Agr. Foo. Nat. Res. J. Volume 4, Issue 2, Page 42 - 50
e-ISSN: 1597-7153 | p-ISSN: 3043-5420

Agriculture, Food and Natural Resources Journal
The Official Journal of the Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Nigeria

Journal homepage: https://journals.unizik.edu.ng/afnrj

Original Article

Evaluation of phytochemicals and anti nutrient changes in canned mushroom (*Calocybe indica*) in different brine solutions

OPEN ACCESS

Zaccheaus Olasupo APOTIOLA*[1], Caroline ANYAKORAH[2] & Olubukola Omoniyi KUFORIJI[2]

[1]Department of Food Science and Technology, Lagos State University of Science and Technology, Ikorodu, Lagos State, Nigeria.
[2]Department of Biological Sciences, Bells University of Technology, Ota, Ogun State., Nigeria.
DOI: https://www.doi.org/10.5281/zenodo.17209527

Editor: *Dr Onyekachi Chukwu, Nnamdi Azikiwe University, NIGERIA*

Received: *December 5, 2024*
Accepted: *April 10, 2025*
Available online: *September 30, 2025*

Peer-review: *Externally peer-reviewed*

Conflict of Interest: *The authors have no conflicts of interest to declare*

Financial Disclosure: *The authors declared that this study has received no financial support.*

ABSTRACT

This study evaluated the effect of storage period on the phytochemical and anti-nutrient contents of fresh and canned milky Calocybe indica stored for six weeks. Freshly cultivated mushrooms were washed, cut into 5 cm sizes, and canned in brine solutions containing varying ratios of salt and citric acid (2.0:1.5, 2.5:1.5, 3.0:1.5, and 3.5:1.5). Each can weigh approximately 190g. Phytochemical and anti-nutrient compositions were analysed weekly using standard methods. Data were subjected to one-way analysis of variance ($p < 0.05$). The phytochemical results (%) ranged as follows: total phenol (7.07–24.54), flavonoids (20.20–29.17), glycosides (0.02–0.34), alkaloids (0.11–2.67), total antioxidants (3.01–4.39), and ferric reducing antioxidant power (0.22–0.68). Anti-nutrient values (%) for tannins, phytates, saponins, and oxalates ranged from 0.04–0.29, 0.11–0.89, 0.06–0.54, and 0.23–0.44, respectively. Canning significantly reduced all parameters except flavonoids, which increased. During storage, most phytochemicals and anti-nutrients decreased significantly, though total phenol, total antioxidants, and ferric reducing antioxidant power increased with higher sodium chloride concentrations in the brine. The brine solution had little effect on other parameters. The study concludes that storage time influenced the anti-nutrient composition, with levels remaining below harmful thresholds, confirming the mushrooms' safety for consumption. Overall, canning and subsequent leaching of phytochemicals into the brine contributed to notable phytochemical losses over time.

KEYWORDS: *Antioxidant, Anti-nutrients, Canning, Mushroom, Phytochemicals*

INTRODUCTION

Mushrooms have been eaten as edible fungi and regarded as plants with infinite life since ancient Egypt and it is believed that the first intentional mushroom cultivation took place approximately 1400 years ago. For a very long time, people have used wild mushrooms as food (Kotowski, 2019). Mushrooms are fleshy fungi that produce spores, which are fruiting bodies, when they grow in soil above ground. They often refer to the fruiting bodies of gill fungi, which, unlike green plants, lack chlorophyll and cannot produce nourishment on their own. These products are extremely nutritious and can be made from lignocellulosic waste materials (Kamthan & Tiwari, 2017). Approximately 200 species of mushrooms have been commercially produced for use in therapeutic formulations and for human consumption out of the more than 14,000 species

*Corresponding author: zoapotiola@yahoo.com

of mushrooms found worldwide. Of these, about 2000 species are edible (Garofalo *et al*., 2017; Mleczek *et al*., 2018).

Maurya *et al*. (2019) and Chelladuraiv *et al*. (2021) documented that edible mushrooms are in high demand as a staple food source due to their potential textural, taste, medicinal, and tonic properties. Mushroom contains necessary phytochemicals (phenols, flavonoids, terpenes, terpenoids, steroids), as well as minerals, protein, fiber, vitamins B_1, B_2, B_{12}, C, D, and E, and ideal nutritional compositions high in copper, zinc, boron, nickel, and chromium. According to Chelladuraiv *et al*. (2021), *Calocybe indica* is a milky-white mushroom that is an excellent source of ascorbic acid, lipids, riboflavin, amino acids, pyridoxine, vitamins, biotin, low fat, nicotinic acid, proteins, minerals (such as arsenic, zinc, potassium, manganese, calcium, phosphorus, magnesium, iron, and sodium), and fibers. It has been shown that *Calocybe indica* is useful in treating cardiovascular and artery-related disorders, atherosclerosis, and hepatic disorders (Roy & Prasad, 2014). *Calocybe indica* is a good source of bioactive polysaccharides, including polyphenols (flavonoids, alkaloids, and triterpenoids) and β-glucans and these active ingredients might be in charge of scavenging processes, improving antioxidant activity (Mirunalini *et al*., 2012; Shashikant *et al*., 2022), and having anti-diabetic, anti-cancer, and anti-lipid peroxidation properties (Ghosh *et al*., 2015). *Calocybe indica* has been reported to have a longer shelf life in tropical climates, it is therefore thought to be a better substitute for *Pleurotus ostreatus* (Chelladurai *et al*., 2021). Ascertaining the effect of storage period on phytochemicals and anti-nutrient characteristics of the canned milky mushroom (*Calocybe indica*) in different brine solution is the specific goal of this study.

