

INFLUENCE OF DRYING ON THE ANTINUTRITIONAL CONTENTS AND ANTIOXIDANT CAPACITIES OF OYSTER MUSHROOMS (*Pleurotussajur-caju*)

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ABSTRACT

This study was conducted to determine the antinutritional contents and antioxidant activities of fresh and dried species of Oyster Mushrooms *Pleurotussajur-caju*. The antioxidant capacity were investigated using Ferricreducing/antioxidant power (FRAP) assay, 2,2'-azino-bis(3-ethylbenzothiazoline6-sulphonic acid (ABTS) and 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assays were used to investigate the antioxidant capacity. The antinutritional compositions were all below level considered harmful and the drying process bring about reduction in the antinutritional compositions of mushroom samples. With drying process there was decrease in the antioxidant content. Generally, processing reduced the antinutrients, antioxidant of the mushroom samples.

INTRODUCTION

Mushrooms has been found to be of important from nutritive, medicinal as well as the economic point of view. There are various varieties however, the edibility, ease of cultivation, nutritional values and the medicinal potential of oyster mushroom makes there highly significant when compared to other mushroom species. The high potassium and low sodium content of the mushroom makes them an ideal diet in the therapeutic treatment of hypertension and heart patients.

Mushrooms have high moisture content of about 85-90% (Khan, 2010) which makes them highly perishable. After harvest their shelf life is only about 2 to 5 days depending on the variety and the problem of seasonal availability makes there cultivation and drying essential to solve these problems. This study is focusing on determining the antinutritional and the antioxidant composition of the mushroom *Pleurotussajur-caju* specie and the effect of drying on these parameters.

MATERIALS AND METHODS

Sources of Materials

The Spawns of *Pleurotussajur-caju* were obtained from the Federal Institute for Industrial Research Oshodi; (FIIRO) in Lagos, Nigeria and were dully identified by Botanists in FIIRO and Nigerian Institute of Horticultural Research and Training, Ibadan, Nigeria (NIHORT)

Methods of data collection

Cultivation of the mushroom

Cultivation of the mushroom was carried out using *Pleurotussajurcaju* spawn, saw-dust and wheat grain. The spawn was used to inoculate sterilized mixtures of saw-dust and wheat grain it remained in the inoculating chamber for a period of 5 weeks for the mushroom to grow. Figure 1 shows the flow-chart for mushroom production.

Mixing (80kg moistened sawdust, 20 kg rice bran, 200g CaCo₃ 100g CaSo₄ and water)

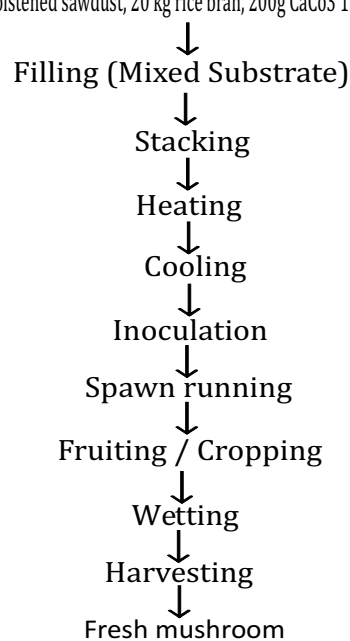


Fig. 1: Flow Chart of the substrate production/Cultivation of Oyster Mushroom Species (*Pleurotus sajur caju*) Source: Bello et al., (2017a)

Production of mushroom flour

Mushroom powder was prepared as illustrated in Fig. 2. Fresh mushrooms were cleaned, cut into slices (about 3 mm thickness) and dry at 60 °C for 8 h. Dried mushroom sample were ground separately in an electric grinder and sifted through an 80 mesh screen to obtain fine powders. The obtained powder were cooled and hygienically packed and stored in airtight container for further use.

