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Effects of various treatments on intrinsic properties of *Agaricusbisporus*

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ABSTRACT

Two types of Agaricusbisporus (Close button and open cup) mushrooms were examined for the possible effect(s) of various treatments on the enzymatic activities and microflora on the mushroom. Different parts of the mushroom were investigated which included: the internal gills, the stem (stipe) and the skin which were also compared. The catalase positive micro-organisms were observed to dominate the microflora. Blanching at different temperatures was carried out to know the effects on enzymes present in the mushrooms, possible reasons for this were advanced. Catalase and peroxidase enzymes were examined for the extent of inactivation in the metabolic activities and catalase was particularly observed to lose activity more than peroxidase. Reasons were given for the differences in rate of activity.

Key words: Catalase, peroxidase, blanching, temperature, enzyme, *Agaricusbisporus.*

INTRODUCTION

Mushrooms are a most popular food product, especially in the diets of Western societies. They are palatable and add flavour and varieties to an otherwise monotonous diet. In particular is *Agaricusbisporus*, a vegetable, which is eaten in many countries all over the world. Microbiological control of manufacturing procedures is essential for an efficient mushroom industry. Mushrooms have been known for several thousand years, but only within the last 3000 years their cultivation began in France. Since this time there have been various works done on mushrooms. Vegetables are living respiring objects when harvested and their basic biochemical processes are catalysed by a variety of enzymes and these in order to preserve nature inherent properties of the food must be inactivated, usually by heat or other processes. In this study heat was used by blanching the mushrooms at different temperatures and the effects later examined. Blanching is the pre-processing thermal treatment commonly applied to fruits and vegetables before freezing, drying and canning for different purposes**[1]**. Mushrooms contain many enzymes, the major ones being catalase and peroxidase. Hydrogen peroxyde is the most stable of the oxygen reactive species (ROS) and is a strong nucleofilic oxidant. Hydrogen peroxide is degraded by catalase and peroxidase, enzymes that act synergistically to protect cells. Fungi are reported to be high producers of catalases **[2, 3],** and different types of catalases and catalase genes have been isolated **[4]**. Recent studies have indicated that retention of a significant proportion of peroxidase activity after blanching is compatible with good quality. This means that a less severe heat treatment will suffice as far as product quality is concerned and that more heat labile enzymes than peroxdase are responsible for deterioration. The physiological function of catalase is still unknown although various theories have

been put forward. Probably the catalase located in the cell organelles plays the role of a specific peroxidase. The enzyme is closely related to peroxidase in structure and function and both enzymes are sometimes considered together as hydroperoxidases. The measurement of peroxidases activities plays an important role in the collection, preparation, stabilization and storage of mushroom. As the presence of peroxidase in foodstuffs often results in unwanted changes (colour changes, smell and taste changes, etc.) the test for their inactivation for example in fruit and vegetable industry or in preparation of oats, is an important requirement for these industries. The velocity of enzyme action is greatly affected by temperature **[5]**. Within certain temperature limits the velocity is increased by rise in temperature until an optimum temperature is reached, further increase in temperature causes a decrease in velocity until the inactivation temperature at which the enzyme is destroyed is attained. The optimum temperature is affected by hydrogen ion concentration ratio of substrate to enzyme, and by other factors. One method of determining the inactivity effect of heat on enzymes is that ascertaining the temperature at which it loses one half of its activity in one hour when heated in aqueous solution at the optimal PH value in the absence of the substrate. Another and perhaps more convenient method is that of determining the minimum temperature at which all activity is destroyed in 5 minutes or in 10 minutes. The objective of this study was aimed at finding out the enzymes present in both the button type and open cup mushrooms and subsequent effects of blanching on these enzymes.

MATERIALS AND METHODS

Catalase analysis

Three different samples of the Open mushrooms were weighed. They were blanched at 70°C; 85°C and 95°C for 10 secs respectively. The above treatments were repeated on different samples for 30 secs, 1 min and 2 mins respectively. Each sample was homogenized and centrifuged. The supernatant liquid was then taken respectively. The 2209 Multi-temp was used to set the temperature at 25°C before starting. Sodium dithionite concentration was used to expel out any oxygen in the system before being used. Three millimeter phosphate buffer pH 7.0; 0.5 m of catalase and 50 µl of the sample were added together and the instrument switched on. The catalase present in each sample was recorded on the graph paper by marker of the oxygen electrode. This was later calculated for each of the samples. The whole procedures were repeated for the Button Cup mushrooms and the readings plotted on the graph paper were later calculated out for each sample.

