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Fermentation of tomato juice improves *in vitro* bioaccessibility of lycopene

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ABSTRACT

The impact of fermentation (*Saccharomyces cerevisiae* ATCC 9763) on the bioaccessibility of lycopene in a model tomato juice was examined. The physicochemical and structural properties of the tomato tissue were determined after fermentation and the bioaccessibility of lycopene was monitored using a simulated gastrointestinal tract. A lycopene concentration of 45.1 mg/100 g was obtained under optimal fermentation conditions. The cell walls of the tomato cells were hydrolyzed and disrupted by fermentation. Cell disruption decreased the pectin content and reduced the tissue fragment size, thereby reducing gravitational separation and facilitating lycopene release. The lycopene bioaccessibility in the tomato juices increased in the following order: unfermented (8.5%) < fermented (11.4%) < unfermented-emulsified (13.6%) < fermented-emulsified (22.7%). These effects were attributed to a combination of greater tomato tissue disruption and enhanced mixed micelle formation. Our results may be useful for the development of functional foods and beverages with improved health benefits.

1. Introduction

China has become the world's largest tomato producer and consumer, with a large market for its products in all regions of the country, as well as in the rest of the world (Nisha, Singhal, & Pandit, 2011). Tomatoes contain various bioactive agents such as polyphenols, carotenoids, and vitamins, which may have a positive impact on human health and wellbeing due to their antioxidant, hypolipidemic, and anticarcinogenic activities (Salehi et al., 2019). Tomatoes, and the products derived from them, may therefore be a good source of health-promoting nutraceuticals and micronutrients. Processed tomatoes are versatile food ingredients that can be used in a broad range of products, including tomato juice, ketchup, salsa, sauces, and soups (Bayod, Willers, & Tornberg, 2008; Porretta, 2019). A variety of processing technologies have been employed to extend the shelf life of these products, to improve their appearance, texture or taste, or to enhance their nutritional attributes (Rojas, Alvim, & Augusto, 2019). For instance, tomato products may be thermally processed, homogenized, and/or fermented to alter their characteristics. Previous studies have shown that fermentation can be used to improve the level of healthy compounds in various fruits and vegetables, as well as to hydrolyze anti-nutrients, thereby enriching the nutritional value of foods (Septembre-Malaterre, Remize, & Poucheret, 2018). However, knowledge of the impact of fermentation on the nutritional value of tomatoes products is currently limited.

Lycopene is one of the most bioactive components found in tomatoes. It is an isoprene compound with 11 conjugated double bonds and 2 non-conjugated double bonds, which belongs to the carotenoid family (Liang, Ma, Yan, Liu, & Liu, 2019). It is relatively abundant in certain types of plant cells such as those found in tomato, watermelon, and guava (Lu et al., 2019; Müller, Caris-Veyrat, Lowe, & Böhm, 2016). In plant cells, lycopene is present in a solid crystalline deposition form (Cooperstone et al., 2015). Before it can be absorbed by the human body, lycopene must be released into the gastrointestinal fluids from these plant tissue structures. Alcoholic or lactic acid fermentations can increase the absorption of lycopene, which is likely due to disruption of the plant tissue facilitating its extractability (Mapelli-Brahm et al., 2020). It has been reported that after yeast fermentation, the cellular structure of tomato is partially disrupted, and the fraction of nutrients released is significantly increased (Bartkiene et al., 2019). Therefore, fermentation may have a positive impact on lycopene extractability of tomato products, which will affect its bioaccessibility. The development of a successful fermentation process requires optimization of processing parameters such as temperature, time, and inoculum level (Hernández-Almanza, Montañez-Sáenz, Martínez-Ávila, Rodríguez-Herrera, & Aguilar, 2014).

The objective of our study was to use response surface design to identify the optimum fermentation conditions required to disrupt the

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structure of tomato cells and release the lycopene, thereby increasing its bioaccessibility. To achieve this goal, the physical and chemical properties of fermented tomato juice exposed to different processing treatments were compared in terms of their ability to improve lycopene release and bioaccessibility. Emphasis was given on elucidating the physicochemical mechanisms impacting lycopene bioavailability. This study therefore provides valuable new information about the benefits of fermentation on improving the potential nutritional value of tomatoes and other products.

2. Materials and methods

2.1. Materials

Tomato powder was acquired from Xi'an Tianrui Bio-Tech (Xi'an, China). *Saccharomyces cerevisiae* ATCC 9763 was obtained from Jinan Meiluwei Biotechnology Technology Co., Ltd. (Jinan, China). Lycopene (> 90%) was purchased from Shanghai Yuanye Bio-Tech (Shanghai, China). Peanut oil was purchased from Shandong Luhua Group Co., Ltd. (Shandong, China). Lipase (30–90 units/mg), mucin, bile salts, and pepsin (250 units/mg) were purchased from the Sigma–Aldrich Company (St. Louis, MO, USA). Glucose and absolute ethanol were provided by Tianjin Zhiyuan Chemical (Tianjin, China). Peptone was supplied by Beijing Aoboxing Bio-Tech (Beijing, China). Ammonia was purchased from Xilong Chemical (Sichuan, China), and other solvents were supplied by Guangdong Huaguang Tech (Guangdong, China). Water purified by a Millipore Milli-Q System (Millipore SAS, Molsheim, France) was used for all the experiments.