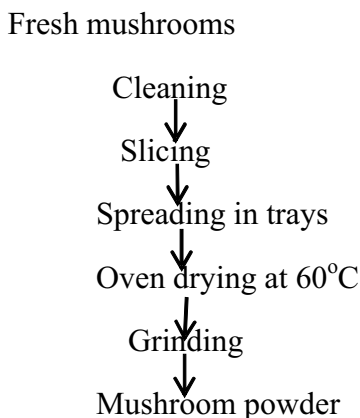


Fig. 2: Flow diagram of the preparation of mushroom powder Source: Bello *et al.*, (2017b)

Method of Data Analyses

Phytochemical Determination

Preparation of aqueous extracts of *Pleurotus sajor-caju*,

The inedible portions of the mushroom samples were removed from the edible portions. The edible portions were subsequently washed in distilled water, chopped into small pieces by table knife, air dried and milled. An amount of 5 g of the milled samples was soaked in 100 ml distilled water for 16 h on an orbital shaker, the mixture was filtered and later centrifuged at 358 g for 10 min to obtain a clear supernatant which was then used for subsequent analysis (Obboh *et al.* 2007).

Preliminary phytochemical screening

Preliminary biochemical tests such as tannins, saponins, total phenols, flavonoids, steroids, terpenoids, alkaloids, glycosides, cardiac glycosides and anthraquinone were carried out on the crude aqueous, and ethanol extract using standard procedures described by Trease and Evans, (1996) and Harborne, (1973). Based on the colour variation they were classified to high (+) and no reaction (-).

Determination of Antinutritional Factors

Determination of tannin

About 0.2 g of finely ground sample was weighed into a

50 ml sample bottle. About 10 ml of 70% aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker and shaken for 2 hours at 30 °C. Each solution was then centrifuged and the supernatant store in ice. About 0.2 solution was pipetted into the test tube and 0.8 ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. Folin ciocateau (0.5 ml) reagent was added to both sample and standard followed by 2.5 ml of 20% Na₂CO₃ the solution were then vortexed and allow to incubate for 40 minutes at room temperature, its absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared (Makkar and Goodchild, 1996).

Determination of saponin

Finely ground sample (2 g) was weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol or (But-2-ol) was added. Shaker was used to shake the mixture for 5 hours to ensure uniform mixing. The mixture was now filtered with No. 1 Whatman filter paper into 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate (MgCO₃). The mixture obtain again was filtered through No 1 Whatman filter paper to obtain a clean colourless solution. The colourless solution (1 ml) was taken into 50 ml volumetric flask using pipette, 2 ml of 5% iron (iii) chloride (FeCl₃) solution was added and made up to the mark with distil water. It was allow standing for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm (Brunner, 1984).

$$\text{Saponin content} = \frac{\text{Absorbance of sample}}{\text{Conc. of sample}} = \frac{\text{Absorbance of STD}}{\text{Conc. of sample}} \dots\dots\dots (1)$$

Determination of Bioactive Components

Determination of total phenol content:

Total phenolic content of the mushroom extracts was estimated using Folin-Ciocalteu reagent according to the method of Singleton *et al.* (1977) with some modifications. Initially, 250 µL of each mushroom extract was mixed with 250 µL of 10% Folin-Ciocalteu reagent, followed with the addition of 500 µL of saturated sodium carbonate (10% aqueous solution) after 2 min of incubation at room temperature. The mixture was kept in the dark for 1 h before absorbance was taken at 750 nm. A calibration curve using gallic acid (2–10 µg/mL) was prepared. Total phenolic content of the mushroom extracts was

expressed as gallic acid equivalents (GAEs), which reflect the phenolic content as the amount of gallic acid (mg) in 1? g of extract.

Determination of total flavonoid content:

The total flavonoids content was determined according to the method Kareti et al., (2012). One hundred micro litter of aliquot of extract was added into a ml volumetric flask containing 4 ml of distilled water. At zero time, 0.3 ml of 5% NaNO₂ was added to the flask. After 5 min, 0.3 ml of 10AlCl₃% was added. At 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the reaction solution was adjusted to 10 ml by adding 2.4 ml of distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm versus blank. The total flavonoids content was expressed in mg catechin equivalents (CE)/g of extracts.

