Research Article

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Quality changes in high hydrostatic pressure treated enriched tomato sauce

Furkan Erdem,[a] Ozan Tas,[a] Nursen Erol,[b] Mecit Oztop[a] and Hami Alpas[a*]

Abstract

BACKGROUND: Use of high hydrostatic pressure (HHP) with reduced processing times is gaining traction in the food industry as an alternative to conventional thermal treatment. In order to enhance functional benefits while minimizing processing losses, functionalized products are being developed with such novel techniques. In this study, changes in quality parameters for HHP treated enriched tomato sauce were evaluated, with the aim to assess its viability as an alternative to conventional thermal treatment methods.

RESULTS: HHP treatments at 500 MPa, 30 °C/50 °C significantly increased the total phenolic and lycopene content of the sauce samples, achieving 6.7% and 7.5% improvements over conventionally treated samples. The antioxidant capacity of the HHP-treated samples was also found to match or be better than conventionally treated samples. Furthermore, a T_2 relaxation time study revealed that pressure–temperature processing treatments were effective in maintaining the structural integrity of water molecules. Microbiological analyses revealed that 500 MPa/50 °C 5 min treatment can offer 8 logs reduction colony formation, matching the results of conventional thermal treatment.

CONCLUSION: Combined pressure–temperature treatments improve results, reduce time consumption. 500 MPa/50 °C treatments provided retention of quality parameters and significant reduction in microbial activity.

Keywords: high hydrostatic pressure; tomato sauce; plant proteins; Mediterranean diet

INTRODUCTION

Tomatoes (*Solanum lycopersicum*) are valued crops that are consumed either raw or in the form of processed products.[1] According to the latest Food Agricultural Organization (FAO) data, tomato production worldwide has nearly doubled since 1993 in terms of area harvested, and its agricultural value has nearly increased ten-fold.[2,3] Consuming them raw or as processed products like tomato juice, sauce, or ketchup has made them valuable for obtaining carotenoids and other beneficial nutrients.[4]

Similarly, olives have attracted significant attention from researchers due to their robust antioxidant properties and positive health impact.[5] By-products such as pits or pomace from olive-oil production have been used to produce powders.[6] However, due to processes they are subjected to and their nutritional properties, such powders are generally non-ideal products. In this context, researchers have explored the production of olive-based powders directly from the fruit to preserve the nutraceutical compounds, increase antioxidant capacity and phenolic compound presence.[7] By incorporating these olive powders into various food products, the health benefits associated with olives can be maintained, and consumer expectations regarding healthier foods can be met.

Due to sustainability concerns, there has been a shift away from animal-based proteins. Pea protein isolates play a crucial role in meeting the demand for sustainable, plant-based protein. With their high nutritional value and eco-friendly production, they offer a viable solution for individuals seeking healthier and more environmentally conscious dietary options.[8] Pea proteins offer a healthy alternative due to low-allergenicity, high nutritional value and ease of availability. Recent trends in pea protein studies generally focus on the effect of processing on the protein isolates themselves without considering the effects of processing in a food system.[8-10]

However, conventional treatment may have negative impacts on quality parameters due to long treatments times and high temperatures, accompanied by high operating costs.[11] In contrast, novel processing technologies may offer improved product quality, with significantly reduced time and energy costs.[11,12] High hydrostatic pressure (HHP) processing is one such method

* *Correspondence to: H Alpas, Middle East Technical University, Department of Food Engineering, Univ. Mah. No. 1, Ankara, 06800, Türkiye. E-mail: imah@metu.edu.tr*

a *Department of Food Engineering, Middle East Technical University, Ankara, Türkiye*

b *Kraft Heinz, Balıkesir, Türkiye*

with increasing industrial applications, where products are subjected to pressures up to 800 MPa at as low as room temperature to inactivate microorganisms and improve nutritional properties.[1,13-15] Pressure processing has been used to evaluate the structural changes in protein gels in the past,[8,16,17] however no studies evaluating pea protein in the tomato matrix have been found.

The purpose of this study was to evaluate the changes in functionally improved tomato sauce samples enriched with pea protein and olive powder for nutritional improvements before and after processing with conventional treatment and combined pressure–temperature processing. Quality of enriched sauce (FS) samples were compared in terms of rheology, phenolic and lycopene content, antioxidant capacity and microbial inactivation. Time domain nuclear magnetic resonance (TD-NMR) experiments in the form of T_2 relaxation times were also measured in relation to changes in water mobility and how water is held within the food matrix to interpret the changes occurred with different processing methods.

MATERIALS AND METHODS

Materials

Roma tomato variety was supplied by Kraft Heinz (Bursa, Türkiye). Raw tomatoes were subjected to hot-break process before being produced into enriched sauce. After de-peeling, hot-breaking was conducted at 85 °C for 3 min using Termomix by Vorwerk (Worverk & Co., Wuppertal, Germany). After hot-breaking, samples were cooled in an ice bath, and kept at −72 °C until further processing. The control sample used in this study was untreated enriched sauce.

