The use of purple yam (*Dioscorea trifida*) as a health-promoting ingredient in bread making

**ABSTRACT:**

The use of purple yam (*Dioscorea trifida*) was evaluated as possible health-promoting ingredient in bread making in the state of Amazonas, Brazil. The centesimal composition, energy, and antioxidant activity of purple yam and its incorporated bread formulations (0%, 10%, 15% and 20%) were determined. An acceptance test and microbiological analysis of the formulations 10%, 15% and 20% were also performed. Except for lipids, the centesimal composition and caloric values revealed no statistically significant differences. An addition of purple yam in natura up to 20%, instead of wheat flour in ordinary bread (0%), can be made with no effect on the diet’s energy. The free radical scavenging, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and lipid per oxidation (LPO) methods revealed that the greater the percentage of purple yam being added into the breads the higher the antioxidant activity detected. The acceptance test applied to compare the three formulations of purple yam breads revealed a significant difference only in the attribute colour. Purple yam breads showed no preferable differences. Results highlight the feasibility of purple yam bread as a health-promoting food in the Amazon region.

**Keywords:**

Purple yam (*Dioscorea trifida*); antioxidant activity; health-promoting food; Amazon region.

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INTRODUCTION

Yams belong to the family Dioscoreaceae, genus Dioscorea (Pedralli, 1988; 1997; Pedralli et al., 2002; Pedralli, 2004). This family is made up by 6 to 9 genera comprising over 600 species distributed throughout the World’s tropical, subtropical and temperate regions (Barroso et al., 1974; Pedralli, 1988; 1997; Melo Filho et al., 2000; Pedralli et al., 2002; Pedralli, 2004). The yams (Dioscorea spp.) yield tubers, which are very important as staple, nutritional and healthy food, and are still used as an ingredient in traditional Chinese herbal medicine. They show a worldwide distribution, and are found in many tropical countries, in South-Eastern Asia and Western Africa, where the species were introduced by cultivators (Rasper and Coursey, 1967; Akanbi et al., 1996; Omonigho and Ikenebomeh, 2000; Lin et al., 2005). They can also be found in some American countries, particularly in Brazil, where one can find them in all regions, from the Amazon down to the Southern part of the country (Chu and Figueiredo-Ribeiro, 1991; Pedralli, 1997; 2004).

Purple yam (Dioscorea trifida) is an American native species, which was domesticated by Amerindians, with the cultivar distribution possibly pointing out its domestication in Brazilian and Guyana border areas, followed by dissemination throughout the Caribbean islands (Pedralli, 1988; Pedralli et al., 2002; Pedralli, 2004). D. trifida shows a wide distribution in Central and South America, from the Caribbean to Peru. In Brazil it is found all the way from the Amazon right down to the Southern region. The species is associated to forest environments-Amazonian highland tropical rainforests, Coastal Atlantic Forest in Southeastern Brazil and, mesophytic (seasonable) and gallery forests (Pedralli, 1997).

Here in the Amazonian region, purple yam (D. trifida) may be consumed in the following ways: baked, boiled, mashed, as ingredients for soups and meat stews, and in the formulation of flour for making cakes, pies and porridges. Nevertheless, this species has undergone little scientific investigation, so little is known about its management techniques, genetic improvement, nutritional potential, industrial use, storage procedures, characterization, uses as natural dye, as well as its use as a health-promoting ingredient, among others.

By and large, the bread consumed throughout the world is made mostly of wheat flour, salt and yeast. Many other ingredients, have been incorporated into bread formulation, so as to increase its diversity and product appeals (Hsu et al., 2004).

A few studies have highlighted the great potential of purple yam in bread making. In this case, yam flour may replace part of the wheat flour, improving bread quality, as well as adding economical advantages to it (Abramo, 1990; Hurtado et al., 1997; Litvin et al., 1998; Omonigho and Ikenebomeh, 2000; Ratti, 2001).