Antioxidant Activity Assay

Determination of DPPH free radical scavenging ability

DPPH scavenging assay was determined as described by Barros et al. (2008). Mushroom extracts (0.3 mL) were mixed with methanolic solution containing DPPH radicals (6 × 10⁻⁵ mM, 2.7 mL). The mixture was shaken vigorously and left to stand for 60 minutes in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the bleaching of the purple-colored methanol solution of DPPH at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the following equation:

$$\% \text{ RSA} = [(A_{\text{DPPH}} - A_s) / A_{\text{DPPH}}] \times 100 \dots\dots\dots(2)$$

where A_s is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

The extract concentration providing 50% of radicals scavenging activity (IC₅₀) was calculated from the graph of RSA percentage against extract concentration. Butylated hydroxyl anisole (BHA) was used as standard. Extract concentration providing 50% inhibition (IC₅₀) was calculated from the plotted graph of inhibition percentage against extract concentration. Tests were carried out in triplicate.

Ferric-Reducing/Antioxidant Power (FRAP)

The procedure of FRAP assay used was according to Benzie and Strain (1996). The FRAP reagent contained 2.5? mL of 10? mmol? L⁻¹ TPTZ (2,4,6-tripyridyl-s-triazine, Sigma) solution in 40? mmol? L⁻¹ HCl plus

2.5? mL of 20? mmol? L⁻¹ FeCl₃ and 25? mL of 0.3? mol? L⁻¹ acetate buffer, pH 3.6, and was prepared freshly and warmed at 37°C. Aliquots of 40? L sample supernatant were mixed with 0.2? mL distilled water and 1.8? mL FRAP reagent. The absorbance of reaction mixture at 593? nm was measured spectrophotometrically after incubation at for 10? min in a microplate (PowerWave XS, BioTek). The 1? mmol? L⁻¹ FeSO₄ was used as the standard solution. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1? mmol? L⁻¹ FeSO₄. Adequate dilution was needed if the FRAP value measured was over the linear range of standard curve.

Statistical Analysis

The experimental results were expressed as mean ± standard error (SE) of three replicates. Data obtained were statistically analysed using Analysis of Variance (ANOVA), a tool in Statistical packages for Social Science (SPSS 14.0). The level of significance was set at p < 0.05. Means were separated with Duncan's New Multiple Range (DNMR) Test.

RESULTS AND DISCUSSION

Phytochemical Screening of Pleurotus sajur-caju Oyster mushroom

Table 1 shows the result of phytochemical screening of the oyster mushroom. The table shows the presence of alkaloids (+); tannins (+); saponins (+); oxalates (+); flavonoids (+); terpenoids (+); and steroids (+); Anthraquinone (+); and Cardiac glycosides (+).

Table 1: Phytochemical Screening of fresh and Cabinet dried Pleurotus saju-caju mushrooms

Parameters	Fresh <i>Pleurotus sajur-caju</i>	Dried <i>Pleurotus sajur-caju</i>
Alkaloids	+	+
Tannin	+	+
Saponin	+	+
Oxalate	+	+
Flavonoid	+	+
Cardiac glycosides	+	+
Anthraquinone	-	-
Steroids	+	+
Terpenoid	+	+
Phlobatannin	-	-

+ = Present

- = Not present

Preliminary phytochemical screening of the oyster mushroom species revealed the presence of different phytochemicals such as tannins, saponins, oxalates, phytates, flavonoids, steroids, alkaloids, glycosides and terpenoids. The result shows all the tested phytochemicals were present (+ve) in the aqueous extract of the three oyster mushroom species.

Antinutritional Composition of *Pleurotus sajor caju* mushrooms

Table 2 shows the result of antinutrient content in mg/100g of both the fresh and dried species of oyster mushroom as follows: tannin (2.43) and (1.08); saponin (13.82) and (2.13); steroid (5.15) and (2.25); Glycosides (27.350) and (17.80); Alkaloids (21.32) and (14.70); oxalates (0.97) and (0.37); phytate (14.83) and (5.65). The Table revealed that there were significant differences in the antinutrient compositions among the fresh as well as the dried samples.

