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Enzyme aided extraction of lycopene from tomato tissues

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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, using cellulase and pectinase enzymes. Various parameters such as concentration of enzymes and time of incubation were optimised, to improve the yield of lycopene from tomatoes. Enzyme aided extraction of lycopene from whole tomatoes under optimised conditions resulted in an increase in the lycopene yield by 132 μg/g (198%) in cellulase treated sample and 108 μg/g (224%) in case of pectinase treated sample. Extraction from tomato peel under optimised conditions showed a remarkable increase in the yield of lycopene by 429 μg/g (107%) and 1104 μg/g (206%), for cellulase and pectinase treated samples, respectively. Likewise, the enzyme aided extraction of lycopene from fruit pulper waste and industrial waste of tomatoes was done to determine the potential for recovering the natural pigment from tomato waste.

Keywords: Lycopene; Cellulase; Pectinase; Extraction; Tomato

1. Introduction

Lycopene, a carotenoid present in high concentration in tomatoes and tomato products, has attracted considerable attention recently, as epidemiological evidence continues to suggest that it may provide protection against cancer and other degenerative diseases, influenced by free radical reactions (Levy, Bosin, & Feldman, 1995). Recent epidemiological studies revealed that the intake of tomatoes and blood lycopene level are inversely associated with the risk of developing cancers at several anatomical sites, including the prostate gland, stomach, and lung. (Stahl & Sies, 1996).

Conjugated carbon–carbon double bonds provide lycopene with its antioxidant properties, by successful delocalization of captured free radical species. Lycopene is in high demand not only by pharmaceutical companies but also for the food, feed, and cosmetic industries.

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 μg/g fresh weight (Baysal, Ersus, & Starmans, 2000). Lycopene is found predominantly in the chromoplast of plant tissues. In tomatoes, lycopene biosynthesis increases dramatically during the ripening process, as chloroplast undergoes transformation to chromoplast. Globulous chromoplast containing mainly β-carotene is found in the jelly part of the pericarp while chromoplast in the outer part of the pericarp contains voluminous sheets of lycopene.

Sharma and Le Maguer (1996) reported 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 and the skin. Tomato extracts and especially skin extracts contain high amounts of lycopene (Sharma & Le Maguer, 1996).

The waste during tomato processing is obtained in the form of seeds and skin residues, which could provide a useful source of lycopene (Sadler, Davies, & Dezman, 1990).

The extraction, handling and analysis of lycopene must be carried out under controlled environmental conditions

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to minimize oxidative degradation and isomer formation. Exposure of lycopene to light should be avoided; and only gold, yellow or red light should be used. Antioxidants such as butylated hydroxytoluene (BHT) should be employed in solvents used for the extraction and separation of lycopene, to control oxidation and isomerization reactions. In addition, nitrogen or argon headspace can be employed to keep exposure to atmospheric oxygen to minimum. Saponification with methanolic potassium hydroxide can be performed to enhance the analysis of lycopene by eliminating chlorophyll and lipid materials, which can interfere with its chromatographic elution and detection. The quantitative estimation of lycopene can be best done by spectrophotometric method (Davis, 1949).

Enzymatic cell wall lysis employing hydrolytic enzymes, that can degrade the cell wall constituents, thus assisting in the release of intracellular contents is a widely reported method for the extraction of various kinds of substances. Enzymes have been employed for the extraction of capsaicinoids and carotenoids from chilli (*Capsicum annuum* L.) using ethanol as solvent (Santamaria et al., 2000). Since the plant cell wall comprises of cellulose and pectins, cellulases and pectinases have been used for this purpose. In the same context, enzymatic pretreatment has also been proposed to increase the extraction of oil from fruits and seeds (Dominguez, Navez, & Lama, 1994).

In this study, for the extraction of lycopene from whole tomatoes, using hydrolytic enzymes such as cellulases and pectinases, process conditions were optimised, with respect to concentration of the enzymes, and incubation time, to increase the yield of lycopene. Furthermore, the concentration of cellulases and pectinases was optimised for lycopene extraction from tomato peel and from the waste derived during tomato processing.

