

## IMPROVED SHELF LIFE OF BELL CAPSICUM FRUITS BY MANIPULATION OF THE ACTIVITIES OF GLYCOSIDASES THROUGH HEAT TREATMENT

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Received on 8 March, 2003, Revised on 25 May, 2004

### SUMMARY

Glycosidases -  $\beta$ -hexosaminidase,  $\alpha$ -mannosidase and  $\alpha$ -galactosidase are likely to play an important role in fruit softening during ripening of fruits. Heat treatment of bell capsicum for a brief period at dark green stage of the fruit decreased the activity of all these three enzymes. But heat treatment of the fruit at light green and climacteric stage resulted in either increased enzyme activities or did not affect the same. SDS-PAGE protein profile revealed a heat stress specific protein of 34 kDa. The carbohydrate / protein ratio was lesser in heat treated capsicum at dark green stage compared to its untreated control. Higher carbohydrate to protein ratio was noticed in heat treated capsicum at light green stage and climacteric stage over their control. Higher water loss was noticed in heat treated capsicum at dark green stage, light green stage and climacteric stage over their respective controls after 72 h storage at ambient temperature following heat treatment. Heat treatment at dark green stage increased the shelf life of the bell capsicum by an average of 9 days. The fruits heat treated at dark green stage retained the luster even after 6 days (144 h), while those of other stages lost the luster. The data suggested that heat treatment at the dark green stage of bell capsicum could probably improve the shelf life of these fruits.

**Key Words :** Bell capsicum, glycosidases, heat treatment, shelf life

### INTRODUCTION

Capsicums are an important vegetable crop among the *Solanaceous* fruits. These are an excellent source of vitamin A and C. High cost of capsicum is mainly due to high perishability and short shelf life. The post-harvest losses are mainly material loss, which include loss of weight, colour, bruise, decay, etc., at various stages from harvest to consumption. The expected shelf life of capsicum depends upon post-harvest handling and storage environment. As of now, cold storage technique (7-9°C) is the only known economically feasible technique for short-term storage of fresh capsicums. In India, the

prevailing tropical climate and lack of adequate cold storage facilities shorten the life of capsicum to only 6-8 days at ambient temperature (27±4°C). Hence there is a need to extend the shelf life of capsicum at ambient temperatures to extend the market season and create distant markets.

Glycosidases -  $\beta$ -hexosaminidase,  $\alpha$ -mannosidase and  $\alpha$ -galactosidase, the carbohydrate hydrolases are understood to be intricately involved in fruit softening of bell capsicum during ripening. In this study, activities of these glycosidases were examined 74 h after a brief exposure of the fruits at different stages to heat and the

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activity profiles were compared with those of untreated fruits. Percent water loss and carbohydrate / protein ratio were also determined in these fruit samples.

## MATERIALS AND METHODS

Bell capsicum, at different stages of fruit ripening were collected from local market and were exposed to heat for exactly 10 min in a hot air oven maintained at 100°C. Acetone dried powders were prepared from the heat exposed fruit samples (entire tissue) with 3 volumes of chilled acetone after three days of storage at ambient temperature.

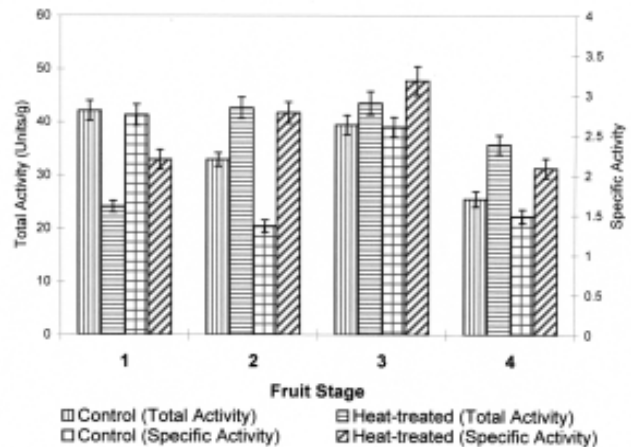
Acetone dried powders of respective samples (1g) were soaked in sodium acetate buffer, pH 6.8 containing 0.2 % polyvinyl pyrrolidone and 0.05% triton x-100 at 4°C with constant stirring for 16 h. The extracts were passed through three layers of cheesecloth and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was dialyzed against double distilled water and concentrated with anhydrous polyethylene glycol. Denatured SDS-PAGE was carried out according to Laemmli (1970) and stained for proteins with silver stain (Porro *et al.* 1982). Protein was estimated by the modified phenol procedure (Hartree 1972) and total carbohydrate in the protein extract was estimated by the method of Dubois *et al.* (1956).

