Lycopene degradation, isomerization and in vitro bioaccessibility in high pressure homogenized tomato puree containing oil: Effect of additional thermal and high pressure processing

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1. Introduction

In the Western diet, tomato is consumed as one of the most important vegetables (although botanically tomato is a fruit) and is consumed both as fresh fruit and in processed form (puree, soup, and sauce). Tomatoes are rich in lycopene, a carotenoid which is important because of its health related properties. The effect of dietary lycopene in reducing the risk of chronic diseases, such as cancer and coronary heart diseases, has already been indicated in epidemiological studies (Giovannucci, 1999; Rao & Agarwal, 1999). The high antioxidant activity of lycopene, which results from its polyene structure with 11 conjugated double bonds, is the main reason for this health related effect. However, some non-oxidative mechanisms like induction of cell–cell communication and suppression of cell proliferation also contribute to the health related effect of lycopene (Sies & Stahl, 1998). Nevertheless, the system of conjugated double bonds makes lycopene also susceptible to isomerization and oxidation (Rodriguez-Amaya & Kimura, 2004). Oxidation is undesirable because it leads to lycopene degradation and a concomitant loss of its health related properties (Rodriguez-Amaya & Kimura, 2004). On the other hand, lycopene isomerization is desirable because the cis-isomers of lycopene have a higher antioxidant activity (Böhm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002) and are more bioavailable (Boileau, Merchen, Wasson, Atkinson, & Erdman, 1999) compared to all-trans-lycopene. The bioaccessibility and the bioavailability of nutrients are more relevant for the nutritional value of foods than the nutrient concentration. The bioaccessibility is defined as the amount of a nutrient that is released from the food matrix during digestion and made accessible for absorption into mucosa and is usually measured using in vitro digestion procedures. Bioavailability is defined as the fraction of an ingested nutrient that is actually absorbed in the intestine and can be used for metabolic processes and storage (Hedrén, Diaz, & Svanberg, 2002).

Processing can affect lycopene bioaccessibility, lycopene isomerization as well as total lycopene concentration. Thermal processing is a conventional method for food pasteurization and sterilization. High temperatures can however have a detrimental effect on quality related properties such as texture, flavour and colour. Therefore, high pressure processing with or without the addition of heat has been suggested as an alternative method for food preservation (Mertens & Knorr, 1992). High pressure processing can be used for food pasteurization because high pressure can...
inactivate vegetative micro-organisms by damaging the cell membranes. As bacterial endospores are known to be very resistant to high pressure, a combination of high pressure and heat is necessary for high pressure sterilization. On the other hand, quality related properties of fruits and vegetables are in general less affected under high pressure when compared to equivalent thermal processing (Balasubramaniam & Farkas, 2008).

In case of tomato products, it has already been shown that lycopene isomerization and oxidation occur during thermal processing. Nevertheless, this mainly happens at extreme processing conditions or when oil is added (Colle, Lemmens, Tolesa, et al., 2010a; Colle, Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010b). A positive effect of thermal processing, especially at high temperatures or when oil is added (Colle, Lemmens, Tolesa, et al., 2010a; Colle, Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010b) is the possibility to inactivate vegetative micro-organisms by damaging the cell membranes. As bacterial endospores are known to be very resistant to high pressure, a combination of high pressure and heat is necessary for high pressure sterilization. On the other hand, quality related properties of fruits and vegetables are in general less affected under high pressure when compared to equivalent thermal processing (Balasubramaniam & Farkas, 2008).

In the present study, it was investigated how equivalent thermal and high pressure processes at different pasteurization and sterilization intensities affect the lycopene concentration, the isomer content and the lycopene in vitro bioaccessibility of high pressure homogenized tomato puree containing 5% olive oil.

2. Materials and methods

2.1. Sample preparation

Tomatoes (Lycopersicon esculentum cv Patrona, Spain) were washed, dried and cut into slices. The slices were vacuum packed and blanched for 8 min at 95 °C (= hot break) to inactivate structure related enzymes like pectin methyl esterase and polygalacturonase. After blanching, the tomato slices were frozen and stored at −40 °C until use.

