Improving extraction of lycopene from tomato waste by-products using ultrasonication and freeze drying

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Abstract

Waste food by-products represent a major disposal problem for the food industry, and they are often used as animal feed or fertilizers. This study examined the possible utilization of tomato waste as good sources of lycopene. Results revealed that lab-prepared tomato waste (LPTW) contains significantly (p<0.05) a larger amount of lycopene (57.87±5.30 µg/g fresh wt.) than industrial tomato waste by-products (ITWBP) (27.11±0.83 µg/g fresh wt.). The average amounts of extracted lycopene obtained from ultrasonication, freeze drying, and their combination were 45.51±1.84, 104.10±1.23 and 138.82±6.64 µg/g fresh wt., respectively. Subjecting ITWBP to freeze drying and to ultrasonication separately increased their lycopene contents by 2.8 and 0.68 folds, respectively. However, applying the combined treatment of freeze drying and ultrasonication (45 min at 50 Hz) increased the yield of extracted lycopene from industrial tomato waste by 4.12 folds. Antioxidants scavenging capacity of FDITW calculated as % reduction in the DPPH and ABTS free radicals using 1.5mg freeze dried industry tomato waste were 49.64±0.44 and 12.3±0.11, respectively.

Keywords: Antioxidants; Lycopene; Ultrasonication; Freeze Drying; Tomato Wastes; Scavenging Capacity.

1. Introduction

A rapidly accumulating and overwhelming evidence from various epidemiological studies around the world indicates that fresh fruits and vegetables are the primary and well known resource of antioxidants. Antioxidants are chemical compounds that can scavenge free radicals in the body [1]. Antioxidants include many compounds such as carotenoids, vitamins, flavonoids, dietary glutathione, endogenous metabolites, and other polyphenolics [2, 3].

The antioxidants, carotenoids, are natural pigments present in various vegetables, plants, birds and marine animals. The presence of conjugated double bonds in carotenoids provide them with the antioxidant properties and their ability to successfully delocalize of captured free radical species [4]. Approximately 600 fat-soluble carotenoids have been identified in nature but only 40 are present in human diet. Almost 90 % of the carotenoids in the diet and human body are represented by β-carotene, α-carotein, lycopene, lutein and cryptoxanthin. Fruits and vegetables are considered as main sources of antioxidant in human diet. Orange fruits provide α-cryptoxanthin, dark green vegetables provide lutein, where tomatoes and tomato products are the primary sources of lycopene [5, 6].

Lycopene (C40H56) is an acyclic carotenoid containing thirteen double bonds and it is the major carotenoid present in tomatoes, accounting for more than 80 % of the total tomato carotenoids in fully red-ripe fruits [7, 8, 9]. Lycopene is shown to be present in high concentrations in tomatoes and tomato products and has received a great interest in the past decade. Epidemiological evidence continues to suggest that lycopene may provide protection against cancer and
other degenerative diseases mediated by free radical reactions [10]. The ability of lycopene to act as a potent antioxidant is believed to be responsible for protecting cells against oxidative damage and thereby decreasing the risk of chronic diseases [8]. Recent epidemiological studies revealed that the intake of tomatoes and blood lycopene levels are inversely associated with the risk of developing cancers at several anatomical sites, including the prostate, skin, stomach, breast and lungs [11, 12, 7, 13, 10, 9] and cardiovascular diseases [14, 5]. Moreover, the consumption of tomatoes has been demonstrated clinically to have beneficial, protective effects against coronary artery disease and several neoplasms [14].