MATERIALS

Materials for Growing Mushroom

The materials for growing mushrooms include Mushroom Spawns, sawdust (*cola nitida*), rice bran, calcium carbonate, metal drum for sterilization, methylated spirit, cotton wool, firewood, black polyethylene sheet, 1kg capacity poly propylene bags, rubber bands, paper sheets, 2cm thick polyvinyl chloride (PVC) pipes as bottle necks.

Experimental Site

This study was conducted at Lagos State University of Science and Technology, Ikorodu, Lagos from February to June 2022.

Experimental Design

Substrate quantity (Sawdust and Rice Bran of ratio 4:1 (40kg: 10kg) was mixed together with 1% of Calcium Carbonate $CaCO_3$ which was also added in order to help balance the pH of the already mixed sawdust and rice bran) was evaluated for growing *Calocybe indica* (Milky Mushroom). 70% of water was added to the mixed substance for homogeneity. The Substrates were packed in a 1kg sized Polypropylene bags and slightly tightened with a rubber band. The Poly-propelene Bag which can withstand more than 100°C. After bagging, it was arranged in the Metal Drum for Sterilization and was allowed to steam for 1hour. The Substrate was brought out and allowed to cool until room temperature so as to prevent the heat from killing the fungi that was introduced into it.

Inoculation and Spawning (7 Days Old Spawn)

The substrate after sterilization was loosed and the spawn (10%) were added into it. The layer was slightly pressed into the straw to make intimate contact of the Spawn Grains with the substrates (figure 3.2). The spawn has a mixture of sorghum and sawdust. The bags were also perforated with a punch to make approximately 20 vents (diameter 1 per bag)

Casing

Casing Materials which include Cassava peels, Molasses, Sawdust and small quantity of Rice bran was mixed together steamed in an Autoclave at 110°C for 60mins. After cooling for 24hrs, the mixture was uniformly spread in layer of 3 -4cm thickness over the Spawn Bags. Regular spraying on the surface of the casing material was done to maintain even moisture nutrient on the bed surface.

Harvesting and Slicing

After the opening of bags, pinning started to appear in the bags (after 14 days) and mushrooms start appearing in flushes. Fruits bodies were then harvested by gently twisting. The substrate end part of the harvest fruit bodies was trimmed off with clean knife (plates 3.5, 3.6 and 3.7). The freshly harvested fruit bodies were collected. After harvesting the fruit bodies, they were washed under tap water and cut into vertical slice.

Plate 1: Sawdust and rice bran

Plate 2: Growth of *Calocybe indica*

Plate 3: Cultivated *Calocybe indica*

Mushroom Cultivation, Brining and Casing

Calocybe indica was cultivated using the bag cultivation method of Singh and Singh (2012) with some modifications while the method of Thakur (2016) was used in harvesting and canning of the mushroom with slight modifications (Figure 1). The canning process is divided into various unit operations. The selected mushrooms were cleaned using running water to remove all the dirt on the mushrooms and cut into smaller 5 cm for cap diameter and stem length. The mushrooms underwent blanching in a 1% citric acid solution for five minutes at 95°C to limit enzymatic activity, inactivate microorganisms, release gases out of the mushroom tissue, and lower bacterial counts. The blanched mushrooms were filled into empty sterile cans with 60ml of brine solution (leaving a ½ inch headspace). After filling the cans, they were labeled and exhausted for 10mins and then hermetically sealed with a semiautomated seamer. The total weight of each can was approximately 190g. The brine solution was prepared with varying amount of (NaCl: Citric acid 2.0: 1.5; 2.5: 1.5; 3.0: 1.5 and 3.5: 1.5). Sterilization was done at 121^{0}C for 1hr using DWS-280 Series portable pressure steam sterilizer and was allowed to cool. After cooling the cans were at room temperature for six weeks (Thakur, 2016). Analysis was carried out after canning at 1, 2, 3, 4, 5, 6 weeks of storage period with the fresh sample as a control.

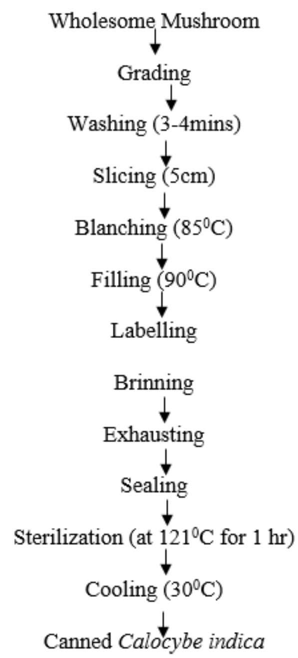

Figure 1: Process flow diagram for the production of canned mushroom

Source: Thakur, (2016).

Determination of Phytochemical

Total flavonoid content (TFC) of the samples was determined using AOAC (2018) colorimetric method. The total phenolic content was determined using spectrophotometric method of AOAC (2018). The reducing power of the extract was evaluated following the AOAC (2018) method. Alkaloid and Glycosides were estimated using the method described by Edet *et al*. (2016).

Determination of Anti-Nutrients

Oxalate, phytate, and tannin contents were determined by the method reported by Aguchem *et al*. (2022) while the method of Adetuyi *et al*. (2011) was used for saponin determination.

Statistical Analysis

Results were expressed as mean values and standard deviation of two (2) determinations. Data were analysed using one-way analyses of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20.0 software 2011 to test the level of significance ($p < 0.05$). Duncan New Multiple Range Test was used to separate the means where significant differences existed.

