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***Research article***

## ANALYSIS OF TISSUE SPECIFIC DIGESTIVE AND ANTIOXIDANT ENZYMES FROM *CUCURBITA PEPO* AND *LANGENARIA SICERARIA* (MOLINA) STANDL

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**ABSTRACT:** Digestive enzymes and antioxidants present in food materials act as a surplus for our digestive system. In present study, five digestive enzymes namely amylase, protease, lipase, pectinase and α -glucosidase and seven antioxidant assays for Catalase, Peroxidase, $H_2O_2$, Super Oxide Dismutase, Malondialdehyde, Carotenoid & radical scavenging were carried out from two members of cucurbitaceae family namely *Cucurbita pepo* (Pumpkin) and *Langenaria siceraria* (bottle gourd). Amongst them amylase, lipase and pectinase activity were found in *C. pepo* and *L. siceraria.* The enzyme kinetics studies revealed their maximum velocities to be 0.2631 and 0.33557 mM $min^{-1}$ for Amylase and Lipase respectively. Our further studies report the presence of proteinase inhibitors and α-gluosidase inducers, while absence of amylase inhibitors and α -glucosidase inhibitors in pumpkin and bottle gourd. Present findings suggest the use of these fruits as an attractive material for further study leading to possible development of digestive syrup.
**Key Words:** *Cucurbita pepo*; *Langenaria siceraria*; amylase; lipase, proteinase inhibitor; antioxidants.

## INTRODUCTION

Vegetables and fruits are one of the most important parts of human diet. Amongst vegetables, family cucurbitaceae occupies around 40% of total production and thus have a tremendous economic importance which also signifies them to be major consumable food. It is the common man's food covering around 90% peoples' associability (Rickman *et.al.*, 2007). Though there is versatile availability and usefulness of these vegetable crops, no work has been reported on the availability of certain digestive enzymes, their inducers, inhibitors antioxidant properties till now. A wide area of research has thus remained unexplored which could lead to remedies over 'modern day' diseases like diabetes, obesity, ulcer, aging, osteoporosis etc.

Interest in the nutritional value of fruits and vegetables is increasing because of recent findings on the high level of obesity and other diet-related health problems in children (Rickman *et.al*, 2007). These studies are mostly focused on antioxidant properties present in these vegetables. Antioxidants are compounds which delay and/or inhibit the oxidation of some other molecules by blocking the initiation and thus the propagation of oxidizing chain reactions (Velioglu *et.al,* 1998). Restricted use of synthetic antioxidants has caused increased interest in natural antioxidant substances (Baardseth., 1989; Gulcin *et.al*, 2002). Therefore, natural antioxidants from extracts plant materials which can replace the synthetic antioxidants needs to be developed (Liyana *et. al,* 2006).

Amylases from microbial and plants sources as food additives, in brewing industry and in preparation of oriental foods have been employed for centuries (Burhan *et.al*, 2003 Tiwari *et.al,* 2007). Lipase has been used for manufacturing process in leather, detergents, and perfumery and most importantly in medicine and synthetic materials which inspired to search cost effective and new sources for this enzyme (Svendsen, 2000).Use of lipase in many of the industrial process such as removing of fat strains from fabrics, and accelerating maturation in cheese and degrading fats in waste products has been investigated (Godfrey *et.al,* 2003). The final step in digestive process of carbohydrates is catalyzed by the enzyme α-glucosidase and hence its inhibitors can retard the use of dietary carbohydrates leading to suppressing of postprandial hyperglycemia (Watanabe *et.al,* 1997). Inducers of this enzyme have been used to treat hypoglycemia (Cornblath *et.al,* 2000).

Pectinases are one of the most important groups of enzymes used in the fruit and vegetable industry and have been used in maceration of vegetable tissue, paper textile industries, coffee and tea processing, clarification of fruit juices and wines, and to improve the cloud stability of fruit nectars (Kashyap *et.al,*2001). The protease inhibitor (PI) proteins are natural antagonists of protease present in all life forms (Ryan *et.al.,* 1998).Researcher also have been reported the anticancer activities of protease inhibitors (Kennedy *et.al,* 2002).
Present work is aimed to screen some of the digestive enzymes, their inducers, inhibitors and antioxidants from pumpkin and bottle gourd to have an insight into their nutrinal value.

## MATERIALS AND METHODS

### Plant Materials and Reagents

Pumpkin (*Cucurbita pepo*) and Bottle gourd (*Lagenaria siceraria*) fruits were brought from local farm field in Ahmednagar, India. All reagents used were of analytical grade of Sigma- Aldrich (Germany) and Qualigens (India) Pvt. Ltd.