For each treatment condition, a new tomato puree (± 1.2 l) was prepared. The frozen tomato slices were thawed, mixed (3 times 5 s) (Büchi Mixer B-400, Flawil, Switzerland) and sieved (pore size 1 mm) to remove seeds and skin. After addition of 5% (w/w) extra virgin olive oil (degree of saturation = 15%, polyunsaturated lipids = 8%), the tomato puree was homogenized at an average pressure of 10 MPa using a high pressure homogenizer (Panda 2K, Gea Niro Soavi, Mechelen, Belgium). The inlet compartment of the homogenizer was kept at 4 °C. Immediately after being homogenized, the puree was cooled in a heat exchanger which was equilibrated at 4 °C.

In addition to this, tomato purees that were not high pressure homogenized (both with and without the addition of 5% olive oil) and a tomato puree which was homogenized at 10 MPa without the addition of oil, were prepared. These purees did not undergo a subsequent thermal or high pressure treatment.

2.2. Dry matter

The dry matter content of the tomato samples was measured gravimetrically. The samples were dried at 100 °C until a constant weight was achieved (Colle et al., 2010b). Analyses were made twice.

2.3. Experimental setup

High pressure homogenized tomato puree with oil was treated at different conditions. Equivalent thermal and high pressure processes at different pasteurization and sterilization intensities were considered. For each treatment condition, a new tomato puree was prepared as described in Section 2.1. After preparation, the puree was divided into 4 parts of which 3 were further treated (triplicate samples at particular treatment conditions) and one was not further treated (= control). For each sample, the in vitro lycopene bioaccessibility, the total lycopene concentration, the concentration of lycopene isomers and the particle size distribution were measured. The results for in vitro lycopene bioaccessibility and total lycopene concentration were expressed relatively to the value of the corresponding control as absolute values varied among the control samples. In Fig. 1, a schematic overview of the experimental setup is given for one particular treatment condition.

2.4. Treatments

2.4.1. Pasteurization

Thermal pasteurization processes were carried out in a temperature controlled water bath at 60 or 90 °C. Stainless steel cylindrical tubes (13 mm internal diameter, 1 mm thickness, 110 mm length) were filled with tomato puree and placed in the water bath. The temperature profile in the centre of a tube was registered using a type T thermocouple connected to a thermocouple box (TR9216, Ellab, Hilleroed, Denmark) and a CMC-92 data acquisition system (Ellab, Hilleroed, Denmark). After the desired process value was obtained (4.3 °C = 1 min or 10 °C = 10 min), the samples were cooled in ice water. Treatments were performed three times.

For high pressure pasteurization processes, a laboratory scale single vessel high pressure equipment (SO. 5-7422-0, Engineered Pressure Systems International, Temse, Belgium) was used. A mixture of 60% DowCaIN in water (Dow Chemical Company, Horgen, Switzerland) was used as a pressure medium. During the treatments, temperature and pressure in the pressure vessel were registered. High pressure processes of 15 min at 450 MPa and 20 °C and of 20 min at 600 MPa and 45 °C were equivalent for the mild and intense thermal pasteurization processes, respectively (see Section 3.1.1). Flexible polyethylene plastic flasks (LDPE, ~100 ml, Medisch Labo Service, Menen, Belgium) were filled with tomato puree and were placed in the high pressure vessel, which was already equilibrated at the process temperature. Pressure was built up automatically to the preset pressure. After the holding time, pressure was manually released to atmospheric pressure and the samples were cooled in ice water. Treatments were performed three times.

2.4.2. Sterilization

Thermal sterilization processes were carried out in a static steri-flow pilot retort (Barriquand, Paris, France) at a process temperature of 117 °C. Both sterilization processes with a process value 10 °C = 1.5 min and with a F0-value = 3 min at the coldest point were performed. Glass jars (90 ml volume, 72 mm height and 40 mm diameter) were filled with tomato puree, leaving a head-space of 1 cm. Temperature profiles of the retort and at the coldest point in the glass jars (3.5 cm above the bottom) were measured using type T thermocouples connected to a thermocouple box.

Fig. 1. Schematic overview of the experimental setup for one treatment condition.
(TR9216, Ellab, Hilleroed, Denmark) and a CMC-92 data acquisition system (Ellab, Hilleroed, Denmark). Tomato puree from glass jars from three different positions in the retort was used for further analysis.

