Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Enzyme and high pressure assisted extraction of carotenoids from tomato waste

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A B S T R A C T

Enzyme (EA) and high pressure (HP) assisted extraction of carotenoids, especially lycopene, from tomato waste using various organic solvents was examined. Total carotenoid and lycopene extraction yields were increased by the use of pectinase and cellulase enzymes, when compared to the non enzyme treated solvent extraction process. The increase of extraction yield depended on the solvent. Maximum total carotenoid (127 mg/kg d.w.) and lycopene (89.4 mg/kg d.w.) extraction yields were obtained in enzyme treated samples extracted with ethyl lactate (solvent:solid = 10:1 mL:g), corresponding to almost 6-fold and 10-fold increase, respectively, with respect to non enzyme treated samples. HP assisted extraction led to higher extraction yields (from 2 to 64% increase depending on the solvent used) compared to conventional solvent extraction process performed at ambient pressure for 30 min. HP assisted solvent extraction was successfully performed at 700 MPa by using significantly ($P < 0.05$) lower ratios of solvent:solid (6:1 and 4:1 mL:g) and reduced processing time (10 min), compared to solvent extraction performed at ambient pressure, solvent:solid ratio 10:1 mL:g and 30 min extraction time.

Keywords: Tomato waste; Carotenoids; Lycopene; Solvent extraction; Enzyme-assisted extraction; High hydrostatic pressure

1. Introduction

By-products of plant food processing represent a major disposal problem for the industry concerned, but they are also a promising source of valuable components. Tomato processing industry generates significant amounts of waste, consisting mainly of tomato skins and seeds, in a proportion depending on the specific process from which it is generated (Del Valle et al., 2006). Tomatoes are a rich source of carotenoids, particularly lycopene. The global market for carotenoids was estimated to US$1.07 billion and is projected to top US$1.2 billion in 2015 (Global Industry Analysts Inc., 2011).

According to Sharma and Le Maguer (1996), at the end of ripening stage, tomato skins contain up to five times more lycopene than the pulp. Conventional food grade solvents, such as hexane, ethanol, and ethyl acetate, have been proposed for the extraction of carotenoids from tomato waste. However, the yield in most cases is low possibly due to the difficulty for the solvent molecules to penetrate the tomato peel tissue and solubilize the pigment, while oxidative degradation of carotenoids is also possible (Lavecchia and Zuorro, 2008).

In that aspect, enzyme-assisted extraction methods are gaining more attention because hydrolytic enzymes break down the structural integrity of cell walls rendering the intracellular materials to be more exposed for extraction. Cellulase and pectinase enzymes were employed by several researchers as a pretreatment step of tomato based products prior to solvent extraction for the recovery of carotenoids, and especially lycopene (Choudhari and Ananthanarayan, 2007; Lavecchia and Zuorro, 2008; Zuorro et al., 2011; Papaioannou and Karabelas, 2012; Ranveer et al., 2013). The enzymatic treatment was followed by extraction with hexane, ethyl acetate, acetone or mixtures of solvents. In all cases, there was an

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Received 13 January 2014; Received in revised form 22 August 2014; Accepted 28 September 2014
Available online 6 October 2014
http://dx.doi.org/10.1016/j.fbp.2014.09.012

increase in extraction yield compared to the untreated samples, which varied from 2- to 18-fold. These differences may be attributed to different raw material, enzyme preparation, experimental conditions and solvents used for the subsequent extraction. The effect of solvent on the extraction yield of carotenoids from enzymatically treated tomato waste might be very important, as different solvents show different penetration abilities and solubilizing effect on carotenoids (Strati and Oreopoulou, 2011).

On the other hand, HP processing can cause some structural changes, such as cell deformation or cell membrane damage that increase cell permeability as well as secondary metabolite diffusion and consequently mass transfer rates, while it has very little effect on low-molecular-weight compounds such as flavour compounds, vitamins and pigments (Knorr, 1993; Corrales et al., 2008). In the literature, earlier studies have been published with regards to the effect of HP on total carotenoid content and carotenoid availability of commonly consumed vegetables (McInerney et al., 2007; Plaza et al., 2012). As an extraction method, this technology was first studied in 2004, and it was found to effectively shorten the extraction time and increase the process efficiency (Zhang et al., 2004). Recently, HP has been implemented to extract bioactive compounds from natural sources, such as anthocyanins from grape by-products (Corrales et al., 2008), or phenolic compounds from *Maclura pomifera* fruits (Altuner et al., 2012), while Xi (2006) applied HP on tomato paste waste and obtained higher yields of lycopene compared to conventional solvent extraction performed at ambient pressure for 30 min.

