1. Pant, D. D. and Rina Banerji, *J. Indian Bot. Soc.*, 1965, **64**, 191.
2. Paliwal, G. S. and Bhandari, N. N., *Phytomorphology*, 1962, **12**, 409.
3. Pant, D. D. and Kidwai, P. F., *Curr. Sci.*, 1966, **33**, 653.
4. Pant, D. D., *On the ontogeny of stomata and other homologous structures*, Plant. Sci. Ser., Allahabad, 1965. p. 1.
5. Bir Bahadur, Rajagopal, T. and Ramayya, N., *J. Linn. Soc. Bot.*, 1971, **64**, 295.
6. Shah, G. L., *J. Indian Bot. Soc.*, 1968, **67**, 305.
7. Philip, T., *Curr. Sci.*, 1983, **52**, 223.
8. Susheelamma, B. N. and Jolly, M. S., *Indian J. Seric.*, 1986, **25**, 6.
9. Metcalfe, C. R. and Chalk, L., *Anatomy of dicotyledons*, Vol. 2, Clarendon Press, Oxford, 1950.
10. Dwivedi, N. K., Sikdar, A. K. and Jolly, M. S., *Indian J. Seric.*, 1987, **26**, 46.

BIOCHEMICAL CHANGES DURING POST-HARVEST STORAGE OF BUTTON MUSHROOM (*AGARICUS BISPORUS*)

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WHITE button mushrooms (*Agaricus bisporus*) are harvested immature (button stage) and have a very short shelf-life. Post-harvest changes resembling growth and maturation cause weight loss, veil opening and external browning. This results not only in low consumer preference[1] but also a loss in nutritive value[2]. Extending the shelf-life of mushrooms is of economic importance and basic studies on post-harvest biochemical changes are required to evolve sound post-harvest practices. Since storage temperature has a profound effect on shelf-life of the mushrooms[3] its effect on protein, amino acid, sugar and phenol content of the mushroom was studied in the present investigation. The results of this analysis necessitated a study of the activities of protease (EC 3.4.22.1) and polyphenol oxidase (PPO, EC 1.10.3.1).

Button mushrooms (3.5 ± 0.2 cm cap diameter) with differentiated but non-stretched velum were harvested, cleaned and packed (200 g) in 100-gauge-thick and perforated (0.5% vent area) polyethylene bags. These were stored at 5°C and 85–90% RH, 10°C and 70–75% RH, and 15°C and 55–60% RH. Chemical composition and protease activity were determined daily till the 4th day.

The protein content was determined by the method of Lowry *et al*[4]. The samples were exhaustively extracted in 80% ethanol for determination of phenols[5], amino acids[6] and sugars. Total soluble sugar and reducing sugars were determined by the anthrone and Nelson–Somogyi methods[7] respectively. Values for non-reducing sugars were derived by the difference. The enzymes were extracted in chilled phosphate buffer (0.1 M, pH 6.8) in a Virtis-type micro-tissue homogenizer. The homogenate was centrifuged at 3000 *g* for 20 min in a refrigerated centrifuge and the supernatant was used as enzyme source. Protease was assayed[8] with haemoglobin as substrate. The reaction mixture for PPO contained, in a final volume of 3 ml, 2 ml of 0.1 m phosphate buffer, (pH 6), 5 μmoles catechol and suitably diluted enzyme source. The change in absorbance was followed at 495 nm in a Spectronic-21 (Bausch and Lomb) colorimeter. The activity of PPO was recorded at 25°C and also at the storage temperatures, i.e. 5, 10 and 15°C, after 96 h of storage.

The chemical composition data are shown in table 1. The decrease in total sugars during storage and at higher temperatures was due largely to a decrease in non-reducing sugars than to decrease in reducing sugars. This may be because, during post-harvest growth, maturation and gill development, the respiratory rate of mushrooms increases and trehalose, the most abundant non-reducing disaccharide, is used as respiratory substrate[9].

The protein content decreased with increasing storage duration. The decrease was more at 15°C than at 10 and 5°C. Since there was a concomitant increase in the protease activity (figure 1), it is evident that proteins were degraded during storage, and the higher the storage temperature, more the degradation[10]. However, there was no significant increase in the total free amino acid content. This suggests that amino acids may be the low molecular weight nitrogenous substances utilized for energy production by the mushrooms during storage, as hypothesized by previous workers[11].

