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Changes in Enzyme Activities of *Termitomyces robustus* (Beeli) Heim and *Lentinus subnudus* Berk during Sporophore Development

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ABSTRACT

The activities of amylases, proteinase, cellulase, lipase, peroxidase, catalase, polyphenol oxidase and glucose-6-phosphatase in mature, young and very young stages of Termitomyces robustus *(Beeli) Heim and* Lentinus subnudus *Berk were determined. Apart from β-amylase and glucose-6-phosphatase, the activities of the assayed enzymes increased from very young to mature fruitbody stages. Moreover, while the young fruitbody showed the highest glucose-6-phosphatase activity out of the three mushroom stages, there was no distinct trend with respect to the β-amylase activities. For cellulase, the activity in the stipe was greater than that of the pileus, while for the other enzymes, the reverse was the case. The mature fruit-bodies show the greatest loss of flavour, whiteness and food nutrients during storage. The findings are discussed in relation to past work.*

INTRODUCTION

Food nutrient metabolism in vegetables is known to be under the control of various enzymes. Baardseth (1979) stated that amylase, cellulase, lipase, proteinase and respiratory enzymes control the levels of carbohydrates, lipids and proteins, while peroxidase and catalase activities alter vegetable flavour. Moore *et al.* (1979) working with *Coprinus cinereus* detected the greatest NADP-glutamate dehydrogenase activity in the most mature stage of the sporophore. Similarly, Paranjpe and Chen (1979) found increasing

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tyrosinase activity but decreasing peroxidase activity with increase in sporophore development of *Agaricus bisporus*.

In our preliminary investigation of *Termitomyces robustus* and *Lentinus subnudus*, we have observed that the mature sporophore stage contains more food nutrients and undergoes browning and decomposition much faster than the young and very young sporophores (Fasidi & Kadiri, 1990). The objective of this study was to investigate changes in enzyme activities of *Termitomyces robustus* and *Lentinus subnudus* during sporophore development.

MATERIALS AND METHODS

The sporophores of *Termitomyces robustus* were collected in the field, while those of *Lentinus subnudus* were harvested from daily-watered logs of *Spondias mombin* Linn. The measurements of the pileus at very young, young and mature stages are given in Table 1. The mushrooms were divided into pilei and stipes, put in water-tight polythene containers and immediately frozen at −9°C.

Cellulase activity

One gram frozen tissue of pileus or stipe was ground with 1/15 M dibasic sodium phosphate (K_2HPO_4) in a mortar maintained at 5°C with ice-blocks (Norkrans, 1957). After centrifuging at 18 000*g* for 30 min, 1 ml of the supernatant was incubated with 9 ml of 1% carboxymethyl cellulose in 0·05M citrate phosphate buffer (pH 5·0) for 1 h at 30°C (Singh & Kunene, 1980). The enzyme action was stopped with 3,5-dinitrosalicylic acid (DNSA) reagent and the amount of reducing sugars formed was determined by taking the optical density at 540 nm against a blank that contained 1 ml of boiled enzyme extract that was similarly treated (Denison & Koehn, 1977).

TABLE 1
The Pileus Dimension of the Sporophores employed as Mature, Young and Very Young Mushrooms

Mushroom species	*Pileus diameter (cm)*		
	Mature stage	*Young stage*	*Very young stage*
Termitomyces robustus	6–13	3–5·9	Less than 3 cm
Lentinus subnudus	4–6	2–3·9	Less than 2 cm

Total amylase (α and β) activity

One gram frozen tissue of pileus or stipe was ground in a cooled mortar with 20 ml 1/10M sodium acetate buffer (pH 5·0) and the ensuing suspension centrifuged at 18 000*g* for 30 min at 2°C. One millilitre of the supernatant was incubated with 1 ml of 1% soluble starch in 1/10 M sodium acetate buffer pH 5·0 in a water-bath set at 27°C for 1 h. The enzymatic action was terminated with DNSA reagent and the quantity of reducing sugars formed determined by taking the optical density at 540 nm against a blank that contained 1 ml of boiled enzyme extract that was similarly treated (Swain & Dekker, 1966).

α-Amylase activity

This was determined by heating 5 ml of the supernatant obtained after centrifuging the total amylase extract at 70°C for 15 min to deactivate the β-amylase (Wilson, 1971). One millilitre of the heated extract was incubated with 1 ml of 1% soluble starch in 1/10M sodium acetate buffer (pH 5·0) in a water-bath set at 27°C for 1 h. The resultant solution was treated with DNSA reagent and the quantity of reducing sugars was determined as above.