2.2. Response surface design

2.2.1. Extraction and quantification of lycopene

The lycopene content in the fermentation liquid was measured using a method described previously (Ilahy, Hdider, Lenucci, Tlili, & Dalessandro, 2011). Briefly, lycopene was extracted using a mixture of hexane/ethanol/ acetone (2/1/1 by vol.) with an ultrasonic oscillator to ensure the lycopene was completely dissolved. The absorbance of the lycopene-loaded solvent solutions was then measured at 502 nm using a UV–visible spectrophotometer (UV-1240, Shimadzu, Japan). The concentration of lycopene was calculated by referring to a standard curve prepared under the same conditions.

2.2.2. Yeast culture and fermentation conditions

The yeast *S. cerevisiae* ATCC 9763 was maintained on Peptone Dextrose Agar slants at 4 °C. Active cultures were prepared by transferring a cell loop from the agar slant into a test tube containing 5 mL of liquid Sabouraud medium. The culture was then incubated overnight at 30 °C with gentle agitation to go through the logarithmic growth phase for 12–16 h. 100 g of tomato powder was dissolved in 370 mL distilled water, the optical density at 600 nm (OD600) of the ATCC 9763 was adjusted to 1.0 cm^{-1} to ensure the yeast was in a stable period of fermentation (Zhou, Sun, Bei, Zahi, & Liang, 2018), where the product formation rate is the highest. Then, the single factor test of temperature, time, and incubation quantity was carried out to identify the optimum levels for each variable. Firstly, the effect of fermentation temperature on lycopene content was analyzed at 15, 20, 25, 30, 35, and 40 °C with a fixed fermentation time of 12 h, and yeast incubation quantity of 6% (v/v). The effect of fermentation time on lycopene content was analyzed using 0, 12, 24, 36, 48, 60 and 72 h incubation times at a fixed fermentation temperature of 25 °C and yeast incubation quantity of 6% (v/v). Finally, the impact of yeast incubation quantity on lycopene content was studied at 3, 4, 5, 6, 7, 8% (v/v) using a fixed fermentation temperature of 25 °C and fermentation time of 12 h.

2.2.3. Experimental design to obtain optimal fermentation conditions

Optimal fermentation conditions were established by the response surface design (Chuyen, Roach, Golding, Parks, & Nguyen, 2017) using commercial software (Design Expert 8.0, Stat-Ease, Inc., Minneapolis, MN). Initially, single-factor experiments were carried out using six fermentation-related parameters (liquid volume, pH, incubation quantity, fermentation temperature, incubator rotating speed, and fermentation time) based on previous literature reports. Three factors were then selected for subsequent experiments based on the significant differences observed in the preliminary experiments: fermentation time, fermentation temperature, and yeast quantity. Three levels were used for each factor to optimize the fermentation process. Each variable was tested in three coded levels: −1, 0 and +1, and the results of the Response Surface Design model were judged using a significance test as described previously. Therefore, a 3-level 3-factor Box-Behnken test protocol was carried out. The range of fermentation times, temperatures, and yeast quantities selected were based on previous studies that have reported that this yeast will grow under these conditions.

2.3. Analysis of lycopene release

2.3.1. Cell morphology observation

The microstructure of the tomato juice was observed using an optical microscope (Motic BA400, Xiamen, China) at a magnification of 400-times, and photographs were taken using a digital camera (Bayod & Tornberg, 2011). Samples were diluted 10-fold with deionized water, a drop was evenly spread on a glass slide, and then the images were acquired. Each sample was analyzed multiple times to obtain representative images.

2.3.2. Detection of pectin content

The pectin content of the tomato tissue was determined by UV–visible spectrophotometry (Bi et al., 2017). Briefly, the pectin in the sample was precipitated with absolute ethanol and hydrolyzed to galacturonic acid, which was then reacted with oxazole in sulfuric acid to form a magenta compound that has a maximum absorption at 525 nm. The pectin content of the test samples was then determined using a standard curve prepared under the same conditions using a range of pectin levels.

2.4. Analysis of physicochemical properties of fermented products

2.4.1. Particle size

The tomato juice samples were diluted 1000-fold with deionized water and then the particle size was measured using a laser particle size analyzer (Nano-ZS90, Malvern Instruments, Malvern, UK) at 25 °C (Rojas, Leite, Cristianini, Alvim, & Augusto, 2016). This instrument is based on dynamic light scattering and determines the particle size from the measured intensity-time relationship of the scattered light. Each individual measurement was the average of 13 runs.