2. Materials and methods

Tomatoes were purchased from the local market and maintained at 2–8 °C for not more than 48 hours. Tomato peel was obtained manually from whole tomatoes. Tomato wastes, comprising of seeds and peel residues, were obtained using a laboratory pulper. Tomato industrial waste was obtained from Mangalam Foods, Nashik, India, and stored at 2–8 °C.

Cellulase and pectinase were obtained from Novozyme, (Bangalore, India), cellulase as Celluclast-1.5 L, produced from a fungal source *Trichoderma reesei* and pectinase as Pectinex Ultra SP-L, produced from a selected strain of *Aspergillus aculeatus*.

Sodium acetate analytical grade was obtained from Himedia limited, Mumbai, India. Sodium sulphate was purchased from Loba Chemie limited, Mumbai.

Iodine, glacial acetic acid and hexane (AR grade) were purchased from S.D. Fine Chemicals Ltd., India.

Petroleum ether, acetone, chloroform and benzene were purchased from Merck Ltd., India.

2.1. Sample preparation

A batch of 60 g of whole tomatoes was homogenized for 5 min, using a domestic blender in 100 ml 0.2 M acetate buffer (pH 4.5 and 5.0 for cellulase and pectinase, respectively) and were distributed equally (3 g each) into tightly-closed glass containers, covered with aluminum foil. Samples were stored at 2–8 °C and were used within 24 hours. Tomato peel samples were prepared in the same way.

Tomato waste was obtained using a fruit pulper (screw type). The whole tomatoes were first washed and then thoroughly sorted and trimmed to remove any visible defects. The tomatoes were passed directly through a fruit pulper to obtain waste, in the form of seeds and peel residues. Tomato industrial waste was obtained, in the form of peel with some tomato tissues and seeds, and was stored in the refrigerator at 2–8 °C, until use. The samples were extracted and lycopene was estimated within 24 hours.

2.2. Enzyme aided extraction of lycopene

Extraction of lycopene from whole tomatoes, tomato peel, laboratory pulper waste and lastly from tomato industrial waste was carried out as detailed below.

Three grams of a well-homogenized sample was taken. To it 20 ml 0.2 M acetate buffer of pH 4.5 (for cellulase) and of pH 5.0 (for pectinase) was added and blended for 3 min using a hand blender. Calculated amounts of cellulase and pectinase enzyme preparations of activities 45 U/ml and 89,574 U/ml, respectively, were dissolved in 0.2 M acetate buffer of appropriate pH and added to achieve the final desired concentration. This mixture was again blended for two minutes. These samples were incubated at 55 °C for 15 min, in the case of the cellulase treated sample, and 60 °C for 20 min, for the pectinase treated sample, and filtered. Filtrate and the residue so obtained were subjected to solvent extraction using petroleum ether and acetone (1:1 v/v). Filtrate and the residue were separated to achieve efficient and complete extraction. Filtrate was extracted using 20 ml petroleum ether and acetone. Residue was extracted first with 30 ml petroleum ether and acetone and re-extracted twice using 10 ml petroleum ether and acetone each time. The extraction was carried out in a separating funnel for 15–20 min each time and allowed to stand for 10 min. 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 filtrate and residue were pooled together and passed through a desiccant, anhydrous sodium sulphate (1 g). Finally, the volume was made up to 40 ml with petroleum ether. A control sample (not enzyme treated) was extracted for each enzyme aided extraction process, to account for any natural variation in lycopene content.

2.3. Lycopene estimation

The standard method of estimation of lycopene is spectrophotometric (Davis, 1949). The absorbance of lycopene extracted in petroleum ether (40 ml) was noted at three different wavelengths, *viz*. 445, 472, 502. The absorbance at maximum wavelength (λ max) of 472 nm for lycopene in petroleum ether was considered. The amount of lycopene (mg) was calculated by a specific extinction coefficient ($E^{1\%}_{1\,\mathrm{cm}} = 3450$ in petroleum ether).

$$\text{Lycopene (mg)} = \frac{A \times \text{dil} \times \text{ml} \times 10}{E^{1\%}_{1\,\mathrm{cm}}}$$

where A, absorbance of the solution in 1 cm cuvette, dil, dilution factor, ml, total ml of the sample $E^{1\%}_{1\,\mathrm{cm}}$, specific extinction coefficient for lycopene in petroleum ether is 3450.

