International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 2123-2125

Received: 25-01-2018
Accepted: 28-02-2018

Ramesha GS
Assistant Horticulture Officer
Department of Horticulture-
Hassan, Karnataka, India

Vasudeva KR
Associate Professor and Head
Department of Postharvest
Technology, College of
Horticulture, UHS Campus,
Bengaluru, Karnataka, India

Krishna HC
Assistant Professor
Department of Postharvest
Technology, College of
Horticulture, UHS Campus,
Bengaluru, Karnataka, India

Amarananjundeswara H
Assistant Professor, Department
of Vegetable Science
Horticulture Research and
Extension Center, Hassan,
Karnataka, India

Correspondence
Vasudeva KR
Associate Professor and Head
Department of Postharvest
Technology, College of
Horticulture, UHS Campus,
Bengaluru, Karnataka, India

Effect of extraction methods on lycopene recovery from culled tomato (*Solanum lycopersicum* L.)

Ramesha GS, Vasudeva KR, Krishna HC and Amarananjundeswara H

Abstract
Tomatoes are prone to rapid quality losses after harvest due to rough handling, poor transportation conditions, poor sanitation, and warm storage temperatures. Utilization of such culled tomato for extraction of lycopene was investigated. Culled tomatoes were disinfected by dipping in hot water (45º C) for five minutes and lycopene is extracted with different methods using cellulase and pectinase at 2 per cent w/w with incubation period of 60 and 120 minutes at 45°C. Later lycopene was extracted with solvent extraction method using solvents like petroleum ether - acetone, ethanol. Among the different treatments, the highest lycopene content (44.46 mg $100g^{-1}$) was obtained in cellulase enzyme (2 per cent) treated for 60 minutes prior to solvent extraction using petroleum ether. When this duration was increased to 120 minutes the lycopene content decreased to 37.50 mg $100g^{-1}$, followed by pectinase enzyme at concentration 2 per cent with incubation time of 120 minutes.

Keywords: Tomato, lycopene, antioxidant, cellulase, pectinase, solvent extraction

Introduction
Tomatoes are prone to rapid quality losses after harvest due to the stage of ripeness at which they are harvested. The effect of the ripening stage on post-harvest tomato quality can be further compounded by rough handling, poor transportation conditions, poor sanitation, and warm storage temperatures. All of these factors provide opportunities for losses at different points along the supply chain. Hence, there is a need to utilize these waste tomatoes for the extraction of lycopene pigment and can be further utilized in drug preparations and fortification. Also during the processing of tomatoes into various products 10 to 30 per cent of their weight becomes waste or pomace which mainly constitutes its peel and seeds (King and Zeidler, 2004) [4]. The by-products of tomato processing industries (peels and seeds) pose a problem in its disposal. Many studies carried out to assess the potential of utilization of several vegetable origin by-products for their inclusion in human diet had shown promising results in reducing the industrial costs and controlling the pollution problem connected with food processing (Lario *et al.*, 2004) [5]. These new ingredients could be of great interest for food and pharmaceutical industries and will helps in waste utilization. Recycling of by-products or processing wastes are of great importance from environmental point of view as well as the health benefits derived from the extracted bio-active compounds. These products may be used as such or after further value addition. Solid and liquid waste recycling is emerging as one of the important areas in research for achieving efficiency in utilization of all the raw material or inputs so as to reduce the cost of production.

Lycopene being a fat soluble pigment, extraction is a challenge limiting its commercial exploitation. Sharma and Maguer (1996) [8] reported that 72 to 92 per cent lycopene in tomato was associated with the water insoluble fraction of the skin; hence several methods have been developed for extraction of lycopene from peel. As the lycopene is embedded in the insoluble fibre matrix, enzymes like cellulase and pectinase have been often employed, as cellulose and pectin form the chief components of many fruits and vegetables including tomato. Among the various methods, solvent extraction using ethanol, super critical fluid extraction (SCFE) with different solvents (Beatriz *et al.*, 2012) [1] and extraction by high pressure process (Naviglio *et al.*, 2008) [6] are some of the important methods for lycopene extraction. Each method has its own advantages and disadvantages. The present study was undertaken to investigate the effect of different extraction methods for extraction of lycopene from culled tomato.

Material and Methods

Sample Preparation

The culled tomatoes *i.e.*, discoloured, infected tomatoes were collected from APMC (*Agricultural Produce Market Committee*) premises, Kolar. Selected fruits from the lot and used for lycopene extraction after disinfection process and all the chemicals used in this investigation were of analytical grade.