In addition to fresh consumption, tomatoes are processed into various products, including juices, ketchup, sauce, paste, puree and powder. The solid waste (70-75 % of fresh tomatoes), remaining after juice/pulp extraction and processing, consists of skin, seeds and fibrous matter. This waste along with damaged tomatoes are dumped as a solid waste or to some extent used as fertilizers [15]. Lycopene contents in the skin, seeds and fibrous matter of tomato waste vary significantly. It is suggested that 72-92 % of lycopene was associated with the water insoluble fraction and the skin [4]. Based on these observations and considering the nutritional value of lycopene and the continuous increase in the production of tomatoes and tomato products, there has been significant interest in recent years to retrieve and utilize tomato waste-by-products. This study investigated the presence and recovery of antioxidant (lycopene) from laboratory and industry generated tomato waste by-products using ultrasonication and freeze drying techniques. Additionally, the antioxidant activities of freeze dried industrial tomato waste by-products were assessed using 2 different in vitro methods, namely: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and [2’.2’-azinobis(3-ethylbenzothiazline-6-sulfonic acid diammonium salt] (ABTS) free radical scavenging assays.

2. Material and methods

2.1. Experimental design

A split blot randomised design was followed in this investigation. The experiments were repeated at three different time intervals. Fresh and frozen tomato waste by-products were handled and prepared following the exact same procedures to minimise variations among repeated experiments. Antioxidant activities were determined in freeze dried tomato waste only, since the main object of this project was targeting mainly the industrial tomato waste.

2.2. Chemicals

All the chemicals used in this study, including lycopene standard (90-95% pure) and HPLC grade methylene chloride, hexane, methanol, acetone, methanol, toluene, Potassium Hydroxide (KOH), Sodium Sulphate (Na₂SO₄), Butylated Hydroxytoluene (BHT), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2’,2’-azinobis(3-ethylbenzothiazline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchro-man-2-carboxylic acid (Trolox), and chloroform were purchased from Sigma Chemical Company (St. Louis, USA).

2.3. Tomato samples

Fresh tomato samples were bought from a local market in central Melbourne at different time intervals to produce lab-prepared tomato waste (LPTW) in laboratory. Efforts were made to choose the fruits of same cultivar, similar size and comparable degree of ripeness and colour. Frozen industrial tomato waste (FITW) by-products were kindly supplied by one of the food processing companies in north Victoria and stored at -20 °C until used.

2.4. Preparation of fresh tomato extract

Tomato tissue waste product was separated following the method of Ajlouni et al., [16] with some modifications. Tomatoes (about 3 kg) were rinsed with distilled water, sliced, and then homogenized in a fruit blender (Breville, Powermax 550 watt) for 1 minute at room temperature. The homogenate was filtered using cheese cloth, and the separated tomato tissues were squeezed many times by hand to remove all juice from the waste by-product.

2.5. Lycopene extraction from laboratory prepared tomato waste by-product using ultrasonication

The laboratory prepared tomato waste (LPTW) was ultrasonicated using a bench top ultrasonication cleaner (Unisonics PTY. LTD,type Fx P8M, at freq 50 Hz, NSW, Australia). About 10 g LPTW was transferred into a 100ml volumetric flask and mixed with 30 ml of hexane:acetone:methanol:toluene, 10:7:6:7 v/v/v/v. That step was repeated 4 times and one of these samples was left as a control, while the remaining flasks (3) were ultrasonicated at room temperature for 15, 30 and 45 min, respectively. Each ultrasonicated sample was then mixed with 6 ml of a 4 % methanolic KOH, Each homogenized for 1 min, and stored in the dark at room temperature for 16 hrs of saponification,
The saponified mixture was then mixed with 30ml of hexane followed by gentle swirling for 1 min, before diluting to a final volume 100 ml with 1 % Na2SO4. The flask was left to stand in the dark for an additional 1 hr, then transferred into a separation funnel. The upper phase was transferred into a rotary evaporator and evaporated at 50 ºC. The rotary evaporated sample was then dissolved in 15 ml of methanol-methylene chloride 45:55 v:v [17]. Within 24 hours of the extraction, all samples were filtered through 0.22 µm membrane and 10 µl of each sample extract were injected into the HPLC using an automatic injector.

2.6. Lycopene extraction from the industrial tomato waste by-product

After the successful extraction of lycopene from LPTW using ultrasonication, the procedures were developed further to extract lycopene from industrial tomato waste by-products (ITWP).

