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Assessment of Endogenous Enzymes of *Volvariella volvacea* Responsible for Its Self-Digestion for Short-Shelf Life

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ABSTRACT

Volvariella volvacea, also known as straw mushroom or paddy straw mushroom is a tropical and subtropical tasty edible mushroom, characterized with the difficulty to store after harvest due to an endogenous enzymatic phenomenon of self digestion (autolysis). The mushroom has been shown to contain complex enzymatic systems which are both endogenous and exogenous in activity. The aim of this study was to assess biochemically the endogenous enzymes responsible for the mushroom self-digestion that gives rise to its short shelf-life. Enzymatic assay method of Cleland was used to determine possible endogenous enzymes in the mushroom. The study revealed eight (8) enzymes; namely: Glucose-oxidase, Hexose-oxidase, Glucotransferase, Lipase tributyrase, Tannase, Cellulase, Decarboxylase and Phosphoglucose-isomerase. These are oxidative, lytic and hydrolytic enzyme groups. The implication is that if *V. volvacea* grows on a substrate made of lignin, its exogenous enzymes will secrete oxidative enzymes. However, as the mushroom attempts to grow in the presence of its endogenous hydrolytic enzymes and while in the struggle to grow, it loses its strength, becomes weak, soft, decomposes and consequently dies, because it lacks the ability to synthesize phenol-oxidizing and lignin transforming enzymes which are supposed to transform the lignin material to substrates for its benefits. Therefore, this study satisfies the search that the presence of endogenous enzymes as mentioned above in *V. volvacea* are truly responsible for the self-digestion (autolysis) that gives rise to the short shelf life of the mushroom.

Keywords: Mushrooms, *V. volvacea*, Complex-Enzyme System, Self-Digestion, Lignin Transforming Enzyme, Shelf Life.

Introduction

Volvariella is a genus of mushrooms with deep salmon pink gills; a filamentous basidiomycete. *Volvariella* is derived from a latin word, volva, meaning; cup-like structure. The volva is located at the base of the mushroom and it is a remnant of its universal veil or peridium that encloses immature fruiting bodies, gasteroid fungi without ring (Chang, 1991).

Although they lack ring, but possess an Amanita-like volva at the base; hence they look alike such that the mushroom could easily be mistaken for Amanita (Kirk *et al.,* 2008). The genus *Volvariella* is estimated to comprise about 50 species, of which *Volvariella volvacea* is the most popular and studied (Kirk *et al.,* 2008).

Volvariella volvacea, also known as straw or paddy straw mushroom is a species of edible wild and cultivated mushroom throughout the tropics and sub-tropics, especially in the East and South-east Asia and extensively in the Asian Cuisine (Chang and Quimo, 1982). It belongs to the family, Pluteaceae of the basidiomycetes. *V. volvacea* is highly perishable with short shelf-life due to cladding temperature and biochemical changes within the tissues caused by endogenous enzymes (Santiago, 1982). It has strong fibrinolytic capacity and consumes abundant agricultural wastes when cultivated.

In nature, it is found growing on paddy straw, cotton wastes, baggase, sorghum straw, oil palm pericarp (that is empty palm bunches) and fibre substrates.

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However, the difficulty to obtain high consistent yield and the inability to enhance its postharvest shelf life; makes it the most difficult mushroom to handle which is the reason its position in the world popularity list dropped from 3rd in 1986 to 5th in 1999 (Chang, 1993b). Straw mushroom is reported to suffer from storage problems as a result of self-digestion or autolysis and browning.

In many agricultural Produce, Polyphenol Oxidase (PPO) activity is a major factor causing browning. The PPO had been found livalised majorly on the mushroom pileus and the outer layer which could be the reason for colour difference between the top and bottom of the fruit bodies. However, it is believed that browning is enzymatic while the mechanism of self-digestion or autolysis is still yet not known (Suen, 1999; Ahlawat, 2003; Patel and Williamson, 2016).

Volvariella volvacea possesses an extreme short shell life, normally 3 hours after harvest and in storage. This implies that the fruit body liquefies, hours after harvest and sometimes before harvest (Chang, 1991). It has been reported that the liquidation of the fruit bodies is related to the activities of hydrolytic enzymes, such as protease, lipase, and chitinase, which are suspected to be endogenous to the mushroom (Chang and Hayes, 1978; Cai, 1994).

