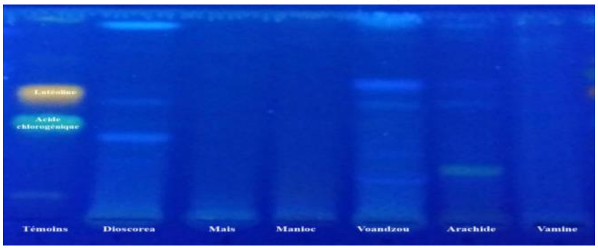

Figure 3. Search for polyphenols (phenol and flavonoid acids). Stationary phase: Silica gel F254 / Mobile phase: Ethyl acetate / Acetic acid / Formic acid / Water (100: 11:11:26); Detection: spray the plate with Neu's reagent and visualize at 366 nm.

Phenol acids appear as blue fluorescent spots and flavonoids appear as yellow, yellow-orange, orange and green fluorescent spots.

3.4. In vitro evaluation of the antioxidant capacity of Vamine® and its constituents

The evaluation of the antioxidant activity of Vamine® and that of its constituents was carried out at ABTS and DPPH, in terms of IC_{50}.The results obtained are reported in table 2.

Table 2. Antiradical and antioxidant activity of Vamine® and its constituents expressed in terms of IC_{50}

Samples	ABTS (µg/ml)	DPPH (µg/ml)
Dioscorea bulbifera (ignames) (1)	150,44 ± 9,64	231,74 ± 24,72
Maïs (2)	179,06 ± 16,37	514,04±121,29
Manioc (3)	218,27 ± 12,4	626,61±304,05
Voandzou (4)	150,31 ± 4,93	430,53 ± 70,66
Arachides (5)	338,06 ± 133,59	2449,1
Vamine® (6)	96,16 ± 5,47	748,82±228,42
Acide gallique	0,71 ± 0,08	1,07 ± 0,10

The evaluation of the anti-free radical and antioxidant activity of Vamine® and its constituents, we found that the scavenging capacity of free radicals of Vamine® is pronounced compared to the constituents evaluated separately. Indeed, Vamine® has an IC50 value at ABTS of 96.16 ± 5.47 µg/ml compared to *Dioscorea bulbifera* (150.44 ± 9.64 µg/ml), Maize (150.44 ± 9.64 µg/ml), Cassava (218.27 ± 12.4 µg/ml), Voandzou (150.31 ± 4.93 µg/ml) Peanuts (338.06 ± 133.59 µg/ml) and control gallic acid (0.71 ± 0.08 µg/ml). In view of these results, we can assume that the trapping capacity of Vamine® comes from the synergy obtained from the mixture of different constituents. The analysis carried out with the DPPH radical did not show good IC_{50} values this could be explained by the fact that for the reactions with the DPPH, only the polar molecules react while the reactions with the ABTS radical concern the polar molecules and non-polar (Arnao et al., 2001; Dejianhuang et al., 2005; Franck et al., 2013).

Bio-guided anti-radical test by thin layer chromatography

This experiment allowed us to locate the compounds exhibiting radical activity which appears in the form of spots colored in yellow.

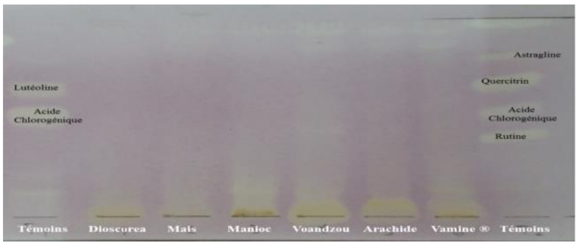

Figure 4. Chromatography Anti-free radical bioautography of Vamine® and its constituents. Stationary phase Silica gel F254 / Mobile phase: Ethyl acetate / Acetic acid / Formic acid / Water (100: 11: 11: 26)

As shown in figure 4 of the chromatogram, the extracts from all samples contain substances with marked anti-free radical activity.

These results corroborate those carried out in solution using the DPPH and ABTS radicals.

Currently, the WHO through its guidelines recommends that infant formulas from 6 months of age may contain constituents capable of fighting against free radicals which have harmful effects on the growth of young children. It is important to remember that breast milk, thanks to its richness in antioxidants (tocopherol, cysteine, carotenoids, glutathione peroxidase, catalase, superoxidase dismutase, coenzyme Q, vitamin A, vitamin E, etc.) allows young children to defend themselves against ROS up to at least 6 months; beyond this age, a young child's metabolism requires additional antioxidants to help fight ROS. The latter can only come for young children from complementary foods (Sadeghi et al., 2010; Lee & Davis; Malvika & Rajinder, 2015).

5. Conclusion

The objective of this work is to assess the antioxidant capacity and to observe the appearance of the different starch grains of Vamine®, a complementary food based on traditional resources. The microscopic examination made us observe the appearance of the various starch grains of polyhedral, oval, ovoid shape according to their origin, with a particular aspect of the starch of voandzou, which will be the key element during the quality control Vamine®.

The FAO / WHO standards, 2006 recommend that complementary foods for young children be rich in antioxidant compounds to supplement those of breast milk. In Vamine®, the content of total polyphenols correlates with their anti-free radical and antioxidant activities

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