Ultrasonication at frequency 50 Hz (a), freeze drying (b), and a combined treatment of freeze drying and ultrasonication (c) were used in these studies.

- Frozen ITWP was delivered to our laboratory at The University of Melbourne in small boxes containing 8-10 kg each. These frozen samples were stored at -20 ºC until analysed. About 100 g of ITWP was thawed at room temperature, squeezed thoroughly by hand to remove remaining juice, and lycopene was extracted after ultrasonication for 45 min as explained before (section 2.5).

- Freeze drying was performed on frozen ITWP. About 8-10 g of frozen tissues were spread in a plastic Petri dish, and 9-12 of these dishes were arranged inside the vacuum chamber of a freeze drier (Dynavac Engineering FD3 freeze-dryer, NSW Australia) and freeze dried at -40 ºC. Freeze dried Industrial tomato waste (FDITW) samples were ground into powder using porcelain pestle and mortar. The powder was packaged in zip lock plastic bags and then stored in a refrigerator until used.

- The freeze dried powder (3 g each) was then subjected to the same procedures of ultrasonication and extraction used with LPTW as explained before (section 2.5).

2.7. Assessment of lycopene contents

Lycopene analysis was performed using a Shimazu High Performance Liquid Chromatography equipped with a workstation computer (Class VP) and a photodiode array (PDA) detector (SPD-M10Avp) following the method of Ajlouni et al. [16]. The column used was a LC18 stainless steel column of 25cm × 4.6 nm packed with C18 reversed-phase material with a particle size of 5µm (SUPELCO). The lycopene standard was dissolved in chloroform containing 0.1 % butylated hydroxytoluene (BHT), divided into 1ml aliquots and stored at -80 ºC until used. All samples (standards and extracts) were filtered through 0.22 µm membrane, and 10 µl from each sample were injected into HPLC. The elution was performed at room temperature with an isocratic solvent, methanol: (methanol : methylene chloride, 45:55 v/v) 99:1 v/v, at a constant flow rate of 2.0 ml/min. The peak response of lycopene was detected at 472 nm and the quantification of the lycopene was performed using data from external standard curves and the linear regression equations (Fig.1).
2.8. Antioxidant activity of the freeze dried industrial tomato waste

Antioxidant extract was prepared by dissolving FDITW samples in 50 % ethanol at room temperature following the method of Tow et al. [18]. The mixture was vortexed for 1 min and centrifuged at 14000×g (Eppendorf centrifuge, Germany) for 4 min before collecting the supernatant for antioxidant activity determination. The antioxidant activities of the lycopene extract were assessed using two different methods.

The 1st method was based on the evaluation of the free radical scavenging capacity of the lycopene extract using 2,2-Diphenyl-1-pirclyhydrazyl (DPPH) as reported by D’Angelo, et al. [19] with some modifications. Reduction of 2,2-Diphenyl-1-pirclyhydrazyl (DPPH) by an antioxidant or by a radical species results in a loss of absorbance at 517 nm. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. The DPPH solution was freshly prepared daily, covered with aluminium foil, and kept in the dark at 4 ºC between measurements. FDITW sample extract (1.5 mg each) was mixed with 60μl 0.5mM Tris-HCl (pH 7.2) in a 96-well micro plate. The reaction was initiated by adding 150 μl/well of DPPH solution in ethanol to each well. The reaction mixture was kept in the dark at room temperature for 30 minutes, before measuring its absorbance at 517 nm using microplate reader (Thermo, Multiskan Spectrum). The percentage reduction of the initial DPPH adsorption was calculated using the following formula:

\[
\text{% Reduciton in absorbence} = \left\{ \frac{\text{absorbance DPPH control} - \text{absorbance sample}}{\text{absorbance DPPH control}} \right\} \times 100
\]