Tomato peel powder samples were obtained by drying (Klarstein Fruit Jerky 9; Berlin Brands Group, Berlin, Germany) of the waste peels leftover from juice production at 55 °C for 2 days. Olive powder samples used in the study were sourced directly from freeze-dried table olives.[7] Pea protein isolate was sourced from Vegrano (Lucca, Italy).

Chemicals

Folin–Ciocalteu reagent, sodium carbonate, potassium persulfate, tryptic soy agar/broth, MacConkey agar, yeast extract (Millipore, Darmstadt, Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)) (Sigma-Aldrich, Steinheim, Germany) were supplied from respective sources.

Production of standard tomato juice (RT) and preparation of enriched tomato sauce (FS)

Frozen, hot-break tomatoes were thawed at room temperature before each treatment. Samples were sieved twice (500 μm) to remove unwanted parts to obtain a homogenous mixture. Samples were stored in a refrigerator at 4 °C until they were produced into enriched sauce.

Preliminary testing was conducted to identify suitable pea protein and olive powder concentrations in terms of impact on viscosity and color properties. Tomato peel powder was added to counter-balance the undesirable color caused by the addition of light-colored pea protein and olive powder, where best concentration was found as 4% (*w/w*) (unpublished data). Samples were prepared by mixing 100 g of standard tomato juice with 2% olive powder, 2% pea protein, and 4% tomato peel powder (*w/w*). Mixture was then high-shear homogenized (IKA T18, Staufen, Germany**)** at 10 000 rpm for 3 min, and kept at 4 °C until treatments.

Basic nutritional composition of the samples, as well as pH, moisture content, and water activities of the ingredients are provided in Table 1 and Table 2.

Conventional thermal treatment

Processing parameters for conventional treatment were sourced from a local industrial partner (Kraft Heinz, Bursa, Türkiye). Conventional treatment was conducted at 94 °C (temperature of the coldest point) with 10 min come-up time and 20 min for microbial inactivation. During the treatment, temperatures of the samples were regularly monitored, and flasks were shaken periodically to stimulate homogenous temperature rise. At the end of the treatment, samples were immediately chilled in an ice-water bath and kept in a refrigerator at 4 °C until analyzed.

High hydrostatic pressure (HHP) treatments

HHP treatments were conducted in the high-pressure system (760.0118 type; SITEC-Sieber Engineering AG, Zurich, Switzerland) equipped with a 100 mL pressure chamber surrounded by a heat jacket, where water was used as the pressure and heat transmitting medium. Samples were pressure treated at 300, 400, and 500 MPa at 30, 40, and 50 °C for 5 min. The system was designed to accommodate two 25 mL polyethylene cryotubes (LP Italiana, Milan, Italy), stacked on top of each other. Before the treatment, samples were transferred with no air bubbles present in the tubes. Samples were then brought to treatment temperature using a hot-water bath. Compression rates were 340 MPa/min and pressure release times were less than 10 s for all conditions, and thus were excluded from total treatment time.

Analyses

Chemical composition

Gross chemical composition analyses were performed on untreated standard tomato juice and enriched tomato sauce samples. Crude protein was determined using the Kjeldahl method following AOAC Official Method 2001.11. Fat analysis was

Table 1. Water activity, pH value, and moisture contents of the ingredients present in enriched tomato sauce (FS)

Name	Water activity	pH	Moisture content
Tomato juice (RT)	0.981 ± 0.007	4.24 ± 0.02	94.967 ± 0.490
Enriched sauce (FS)	0.912 ± 0.005	4.20 ± 0.01	89.802 ± 0.425
Pea protein	0.297 ± 0.001	5.61 ± 0.06	5.811 ± 0.047
Olive powder	0.118 ± 0.001	3.71 ± 0.07	4.105 ± 0.055
Tomato peel powder	0.332 ± 0.002	4.24 ± 0.12	7.084 ± 0.083

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Table 2. Proximate composition of standard tomato juice (RT) and enriched sauce (FS) products (%, dry basis)

Name	Tomato juice (RT)	Enriched sauce (FS)
Fat	2.561 ± 0.161	23.770 ± 0.993
Crude protein	20.708 ± 1.288	25.789 ± 0.448
Carbohydrates	71.670 ± 1.443	45.051 ± 1.097
Ash	5.065 ± 0.632	5.392 ± 0.127

conducted using Soxhlet extraction, adhering to AOAC Official Method 920.39. Moisture content was assessed according to AOAC Official Method 925.10, and ash content was measured using AOAC Official Method 942.05.[18] Total carbohydrates were determined by 'difference' method, where crude protein, fat, ash, and moisture was subtracted from the total weight according to the instructions provided in AOAC Official Method 2020.07. Water activity of the samples was determined using a water activity analyzer (AQUALAB 4TE; Aqualab, Pullman, WA, USA) model water activity meter. The pH values of the samples were determined using a laboratory pH meter (FiveEasy Plus; Mettler Toledo Columbus, OH, USA).