Lycopene Extraction Methods

Acetone and petroleum ether method

Five to ten gram of wet peel was homogenized repeatedly using vertex homogenizer with acetone as solvent until the peel became colorless. The acetone extract was then pooled and transferred into a separating funnel containing 20 ml of petroleum ether and then gently shook to mix the contents in it. About 100 ml of 20 per cent sodium sulphate solution was added to the separating funnel, shook gently and allowed to stand still for 10 minutes. Two phases were formed with the colorless lower phase and colored upper phase. The colored upper phase containing lycopene was stored in amber colored bottle and lower phase was again used to re-extract lycopene with additional 20 ml petroleum ether until aqueous solution became colorless. The petroleum ether extract was pooled in 100 ml volumetric flask and the volume was made up using petroleum ether and the absorbance was measured in a spectrophotometer (Model SP 3000 PLUS) at 503 nm using petroleum ether as blank.

Ethanol Method

Five to ten gram of wet peel was homogenized repeatedly using vertex homogenizer with ethanol as solvent until the peel became colorless. The ethanol extract was then pooled and transferred into 100 ml volumetric flask and the volume was made up using ethanol and the absorbance was measured in a spectrophotometer (Model SP 3000 PLUS) at 503 nm using ethanol as blank.

Pectinase Enzyme Method

Five to ten gram of wet peel was homogenized repeatedly with water using vertex homogenizer and the volume was made up to 50 ml. Pectinase enzyme (2%) was taken and mixed well in the homogenized sample and incubated for different time periods (60 and 120 minutes). After completion of appropriate time periods, the samples were cooled and homogenized again. The homogenized extract sample and was used for lycopene extraction by using standard solvent extraction method as explained above.

Cellulase Enzyme Method

Five to ten gram of wet peel was homogenized repeatedly with water using vertex homogenizer and the volume was made up to 50 ml. Cellulase enzymes (2%) was taken and mixed well in the homogenized sample and was incubated for different time periods (60 and 120 minutes). After completion of appropriate time periods, the samples were cooled and homogenized again. The homogenized extract sample was used for lycopene extraction by using standard solvent extraction method as explained above.

Results and Discussion

Lycopene content and antioxidant activity

The details on lycopene content and antioxidant activity of culled tomato by different extraction methods were presented in table 1.

The results indicate that method of lycopene extraction significantly affected its content. Among the organic solvents, petroleum ether-acetone gave better extraction than ethanol and pectinase enzyme pre-treatment increased lycopene content in the range of 32.60 to 44.46 mg $100g^{-1}$.

Table 1: Efficacy of different extraction methods on lycopene recovery and antioxidant activity from culled tomato

Treatment	Lycopene recovery (mg $100g^{-1}$)	Antioxidant activity (mg AAE $100g^{-1}$)
T_1- Petroleum ether and Acetone	19.72	12.46
T_2- Ethanol	16.10	10.80
T_3- Pectinase 2.0 per cent for 60 minutes incubation at 45°C	32.60	15.13
T_4- Pectinase 2.0 per cent for 120 minutes incubation at 45°C	40.33	18.03
T_5- Cellulase 2.0 per cent for 60 minutes incubation at 45°C	44.46	20.76
T_6- Cellulase 2.0 per cent for 120 minutes incubation at 45°C	37.50	17.10
T_7- Petroleum ether and acetone method (fresh tomato - control)	21.40	13.03
SEm±	0.46	0.26
CD @ 1 %	1.95	1.10

Among the different treatments, the highest lycopene content (44.46 mg $100g^{-1}$) was obtained in cellulase enzyme 2 per cent pre-treatment for 60 minutes prior to solvent extraction using petroleum ether. When this duration was increased to 120 minutes the lycopene content decreased to 37.50 mg $100g^{-1}$, followed by pectinase enzyme at concentration 2 per cent with incubation time of 120 minutes.The increase in the lycopene content recovery in samples mixer of pulp and peel pre-treated with cellulase and pectinase enzyme in the present study is due to degradation and disintegration of the fruit tissue making lycopene easily accessible to organic solvent for dissolution (Choudhari and Ananthanarayan, 2007; Cinar, 2005 and Seymour, 1993). [2]

The highest antioxidant activity was observed in bright red colored ripe tomato. Among enzyme extraction process, cellulase 2.0 per cent for 60 minutes incubation at 45 °C got maximum antioxidant activity of 20.76 mg $100g^{-1}$.