### Preparation of cell free extracts and enzymatic analysis

### Amylase and their inducer and inhibitor activities

For α-amylase assay, sample was crushed in physiological saline (0.85%) and centrifuged at 10,000 rpm for 15 min at 4 $^{0}$C. The supernatant was desalted through Sephadex G-25 gel (P10 column) and used as an enzyme source. A 0.7 cm bore on starch agar plate (2.5% agar and 0.5% starch) were used for different concentration of sample loading (50 µl, 100 µl, 150 µl) with control saline in one well. Zone of clearance were measure after incubation of 2 hrs by adding iodine. The Screening of amylase inducers or inhibitors was carried out by using diastase as standard enzyme. On four wells of starch agar plate saline (control), diastase, sample with diastase and sample were loaded separately. The plates were incubated at 4 $^{0}$C and room temperature for 2-2 hrs before observing it under iodine treatment. Reducing sugar was estimated by standard DNSA method (Miller, 1959).

### Assay of lipase

Lipase assay was performed with olive oil emulsion. The plant tissue were extracted in 50 mM phosphate buffer, pH 7.0 and assayed by titration of fatty acid released from hydrolysis of fat catalyzed by lipase (Sarita *et.al.,* 2000). The Quantity of fatty acid released in unit times is measured by the quantity of NaOH required to maintain pH constant. One unit of lipase (U) is the amount of enzyme which releases 1 µmol of titrable free fatty acids per minute under the described conditions. Substrate solution: olive oil (2 ml), Sodium taurocholate (200 mg) and Arabic hasten or Sodium dodecyl sulfate (2 gm); Analysis conditions: 4 mL of olive oil emulsion, 5 mL of 0.05 M tris buffer and 1 mL of enzyme solution were mixed and incubated at 40 °C for 15 min. The reaction was terminated with the addition of 95 % ethanol (15 mL) after incubation, and the liberated fatty acids were titrated with 0.05 M NaOH with 1% phenolphthalein indicator. Blanks were measured with a heat-inactivated enzyme sample, for which an enzyme stock solution was kept at 100 °C for 15 min. After cooling to ambient temperature, the solution was used as described for the active enzyme sample.

### Pectinase Enzyme Assay

A 30 gm macerated tissue and 20 ml NaCl-EDTA salt mixture was stirred for 15min and the supernatant was collected after centrifugation at 10,000 rpm for 15 min. Above steps were repeated with residue and 20 ml of salt mixture solution for twice. The precipitate were collected from the supernatant by adding 100 ml of cold ethanol and dissolved in 1.5 M NaCl and used as crude enzyme source. A stock of 2% of pectin prepared in 1.5M NaCl and was diluted according to get 1% solution in 0.1M NaCl. Reaction mixture i.e. 100 ml of above pectin solution, 30 ml of 1M NaCl and 60 ml of distilled water was placed in water bath at 30 $^{0}$C. Under continuous magnetic stirring pH 7.5 was maintained (by 0.02N NaOH). A 10 ml of enzyme solution was added in reaction mixture, pH was maintained constant to 7.5 by adding 0.02N NaOH for a definite period of time. The alkali consumption was measured as a function of time with suitable heat inactivated enzyme as blank (Hultin *et.al,* 2006).

### α- Glucosidase activity, inducers and inhibitors

The sample was crushed in physiological saline (0.85 %) and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was used as crude enzyme source or inhibitor or inducer source for further experiments. Three reaction mixtures were prepared separately for α-Glucosidase activity one with (1.5 ml of phosphate buffer, 0.5 ml of sample and 0.5 ml of starch solution), other was substrate blank and enzyme blank and were incubated at 37 °C for 30min.

A 0.5 ml from each reaction mixture, 2.5 ml phosphate buffer and 1 ml GOD-POD reagent was incubated at room temperature for 18 min and absorbance was recorded at 530 nm. Same procedures were followed for their inducer and inhibitor activity (Chougale *et.al,* 2009).

## Protease, their inhibitors and inducers

Extraction of plant proteases and their inhibitors (PIs) was performed (Ryan *et.al,* 1998). Briefly, seeds of pumpkin and bottle gourd were ground in a pre-chilled mortar and pestle and washed with acetone followed by hexane for de-pigmentation and de-fatting. Air dried powdered of all tissues was extracted over night in 1% PVP. Centrifuged for min at 10,000 × g at 4 °C and supernatant obtained was used as a source of enzyme, inducer and inhibitors. Similarly pulp, peel, fiber were processed to get powder.

Three varying concentrations of tris-buffer, enzyme (Tripsin 0.1 mg/ml tris-HCl buffer pH 7.8) and powder extract in ratios (20: 14: 6, 14: 12: 14, 6: 14: 20) were prepared. Final adjusted volume of 20µl by tris-buffer was loaded on X-ray photographic film. The film with spots again incubated for 20 min. Hydrolysis of gelatin was visually monitored. Spots appeared as un-hydrolyzed gelatin against background that reveals presence of inhibitory activity. X-ray film washed with tap water and scanned (Pichare *et.al,* 1994). Quantitative estimation of protein was carried out by Bradford's method (Bradford. 1976).