Preparation of hydrophilic extracts

Extracts were prepared using methods from the literature with slight modifications.[13,19] Approximately 10 g of enriched sauce sample was centrifuged at 6000 × *g* for 15 min at 4 °C and filtered through Whatman No. 1 filter paper. The pellet was mixed with 10 mL 80% methanol and vortexed for 60 s before being centrifuged at 6000 × *g* for 15 min. Supernatants were then combined to obtain the extract.

Determination of total phenolic content

Total phenolic contents (TPCs) were analyzed with Folin–Ciocalteu method, with slight modifications.[19] Briefly, 500 µL of extract was mixed with 500 µL of Folin–Ciocalteu reagent and was left to sit for 3 min. Next, 10 mL of 20% sodium carbonate solution was added, and the mixture was allowed to stand for 1 h in the dark. The absorbance of the samples was measured at 725 nm with a spectrophotometer (Optizen Pop; Mecasys, Daejeon, Republic of Korea). Absorbance results were compared with a calibration line built with the concentrations of 0, 10, 25, 50, 75, 100, and 150 mg GAE/100 mL ($R^2 = 0.997$). Results were expressed as milligram gallic acid equivalent (GAE) per 100 mL of sample.

DPPH assay

DPPH assay was conducted using methods from the literature with slight modifications.[20] Briefly, 0.1 mL aliquot of extract was mixed with 3.9 mL of methanolic DPPH$^{\bullet}$ solution prepared by dissolving 0.1 mmol/L DPPH in 80% methanol, and was allowed to sit in the dark for 30 min at room temperature. Absorbance values were measured in a spectrophotometer at 517 nm against a blank of 80% methanol solution. Changes were evaluated based on the retention of absorbance at $t = 0$ and $t = 30$ min, and presented in terms of maximum scavenging capacity (SC_{max}).

$$SC_{max} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (1)$$

ABTS assay

ABTS assay was performed using methods from the literature with slight modifications.[1] To obtain an $ABTS^{\bullet+}$ solution with a long shelf-life, 7 mmol/L ABTS solution was mixed with 2.45 mmol/L potassium persulfate and incubated in a dark room for 16 h. After incubation, the mixture was adjusted to absorbance of 0.7 ± 0.01 measured at 734 nm. The mixture was kept in the refrigerator at 4 °C until use and made fresh, weekly. Approximately 25 µL of sample was added to 4 mL of $ABTS^{\bullet+}$ reagent and was allowed to stand in the dark for 5 min. Absorbance was measured at 734 nm against the blank reagent.

$$SC_{max} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (2)$$

Extraction and quantification of lycopene

Extraction and quantification of lycopene were performed immediately after the treatments using methods from the literature with slight modifications.[21] Immediately before the analyses, 100 g of sample was high-shear homogenized at 10 000 rpm for 3 min. Next, 100 mg of sample was mixed with 8 mL of 2:1:1 hexane–ethanol–acetone mixture, vortexed vigorously and then left to stand for 30 min. After 30 min, 1 mL of distilled water was added into the mix, vortexed, and let sit for 10 min. After 10 min, the upper layer was read at 503 nm, where hexane–ethanol–acetone mixture was used as blank. Lycopene content of the samples (in mg/kg sample) was then determined using the following formula.

$$\text{Lycopene content} = \frac{Abs_{503} \times MW_{lyc} \times R \times V}{m_{sample} \times F} \quad (3)$$

where Abs_{503} is the absorbance of sample at 503 nm, MW_{lyc} is the molecular weight of lycopene, R is the volumetric ratio of the upper layer compared to whole, V is the volume of hexane–ethanol–acetone mixture, m_{sample} is the weight of sample, and F is the molar constant for lycopene in hexane (172 L/mmol).

Rheological properties of enriched sauce samples

To obtain rheological parameters of the system, steady shear experiments were conducted. Rheological measurements were performed in bob and cup rheometer (Brookfield RST; Ametek Inc., Middleboro, MA, USA).

Samples were homogenized before the analyses and let to sit for 10 min to allow the sample to restore its structural network. Steady shear experiments in the range of 100 to 1000 1/s for minimal noise and clear output with 240 s duration and 20 data points were conducted at room temperature (maintained at 22 °C). With 5 min waiting period, each sample was measured twice. Rheological parameters of the samples were evaluated using OriginPro 2019 (OriginLab Corporation, Northampton, MA, USA) and fit to Herschel–Bulkley model.

Assessing hydration dynamics in tomato sauce using TD-NMR

The T_2 relaxation times were measured using a 0.5 Tesla (20.34 MHz) benchtop TD-NMR system (Spin Track; Resonance Systems GmbH, Kirchheim/Teck, Germany). Briefly, 400 mg of sample was placed in NMR tubes 10 mm diameter, and T_2 relaxation times of the samples were measured using

Carr–Purcell–Meiboom–Gill (CPMG) sequence with 4000 μs echo time, 700 echoes, 16 points, and 8 scans. Obtained data were fitted mono- and bi-exponentially using XPfit (Soft Scientific, Tirat Carmel, Israel). Analysis was conducted with five replicates.