RESULTS AND DISCUSSION

The phytochemical contents of the fresh and canned milky mushroom (Calocybe indica) stored for six weeks in different brine salt solutions are presented in Table 1. The phenol and flavonoid contents of the fresh and canned Calocybe indica mushroom ranged from 7.07 - 24.54% and 20.32-29.17% respectively. Significant difference ($p<0.05$) exists among the samples. According to Finimundy *et al*. (2013), Liu *et al*. (2017), and Abdelshafy *et al*. (2021), mushrooms contain phenolic compounds that have anti-inflammatory, anti-cancer, and anti-microbial properties. They also provide protection against a number of degenerative conditions, such as aging, cardiovascular disease, and brain dysfunction. Both phenols and flavonoids, which are effective natural antioxidants, were found in this study, indicating that this particular species of mushroom may be used as a potential agent in the treatment of medical conditions such as inflammatory, cancer, cardiovascular disease and brain dysfunction.

According to earlier research, phenols, flavonoids, tannins, and terpenoids have a variety of pharmacological and therapeutic advantages, such as anti-inflammatory, anti-cancer, and anti-malaria properties (Wandati and Kenji, 2013; Pandimeena *et al*., 2015; Okorie *et al*., 2021). The glycoside and alkaloid content ranged from 0.02 - 0.34% and 0.11 - 2.67% respectively with the fresh sample having the highest value. Alkaloids have been shown to have antipuretic properties, strong pain relievers, and stimulating effects. They also function as tropical anesthetics in ophthalmology (Ade-Ogunnowo *et al*., 2021). Ade-Ogunnowo *et al*. (2021) also found the presence of alkaloids in numerous mushroom species. The values obtained in this study were lower when compared with the value (3.42 mg/g) reported by Shyni *et al*. (2018) for edible mushroom Calocybe indica using solvents extracts. The total antioxidant and ferric reducing antioxidant power ranged from 2.78 - 4.39% and 0.22 - 0.68%. According to Vimalkumar *et al*. (2014), antioxidants have numerous applications that improve human health as a means of disease prevention and management. Antioxidant compounds in food have the ability to protect against chronic disease (Oluwajuyitan & Ijarotimi 2019). The hydroxyl group of the phenolic compounds may be the cause of the antioxidants' reducing power in the canned milky mushroom samples. However, a compound's reducing capacity could be a useful predictor of its possible antioxidant action (Badejo *et al*., 2017). The results of this research showed that the fresh sample had the highest ferric reducing antioxidant power. However, the total antioxidants and ferric reducing antioxidants power was observed to increase with increase in sodium chloride of the brine solution but decrease with storage time.

In addition, the phenol content was only observed to increase with increase in sodium chloride of the brine solution for the first two weeks followed by a gradual decline up to sixth week. A slight increase was also observed in the flavonoid content with increase in sodium chloride of the brine solution in fifth and sixth week respectively. While alkaloids and glycosides gradually decline both with increase in sodium chloride of the brine solution and as well as increase in storage period. However, a study by Abacan *et al*. (2017) examined the impact of cooking time, temperature, and salt concentration on the phenolic content and antioxidant activity of various edible mushrooms, including Agaricus bisporus, Pleurotus florida, Pleurotus ostreatus, and Termitomyces cartilagineus. The researchers found that variations in salt concentration (ranging from 0 to 6 g/L) did not significantly affect the levels of phenolics or antioxidant activity. The decline patterns of phenol, alkaloid, glycosides, antioxidants and ferric reducing antioxidants power levels after canning and during storage is in line with research by Putriani *et al*. (2020). Food processing might change the matrix composition and chemical structure, which could impact the bioavailability of polyphenols (Ribas-Agustí *et al*., 2018; Shahidi and Peng, 2018). The bioactive metabolites can be absorbed by the circulatory system and subsequently exhibit beneficial antioxidant activity for human health (Palafox-Carlos *et al*., 2011). It was found that canning the mushrooms flavonoid content significantly ($p<0.05$) increased after canning but then declined with longer storage times. Total flavonoid content in this research was higher than that of A. hypochondriacus (13.4 – 14.3 mg CE/100g) and oat (17.7 mg/100g) as reported by Pamela *et al*. (2017). According to Olajuyigbe *et al*. (2011); Khanam *et al*. (2012); Abushouk *et al*. (2017) and Uddin *et al*. (2018) flavonoids are a class of hydroxylated phenolics that have been shown to have heart-protective, anti-inflammatory, anti-cancer, anti-bacterial, and antioxidant properties. Leaching of the phytochemicals into the brine solutions during the storage time or the heat the canned samples were exposed to may have caused the reduction seen after canning and during the storage period. More so, higher cooking temperatures were associated with reduced phenolic content but increased antioxidant activity, indicating that temperature plays a more critical role than salt concentration in influencing these compounds as reported by Abacan *et al*. (2017). According to Khan *et al*. (2013), milky mushrooms have been identified as a possible natural source of dietary flavonoids, exhibiting a wide variety of chemicals in considerable concentration and exhibiting healthful qualities.

The anti-nutritional contents of the fresh and canned milky mushroom (Calocybe indica) stored for six weeks are presented in Table 2. The tannin, phytate, saponin and oxalate contents varied between 0.04 - 0.47%, 0.11 - 0.89%, 0.06 - 0.54%, 0.23 - 0.44%, respectively. The levels of anti-nutrients in the canned milky mushroom significantly ($p > 0.05$) decreased from first week to the 6th week. This observation is in good agreement with the findings of Nketia *et al*. (2020) who also reported decrease in soluble and solids substances with storage period due to leaching.