Hsu et al., (2004) demonstrated the presence of antioxidants in the flour of purple yam (Dioscorea purpurea), in five formulations of breads prepared with this tuber’s flour, with excellent acceptance in Taiwan supermarkets. Contado et al., (2009) showed yam (Dioscorea spp.) mucilage-based loaf to present good public acceptance as to flavor, aroma and texture with sensory attributes, demonstrating the use of this tuber to be feasible as improvers in bread making.

The following aspects motivated the use of purple yam (Dioscorea trifida) in natura as a bread manufacturing health-promoting ingredient, in the present work: 1) its significant world consumption, presenting a considerable, expanding tillage alternative (Rasper and Coursey, 1967; Abramo, 1990; IITA, 2007); 2) although, as yet incipient, an increase on the production of this tuber in the State of Amazonas, Brazil, especially in Caapiranga and Careiro Castanho municipalities is being observed. According to the Instituto de Desenvolvimento Agropecuário do Estado do Amazonas (IDAM) in 2008, 110 families of the
Caapiranga municipality yielded 2,475 tonnes in an area of 165 ha; and 3) the presence of antioxidants in purple yam, which increases the nutritional capacity in breads made from this tuber (Hsu et al., 2004).

The main aim of the present study was to evaluate the potential of purple yam yield in the State of Amazonas, Brazil as a health-promoting ingredient in bread making. On this context, it determined the centesimal composition, caloric value, and antioxidant properties of purple yam as well as of breads made from this tuber in natura. Then, it undertook an organoleptic characteristic assessment of the breads, following tasters’ panel acceptance criteria. This purple yam species is, for the very first time, being used in the Amazonian region, as a feasible alternative for bread making.

MATERIALS AND METHODS
Species identification and purple yam tuber (Dioscorea trifida) collection

Identification of the species Dioscorea trifida was accomplished by comparisons with a voucher herbarium specimen (Exsicata number 1353) deposited at the National Research Institute of Amazonia (INPA) Herbarium. It is very common to find the purple yam (D. trifida) exhibiting several color hues of its flesh (edible portion), in Amazonas State Townships. The types most easily identified are: roxinho (light purple flesh); roxo (mid purple flesh); roxão (dark purple flesh); branco (white flesh); and misto (white-purple flesh) (Figure 1).

Purple yam samples were collected at two Amazonas Townships: Caapiranga and Careiro Castanho. Due to the seasonality and availability of these tubers in the region, the centesimal composition analyses of yams and breads were performed with Caapiranga samples. Yam and bread antioxidant and bread sensory and microbiological analyses were carried out with Careiro Castanho samples.

Purple yam bread elaboration

On account of the probability of getting breads with higher antioxidant concentration (Hsu et al., 2004), roxão (dark purple flesh) type samples were used in the present study (Figure 1C). Yams in natura, for replacing wheat flour, were washed, peeled, weighed, ground in the liquidizer together with yeast, oil and water. Then, this mixture was added to the previously mixed dry ingredients (wheat flour, powdered milk, sugar and salt). Bread manufacturing formulations can be seen at (Table 1). Homogenization (30 min.), dough underwent initial fermentation (60 min.), intermediate time for

Figure 1. Flesh color varieties of the kinds of purple yam (Dioscorea trifida) commonly found in fairs and markets of Manaus-AM. A) roxinho (light-purple flesh); B) roxo (mid-purple flesh); C) roxão (dark purple flesh); D) branco (white flesh); and E) misto (white-purple flesh).
bread shaping (25 min.), final fermentation (60 min.), time for baking (30 min.) are followed for making yam bread. After being prepared the breads were cooled to room temperature and packed in polyethylene bags displaying the product’s labeling.

**Centesimal composition analyses of purple yam and its incorporated breads**

Centesimal composition analyses of purple yam (*Dioscorea trifida*), and purple yam incorporated breads in four formulations: 0%, 10%, 15% and 20%, were done in triplicate. Moisture, ashes, lipid, proteins and crude fiber contents were determined according to procedures described by the Instituto Adolfo Lutz-IAL (2008). Carbohydrate and caloric values were determined according to the method of (AOAC, 2005).