Tannin content

The tannin content obtained in *Pleurotus sajor -caju*, was within the range reported of 0.21-0.31% reported by Akindahunsi and Oyetayo (2006) in *Pleurotus tuber-regium* parts and range of 0.27 – 0.30% reported by Kayode et al. (2013). These levels might not affect the nutritional potentials of the mushroom parts since they were all less than 10% of the total dry weight of the samples (Osagie, 1988). The low tannin content of the mushroom could be responsible for the absence of bitter taste in the samples. The reduction in dried sample may be due to tannin being labile and its sensitivity to oxidation. Drying showed significant reduction in tannin concentration at ($p < 0.05$) making it a good method for the reduction of antinutritional factors.

Table 2: Antinutritional composition of *Pleurotus sajor -caju* mushrooms (mg/100g)

Parameters	FPS	DPS	SL(WHO, 2003)
Saponin	13.82 ^a ± 0.01	2.13 ^c ± 0.05	48.05
Tannin	2.43 ^a ± 0.01	1.08 ^a ± 0.02	10%
Steroid	5.15 ^a ± 0.04	2.25 ^b ± 0.02	-
Glycosides	27.35 ^a ± 0.09	17.80 ^b ± 0.03	-
Alkaloid (%)	21.32 ^a ± 0.07	14.70 ^b ± 0.03	-
Oxalates	0.97 ^a ± 0.02	0.37 ^b ± 0.01	105.00
Phytates	14.83 ^a ± 0.02	5.65 ^b ± 0.20	22.10

Data represent mean ± standard deviation of three replicates. Mean values with the same superscript in a row are not significantly different [$p > 0.05$]

KEY:

FPS = Fresh *Pleurotus sajor -caju*

SL = Safe Limit

DPS = Dried *Pleurotus sajor -caju*

WHO = World health organisation

Phytate content

The phytate contents of the studied mushrooms were in the range of 12.36 - 14.83 mg/100g for the fresh samples while there was reduction in the values of the dried samples in the range of 4.81 – 5.65 mg/100g. The reduction in the phytate content of the dried samples is an indication that phytate is liable to heat like all other antinutrients. These values are lower than the safe limit (22.10 mg/ 100g) (WHO, 2003). Phytate concentration (mg/100g) obtained in this study were lower than the values 18.15-38.5 mg/100g reported by Akindahunsi and Oyetayo (2006) and equally lower when compared with green leafy vegetables whose phytate contents were found to be exceptionally high (Akindahunsi and Obobo, 1999).

Saponin content

The saponin content of the oyster mushroom species in this study was found to be in the range of 13.82 – 15.70 mg/100g for the fresh samples while for the dried samples, 2.13 – 2.45 mg/100g were recorded. Drying caused significant ($P < 0.05$) reductions in saponin content. Being glycosides, they are heat labile. The values were lower than the maximum permissible limit of 48.05mg/100g set by WHO (2003). The values were lower than 4.05% reported of Kayode et al. (2013) but comparable to 1.26% reported by Ogbe and Obeka (2013). They were however higher than 0.02 – 0.51% reported by Afiukwa et al. (2013).

Oxalate content

The oxalate content in both the fresh and dried oyster mushroom in this study was found to be within the range of 0.97 – 1.59 mg/100 g, as shown in Table 4.6. These values were lower than the tolerable limit of (105 mg/ 100 g) given by WHO (2003) and within the range of 0.57 reported by Ogbe and Obeka (2013). There were significant differences among both the fresh and dried samples in terms of their content of oxalates.

Antioxidant Properties

Total phenolic and flavonoid contents

Total Phenol content

Table 3 shows the total phenolic content of fresh and dried mushroom *Pleurotus sajor -caju*. Drying reduced the total phenol content of the samples. The total phenolic content obtained in this study for both the fresh and the dried *Pleurotus sajor -caju* were as follows: 28.70 and 22.40 mg/g respectively.

The fresh mushroom had higher phenolic content than the dried samples. This reduction of phenolics by drying process was supported by Praveen et al. (2007) as phenolic compounds are heat labile and other

researchers reported that phenolic compounds are unstable. Heat used in the drying procedure could destroy the structures of polyphenols and cause a decrease in their antioxidant activity (Yen and Hung, 2000; Leffer, 1993). Comparison with the previous

research works showed that the results of this study were lower than the reported values of in antioxidant properties in the oyster and split gill mushrooms (Arbaayah and Umi, 2013).