Table 1: Phytochemical Contents of Fresh and Canned Milky *Calocybe indica* Stored for Six Weeks

Week	Sample	Phenol (%)	Flavonoids (%)	Glycoside (%)	Alkaloids (%)	Total Antioxidant (%)	FRAP (%)
0	S10	24.54±3.54[a]	22.18±0.00[d]	0.34±0.01[a]	2.67±0.01[a]	4.39±0.00[a]	0.66±0.01[a]
1	S20	11.69±0.00[b]	29.17±0.01[a]	0.08±0.00[c]	0.66±0.01[b]	3.42±0.31[c]	0.34±0.02[d]
	S25	13.43±0.00[b]	28.44±0.02[b]	0.06±0.01[b]	0.61±0.01[c]	3.98±0.00[b]	0.39±0.02[c]
	S30	13.27±0.00[b]	24.92±0.00[c]	0.07±0.01[c]	0.57±0.00[d]	4.16±0.00[ab]	0.42±0.03[b]
	S35	14.28±0.00[b]	22.76±0.01[d]	0.05±0.00[d]	0.53±0.00[e]	4.23±0.23[ab]	0.68±0.01[a]
2	S20	10.13±0.00[d]	25.84±0.00[b]	0.06±0.00[a]	0.53±0.01[a]	3.18±0.00[c]	0.27±0.01[c]
	S25	10.29±0.00[c]	23.27±0.00[c]	0.06±0.00[a]	0.48±0.00[b]	3.33±0.00[bc]	0.31±0.01[ab]
	S30	10.98±0.01[b]	22.29±0.01[c]	0.06±0.00[a]	0.41±0.01[c]	3.38±0.18[ab]	0.34±0.02[ab]
	S35	11.39±0.00[a]	20.32±0.00[d]	0.05±0.00[b]	0.21±0.01[d]	3.51±0.00[a]	0.55±0.01[a]
3	S20	10.69±0.00[a]	25.16±0.01[b]	0.03±0.00[a]	0.28±0.01[ab]	3.18±0.01[c]	0.25±0.00[c]
	S25	10.46±0.00[d]	21.22±0.02[d]	0.03±0.00[a]	0.26±0.01[ab]	3.32±0.01[b]	0.31±0.03[b]
	S30	10.58±0.01[b]	21.18±0.02[d]	0.03±0.01[a]	0.51±0.39[a]	3.34±0.14[b]	0.33±0.02[b]
	S35	10.53±0.00[c]	20.54±0.04[e]	0.03±0.01[a]	0.27±0.05[ab]	3.50±0.01[a]	0.55±0.00[a]
4	S20	9.43±0.00[a]	20.00±0.22[e]	0.03±0.00[a]	0.23±0.00[a]	3.08±0.16[b]	0.25±0.00[c]
	S25	9.42±0.00[b]	20.32±0.03[e]	0.03±0.00[a]	0.23±0.00[a]	3.32±0.01[a]	0.31±0.03[b]
	S30	7.95±0.00[c]	21.21±0.31[d]	0.02±0.00[b]	0.21±0.00[b]	3.34±0.14[a]	0.33±0.02[b]
	S35	7.22±0.00[d]	22.02±0.22[d]	0.02±0.00[b]	0.21±0.00[b]	3.50±0.01[a]	0.55±0.00[a]
5	S20	9.33±0.00[a]	20.20±0.12[e]	0.03±0.00[a]	0.20±0.00[a]	3.01±0.11[b]	0.24±0.01[c]
	S25	9.31±0.01[b]	21.41±0.20[d]	0.03±0.00[a]	0.13±0.00[b]	3.24±0.03[a]	0.28±0.05[bc]
	S30	7.69±0.00[c]	22.03±0.04[d]	0.02±0.00[b]	0.13±0.00[b]	3.19±0.04[a]	0.29±0.02[b]
	S35	7.21±0.01[d]	21.30±0.02[d]	0.02±0.00[b]	0.11±0.00[c]	3.32±0.05[a]	0.54±0.00[a]
6	S20	8.95±0.00[a]	22.04±0.33[d]	0.03±0.00[a]	0.21±0.01[a]	2.78±0.47[a]	0.22±0.01[c]
	S25	8.65±0.00[b]	20.32±0.23[e]	0.03±0.00[a]	0.12±0.00[b]	3.19±0.06[a]	0.28±0.05[b]
	S30	7.24±0.00[c]	20.22±0.33[e]	0.02±0.00[b]	0.11±0.00[c]	3.19±0.10[a]	0.27±0.00[b]
	S35	7.07±0.00[d]	21.20±0.03[d]	0.02±0.00[b]	0.11±0.00[c]	3.26±0.08[a]	0.53±0.00[a]
	WHO/ FAO		52.02		61.00		

*Mean values with same superscripts in the same column are not significantly different at p>0.05. FRAP: Ferric reducing antioxidants power; S10-Fresh *Calocybe indica*; S20 - Canned *Calocybe indica* (2.00g sodium chloride; 1.50 g citric acid); S25- Canned *Calocybe indica* (2.5 sodium chloride; 1.50 g citric acid); S30 - Canned *Calocybe indica* (3.00g sodium chloride; 1.50 g citric acid); S35 - Canned *Calocybe indica* (3.50g sodium chloride; 1.50 g citric acid).