All the statistical analysis was performed by using software Statistical Package for the Social Science (SPSS 14.0) (Illinois, USA) for windows program. All data represents mean of three separate experiments ± standard error (n = 3). The data were analyzed by student's t-test at $P \leq 0.05$ significance level.

## Catalase activity

In cold condition 1 g of plant sample was homogenized with 10 ml of 0.067 M Sodium phosphate buffer (pH 7.0) and homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was used for the assay of catalase activity. The absorbance ($A_{240}$) of reaction mixture contained $H_2O_2$- $PO_4$ buffer (3 ml) and 20 µl of enzyme extract. Activity of catalase was calculated as described by (Luck 1965).

## Peroxidase activity

The enzyme was extracted from fresh sample at 4°C with 0.1 M sodium phosphate buffer (pH 7.4). The extract was centrifuged at 5000 rpm for 15 min. the supernatant was used as enzyme preparation. Assay medium consisted of 1.0 ml of 100mM sodium phosphate buffer (pH 6.4), 1 ml of guaiacol (0.29 ml/100 ml), 2.0 ml of distilled water and 1.0 ml of enzyme preparation in a total volume of 5 ml. Reaction was started by adding 1 drop of 3% $H_2O_2$ and was recorded spectrophotometerically at 470 nm at 15 second interval till absorbance increase. The enzyme activity was expressed as $\Delta$OD $min^{-1}gm^{-1}$FW and was calculated by taking the difference of absorbance at 1 minute time interval (Putter, 1974).

## Super oxide dismutase activity

For Super Oxide Dismutase (SOD) activity 0.5 gm of fresh same was homogenized with 1ml of buffer (pH 7.0), 0.5 ml PVP (0.2%), 0.5 ml EDTA (0.1mM) and 0.5 ml $MgCl_2$ (3 mM). After grinding, mixture was centrifuged at 2500 rpm for 10 minutes at ± 4°C. the supernatant was prepared by taking 0.7 ml buffer (pH 7.8), 0.5 ml Methionine (0.0970 gm in 50 ml DW), 0.7 ml NBT (0.031 gm in 50 ml DW), 0.5 ml EDTA, 0.1 ml enzyme extract and 0.3 ml Riboflavin (50 µM), at last in test tube. The test tube was kept 30cm below a light source consisting of two 15 watts tube light for 15 minutes, to develop the colour. A non-irradiated reaction mixture was run in parallel, which did not develop colour served as control (Beauchamp *et.al,* 1971). Absorbance was taken spectrophotometerically (Visiscan 167) at 560 nm.

### Hydrogen peroxide scavenging activity

A solution of $H_2O_2$ (40 mM) was prepared in phosphate buffer (pH 7.4). Tissues of *C. pepo* and *L. siceraria*, at the 30 µg $ml^{-1}$ concentration in 3.4 ml of phosphate buffer, were added to an $H_2O_2$ solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. A blank solution contained the phosphate buffer without $H_2O_2$. The hydrogen peroxide scavenging capacity of these tissues was calculated from the calibration curve determined by linear regression. The percentage of $H_2O_2$ scavenging of both plant tissues and the standard compounds was calculated (Liyana *et.al,* 2006).

## DPPH free radical scavenging activity

To determine the IC50 value of the active component, the technique using 96-well microplates was employed. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of methanol, and the solution was kept in the dark at 4 °C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 µl of methanolic DPPH solution (final concentration 300 µm) was added to each well.

The plate was shaken to ensure thorough mixing before being wrapped with aluminium foil and placed into the dark. After 30 min, the optical density (OD) of the solution was read using an ELISA Reader (EL340 Biokinetic reader, Bio-Tek Instrumentation, USA) at the wavelength 517 nm. A methanolic solution of DPPH served as a control. Percentage inhibition was calculated using the following formula:

% Inhibition = [1-$A_{517}$ (DPPH + sample)/OD (DPPH)] X 100%

A dose response curve was plotted to determine the $IC_{50}$ values. $IC_{50}$ is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged. The standard used in this assay was L-ascorbic acid (Sigma, USA) (Gulcin *et.al,*2002, Gulcin *et.al,*2003).

### Enzyme Kinetics of amylase and lipase activities

For enzyme kinetics of amylase, Michaelis-Menten constant ($K_m$) and maximum rates ($V_{max}$) were determined by using starch solution in the range of concentrations 0.5-2 %, pH 6.8 at $37^0$ C. The reaction was followed in a spectrophotometer (Visiscan 167) and data were plotted according to Lineweaver-Burk.
For enzyme kinetics of Lipase, different concentration (0.5 to 2 ml) of olive oil were taken and assayed for lipase activity. Line weaver-Burk plot were used to calculate $K_m$ and $V_{max}$ under different concentration of substrate.