Peroxidase analysis

Three different samples of the open cup mushroom were weighed. They were then blanched at 70°C, 85°C and 95°C for 10 secs respectively. This treatment was repeated for 30 secs, 1 min and 2 mins respectively. Each sample was homogenized using stomacher and later centrifuged. The aliquot of each sample was aseptically pipetted into different sterile 100 ml conical flasks. Phosphate buffer pH 7.0 was used as blank to measure the absorbance at 436 nm using the visible spectrophotometer. Each sample was sucked by the spectrophotometer and the reading taken. This was done five times at zero, 15 secs, 30 secs, 1 min and 2 mins intervals. The absorbance readings obtained were later plotted against time of assay. Similar experiment was conducted on Button type mushrooms and the readings obtained were plotted against time.

Determination of Peroxidase Activity

The peroxidase enzyme activity was determined by assay method using the visible spectrophotometer with 436 nm wavelength for the close button and open cup mushrooms respectively. The different absorbance readings obtained were plotted against time of assay. The slope of each curve was determined and then used to determine the enzyme activity for the respective blanching temperature of 70°C, 85°C and 95°C.

Determination of Decimal reduction Time ‘D’ of microbial loads

Death time, the rate of destruction ‘D’ was determined by considering the Increase in temperature above that at which the growth of micro-organisms stop results in injury. The D values were determined for the peroxidase and catalase activities in the close button and open cup mushrooms.

RESULTS AND DISCUSSION

Decimal reduction Time ‘D’.

The graphs below (Figures 1a.1b,1c and 2a, 2b, 2c) show the rate of destruction ‘D’ which was determined by considering the Increase in temperature above that at which the growth of micro-organisms stop.

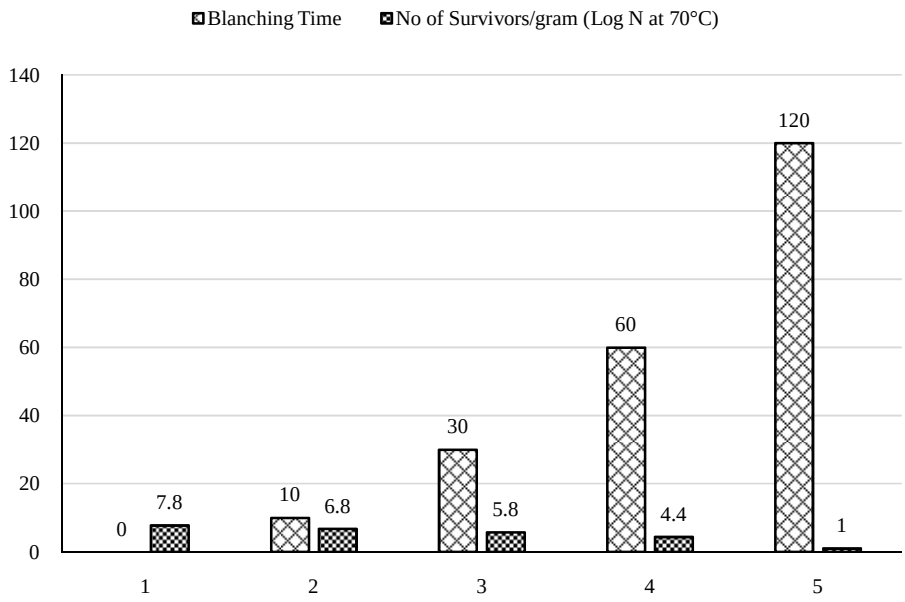

Figure 1a: The Decimal reduction Time 'D' of microbial loads in close button mushroom blanched at 70°C