High pressure sterilization was performed in a laboratory scale 6-vessel high pressure equipment (HPIU-10.000, serial No. 95/1994, Resato, Roden, The Netherlands). A propylene glycol fluid (PG fluid, Resato, Roden, The Netherlands) was used as a pressure medium. High pressure sterilization processes were performed at 117 °C and 600 MPa. Polyoxymethylene acetal cylindrical tubes (12 mm internal diameter, 4 mm thickness, 85 mm length) filled with tomato puree were placed in the vessels, which were already equilibrated at process temperature. The temperature in the centre of a tube was measured using a type J thermocouple attached to the pressure vessel stopper. Initially, the temperature of the puree increased as a result of conductive heat transfer. When the puree reached a temperature of 74 °C, pressure was built up to 600 MPa (initial step of 150 MPa followed by a pressure increase at a rate of 10 MPa/s). After a certain holding time, pressure was released, the samples were removed from the vessels and immediately cooled in ice water. Treatments were carried out three times.

2.5. Particle size distribution

The particle size distribution of the samples was measured by laser diffraction using a Malvern Mastersizer S long bench instrument (Malvern Instrument Ltd., Worcestershire, UK). Light from a laser (He–Ne laser, wavelength 633 nm, 18 mm diameter) was shone into a suspension of tomato puree (± 5 g) in water and the scattered light, the particle size distribution was calculated from the intensity distribution of the scattered light, the particle size distribution was calculated described by Hedrén et al. (2002) with major modifications. The particle size distribution of the samples was measured using a Malvern Mastersizer S long bench instrument (Malvern Instrument Ltd., Worcestershire, UK). Light from a laser (He–Ne laser, wavelength 633 nm, 18 mm diameter) was shone into a suspension of tomato puree (± 5 g) in water and the scattered light, the particle size distribution was calculated described by Hedrén et al. (2002) with major modifications.

2.6. Determination of total lycopene content

The extraction of lycopene from the tomato puree was based on the method described by Sadler, Davis, and Dezman (1990) with some modifications. To 1 g tomato puree, 0.5 g NaCl and 50 ml extraction solvent (50% hexane, 25% acetone, 25% ethanol, containing 0.1% BHT) were added. After stirring for 20 min at 4 °C, 15 ml reagent grade water was added and the solution was stirred for another 10 min at 4 °C. The organic phase, containing the carotenoids, was separated from the water phase using a separation funnel.

After filtration (Chromafil PET filters, 0.20 µm pore size – 25 mm diameter, Macherey–Nagel, Düren, Germany), the absorbance of lycopene was measured spectrophotometrically at 472 nm (= λmax for lycopene in hexane). The total carotenoid concentration was calculated using following equation:

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\text{Carotenoid concentration (µg/g) = \frac{A \times \text{volume (ml)} \times 10^4}{E_{1\text{cm}} \times \text{sample weight (g)}}
\]

where \(A\) = absorbance at \(\lambda_{\text{max}}\), \(\text{volume} = \text{total volume of extract,}
\(E_{1\text{cm}}\) = extinction coefficient (3450 for lycopene in hexane (Hart & Scott, 1995)). During the whole procedure, light was excluded as much as possible. Analyses were made three times.

2.7. Determination of lycopene isomers

To measure the lycopene isomer content, the extraction procedure described in Section 2.6, was used. The filtrate was concentrated under vacuum using a rotary evaporator at 30 °C. β-Apo-ß-carotenal was added to calculate the concentration factor. After evaporation, the residue was redissolved in hexane:dichloromethane (4:1).