Microbiological analysis

Escherichia coli O157:H7 ATCC 43888 (provided by Food Safety Laboratory, METU, Ankara, Türkiye) was used in this study. *Escherichia coli* O157:H7 is a known food pathogen and has been shown to be relatively pressure resistant.[22,23] The strains were cultivated in tryptic soy broth (Millipore, Merck, Bedford, MA, USA) with 0.6% yeast extract (Millipore, Merck) and transferred to a fresh broth every 2 days.

Samples were inoculated with approximately 4.5×10^8 CFU/mL of *E. coli* O157:H7 harvested from early stationary phase after an 11 h incubation period at 37 °C for maximum pressure resistance.[24] Samples were subjected to 500 MPa treatments at 30, 40, and 50 °C for 5 min, and also conventional treatment at 94 °C for 30 min (0.1 MPa). MacConkey and tryptic soy agars (0.6% yeast extract) were used as selective and non-selective agar medium. Samples were serial diluted using buffered peptone water and spread plated onto selective and non-selective agar plates at 0.1 mL volume, and incubated at 37 °C for 18–24 h and 36–48 h for non-selective and selective agars, respectively. Moreover, 1 mL of undiluted samples were plated on agar plates to obtain a minimum detection limit of colonies to approximately 30 CFU/mL. Pressurization and plating were conducted in duplicates.

Statistical analysis

Data obtained from the study were processed using Minitab 19 (Minitab LLC, State College, PA, USA). Significance of difference between combined pressure–temperature treatments was compared via two-way analysis of variance, and comparisons for the means of treatment factors were evaluated with Tukey's test at 95% confidence level ($P < 0.05$). All analyses were conducted in three replicates unless stated otherwise.

RESULTS AND DISCUSSION

Effect of combined pressure–temperature treatments on total phenolic content (TPC)

Changes in TPC values of FS samples are provided in Table 3 and Fig. 1. Quantity of total phenols in untreated standard tomato juice (RT) and tomato sauce (FS) samples were 21.044 ± 0.479 and 38.994 ± 0.359 mg GAE/100 g sample, where standard tomato juice conformed with previous studies.[19,25] The 50 °C pressure treated samples had the highest TPC amongst all samples ($P < 0.001$), likely due to intensified cell wall disruption at that temperature. Conventional treated samples retained their phenolic content and did not show a significant difference to untreated samples ($P > 0.05$).

Relative resistance of polyphenols to the treatments was documented by past studies.[26] While the impact of pressure alone was found to be non-significant ($P > 0.05$), combined effect of pressure–temperature was significant ($P < 0.05$). Non-significant impact of elevated pressures was also documented for tomato and other fruit juices.[12,27]

Changes in antioxidant capacities of samples

Antioxidant capacities of hydrophilic extracts determined with $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays are presented in Table 3.

Both pressure and temperature had significant effects on the antioxidant capacities of samples ($P < 0.001$). While pressure increase led to higher antioxidant capacities regardless of assays, temperature increase had a negative impact; the highest antioxidant capacities were observed at 500 MPa and 30 °C. Detrimental impact of increased temperatures was also seen for other materials such as orange juice and aqueous extracts.[28,29]

Conventionally treated samples had shown significant loss in inhibition with $ABTS^{\bullet+}$ assay. Antioxidant capacity of combined pressure–temperature treated samples were comparable to untreated and conventionally treated samples with 30 °C pressure treatments. While high temperatures and pressures favor cell wall disruption, it may also enable the release of oxidative enzymes. With 94 °C treatment, these enzymes would be completely inactivated.[30]

Despite having higher TPC values, 50 °C treatments had significantly lower antioxidant capacities ($P < 0.05$), which indicates reduced impact of phenolics on antioxidant capacity with increased contribution of other antioxidative compounds like vitamins and fatty acids. It is possible that 50 °C treatments negatively impacted the compounds interacting with these radicals, as increased cell wall disruption would increase release of oxidative enzymes, despite the hot-break process. Enzymatic inactivation of several enzymes with pressure treatments were shown to increase just after 400 MPa.[14,15,31] Pressurization was expected to positively impact antioxidant capacity of olive-based additive, as well as on pea protein isolate.[9,32]

Lycopene content

Results of enriched sauce (FS) and untreated standard tomato juice are provided in Table 3 and Fig. 2. As olive powders used in this study are made from table olives themselves, oil content is completely preserved and was anticipated to increase lycopene availability due to lycopene's hydrophobic properties.[33] Protein addition was expected to aid the incorporation of non-polar and liposoluble components, like lycopene, into surrounding lipid droplets and increase extractability.[34]

Highest and lowest lycopene contents found in pressure–temperature treated samples were 50 °C/300 MPa (150.14 ± 1.094 mg/kg sample) and 30 °C/300 MPa (114.52 ± 2.380 mg/kg sample). Including thermal, none of the treatments resulted in higher lycopene content when compared to untreated samples ($P < 0.05$).