However, the result confirmed the presence of tannins, phytate, saponin and oxalate of the canned *Calocybe indica* during the storage period to be in moderate percentage. According to Khan, (2023) tannin precipitate and bound proteins and other organic substances from our diet, such as amino acids and alkaloids which are polyphenolic molecules that prevent human cells from using them. The results of this investigation are within the range of 0.03 to 1.72 mg/100g that Ekpa & Sani (2018) reported. Phytate can also form chelates with di- and trivalent metallic ions, including Cd, Mg, Zn, and Fe, to create poorly soluble compounds that are difficult to absorb from the gastrointestinal system, hence decreasing their bioavailability (Wasagu *et al*., 2013). The phytate results observed in this study were relatively lower compared to ideal phytate content of 25 mg/100g for food products as reported by Bello *et al.* (2013). The phytate range were also lower than the standard safe limit (22.10mg/100g) (WHO, 2003). Saponin on the other hand has a protective property, including hepatoprotective, gastro-protective, and hypolipidemic effects (Banno *et al*., 2004). The study's saponin results are comparatively below the WHO maximum allowable limit of 48.50 mg/100g. According to Kao *et al*. (2008), saponins are useful in preserving liver function, reducing blood cholesterol, and averting peptic ulcers. According to the study's findings, the amount of oxalate reduced as storage time and citric acid concentration rose from 2.00 g to 3.50 g. The results are significantly lower than the WHO acceptable limit of 105.00 mg/100 g, which is supported by Haden's (2007) findings of 0.412%. According to Bolarin *et al*. (2023), an elevated oxalate content in the human diet may raise the risk of kidney stones and renal calcium absorption. Awolu *et al*. (2017) reported that the toxicity limit for oxalates and phytates in humans is 2 – 5 g/day, while the average mean value of oxalates (0.42 mg/g) reported by Polycarp *et al*. (2012) was higher than the results obtained in this investigation. Disparities in the results may result from differing raw material sources and processing techniques used. The samples of mushrooms had saponin levels below the range of 0.6656 to 1.001 mg/g, as well as below the ranges of 4.05% and 3.03% for the wild and exotic oyster mushroom species reported by Kayode *et al*. (2013) and 1.26% by Ogbe and Obeka (2013). Additionally, it is less than the WHO-established upper limit of 48.05 mg/100 g (2003). The study found that the antinutritional factors (oxalate, tannin, phytate, and saponin) had little or no significant different at p>0.05 but decreased as the storage period increased as well as increase in sodium chloride of the brine solution, though this decrease does not follow regular patterns. The reduction was found to be below the acceptable limits for consumption and as such poses no danger to safety.

Table 2: Anti-nutritional Content of Fresh and Canned *Calocybe indica* Stored for a Period of six weeks

Week	Sample	Tannin (%)	Phytate (%)	Saponin (%)	Oxalate (%)
1	S10	0.29±0.02^{b}	0.89±0.00^{a}	0.54±0.03^{a}	0.44±0.03^{a}
	S20	0.27±0.01^{b}	0.17±0.01^{d}	0.49±0.05^{ab}	0.39±0.04^{ab}
	S25	0.21±0.02^{c}	0.18±0.00^{c}	0.47±0.01^{ab}	0.38±0.00^{ab}
	S30	0.14±0.01^{d}	0.19±0.01^{b}	0.45±0.08^{c}	0.38±0.07^{ab}
	S35	0.47±0.01^{a}	0.19±0.00^{c}	0.43±0.01^{c}	0.35±0.00^{c}
2	S20	0.14±0.00^{a}	0.15±0.00^{a}	0.13±0.01^{a}	0.31±0.03^{a}
	S25	0.12±0.07^{a}	0.15±0.02^{a}	0.10±0.06^{a}	0.30±0.00^{a}
	S30	0.09±0.01^{ab}	0.12±0.00^{b}	0.11±0.01^{a}	0.29±0.05^{a}
	S35	0.05±0.01^{b}	0.11±0.01^{b}	0.14±0.01^{a}	0.28±0.00^{a}
3	S20	0.14±0.00^{a}	0.15±0.01^{a}	0.13±0.00^{a}	0.29±0.03^{a}
	S25	0.12±0.07^{a}	0.15±0.02^{a}	0.08±0.02^{b}	0.29±0.01^{a}
	S30	0.09±0.01^{ab}	0.12±0.00^{a}	0.12±0.01^{a}	0.24±0.05^{a}
	S35	0.05±0.01^{b}	0.13±0.01^{a}	0.13±0.00^{a}	0.27±0.01^{a}
4	S20	0.14±0.00^{a}	0.15±0.01^{a}	0.13±0.00^{a}	0.29±0.03^{a}
	S25	0.12±0.07^{a}	0.15±0.02^{a}	0.08±0.02^{b}	0.29±0.01^{a}
	S30	0.09±0.01^{ab}	0.12±0.00^{a}	0.12±0.01^{a}	0.24±0.05^{a}
	S35	0.05±0.01^{b}	0.13±0.06^{a}	0.13±0.00^{a}	0.27±0.01^{a}
5	S20	0.13±0.00^{a}	0.14±0.02^{a}	0.13±0.01^{a}	0.28±0.05^{a}
	S25	0.10±0.02^{b}	0.14±0.02^{a}	0.07±0.01^{b}	0.29±0.02^{a}
	S30	0.09±0.01^{b}	0.12±0.01^{a}	0.12±0.01^{a}	0.24±0.06^{a}
	S35	0.04±0.01^{c}	0.12±0.05^{a}	0.12±0.00^{a}	0.23±0.02^{a}
6	S20	0.13±0.00^{a}	0.13±0,01^{a}	0.11±0.00^{a}	0.28±0.06^{a}
	S25	0.09±0.05^{ab}	0.13±0.01^{a}	0.06±0.01^{b}	0.27±0.03^{a}
	S30	0.08±0.02^{ab}	0.11±0.01^{a}	0.12±0.01^{a}	0.24±0.06^{a}
	S35	0.04±0.01^{b}	0.12±0.05^{a}	0.12±0.00^{a}	0.23±0.02^{a}
	WHO/FAO mg/100g		22.10	48.50	105.00

*Mean with same superscripts in the same column are not significantly different at p>0.05. S10-Fresh *Calocybe indica*; S20 - Canned *Calocybe indica* (2.00g sodium chloride; 1.50 g citric acid); S25- Canned *Calocybe indica* (2.5 sodium chloride; 1.50 g citric acid); S30 - Canned *Calocybe indica* (3.00g sodium chloride; 1.50 g citric acid); S35 - Canned *Calocybe indica* (3.50g sodium chloride; 1.50 g citric acid).