Mycolitic enzymes that digest away fungal cell wall to replace protoplasts are mostly of microbial origin. Currently, available information on major components of *V. volvacea's* chitin is β-glucan, and α-glucan which indicate the requirements of corresponding hydrolytic enzymes for self-digestion (Suen, 1999).

Lytic enzymes exist as a mixture of several hydrolytic enzymes such as chitinase, and β-glucanase according to Hamlyn *et al.* (1981). Although the activities of lytic enzymes may be subject to variation, but in autolysis or self-digestion complex, their activities may be affected by factors like osmotic stabilization (Yu and Chang, 1987, Perberdy, 1991). Perberdy (1991), further suggested that osmotic stabilizer and lytic enzymes interact in the digestion mixture of straw mushroom. However, Yu and Chang (1987) were of the opinion that Chitinase is the most sensitive to inhibition that increases in an order: NO_3, CT < SO_4^{2-} < PO_4^{3-}, Na^+, K^+ < Mg^{2+} and Ca^{2+}, although $MgSO_4$ has been shown to be equally efficient as KCl and NH_4Cl for protoplast liberation. In fact, Zhen (2005) reported that changes in nutrients are indications of degeneration and discoloration in *V. volvacea*.

Straw mushroom (*V. volvacea*) is also reported to produce a multi-component enzyme system, consisting of endo-1, 4 β - glucanase, cellobiohydrolase and β-glucosidase for conversion of cellulose to glucose. It has been reported that, though straw mushroom is cultivated on a variety of lingo-cellulosic wastes, it thrives better on woody materials with substantial lignin content to achieve increased yield (Chang, 1974). It was reported in a study of traditional cultivating substrate of the mushroom that it produced several enzymes for the hydrolysis of cellulose and hemicellulose including endo and exoglucanases, β-glucanase, xylanase,β-xylosidase, and β-xylosidase; but lacked the ability to synthesize phenol-oxidizing and lignin-transforming enzymes (Wang *et* al., 1980; Wang, 1982; Buswell *et al.,* 1993; Cai, 1994). They also reported that the mushroom thrives well on rich cellulose substrates such as cotton wastes and straws in which the amount of lignified components are negligible. A study that investigated the lignocellulosic enzymes of the mushroom revealed that cellulases were partly cell free and bond. It also revealed that the mushroom could not digest the lignin portion of the substrate because of its inability to produce lactase (P-diphenol oxidase); an enzyme well distributed in fungi for lignin degradation (Mandels & Andreotti, 1978; Bourbonnais and Paice, 1990; Archibald & Roy, 1999; Thurston, 1994; Whitaker, 2003). Patel and Williamson (2016) also reported that mannitol is found in the hyphae of basidiomycetes and in the sporophores, possessing biological function to keep fungi under stress such as osmotic pressure and removal of reactive oxygen species (ROS).

The productivity of *V. volvacea* has been attributed to hydrolytic enzyme production potential and quality of the substrate used for its cultivation over the years. Additionally, there has been difficulty obtaining high and consistent yield; coupled with the constraint to enhance its postharvest shelf-life due to self-digestion, also known as autolysis.

Based on the fore-goings, this study was designed to biochemically assess the enzymes responsible for self-digestion that gives rise to short shelf-life; and perhaps, they are endogenous in the mushroom.

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Materials and Methods

Study Area

This study was carried out in the Biology Laboratory of Faculty of Science, Ignatius Ajuru University of Education, within Port Harcourt Metropolis in the Niger Delta sedimentary Basin of Nigeria, covered on the surface by the Benin formation. The study area is situated 15.0 meters above sea level and relatively low land area.

Source of Sample and Sample Collection and Processing

The sampling area for the study was Omuanwa community in Ikwerre Local Government Area of Rivers State, Nigeria. *Volvariella volcacea* samples was obtained from a garden near a local palm oil mill; from where empty palm fibers are dumped.it was from the large dump of empty palm oil fiber that sample was hand-picked and put in a sterile paper.