Lycopene and its isomers were separated and quantified using an HPLC system equipped with a diode array detector (Agilent Technologies 1200 Series, Diegem, Belgium) and a reversed phase C18-column (3 µm × 150 mm × 4.6 mm, YMC Europe, Dinslaken, Germany). During analysis, the autosampler and the column were kept at 4 and 25 °C, respectively. For the separation, linear gradient elution with reagent grade water (A), methanol (B) and methyl-t-butyl-ether (C) was applied. In the first step, the gradient was built up in 5 min from 4% (A), 81% (B) and 15% (C) to 4% (A), 36% (B) and 60% (C) at a flow rate of 1 ml/min. In the second step, the gradient was built up further in another 25 min to 4% (A), 28% (B) and 68% (C). The absorbance was measured at 472 nm (= absorbance of lycopene). As external standards are only commercially available for all-trans- and 5-cis-lycopene, only these isomers could be identified with certainty. Two other peaks were tentatively identified as 9-cis- and 13-cis-lycopene based on their spectral characteristics. Furthermore, some unidentified cis-isomers were taken together in one group (= sum x-isomers). To quantify the concentration of all-trans-lycopene and 5-cis-lycopene, calibration curves of corresponding standards (CaroteNature, Lupsingen, Switzerland) were used. To quantify the concentration of the other cis-isomers, the calibration curve of all-trans-lycopene was used.

2.8. Measurement of in vitro lycopene bioaccessibility

The in vitro lycopene bioaccessibility of tomato puree was measured using the in vitro digestion procedure based on the method described by Oomen et al. (2003), with minor modifications. To 5 g tomato puree, 5 ml NaCl/ascorbic acid solution (0.9% NaCl, 1% ascorbic acid in water) and 5 ml stomach electrolyte (0.3% NaCl, 0.11% KCl, 0.15% CaCl2·2 H2O, 0.05% KH2PO4, 0.07% MgCl2·6 H2O in water) were added. After adding 5 ml pepsin solution (0.5% pepsin from porcine gastric mucosa in electrolyte), the pH of the samples was adjusted to pH 4 ± 0.05, the headspace of the tubes was flushed with nitrogen and the samples were incubated for 30 min at 37 °C while rotating end over end. This step imitates the first part of the digestion in the stomach. Eating a meal results in a temporary increase of the pH as a result of diluting effects of the ingested food (Oomen et al., 2003). Afterwards, the pH was adjusted to pH 2 ± 0.05, the headspace of the tubes was flushed with nitrogen again and the samples were incubated for another 30 min at 37 °C. This step simulates the second part of the digestion in the stomach, when the pH is decreased again as a result of the excretion of HCl. The passage through the small intestine was simulated by adjusting the pH to 6.9, adding 3 ml pancreatin/bile solution (0.4% porcine pancreas pancreatin, 0.2% porcine pancreas lipase, 2.5% porcine bile extract, 0.5% pyrogallol, 1% 5-tocopherol in water), flushing the headspace with nitrogen and incubating the samples for 2 h at 37 °C. After incubation, the samples were filtered under vacuum (Macherey–Nagel 615, paper filter, 90 mm diameter, Macherey–Nagel, Düren, Germany) to remove the non-digested particles. The lycopene concentration in the filtrate was then measured using the spectrophotometric method described in Section 2.6. Analyses were made three times.

2.9. Data analysis

SAS software (SAS 9.2) was used to statistically analyze the results of the in vitro lycopene bioaccessibility, the total lycopene concentration and the lycopene isomer concentration. The mean value of three independent treatments was calculated. A mixed model with one fixed factor (treatment condition) and one random factor (repetition of treatment) and with a Tukey correction for pairwise comparison was used for statistical comparison of the
means of the in vitro lycopene bioaccessibility and the total lycopene concentration. Statistical analysis of the lycopene isomer concentration was performed using two factor ANOVA. Significant differences among the means were analyzed using the Tukey test. In both statistical tests, the significance level was set at $\alpha = 0.05$.

3. Results and discussion

3.1. Process conditions

3.1.1. Pasteurization

Both a mild and an intense pasteurization process were performed. The purpose of a mild pasteurization is to achieve a microbiologically safe product. For acid foods (e.g. tomatoes), a 5 log reduction of Salmonella is necessary to reach this microbiological safety (FDA, 2004). For the mild thermal pasteurization, a process value of $10^{3.6} \text{C}_{0.0-0.1} = 1 \text{ min} (\text{D}_{0.0-0.1} = 0.2 \text{ min} \text{ for Salmonella enteritidis (Breeuwer, Lardeau, Peterz, & Joosten, 2003)})$ was chosen. A process of 15 min at 450 MPa and 20 °C was chosen as an equivalent high pressure process. These conditions also induce a 5 log reduction of S. enteritidis (Cheffel, 1995).