### Measurement of Carotenoids and Malondialdehyde content (MDA)

For measurement of Carotenoids, 1 g samples were extracted in a pre-cooled mortar and pestle chilled at 4ºC using chilled 10 ml 80% Acetone. Filtrate was washed with acetone until the supernatant become colorless and further volume was made up to 100m with acetone. The extracts were then centrifuged at 5000 rpm for 15 min. Absorption of clear supernatant was determined with spectrophotometer (Visiscan 167) at 440nm for carotenoids content and calculated using the formula proposed by Arnon (1949) .
For measurement of Malondialdehyde content, tissue sample weighing 0.2 gm was homogenized in motor and pestle. The content of tissue MDA was then determined using the method described by Heath and Packer, 1968. A sample of 0.5 ml was acidified with 2.5 ml of 1.22 mol $L^{-1}$ trichloroacetic acid in 0.5 ml $L^{-1}$ HCl. The mixture was left to stand for 15min. After this time, 1.5 ml of 0.6 % thiobarbituric acid in 0.05 mol NaOH was added. The samples were incubated in 100 °C water bath for 30 min. Subsequently it was cooled under running tap water and 4 ml of n-butanol was added. After thorough mixing, the mixture was centrifuged for 10 min at 1500 g. The absorbance of the upper phase was read at 535 nm by spectrophotometer (Visiscan 167).

## RESULTS AND DISCUSSION

The screening of digestive enzymes in pumpkin (*Cucurbita pepo*) and Bottle guard (*Lagenaria siceraria*) was studied along with their inducer and inhibitor activity and were summarized in Table 1.

### Amylase activity it's inducers, inhibitors and kinetics

In this assay Amylase, inducers & inhibitors were screened by Starch Agar plate using diastase as a standard enzyme. Amylase is the initial enzyme to hydrolyze the starch component of food from internal α, 1-4 glycosidic bond with the retention of α anomeric configuration in the product (Satyanarayana *et.al,* 2005). Maximum amylase activity i.e. 610 U/ml was observed in Pumpkin seed by DNSA test (Fig. 1a). In general, amylase activity was obtained maximum during germinating seeds (Norastehnia *et.al,* 2007). Bottle gourd seeds exhibits no amylase activity. Amylase activity can be inhibited by metal chelators and chemical compound which help to study characteristics of the enzymes (Najafi *et.al,* 2005). Amylase inducer and inhibitor activity are absent both in pumpkin and bottle gourd (Table-1).
The desalted enzyme was used for kinetic study under different concentration of starch. In Line weaver and Burk plot, the value of $K_m$ and $V_{max}$ of amylase was -1.66 and 0.2631 mM $min^{-1}$ respectively (Fig. 2). The $K_m$ for α-amylase obtained from *Pichia burtonii* was reported very high under different experimental conditions and in molecular cloning studies (Kato *et.al,* 2007). The $K_m$ value obtained revealed that the pumpkin amylase present in natural form have more affinity for substrate. This suggest that amylase extraction from pumpkin is cost effective than fermentation through microbes.

### Lipase activity

It is well known that in plants lipases are present mostly in food reserve tissue of growing seedling and especially in those which contains large amount of triacylglycerols are localized in the organelles called lipid bodies (Pahoja *et.al,*2002). Maximum lipase activity was observed in bottle gourd seed (Table 2).

The absence of lipase activity in pumpkin could be due to physiological condition, sensitivity of tertiary structure of enzyme (Pahoja *et.al,* 2001). or presence of other enzymes which hamper the activity of lipase in the sample (Saven, 2000).
Lipids are the major content of the fast foods and play a key role in the release of fatty acids from the triacylglycerols of lipid (Sharma *et.al,* 2001). By using varying concentration of substrate oil (0.5 ml, 1 ml, 1.5 ml, 2 ml), it has been shown that the reaction rate followed the Michaelis-Menten kinetics. Line weaver and Burk plot in Fig. 3 represents $K_m$ value of lipase as 0.2173 and $V_{max}$ value as 0.33557 mM $min^{-1}$. The specific activities of the enzyme depicted are also reflected here through the values for the maximum velocity ($V_{max}$). Natural form of lipase present in food having high notification value. It was observed in other studies the esterification of lipids is higher by free form of lipase (as present in vegetables) than the immobilized one (Rahim *et.al,* 2008).