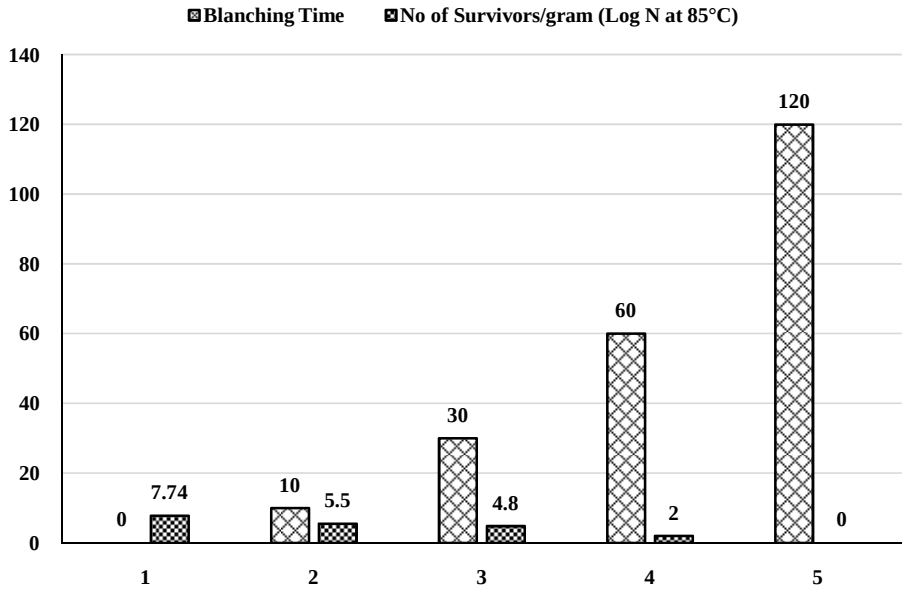

Figure 1b: The Decimal reduction Time 'D' of microbial loads in close button mushroom blanched at 85°C

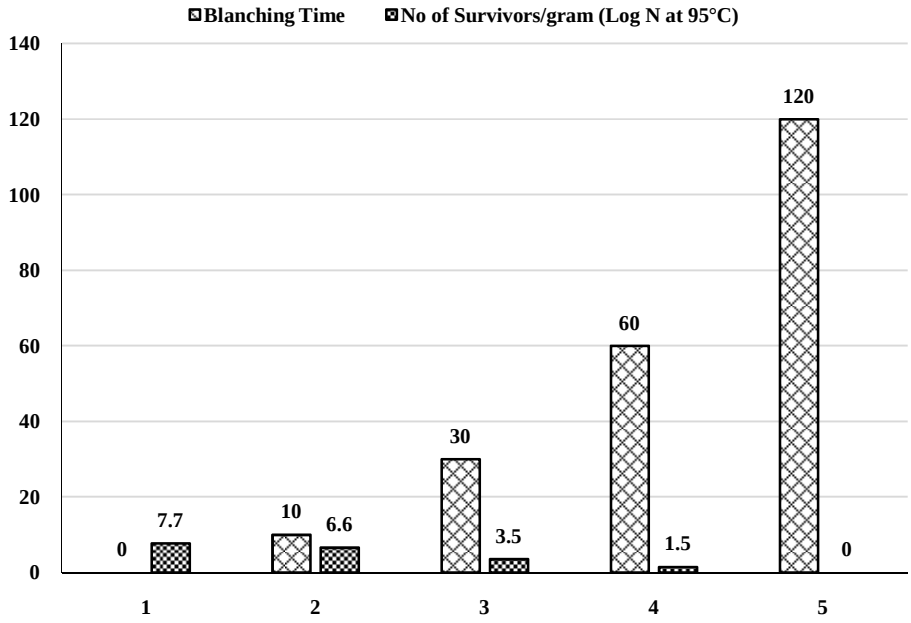

Figure 1c: The Decimal reduction Time 'D' of microbial loads in close button mushroom blanched at 95°C

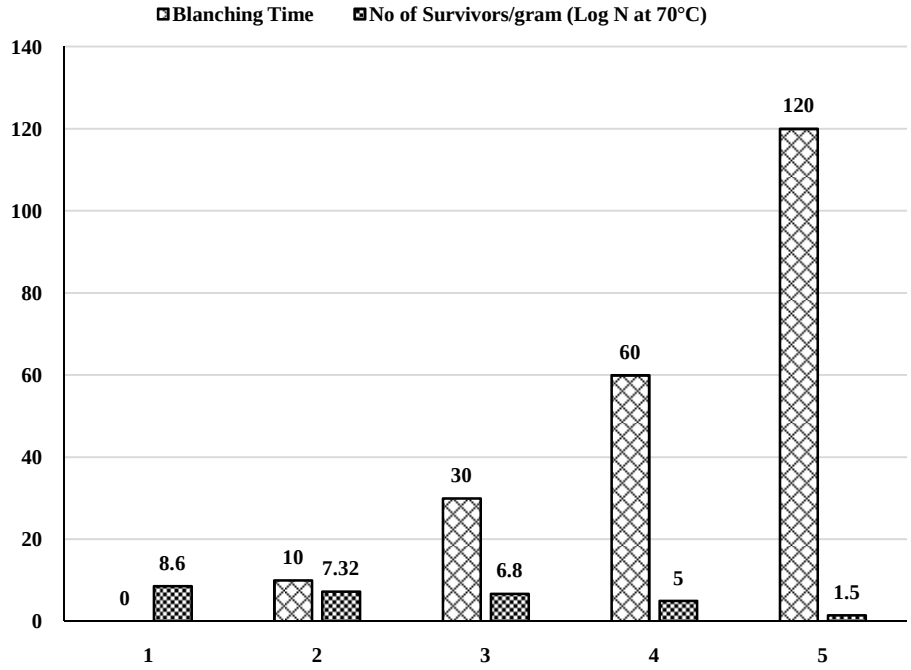

Figure 2a: The Decimal reduction Time 'D' of microbial loads in Open cup mushroom blanched at 70°C