An intense pasteurization is necessary to achieve a microbiologically stable product with a shelf life of some weeks at 5 °C. A process value of $10^{10} \text{C}_{0.0-0.1} = 10 \text{ min}$ was chosen. These conditions induce a 6 log reduction of non-proteolytic Clostridium botulinum type E spores (ECFF, 2006). By extrapolation from available literature data (Reddy et al., 1999), a process of 20 min at 600 MPa and 45 °C was chosen as an equivalent high pressure pasteurization process.

3.1.2. Sterilization

Sterilization processes are applied in order to produce shelf stable products by inactivating spores rather than only vegetative micro-organisms. A sterilization process with a $F_d$-value of 3 min (= process that results in a 12 log reduction of proteolytic C. botulinum type A spores) is generally recognized as a safe minimum public health sterilization value for low acid food products (Pflug & Odlaug, 1978). However, as the pH of the product decreases, the minimum public health sterilization $F_d$-value decreases too. Pflug, Odlaug, and Christensen (1985) listed the minimum $F_d$-value for public health as a function of pH for pH values between 4.6 and 7. In the present study, both a sterilization process with a $F_d$-value of 1.5 min (corresponds to pH 4.9) and a conventional sterilization process with a $F_d$-value of 3 min were aimed for.

High pressure sterilization was performed at 600 MPa and no additional effect of high pressure on the inactivation of C. botulinum spores was considered because synergy between temperature and high pressure was not consistently observed among strains of C. botulinum or among products (Bull, Olivier, van Diepenbeek, Kormelink, & Chapman, 2009). Therefore, high pressure sterilization processes were designed to be thermally equivalent to $F_d$-values of 1.5 and 3 min.

In Fig. 2, the temperature profiles of the retort and of the tomato puree during thermal sterilization and of the tomato puree during high pressure sterilization are presented for sterilization with a $F_d$-value of 3 min. Similar profiles were obtained for sterilization with a $F_d$-value of 1.5 min.

In the temperature profile of the retort, 3 different phases can be distinguished. Initially, the temperature of the retort was raised to the process temperature (117 °C) during the coming-up phase. During the holding phase, the temperature of the retort was held at the process temperature for a certain time (19.5 and 24.3 min for thermal sterilization treatments with $F_d$-value of 1.5 and 3 min, respectively). Finally, the temperature of the retort was decreased again during the cooling phase. During thermal sterilization, the temperature of the tomato puree increases by a combination of conduction and convection which are rather slow processes. Due to these heat transfer limitations, the temperature of the tomato puree increased much more slowly compared to the retort and the process temperature of 117 °C was never reached. At the end of cooling, $F_d$-values of 1.53 ± 0.01 min and of 2.92 ± 0.09 min were reached.

The high pressure sterilization processes began with a pre-heating phase followed by the pressure build up. When the pressure was built up to 600 MPa, the temperature of the tomato puree increased very fast to the process temperature of 117 °C as a result of adiabatic heating. Due to the fast heating up, the holding phase of the high pressure sterilization was much shorter (1.7–3 min or 4.5–10 min in order to reach the desired $F_d$-value of 1.5 min and 3 min, respectively), in comparison with the thermal sterilization. The obtained variability in the holding times within each high pressure sterilization process is a consequence of small differences in the temperature reached after pressure build up. Finally, the cooling of the samples was almost immediately as a result of adiabatic decompression. For the different high pressure treatments, $F_d$-values of 1.53 ± 0.06, 1.49 ± 0.11, 1.53 ± 0.12 min and of 3.01 ± 0.06, 3.05 ± 0.18, 3.03 ± 0.12 min were reached.

3.2. Effect of processing on particle size distribution of tomato purees

In Fig. 3A, the particle size distribution of untreated tomato puree is given. Most of the particles have a diameter between 100 and 800 μm. A very small peak around 10 μm (see Fig. 3B) represents the oil droplets present in the tomato puree. The parameters $d(v,0.1)$, $d(v,0.5)$ and $d(v,0.9)$ are 152 ± 6, 408 ± 10 and 675 ± 17 μm, respectively.