Proteinase activity

This was determined using the Lowry Folin–Ciocalteu method of McDonald and Chen (1965). Enzyme extracts were prepared in the same way as that of amylase assay but using 20 ml of 0·05M sodium phosphate buffer (pH 6·0) as the extracting buffer.

Lipase activity

This was determined using the method of Young and Wood (1977). Enzyme extracts were prepared in the same way as those for amylase activity.

Peroxidase activity

This was determined using the purpurogallin method as described by Keilin and Hartree (1951). Enzyme extracts were obtained in the same manner as those of amylase activity but using 0·3M phosphate buffer (pH 6·8) as the extracting medium.

Catalase activity

This was measured using the sodium perborate method of Feinstein (1949). Enzyme extracts were prepared in the same way as those for peroxidase activity.

Polyphenol oxidase activity

The enzyme activity was assayed by determining the amount of quinone formed (Yamaguchi *et al.*, 1970). The substrate of the enzymatic reaction is catechol and quinone. The product formed reacts with proline to produce a red compound measurable at 525 nm.

Glucose-6-phosphatase activity

This was determined using the method described by Colowick and Kaplan (1955) using glucose-6-phosphate solution as the substrate. The inorganic phosphate formed was assayed using the method of Russell (1940).

Protein content

This was estimated using the Folin–phenol method of Lowry *et al.* (1951) with casein as the standard protein.

RESULTS AND DISCUSSION

Cellulase, total amylase, α-amylase, proteinase, lipase, peroxidase, catalase and polyphenol oxidase activities of both *T. robustus* and *L. subnudus* increased from very young to mature stages (Tables 2 and 3). However, for glucose-6-phosphatase the young sporophore stage showed greater activity than the very young or the mature stages (Table 3). For β-amylase activity, there was no distinct trend (Table 2). Furthermore, the activities of the various enzymes were usually greater in the pilei of the mushrooms than in their corresponding stipes (Tables 2 and 3). For cellulase activity, the converse was obtained (Table 2). Some of the differences in enzyme activities are statistically significant (Tables 2 and 3).

For cellulase and polyphenol oxidase, *L. subnudus* showed consistently higher activity than *T. robustus* in all the sporophore stages but, for the other enzymes, no clear-differences were observed between the two mushrooms (Tables 2 and 3).

For cellulase, amylase, proteinase and lipase, the mature fruitbodies

TABLE 2

Intracellular Cellulase, Total Amylase, α-Amylase, β-Amylase and Proteinase Activities in the Various Fruitbody Stages of *L. subnudus* and *T. robustus*

(Data are means of three determinations)

Mushroom species and stage	*Cellulase activity (mg glucose/h/ mg protein)*	*Total amylase activity (mg maltose/h/ mg protein)*	*α-Amylase activity (mg maltose/h/ mg protein)*	*β-Amylase activity (mg maltose/h/ mg protein)*	*Proteinase activity (mg tyrosine/h/ mg protein)*
L. subnudus					
Mature pileus	1·22*bc*	4·83*a*	4·12*a*	0·76*a*	5·54*a*
Mature stipe	1·33*a*	2·12*d*	1·71*d*	0·43*c*	2·63*c*
Young pileus	1·04*d*	3·54*b*	3·03*b*	0·54*b*	3·62*b*
Young stipe	1·14*cd*	2·02*d*	1·74*d*	0·32*d*	2·52*c*
Very young pileus	1·01*d*	3·03*c*	2·52*c*	0·48*c*	1·94*d*
Very young stipe	1·11*cd*	1·72*d*	1·43*d*	0·33*d*	1·41*d*
T. robustus					
Mature pileus	0·48*c*	4·62*a*	4·33*a*	0·34*ab*	3·42*a*
Mature stipe	0·74*a*	4·41*a*	3·73*b*	0·31*b*	2·63*b*
Young pileus	0·46*c*	4·53*a*	4·31*a*	0·24*c*	3·12*a*
Young stipe	0·64*b*	3·82*b*	3·64*b*	0·21*c*	2·54*b*
Very young pileus	0·38*d*	2·42*c*	2·02*c*	0·36*a*	1·62*b*
Very young stipe	0·54*c*	1·83*d*	1·62*c*	0·17*d*	1·34*b*

Means ± SE followed by the same letter(s) within any mushroom group are not significantly different at $P = 0{\cdot}01$ by Duncan's multiple range test.

