PAPER

EFFECT OF HIGH FREQUENCY ULTRASOUNDS ON LYCOPENE AND TOTAL PHENOLIC CONCENTRATION, ANTIOXIDANT PROPERTIES AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF TOMATO JUICE

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ABSTRACT

Tomato juice was subjected to high frequency ultrasounds (378 and 583 kHz) at increasing energy densities (up to 250 MJ/m^3). Results relevant to the treatments at high frequency providing an energy density of 250 MJ/m^3 were compared with those obtained at 24 kHz delivering the same energy density. Lycopene and total phenolic concentration, as well as the α-glucosidase inhibitory activity of tomato juice, were not affected by ultrasound regardless the frequency and energy density. However, the antioxidant properties were negatively affected by high frequency ultrasounds.

Keywords: antioxidant properties, α-glucosidase inhibitory activity, high frequency ultrasounds, tomato juice, total phenolic concentration

1. INTRODUCTION

The consumer's interest towards functional food which claims to have health-promoting or disease-preventing properties is increasing. This has led to the study and development of processes able to preserve the food nutritional and sensory quality as well as to enhance the bioactivity of certain constituents (KNORR *et al.,* 2004).
Amongst these, ultrasound (US) processing, which is a non-thermal technology characterized by low energy consumption, has been found to be suitable for different applications in the food industry. Ultrasound technology is widely used to emulsify, encapsulate ingredients, extract bioactive molecules and inactivate microorganisms (PIYASENA *et al.*, 2003; CANSELIER *et al*., 2002; SORIA and VILLAMIEL, 2010; KENTISH and ASHOKKUMAR, 2011; CHEMAT *et al.*, 2011; MANE *et al.*, 2015).
Ultrasounds can be considered as waves with a frequency range higher than 18 kHz. During the wave propagation into a liquid medium, cavitation phenomenon occurs. In particular, cavitation involves the formation and violent collapse of small bubbles, generating shock waves associated to high local temperatures (1000-5000 K) and pressures (100-50000 bar) inside the collapsing bubbles (LEIGHTON, 1994). These extreme conditions lead to the occurrence of physical phenomena (microject, turbulence, shear forces) and formation of free radicals (GOGATE *et al.*, 2003).
High frequency (100 kHz to 1000 kHz) low power ultrasounds is used for food quality monitoring and diagnostic purposes. However, recent studies have found that free radicals generated by high frequency ultrasounds can react with bioactive compounds and enhance their functionality. In particular, hydroxyl radicals generated during ultrasonication can enhance the hydroxylation degree of food materials and consequently modify their antioxidant activity (ASHOKKUMAR *et al.*, 2008).
Tomatoes, an important agricultural commodity worldwide, are well known for their nutritional properties. In fact, they are a good source of bioactive compounds such as carotenoids and phenolic compounds (GIOVANELLI *et al.*, 1999; LENUCCI *et al*., 2012; ZANFINI *et al*., 2017). Lycopene is the most abundant carotenoid in tomatoes and its chemical structure confers important properties, such as oxygen-radical scavenging and quenching capacity (DI MASCIO *et al.*, 1989; SHI and LE MAGUER 2000; RAO and RAO, 2007). Moreover, phenolic compounds, although present in lower amount, may also contribute to beneficial effects of tomato products, because of their high antioxidant activity and ability to inhibit carbohydrate hydrolysing enzymes, such as α-glucosidase (HANHINEVA *et al.*, 2010; NAIR *et al.*, 2013; TOMAS *et al*., 2017). The latter, located in the brush-border surface of intestinal cells, catalyses the hydrolysis of complex carbohydrates and disaccharides to absorbable monosaccharides (KIM *et al.*, 2010). As a consequence, the α-glucosidase inhibition can suppress the influx of glucose from the intestinal tract to blood vessels resulting in an important strategy in the management of postprandial hyperglycemia (KWON *et al.*, 2008).
Nevertheless, the nutritional value due to carotenoids and phenolic concentration might be modified during processing. In fact, there are several studies dealing with the effect of thermal and non-thermal processing (high pressure homogenization, pulsed electric field and ultrasounds) on bioactive molecules naturally occurring in tomato derivatives in order to obtain fruit and vegetables derivatives able to accomplish desired nutritional functions (COLLE *et al.*, 2010; ODRIOZOLA-SERRANO *et al.*, 2009; ANESE *et al.*, 2015). However, the effect of high frequency ultrasound treatment on tomato properties has been less studied (GOLMOHAMADI *et al.*, 2013). Because of this, the aim of the present study was to evaluate whether the high frequency ultrasounds might modify nutritional functions of the main bioactive compounds in tomato extract. To achieve this aim, the objectives of this work were to investigate the effect of high frequency (378 and 584 kHz)