**Enzyme activity determinations:** The reaction mixture consisted of 1.25 mM p-nitro-phenyl- $\beta$ -D-N-acetyl glucosaminopyranoside, p-nitrophenyl- $\alpha$ -D-mannopyranoside or p-nitrophenyl- $\alpha$ -D-galactopyranoside, 100mM sodium acetate buffer (pH: 5.0) and suitable aliquot of the enzyme (Priyasethu and Prabha 1997). Incubation was carried out for 15 min at 37°C and the reaction stopped with the addition of 500 mM sodium bicarbonate to the reaction mixture. The colour intensity of the liberated p-nitrophenol was measured at 405 nm. One unit of the enzyme is defined as the amount of enzyme required to liberate one mmol of p-nitrophenol per min.

## RESULTS

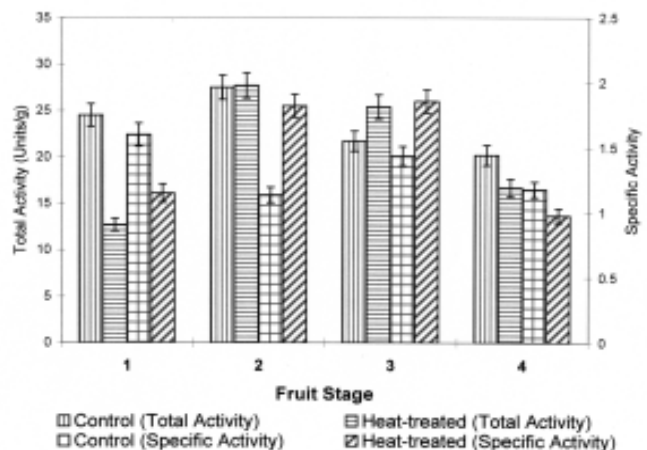
**Activities of glycosidases :** Fig.1 depicts the effect of heat stress on capsicum at different stages of fruit

ripening on the activity of  $\beta$ -hexosaminidase. Heat treatment of capsicum at dark green stage of the fruit decreased this enzyme activity (total activity by 42% and specific activity by 20%). The enzyme activity was higher than the control at all other stages of fruit ripening.



**Fig.1.  $\beta$ -hexosaminidase enzyme activity (Total and specific) in heat-treated capsicum. Values represent mean  $\pm$  SE of 5 independent determinations Fruit stages: 1. Dark green stage 2. Light green stage 3. Climacteric stage 4. Red ripe stage**

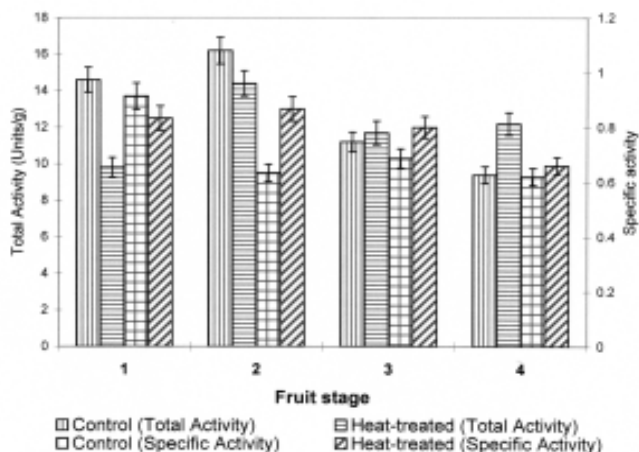
Fig. 2 depicts the influence of heat treatment given to capsicum at different stages of fruit ripening on its  $\alpha$ -mannosidase activity. Heat-treated capsicum at dark