TABLE 3

Intracellular Lipase, Peroxidase, Catalase, Polyphenol Oxidase and Glucose-6-Phosphatase Activities in the Various Fruitbody Stages of *L. subnudus* and *T. robustus*
(Data are means of three determinations)

Mushroom species and stage	*Lipase activity (vol. of 0·2M NaOH in cm^3/h/mg protein)*	*Peroxidase activity (mg purpurogallin/h/mg protein)*	*Catalase activity (mg $NaBO_3 4H_2O$/h/mg protein)*	*Polyphenol oxidase (activity mg quinone/h/mg/protein)*	*Glucose-6-phosphatase (activity mg inorg. phosph./h/mg protein)*
L. subnudus					
Mature pileus	5·94*a*	2·11*a*	1·33*a*	4·54*a*	2·73*a*
Mature stipe	4·83*b*	1·52*bc*	0·92*bc*	2·43*c*	1·54*c*
Young pileus	4·72*b*	1·63*b*	1·04*b*	3·12*b*	2·82*a*
Young stipe	4·61*b*	1·44*bc*	0·83*c*	2·32*c*	2·13*b*
Very young pileus	4·63*b*	1·42*bc*	0·94*bc*	3·02*b*	2·10*b*
Very young stipe	4·42*b*	1·34*c*	0·80*c*	2·22*c*	1·44*c*
T. robustus					
Mature pileus	4·53*a*	1·54*a*	1·04*a*	2·22*a*	3·04*a*
Mature stipe	4·02*b*	1·43*ab*	0·73*bc*	1·92*bc*	2·22*bc*
Young pileus	4·41*a*	1·52*a*	0·84*b*	2·10*ab*	3·12*a*
Young stipe	3·94*bc*	1·41*ab*	0·63*cd*	1·83*c*	2·33*b*
Very young pileus	4·22*ab*	1·43*ab*	0·60*cd*	1·44*d*	2·14*bc*
Very young stipe	3·64*c*	1·34*b*	0·52*d*	1·22*d*	1·93*c*

Means ± SE followed by the same letter(s) within any mushroom group are not significantly different at $P = 0·01$ by Duncan's multiple range test.

showed the highest activity in both mushrooms (Tables 2 and 3). This result is consistent with our earlier observation that sugar, glycogen, protein, amino acids and crude fibre contents of the two mushrooms increased from very young to mature sporophore stages (Fasidi & Kadiri, 1990). The stipes of *T. robustus* and *L. subnudus* exhibited higher cellulase activities than their corresponding pilei (Table 2). This may be due to the greater preponderance of crude fibre in the stipes (Fasidi & Kadiri, 1990). Since the stipe forms the upright support for the entire fruitbody, it is not surprising that it contains a greater amount of crude fibre than the pileus. Similarly, the observed higher cellulase activity in *L. subnudus* also confirms the earlier result that the fruitbody of *L. subnudus* is more fibrous than that of *T. robustus*.

Peroxidase, catalase and polyphenol oxidase activities increased from very young to mature fruitbodies of *T. robustus* and *L. subnudus* (Table 3). This result agrees with the findings of Yamaguchi *et al.* (1970) on the polyphenol oxidase activities of tight-button, veil-stretched and veil-broken stages of *A. bisporus*. Paranjpe and Chen (1979) also observed greater polyphenol oxidase activity in the young fruiting body of *A. bisporus* than in the pin stage.

Glucose-6-phosphatase is a respiratory enzyme. Its greater activities in the pilei of both *T. robustus* and *L. subnudus* indicate greater respiration rates in the pilei than in the stipes (Table 3). This is not surprising because the pileus is the zone of cell divisions and spore formation. The present investigation also reveals that respiration rate reaches its peak at the young fruitbody stage and then declines (Table 3). The young fruitbody stage is a developmental stage known for its high respiratory rate and metabolite utilization. Moore *et al.* (1979) working on NADP–glutamate dehydrogenase activities of five stages of *C. cinereus* obtained a similar result of greatest activity in the stage 3 (young mushroom) and the least activity in the stage 1 (very young mushroom).

The significance of the findings in the present study is that the mature fruitbodies of *T. robustus* and *L. subnudus*, which appear to be the most nutritious out of the three mushroom stages employed, would be expected to undergo a faster decline in flavour and food nutrients and to show more browning than the other two stages during storage or after harvest. This is due to their having greater activity of amylase, proteinase, lipase, peroxidase, catalase and polyphenol oxidase.

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