ultrasounds on bioactives present in tomato (lycopene and total phenolic compounds) and the effects of extracts on the antioxidant properties and α-glucosidase inhibitory activity. Moreover, the treatment was performed for increasing length of time (up to 60 min), thus providing increasing energy densities (up to 250 MJ/m^3). The results obtained by providing the maximum energy density value were compared with those obtained for samples subjected to low frequency ultrasounds (24 kHz). Such investigation represents a preliminary study aimed to understand whether high frequency US could represent a suitable technological tool for steering tomato juice functionality. No industrial application was considered at this stage of the research.

2. MATERIALS AND METHODS

2.1. Sample preparation

Commercial pasteurized tomato juice (7.5 °Brix) was sieved (20 mesh) to separate seeds and coarse particles, and submitted to ultrasound treatment. Tomato juice not subjected to ultrasound treatment was used as a control.

2.2. Sonication

Ultrasound treatments were conducted using two ultrasonic processors operating at either 378 kHz or 583 kHz, and 24 kHz.

2.2.1. Ultrasound treatment at 378 kHz and 583 kHz

An ultrasonic processor (Meinhardt Ultraschlltechnik Leipzig, Germany) equipped with two transducers (operating at 378 kHz and 584 kHz) was used. Aliquots of 250 mL of tomato juice were introduced into a glass reaction vessel (63 mm internal diameter) with a cooling jacket (wall thickness 5 mm) connected to a cryostatic bath (Fisher Scientific, ISOTEMP Thermostatic bath). Samples were subjected to sonication for increasing length of time up to 60 min. During the ultrasound treatment, the temperature never exceeded 20 °C.

2.2.2. Ultrasound treatment at 24 kHz

An ultrasonic processor (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) with a titanium horn tip diameter of 22 mm operating at 24 kHz was used. Aliquots of 100 mL of tomato juice were introduced into 250 mL capacity (110 mm height, 60 mm internal diameter) glass vessels. The tip of the sonicator horn was placed in the centre of the tomato juice, with an immersion depth in the fluid of 50 mm. Sample were subjected to sonication up to 3 min. During the ultrasound treatment the temperature never exceeded 40 °C.

2.3. Determinations

2.3.1. Power and energy density computation

The power density (P_v, W/m^3) transferred from the ultrasounds probe to the sample was determined calorimetrically during preliminary trials by recording the temperature (T, K) increase during the treatment, following eq. (1) (RASO *et al.*, 1999).

$$P_v\,(T) = dc_p(\partial T/\partial t) \quad (1)$$

where d is the sample density (kg/m^3), c_p is the water heat capacity (4186 J/kg K).
The energy density (MJ/m^3) was calculated by multiplying the power density value by the duration of the treatment (HULSMANS *et al.*, 2010).

2.3.2. Lycopene concentration

The lycopene extraction was performed following the procedure of SADLER *et al.*, (1990), with minor modification, under subdued light to prevent carotenoid degradation and isomerisation. Aliquots of 25 mL of extraction solution (hexane:acetone:ethanol, 2:1:1 v/v/v) were added to 1 g of tomato juice. The mixture was stirred at room temperature for 20 min. Reagent grade water (7.5 mL) was added and stirring was continued for 10 min. The hexane phase, containing lycopene, was separated from the polar phase using a separation funnel. Immediately after extraction, the absorbance of lycopene was measured at 472 nm with a spectrophotometer (Thermo Fischer Scientific, Genesys 10S UV/VIS Spectrophotomer) using hexane as reference. The total lycopene concentration was calculated using the Beer-Lambert law, considering the extinction coefficient of lycopene in hexane equal to 1.8 x 10^5 L/mol cm.