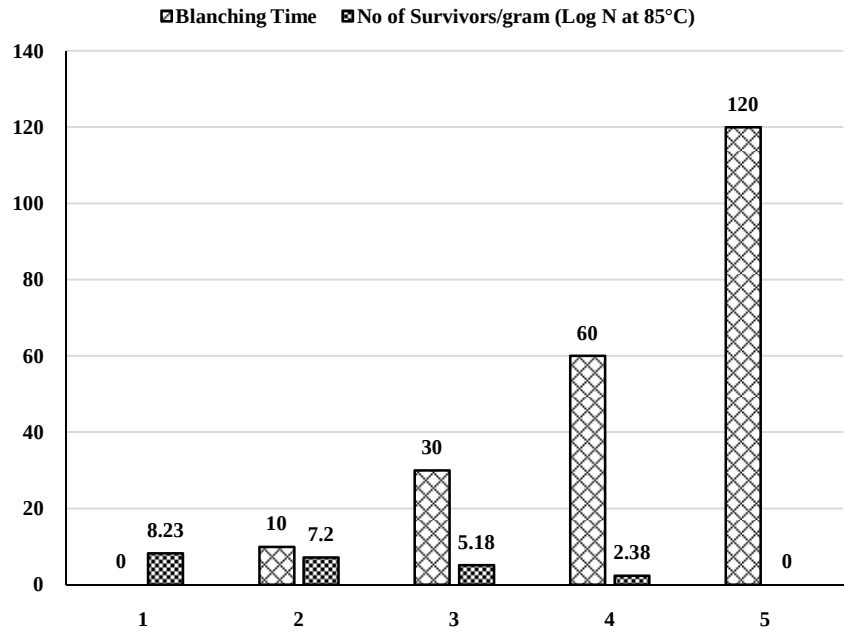

Figure 2b: The Decimal reduction Time 'D' of microbial loads in Open cup mushroom blanched at 85°C

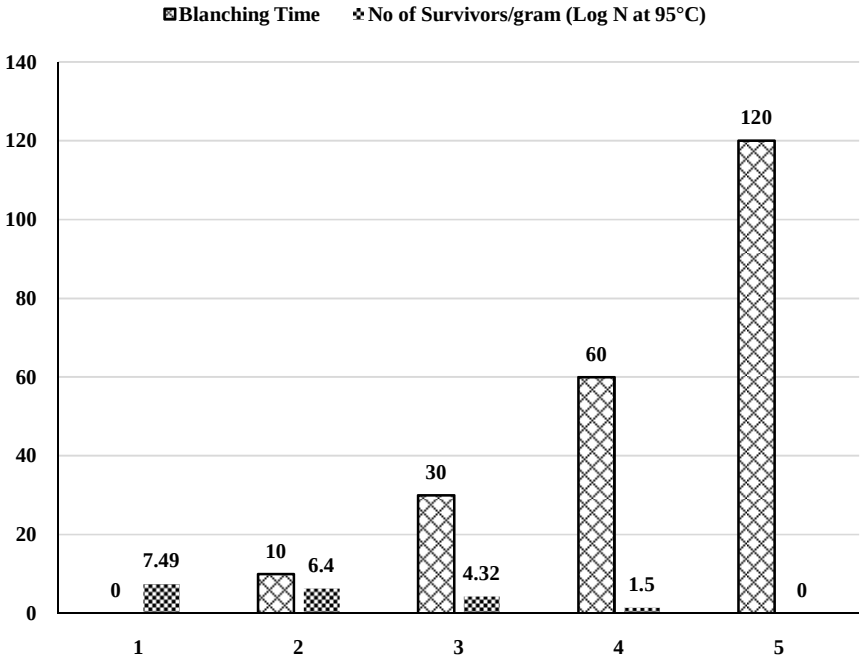

Figure 2c: The Decimal reduction Time 'D' of microbial loads in Open cup mushroom blanched at 95°C

Effect of blanching on catalase enzyme on close button mushrooms

The percentage residual enzyme for each blanching temperature was worked out and recorded as shown below (Table 1a and 1b)

Table 1a Residual catalase enzyme in close button mushrooms after blanching at 70°C

Time of Blanching (sec)	Residual Enzyme (μ moles O_2 min^{-1})	% Residual
10	8.1± 0.04	65.3± 0.03
30	5.6± 0.02	20.1± 0.02
60	2.49± 0.01	45.2± 0.02
120	**0**	**0**

Data are mean ±SE values (n = 3).