Table 3: Antioxidants content of fresh and dried *Pleurotus sajur -caju* mushroom

Parameters	Fresh	Dried
	<i>Pleurotus sajur -caju</i>	<i>Pleurotus sajur -caju</i>
DPPH (%)	70.16 ^a ±0.08	52.19 ^c ±0.04
FRAP ((mg AAE/100g)	18.81 ^a ±0.02	12.20 ^e ±0.06
ABTS (mg TEAC/100g)	0.04 ^a ±0.01	0.03 ^a ±0.02
Total phenol (mg/g)	2.87 ^a ±0.04	2.24 ^e ±0.03
Flavonoid (mg/g)	0.55 ^a ±0.02	0.29 ^e ±0.01

Data represent mean ± standard deviation of three replicates.

Mean values with the same superscript in a row are not significantly different [p>0.05]

Total flavonoid content

The total flavonoid content of the fresh and dried *Pleurotus sajur -caju* were; 8.52 and 5.93 mg/g respectively. The result indicated the fact that flavonoid content was equally affected by drying. Flavonoids are large secondary metabolites that are widely found in plant species. Several studies have demonstrated that flavonoid may act as an antioxidant by breaking the free radical chains into more stable forms and also plays an important role to provide instinctive protection against oxidative stress (Arbaayah and Umi, 2013). The flavonoid content of the three species of oyster mushrooms shows similar trend to their phenolic content and this could be due to the fact that flavonoids are major parts of phenolic compounds (Mai, 2007). The result obtained in this study is comparable to the values reported by Arbaayah and Umi (2013) in the first flush of Oyster mushrooms and split gill mushrooms. The results equally showed that the three oyster mushroom species had lower flavonoid content than Persian sour summer pomegranate, the flavonoid content reported by Olajire and Azeez (2011) in cabbage leaves, Onion bulb and in two varieties of ginger as reported by Ayodele et al. (2011).

Antioxidant activities of *Pleurotus sajur -caju*

DPPH activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability of the extracts of the oyster mushrooms are shown in Table 6. The antioxidant activity as % DPPH in the oyster mushrooms followed

this trend for both fresh and dried samples: *Pleurotus sajur caju* showed a strong antioxidant activity of 70.16%, followed by the dried *Pleurotus sajur -caju* of 52.19. There was reduction in the antioxidative properties as measured by the scavenging abilities of the extracts. This is comparable to the report of Antia et al. (2013) on the effect of drying on phenolic and antioxidative capacity of spearmints; that of Priyanka et al. (2016) on the effect of drying techniques on antioxidant capacity of guava fruits; and of Bindvi (2014) on the effect of cooking on antioxidant activity and phenolic contents of various mushrooms of India. The reduction in the antioxidative activity of the species might be due to cell structure damage during heating. This is comparable to the report of Puupponen-Pimea et al. (2013). The values obtained in this study were however higher than the values reported by Bindvi (2004) but were within the range reported by Bindvi (2014) in antioxidant activities of various vegetables.

Ferric reducing antioxidant power (FRAP)

There was significant difference in the reducing power of the fresh and the dried *Pleurotus sajur -caju*, in which higher values were recorded for the fresh samples., *Pleurotus sajur -caju* mushroom recorded the highest reducing antioxidant power (18.81mg AAE/100g), followed dried *Pleurotus sajur -caju* (12.20 mg AAE/100g), The presence of reductants (i.e. antioxidants) in the sample (mushroom samples) resulted in the F³⁺ to Fe²⁺ by donating an electron. The resulting properties are generally associated with the

presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain via donation of hydrogen atom. Reductones have also been reported to react with certain precursors of peroxide thus preventing peroxide formation (Suaib, 2012). The mushroom samples thus showed the presence of this powerful tool called reductones.

CONCLUSION

The antinutritional composition of the samples were well below the acceptable limits hence, will not pose any health threat and will not hinder the availability of macronutrients to the consumer. Mushroom may be regarded as a good source of antioxidant however they are heat liable hence minimal amount of heat should be used in the drying process so as to prevent loss of the essential components.

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