It has been reported that storage duration and higher storage temperatures are correlated with the browning of the mushrooms[3]. The decrease in total phenol content during storage and at higher temperature (table 1) indicates that phenols were utilized for the browning reaction catalysed by the enzyme

Table 1 *Effect of storage temperature on composition of Agaricus bisporus*

	Days of storage					
	0	1	2	3	4	CD (0.05)
Protein						
5°C	315.7	311.5	301.9	280.2	247.4	
10°C	315.7	295.2	280.2	248.7	223.0	7.2
15°C	315.7	286.7	267.9	221.3	193.3	
Free amino acids						
5°C	52.0	51.9	50.5	46.9	44.7	
10°C	52.0	54.8	54.7	52.9	54.9	4.5
15°C	52.0	55.2	58.7	53.5	54.1	
Total sugars						
5°C	92.2	88.5	85.7	80.2	71.9	
10°C	92.2	86.5	81.1	70.1	65.6	6.2
15°C	92.2	82.9	75.2	60.6	53.3	
Reducing sugars						
5°C	21.0	20.2	21.0	18.0	17.5	
10°C	21.0	20.2	18.9	16.2	15.6	1.8
15°C	21.0	19.1	17.4	14.2	13.3	
Non-reducing sugars						
5°C	70.6	68.3	64.8	71.2	54.4	
10°C	70.6	66.3	62.3	53.9	50.0	4.4
15°C	70.6	63.8	57.8	46.5	40.0	
Phenols						
5°C	8.4	7.9	7.7	7.0	6.2	
10°C	8.4	7.5	6.8	6.0	5.3	0.6
15°C	8.4	6.8	5.9	4.8	3.9	

Values expressed as mg/g dry weight are mean of 4 replicates.

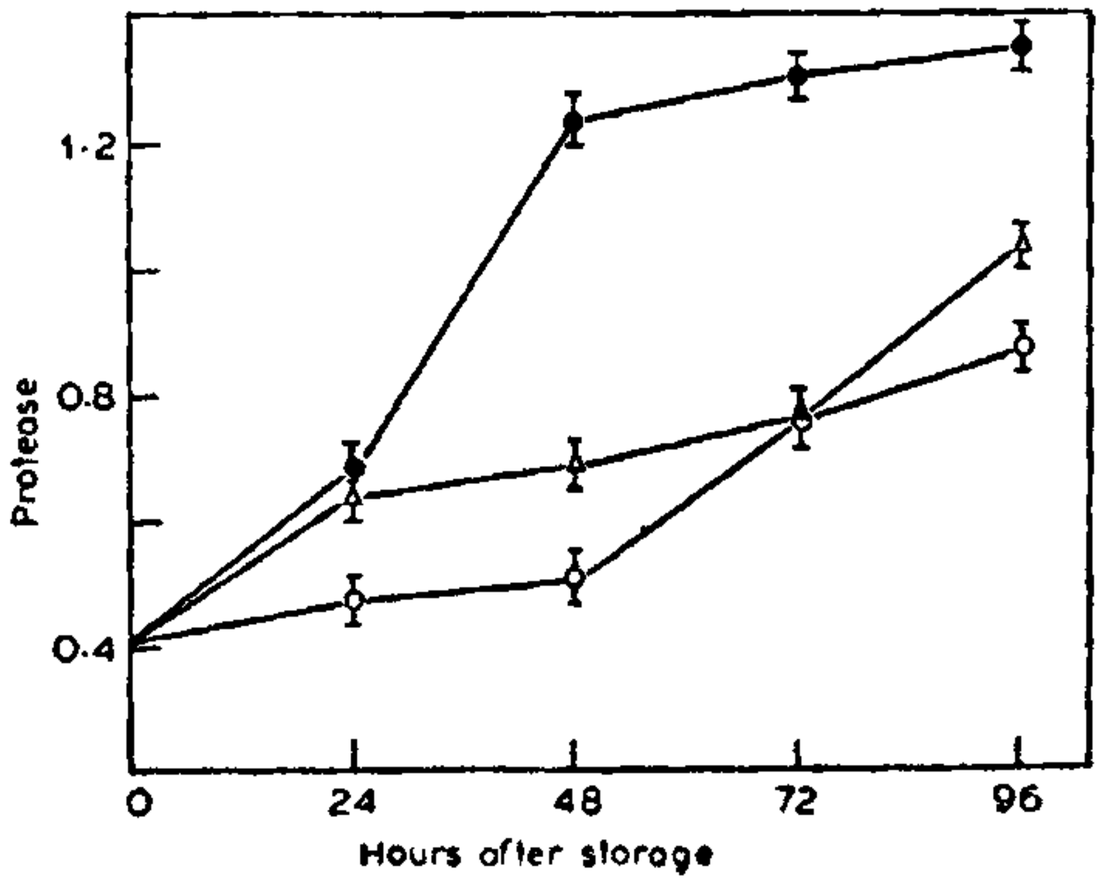

Figure 1. Effect of storage temperature on protease activity (μg tyrosine released/min/g dry weight) of the mushroom *Agaricus bisporus* (mean ± SD of 4 replicates).

PPO concentrated in the skin of the mushroom[1]. The activity of PPO has been reported to increase with increasing storage temperature[12]. In the present study, when the PPO activity was measured at 25 C, the activity in mushrooms stored at 15 C was slightly less than in those stored at 10 or 5 C. When the activity was monitored at the temperature at which the samples were stored, it was found to be almost double at 15 C than at 10 and 5 C (table 2).