Data observed from this study for lycopene content of untreated standard tomato juice (RT) samples are conformed by several studies where lycopene content of Roma tomato was reported as 70.9 mg/kg sample and 65–70 mg/kg.[15,19] Past studies also noted that the change in lycopene content between raw tomato, unprocessed tomato juice, and hot-break tomato products were non-significant.[4,15,35] Pressure increase led to significant loss of lycopene ($P < 0.05$), except for 30 °C treatments were pressure change was found to be non-significant ($P > 0.05$). Decrease in lycopene content with increasing pressures is also reported in the literature[15,36] and is explained by preventative effect of pressure on protein thermal denaturation. It is accepted that increasing temperatures have a positive impact on the availability of lycopene in tomatoes due to modification of components bound with carotenoids.[14,30,37] Considering that protein structure is bound to carotenoids, decreased disruption of these bonds could lower extractability of lycopene in the sample.[14]

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Table 3. Phenolic contents, maximum scavenging capacities, and lycopene contents of standard tomato juice (RT) and untreated or treated enriched sauce (FS)

Tomato Sample	TPC (mg GAE/100 g sample)	SC_{max} (% loss of $DPPH^{\bullet}$)	SC_{max} (% loss of $ABTS^{\bullet+}$)	Lycopene content (mg lycopene/kg sample)
Untreated RT	21.044 ± 0.479	75.627 ± 1.015	80.315 ± 1.012	77.43 ± 3.060
Untreated FS	38.994 ± 0.359^{b,c}	89.333 ± 0.743^{a}	89.428 ± 1.01^{a,b}	154.10 ± 5.160^{a}
94 °C/30 min	37.540 ± 0.343^{c,d}	86.880 ± 0.261^{a,b}	87.318 ± 0.215^{c,d}	139.72 ± 3.030^{c,d}
300 MPa/30 °C	36.853 ± 0.121^{d}	83.719 ± 0.152^{c,d}	88.464 ± 0.001^{b,c}	114.52 ± 2.380^{f}
400 MPa/30 °C	37.091 ± 0.199^{d}	85.790 ± 1.021^{b,c}	89.473 ± 0.456^{a,b}	116.56 ± 1.920^{f}
500 MPa/30 °C	37.513 ± 0.692^{c,d}	86.358 ± 1.842^{a,b,c}	90.306 ± 0.158^{a}	119.81 ± 1.307^{e,f}
300 MPa/40 °C	36.601 ± 0.421^{d}	84.952 ± 1.245^{b,c}	84.826 ± 0.289^{e,f}	139.39 ± 1.355^{c,d}
400 MPa/40 °C	36.923 ± 0.186^{d}	86.202 ± 0.729^{b,c}	86.080 ± 0.543^{d,e}	135.59 ± 0.583^{d}
500 MPa/40 °C	36.676 ± 0.599^{d}	87.372 ± 0.323^{a,b}	87.237 ± 0.254^{c,d}	124.73 ± 1.259^{e}
300 MPa/50 °C	41.638 ± 0.351^{a}	77.638 ± 0.438^{f}	83.146 ± 0.585^{g}	150.14 ± 1.094^{a,b}
400 MPa/50 °C	40.999 ± 0.929^{a}	78.506 ± 0.610^{e,f}	83.966 ± 0.070^{f,g}	145.81 ± 0.296^{b,c}
500 MPa/50 °C	40.176 ± 0.759^{a,b}	80.765 ± 0.182^{d,e}	86.051 ± 0.354^{d,e}	142.91 ± 1.301^{c,d}

Note: Different letters in the same column represents significant difference ($P \leq 0.05$). TPC, total phenolic content; SC_{max}, maximum scavenging capacity.