In literature, the average diameter of a tomato cell has been estimated from 350 up to 1000 μm (Lopez-Sanchez, Svelander, Bialek, Schumm, & Langton, 2011; Redgwell, Curti, & Gehin-Delval, 2008). Consequently, after the preparation of the tomato puree (= hot break followed by high pressure homogenization at 10 MPa), most of the particles are present as single or broken cells. The largest sizes correspond to clusters of maximum 2 cells. Lopez-Sanchez et al. (2011) showed that high pressure homogenization of a tomato puree at a pressure of 10 MPa is enough to separate the cells and break some of the cells into smaller fragments.

None of the subsequent thermal or high pressure processes had an effect on particle size distribution or on the different parameters (results not shown).

3.3. Effect of processing on health related properties of tomato purees

3.3.1. Effect on total lycopene concentration

The preparation of the starting puree (= addition of oil + homogenization at 10 MPa) did not have an effect on the total lycopene concentration (results not shown). The total lycopene concentration of the different processed tomato purees expressed relatively to the total lycopene concentration of the corresponding control sample (= untreated tomato puree) is represented in Fig. 4. The relative concentration of the control samples equals 100%. The different pasteurization processes did not affect the total lycopene concentration. For the thermal pasteurization processes, this can be explained by the low treatment temperatures and short treatment times applied. The thermal stability of lycopene in tomato puree during conventional thermal pasteurization processes has repeatedly been shown (Hsu, 2008; Kребbers et al., 2003; Sanchez-Moreno, Plaza, de Ancos, & Cano, 2006). Additionally, Colle et al. (2010b) did not observe a significant decrease in total lycopene content of tomato purees treated at temperatures below 130 °C.
Qiu, Jiang, Wang, and Gao (2006) did not observe either a significant change in total lycopene concentration of tomato purees after treatments of 12 min at pressures of 400 or 600 MPa at 20 °C whereas an increase in the extractability of lycopene from tomato puree after high pressure processing has also been described in literature (Hsu, 2008; Sánchez-Moreno et al., 2006). The increase in lycopene extractability in high pressure treated tomato purees, observed in the latter studies, possibly results from an incomplete extraction of lycopene from the untreated tomato puree by the extraction method used.

In contrast to pasteurization, sterilization of the tomato purees resulted in a significant decrease in total lycopene concentration of 20% up to 30%. No significant differences in lycopene loss between all sterilization conditions were observed. The decrease can be explained by lycopene degradation due to oxidation. Oxidation occurred at a temperature of 117 °C and after short treatment times, which can be explained by the presence of oil in the tomato samples. Crystalline lycopene can be solubilized in the oil which makes it more susceptible to degradation. At temperatures above 100 °C, Colle et al. (2010a) also observed oxidative degradation of lycopene during thermal processing of an olive oil/tomato emulsion.

### 3.3.2 Effect on lycopene isomers

The preparation of the starting puree did also not result in formation of cis-isomers of lycopene (results not shown). In Table 1, the concentration of the different lycopene isomers expressed as percentage of the total lycopene concentration is given for the untreated tomato puree and for the tomato purees after different thermal and high pressure processes at different pasteurization and sterilization intensities.

On average, the untreated tomato purees contained 88 ± 19 mg/100 g DM total lycopene of which 85.43 ± 2.59% was present in the all-trans-form. All-trans-lycopene is known to be thermodynamically the most stable form (Rodríguez-Amaya & Kimura, 2004). This result is similar to the result of Colle et al. (2010b) who found that 89% of the total lycopene present in untreated tomato pulp is all-trans-lycopene. In untreated tomato purees, 3 lycopene cis-isomers could be identified as 5-cis-lycopene (4.73 ± 1.2%), 9-cis-lycopene (2.77 ± 1.34%) and 13-cis-lycopene (1.97 ± 0.76%). Additionally, 5.10 ± 0.85% of lycopene was present as unidentified cis-isomers.

