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Research Paper :

Enzymatic extraction of lycopene from tomato skin

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ABSTRACT

Lycopene is a natural carotenoid pigment and a high value nutraceutical having wide use. The objective of the present work was to obtain a good yield of lycopene from tomato tissues pectinase enzyme derived from *Aspergillus niger*. Various parameters such as concentration of enzymes and by varying the degree of incubation temperature were optimized for incubation time of 20 min, to improve the yield of lycopene from blanched and unblanched tomato skin. Enzymatic extraction of lycopene from tomato skin under optimized conditions showed a remarkable increase in the yield of lycopene by 56.92 mg/100g and 42.32 mg/100g for unblanched and blanched tomato skin, respectively at 2.0% w/w of pectinase enzyme (*Aspergillus niger*) at 50^{0}C for 20 minutes incubation time.

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Key words : Lycopene, Blanched, Enzyme, Extraction, Tomato, Aspergillus niger

Lycopene, the pigment principally responsible for the characteristic deep-red colour of ripe tomato fruits and tomato products, has received much attention in recent years because of its beneficial effect in the treatment of diseases. Tomatoes and tomato products are considered as one of the best sources of lycopene. As determined by Gross (1987), the total lycopene content in tomatoes varies between 90 and 190 lg/g fresh weight (Baysal *et al.,* 2000).The occurrence of lycopene in different fractions of tomato fruit such as tomato skin, the water insoluble fraction, and the fibrous fraction including the fibre and soluble solids. Their results indicated that 72–92% lycopene was associated with the water-insoluble fraction of the skin. Tomato extracts and especially skin extracts contain high amounts of lycopene (Sharma and Le Maguer, 1996). The amount of lycopene in fresh tomato fruits depends on the variety, maturity, and environmental conditions under which the fruit matured. More than 80% of processed tomatoes are consumed in the form of tomato juice, paste, puree, ketchup, sauce, and salsa (Shi and Le Maguer, 2000).

METHODOLOGY

Tomatoes (Bangalore variety) were procured from local market and were maintained at 2 - 8^{0}C for 24 hrs. The whole tomatoes were divided into two batches. In the first batch the tomato skin was obtained manually from whole tomatoes. Whereas, in the second batch tomatoes were blanched at 88-90^{0}C for 2min to obtain skin. Pectinase was obtained from Fluka (Denmark), produced from a selected strain of *Aspergillus niger*. Sodium acetate and acetone (AR) were purchased from Universal laboratories private limited, Mumbai. Sodium sulphate was purchased from Merck private limited, Mumbai. Sodium Sulphate anhydrous and glacial acetic acid were purchased from Loba Chemie limited, Mumbai. Petroleum ether (AR) grade was obtained from S.D. Fine chemicals limited, Mumbai.

Sample preparation:

The whole tomatoes were washed and then thoroughly sorted and trimmed to remove any visible defects. A batch of 100g of each unblanched and blanched tomato skin excluding pulp, seeds and juice was taken and ground in the mixer for two min. A 60g of tomato skin paste of each unblanched and blanched was placed in the flask containing 100 ml of 0.2M acetate buffer (pH 5.0). One gram sample of each unblanched and blanched was distributed in the beaker and were covered with aluminium foil. The samples were stored in the refrigerator (2 - 6^{0}C) and these samples were used within 24 hours.

Enzyme aided solvent extraction of lycopene:

One gram of well homogenised samples was taken. To it 20 ml of 0.2 M acetate buffer of pH 5.0 was added and blended for 5 min using a pestle and mortar. Calculated amounts of pectinase (*Aspergillus niger*) enzyme was prepared at different concentrations of 1.5%, 2.0% and 2.5% w/w, respectively and were dissolved in 0.2M acetate buffer of appropriate pH and added to achieve the final desired concentration. This mixture was again blended for two minutes in pestle and mortar. These samples were incubated at varying temperatures at 40°C, 50°C and 60°C for 20 min. The mixture was then transferred to centrifuge tubes and centrifuged (JH Bio Model No. MPW-350R) at 2500 rpm for 10 minutes to obtain supernatant and residue. The residue obtained was subjected to solvent extraction using 20 ml acetone and re-extracted until a colourless residue was obtained using 20 ml acetone each time.

The extraction was carried out in a separating funnel using 5 ml (5% sodium sulphate) solution and petroleum ether, the flask was shaked well and allowed it to stand for 10-15 min each time. Upper phase was non polar in nature, comprising mostly lycopene and other lipophilic carotenoids. The lower aqueous phase was discarded. The petroleum ether extracts of lycopene from the residue were pooled together and passed through a desiccant, anhydrous sodium sulphate (1g). Finally the volume was made up to 100 ml with petroleum ether. A control sample (without enzyme treated) was extracted for each enzyme aided extraction process, to account for any natural variation in lycopene content. The non-polar layer, containing lycopene was obtained and the absorbance was measured using spectrophotometer at 503 nm (λ_{max}). The experiment was replicated thrice.