2.3.3. Total phenolic concentration

The total quantity of phenolic components was measured by the Folin-Ciocalteau method (SINGLETON and ROSSI, 1965). Aliquots of 10 mL of methanol:water (1:1 v/v) were added to 1 g of tomato juice and the mixture was stirred for 5 min. The solution was filtered (QL10, size 150 mm) and 100 μL was added to 5 ml of a 1:10 dilution of Folin-Ciocalteau reagents and 0.9 mL of distilled water. After 5 min, aliquots of 3.5 mL of Na_2CO_3 (115 g/L) were added and the mixture left in the dark, at room temperature for 2 hours. The absorbance of the solution was measured at 765 nm. The optical density was compared to a standard curve prepared with 0 to 500 mg/L of gallic acid and the results were expressed as mg GAE (gallic acid equivalents)/100 g.

2.3.4. Optical microscopy

Tomato juice microstructure was analysed by using an optical microscope (Leica DM 2000, Leica Microsystems, Heerburg, Switzerland). The pictures were taken by a digital camera (Leica EC3, Leica Microsystems, Heerburg, Switzerland), using the Leica Suite LAS EZ software (Leica Microsystems, Heerburg, Switzerland).

2.3.5. Antioxidant activity

The antioxidant activity was determined following the procedure of BENZIE and STRAIN (1996) determined using ferric reducing antioxidant potential (FRAP). Aliquots of 20 mL of acetone:water (80:20 v/v) mixture were added to 1 g of tomato juice and the mixture was stirred for 5 min. Aliquots of 90 μL of this mixture were added to 3 mL of FRAP solution and incubated in a water bath at 37 °C for 4 min followed by measurement of absorbance at 593 nm against a blank. The optical density was compared to the standard curve for ferrous sulphate ($FeSO_4$) solution, with concentrations between 0 and 1 mM. Results were expressed as FeII (mM) produced/100 g sample.

2.3.6. α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of tomato juice was determined spectrophotometrically (UV-2501PC, UV-Vis recording Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) following the method of SING *et al.*, (2014) with some modifications. The tomato juice was first centrifuged at 2200 g for 3 min at 20 °C. Aliquots of 30 μL of 1 U/mL α-glucosidase in phosphate buffer (100 mM, pH 7.0) were introduced into 1 mL capacity cuvette in the presence of 100 μL of tomato extract and a volume of 100 mM phosphate buffer (pH 7.0), giving a final volume of 900 μL in the cuvette, and mixed thoroughly. After 10 min of incubation at 37 °C, the reaction was started by the addition of 100 μL of 5 mM 4-nitrophenyl- α-D-glucopyranoside (pNGP) solution (Sigma-Aldrich, Milano, Italy) in 100 mM phosphate buffer (pH 7.0) as substrate. The release of p-nitrophenol from pNGP was monitored at 405 nm for 15 min at 37 °C. The changes in absorbance per min were calculated by linear regression, applying the pseudo zero order kinetic model. The eventual stationary phase was excluded from the regression of data. Control samples were run in the absence of tomato extract. The inhibitory activity (*IA%*) of samples on α-glucosidase was calculated according to the following equation:

$$\mathrm{IA\%} = 100 - \left(\frac{k_s}{k_c} \cdot 100\right) \qquad (2)$$

where k_s and k_c are the constant rates (Abs/min) of the enzymatic activity in the presence or in the absence of the inhibitor.

2.4. Data analysis

The results reported here are the averages of at least two measurements carried out on two replicated experiments (n ≥ 4). Data are reported as mean value ± standard deviation. Statistical analysis was performed using R v. 2.15.0 (The R foundation for Statistical Computing). Bartlett's test was used to check the homogeneity of variance, one way ANOVA was carried out and Tukey test was used to determine statistically significant differences among means ($p < 0.05$).