2.4. Optimisation of extraction of lycopene from whole tomatoes

The extraction procedure was used to determine the optimum concentration of cellulase and pectinase enzyme for extraction of lycopene pigment from whole tomatoes. The concentrations of the cellulase and pectinase enzyme screened were from 4% to 9% w/w and 0.25% to 4% w/w of whole tomatoes, respectively.

The extraction of lycopene from whole tomatoes was carried out at optimised concentrations of cellulase and pectinase, i.e., 6% and 0.5% w/w of whole tomatoes, respectively. Incubation times of 5 to 180 min (for cellulase) and 10 to 120 min (for pectinase) were varied, in order to have optimum enzyme action in the cell.

2.5. Optimisation of extraction of lycopene from tomato peel and tomato wastes

The extraction of the lycopene pigment from tomato peel was carried out, varying the concentration of cellulase from 1% to 5% w/w and pectinase from 0.5% to 4% w/w of tomato peel. The control sample was also extracted under similar conditions.

The enzyme-aided extraction of lycopene from fruit pulper waste and industrial wastes was carried out using the optimised parameters, i.e., 3% w/w cellulase at pH 4.5 and 55 °C for 15 min, and 2% w/w pectinase at pH 5.0 and 60 °C for 20 min.

3. Results and discussion

The effect of using different concentrations of enzymes on the maximum extraction of lycopene from whole tomatoes is shown in Figs. 1 and 2. Cellulase, a hydrolytic enzyme, showed highest lycopene extraction when used at 6% w/w of whole tomatoes, as seen in Fig. 1. Results of the enzyme-aided extraction showed an increase in lycopene yield by 96.3 μg/g (144%), in the case of cellulase treated sample. Cellulase acts on cellulose, which is present in the primary wall beneath the first layer of middle lamella of the plant cell wall. Primary wall consists of a rigid skeleton of cellulose embedded in a gel-like matrix composed of pectic compounds, hemi-cellulose and glycoprotein. The Cellulase enzyme catalyzes the breakdown of cellulose into glucose, cellobiose and higher glucose polymers.

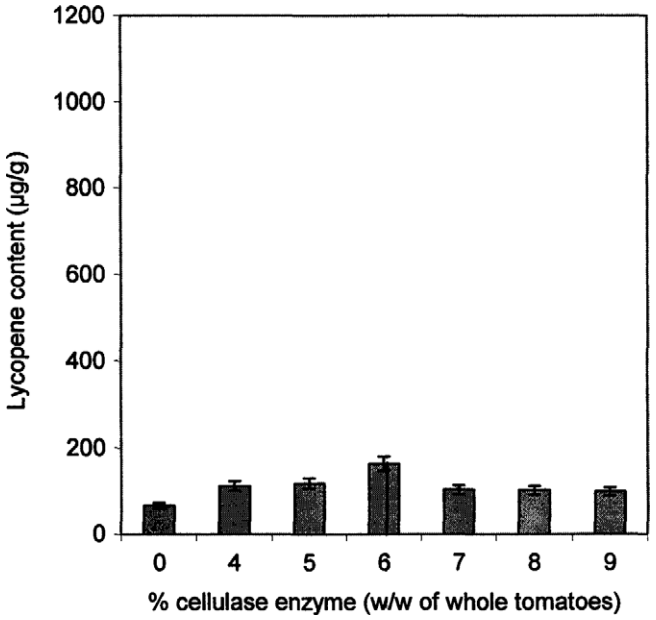

Fig. 1. Effect of concentration of cellulase on lycopene extraction from whole tomatoes.