Several solvents have been examined in a previous work (Strati and Oreopoulou, 2011) for the extraction of carotenoids, and especially lycopene, from tomato waste and the results were highly dependent on the solvent. In the present study we further investigated the possibility to enhance carotenoid extractability by enzyme pre-treatment or HP assisted extraction. Tomato waste was treated with pectinase and cellulase enzymes prior to extraction with various solvents, from the non-polar – hexane to the highly polar – ethanol so as to define the combined effect on extraction yield. Additionally, HP processing, in the pressure range of 100–800 MPa with the initial (prior to pressurization) temperature of 25 °C was explored in order to obtain the maximum extraction yields of carotenoids and lycopene from tomato waste.

2. Materials and methods

2.1. Chemicals and enzymes

Hexane and ethyl acetate, analytical grade, were purchased from Thermo Fisher Scientific (Fair Lawn, NJ). Acetone and ethanol, p.a., were purchased from Sigma Chemical Co. (Sigma–Aldrich Company, St. Louis, MO). (-)-Ethyl L-lactate, p.a., was purchased from Fluka Analytical (Sigma–Aldrich Chemie Gmbh, Munich, Germany). All solvents used for HPLC analysis (acetonitrile, 1-butanol and methylene chloride) were of HPLC grade and were obtained from Merck (Darmstadt, Germany). REDIVIVO Lycopene 10% FS (10% microcrystalline lycopene in corn oil containing a-tocopherol as antioxidant) from DSM Nutritional Products (Kaiseraugst, Switzerland) was used for the preparation of standard solutions for spectrophotometric measurements. All-trans lycopene standard was purchased from Sigma Chemical Co. (Sigma–Aldrich Company, St. Louis, MO).

Cellulyve AN 3500, a powder enzyme preparation of *Aspergillus niger* cellulase, was obtained from Lyven S.A. (Colombelles, France), with enzyme activity of 3500 ± 200 U/g and pH 4–6 (concentration 1%). Pectinex Ultra AFP, a liquid enzyme preparation produced from *Aspergillus aculeatus* and *A. niger*, containing pectin lyase and polygalacturonase activities of 10,000 Units/mL, was obtained from Novozymes (Bagsvaerd, Denmark). Both products were stored at 4 °C and prior to use, calculated amounts of enzyme preparations were diluted with acetate buffer solution of appropriate pH (4.8 for cellulase and 5.0 for pectinase) to obtain the desired enzyme concentration.

2.2. Plant material

Tomato processing waste, composed of skin and seeds of tomato cultivar Red Sea, was collected from a Greek tomato processing plant (NOMIKOS S.A., Aliartos, Viotia, Greece). Moisture content was determined at fresh tomato processing waste upon arrival at the laboratory and found to be 80.48 ± 0.35%. Tomato waste material was air dried at 25 °C, homogenized in a domestic blender and finally ground in a laboratory mill (Cutting Mill Pulverisette 15, Fritsch Gmbh, Idar-Oberstein, Germany) equipped with a 0.5 mm sieve. Moisture content of ground dry tomato waste was 7.65 ± 0.21%. The dry ground material was kept in glass jars wrapped with aluminium foil at −20 °C before further processing.

The critical steps involved in the EA and HP assisted extraction of carotenoids from tomato waste are described in Fig. 1.

2.3. Enzyme aided extraction of carotenoids

Dry, ground and homogenized tomato waste was distributed (1.0 g each) in tightly-closed glass test tubes, covered with aluminium foil. To each test tube, 7.0 mL of enzyme solution of the appropriate enzyme concentration were added and the mixture was vortexed for 3 min. The samples were then incubated under agitation in a Memmert shaking waterbath WB 14 with drive SV 1422 (Memmert GmbH + Co. KG, Schwabach, Germany). The incubation temperature for both enzymes (pectinase and cellulase) was chosen according to that reported by the supplier and literature review as optimal for enzyme activity and was 45 °C and 55 °C for Pectinex Ultra AFP and Cellulyve AN 3500, respectively.

At the end of the incubation period the enzymes were inactivated by immersing the test tubes in boiling water for 3 min. After filtration, the tomato waste solid residue was placed in screw-top conical flasks in a thermostated water bath (25 ± 0.1 °C) and was subjected to solvent extraction, using 10 mL of each solvent. The whole system was kept under agitation for 30 min. On completion of the extraction procedure, the mixture was centrifuged at 1000 × *g* for 10 min (Centrifuge Thermo scientific Heraeus Megafuge 16R, DJB Labcare Limited, UK), to separate the supernatant.

Blank (enzyme free) samples were prepared for each solvent extraction process, by adding buffer solution in the same amount as the enzyme solution, prior to solvent extraction.

2.4. High pressure treatment

Tomato waste samples (2.5 g each) were weighed, mixed with the appropriate volume of different solvents or solvent mixtures and packed into polypropylene pouches for HP experiments. The pouch was sealed after eliminating air from the inside and placed into the HP vessel. HP samples