**Table 1. Screening of antioxidative, digestive enzymes activities, their inducers and inhibitors in pumpkin and bottle gourd sample. Value representing mean of n=5 ± S.E.**

| Sample / Assay | *Cucurbita pepo* | | | | *Lagenaria siceraria* | | |
|---|---|---|---|---|---|---|---|
| | Seed | Pulp | Peel | Fiber | Seed | Pulp | Peel |
| Amylase Activity ($Uml^{-1}$) | 611±5.19 | 89.5±2.52 | 167±6.11 | 192±9.32 | - | 93.7±2.92 | 152.82±9.91 |
| Amylase Inducer ($Uml^{-1}$) | - | - | - | - | - | - | - |
| Amylase Inhibitor ($Uml^{-1}$) | - | - | - | - | - | - | - |
| Lipase Activity ($Umin^{-1}g^{-1}$) x $10^{-5}$ | - | - | - | - | 3.571 ±0.05 | 3.12 ±0.017 | - |
| α-glucosidase Activity | - | - | - | - | - | - | - |
| α-glucosidase Inducer | + | + | + | + | + | + | + |
| α-glucosidase Inhibitor | - | - | - | - | - | - | - |
| Pectinase Acitvity ($Umin^{-1}g^{-1}$) x $10^{-3}$ | 3.6 ±0.021 | 2.9 ±0.041 | 4.8 ±0.23 | 2.6 ±0.001 | 4.8 ±0.048 | 2.8 ±0.067 | 4.8 ±0.083 |
| Proteinase Activity | - | - | - | - | - | - | - |
| Proteinase Inducer | - | - | - | - | - | - | |
| Proteinase Inhibitor | + | + | + | + | + | - | + |
| Protein Concentration ($\mu gg^{-1}$FW) | 830±9.32 | 820±1.92 | 727±5.12 | 910±5.19 | 910±9.91 | 870±2.17 | 812±2.92 |
| Catalase (CAT) (U g-1 FW) | 54.65±1.23 | 32.92±0.81 | 18.52±0.21 | 26.97±2.92 | 12.76±0.22 | 18.83±0.16 | 15.91±0.098 |
| Guiacol Peroxidase (POX) (∆ OD min-1gm-1FW) | 1.23±0.002 | 1.04±0.010 | 0.45±0.001 | 0.83±0.007 | 1.02±0.003 | 0.75±0.004 | 0.32±0.001 |
| H2O2 (µmole/ml) | 1.42±0.01 | 0.95±0.03 | 0.70±0.005 | 0.72±0.007 | 1.14±0.01 | 1.09±0.006 | 0.93±0.006 |
| Super Oxide Dismutase (SOD) (U min-1 gm-1 FW) | 74.95±1.32 | 55.96±2.93 | 66.64±2.35 | 34.71±3.82 | 84.97±4.01 | 60. 27±2.9 | 73.96±1.21 |
| MDA(n mol g-1DW) | 0.093±0.01 | 0.088±0.07 | 0.063±0.001 | 0.029±0.001 | 0.099±0.001 | 0.073±0.001 | 0.051±0.001 |
| Carotenoid (mg g-1 FW) | 0.69±0.02 | 4.85±0.21 | 6.43±0.12 | 5.03±0.11 | 1.32±0.01 | 3.20±0.003 | 5.91±0.15 |
| DPPH (%) | 32.42±0.003 | 55.83±0.19 | 72.32±0.31 | 57.93±0.13 | 41.92±0.21 | 50.73±0.11 | 70.58±0.37 |

Where, (+): presence of activity; (-): absence of activity

**Table 2. Pectinase and Lipase activity in *Cucurbita pepo* and *Lagenaria siceraria*.**

| Test sample | Pectinase activity (U/min/gm) | Lipase activity (U/min/gm) |
|---|---|---|
| Pumpkin Peel | 4.8 x $10^{-3}$ | - |
| Pumpkin Fiber | 2.6 x $10^{-3}$ | - |
| Pumpkin Pulp | 2.9 x $10^{-3}$ | - |
| Pumpkin Seed | 3.6 x $10^{-3}$ | - |
| Bottle gourd Peel | 4.8 x $10^{-3}$ | - |
| Bottle gourd Pulp | 2.8 x $10^{-3}$ | 3.12 x $10^{-5}$ |
| Bottle gourd Seed | 4.8 x $10^{-3}$ | 3.571 x $10^{-5}$ |

## Pectinase activity

Pectic substances are complex colloidal acid polysaccharides accounting 30% of the dry weight of plant tissue (Celestino *et.al,* 2005). Pectinase enzyme is involved in degradation of pectins which is the major component of cell wall of plant tissues (Satyanarayana *et.al*, 2002). High value of it in food implicit their importance in digestivity of vegetables. Pectinase produces methanol and free carboxylic group in the pectin acid either methanol or carboxylic group is measured as function of enzyme. Pectinase activity is found in all test samples. Maximum pectinase activity 4.8 x $10^{-3}$ $U^{-1}min^{-1}$gm was observed in bottle gourd peel, seed and pumpkin peels (Table 2).

The importance in cucumber brining of the softening problem caused by enzymatic degradation of cell wall pectin (Beaupre *et.al.* 2001). Pectinases are one of the most important groups of enzymes used in the fruit and vegetable industry, and have been employed in clarification of fruit juices and wines, coffee and tea processing, maceration of vegetable tissue and papermaking (Kashyap *et.al,*2001).