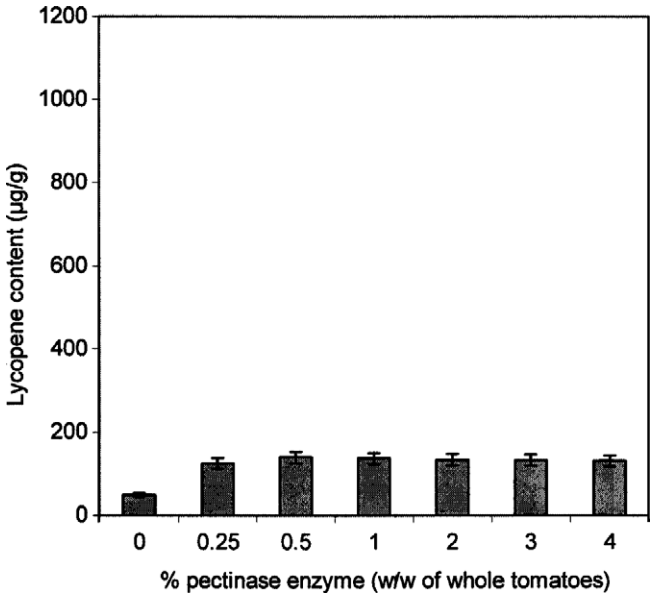

Fig. 2. Effect of concentration of pectinase on lycopene extraction from whole tomatoes.

Pectinase at 0.5% w/w proved to be very effective in extracting a high amount of lycopene as shown in Fig. 2. An increase in the yield of lycopene by 90.6 μg/g (188%) is observed in the sample treated with pectinase. Pectinase being pectolytic and hemicellulolytic has the ability to dis-

integrate pectic compounds and pectin, the latter a polymer of 100-200-galacturonic acids, found in the middle lamella and primary walls.

An incubation time of 15 min was found to be optimum for cellulase to degrade the cell wall (Fig. 3). A decrease in the lycopene content with increasing incubation time may be due to oxidation of lycopene. An incubation time of 20 min was optimum for pectinase (Fig. 4).

A concentration of 3% w/w cellulase enzyme proved to be effective in extracting lycopene from tomato peel (Fig. 5). An increase in the extraction yield of lycopene by 424 μg/g (128%) was observed for the cellulase treated sample. Similarly, 2% w/w pectinase enzyme showed increased extraction of lycopene of 712 μg/g from tomato peel, as shown in Fig. 6. The results obtained for enzyme-aided extraction of lycopene from tomato peel differ from those obtained using whole tomatoes, on account of the differences in the chemical composition of the peel and the whole fruit, as well as due to the fact that lycopene is reported to occur in higher concentrations in tomato

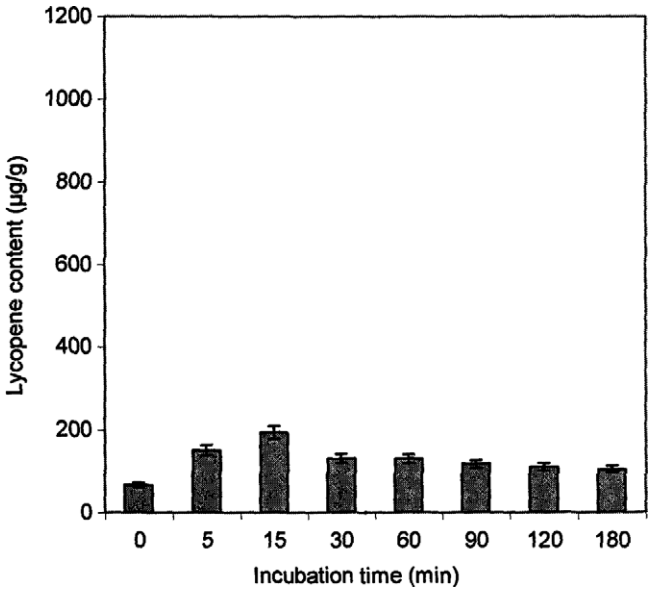

Fig. 3. Effect of incubation time on lycopene extraction from whole tomatoes, using cellulase.