Initial catalase enzyme in the samples before blanching was 12.4 (μ moles O_2 min^{-1}).

Table 1b Residual catalase enzyme in close button mushrooms after blanching at 85°C

Time of Blanching (sec)	Residual Enzyme (μ moles O_2 min^{-1})	% Residual
10	5.2± 0.02	41.9± 0.02
30	3.3± 0.01	26.6± 0.01
60	0.8± 0.01	6.45± 0.01
120	**0**	**0**

Data are mean ±SE values (n = 3).

Initial catalase enzyme in the samples before blanching was 12.4 (μ moles O_2 min^{-1}).

Effect of blanching on catalase enzyme on open cup mushrooms

The catalase enzyme was less in open cup than in close button mushrooms as shown below (Table 2a and 2b).

Table 2a Residual catalase enzyme in open cup mushrooms after blanching at 70°C

Time of Blanching(sec)	Residual Enzyme (μ moles O_2 min^{-1})	% Residual
10	5.2± 0.02	73.2± 0.04
30	2.9± 0.03	40.8± 0.02
60	1.1± 0.01	15.5± 0.02
120	**0**	**0**

Data are mean ±SE values (n = 3).

Table 2b Residual catalase enzyme in open cup mushrooms after blanching at 85^0C

Time of Blanching (sec)	Residual Enzyme (μ moles O_2 min^{-1})	% Residual
10	4.8± 0.02	67.6± 0.03
30	2.1± 0.01	29.6± 0.01
60	0.2± 0.01	2.82± 0.02
120	**0**	**0**

Data are mean ±SE values (n = 3).

The effect of blanching on catalase enzyme was examined on the close button mushrooms.The initial catalase enzyme present before blanching was determined and found to be 12.4 (μ moles O_2 min^{-1}). After blanching for 1 minute the residual catalase was found to be 20.1% whereas blanching at 95°C, there was no residual activity got even at 10 secs. This observation agrees with the view of **[6]**that blanching mushrooms at a high temperature for 5 secs inactivates catalase. The results showed that it took about 120 secs. (2mins) to completely inactivate the enzymes in the samples at 70°C, 85°C and 120°C respectively. However, the higher the time of blanching, the lower the no of microbial survivors becomes. Therefore, most of the micro-organisms in the samples are likely to be mesophiles which would have been killed by the time it reaches a high temperature of 120°C. Also, since a large proportion of the microflora are coliforms and these would also die off with the high temperature used for the blanching. It has been found that blanching mushrooms at a temperature of 95°C for 5 seconds inactivates catalase, though some peroxidases are still present after 20 seconds blanching. This is supported by the work of **[7]** which

indicates that a considerably longer period of blanching is required than that barely necessary to destroy catalase in order that enzymic deterioration may not occur after blanching. Peroxidase was also observed to have activity even at the high temperature of 95°C after the 10 secs blanching time, though the activity was very low. This result is supported by previous work done by**[8]** who proved that a significant proportion of peroxidase is retained after sufficient proportion heat treatment. Also, similar report was given by **[9]** that peroxidase in carrot extract was inactivated by approximately 29% after 15 min of heating at 45°C and complete inactivation was obtained after 10 min heating at 75°C. This is also in support of **[6]** who reported that it is desirable to minimize the heat treatment impact, however keeping the peroxidase enzyme inactivation to a suitable residual level. Also, **[6]** reported that blanching mushrooms at a temperature of 95°C for 5 secs inactivates catalase, though some peroxidase are still present after 20 secs blanching. The two enzymes peroxidase and catalase examined were observed to be inactivated by heat, however, the effect of the blanching was greater on catalase than peroxidase. This is because catalase is more easily inactivated than peroxidase. The decimal reduction time of microbial loads in this study differ for both close button and open cup mushrooms and this could be attributed to their morphological differences. Also, the hot water used for blanching could easily penetrate the open cup mushrooms more than the close button ones.

CONCLUSION

In conclusion, since blanching is a thermal treatment needed to stabilize items like vegetables, through the inactivation of given enzymes (e.g peroxidase and catalase) that can affect products quality during storage, blanching of mushrooms therefore has several advantages, but also a number of disadvantages. Some of the advantages include inactivation of enzymes, stabilization of organoleptic properties, texture, flavor and nutritional qualities and also reduction in numbers of microbial density which all together give the desirable and expected results after mild thermal processing.

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