**Table 3. Proteinase inhibitors in *Cucurbita* sp. Where A, B and C is concentration of reaction mixture (Tris Buffer: Trypsin: Sample). A 30µl of sample implemented on each spots. Number of sign represents their intensity of inhibitor actions.**

| Test sample | A (14: 20:6) | B (0:20: 20) | C (14: 6: 20) |
|---|---|---|---|
| Control | - | - | - |
| *C. pepo* Peel | - | ++ | ++ |
| *C. pepo* Pulp | - | - | ++ |
| *C. pepo* Seed | ++++ | ++++ | ++++ |
| *C. pepo* Fiber | - | ++ | ++ |
| *L.siceraria* Peel | ++++ | ++++ | ++++ |
| *L.siceraria* Pulp | - | - | - |
| *L.siceraria* Seed | - | - | + |

**Table 4. Antioxidant Properties of the *Cucurbita pepo* and *Lagenaria siceraria*.**

| Sample / Assay | *Cucurbita pepo* | | | | *Lagenaria siceraria* | | |
|---|---|---|---|---|---|---|---|
| | Seed | Pulp | Peel | Fiber | Seed | Pulp | Peel |
| Catalase (CAT) (U g$^{-1}$ FW) | 54.65±1.23 | 32.92±0.81 | 18.52±0.21 | 26.97±2.92 | 12.76±0.22 | 18.83±0.16 | 15.91±0.098 |
| Guiacol Peroxidase (POX) (≃OD min$^{-1}$gm$^{-1}$FW) | 1.23±0.002 | 1.04±0.010 | 0.45±0.001 | 0.83±0.007 | 1.02±0.003 | 0.75±0.004 | 0.32±0.001 |
| $H_2O_2$ (µmole/ml) | 1.42±0.01 | 0.95±0.03 | 0.70±0.005 | 0.72±0.007 | 1.14±0.01 | 1.09±0.006 | 0.93±0.006 |
| SOD (U min$^{-1}$ gm$^{-1}$ FW) | 74.95±1.32 | 55.96±2.93 | 66.64±2.35 | 34.71±3.82 | 84.97±4.01 | 60. 27±2.9 | 73.96±1.21 |
| MDA(n mol g$^{-1}$DW) | 0.093±0.01 | 0.088±0.07 | 0.063±0.001 | 0.029±0.001 | 0.099±0.001 | 0.073±0.001 | 0.051±0.001 |
| Carotenoid (mg g$^{-1}$ FW) | 0.69±0.02 | 4.85±0.21 | 6.43±0.12 | 5.03±0.11 | 1.32±0.01 | 3.20±0.003 | 5.91±0.15 |
| DPPH (%) | 32.42±0.003 | 55.83±0.19 | 72.32±0.31 | 57.93±0.13 | 41.92±0.21 | 50.73±0.11 | 70.58±0.37 |

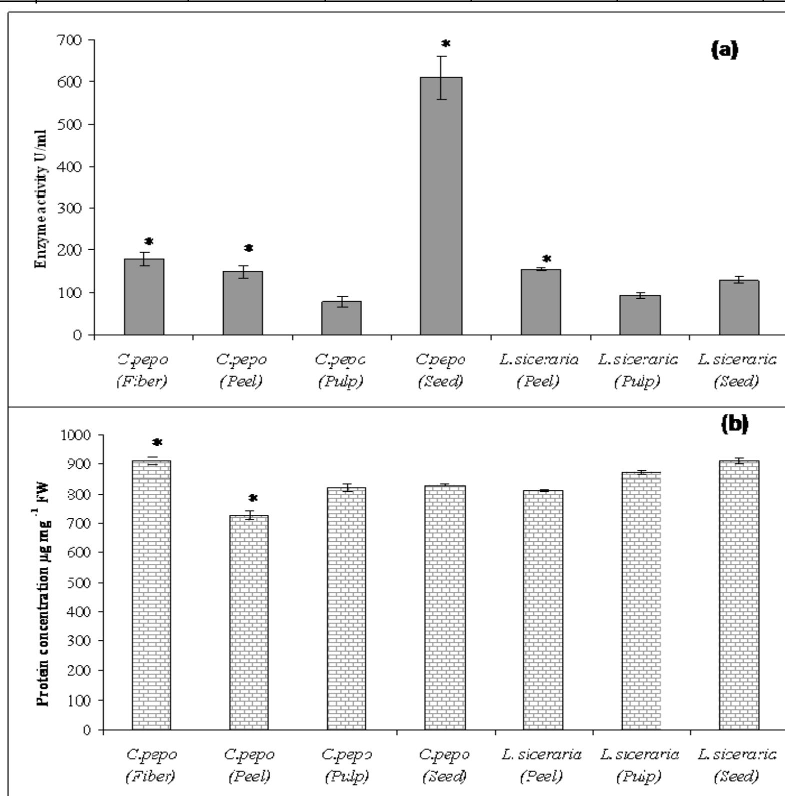


**Fig 1. (a) Amylase activity in different samples of *Cucurbita* sp. and (b) Protein concentration in different pumpkin and bottle gourd sample. Data representing with significance (*) at P≤0.05.**