2.4.2. Sedimentation index

Samples were transferred into 10 mL test tubes and stored at 25 °C for 12 days. The sedimentation index (IS) were calculated from the ratio of the volume of the sediment (V_S) to the total volume of the sample (V_T) according to the following formula:

$$IS\% = V_S/V_T \times 100 \quad (1)$$

2.4.3. Turbidity

The turbidity of the supernatant collected from the samples was determined using a method described previously (Rojas et al., 2016), with some slight modifications. Briefly, 25 mL of sample was placed in a centrifuge tube and then centrifuged at 320 *g*, 4 °C for 10 min. The supernatant was then removed, and its absorbance was measured at 660 nm over 11 days. These measurements provide information about the presence of any small colloidal particles within the samples.

2.4.4. Color

Changes in the color of the tomato juice during storage at 25 °C were measured periodically (Campoli, Rojas, do Amaral, Canniatti-Brazaca, & Augusto, 2018). An instrumental colorimeter was used to quantify the color (Konica Minolta, Chroma Meter, CR-400, Mahwah, NJ, USA) using the CIELAB technique: L* corresponds to lightness; a* corresponds to redness (from green to red); and b* corresponds to yellowness (from blue to yellow). A measurement area of 64 mm^2 and an observation angle of 10° were used for these measurements. A cylindrical aluminum container (h = 6.5 cm and d = 3.7 cm) was filled with 5 mL of tomato juice and three readings were performed for each sample after calibration with standard white porcelain.

2.5. Analysis of lycopene bioaccessibility

Lycopene *in vitro* bioaccessibility was determined after samples had passed through a simulated gastrointestinal model, which included oral, gastric, and intestinal phases (Berni, Chitchumroonchokchai, Canniatti-Brazaca, De Moura, & Failla, 2015). To determine the impact of oil on lycopene bioaccessibility, we also prepared two samples containing emulsified oil. These samples were prepared by blending 2 wt% peanut oil with the tomato juice using a high shear mixer operating at 3500 rpm for 10 min (Ultra-Turrax IKA, T25, Werke, Germany).

Ensuring the vitality of digestive enzymes is a very important part of *in vitro* digestion experiments, according to the instructions for use, the optimum pH and reaction temperature are adjusted to ensure the vitality of the enzyme. As described by previous reports (Bai et al., 2019), we kept the lipase solution and pepsin solution at 5 units/mL and 10 units/mL respectively. The samples were then passed through the simulated gastrointestinal tract consisting of mouth, stomach, and small intestine phases (37 °C). As the method used by Qian, Decker, Xiao, and McClements (2012), we determined the bioaccessibilty of lycopene in samples. The bioaccessibility (%) of lycopene was calculated from the ratio of the lycopene in a bioaccessible form in the supernatant (C_B) to that in the initial sample (C_I) according to the following formula:

$$\text{Bioaccessibility}\,(\%) = (C_B/C_I) \times 100 \quad (2)$$

2.6. Statistical analysis

All measurements were conducted in triplicate and results are reported as the mean and standard deviation. All the data were subjected to statistical analysis of variance (ANOVA) and a Tukey HSD test (SPSS 18.0, Chicago, USA). A *P*-value of < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Effects of processing parameters on lycopene content in tomato juice

Several important factors must be considered for the development of a successful bioprocess for lycopene extraction, including time of incubation, temperature, inoculum density, *etc.* (Azabou et al., 2016). Initially, the impact of selected processing parameters on the lycopene content of the tomato juice was determined. The optimum temperature range for carrying out fermentation using *Saccharomyces cerevisiae* ATCC 9763 was found to be between 20 and 30 °C, as this gave the highest measured lycopene content (Fig. 1a). The reproduction and growth of the yeast is inhibited when the fermentation temperature is either too low or too high, which reduces the hydrolytic activity of the yeast because less pectinase is secreted and/or the pectinase is deactivated. Enzyme deactivation occurs due to irreversible changes in the 3D-structure of the protein when the temperature is too high or too low. As a result, the ability of the yeast to disrupt the cellular structures of the tomatoes and release the lycopene is reduced.

The impact of fermentation time on the lycopene content extracted from the tomato juice was then measured (Fig. 1b). There was an appreciable increase in lycopene release when the incubation time was increased from 12 to 24 h but then it remained relatively constant. Presumably, the pectinases secreted by the yeast progressively broke down the cellular structure of the tomato tissues and released the lycopene. This process was complete after about 24 h incubation since there was no significant difference between the 24, 36, 48, 60 and 72 h treatment groups. Alternatively, the enzyme may have lost some of its activity after prolonged storage.

The impact of the level of yeast used to carry out the fermentation process was also examined (Fig. 1c). Interestingly, the amount of lycopene released initially increased with increasing yeast content, but then decreased. When the yeast inoculation is too low, there is insufficient pectinase secreted to disrupt all the tomato tissues and fully release the lycopene. Conversely, when the yeast inoculation is too high, there are not enough nutrients available for all the yeast cells to function properly and so they do not produce enough pectinase. Our data suggests that the liberated lycopene content in the tomato juice was highest when the yeast incubation quantity was between about 4% and 5% during fermentation (Fig. 1c).