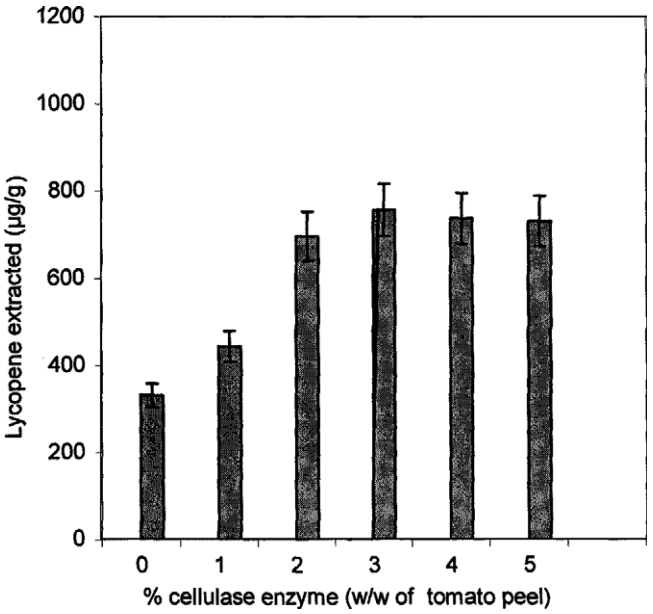

Fig. 5. Effect of concentration of cellulase enzyme used on the extraction of lycopene from tomato peel.

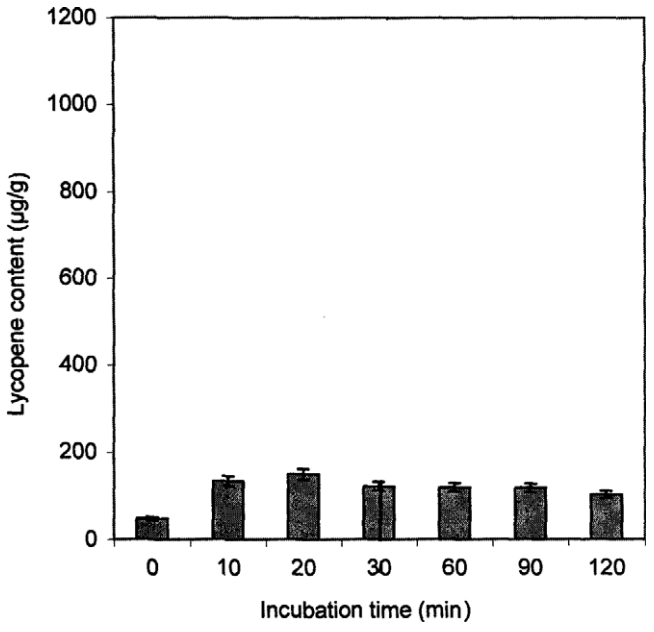

Fig. 4. Effect of incubation time on lycopene extraction from whole tomatoes, using pectinase.

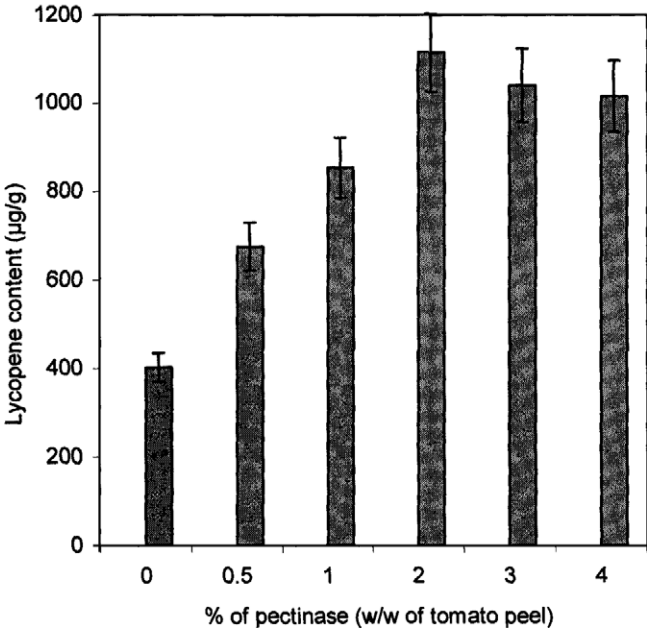

Fig. 6. Effect of concentration of pectinase enzyme used on the extraction of lycopene from tomato peel.

