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Effect of ultrasound treatments of tomato pulp on microstructure and lycopene *in vitro* bioaccessibility

Monica Anese *, Giorgio Mirolo, Paola Beraldo, Giovanna Lippe

Dipartimento di Scienze degli Alimenti, University of Udine, Via Sondrio 2/A, 33100 Udine, Italy

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ABSTRACT

The influence of ultrasound treatments of tomato pulp on microstructure and lycopene *in vitro* bioaccessibility was investigated. To this purpose, samples were subjected to ultrasound at a frequency and amplitude of 24 kHz and 100 μm, respectively, for increasing lengths of time. Results showed that ultrasound was responsible for loss of tomato cell integrity, as well as a decrease in the degree of pectin esterification. In contrast, rheological measurements showed that ultrasonically treated tomato pulp had greater gel-like properties than an untreated sample. It was inferred that ultrasound promoted the formation of a new network due to hydrogen bonding and hydrophobic interactions among the de-esterified pectin molecules. Such a reinforcement of the tomato pulp structure resulted in a decrease in lycopene *in vitro* bioaccessibility of the ultrasonically treated tomato pulp, probably due to the fact that the presence of a stronger network may make lycopene less available to the digestion process.

1. Introduction

High power ultrasound induced changes of some physical and chemical properties of molecules are nowadays exploited at an industrial level for food processing and preservation purposes. For example, ultrasound treatments are used to generate emulsions, disrupt cells, tenderise meat, modify crystallisation processes, promote chemical reactions and inhibit enzymes (Chemat, Huma, & Khan, 2011; Mason, Paniwnyk, & Lorimer, 1996). Ultrasound was also shown to induce changes in the physical properties (e.g., viscosity, water binding capacity, etc.) of biopolymers such as pectin, starch and proteins (McClements, 1995). As far as is known, the mechanism of action lies in the rapidly alternating compression and decompression zones propagating into the material being treated and the cavitation that these zones cause. Cavitation involves the formation and collapse of small bubbles, generating shock waves with associated local very high temperatures and pressures, as well as micro-streaming, i.e., micro-currents generated around the bubbles that can catalyse chemical reactions and disrupt animal, plant and microbial cells (Barbosa-Cánovas & Rodriguez, 2002; Leighton, 1995; Mason, 1998). The use of this process to induce structural modification of biopolymers is reported to be highly dependent on the ultrasound intensity and the nature of the material (Seshadri, Weiss, Hulbert, & Mount, 2003; Toubal, Nongaillard, Radziszewski, Boulenguer, & Langendorff, 2003; Vercet, Sànchez, Burgos, Montanes, & López-Buesa, 2002; Wu, Gamage, Vilkhu, Simons, & Mawson, 2008). In particular, the power of the ultrasound may induce opposite effects on macromolecules. For example, pectin, starch or protein-containing systems that were subjected to high ultrasound power have been shown to undergo a viscosity decrease, probably due to depolymerization (Huang, Li, & Fu, 2007; Jambrak, Lelas, Mason, Krešić, & Badanjak, 2009; Price, 1993; Seshadri et al., 2003; Zuo, Knoerzer, Mawson, Kemtish, & Ashokkumar, 2009). On the other hand, a viscosity increase was observed in ultrasound treated food matrices such as tomato puree and yoghurt (Bates, Bagnall, & Bridges, 2006; Krešić, Lelas, Jambrak, Herceg, & Brnčić, 2008; Vercet, Oria, Marquina, Crelier, & López-Buesa, 2002; Vercet, Sànchez et al., 2002; Wu et al., 2008). As has been suggested, ultrasound treatment can give rise to a different type of network, which may be accompanied by an enhancement of rheological properties (Seshadri et al., 2003).

Tomato is a product of great economic importance worldwide. Most of the world's tomato crop is processed into derivatives, such as tomato juice, paste, concentrate and powder (Gould, 1991), that are used for direct consumption or as ingredients for the manufacture of a wide range of formulated foods. Several human studies have indicated a positive relationship between a high intake of tomato products and a decreased risk of chronic and degenerative diseases, such as cardiovascular diseases (Mordente et al., 2011), and some types of cancer (Giovannucci, 1999). The beneficial properties of tomato are often attributed to the antioxidant and anti-inflammatory properties of lycopene (Porrini et al., 2005; Singh & Goyal, 2008). To exert its health effects, lycopene has to be absorbed into the blood stream and reach its site of action. This

* Corresponding author. Tel.: +39 0432 558153; fax: +39 0432 558130.
E-mail address: monica.anese@uniud.it (M. Anese).

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bioavailability is related to the release of lycopene from the tomato matrix, or its bioaccessability. It has been demonstrated that processing operations, such as those involving heat treatment rupture the cell walls, favouring the release of lycopene from tomato chromoplasts and hence enhancing lycopene bioavailability (Gartner, Stahl, & Sies, 1997; Svelander et al., 2010). The eventual presence of an oily phase in food formulations incorporating tomato products would lead to further improvements in lycopene extraction and so increase its levels in human serum (Colle, Van Buggenhout, Lemmens, Van Loey, & Hendrickx, 2012; Stahl & Sies, 1992). Recently, Colle, Van Buggenhout, Van Loey, and Hendrickx (2010) demonstrated that high pressure homogenisation treatments negatively affected the bio-accessibility of lycopene *in vitro*. To our knowledge, no data are available on the effect of ultrasound on lycopene bioavailability.