Mild pasteurization (both thermal and high pressure) and intense high pressure pasteurization did not significantly affect the relative concentration of lycopene cis-isomers except for a significant increase in relative 5-cis-lycopene concentration after intense high pressure pasteurization. Actually, the process temperature for all these treatments was too low (<60 °C) for isomerization to be significant. Similar to the present results, Qiu et al. (2006) observed no significant difference in percentage of 13-cis-lycopene between untreated tomato puree and tomato purees processed during 12 min at pressures from 100 to 600 MPa and 20 °C. On the other hand, Varma, Karwe, and Lee (2010) noticed a small but non-significant increase in total cis-lycopene concentration after high pressure processing of a tomato homogenate during 3 min at pressures from 310 up to 620 MPa and ambient temperature.

![Fig. 2. Temperature profiles of the retort (dashed line) and of the tomato puree (black line) during thermal sterilization and of the tomato puree (grey line) during high pressure sterilization. Both processes have a \( F_0 \)-value of 3 min.](image)

![Fig. 3. (A) Particle size distribution of a tomato puree after high pressure homogenization at 10 MPa with 5% added olive oil without any subsequent thermal or high pressure treatment. (B) Detail of the particle size distribution at very small particle sizes (= oil droplets).](image)
In contrast to the intense high pressure pasteurization, a significant increase in the relative concentration of all cis-isomers (except 5-cis-lycopene) was observed after intense thermal pasteurization and the change in the percentage of 13-cis-lycopene was the most pronounced. As stated before, lycopene is normally quite thermostable, but the oil in the tomato puree probably makes lycopene more sensitive to isomerization. Similar to the present results, Colle et al. (2010a) also found a remarkable increase in 13-cis-lycopene concentration after a thermal treatment of a tomato puree with 5% olive oil during 10 min at 90 °C whereas the change in the other isomers was less pronounced.

After all sterilization processes, a significant decrease in the relative all-trans-lycopene concentration and a simultaneous increase in the relative total cis-lycopene concentration were observed which were more pronounced for the more intense sterilization processes (F0 = 3 min). Nevertheless, less isomers were formed during the high pressure sterilization processes compared to the thermal sterilization processes. The shorter process time for high pressure sterilization compared to thermal sterilization can be a possible explanation. Another hypothesis is that high pressure retards the isomerization reaction. Gupta, Kopec, Schwartz, and Balasubramaniam (2011) hypothesized that pressure possibly favours the formation of compact lycopene aggregates, which might inhibit the conversion of all-trans-lycopene to non-linear cis-isomers. As the cis-isomers of lycopene are more bioavailable than all-trans-lycopene, it seems that high pressure processing cannot have an advantage over thermal processing. However, very limited information about the effect of high pressure on isomerization of carotenoids is currently available.

When looking at the different cis-isomers individually, the same trends as described above can be observed. Thermal sterilization and longer treatment times resulted in higher individual cis-isomer concentrations compared to high pressure sterilization and shorter treatment times, respectively. Only 5-cis-lycopene seems to behave differently. Whereas high pressure sterilization resulted in an increase in the relative 5-cis-lycopene concentration, a small but non-significant decrease in relative concentration due to thermal sterilization was observed. The degradation of 5-cis-lycopene during thermal processing of tomato puree has already been shown in literature (Colle et al., 2010a). On the other hand, it seems that high pressure favours the formation of 5-cis-lycopene.

Of the different cis-isomers, 9-cis-lycopene increased the most (up to 8 times), followed by 13-cis-lycopene (up to 6.4 times) but the formation of 13-cis-lycopene was already more pronounced at pasteurization conditions compared to 9-cis-lycopene which was mainly formed at sterilization conditions. The relative concentration of 13-cis-lycopene already increased 5 times after the intense thermal pasteurization but only a slight difference between the relative concentration after intense thermal pasteurization and after thermal sterilization, a large increase in the relative concentration was observed. This can be explained by the fact that the rotational barrier for conversion of all-trans- to 13-cis-lycopene is smaller than that for the conversion of all-trans- to 9-cis-lycopene (Guo, Tu, & Hu, 2008). Colle et al. (2010a) observed similar results in a kinetic study of lycopene isomerization and degradation during thermal processing of an olive oil/tomato emulsion.
3.3.3. Effect on in vitro lycopene bioaccessibility