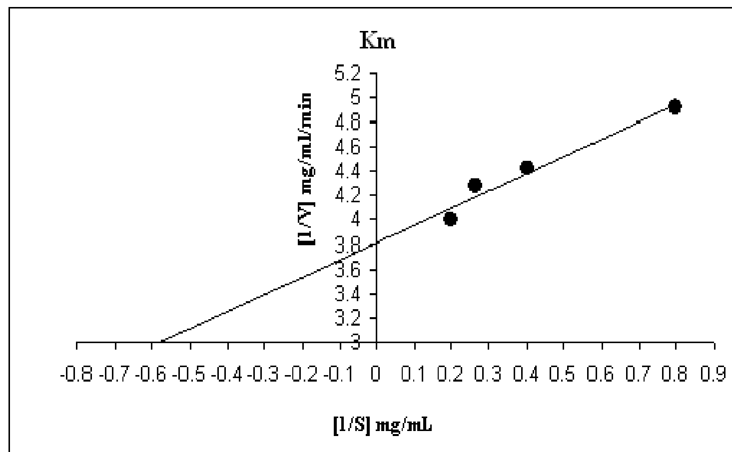


**Fig. 2. Line weaver and Burk's plot for $K_m$ and $V_{max}$ of amylase.**

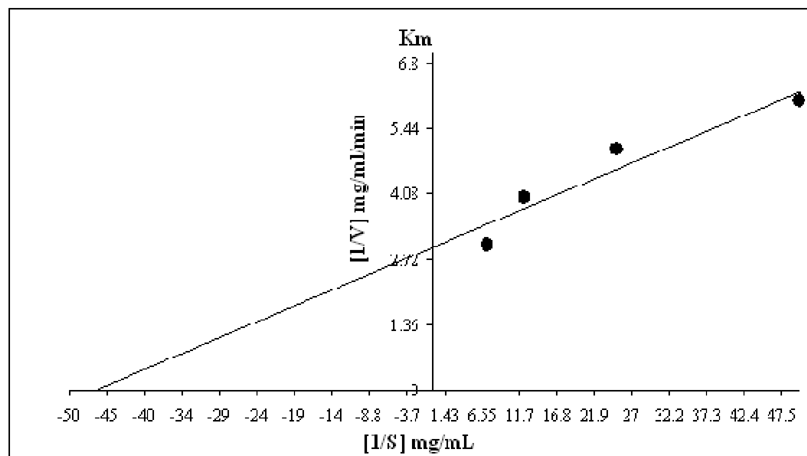


**Fig. 3. Line weaver and Burk's plot for $K_m$ and $V_{max}$ of lipase.**

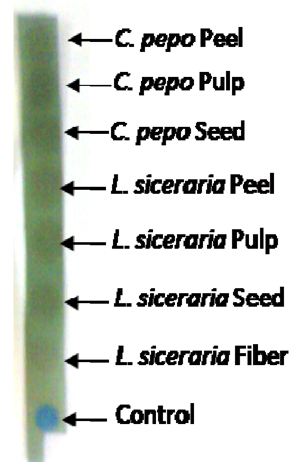


**Fig 4. Proteinase activity in *C. pepo* and *L. siceraria*.**

## Proteinase, Proteinase inhibitors and inducers

Proteinase activity is absent in all sample of pumpkin and bottle gourd. Strong proteinase inhibitors are present in pumpkin seed and bottle gourd peel (Table 3). Under varying concentration of sample and enzyme trypsin, the inhibitory activity is also observed in pumpkin peel, pulp, fiber and bottle gourd seed. While proteinase inducer activity is absent in all the sample of pumpkin and bottle gourd when hydrolyzed spots are compared with Trypsin as control (Fig. 4). Natural protease inhibitors include the family of lipocalin proteins, which play a role in cell regulation & differentiation (Jayalakshmi *et.al,* 2009).

Lipophilic ligands attached to lipocalin proteins, have been found to be possess tumor protease inhibiting properties. The natural protease inhibitors used in antiviral therapy. Some viruses depend on proteases in their reproductive cycle. Quantification of protein is carried out by Bradford method. Significantly higher concentration of protein ($P \leq 0.05$) i.e. 910 μg $mg^{-1}$ FW presents in Pumpkin fiber and Bottle Gourd seed (Fig. 1b).