Therefore, this work was designed to study the effects of ultrasound treatments on the microstructure of tomato pulp and the *in vitro* bioaccessibility of lycopene.

2. Materials and methods

2.1. Sample preparation

Commercial tomato pulp (8.6 ± 0.8% dry matter) was sieved to separate seeds and coarse particles, divided into 130 g aliquots and stored at −18 °C until use. Aliquots of 60 g of the previously thawed samples were introduced into 250 mL (90 mm height, 75 mm diameter) capacity glass vessels and immediately processed with the ultrasound treatments. Unprocessed tomato pulp was taken as a control.

2.2. Ultrasound treatments

An ultrasonic processor (400 W, 24 kHz) (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) with a titanium horn tip diameter of 22 mm was used. Treatments were performed for 15, 30 and 60 min at an ultrasound amplitude of 100 μm and intensity of 105 W/cm^2.

The horn was placed in the centre of the vessel and immersed 5 mm in the tomato pulp. In order to minimise water evaporation during sonication, the vessel was closed with a Plexiglas lid fitted with holes allowing horn and thermocouple probes to be placed at the desired positions in the tomato pulp. During ultrasound treatments, samples were maintained under constant stirring to allow temperature to be as uniform as possible in each sample. The temperature was recorded as a function of time using a copper-constantan thermocouple probe (Ellab, Denmark), connected to a data-logger (CHY 502A1, Tersid, Milano, Italy). Temperature never exceeded 90 °C.

2.3. Extraction of water-soluble pectins

Extraction of water-soluble pectins was carried out using the method of Chou and Kokini (1987). Tomato pulp (60 g) was centrifuged (Beckman, Avant J-25 centrifuge, Palo Alto, California, USA) at 7500*g* for 15 min at 20 °C. The supernatant was filtered under vacuum through filter paper (RPE ACS, Carlo Erba, Milano, Italy). The filtrate was mixed thoroughly with an equal volume of 2-propanol to precipitate the isopropanol-insoluble pectins. After 15 min stirring, the suspended solids in the water-isopropanol mixture were separated using continuous centrifugation at 7500*g* for 15 min at 20 °C. Isopropanol was removed by means of vacuum dehydration (Laborota 4001 Efficient, Hedolph Instruments, Schwabach, Germany).

2.4. Analysis of viscoelastic properties

Viscoelastic properties were analysed at 20 °C constant temperature according to the method described by Vercet, Sànchez et al. (2002). In particular, a Stresstech Rheometer (ReoLogica Instruments AB, Lund, Sweden) equipped with a concentric cylinder geometry (C25) was used.

Dynamic oscillatory tests were performed in a controlled strain mode. Prior to a frequency sweep, a strain sweep was carried out at an angular frequency of 1 Hz to determine the linear viscoelastic range. Then, oscillatory measurements of storage (G′) and loss (G″) moduli, which are the elastic and viscous components of the sample, respectively, were carried out in the frequency range of 0.1–10 Hz using a constant stress amplitude of 0.4 Pa (i.e., in the linear viscoelastic region of the material).

Creep and recovery tests were performed following the method described by Dolz, Hernández, and Delegido (2008). A constant stress amplitude of 0.4 Pa was instantly applied and maintained for a period of 300 s. After load removal, recovery compliance was measured, also over 300 s. Data were expressed as creep (J_C) and recovery (J_R) compliances according to Burger's model.

2.5. Determination of total solids content

Total solids content was determined gravimetrically by drying the samples in a vacuum oven (1.32 kPa) at 75 °C to constant weight.

2.6. Determination of degree of esterification

Degree of esterification (DE) was determined using the titration method of Chou and Kokini (1987), with minor modifications. 10 mL 1% pectin solution were titrated with 0.05 N NaOH (titration A). Then, 20 mL 0.5 N NaOH were added to de-esterify the pectin and, after 30 min, 20 mL 0.5 N HCl were added to neutralise the NaOH. This mixture was titrated with 0.1 N NaOH (titration B), using phenolphthalein as indicator. The degree of esterification (DE), expressed as a percentage, was calculated using the following equation:

$$DE = [B/(A + B) \times 100]$$

2.7. Precipitate weight ratio

Precipitate weight ratio was determined using the method of Colle et al. (2010), with minor modifications. Tomato pulp (25 g) was centrifuged (Beckman, Avant J-25 centrifuge, Palo Alto, California, USA) at 45,000*g* for 30 min at 15 °C. The percentage of precipitate weight ratio of the pellet was calculated as:

$$P = \frac{W_p}{W_t} \times 100$$

where W_p and W_t are the precipitate and tomato pulp weights, respectively.

2.8. Microscopy analysis

Pulp microstructure was visually analysed using an optical microscope (Leica DRMB, Leica Microsystems GmbH, Solms, Germany). The pictures were taken by a digital camera (Leica, ICC50, Solms, Germany) using the software LAS-EZ (Leica, Solms, Germany).

2.9. Determination of lycopene concentration

Lycopene content was determined according to the method of Sadler, Davis, and Dezman (1990). Briefly, 1 g tomato pulp was