The 2nd method was based on the measurement of the rate of radical [2.’2’-azinobis(3-ethylbenzothiazline-6-sulfonic acid diammonium salt) (ABTS) decolourisation. The assay was performed according to the method of Seeram et al. [20] with minor modification. The radical cations ABTS were prepared by adding 80 mg of solid manganese dioxide to 20 ml of 5 mM ABTS+ aqueous stock solution (using a 75 mM Na/K buffer at pH 7). Excess manganese dioxide was removed from the filtrate by passing through a 0.22 µm membrane. This solution was then diluted with 5 mM phosphate buffered saline (PBS) pH 7.4 to an absorbance of 0.70±0.02 at 734 nm, and pre-incubated at 30 °C prior to use. Fresh ABTS+ radical cation solution was prepared each day. Antioxidant extract of FDITW samples (1.5 mg) was mixed with 200 μl of ABTS+ radical cation solution in 96-well micro plates. The absorbance was read at 734 nm after 6 min of incubation at 30 °C. The percentage reduction of the initial ABTS free radical absorption) was calculated using the following formula:

\[
\text{% Reduction in absorbence} = \left\{ \frac{\text{absorbance ABTS control} - \text{absorbance sample}}{\text{absorbance ABTS control}} \right\} \times 100
\]

2.9. Statistical Analysis

The statistical analysis was done using Statistical Analysis System (SAS) program (1990, SAS/STAT User's guide, Version 6.06, 4th ed. Cary, NC.). Data were analysed using the one –way ANOVA. The LSD was used to test differences between experiment means. Differences among means with p < 0.05 were accepted as representing statistically different samples.
3. Results and discussion

3.1. Impact of Ultrasonication on lycopene contents in lab-prepared tomato waste (LPTW)

Ultrasonicating of LPTW for 15, 30 and 45 minutes using 50 Hz at room temperature showed significant (p<0.05) improvement in the amount of extracted lycopene. Ultrasonication of LPTW also indicated a positive correlation between ultrasonication for 45 min and the amount of extracted lycopene (Fig. 2). Non-ultrasonicated (control) LPTW showed average lycopene contents of 57.87± 5.30 µg/g fresh wt. Increasing ultrasonication time from 15 min to 30 min showed insignificant improvement (P>0.05) in lycopene contents. However, data revealed significant (p<0.05) increment in the amounts of extracted lycopene when the ultrasonication time was increased to 45 minutes at room temperature. The amount of extracted lycopene from LPTW after 45 min ultrasonication was 87.68±1.59 µg/g fresh wt (Fig.2), which represented an increment of 51.51 %, in comparison to the control. These findings clearly indicated that tomato waste tissues could be utilized as an excellent source of lycopene when treated properly. Additionally, these results confirmed that ultrasonication (50 Hz) for 45 min could be considered a good method to improve lycopene extraction from tomato waste tissues. It was observed also that those ultrasonication treatments did not affect the integrity of the treated tomato waste tissues. Consequently, it was assumed that modifying the ultrasonication process to help rupturing the cellular structure of the tomato tissues could be an essential requirement to improve the yield of lycopene extraction. To test that assumption, lycopene was extracted from the industrial tomato wastes as thawed samples, after freeze drying, and after freeze drying and ultrasonication.

![Figure 2](image-url) Lycopene contents in lab-prepared tomato waste (LPTW) ultrasonicated for 15, 30 and 45 min at room temperature. Results represent the average of 9 measurements ±SD. Columns with different superscripts (a, b, c, d) were significantly different (p<0.05).