Optimization of extraction of lycopene from tomato skin:

The extraction of the lycopene pigment from tomato skin was carried out, at the different concentrations of enzymes at 1.5%, 2.0% and 2.5% w/w (*Aspergillus niger*) by varying the temperatures (40^{0}C, 50^{0}C and 60°C) with 20 minutes incubation time. The control sample was also extracted under similar conditions.

Lycopene estimation:

The standard method of estimation of lycopene was spectrophotometric. The absorbance of lycopene extracted in petroleum ether was noted at 503 nm. The amount of lycopene was calculated by using following formula:

$$\text{Lycopene content (mg/100g of sample)} = \frac{31.206 \times \text{Absorbance reading}}{\text{Sample weight (g)}}$$

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussion have been summarized under following heads:

Extraction optimization:

The effect of using different concentration of pectinase enzyme (*Aspergillus niger*) on the maximum recovery of lycopene from tomato skin unblanched, blanched and control at 40^{o}C incubation temperature for 20 min is shown in Fig.1. Pectinase (*Aspergillus niger strain*) showed highest lycopene recovery when used at 2.0% w/w of tomato skin, as seen in Fig. 1. Results of the enzyme aided extraction revealed that an increase in the yield of lycopene by 51.27 mg/100g and 30.08 mg/100g in case of unblanched and blanched tomato skin for incubation time of 20 minutes, when compared to the control unblanched and blanched (26.87 mg/100g and 21.34 mg/100g), respectively. This might be due to the fact that lycopene in higher concentration in tomato skin.

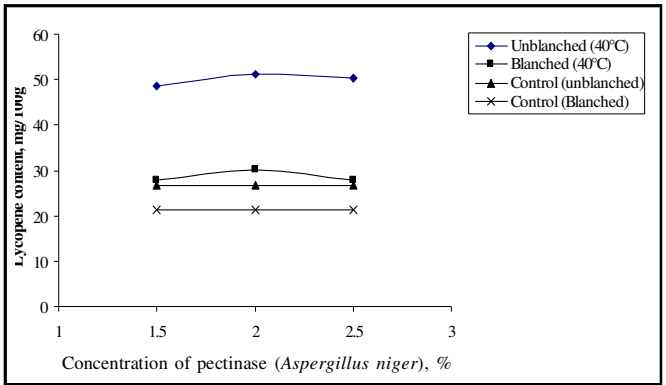

Fig. 1 : Effect of concentration of pectinage *Aspergillus niger* on lycopene extraction from tomato skin at 40^{0}C for incubation time of 20 min

A concentration of 2% w/w pectinase (*Aspergillus niger*) enzyme proved to be effective in extracting lycopene from tomato skin at 50^{o}C for incubation time of 20 min as shown in Fig. 2. An increase in the yield of lycopene extract was observed to be 56.92 mg/100g and 42.32 mg/100g for unblanched and blanched tomato skin, when compared to control 26.87 mg/100g for unblanched and for the blanched the value was found to be 21.34 mg/100g, respectively. The decrease in yield of lycopene extract in case of blanched may be due loss of lycopene pigment while blanching tomatoes when compared to the

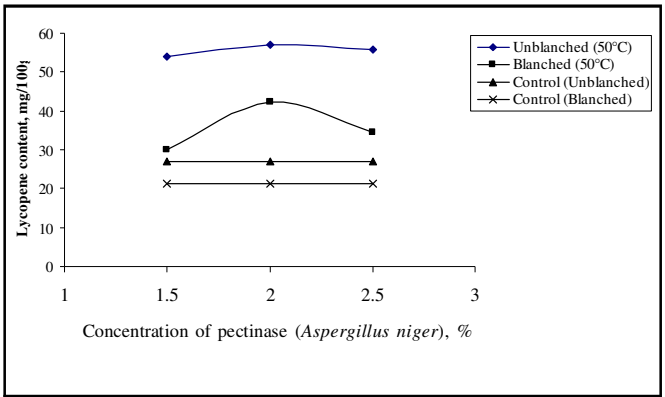

Fig. 2 : Effect of concentration of pectinase *Aspergillus niger* on lycopene extraction from tomato skin at 50^{0}C for incubation time of 20min