peel. The outer pericarp of tomatoes has the highest total carotenoid concentration, and 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 summary of the lycopene extractions using cellulases and pectinases from various tomato fractions and wastes (Figs. 7a and 7b, respectively) shows that both cellulase and pectinase were effective in increasing the lycopene yield. For whole tomatoes, pectinase was more effective than cellulase, with an increase in lycopene yield of 108 μg/g (224%). For tomato peel used as a lycopene source, pectinase was also found to be more effective than cellulase, with an increase in lycopene yield of 1104 μg/g (206%). Fruit pulper wastes showed increase in extraction yields of lycopene of 119 μg/g (23%) for cellulase and 190 μg/g (52%) for pectinase treated samples. Again pectinase proved to be more effective than cellulase for lycopene extraction from fruit pulper wastes. However, there is increase in yield of lycopene by 202 μg/g (61%) and 156 μg/g (45%) for cellulase and pectinase treated samples of industrial wastes, respectively. Hence, cellulase enzyme is more effective than pectinase for lycopene extraction from industrial wastes. Of all the tomato fractions and wastes studied as a source of lycopene, tomato peel was found to show the highest increase in lycopene yield using pectinase enzyme. Both the enzymes employed in the present study were found to enhance the recovery of lycopene from tomato wastes. In conclusion, the valuable quantities of lycopene pigment in tomatoes, which is lost as waste in processing, can be recovered in high yields by extraction using cellulases and pectinases.

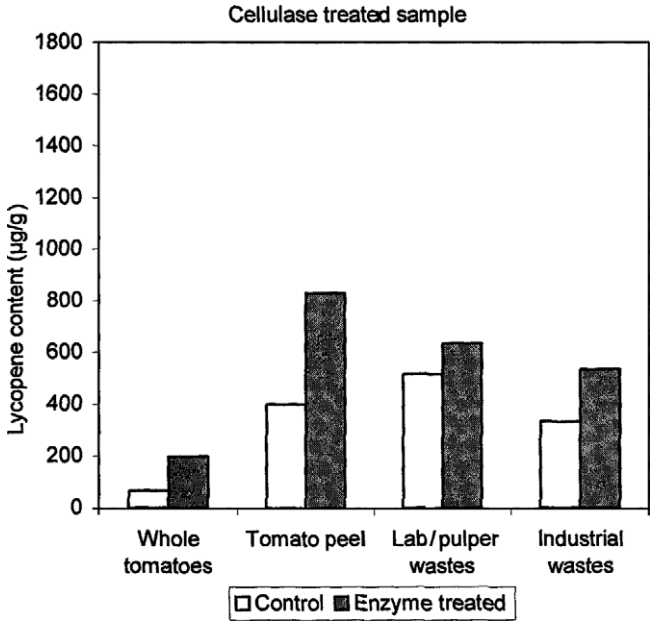

Fig. 7a. Summary of the use of cellulase for the extraction of lycopene from various raw materials under optimal conditions.

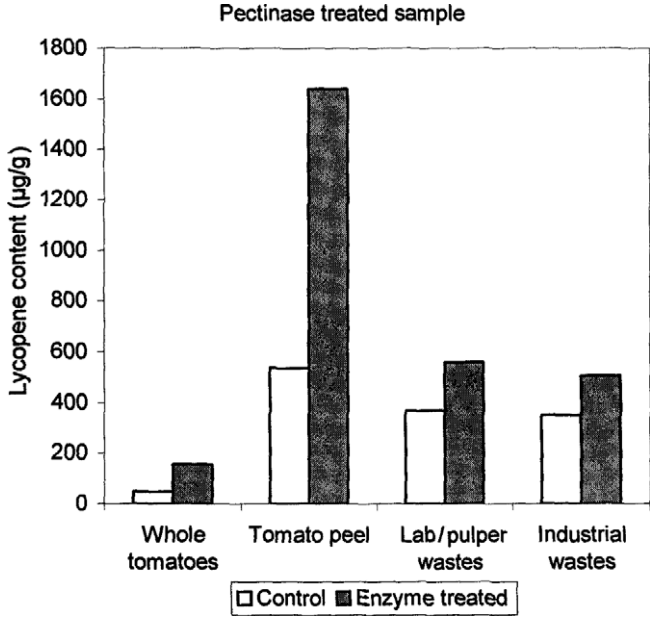

Fig. 7b. Summary of the use of pectinase for the extraction of lycopene from various raw materials under optimal conditions.

Acknowledgement

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