Findings obtained on the bread formulation centesimal composition analyses were subjected to post hoc tests are shown.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Yam (D. spp.)</th>
<th>Yam (D. alata)</th>
<th>Purple Yam (D. trifida)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>72.60</td>
<td>73.70</td>
<td>76.43 ± 0.50</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>0.20</td>
<td>0.10</td>
<td>1.13 ± 0.69</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>2.00</td>
<td>2.30</td>
<td>1.83 ± 0.13</td>
</tr>
<tr>
<td>Crude Fiber (%)</td>
<td>0.60</td>
<td>7.30</td>
<td>1.80 ± 0.05</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.90</td>
<td>0.90</td>
<td>0.78 ± 0.02</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>24.30</td>
<td>23.00</td>
<td>18.04 ± 0.66</td>
</tr>
<tr>
<td>Caloric value</td>
<td>100.00</td>
<td>96.00</td>
<td>89.64 ± 4.52</td>
</tr>
</tbody>
</table>

*(Montaldo, 1977), **(TACO 2006), ***Present study*
statistical analysis through the statistical software package (Statsoft STATISTICA 8.0 2007). Given to the number of sampled observations (n=3), Kruskal-Wallis ANOVA and post hoc tests were applied as a non-parametric alternative to Fisher ANOVA, for independent data, in the comparison among the bread formulations.

Findings showing significance level of \( P<0.05 \) were considered as statistically significant.

**Preparation of purple yam and its incorporated breads methanolic extract**

Samples of purple yam (Dioscorea trifida), were peeled and ground with the aid of a knife. They were then dehydrated in a laboratory oven at 60°C for 24 h. Purple yam incorporated breads of four formulations: 0%, 10%, 15%, and 20%, were cut into 1 cm thick slices, and dehydrated in a laboratory oven at 40°C for 24 h (Hsu *et al*., 2004). Dehydrated yams and breads were ground with pestle and mortar, weighed at 0.125, 0.25, 0.5 and 1.00 g (40, 80, 160 and 330 mg/mL, respectively). They were placed into small test tubes added with 5 mL of methanol and left in a rotary shaker for 24 h. The material was centrifuged at 2,500 RPM for 10 min so as to obtain the supernatant (methanolic extract). The antioxidant activity of the samples was determined by the free radical scavenging, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and lipid peroxidation (LPO) methods. The latter method evaluates the inhibition of free radicals generated during the linoleic acid peroxidation, and is based on spectrophotometric measurements of discoloration (oxidation) of ß-carotene, induced by linoleic acid oxidative degradation products (Marco, 1968; Miller, 1971; Duarte-Almeida *et al*., 2006).

**Antioxidant activity determination through free radicals scavenging methods (DPPH) in purple yam and its incorporated breads**

DPPH method, following the methodologies described by Shimada *et al*., (1992) and Hsu *et al*., (2004), with some modifications, where 2 mg of DPPH were dissolved into 15 mL of methanol, and applied so as to determine the antioxidant activity of samples of purple yam and its incorporated breads in the four aforementioned formulations. A micro plate bearing 96 well was used. Thirty microliters (30 µL) of the methanolic extract, plus 170 µL of methanol (used as the blank) were placed in the wells. The reading was performed on an Elisa reader (DXL 800-BECKMAN COULTER) at a wavelength of 492 nm, using triplicate samples. Then, 100 µL of the DPPH solution were added, and the material was stored in a dark place for 30 min, and the reading was repeated as soon as this time was over. Two hundred microliters (200 µL) of methanol added to 100 µl of the DPPH solution were used as the control. Thirty microliters (30 µL) of quercetin (10 µg/mL), 170 µL of methanol and 100 µl of the DPPH solution, were used as the standard. The following formula was used so as to calculate the antioxidant activity percentage

\[
\% \text{ AA} = \frac{\text{A sample} - \text{A blank}}{\text{A control}} \times 100
\]

**Antioxidant activity determination through the lipid peroxidation (LPO) method in purple yam and its incorporated breads**

The determination of the antioxidant activity of the samples through the LPO method was carried out according to the method reported by Duarte-Almeida *et al*., (2006), based on the methodology originally described by Marco (1968), and later modified by Miller (1971). The reactive mixture was prepared in an Erlenmeyer flask, containing 50 µL of linoleic acid, 200 µL of tween 80 (emulsifying agent), 150 µL of ß-carotene solution at 2 mg/mL in chloroform, and 500 µL of chloroform. The mixture was then subjected to evaporation in nitrogen till there was no more chloroform left. Later, the mixture of 25 mL of previously oxygen saturated water was added, and during
a period of 30 min it was homogenized through vigorous shaking.