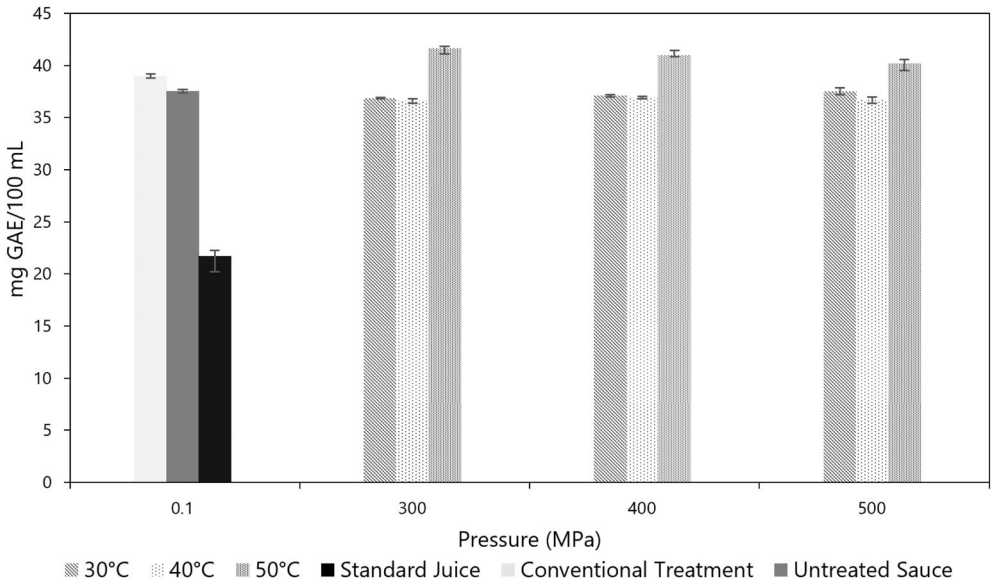

Figure 1. Total phenolic contents of processed and unprocessed samples. (▨) 30 °C, (⁘) 40 °C, (▩) 50 °C pressure treatments of enriched sauce, (□) conventional thermal treated sauce, (■) untreated sauce, (■) untreated standard juice. Error bars presented are standard deviations of three replicates.

Rheological changes in enriched sauce (FS) samples

Previous research indicates that the Herschel–Bulkley model can sufficiently describe tomato products, and therefore no other models were tested in this study.[38-40]

As seen from Table 4, all samples exhibited shear thinning behavior with a flow behavior index (*n*) less than 1. Yield stresses increased with temperature decrease, possibly due to the compounding effect of the additives, while consistency coefficients decreased with increased pressures. Several studies emphasize a loss of rigidity and consistency in protein gels at lower concentrations (up to 8 g protein/100 g solution) when subjected to increased pressures.[8,16,41,42] Pressure application can be seen to cause a decrease in pseudoplasticity, and thus lead to consistency loss and increased flow behavior indices.[8,16,42] However, presence of free calcium ions in tomato were observed to cause the formation of gel like structures.[43] With the complex matrix present in sauce samples, binding of these ions and their interaction with the additives could have led to a decrease in consistency. It is likely that changes in rheological parameters were due to structural changes occurring with added pea protein.

Changes in relaxation spectra and T_2 relaxation times

The T_2 measurements provide insights into water content, water–macromolecule interactions, and water binding within the sample.[44-46] Loss of water mobility in the cell wall increases proton exchange, reducing T_2 relaxation times. In plant cells, shorter T_2 times are linked to water bound in the cell wall, while longer T_2 times relate to cytoplasm and vacuoles with more free or mobile water.[47,48]

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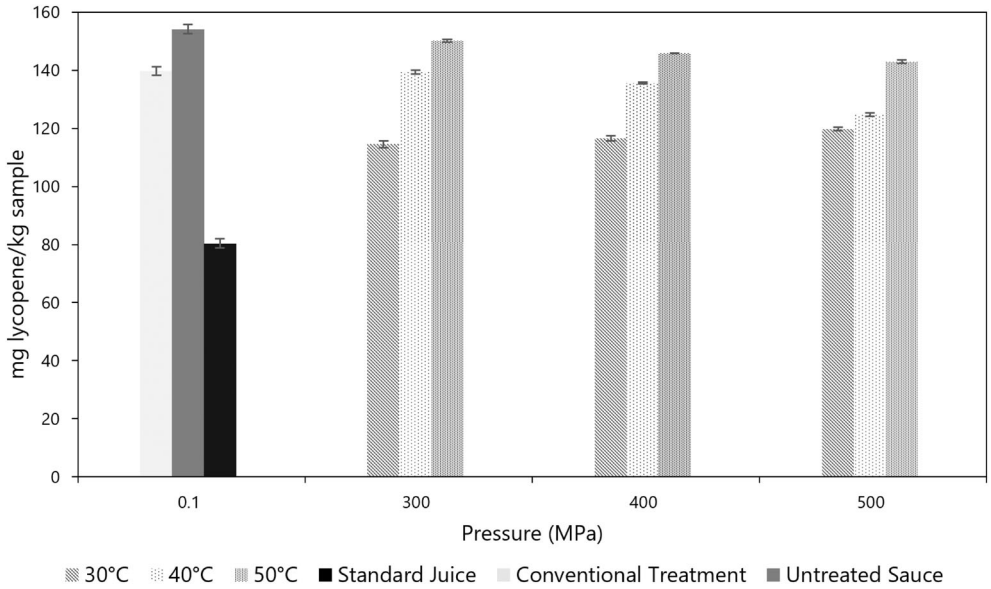

Figure 2. Available lycopene in the processed and unprocessed samples. (▨) 30 °C, (⁘) 40 °C, (▩) 50 °C pressure treatments of enriched sauce, (▩) conventional thermal treated sauce, (▩) untreated sauce, (■) untreated standard juice. Error bars presented are standard deviations of three replicates.