**Fig.2.  $\alpha$ -Mannosidase enzyme activity (Total and specific) in heat-treated bell capsicum. Values represent mean  $\pm$  SE of 5 independent determinations Fruit stages: 1. Dark green stage 2. Light green stage 3. Climacteric stage 4. Red ripe stage.**

green stage exhibited significantly lesser activity of  $\alpha$ -mannosidase compared to its control. Total  $\alpha$ -mannosidase activity was more or less unaffected in the fruits exposed to heat at light green stage. In heat treated fruits  $\alpha$ -mannosidase activity at climacteric stage was slightly higher than of the corresponding control.

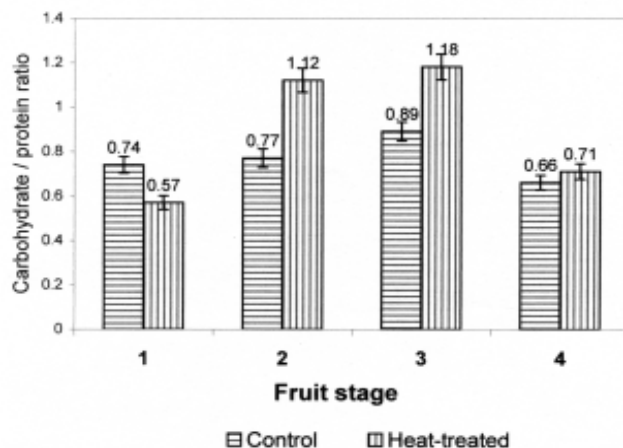
Fig. 3 depicts the influence of heat treatment of capsicum at different stages of fruit ripening on its  $\alpha$ -galactosidase activity. Total  $\alpha$ -galactosidase activity was found to be lesser in heat treated capsicum at dark green stage and light green stage compared to untreated fruits. The enzyme activity was unaffected by heat stress at climacteric stage.



**Fig.3.** Total and specific activity of  $\alpha$ -galactosidase enzyme in heat-treated bell capsicum. Values represent mean  $\pm$  SE of 5 independent determinations Fruit stages: 1. Dark green stage 2. Light green stage 3. Climacteric stage 4. Red ripe stage

**Carbohydrate/protein ratio:** Fig. 4 presents the influence of heat treatment on capsicum at different stages of fruit ripening on carbohydrate/protein ratio of aqueous enzyme extracts. The carbohydrate/protein ratio was lesser in heat treated capsicum at dark green stage compared to its untreated control. Higher carbohydrate to protein ratio was noticed in heat treated capsicum at light green stage and climacteric stage over their control.

**Water loss:** Table 1 presents the influence of heat treatment on capsicum at different stages of ripening on



**Fig.4.** Carbohydrate/protein ratio of aqueous extracts from bell capsicum. Values represent mean  $\pm$  SE of 5 independent determinations Fruit stages: 1. Dark green stage 2. Light green stage 3. Climacteric stage 4. Red ripe stage

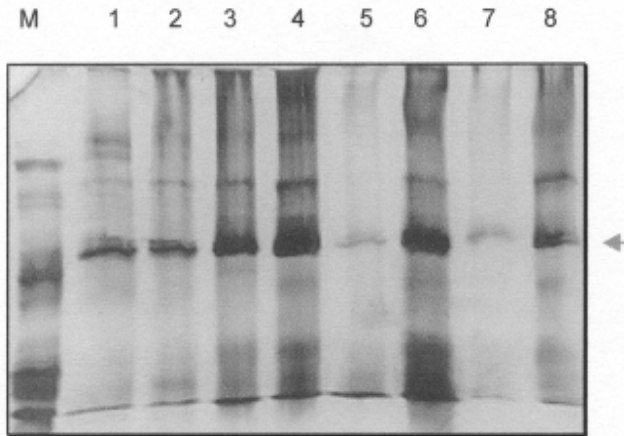
its water content. Higher water loss was noticed in heat treated capsicum at dark green stage, light green stage and climacteric stage over their respective controls after 72 h storage at ambient temperature following heat treatment. Water loss at red ripe stage remained more or less the same for both heat-treated capsicum and its control. Water loss from the fruit was almost equal in all the stages at the end of 144 h.