3.2. Optimization of fermentation conditions

A three-factor, three-level response surface design was performed using a statistical software program. The fermentation temperature (A), fermentation time (B) and yeast incubation quantity (C) were used as independent variables, while lycopene content (Y) was the response value, and a 3-level 3-factor Box-Behnken test protocol was used (Tables 1 and 2). Response surface images are also presented in the text (Fig. 2). The multiple nonlinear quadratic regression model established using this approach was:

$$\text{Lycopene content} = 317.31 - 26.63A - 0.83B + 20.21C - 0.00083AB + 0.32AC + 0.40BC + 0.51A_2 - 0.008B_2 - 4.55C_2 \quad (3)$$

The *P*-value was significant and demonstrated that the model fitted the data well (Table 2). This model was found to be significant at the 95% confidence level and the Model F-value of 3.77 implies the model is appropriate: there was only a 4.7% chance that the "Model F-Value" could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The order of influence of each factor on lycopene concentration was: fermentation time > fermentation temperature > yeast incubation quantity. After analysis, the optimal fermentation conditions were determined as: 30 °C, 36 h, and 4.9% (v/v) inoculum. The maximum concentration of lycopene release predicted by the model was 46.7 mg/100 g. This value is close to the level found in the subsequent verification test, where the lycopene concentration was 45.1 mg/100 g under the optimal treatment conditions. After calculation, the error between the measured value and the predicted value was 3.3%, which indicated that the tomato juice fermentation process had been optimized by the response surface method. The optimized conditions were used in the subsequent experiments.

3.3. Changes in pectin content and cell morphology before and after fermentation

3.3.1. Observation of cell morphology

Lycopene is located primarily within the chromoplasts of the tomato cells (Ladole, Nair, Bhutada, Amritkar, & Pandit, 2018). The presence of this carotenoid can be detected using optical microscopy due to its intense pigmentation.

Representative microstructures (from around 100 images/sample) of the tomato juices after 0, 12, 24 and 36 h of fermentation are shown (Fig. 3a). These results suggest that the presence of the yeast promoted

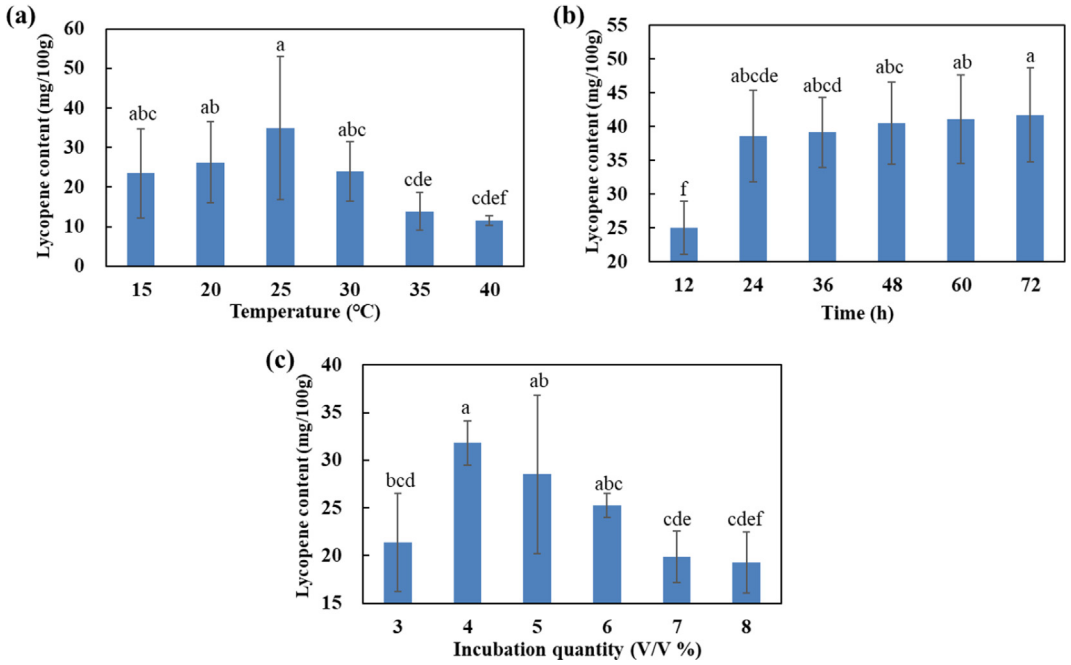

Fig. 1. Effect of three factors on lycopene content. Fermentation temperature (a), fermentation time (b), and yeast incubation quantity (c); Vertical bars represent the standard deviation in each value; different letters indicate significant differences ($p < 0.05$).

Table 1
Actual and predicted responses of lycopene content using the central composite experimental design.