3.2. Impact of Ultrasonication on lycopene contents in thawed industrial tomato waste (TITW)

The initial lycopene contents in the thawed industrial tomato waste (TITW) was 27.11±0.83 µg/g fresh wt. (Fig. 3). This value was significantly (P<0.05) smaller than the value (57.87± 5.30 µg/g fresh wt.) obtained from lab-prepared tomato waste (Fig. 2). Such variations may be attributed to the high pressure processing conditions used by the industry to extract tomato juice, and the squeeze out of most of the lycopene from the tissues. Aside from these variations, data generated from thawed industrial tomato wastes (TITW) clearly confirmed the positive impact of ultrasonication on the amounts of extracted lycopene from tomato waste by-products. Ultrasonicating (50 Hz) TITW for 45 min increased the amounts of extracted lycopene significantly (P<0.05). The amount of lycopene isolated from ultrasonicated thawed industrial tomato waste was 45.51±1.84 µg/g fresh wt, as compared to 27.11±0.83 in untreated (control) samples (Fig.3).
Comparing the percentage increment in lycopene contents in both laboratory prepared and industry generated tomato waste by-products as a result of ultrasonication (45 min) clearly revealed that the % increment in industry tomato waste (67.87 % = 0.67 fold) was significantly (P<0.05) larger than that in laboratory prepared tomato waste (51.51 %). Such variations could be attributed to many factors, including tomato cultivar, degree of ripeness, level of damaged tissues, and most importantly, the method of processing [21]. Additionally, as mentioned earlier, the high pressure used by the industry to extract tomato juice left the remaining tissue with less lycopene content (27.11±0.83 µg/g fresh wt.) (Fig.3) in comparison to laboratory prepared samples (57.87±5.30 µg/g fresh wt.) (Fig. 2). These variations could indicate that the larger initial lycopene content in laboratory prepared tomato waste (57.87±5.3 µg/g fresh wt.) (Fig 2) than the industrial tomato waste samples (27.11±0.83 µg/g fresh wt.) (Fig 3) reduced the chance of percentage lycopene increment as a result of ultrasonication. In another word, the gap between the initial (27.1±0.83 before ultrasonication) and final lycopene content (45.51±1.84 µg/g fresh wt.) in the industrial waste was larger than that in laboratory samples and led to larger percentage increment of lycopene content.

3.3. Impact of freeze drying on lycopene contents in industrial tomato waste

Freeze drying of industrial tomato waste by-products significantly (P<0.05) improved the lycopene yield. The average amounts of lycopene based on fresh weight ranged from 27.11±0.83 µg/g in the control samples (none freeze dried) to 104.10±1.23 µg/g in freeze dried sample (Fig 3). Consequently, it could be concluded that that freeze drying treatment could increase the yield of lycopene extraction from tomato waste by-products by 2.8 fold. These results clearly emphasised the previously proposed theory (section 3.1) that rupturing the cellular structure of tomato tissues might be essential treatment to improve the yield of extracted lycopene. Freeze drying could be considered an excellent technique to improve antioxidants extraction, such as lycopene, without exposing the tissue to any heat treatment. However, the amount of energy needed during the processes of freezing and vacuuming would increase the cost of extraction much more than ultrasonication and might become a limiting factor.

3.4. Impact of freeze drying and Ultrasonication (50 Hz and 45 min) on lycopene contents in freeze dried industrial tomato wastes (FDITW)

Subjecting FDITW tissue to ultrasonication (50 Hz) for 45 min caused additional significant (P<0.05) improvement in the amounts of extracted lycopene (Fig.3). Results from ultrasonication freeze dried tomato waste showed an increment in lycopene content from 104.10±1.23 to 138.82±6.64 µg/g fresh wt. after ultrasonication (Fig.3). The calculated additional percentage increment in extracted lycopene as a result of ultrasonication reached 33.35 %. These results confirmed our previous speculation (section 3.3) that freeze drying and grinding of tomato waste would rupture and damage the tissues cellular structure, consequently, facilitate and improve the efficacy of ultrasonication.

These findings showed that industrial tomato wastes can be a significant source of lycopene when subjected to freeze drying, ultrasonication and their compensation. Freeze drying appeared to be more efficient than ultrasonication, and their combination was proved to be the best treatment for the largest lycopene yield. As several previous studies [4, 13, 22, 23], confirmed that tomato wastes contains more lycopene than tomato juice it is strongly recommended that tomato wastes...
waste by-products should be recycled using these new techniques and utilized as additional nutritional sources of lycopene.