**Table 1.** Influence of heat stress on water loss from fresh capsicum during storage

Fruit stage	% Water loss*	
	Control	Heat treated
<i>Stored for 72 h</i>		
1. Dark green stage	6.7 $\pm$ 0.4	8.2 $\pm$ 0.6
2. Light green stage	6.9 $\pm$ 0.4	7.9 $\pm$ 0.4
3. Climacteric stage	6.3 $\pm$ 0.3	7.5 $\pm$ 0.4
4. Red ripe stage	5.5 $\pm$ 0.4	5.0 $\pm$ 0.3
<i>Stored for 144 h</i>		
1. Dark green stage	4.0 $\pm$ 0.3	4.5 $\pm$ 0.2
2. Light green stage	4.2 $\pm$ 0.2	4.7 $\pm$ 0.3
3. Climacteric stage	3.9 $\pm$ 0.2	3.4 $\pm$ 0.2
4. Red ripe stage	3.6 $\pm$ 0.3	2.0 $\pm$ 0.1

Values are mean  $\pm$  SE of 5 independent determinations  
\* On fresh weight basis

**Protein profile:** SDS-PAGE protein profile of heat treated and control (unheated) bell capsicum at different stages of fruit ripening (Fig. 5) revealed a protein of approx. m.w. 34 kDa in heat-treated capsicum in 50 % acetone precipitated protein samples.

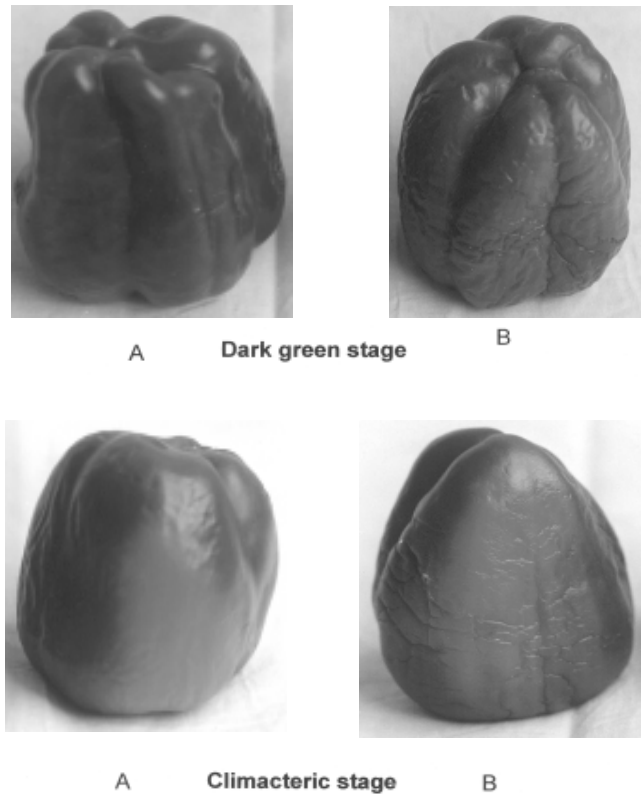


**Fig.5.** SDS-PAGE profile of bell capsicum at different stages of fruit development. Lane: M. Molecular weight markers 1. dark green control 2. dark green heat treated 3. light green control 4. light green heat treated 5. climacteric control 6. climacteric heat treated 7. red ripe control 8. red ripe heat treated