Test	X1	X2	X3	Lycopene content (mg/100 g)	
				Observed	Predicted
1	20	12	4	30.29 ± 2.14	30.18
2	30	12	4	38.61 ± 6.12	31.58
3	20	36	4	36.63 ± 0.66	38.60
4	30	36	4	44.75 ± 3.70	39.80
5	20	24	3	36.58 ± 1.61	31.24
6	30	24	3	31.65 ± 1.44	29.34
7	20	24	5	33.60 ± 2.90	30.86
8	30	24	5	35.08 ± 5.18	35.36
9	25	12	3	17.00 ± 3.14	16.97
10	25	36	3	17.79 ± 1.84	15.69
11	25	12	5	12.93 ± 3.09	10.19
12	25	36	5	32.97 ± 2.41	28.11
13	25	24	4	21.42 ± 1.24	23.50
14	25	24	4	19.18 ± 1.10	23.50
15	25	24	4	28.22 ± 4.91	23.50
16	25	24	4	26.48 ± 3.27	23.50
17	25	24	4	34.35 ± 6.14	23.50

the decomposition of the internal structure of the tomato cells. This phenomenon may therefore account for the observed increase in liberated lycopene after fermentation, as well as for the observed improvement in bioaccessibility.

3.3.2. Analysis of pectin content in tomato juice.

It has been proposed that *Saccharomyces cerevisiae* secretes pectinase during fermentation (Ejaz, Ahmed, & Sohail, 2018), which then hydrolyzes the plant cell walls and facilitates carotenoid release. The hydrolysis of the cell walls can be indirectly monitored by measuring the decrease in the pectin content. To provide some evidence for this hypothesis, the pectin content of the tomato juice was therefore determined before and after fermentation (Fig. 3b). The pectin content in the tomato juice after fermentation was less than half of that before fermentation, indicating that some of the pectin in the plant cell walls had been degraded by the pectinase during fermentation. Our results are therefore consistent with a recent study on the fermentation of grape pomace using yeast (Williams, Schückel, Vivier, Buffetto, & Zietsman, 2019). In that study, yeast not only digested the cell wall polysaccharides and disintegrated the internal cell structure, but also produced a series of by-products, including alcohol. It was postulated

Table 2
Significance test of response surface design.

Source	M^2	df	Square	Value	P-Value Prob > F	Significance
Model	1040.12	9	115.57	3.77	0.0471	*
A-Temperature	21.09	1	21.09	0.69	0.4343	
B-Time	138.69	1	138.69	4.52	0.0710	
C-Incubation Quantity	16.70	1	16.70	0.54	0.4845	
AB	0.01	1	0.01	0.00	0.9861	
AC	10.27	1	10.27	0.33	0.5809	
BC	92.64	1	92.64	3.02	0.1258	
A^2	694.98	1	694.98	22.66	0.0021	
B^2	6.14	1	6.14	0.20	0.6681	
C^2	87.17	1	87.17	2.84	0.1357	
Lack of Fit	72.37128	3	24.12376	0.677893	0.6097	Not significant
$R^2 = 0.8289$	R^2 (adj.) = 0.8029					

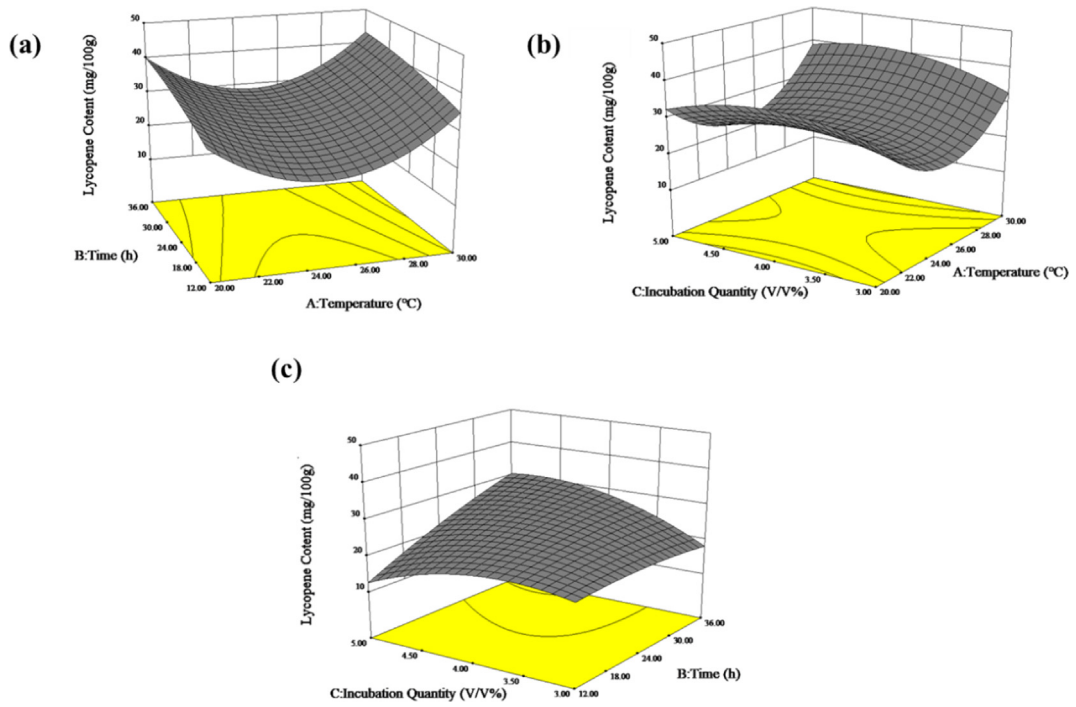

Fig. 2. Response surface plot of lycopene content against fermentation temperature, fermentation time, and yeast incubation quantity.

that the presence of the alcohol promoted the leaching out of lycopene by increasing its solubility in the external fluids.

