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# Enhanced lycopene extraction from tomato industrial waste using microemulsion technique: Optimization of enzymatic and ultrasound pre-treatments

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ABSTRACT

Microemulsion technique (MET) is a green scalable method allowing selective extraction of components of biological interest. This report deals with application of MET using natural surfactant, saponin, for lycopene extraction from industrial waste of tomato paste plants where the effects of ultrasound and enzyme treatments, surfactant:lycopene and surfactant:co-surfactant ratios as well as waste powder particle size on extraction efficiency were examined. Based on the results, a multi-step extraction scheme including ultrasound pre-treatment followed by enzymatic hydrolysis and then microemulsion extraction of tomato waste was led to an extended separation of the lycopene with minor surfactant use (mass ratio of saponin to lycopene 20:1). Under the optimized conditions, the efficiency showed an increase of just over 100% compared to initial experiment outcome. MET using saponin along with mild pre-treatments can be a simple, rapid, green and low-priced technique for lycopene extraction from tomato industrial waste and possibly other vegetable sources.

*Significance of the research data presented towards potential industrial applications:* Saponin is a renewable natural glycoside distributed in a wide variety of plants, low-priced to produce, stable over a wide range of pH values (from 2 to 11), already recognized as safe for use in foods and drinks by the FDA and therefore promising for its large scale industrial use in extracting applications as an appropriate alternative for synthetic surfactants. On the whole, simplicity and low energy requirements of the microemulsion technique as well as the low price of commercial saponin compared to biosurfactants and synthetic surfactants, make microemulsion technique a very promising technique to be scaled up.



## 1. Introduction

Lycopene has been credited as a key biosynthetic precursor of many carotenoids with a strong antioxidant effect twice than that of β-carotene (Urbonaviciene, Viskelis, Viskelis, Jankauskiene, & Bobinas, 2012). The free radical quenching ability of lycopene has led to promising outcomes with respect to protection against coronary heart disease, diabetes and cancer incidence, making the demand for this nutraceutical expeditiously growing (Ordóñez-Santos & Vázquez-Riascos, 2010).

Lycopene naturally occurs in tomato and tomato based products which account for more than 85% of human dietary intake of lycopene (Rao & Agarwal, 2000). Tomato pomace contains a sizable amount of lycopene (70–90%) which is five times more compared to tomato whole pulp. Moreover, many studies have shown that lycopene from processed tomato-based products including tomato juice, paste, sauce, puree and ketchup which is submitted to heat and mechanical treatments is more bioavailable than that found in the fresh tomatoes (Dewanto, Wu, Adom, & Liu, 2002; Nobre, Palavra, Pessoa, & Mendes, 2009).

It is worth noting that Iran is one of the leading producers of tomato in the world with annual production of 4 million tons, most of which is used in processing industries mainly tomato paste production (Kohansal & Zamaninejad, 2013). Moreover, 10–40% of the whole tomato processed in the plants is believed to be a mixture of peels and seeds, also known as tomato pomace (Nobre et al., 2009). The pomace is presently thrown out in the environment or used to feed animals while it can be more effectually used as a low price fresh matter containing lycopene. Since the pomace contains most of the lycopene and has been exposed to thermomechanical treatments, therefore it can potentially be considered as an interesting low-price source of lycopene (Naviglio et al., 2008).

In the recent years, defects of the existing extraction methods, such as environmental hazards due to the consumption of significant quantities of volatile organic solvents, presence of solvent traces in final product, human health problems, high working temperatures, long process

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time and high energy consumption, have forced the food technologists to consider green and sustainable techniques to substitute conventional extraction techniques (Chemat, Huma, & Khan, 2011). Extraction methods using different surfactants to extract biomolecules from fluid mixtures have become increasingly attractive, as water is often used as the main solvent in such techniques instead of extensive use of harmful chemicals in the current solvent-based processes. Moreover, a wide variety of biodegradable surfactants could be applied in such techniques which have no environmentally detrimental impact. Among various surfactant-based extraction approaches, microemulsion technique (MET) is a promising new method having the potential to be used for extraction purposes. The rising interest in application of microemulsion systems in the area of food and chemical industry derives, above all, from their outstanding physicochemical properties such as unique optically transparent appearance, perfect stability, low viscosity, large solubilization capacity for both hydrophilic and lipophilic compounds, increasing bioavailability of nutraceuticals, generating an extremely large oil–water interfacial area and ultralow interfacial tension. In addition, microemulsion systems provide nanodroplets that can be considered as receptors for extraction of different analytes at a nanoscale level (Abbasi & Scanlon, 2016; Berry, 2011; Gadhave & Waghmare, 2014). MET, using a wide variety of surfactants, has been already applied successfully in extraction of diverse nutraceuticals and organic compounds namely phenols, enzymes and proteins from liquids (Materna & Szymanowski, 2002), oil (Abbasi & Radi, 2016; Radi, Abbasi, Hamidi, & Azizi, 2013), proteins, and glucosinolates from cruciferous oilseed meals (Ugolini, De Nicola, & Palmieri, 2008) and lycopene from watermelon (Jin et al., 2008).

Pre-treatments such as high power ultrasound and enzymatic digestion are now recognized as efficient techniques in the chemical and food industry for extraction purposes, as they can effectively improve mass and heat transfer leading to shorter extraction time, consuming less solvents, saving in energy, improving yields and giving better quality and higher purity of the extracted compounds (Chemat et al., 2011; Choudhari & Ananthanarayan, 2007; Kadam, Tiwari, & O'Donnell, 2013; Zhao et al., 2006).