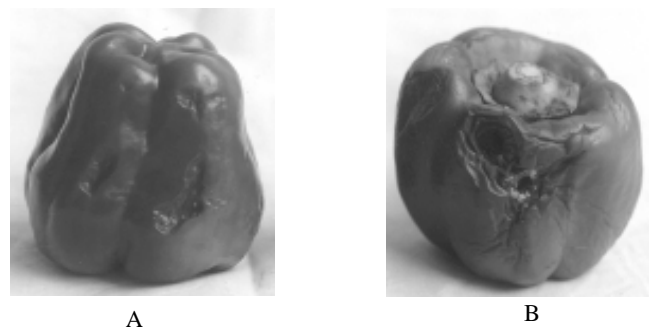
**Shelf life studies:** Shelf life of the capsicum was assessed based on its appearance, color and luster. Fig. 6 depicts the appearance of heat-treated and control capsicum fruits at dark green stage and climacteric stage after 72 h of storage at room temperature following the heat treatment. Heat treated capsicum at all stages of ripening except red ripe stage retained the luster, but shrivelled to some extent compared to the fruits heat treated at dark green stage. The fruits heat treated at dark green stage retained the luster even after 6 days (144 h) while those of other stages lost the luster. Heat treated capsicum fruits at dark green stage not only retained its appearance after 9 days of storage at room temperature but also appeared to be free from pathogen attack in spite of mechanical damage (Fig. 7).

**DISCUSSION**

Heat treatment of capsicum at dark green stage has resulted in a significant decrease in the activity of all the three glycosidase enzymes, viz.,  $\beta$ -hexosaminidase,  $\alpha$ -



**Fig.6.** Appearance of bell capsicum 3 days after heat treatment at dark green / climacteric stage. A. Heat treated B. Control



**Fig.7.** Appearance of bell capsicum 9 days after heat treatment at dark green stage. A. Heat-treated B. Control

mannosidase and  $\alpha$ -galactosidase. But heat treatment at later stages viz., light green stage, climacteric stage and red ripe stage increased the activity of  $\beta$ -hexosaminidase. Such a reduced enzyme activity after heat stress has been reported for polygalacturonase enzyme protein and activity and the mRNA at temperature 32°C and above in

tomato (Kagan-Zur *et al.* 1995). In the tomato system, the exogenous ethylene treatment did not reverse heat stress induced inhibition of polygalacturonase gene expression (Kagan-Zur *et al.* 1995). The polygalacturonase gene expression was gradually and irreversibly shut off during heat stress (for 24 h). Since  $\beta$ -hexosaminidase,  $\alpha$ -mannosidase and  $\alpha$ -galactosidase are understood to have a positive role in fruit softening during ripening, a reduction in these enzyme activities may prove advantageous from the point of view of shelf life of the fruit. Such a beneficial reduction in enzyme activity has been achieved in capsicum by a brief exposure of the fruits to heat at the dark green stage.

The reduced activity of glycosidases in heat treated capsicum at dark green stage could have resulted from switching over to senescence phase, where gene expressions along with other biochemical changes alters fruit biochemistry, hence heat stress along with its changing gene expression might have brought down the expression of enzyme or the heat stress might have altered the enzyme activity. The increased or unaltered enzyme activity of these glycosidases in heat treated capsicum at later stages might be due to the already switching on of the ripening gene and subsequent expression of the proteins responsible for fruit ripening. This is evident from the increased specific activity and increased carbohydrate/protein ratio at the later stages.

Heat treated capsicum at dark green stage was found to have lost higher amount of water (g % of fresh weight) compared to its control and this water loss was higher than the heat treated fruits at rest of the stages. The percent water loss was almost similar in both heat-treated and control capsicum after 72 h to 144 h of storage.

The shelf life of the heat treated capsicum at dark green stage was 9 days on an average compared to heat treated capsicum at light green stage, which was 6 days. Heat treatment at climacteric and red ripe stage did not have significant effect on the shelf life. Heat treatment at dark green stage also prevented the spoilage of fruit due to pathogen attack following mechanical damage. The significant improvement of shelf life by the heat treatment

of capsicum fruits at the dark green stage is probably attributable to the decrease in the activity of glycoprotein glycosidases —  $\beta$ -hexosaminidase and  $\alpha$ -mannosidases as observed in this investigation, since these two enzymes are understood to have a role in fruit softening associated with ripening of the fruit (Jagadeesh *et al.* 2004, 2004a). This observation that a brief heat exposure at dark green stage of capsicum extends its shelf life by 9 days assumes importance from the point of view of a possible application of this strategy to enhance the shelf life of these perishable fruits.

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