CONCLUSION AND RECOMMENDATION

Significant reduction occurred in the phytochemicals and antinutrients properties of the *Calocybe indica* after canning. Additionally, as storage times increased, it was shown that the phytochemical and antinutrients contents of the canned *Calocybe indica* dramatically decreased as compared to the fresh sample. A non-significant increase was noticed only in the phenol, total antioxidants and ferric reducing antioxidants power with increase in sodium chloride of the brine solution while the brine solution to large extent has no effect on other parameters during the storage period.

Based on the study's findings, it can be concluded that the storage time affected the anti-nutrients composition of the mushroom samples which are relatively below recommended value indicating that they are safe for human consumption.

This study has shown that, canning as well as leaching of phytochemicals into the brine solution due to the succulent nature of *Caocybe indica* mushroom potentially resulted in significant losses of phytochemicals over the storage period. Finally, in order to reduce the loss of phytochemical components, processing conditions should be adjusted.

Acknowledgement

The authors greatly appreciate the constructive contribution of the Lagos State University of Science and Technology for giving us the opportunity to carry out the analysis in their laboratories and other valuable assistance.

Competing Interests

The authors declare no conflict of interest

Author contributions

CA: Conceptualization, Methodology and Supervision; OOK: Visualization and Supervision; ZOA: Writing of the original draft, Statistical analysis and interpretation of data results, formal analysis, writing review and editing. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Abacan, S. F., Hurtada, W. A., & Devanadera, M. A. R. (2017). Effects of cooking time, temperature, and salt concentration on the phenolic content and antioxidant activity of selected edible mushrooms. *International Food Research Journal*, 24(5), 2028–2032.

Abdelshafy, A. M., Belwal, T., Liang, Z., Wang, L., Li, D., Luo, Z., & Li, L. (2022). A comprehensive review on phenolic compounds from edible mushrooms: Occurrence, biological activity, application and future prospective. *Critical Reviews in Food Science and Nutrition*, 62(22), 6204–6224. https://doi.org/10.1080/10408398.2021.1898335

Abushouk, A. I., Salem, A. M. A. & Abdel-Daim, M.M. (2017). Berberis vulgaris for cardiovascular disorders: a scoping literature review. *Iran Journal of Basic Medical Science*, 20(5), 503–510. https://doi.org/10.22038/IJBMS.2017.8674

Ade-Ogunnowo, F.E., Adejoye, O.D. & Adesokan, K. (2024). Phytochemical Screening, Proximate Composition and Mineral Activities of Four Selected Nigerian Mushrooms. African Scientist 25 (20): 124-134.

Adetuyi, F. O., Osagie, A. U., & Adekunle, A. T. (2011). Nutrient, antinutrient, mineral and zinc bioavailability of okra (*Abelmoschus esculentus* (L.) Moench) variety. *American Journal of Food and Nutrition*, 1(2), 49–54. https://doi.org/10.5251/ajfn.2011.1.2.49.54

Aguchem, R.N., Chibuogwu, C.C., Okolo, B.O., Oyeagu, U., Etim, V.E., Anaele, E.N. & Njoku, O.U. (2022). Nutrient and Antinutrient Composition of *Pleurotus ostreatus* Grown on Different Substrates. *Biological Life Science. Forum*, 11: 69. https://doi.org/10.3390/IECPS2021-11955

AOAC. (2018). Official Methods of Analysis, Association of Official Analytical Chemists, 21st Edition (New York).

Badejo, A. A., Osunlakin, A. P., Famakinwa, A., Idowu, A. O., & Fagbemi, T. N. (2017). Analyses of dietary fibre contents, antioxidant composition, function and pasting properties of plantain and moringa oleifera composite flour blends. *Journal of Cogent Food and Agriculture,* 3:1278871. https://doi.org/10.1080/23311932.2017.1278871

Banno, N., Akihisa, T., Yasukawa, K., Higashihara, H., Ukiya, M. & Watanabe, K. (2004). Triterpene acids from the leaves of Perilla frutescenes and their anti-inflammatory and antitumour-promoting effects. *Bioscience Biotechnology and Biochemistry*, 68, 85 – 90. https://doi.org/10.1271/bbb.68.85

Bello, F. Salami-Jaji, J.I. Sani, I. Abdulhamid, A. & Fakai, I.M. (2013). Evaluation of Some Antinutritional Factors in Oil-Free White Sesamum indicum L. Seed Cake. *International Journal of Food Nutrition and Safety,* 4(1), 27-33.