3.5. Antioxidant activity of freeze dried industrial tomato waste (FDITW)

*In vitro* antioxidant analysis to assess the antioxidants capacity of FDITW was performed using two different methods, namely, 2,2-diphenyl-1-picyryldrazyl (DPPH) radical reduction and [2',2'-azinobis(3-ethylbenzothiazline-6-sulfonic acid diammonium salt)] (ABTS) radical decolourisation. Results from these analyses revealed that DPPH method was more sensitive than ABTS. Only 1.5 mg freeze dried sample was sensitive enough with the DPPH method, while 10 mg was needed with the ABTS. The percentage reduction of the initial DPPH via the FDITW antioxidant scavenging capacities ranged from 48.87±2.39 to 50.25±1.09 in the presence of 1.5 mg freeze dried tomato tissue (Table 1). The calculated scavenging activity of FDITW using ABTS radicals revealed an average reduction in the absorbance of the initial ABTS radical by 12.30 % (Table 1). Consequently, comparing the % scavenging capacity of DPPH and ABTS showed that antioxidants from FDITW were more active in the presence of DPPH free radicals. These results were in disagreement with those reported by Chen *et al.* [24], who reported that antioxidants extract from persimmon and tomato fruits had almost identical ability to scavenge both ABTS and DPPH radicals. However, data by same authors showed significant differences between DPPH and ABTS results when testing grape and apple antioxidant extracts. Consequently, our results were in agreement with those reported on grape and apple and could suggest that antioxidants from different fruits may show different rate of scavenging activities to DPPH and ABTS free radicals. The ABTS scavenging capacity could also be reported using as the micromolar Trolox equivalent (mM TEAC) as suggested by D’Angelo, *et al.* [19].

The antioxidant properties of phytochemicals have been suggested to reflect the rates of free radical scavenging [25]. The properties underlying the activities of antioxidants towards free radicals and their scavenging effects relate particularly to their abilities to donate electrons or hydrogen atoms and to their relative propensities to undergo oxidation [25]. Another study by Chang *et al.* [26] reported that the reducing power could be attributed mainly to the bioactive compounds associated with some antioxidant activity, such as lycopene, present in tomatoes. Lycopene are good electron donors and could terminate the radical chain reaction by converting free radicals to more stable products.

**Table 1 Antioxidant activities of freeze dried industry tomato waste (FDITW) using DPPH and TEAC assays**

| Trials | Percentage reduction of the initial absorption in the presence of 1.5 mg FDITW* |
|--------|---------------------------------------------------------------------------------|
|        | DPPH                | ABTS                         |
| 1      | 50.25±1.09          | 12.15±1.50                   |
| 2      | 48.87±2.39          | 12.33±0.11                   |
| 3      | 49.64±0.44          | 12.24±0.16                   |

*Results represent the average of 9 measurements ±SD.

4. Conclusion

Results from this study have clearly demonstrated that freeze drying, ultrasonication (50 Hz, 45 min), and their combination could be excellent methods to improve lycopene extraction from tomato wastes. Additionally, this study also demonstrated that the yield of antioxidant extraction form industrial tomato wastes can be significantly improved *via* freeze drying alone or in combination with ultrasonication. The current industrial practices use food wastes, in general, as animal feed and/or fertilizers, which may require additional costs to transfer the wastes into the farm. However, based on this investigation it is anticipated that these developed methods, especially the combination of freeze drying and ultrasonication, could be applied to extract antioxidants from various food wastes, and will provide the food industry with better alternative options to re-utilize such wastes. Further investigation is continuing to examine the possible use of extracted lycopene as a functional ingredient in food mixes and/or in the pharmaceutical products.

Compliance with ethical standards

The authors agree to comply with the standards of expected ethical behaviour for all parties involved in the act of publishing.
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Disclosure of conflict of interest
All authors of the manuscript have no conflict of interest to declare.

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