The quantity obtained was 500g. This was air-dried at 24ºC room temperature by spreading them on two layered clean A4 plain paper and placed on a table. The papers were changed at intervals when soaked. The samples were aseptically handled and were allowed to air-dry for three (3) days at room temperature and later grounded to powder form for analysis.

Extraction and Detection of endogenous enzymes in straw mushroom (*Volvariella volcacea*)

The method of Cleland (1979) was adopted for this study. Ten grams of the grounded sample was homogenized using common saline buffer concentration of 50mM sodium citrate at pH (4.5). The buffer was used in the ratio of 1pg ground mushroom tissue to 100ml of the buffer. This was filtered to release the enzymes.

A control standard of the pure enzyme solution was also prepared and ran alongside the unknown enzymes. A colour change, disappearance of substrate and appearance of product were the basis used for identification of the various endogenous enzymes reaction in the colourimetric assay.

Identification of endogenous enzymes in straw mushroom

The enzymes implicated in this study were identified by their specificity in breaking down or converting a particular substrate into a product. Enzyme Kinetics was used to monitor the substrate disappearance rate to product formation. This was monitored using spectrophotometric methods (A UV-VIS-NIR spectroscopy (32) spectrophotometer manufactured in Shimadzu, China), where absorbance was monitored overtime at an interval of 60 seconds. Beside optical method, electrochemical-methods, especially pH determinations for reactions with pH changes, such as the liberation of acids by lipase or choline esterase were also employed with the aid of Bench top pH meter indicator. Since changes in pH influence enzyme activity, a pH stat connected with an auto-burette was used which kept the pH constant by adding a neutralizing solution; its amount being direct measure of the proceeding reaction (MacDonald and Tipton, 2022).

Results

The results of this study as presented on Table 1 revealed that *Volvariella volvacea* (Straw mushroom) contains various digestive enzymes. The results revealed eight enzymes, namely: glucose-oxidase, hexose-oxidase, glucano transferase, lipase tributyrase, Tannase, cellulase, Decarboxylase and phospho-glucose-isomerase as contained in the mushroom. The enzymes possess medicinal and biological values in activity. The results also indicated that they all had enzyme classification number (EC). It also showed that glucose and hexose oxidase(s) belong to the reaction class; oxidoreductases which catalyse oxidation and reduction reactions, bearing number, '1'.

Glucano-transferase with enzyme number '2' belongs to the class transferase. The enzymes: Lipase tributyrase, tannase and cellulasewere shown as hydrolytic enzymes; bearing the number '3', while decarboxylase bearing number '4' is a lytic enzyme. And phospho-glucose-isomerase with number '5' was shown as an isomerase enzyme that isomerises molecules. All the eight enzymes belonged to subclasses and sub-sub-classes; bearing number 4; an indication that they all zeroed on specific metabolites of the sample and the co-factors involved.

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Table 1: Digestive Enzymes in the Straw Mushroom (*Volvariella volvacea*) and Classification Code

Digestive Enzymes in *Volvariella volvacea*	Enzyme Classification Code (EC)
Glucose oxidase	1.1.3.4
Hexose oxidase	1.1.3.4
Glucano transferase	2.4.1.1.9
Lipase tributyrase	3.1.1.3
Tannase	3.1.1.2.0
Cellulase	3.2.1.4
Decarboxylase	4.1.1.5
Phospho-glucose isomerase	5.3.1.9

Their EC(s) indicate the isolated enzymes as endogenous to the mushroom.

Discussion

The results of this study indicated that the mushroom sample, *Volvariella volvacea* contained eight endogenous enzymes, namely; glucose-oxidase, hexose-oxidase, glucanotransferase, lipase tributyrase, tannase, cellulase, decarboxylase and phospho-glucose-isomerase. It also revealed that glucose and hexose-oxidases belong to the reaction class; oxio-oreductases (as indicated by their classification number) which catalyze oxidation and reduction processes by the possession of the enzymatic number 'I'. It is possible that these oxidative enzymes may be the cause of colour change in the mushroom such as browning. This finding seems to be in line with the suggestion of Ahlawat (2003), who reported that *V. volvacea* exhibits browning, shortly after harvest which was shown to be related to oxidation of phenolic compounds by the mushroom endogenous enzymes.