3.3.3. Comparative analysis of particle size of tomato juice

One would expect that the bioaccessibility of the carotenoids would increase as the size of the pulp fragments in the tomato juice decreased, because this would lead to an increase in exposed surface area. For this

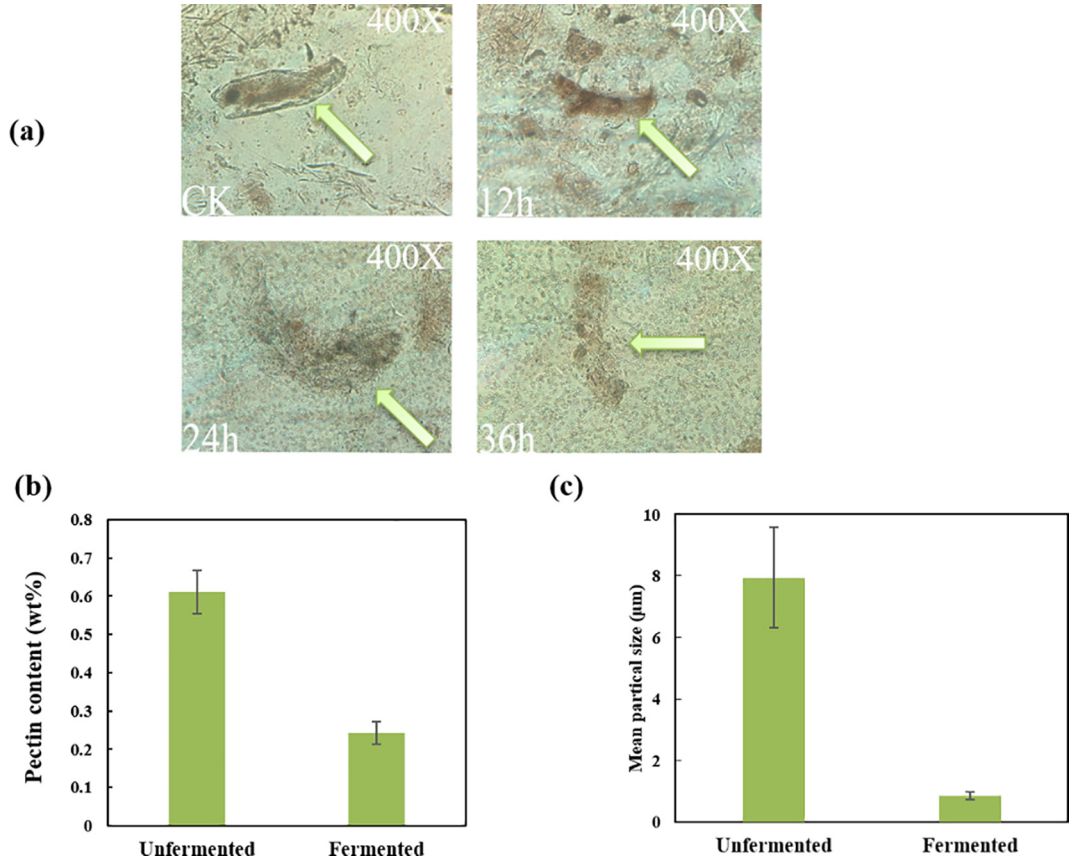

Fig. 3. Comparison of microstructure in terms of time (a), pectin content (b) and mean particle sizes (c) between fermented and unfermented cells.