The reactive mixture showed to be clear with absorbency ranging from 0.6 to 0.7 at a wavelength of 492 nm. A 96 well bearing micro plate was used. Two hundred forty microliters (240 µL) of the reactive mixture and 10 µL of the methanolic extract samples were placed in the wells. Ten microliters (10 µL) of methanol and an equal volume of butylhydroxytoluene (BHT) at a concentration of 40 µg/mL were used as control and standard, respectively. The micro plate was incubated at 50ºC to speed up the oxidation reactions and start β-carotene discoloration. Discoloration slope readings of samples, control and BHT (in triplicate) were performed readily, in an Elisa reader at a wavelength of 492 nm every 15 min for 135 min. The following formula was used so as to calculate the oxidation inhibition percentage:

\[
% I = \frac{100 \cdot (A2 \text{ sample} - A1 \text{ sample})}{A2 \text{ control} - A1 \text{ control}} \times 100
\]

**Sensory analysis of purple yam incorporated breads**

The acceptance test of purple yam in natura incorporated breads counted with the participation of 78 non-trained volunteer judges. Each one of them was provided with an answering card bearing a 9 point hedonic scale (9-like extremely to 1-dislike extremely), adapted from Stone et al., (1993) and Silva et al., (2005). The judges were provided with three purple yam incorporated bread samples, produced from three formulations (10%, 15% and 20%) (Table 1). Samples were served in white, disposable plastic plates; encoded with three randomly chosen numbers. Samples were evaluated according to their sensory qualities: global feel, aroma, flavor, color and texture. Judges were advised to always rinse their mouth with water before testing the next sample.

The findings obtained on the acceptance test were submitted to statistical analysis through statistical software package (Statsoft STATISTICA 8.0 2007). The Shapiro-Wilk test rejected the frequency distribution normality of the three tested bread formulations, in all their sensory attributes. However, the Levene test accepted the homocedasticity (homogeneity of variances) among the formulations for all sensory attributes. As frequency distribution normality and variance homogeneity are basic assumptions made for the application of parametric tests, such as Fisher’s ANOVA, and as these assumptions were not attended to, the Friedman ANOVA followed by post hoc tests were applied as a non-parametric alternative for paired data in bread comparisons. Findings presenting significance level of \((P<0.05)\) were considered as statistically significant.

**Microbiological analysis of purple yam breads**

Following the recommendation of the Brazilian National Health Surveillance Agency (in Portuguese, Agência Nacional de Vigilância Sanitária, ANVISA), based on Ruling Number 12 (RDC, 2001), we carried out the microbiological analysis so as to verify Coliforms and Salmonella in samples of the three purple yam bread formulation samples through the membrane filtration method (APHA, 2001).

**RESULTS AND DISCUSSION**

**Centesimal composition and caloric value of purple yam**

Moisture (76.43±0.50), protein (1.83±0.13) and ash (0.78±0.02) contents, as well as the caloric value (89.64±4.52) of purple yam \((D. \text{ trifida})\) samples analyzed in the present study (Table 2) show to be near those presented by Montaldo (1991) for yam \((Dioscorea\) spp.) and those found in the Brazilian Food Composition Table TACO (2006), for the yam \((D. \text{ alata})\). Lipid content (1.13±0.69) stayed well above that presented by Montaldo (1991) and TACO (2006). Crude fiber content (1.80±0.05) is above the value observed by Montaldo (1991), and well below that

The high fiber content presented in TACO (2006) might be due to the enzymatic gravimetric method employed in the analyses. That method warrants a higher precision for determining the dietary fiber as compared to the acid digestion methodology used in the present study as well as by Montaldo (1991). Total carbohydrate content (18.04 ± 0.66) is well below Montaldo (1991) and TACO (2006) values. The remaining differences in centesimal composition values presented by Montaldo (1991) and in the present study might be related to the different soil types being employed on planting the tubers and/or to the different species being utilized. Nevertheless, the different values presented in TACO (2006) may be related to the different yam species being analyzed.