3. RESULTS AND DISCUSSIONS

3.1. Effect of ultrasounds on lycopene and total phenolic concentration of tomato juice

Table 1 shows lycopene and phenolic concentration of tomato juice subjected to high frequency ultrasounds (378 and 584 kHz) with increasing energy density. No differences in lycopene concentration were found among the untreated and ultrasonically treated samples, regardless of the frequency and energy density. The total phenolic concentration was slightly affected by the application of high frequency ultrasounds. A significant increase of the phenolic concentration was observed for the tomato juice subjected to ultrasounds at 378 kHz, providing an energy density of 250 MJ/m^3. However, from a practical point of view, this result does not appear to be relevant in terms of total phenolics recovery. To our knowledge, only GOLMOHAMADI *et al.* (2013) have investigated the effect of high frequency ultrasounds (490 kHz) on the total phenolics in red raspberry puree. They found that ultrasonication did not affect the total phenolic concentration. However, results of GOLMOHAMADI *et al.* (2013) cannot be directly

compared with those obtained in this study, due to discrepancies in the frequencies as well as the lack of information concerning the energy density applied.
Levels of lycopene and total phenolics presented in the tomato juice after treatment at 378 and 583 kHz (250 MJ/m^3) were compared with those obtained at 24 kHz with the same energy density (Table 1). In fact, although the application of low frequency ultrasounds is commonly used in food processing, no data are present in the literature comparing the effect of high and low frequency ultrasounds at the same energy density. It can also be observed that the ultrasound treatment at 24 kHz did not cause any significant change in lycopene concentration. This result is in agreement with previously published data (ANESE *et al.*, 2013) relating to tomato juice ultrasonically treated at 24 kHz with an energy density of 731 MJ/m^3. Moreover, the low frequency ultrasound treatment did not significantly modify the total phenolic concentration of tomato juice. This result is, however, contrary to that of CHEMAT *et al.* (2011) and the discrepancy may be attributed to differences in the process parameters.

Table 1. Lycopene and total phenolic concentrations, antioxidant activity and α-glucosidase inhibitory activity of untreated and ultrasonically treated tomato juice. Data are referred to increasing energy density provided at 24 kHz, 378 kHz and 583 kHz.

Frequency (kHz)	Time (min)	Energy density (MJ/m^3)	Lycopene (mg/g)	Total phenolic (GAE mg/100 g)	Antioxidant activity (FeII mM/100 g)	α-glucosidase inhibitory activity (%)
Untreated	0	0	0.35±0.01^a	192.1±4.0^{bc}	25.0±2.7^a	72±4^a
24	3	250	0.36±0.01^a	193.9±1.0^{bc}	n.d.	74±2^a
378	15	15	0.35±0.01^a	192.9±1.0^{bc}	17.8±0.5^b	n.d.
	60	59	0.35±0.02^a	191.4±1.0^{bc}	15.7±0.3^b	78±2^a
	15	62	0.36±0.01^a	205.0±2.0^{ab}	19.2±0.5^b	n.d.
	60	250	0.37±0.02^a	212.2±6.0^a	18.8±1.1^b	78±2^a
583	15	8	0.35±0.00^a	197.1±3.0^{bc}	18.7±0.6^b	n.d.
	60	31	0.35±0.01^a	195.0±2.0^{bc}	17.8±2.2^b	76±1^a
	15	62	0.33±0.00^a	194.3±3.0^{bc}	19.3±1.8^b	n.d.
	60	250	0.35±0.01^a	187.1±5.0^c	18.7±0.3^b	79±3^a

Means with different letters within the same column are significantly different ($p<0.05$).
n.d.: not determined.

Although the application of high and low frequency ultrasounds did not affect the levels of bioactive components, slight differences in the tomato microstructure were observed. Fig. 1 shows the micrographs of tomato juice subjected to ultrasounds at 24 kHz, 378 kHz, 583 kHz at an energy density of 250 MJ/m^3. When compared to the untreated tomato juice, the low frequency ultrasonically processed samples showed a partial disruption of cell membranes with carotenoids distributed into the matrix. However, no differences between the untreated sample and those processed at 387 and 583 kHz were observed. The differences in the microstructure of samples treated at low and high frequency can be attributed to cavitation phenomena occurring during ultrasound treatment. In fact, during low frequency (24 kHz) ultrasounds, transient cavitation phenomena are responsible for the rapid change in fluid pressure and temperature, which might cause cell wall disruption and carotenoids released. By contrast, microstreaming phenomena are

generated by stable cavitation at high frequency (378 kHz and 583 kHz) ultrasounds; these are reported not to cause dramatic structure changes (MCCLEMENTS, 1995).