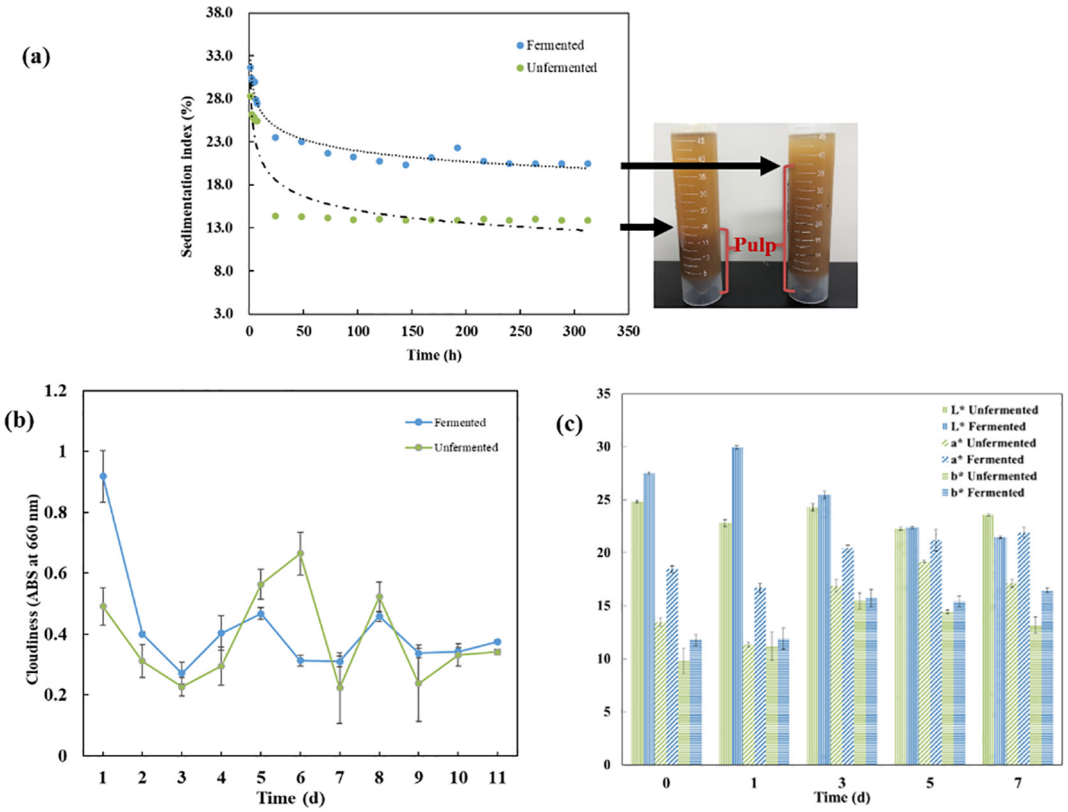

Fig. 4. Comparison of sedimentation indexes (a), turbidity (b) and changes of color (c) of fermented and unfermented samples.

reason, the particle size characteristics of the tomato juice samples were measured using light scattering (Fig. 3c). Our results showed that fermentation greatly reduced the size of the plant tissue fragments in the tomato juice. Indeed, the mean particle diameter after fermentation was about 1/10th of that before fermentation. These results suggest that the fermentation process was able to break down the tomato tissue fragments, which was most likely due to partial disruption of the pectin molecules holding the plant cells together.

3.4. Physical and chemical properties of fermented tomato juice

3.4.1. Sedimentation index

The sedimentation index of the tomato juice samples was measured when they were stored at 25 °C for 14 days (Fig. 4a). During storage, the samples separated into a sediment layer at the bottom and a clear serum layer at the top. The unfermented control group exhibited significant sedimentation during the first few hours, resulting in the formation of a sediment layer of about 15% of the total volume of the system after one day. The fermented control group settled to about 23% after the first day, which suggested that the sediment layer was less densely packed. This phenomenon may have occurred because a more open three-dimensional network of aggregated particles was formed by the smaller particles. A similar result has been reported in previous study (Campoli et al., 2018).

3.4.2. Turbidity analysis

The initial turbidity of the fermented group was higher than that of the unfermented group (Fig. 4b), which may have been due to greater light scattering by the smaller suspended particles in this sample (Saricaoglu, Atalar, Yilmaz, Odabas, & Gul, 2019). The turbidity of the fermented group decreased rapidly during the first day of storage, but then remained relatively constant, which suggests that there may have been some change in the aggregation state of the particles. The turbidity of the unfermented group remained relatively constant during storage. As described in the previous study (Campoli et al., 2018), a higher fraction of small particles in a juice can lead to an increase in the cloudiness of the serum phase because these particles are stable to sedimentation during centrifugation. However, these small particles can aggregate and precipitate during storage leading to an increase in turbidity (Kubo, Augusto, & Cristianini, 2013). The observed increase in turbidity after fermentation observed in our study is, therefore, consistent with the findings of this previous study.

3.4.3. Color analysis

Changes in the appearance of the different samples during storage was assessed by measuring their tristimulus color coordinates (L*, a* and b*) using an instrumental colorimeter. Both treated and untreated groups had similar appearances throughout storage (Fig. 4c). These results suggest that fermentation did not have a major impact on the appearance of the tomato juice because there are only small differences in the L*, a* and b* values between the samples. Changes in color might have been due to changes in tomato tissue structure (light scattering) and/or free lycopene content (light absorption) during fermentation. In comparison, Kwaw et al. (2018) measured changes in the color of lactic-acid-fermented mulberry juice throughout fermentation. They observed a decrease in L* and b* values and an increase in a* values, which was attributed to changes in the type and levels of different chromophores and colloidal particles present in the samples during fermentation.

3.5. Bioaccessibility of lycopene in tomato juice

The bioaccessibility of lycopene from the different tomato juice samples was determined after they were subjected to the full simulated GIT model: mouth, stomach, and small intestine. These studies were designed to assess the impact of both fermentation and emulsification with oil on lycopene bioaccessibility. There were clear differences in carotenoid bioaccessibility depending on whether the samples were fermented and/or emulsified (Fig. 5). The lycopene bioaccessibility in