Bolarin F.M., Akande E.A. & Oke M.O. (2023). Effects of Processing Methods on the Yield, Functional and Pasting Properties of "Garri Analogue" Produced from Different Varieties of Orange Flesh Sweet Potato. *African Journal of Agriculture and Food Science* 6(1), 53-68. http://dx.doi.org/10.52589/AJAFS-WOKXWL42

Chelladurai G., Yadav T.K. & Pathak R.K. (2021). Chemical Composition and Nutritional Value of Paddy Straw Milky Mushroom (*Calocybe indica*). *National Environmental Pollution Technology.* 20:1157–1164. http://dx.doi.org/10.46488/NEPT.2021.v20i03.023

Edet, U.O., Ebana, R.U.B., Etok, C.A & Udoidiong1, V.O. (2016). Nutrient Profile and Phytochemical Analysis of Commercially Cultivated Oyster Mushroom in Calabar, South-South Nigeria. *Advances in Research*, 7(3): 1-6. DOI: 10.9734/AIR/2016/26196

Ekpa, E. & Sani, D. (2018). Phytochemical and anti-nutritional studies on some commonly consumed fruits in lokoja, kogi state of Nigeria. *General Medicine Open*, 2(3), 1-5. http://dx.doi.org/10.15761/GMO.1000135

Finimundy, T. C., Gambato, G., Fontana, R., Camassola, M., Salvador, M., Moura, S. & Roesch-Ely, M. (2013). Aqueous extracts of Lentinula edodes and Pleurotus sajor-caju exhibit high antioxidant capability and promising in vitro antitumor activity. *Nutrition Research*, 33(1), 76–84. http://dx.doi.org/10.1016/j.nutres.2012.11.005

Garofalo, C., Osimani, A., Milanovi´c, V., Taccari, M., Cardinali, F., Aquilanti, L. & Clementi, F. (2017). The microbiota of marketed processed edible insects as revealed by high-throughput sequencing. *Food Microbiology*, 62, 15–22. http://dx.doi.org/10.1016/j.fm.2016.09.012

Ghosh, S.K. (2015). Study of anticancer effect of Calocybe indica mushroom on breast cancer cell line and human Ewings sarcoma cancer cell lines. *NY Sci. J.* 8, 10–15. http://www.sciencepub.net/newyork

Haden, M. (2007). Chlorophylls, in: chemistry and biochemistry of plant pigment, 6th edition, (Godwin, T.W., editor). *Academic press London*; 468-490.

Kamthan, R. & Tiwari, I. (2017). Agricultural wastes-potential substrates for mushroom cultivation. *Eur Exp Biol.* 7(5), 31. http://dx.doi.org/10.21767/2248-9215.100031

Kao, T.H., Huang, S.C., Inbaraj, B.S. & Chen, B.H. (2008). Determination of flavonoids and saponins in Gynostemmapentaphyllum (Thunb) Makino by liquid chromatography – mass spectrophotometry. *Analytical Chimica Acta*, 626, 200 – 211. DOI: 10.1016/j.aca.2008.07.049

Kayode R.M.O., Olakulehin T.F., Annongu A.A., Sola-Ojo F.E., Oyeyinka S.A. & Kayode B.I. (2013). Evaluation of the nutritional composition and phytochemical screening of an exotic and wild species of oyster mushrooms (*Pleurotus sajor-caju*). *In: Nigerian Journal of Agriculture Food and Environment.* 9(3), 48-53.

Khan, M.A., Tania, M., Liu, R., & Rahman, M.M. (2013). Hericium erinaceus: An edible mushroom with medicinal values. *Journal of complementary and integrative medicine.* 10. https://doi.org/10.1515/jcim-2013-0001

Khan, U., Hayat, F., Khanum, F., Shao, Y., Iqbal, S., Munir, S., Abdin, M., Li, L., Ahmad, R. M., Qiu, J., & Xin, Z. (2023). Optimizing extraction conditions and isolation of bound phenolic compounds from corn silk (Stigma maydis) and their antioxidant effects. *Journal of Food Science*, 88(8), 3341–3356. https://doi.org/10.1111/1750-3841.16682

Khanam, U.K.S., Oba, S. & Yanase, E. (2012). Phenolic acids, flavonoids and total antioxidant capacity of selected leafy vegetables. *Journal of Functional Foods*. 4(4), 979–987. https://doi.org/10.1016/j.jff.2012.07.006

Kotowski, M.A. (2019). History of mushroom consumption and its impact on traditional view on mycobiota–an example from Poland." *Microbial Biosystems*. 4(3). 1–13. http://dx.doi.org/10.21608/mb.2019.61290

Liu, K., Xiao, X., Wang, J., Chen, C. Y. O., & Hu, H. (2017). Polyphenolic composition and antioxidant, antiproliferative, and antimicrobial activities of mushroom Inonotus sanghuang. *LWT-Food Science and Technology*, 82, 154–161. d: https://www.sciencedirect.com/science/article/pii/S0023643817302670

Maurya A.K., John V., Murmu R. & Simon S. (2019). Impact of different substrates for spawn production and production of milky mushroom (Calocybe indica). *International Journal of Pharmacology and Biological Science.* 10:5–10. http://dx.doi.org/10.22376/ijpbs.2019.10.3.b5-10

Mirunalini, S., Dhamodharan, G. & Deepalakshmi, K. (2012). Antioxidant potential and current cultivation aspects of an edible milky mushroom-*Calocybe indica*. *International Journal of Pharmacology and Science*, 4, 137–143.

Mishra, K.K., Pal, R.S., Arunkumar, R., Chandrashekara, C., Jain, S.K. & Bhatt, J.C. (2013). Antioxidant properties of different edible mushroom species and increased bioconversion efficiency of *Pleurotus eryngii* using locally available casing materials. *Food Chemistry*., 138(2–3),1557–63. https://doi.org/10.1016/j.foodchem.2012.12.001

Mleczek, M., Rzymski, P., Budka, A., Siwulski, M., Jasi ́nska, A., Kalaˇc, P. & Niedzielski, P. (2018). Elemental characteristics of mushroom species cultivated in China and Poland. *Journal of Food Composition and Analysis*, 66, 168–178. http://dx.doi.org/10.1016/j.jfca.2017.12.018

Nketia, S., Buckman, E.S., Dzomeku, M. & Akonor, P.T. (2020). Effect of processing and storage on physical and texture qualities of oyster mushrooms canned in different media. / Scientific African 9 (2020) e00501.