Centesimal composition and caloric value of purple yam incorporated breads

Based on data from Kruskal-Wallis (ANOVA) followed by post hoc tests (Table 3), it may be asserted that, except for the lipids (P<0.05), all other centesimal composition and caloric values of the four purple yam incorporated bread formulations (0%, 10%, 15% and 20%) showed to be statistically similar (P>0.05). That is, replacing wheat flour by purple yam in natura in up to 20% neither modifies bread centesimal composition nor caloric value. As for lipid, statistically significant difference was only observed for 10% and 20% formulations; this negligible 0.5% difference may be neglected in technological applications.

Purple yam incorporated breads centesimal composition and caloric value were compared to those of ordinary bread loaf (OBL) (Anton et al., 2006) and whole bread loaf (WBL) (TACO, 2006) (Table 4). One notices, a high fiber content (6.90%) in the whole bread loaf (WBL) (TACO, 2006), relative to the remaining breads. It can be highlighted that in whole bread composition, we have the presence of grain-composed whole flour, almost wholly made up of bran, germ and endosperm (FDA, 2006). By and large, all other values show to be approximate. All differences found may be related to formulations employed in the preparation of those breads.

**Antioxidant activity determination through the free radical scavenging method (DPPH) in purple yam and its incorporated breads**

Antioxidant activity (% AA) of the methanolic extract pertaining to purple yam (*Dioscorea trifida*) and its incorporated breads are shown in Figure 2. The quercetin was used as standard control. Bars indicate standard deviation.

![Figure 2](image-url)
samples in the concentrations of 330, 160, 80 and 40 mg/mL, as determined by the DPPH method, were higher than 70%, reaching a maximum of 88.13±0.12. This plainly shows this species to exert DPPH radical scavenging activity (Figure 2). This same figure reveals purple yam incorporated breads prepared in 10%, 15% and 20% formulations, to also present a certain antioxidant activity, reaching 43.32±1.18; 48.13±1.17 and 53.71±1.01 maximum percentile values, respectively. Those findings are above the values presented by Hsu et al., (2004) (20-40% approximately), who used breads of several formulations prepared with flour from the purple yam tuber (Dioscorea purpurea) representing the one with the widest variety in Taiwan, for substituting part of the wheat flour. Bread prepared with no purple yam at all (0%) showed certain antioxidant activity, as well, probably due to Maillard reaction products, where, some hot processed foods, present free radical scavenging activity (Kim et al., 2007; Jing and Kitts, 2000; Hsu et al., 2004; Michalska et al., 2008). Corroborating data from Hsu et al., (2004), it was confirmed that the antioxidant activity rose as the percentage of purple yam substituting wheat flour increased. The high free radical scavenging activity observed by Hsu et al., (2004) in flour of Taiwan purple yam (D. purpurea), was also detected in the Amazonian region’s purple yam (D. trifida).

Antioxidant activity determination through the lipid per oxidation (LPO) method in purple yam and its incorporated breads

Discoloration slope (Figure 3) and free radical inhibition activity (Figure 4) determined through the LPO method confirmed the antioxidant activity (% I) in purple yam (55.80±4.85) and its breads from the three formulations (10%, 15% and 20%), with the values of 46.16±4.90; 48.20±3.72 and 49.13±2.79, respectively.