There is a correlation between an increased content of trypsin inhibitors and chymotrypsin inhibitors and resistance to the pathogen (Valueva *et.al,* 2004, Dunaevsky *et.al,* 2005).. Protective plant proteins specifically induced in pathological or related situations have been intensively studied from an agricultural perspective, and are called "pathogenesis-related proteins" (PR proteins). Proteinase inhibitors in plants are able to suppress enzymatic activities of phytopathogenic microorganisms (Ferreira *et.al,* 2007). The protinase inhibitors present in pumpkin and bottle gourd are proteinaceous in nature. This will not going to effect on nutritive value of fruit as it naturally present in fruit material to protect themselves from microbial attack. Further these will digest and cleaned up during the process of digestion.

### α-glucosidase activity, and it's inducer and inhibitor

Glucosidase play physiologically important role which includes the digestive process for carbohydrates and processing steps for antigenic sugar chain expressed on cell surface **(**Philip, 2003). GOD-POD assay is used to determine the α-glucosidase activity, their inducers and inhibitors. All samples of pumpkin and bottle gourd exhibited α-glucosidase inducers activity. Enzyme α-glucosidase inducer has biological importance in carbohydrate digestion and post translational processing of glycoprotein glycans. Fruits of Pumpkin and Bottle guard might be used to increase blood sugar level and act as instant energy resource **(**Horwitz, 1989). The falling glucose levels clearly affect the nervous, hormonal and metabolic responses making importance of an adequate supply of glucose to the brain **(**Cornblath *et. al,* 2000).

### Antioxidant assays

The screening of antioxidant activity in pumpkin (*Cucurbita pepo*) and Bottle guard (*Lagenaria siceraria*) was studied and their results were summarized in Table 1. The cucurbita seed contains 54.65 U $g^{-1}$ FW catalase activity, while peel contains 18.52 U $g^{-1}$ FW. The catalase activity in *Lagineria* is lesser than *Cucurbita*. In *Lagenaria* maximum activity was observed in pulp. The presence of catalase enzyme can be monitored by its ability to convert hydrogen peroxide into oxygen and water. Low catalase activity in pulp could be because of their high water content in pulp and there may be a strategy to protect photosynthetic function from stress induced oxidative damage (Chaoui *et.al,*1997). Peroxidase activity (POX) was found maximum in seeds of both plants i.e in *Cucurbita* (1.23 ΔOD $min^{-1}gm^{-1}$FW) and in *Lagineria* (0.02 ΔOD $min^{-1}gm^{-1}$FW). The least activity was noticed in peel 0.70 ΔOD $min^{-1}gm^{-1}$FW in *Cucurbita* and 0.93 ΔOD $min^{-1}gm^{-1}$FW in *Lagineria*. The high value of peroxides is indicative of enhanced production of toxic oxygen species. The content of MDA observed high in the tissues of plants which have high peroxidase value. The value of MDA increase in the tissue that loses their cell permeability (Bhattacharya *et.al.,* 1995). The $H_2O_2$ is high in seeds as compared to other tissues of plant (Table 1). Excessive contents of $H_2O_2$ could be minimized through the activities of CAT and POX. Our results showed that activities of CAT and GPOX are least in the peel of fruit compared to seeds. The superoxide dismutase (SOD) activity is also high in seed and peel of both the plants. In Inter species comparison *Lagineria* has maximum SOD activity than *Cucurbit*. SOD is generally considered to be an antioxidant. Carotenoid content also shows a significant presence in both the plants and were maximum in peel, which are the content of antioxidative defence mechanism as reported in other plants (Saxena *et.al,* 2009). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Parr *et.al,* 2000). In addition, it was reported that phenolic compounds are associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. The *in vitro* antioxident activity i.e. radical scavenging activity is very high in peel as compare to seed of both the plants it was 72.32 % and 70.58 % in *Cucurbita* & *Lagineria* peel respectively. Antioxidants react with $DPPH^{\bullet}$, which is a stable free radical, and convert it to 1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the radical scavenging potential of the antioxidant (Singh *et.al,* 2002). The DPPH radical scavenging effect of *Cucurbita pepo* peel, fiber, pulp and seed decreased in the following order: 72.32%, 57.93%, 55.83% and 32.42 %, at the concentration of 30 μg $ml^{-1}$, respectively. In case of *Lagenaria siceraria* peel, pulp and seed it was 70.58 %, 50.73 % and 41.92 %, at the concentration of 30 μg $ml^{-1}$, respectively.

## CONCLUSIONS

The food containing digestive enzymes and inducer of the digestive enzymes may help to reduce indigestion problems. The development of safer natural antioxidants from plant materials can replace synthetic antioxidants is of interest. The undertaken fruits can be used as easily accessible sources of natural antioxidants and possible food supplements, or in pharmaceutical applications.

Thus scientifically verifying the traditional claim that pumpkin and bottle gourd are appears to be an attractive material for further study leading to possible development for digestive syrup. Work on these reported enzyme from pumpkin and bottle gourd for their greater kinetic stability and role of different inhibitors on its activity could be an objective of our future research.

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