The lowest amount of bioaccessible lycopene was found in tomato purees that were not high pressure homogenized (both with and without the addition of 5% olive oil). High pressure homogenization without oil could improve the in vitro lycopene bioaccessibility. In addition to this, high pressure homogenization in combination with 5% olive oil resulted in the highest amount of bioaccessible lycopene (397 ± 74 μg/g DM). Tomato puree homogenized at 10 MPa with 5% olive oil was chosen as a starting puree (= control) for subsequent thermal and high pressure treatments. In Fig. 5, the in vitro lycopene bioaccessibility expressed relatively to the in vitro lycopene bioaccessibility of the corresponding control sample was given. The relative in vitro lycopene bioaccessibility was not affected by the different pasteurization processes. After thermal and high pressure sterilization processes at different intensities, a significant decrease in the relative in vitro lycopene bioaccessibility was observed. This means that the in vitro lycopene bioaccessibility of high pressure homogenized tomato puree with oil can be decreased during subsequent thermal or high pressure processing, especially during intense thermal or high pressure processing. The latter decrease can be explained by the decrease in the relative total lycopene concentration due to lycopene oxidation during the different sterilization processes (see Section 3.3.1). When the relative in vitro lycopene bioaccessibility was expressed to the relative total lycopene concentration, no more differences between the untreated and treated tomato purees were observed.

In literature, the positive effect of processing on the lycopene bioavailability in tomato products has been observed in several studies (Gärtner, Stahl, & Sies, 1997; Porrini, Riso, & Testolin, 1998). Nevertheless, often the combined effect of different processing steps has been investigated. The current study has on the one hand proved the positive effect of high pressure homogenization in combination with the addition of oil and on the other hand, it has investigated the effect of thermal and high pressure processing as such for the first time. The results clearly showed that the positive effect of high pressure homogenization in combination with oil on the lycopene bioaccessibility can be counterbalanced by the negative effect of subsequent intense thermal processes inducing lycopene degradation via oxidation. Hereby, the intensity of the thermal process seemed to be an important factor and high pressure sterilization processes were proved to have a similar effect as their equivalent conventional thermal processes. Discrepancies between the results obtained in this study and results of Colle et al. (2010b) suggesting the positive effect of intense thermal processing on lycopene bioaccessibility in tomato products lead to the hypothesis that the solubilization of lycopene in the oil prior to thermal processing is a critical factor in the observed negative effect on lycopene concentration as well as bioaccessibility.

4. Conclusion

When comparing equivalent thermal and high pressure pasteurization and sterilization processes of high pressure homogenized tomato puree with olive oil, it could be concluded that total lycopene concentration was not affected during pasteurization processes as result of the low temperatures and short treatment times. During sterilization processes, total lycopene concentration significantly decreased due to lycopene oxidation. No significant differences between thermal and high pressure sterilization processes were observed.

The relative total cis-lycopene content was significantly affected during processing. For thermal processing, the increase in total cis-lycopene content was already significant after an intense pasteurization process whereas in case of high pressure processing, sterilization conditions were required to significantly increase total cis-lycopene concentration. Moreover, significantly less cis-isomers were formed during high pressure sterilization compared to thermal sterilization. The shorter treatment time for high pressure sterilization or inhibition of isomerization by high pressure might possibly explain this observation. For the individual cis-isomers, the same trends could be observed. Moreover 9-cis-lycopene was formed in the highest concentration whereas 13-cis-lycopene was already formed at less intense conditions compared to 9-cis-lycopene. In contrast to this, the concentration of 5-cis-lycopene decreased during thermal sterilization whereas it increased during high pressure sterilization.

The in vitro lycopene bioaccessibility of a high pressure homogenized tomato puree containing oil was decreased during subsequent thermal or high pressure processing. The decrease was significant for all sterilization processes studied. It was hypothesized that lycopene was transferred to the oil phase by high pressure homogenization, making it very susceptible to degradation and causing a decrease in absolute lycopene bioaccessibility values. These results can be of great importance for the bioaccessibility of industrially processed tomato based products for which a preservation process is generally applied to obtain microbiologically safe products.
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