![Figure 3. Discoloration slope of purple yam (Dioscorea trifida) and its incorporated bread extracts in four formulations: 0%, 10%, 15%, 20%, blank and BHT, as determined through the LPO method.](image-url)

![Figure 4. Inhibition percentage of free radicals of purple yam (Dioscorea trifida) and its incorporated bread extracts in four formulations: 0%, 10%, 15%, 20%, and BHT as determined by LPO method. Bars indicate the standard deviations.](image-url)

Table 5. Probability (P) values calculated from Shapiro-Wilk and Levene tests for evaluating frequency normality and homogeneity of variances, respectively, of the data obtained in the sensory analysis of the three tested purple yam incorporated bread formulations.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Bread%</th>
<th>Color</th>
<th>Aroma</th>
<th>Flavor</th>
<th>Texture</th>
<th>Overall impression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shapiro-Wilk P</td>
<td>10</td>
<td>0.0009</td>
<td>&lt; 0.0001</td>
<td>0.0023</td>
<td>0.0044</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>&lt; 0.0001</td>
<td>0.0009</td>
<td>0.0008</td>
<td>0.0019</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>&lt; 0.0001</td>
<td>0.0001</td>
<td>0.0002</td>
<td>0.0090</td>
<td>0.0005</td>
</tr>
<tr>
<td>Levene P</td>
<td></td>
<td>0.0519</td>
<td>0.5580</td>
<td>0.2306</td>
<td>0.8415</td>
<td>0.5184</td>
</tr>
</tbody>
</table>

Values were considered statistically significant at \((P< 0.05)\).
As it was observed by the DPPH method, the antioxidant activity rose as the percentage of purple yam substituting wheat flour in the breads increased. Moreover, bread with no addition of purple yam (0%) presented some antioxidant ability which might have resulted from the development of Maillard reaction products (Kim et al., 2007; Jing and Kitts, 2000; Hsu et al., 2004; Michalska et al., 2008). Anthocyanins might be partly responsible for the antioxidant activities detected in the purple yam (D. trifida) and its incorporated breads analyzed in the present study, since these pigments were detected in purple yams, D. alata (Rasper and Coursey, 1967) and D. trifida L. (Carreno-Diaz and Grau, 1977; Escudero et al., 2010). In fact, polyphenols and anthocyanins, usually detected in plants, might be the active components for this antioxidant activity in yams (Hou et al., 2001; Hsu et al., 2004).

**Sensory analysis of purple yam incorporated breads**

Table 5 shows the rejection of the frequency distribution normality of the three tested purple yam incorporated bread formulations (10%, 15% e 20%) through the Shapiro-Wilk test, and the acceptance of the homoscedasticity among the formulations through the Levene test on all sensory attributes evaluated in the acceptance test (i.e. statistically significant values at (P <0.05).

Friedman ANOVA followed by post hoc tests applied for comparing the three purple yam incorporated bread formulations revealed a significant difference (P<0.05), only for the colour attribute (Table 6). The bread at 20% presented a better evaluation regarding the remaining ones, probably due to the higher purple yam concentration, which gives the final product a more attractive kind of color. It was observed that the larger the purple yam amount being added to the bread the higher the mean score obtained (values ranging from 6.15 to 6.97).

Choosing a determined food should depend
mainly on its nutritional value. Nevertheless, color, aroma and texture are the factors usually guiding the consumer’s preference rate. Of these three factors, color interferes the most on the product’s preference (Bobbio and Bobbio, 2001).

Given that there were no preferential differences among the other sensory attributes, the three breads evaluated can be considered approved.

Microbiological analysis

Considering that the microbiological analysis was negative for *Coliforms* and *Salmonella* (Table 7), the purple yam incorporated breads may be considered proper for human consumption, as long as they have been properly handled.

CONCLUSIONS

Through such findings, one concludes that any of the purple yam incorporated breads tested in the present study (10%, 15% and 20%), can substitute ordinary bread (0%), with no effect on the diet’s caloric value, since their centesimal compositions are similar. Due to the presence of antioxidants in purple yam incorporated bread, and to their ability to fight free radicals, those breads can be considered as health-promoting food. Furthermore, these findings point out the feasibility of the consumption of purple yam incorporated bread, as an alternative in the local bread making industry, and an incentive to a larger production of this tuber in the Amazonian region.

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