According to Ahlawat (2003), oxidative browning of *V. volvacea* involves the action of a group of enzymes; often referred to as polyphenol oxidase (PPO) which together with phenolic compounds is directly responsible for enzymatic browning reactions of mushrooms. Although the mechanism of actions of PPO is based on its capacity to oxidise the phenolic compounds hence it can use monophenols and polyphenols; as well as oxygen to act.

This result is in line with the report of Whitaker who supported the submission of Ahlawat (2003). According to their reports, mushrooms are shown to contain a large amount of phenolic compounds which are readily oxidized during homogenization of the mushroom.

This agreement in findings also relates and supports the report of Haghbeen *et al.* (2004) and Zaidi *et al.* (2004); who reported that after successive oxidation and polymerization of phenolic compounds in the mushroom *V. volvacea;* the macro-molecule in melanin becomes prominent; little wonder, why, shortly after harvest, the mushroom tissues, especially the pileus could turn to blue-black colour.

The results also revealed that for the mushroom to successfully be oxidized by its endogenous enzymes, it could be that the mushroom was under osmotic stress. Thus, it seems to be in line with the suggestion of Zheng (2005) ; Patel and Williamson, (2016) who reported that mannitol is found in the hyphae of basidiomycetes and their sporophores are important polyol and that mannitol has biological function in fungi under stress, such as regulation of osmotic pressure and removal of Reactive Oxygen Species (ROS).

The results also revealed that glucanotransferase belongs to the class transferase, hence it bears the classification number 2; while lipase tributyrase, tannase and cellulase bear the classification number 3.

This is an indication that they are hydrolytic enzymes, but classification number 4 is a lytic enzyme. All these enzymes are found to be endogenous to the mushroom *V. volvacea;* in line with the findings of Wang (1982); Buswell *et al.* (1993); and Cai *et al*. (1994). All these researchers reported from a study on traditional cultivating substrate of *V. volvacea* in which the substrate degrading enzymes produced by the mushroom included those of hydrolytic enzymes such as endo and exoglucanases, beta-glucanase, xylanase and beta xylosidase.

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However, the mushroom lacked the ability to synthesize phonol-oxidizing and lignin transforming enzymes, which could have catalyzed the hydrolytic enzymes to save the mushroom from hydrolysis.

This finding is clearly in line with the submission; that also suggested that *V. volvacea* thrives better on a rich cellulosic substrate such as cotton wastes in which the amount of lignified components is negligible. This implies that if *V. volvacea* grows on a substrate made up of lignin, and in the cause of the mushroom struggle to grow in the presence of its endogenous hydrolytic enzymes, it loses its tissues to hydrolysis, weakness of the tissue, and subsequent death. Hence it lacks the ability to synthesize phenol-oxidizing and lignin-transforming enzymes. This could really be the true reason why the mushroom tissues become soft, decompose, loose vitality and consequently dies; in line with the submission of Chang and Hayes (1978) and Wang *et al.* (1980). It could also be that there were changes in nutrients of the mushroom that led to its liquidation, decomposition and death few hours after harvest. This is in line with the report of Chang (1995) who stated that changes in nutrients are important indication of *V. volvacea's* degeneration and that as the degeneration of the mushroom progresses; it gets decolourized, become weak and subsequently dies. This was exactly the findings of this study as is being backed up by previous reports and submissions of scholars.

In conclusion, the results obtained from this study shows that variations in *Volvariella volvacea* do exist and that the various endogenous enzymes inherent in the mushroom are responsible for its self digestion that results to its short shelf life. It also revealed that *V. volvacea,* as a typical edible straw mushroom with high temperature tolerance can not be stored under low temperature. And sub-culturing of the mycelia facilitates its degeneration. The findings also indicate that the cause of browning of the fungus is enzymatic and polyphenol oxidase (PPO) is responsible. Additionally, the findings of this study satisfy the search that endogenous enzymes in the fungus are the reasons why the mushroom undergoes self digestion; hence it lacks the ability to synthesize phenol-oxidizing and lignin transforming enzymes. And that in the course of trying to grow using lignin materials, which it cannot digest, it will begin to self-digest itself which manifests as autolysis.

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