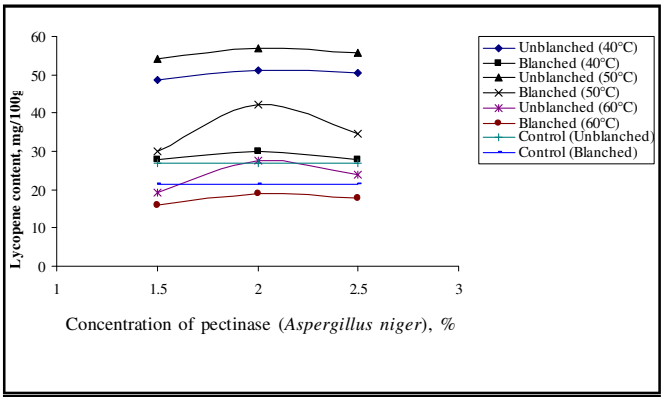

Fig. 4 : Summary of the use of pectinase enzyme derived from *Aspergillus niger* at different incubation temperature for 20 min

yield of lycopene from unblanched tomato skin. An increase in yield of lycopene extract may be due to concentration of carotenoid on the outer pericarp and also the locular contents have the highest carotene content. It has been reported that lycopene represents a substantial proportion of the total carotenoid content of tomato products. It is estimated as much as 60-64% of the total carotenoid content consists of lycopene.

A decrease in the concentration of the enzyme treated unblanched and blanched tomato skin at 60^{0}C for incubation time of 20 min is shown in Fig. 3. There was a remarkable decline noticed in the extraction of lycopene from tomato skin at 60^{0}C when compared to other temperatures at 40^{0}C and 50^{0}C for incubation time of 20min. A decrease in the concentration of pectinase (*Aspergillus niger*) enzyme treated at 2% w/w of lycopene extract for unblanched was observed to be 27.62 mg/100g.Whereas, in case of blanched, it was found to be 19.07 mg/100g and the values obtained were blanched less than that of the control (21.34 mg/100g). This might be due to inactive enzyme at higher temperature.

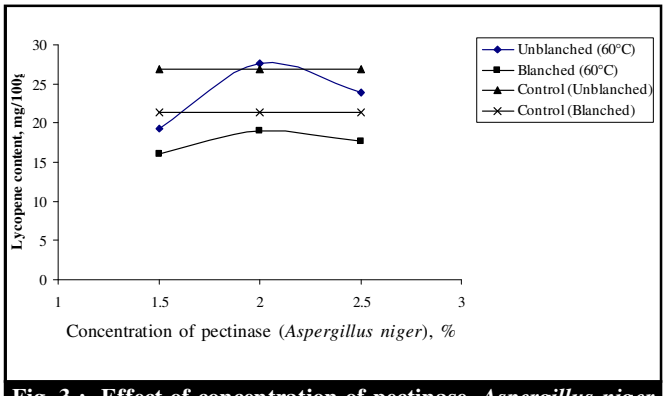

Fig. 3 : Effect of concentration of pectinase *Aspergillus niger* on lycopene extraction from tomato skin at 60^{0}C for incubation time of 20 min

Confirmative studies:

A summary of lycopene extractions using pectinase enzyme of form *Aspergillus niger* is shown in Fig. 4. Pectinase proved to be more effective at 2% w/w at 50°C for incubation time of 20 min, for an increase in the yield of lycopene by 56.92 mg/100g for unblanched and 42.32 mg/100g for blanched in comparison with 40^{0}C and 60^{0}C temperature at different concentrations of the enzyme. The enzyme was inactivated at 60°C and thus, there was a reduction in the yield of lycopene from tomato skin. The variation incubation of temperature revealed that recovery of lycopene was maximum at 50^{0}C for incubation period of 20 min using enzyme pectinase.

A summary of enzymatic extraction of lycopene using pectinase (*Aspergillus niger*) from tomato skin/ waste. Pectinase was more effective with an increase in lycopene yield of 108 µg/g (224%). Tomato peel was found to show the higher increase in lycopene yield using pectinase enzyme.

The solvent extraction of the lycopene pigment from tomato skin was carried out, at the different concentration of enzyme at 1.5%, 2.0% and 2.5% w/w (*Aspergillus niger*) pectinase, by varying the incubation temperature at 40^{0}C, 50^{0}C and 60^{0}C for 20 minutes incubation time. The control samples were also extracted under similiar conditions.

The maximum yield of lycopene was obtained at 50^{0}C for incubation period of 20 min. Therefore, the valuable quantity of lycopene pigment in tomatoes, which is lost in the tomato waste in processing can be, recovered by solvent extraction method using pectinase derived from *Aspergillus niger*.

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