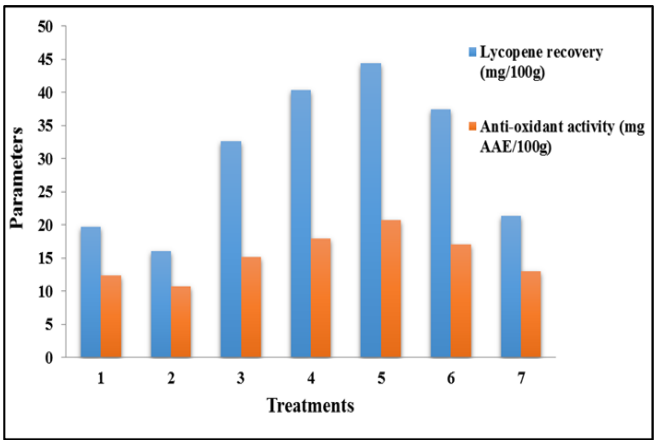

Fig 1: Effects of different extraction methods on lycopene recovery (mg $100g^{-1}$) and antioxidant activity (mg AAE $100g^{-1}$) from culled tomato

The extracted lycopene pigment was used for analysis of antioxidant activity. The antioxidant activity was more (20.76 mg AAE $100g^{-1}$) in cellulose 2.0 per cent for 60 minutes incubation at 45 °C (T_5 - in table 7), then followed by treatment T_5 than T_4, which could be due to reduced level of lycopene in cellulase 2.0 per cent for 120 minutes incubation at 45 °C. The reason for the loss of lycopene is due to incubating or heating. Shi and Maguer (2000) [9] reported that no *trans-cis* isomerisation was observed during prolong incubation. They found that prolonged incubating or heating of lycopene resulted initially in isomerisation of *trans* forms to *cis* forms and then after reaching an equilibrium, further incubating or heating lead to degradation. In the present study the tomato pulp and peel was incubating for 120 minutes with temperature of 45 °C, due to which it might have degraded. The reduction in yields (17.10 mg $100g^{-1}$) of lycopene observed at prolonged incubation time could be the result of progressive lycopene loss due to oxidation as compared to cellulace at 2 per cent incubating for 120 minutes with temperature of 45°C (Choudhari and Ananthanarayan, 2007) [2]. Therefore, the vast majority of lycopene molecules present in the plant tissue is likely to be rapidly released from the protective chloroplast structures and exposed to the conditions of the external environment and the released lycopene molecules can undergo rapid oxidative degradation (Xianquan *et al*., 2005) [10].

The solvents are also used for extraction of the lycopene. In presence of high temperature solvent are having different hydroxyl ions concentration. Though the antioxidant activity content *per se* reduced, its bioavailability must have been more compared to different extraction method due to isomerization from *trans* to *cis* form (Shi and Maguer, 2000) [9]. However, this aspect of variation in bioavailability in different types of tomato peel needs further investigation.

Conclusion

Culled tomatoes can be utilized for extraction of lycopene and among the different extraction methods, highest lycopene content (44.46 mg $100g^{-1}$) with maximum antioxidant activity was obtained from culled tomatoes disinfected by dipping in hot water (45º C) for five minutes and further extracted by cellulase enzyme 2 per cent pre-treatment for 60 minutes prior to solvent extraction using petroleum ether. Study indicated that enzyme extraction method can be utilized for extraction of lycopene from culled tomatoes for effective and for better recovery.

References

1. Beatriz NP, Gouveia L, Patricia MGS, Ana CF, António FP Rui LM. Supercritical extraction of lycopene from tomato industrial wastes with ethane. *Molecules*, 2012; 17:8397-8407.
2. Choudhari SM, Ananthanarayan L. Enzyme aided extraction of lycopene from tomato tissues. Food Chem, 2007; 102:77-81.
3. Cinar I. Effects of cellulase and pectinase concentrations on the colour yield of enzyme extracted plant carotenoids. Proc. Biochem, 2005; 40:945-949.
4. King AJ, Zeidler G. Tomato pomace may be a good source of vitamin E in broiler diets. *Californiea Agri*, 2004; 58:59-62.
5. Lario Y, Sendra Garci A, Perez J, Fuentes C, Sayas Barbera E, Ferna Vdez Lo, *et al*. Preparation of high dietary fiber powder from lemon juice by-products. Innovative Food Sci. Emerging Technol. 2004; 5(1):113-117.
6. Naviglio DA, Pizzolongo F, Alejandro LF, Arago, Santini A. Extraction of pure lycopene from industrial tomato by-products in water using a new high pressure process. J Sci. Food Agri. 2008; 88:2414-2420.
7. Seymour GB. Biochemistry of fruit ripening (Eds. Seymour GB, Taylor JE, and Tucker GA), Chapman and Hall, 2-6 Boundary Row, London, 1993, 83-101.
8. Sharma SK, Maguer ML. Lycopene in tomatoes and tomato pulp fractions. Italian J Food Sci. 1996; 2:107-113.
9. Shi J, Maguer MLE. Lycopene in tomatoes: Chemical and Physical properties affected by food processing. J Food Sci. Nutri. 2000; 40(1):1-42.
10. Xianquan S, Shi J, Kakuda Y, Yueming J. Stability of lycopene during food processing and storage. J Med. Food. 2005; 8(4):413-422.