Table 2 *Effect of storage temperature on polyphenol oxidase activity of Agaricus bisporus*

Storage[a] temperature (C)	Activity[b] measured at 25 C	Activity[b] measured at storage temperature
5	7.05 ± 0.45	2.67 ± 0.10
10	5.80 ± 0.49	3.56 ± 0.10
15	4.40 ± 0.36	5.28 ± 0.43

[a]Duration four days; [b]Unit: Change in A_{495} by 0.001 min/g dry weight (mean ± SD of 4 replicates).

indicating thereby that the browning is due to the direct effect of temperature on enzyme activity.

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1. Burton, K. S., *Mushroom J.*, 1986, **158**, 68.
2. Rai, R. D. and Saxena, S., *Curr. Sci.*, 1988, **57**, 434.
3. Saxena, S. and Rai, R. D., *Mushroom J. Tropics*, 1988, **8**, 15.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
5. Singh, M., Singh, S. S. and Sanwal, G. G., *Indian J. Exp. Biol.*, 1978, **16**, 712.
6. Moore, S. and Stein, W. H., *J. Biol. Chem.*, 1948, **176**, 367.
7. Spiro, R. G., *Methods Enzymol.*, 1966, **8**, 3.
8. Davis, N. C. and Smith, E. L., *Methods Enzymol.*, 1955, **2**, 215.
9. Hammond, J. B. W. and Nichols, R., *Ann. Appl. Biol.*, 1977, **85**, 162.
10. Eby, D. L., McCardle, F. J. and Beelman, R. B., *J. Fd. Sci.*, 1977, **42**, 22.
11. Murr, D. P. and Morris, L. L., *J. Am. Soc. Hortic. Sci.*, 1975, **100**, 16.
12. Goodenough, P. W. and Ricketts, V., *Ann. Appl. Biol.* 1977, **85**, 447.

ALKALOID PRODUCTION BY THE IMMOBILIZED CELLS OF *SOLANUM XANTHOCARPUM*

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HIGHER plants produce a variety of secondary metabolites, some of which are an indispensable source of important pharmaceuticals. However, due to the uncontrolled exploitation of natural habitat and the fluctuations in the supply of raw materials the production of secondary compounds by plant cell cultures *in vitro* may prove a viable alternative[1]. It is also possible to increase the secondary metabolite production in cell cultures by various methods. However, when scaling-up of production technology from culture vessels to industrial fermentor is attempted, plant cells pose a series of problems, some of which are: instability of production, susceptibility to shearing force, lack of secretion of active principle in the medium and contamination[2]. These problems could be overcome by immobilizing the cells by entrapping them in an inert matrix and suspending them in the medium. Due to these advantages, we decided to use the immobilized cells of *Solanum xanthocarpum* to produce steroidal alkaloid called solasodine which is a good substitute for diosgenin from *Dioscorea* tubers for the synthesis of corticosteroidal drugs[3]. In this communication we present data on viability and the solasodine production by immobilized cells from the suspension culture of *S. xanthocarpum*.

A batch culture of cell suspension of *S. xanthocarpum* was generated from the leaf callus, developed on Murashige and Skoog's (MS) medium[4] containing 3% w/v sucrose, 0.025% w/v casein hydrolysate, 0.5 ppm, 2,4-D and 0.1 ppm Kn.

About 12–14 month old cell suspension cultures maintained through regular subculturing (once in twelve days) were used for analysis. Five milliliter cell suspension (250–280 mg) was pippetted into 30 ml of fresh culture medium and the cells were harvested at three-day-intervals for fresh weight and dry weight determination. Three replicates were used for each observation and the experiment was repeated (figure 1B).

Our initial experience on the use of various matrices to immobilize *S. xanthocarpum* cells indicated that sodium alginate is a better matrix than agar-agar or agarose as it gave sustained production of the alkaloid for 20 days[5]. Therefore in the present experiment only sodium alginate was used for the immobilization experiment using the protocol of Brodelius[6]. Briefly, the cell suspension of lag, exponential and stationary phases was filtered separately through a sterile stainless steel mesh of 0.7 mm^2. At least 2 g cells in 10 ml medium of each growth phase were mixed separately with an equal volume of 2% (w/v) aqueous solution of sodium alginate. This cell suspension-alginate mixture was pippetted through a wide mouth (4 mm dia) 10 ml pippette into sterile 30 mM aqueous calcium chloride solution.

Beads of 4–5 mm in diameter formed instantly at this step and were left in this solution for 15 min for hardening. Finally, the beads were incubated in 250 ml Erlenmayer flask that contained 10 ml of suspension culture medium. Six sets for each type of cells were prepared. Of the six sets, three flasks were used to determine cell viability. From the remaining