Enzymatic breakdown of cell walls employing food-grade enzymes with cellulolytic, pectinolytic and hemicellulolytic activity, that can break the plant cell walls releasing intracellular components from degraded cells, has been reported widely for enhancing the extraction efficiency of different biomolecules from vegetable matrices (Choudhari & Ananthanarayan, 2007; Cuccolini, Aldini, Visai, Daglia, & Ferrari, 2013).

However, practical application of MET to assist the recovery of lycopene from tomato industrial waste preceded by enzymatic and ultrasound pretreatments is, so far, a neglected field in food technology that has never been examined before.

Moving towards green extraction, the main objective of this study was to examine the impacts of optimized ultrasound and enzymatic pre-treatments as well as optimized experimental parameters of microemulsion extraction process on extraction efficiency of lycopene from tomato industrial waste using MET where saponin was used as a natural surfactant.

## 2. Materials and methods

### 2.1. Materials

Tomato industrial waste (tomato pomace) was supplied from Yara Tomato Paste Plant (Shahryar, Tehran, Iran). Saponin (pharmaceutical grade, HLB: 13.5) was purchased from Pioneer Biotech Co. (Shaanxi, China). Glycerol, ethanol, acetone, hexane, acetic acid, sodium acetate, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide and sodium bicarbonate were purchased from Merck Chemical Co. (Darmstadt, Germany). The commercially available Endozym®-Pectofruit, derived from microbial sources and in liquid form, was provided by AEB Group (Spindal Co., Brescia, Italy). For preparation of solutions and extraction mixtures double distilled water was used.

### 2.2. Methods

#### 2.2.1. Sample preparation

Tomato industrial waste, with an initial moisture content of 83% wt., was dried away from light in an oven at 35 °C for 64 h until moisture content of 7.6% wt. The dried pomace was pulverized using a domestic mill (Hamilton, FH-140, 230 W, China), passed through nest of sieves (2000, 1410, 1000, 707, 595, 400, 250 and 210 μm) and classified to 8 groups: (a) ˃2000; (b) 1410–2000; (c) 1000–1410; (d) 707–1000; (e) 595–707; (f) 400–595; (g) 250–400 and (h) 210–250 μm.

#### 2.2.2. Surfactant assisted extraction

One gram of tomato industrial waste powder was added to the centrifuge tubes (15 ml) containing saponin:glycerol, already dissolved in distilled water at various mass ratios of surfactant:lycopene. The optimum type of surfactant and co-surfactant was determined in our previous study where the natural surfactant saponin having HLB of 13.5 and glycerol as co-surfactant, showed the best performance for lycopene extraction (Amiri-Rigi & Abbasi, 2016). Consequently, in order to optimize operating conditions, the influence of surfactant:lycopene ratio (10:1, 20:1, 30:1, 40:1, 50:1 and 60:1), surfactant:co-surfactant ratio (2:1, 1:1 and 1:2) and particle size of industrial waste powder on lycopene extraction efficiency was investigated. In all experiments, at first the saponin:glycerol mixture was combined with double distilled water and thoroughly mixed for about 30 s. Afterward, tomato industrial waste powder was added to the mixture and agitated (at 25 °C, 250 rpm for 30 min) using a shaking incubator. Subsequently, samples were centrifuged (10,000 *g* for 15 min) and a sample of the upper phase was withdrawn, filtered and analyzed for lycopene content.

#### 2.2.3. Lycopene analysis

The concentration of lycopene in initial pomace powder and microemulsion phase was determined according to the procedure outlined by Fish, Perkins-Veazie, and Collins (2002) with some modifications. Extraction of lycopene from pomace (0.5 g) was conducted in a 50 ml Erlenmeyer flask using a mixture of hexane:ethanol:acetone (2:1:1) in 20 ml final volume at room temperature under reduced light conditions. Erlenmeyer flask was placed on a stirrer plate at a speed of about 300 rpm for 15 min. After that, 3 ml of double distilled water was added to the vessel, and the solution was shaken for another 5 min. Thereafter, shaking was stopped and the suspension was allowed to stand at room temperature for 5 min until spontaneous separation of polar and nonpolar phases was complete. The lower phase was re-extracted until the residue became colorless. Subsequently, the upper phases were transferred into a vial and combined. The same procedure was applied for all microemulsion preparations. The absorbance of the upper, nonpolar phase at a wavelength of 503 nm was recorded versus hexane as blank using a spectrophotometer (U-1700, Shimadzu Corp., Kyoto, Japan, path length = 1 cm). The absorbance at 503 nm was monitored in order to reduce interference levels from other tomato carotenoids. The lycopene content of the tissue was then calculated through Eq. (1):

$$\begin{aligned}\text{Lycopene}(\mu g/g) = A_{503}/\left(17.2\times10^4\ \text{L mol}^{-1}\ \text{cm}^{-1}\right)\times 1/\text{b cm}\\ \times 536.9\ \text{g/mol}\times 1\ \text{L}/10^3\ \text{ml}\times 10^6\ \mu g/1\\ \times \text{V ml/kg tissue}\times \text{kg}/10^3\ \text{g}\end{aligned} \quad (1)$$

where $A_{503}$ is absorbance of nonpolar phase at 503 nm, b is path length (cm), 536.9 is molecular weight of lycopene (g $mol^{-1}$), V is volume of nonpolar phase (ml) and $17.2\times10^4$ is the molar extinction coefficient for lycopene in hexane (L $mol^{-1}$ $cm^{-1}$) (Amiri-Rigi & Abbasi, 2016; Fish et al., 2002).