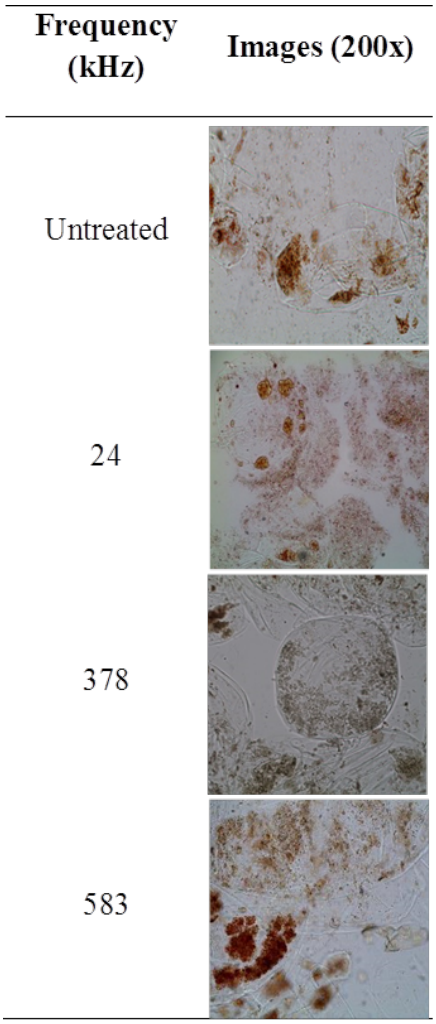

Figure 1. Images of tomato juice subjected to ultrasounds at 24 kHz, 378 kHz and 583 kHz (at 250 MJ/m^3 corresponding to 3 min for ultrasonically treated sample at 24 kHz and 60 min for 378 and 583 kHz ultrasonically treated sample) along with an untreated sample.

3.2. Effect of ultrasounds on antioxidant properties and α-glucosidase inhibitory activity of tomato juice

Table 1 shows the effect of high frequency ultrasounds at 378 kHz and 583 kHz on the antioxidant activity of tomato juice. As compared with the untreated sample, the application of the high frequency ultrasounds caused a significant reduction in the antioxidant activity. This seems to be in contradiction with the levels of lycopene and total phenolic compounds which were affected by ultrasounds. It should be kept in mind that these are not the only compounds with antioxidant properties present in tomato product. For instance, ascorbic acid is a well-known antioxidant and this vitamin has been destroyed by high frequency ultrasounds (GOLMOHAMADI *et al.*, 2013; PORTENLÄNGER and HEUNSIGER, 1992). This result is in contrast with data reported

by ASHOKKUMAR *et al.* (2008) for a phenol model aqueous solution subjected to 358 kHz. The authors attributed the increase in antioxidant properties of the phenolic component in the model solution to the generation of $OH^{\cdot}$ radicals due to the homolysis of water molecules and subsequent phenol hydroxylation. The decrease in antioxidant capacity observed under our experimental conditions may be attributed to the addition of the hydroxyl radicals in a non-preferred position of the aromatic ring. This has previously been observed for a cyanidin 3-glucoside model system (ASHOKKUMAR *et al.*, 2008). These results suggest that controlled hydroxylation is needed to promote an increase in the antioxidant properties of phenolic compounds, which is indeed difficult to achieve. Increasing attention has been recently paid towards the capability of phenolic compounds to control blood glucose levels related to type 2 diabetes (HANHINEVA *et al.*, 2010; LORDAN *et al.*, 2013). In particular, phenolics were found to inhibit digestive enzymes involved in starch breakdown, such as α-glucosidase. Table 1 shows the α-glucosidase inhibitory activity of tomato juice subjected (or not) to ultrasounds at 24 kHz, 378 kHz and 583 kHz at an energy density of 250 MJ/m^3. It can be observed that in our experimental conditions for the untreated sample α-glucosidase inhibition was about 72%. Moreover, no significant changes in α-glucosidase inhibition were found when considering the ultrasound treated samples.

4. CONCLUSIONS

The results of this study showed that ultrasound treatments at 24, 378 and 583 kHz had no effect on major bioactive compounds (i.e. lycopene and total phenolics) as well as on α-glucosidase inhibitory activity of tomato juice regardless the frequency and the energy density. On the other hand, the antioxidant properties appear to be reduced by high frequency ultrasounds. Thus, we conclude that high frequency ultrasound treatments do not seem to effectively alter the bioactive concentration or improve the functionality of tomato juice.

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