Table 4. Rheological properties of standard tomato juice (RT) and untreated or treated enriched sauce (FS)

Tomato sample	K (Pa s^n)	n	Yield stress (Pa)
Untreated RT	$0.031 \pm 0.0022^{b,c}$	0.795 ± 0.0146^{a}	$5.379 \pm 0.270^{b,c}$
Untreated FS	1.004 ± 0.0629^{b}	0.547 ± 0.0078^{f}	14.583 ± 0.238^{g}
94 °C/30 min	1.214 ± 0.0213^{a}	$0.552 \pm 0.0041^{e,f}$	$17.576 \pm 0.629^{d,e}$
300 MPa/30 °C	1.005 ± 0.0204^{b}	$0.552 \pm 0.0148^{e,f}$	$19.576 \pm 0.942^{b,c,d}$
400 MPa/30 °C	$0.709 \pm 0.0187^{c,d}$	$0.602 \pm 0.0196^{d,e}$	$14.928 \pm 0.267^{f,g}$
500 MPa/30 °C	$0.473 \pm 0.0132^{e,f,g}$	$0.653 \pm 0.0101^{b,c,d}$	$16.764 \pm 0.413^{e,f}$
300 MPa/40 °C	0.757 ± 0.0387^{c}	$0.599 \pm 0.0190^{d,e}$	$19.901 \pm 0.88^{b,c}$
400 MPa/40 °C	$0.595 \pm 0.0462^{d,e}$	$0.628 \pm 0.0161^{c,d}$	$16.656 \pm 0.176^{e,f,g}$
500 MPa/40 °C	$0.374 \pm 0.0133^{f,g}$	$0.695 \pm 0.0153^{a,b}$	$17.734 \pm 0.912^{d,e}$
300 MPa/50 °C	0.522 ± 0.0435^{e}	$0.608 \pm 0.0259^{c,d}$	$20.338 \pm 0.94^{a,b}$
400 MPa/50 °C	$0.470 \pm 0.0453^{e,f}$	$0.657 \pm 0.0171^{b,c}$	22.159 ± 0.441^{a}
500 MPa/50 °C	0.322 ± 0.0241^{g}	0.717 ± 0.0260^{a}	$18.057 \pm 1.105^{c,d,e}$

Note: Different letters in the same column represents significant difference ($P \leq 0.05$). For all samples the R^2 value was at least 0.99.

Table 5 presents the mono- and bi-exponential T_2 times of the samples. Mono-exponential analysis provides information on compounding impact of components on T_2, and therefore water status. Bi-exponential analysis would identify individual components. A representative output of the bi-exponential analysis is given in Fig. 3. The T_2 values of mobile molecules are influenced by cytoplasts, solids, and their interactions, as the T_2 value of free water would be north of 1000 ms.[48]

The addition of pea protein and olive powder led to a significant reduction in T_2 times, as anticipated ($P < 0.05$). Peak 1 is likely attributed to the proton–proton interactions of the cytoplasm and bound water, while Peak 2 is associated with proton–proton interactions between free water and other components. The addition of pea protein and olive powder resulted in increased binding of free water and a higher magnitude and impact of dry components. The T_2 times of thermally treated samples were significantly shorter likely due to increased proton interactions ($P < 0.05$), as the Brix value of the samples did not change after treatments (unpublished data). Increased pressures led to an overall decrease in T_2 times ($P < 0.05$). This could be attributed to the addition of proteins, whose ability to hold water was seen to increase with increasing pressure levels and particularly with 500 MPa/50 °C treatment.[7,16,49] The pressure treatment's impact on T_2 times was mostly insignificant when compared to untreated sauce samples, suggesting that there were limited alterations in water mobility. This observation implies that the changes in rheological parameters were not primarily influenced by changes in water mobility but rather by structural modifications in the added compounds.

Microbiological impact of the treatments

As 500 MPa treatments generally yielded better results compared to other pressure treatments, microbial inactivation analyses were conducted with 500 MPa chosen as a viable processing pressure.

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Table 5. Time domain nuclear magnetic resonance (TD-NMR) T_2 relaxation times of standard tomato juice (RT) and enriched sauce (FS)

Tomato sample	Mono-exponential T_2 (ms)		Bi-exponential T_2 (ms)	Relative area (%)
Untreated RT	444.050 ± 34.00	Peak 2	499.27	81.77
		Peak 1	194.50	18.23
Untreated FS	188.31 ± 4.810[b]	Peak 2	252.95	50.07
		Peak 1	101.42	49.93
94 °C/30 min	171.24 ± 1.419[d]	Peak 2	224.18	52.78
		Peak 1	97.27	47.22
300 MPa/30 °C	190.233 ± 0.950[b]	Peak 2	264.37	57.37
		Peak 1	94.33	42.63
400 MPa/30 °C	180.8 ± 1.229[c]	Peak 2	258.63	53.60
		Peak 1	95.73	46.40
500 MPa/30 °C	186.567 ± 0.945[b,c]	Peak 2	264.50	55.40
		Peak 1	95.60	44.60
300 MPa/40 °C	189.933 ± 0.651[b]	Peak 2	258.80	59.03
		Peak 1	93.99	40.97
400 MPa/40 °C	186.367 ± 1.002[b,c]	Peak 2	260.83	56.20
		Peak 1	91.87	43.80
500 MPa/40 °C	186.767 ± 0.681[b,c]	Peak 2	267.50	53.80
		Peak 1	91.87	46.20
300 MPa/50 °C	197.73 ± 2.810[a]	Peak 2	264.40	59.97
		Peak 1	88.28	40.03
400 MPa/50 °C	186.03 ± 3.040[b,c]	Peak 2	260.77	55.27
		Peak 1	88.22	44.73
500 MPa/50 °C	172.93 ± 3.150[d]	Peak 2	251.97	48.63
		Peak 1	92.79	51.37