Ogbe A.O. & Obeka A.D. (2013). Proximate, mineral and antinutrient composition of wild Ganodermalucidum: Implication on its utilization in poultry production

Okorie, P.C., Ofoegbu, V.O., Akinyemi, A.O., Abikoye, E.T., Okere, V.O., Lawal, A.K & Asagbra, A. E. (2021). Evaluation of phytochemical content of *calocybe indica* isolated in Nigeria for a possible theraputic use. *International Journal of Scientific & Engineering Research,* 12:12.

Olajuyigbe, O.O. & Afolayan, A.J. (2011). Phenolic content and antioxidant property of the bark extracts of Ziziphus mucronata Willd. subsp. mucronata Willd. *BMC Complement Altern Med*. 11(1), 130. http://www.biomedcentral.com/1472-6882/11/130

Oluwajuyitan, T.D. & Ijarotimi, O.S. (2019). Nutritional, antioxidant, glycaemic index and antihyperglycaemic properties of improved traditional plantain-based (Musa AAB) dough meal enriched with tigernut (*Cyperus esculentus*) and defatted soybean (*Glycine max*) flour for diabetic patients. *Heliyon*, 5(4): e01504. https://doi.org/10.1016/j.heliyon.2019.e01504

Palafox-Carlos, H., Ayala-Zavala, J. F. & González-Aguilar, G. A. (2011). The Role of Dietary Fiber in the Bioaccessibility and Bioavailability of Fruit and Vegetable Antioxidants. *Journal of Food Science*, 76(1), 6–15. https://doi.org/10.1111/j.1750-3841.2010.01957.x

Pamela, E.A.I., Olufemi, T.A., Yemisi, O.O., Aduloju, O.A. & Usifo, G.A. (2017). Phytochemical Content and Antioxidant Activity of Five Grain Amaranth Species. *American Journal of Food Science and Technology*, 5, 249–255. https://doi.org/10.12691/ajfst-5-6-5.

Pandimeena, M., Prabu M., Sumathy R. & Kumuthakalavalli R., (2015). Evalution of Phytochemicals and in vitro anti-inflammatory, anti-diabetic activity of the white Oyster mushroom, Pleurotus florida. *International Research Journal of Phharmaceutical and Applied Sciences (IRJPAS).* 5:18 – 19. https://www.scienztech.org/index.php/irjpas/article/view/638

Polycarp, D., Afoakwa, E.O., Budu, A.S. & Otoo, E. (2012). Characterization of Chemical Composition and Anti-Nutritional Factors in Seven Species within the Ghanaian Yam (Dioscorea) Germplasm. *International Food Research Journal*, 19, 985-992.

Putriani, N., Perdana, J., Meiliana, & Nugrahedi, P. Y. (2020). Effect of Thermal Processing on Key Phytochemical Compounds in Green Leafy Vegetables: A Review. *Food Reviews International,* 38(4), 783–811. https://doi.org/10.1080/87559129.2020.1745826

Ribas-Agustí, A., Martín-Belloso, O., Soliva-Fortuny, R. & Elez-Martínez, P. (2018). Food Processing Strategies to Enhance Phenolic Compounds Bioaccessibility and Bioavailability in Plant-Based Foods. *Critical Revision of Food Science and Nutrition*, 58(15): 2531–2548. https://doi.org/10.1080/10408398.2017.1331200

Roy A. & Prasad, P. (2014). Properties and uses of an Indigenous Mushroom: Calocybe indica. Asian J. Pharm. Tech. 4(1): 17-21.

Shahidi, F. & Peng, H. (2018). Bioaccessibility and Bioavailability of Phenolic Compounds. *Journal of Food Bioactive*. 4: 11–68. http://dx.doi.org/10.31665/JFB.2018.4162

Shashikant, M., Bains, A., Chawla, P., Fogarasi, M. & Fogarasi, S. (2022). The Current Status, Bioactivity, Food, and Pharmaceutical Approaches of *Calocybe indica*: A Review. *Antioxidants*, 11, 1145. https://doi.org/10.3390/antiox11061145

Shyni, A.R., Irene, W.J. & Reginald, A.M. (2018) Qualitative and Quantitative Analysis of Edible Mushroom *Calocybe indica* using Solvent Extracts. *International Journal of Science and Research,* 8(5): 70-72.

Thakur, N. K. (2016). Training manual on cultivation of tropical mushroom and it's value addition. 1-25.

Uddin, M.S., Hossain, M.S. & Al Mamun, A. (2018). Phytochemical analysis and antioxidant profile of methanolic extract of seed, pulp and peel of Baccaurea ramiflora Lour. *Asian Pakistan Journal of Tropical Medicine*. 11(7): 443–450. http://dx.doi.org/10.4103/1995-7645.237189

Vijaykumar, G., John, P., & Ganesh, K. (2014). Selection of different substrates for the cultivation of milky mushroom (*Calocybe indica* P and C). *Indian Journal of Traditional Knowledge*, 13(2): 434-436.

Wandati, T. W. & Kenji G. M. (2013). Phytochemicals in Edible wild mushrooms from selected areas in Kenya. *Journal Food Research*. 2(3): 139-141. https://doi.org/10.5539/jfr.v2n3p137

Wasagu, R.S.U., Lawal, M., Shehu, S., Alfa, H.H. & Muhammad, C. (2013). Nutritive values and Antioxidant properties of *Pistia stratiotes* (Water lettuce). *Nigerian Journal Basic and Applied Sciences* 21: 253. https://doi.org/10.4314/njbas.v21i4.2