Note: Different letters in the same column represents significant difference ($P \leq 0.05$).

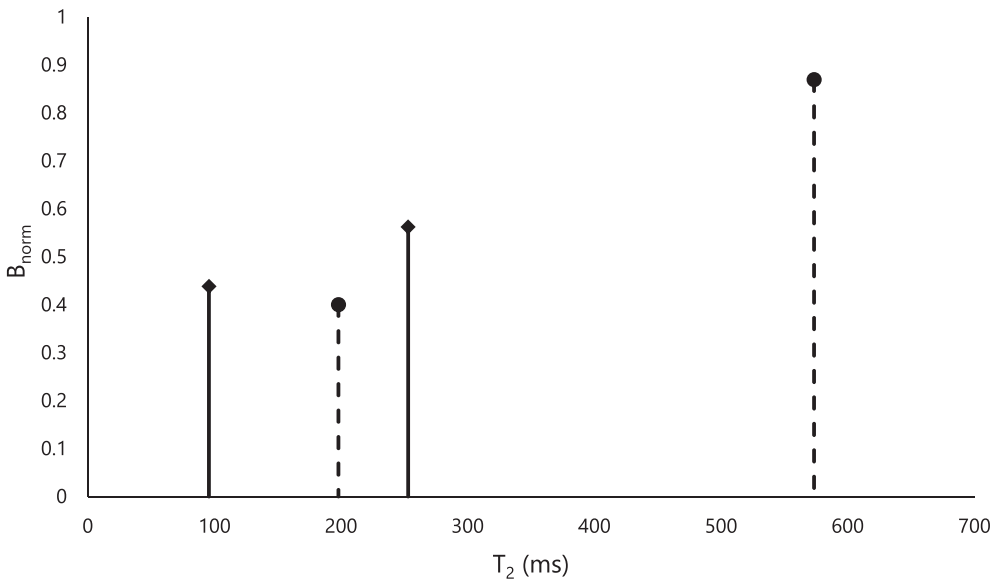

Figure 3. Discrete analysis of components present in standard tomato juice (dashed line) and reformulated sauce (constant line).

The 500 MPa treatments are also anticipated to be marginally better at microbial inactivation compared to 300 and 400 MPa treatments.[22,23]

Combined pressure–temperature treatments enhanced log reduction of viable cells, particularly at higher temperatures (Table 6). Previous studies use non-selective agar to indicate cell injury and selective media for more challenging growth conditions, reflecting realistic scenarios.[50] Ideal conditions are indicated by colonies forming on non-selective media.

Conventional treatment resulted in foreseeably complete inactivation of bacteria. Samples treated with at least 40 °C had shown minimum of 5 log CFU/mL reductions in microbial load, and 50 °C pressure treatments yielded results comparable to conventional treatment, where no colonies were seen.

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Table 6. Microbiological analyses of the inoculated samples

Tomato sample	Colony formation (non-selective/selective)	Viable cells in non-selective media (log CFU/mL)	Viable cells in selective media (CFU/mL)
Enriched sauce			
Untreated	+/+[†]	8.65	
90 °C/30 min	–/–	ND[‡]	ND
500 MPa/30 °C	+/+	4.47	2.32
500 MPa/40 °C	+/–	3.30	ND
500 MPa/50 °C	–/–	ND	ND

[†] Presence (+) and absence (–) of colonies on respective non-selective and selective agar.
[‡] ND, not detected. No microorganism growth was observed.

CONCLUSIONS

Compared to conventional treatment (94 °C, 30 min), HHP treatments yielded similar or better results depending on the temperature (30, 40, 50 °C) treatment. Enriched sauce samples treated at 500 MPa had higher TPC, ABTS, DPPH, and lycopene values than conventional treatment at 30 or 50 °C, with TPC and lycopene content increasing up to 6.7% and 7.5%. In the case of pasteurization, 500 MPa treatment achieved 8 log reductions in microbial load when treated at 50 °C. Non-significant changes in T_2 times indicate that consistency loss was due to structural changes.

The 5 min, 500 MPa HHP treatments of enriched tomato sauce allowed quality parameters to be preserved at levels comparable to conventional treatments. Conservation of nutritional value using HHP with the fraction of time costs present in conventional treatments demonstrates the efficiency and use-case of HHP treatments.

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DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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