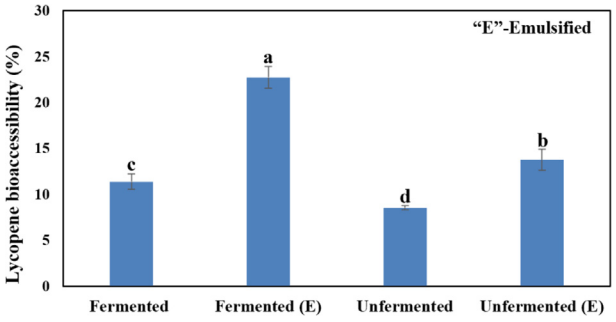

Fig. 5. Influence of fermentation and emulsification on the bioaccessibility (%) of lycopene after *in vitro* digestion. E: Emulsified; Different superscript letters show significant differences ($p < 0.05$) between samples.

the tomato juices increased in the following order: unfermented (8.5%) < fermented (11.4%) < unfermented-emulsified (13.6%) < fermented-emulsified (22.7%) (Fig. 5). Previous studies have reported increases in lycopene bioaccessibility after guava juice was treated with high-intensity ultrasound (Campoli et al., 2018), which was attributed to fragmentation of the guava tissue structure and chromoplasts by sonication. As a result, the release of lycopene from the plant cells was facilitated, leading to an increase in its *in vitro* bioaccessibility. A recent study also reported that the bioaccessibility of hydrophobic bioactives can be enhanced by reducing the particle size and adding emulsified oil (Liu et al., 2019). Our results showed that fermentation can also be used to increase the bioaccessibility of lycopene in tomato juice because of the higher free lycopene content and smaller particle size, especially when used in combination with emulsification.

4. Conclusions

In summary, fermentation of tomato juice improves both the physical properties of the juice and the *in vitro* bioaccessibility of lycopene. The digestive enzymes released by *Saccharomyces cerevisiae* ATCC 9763 during fermentation break down the tomato cells, thereby releasing the intracellular lycopene and increasing its bioaccessibility. Fermentation also reduces the size of the pulp particles in tomato juice, which improves its stability to sedimentation during storage. Hence, yeast fermentation is a potential bioprocess for not only enhancing the physical proprieties of tomato juice, but also increasing their nutritional value. Nevertheless, further research is needed to determine the impact of fermentation on the sensory properties of tomato juice, as well as to establish the *in vivo* bioavailability of the lycopene using animal and/or human feeding studies.

Ethics statement

Our research did not include any human subjects and animal experiments.

Author statement

Yuyan Lu and Fuguo Liu conceived and designed the experiments. Yuyan Lu, Kaiyu Mu and Xiuping Liang performed the experiments. Xuebo Liu contributed helpful discussion during the experiment. Yuyan Lu and Fuguo Liu analyzed the data and wrote the manuscript. David Julian McClements, Xuebo Liu and Fuguo Liu reviewed and revised the manuscript. All persons who have made substantial contributions to the work are named in the manuscript.

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Declaration of Competing Interest

There are no conflicts to declare.

References

Azabou, S., Abid, Y., Sebii, H., Felfoul, I., Gargouri, A., & Attia, H. (2016). Potential of the solid-state fermentation of tomato by products by Fusarium solani pisi for enzymatic extraction of lycopene. *LWT-Food Science and Technology, 68*, 280–287.

Bai, L., Lv, S., Xiang, W., Huan, S., McClements, D. J., & Rojas, O. J. (2019). Oil-in-water Pickering emulsions via microfluidization with cellulose nanocrystals: 2. Vitro lipid digestion. *Food Hydrocolloids, 96*, 709–716.

Bartkiene, E., Bartkevics, V., Mozuriene, E., Lele, V., Zadeike, D., & Juodeikiene, G. (2019). The safety, technological, nutritional, and sensory challenges associated with lacto-fermentation of meat and meat products by using pure lactic acid bacteria strains and plant-lactic acid bacteria bioproducts. *Frontiers in Microbiology, 10*, 1036.

Bayod, E., & Tornberg, E. (2011). Microstructure of highly concentrated tomato suspensions on homogenisation and subsequent shearing. *Food Research International, 44*(3), 755–764.

Bayod, E., Willers, E. P., & Tornberg, E. (2008). Rheological and structural characterization of tomato paste and its influence on the quality of ketchup. *LWT-Food Science and Technology, 41*(7), 1289–1300.

Berni, P., Chitchumroonchokchai, C., Canniatti-Brazaca, S. G., De Moura, F. F., & Failla, M. L. (2015). Comparison of content and in vitro bioaccessibility of provitamin A carotenoids in home cooked and commercially processed orange fleshed sweet potato (Ipomea batatas Lam). *Plant Foods for Human Nutrition, 70*(1), 1–8.

Bi, Y., Zhang, Y., Jiang, H., Hong, Y., Gu, Z., Cheng, L., ... Li, C. (2017). Molecular structure and digestibility of banana flour and starch. *Food Hydrocolloids, 72*, 219–227.

Campoli, S. S., Rojas, M. L., do Amaral, J. E. P. G., Canniatti-Brazaca, S. G., & Augusto, P. E. D. (2018). Ultrasound processing of guava juice: Effect on structure, physical properties and lycopene in vitro accessibility. *Food Chemistry, 268*, 594–601.

Chuyen, H. V., Roach, P. D., Golding, J. B., Parks, S. E., & Nguyen, M. H. (2017). Optimisation of extraction conditions for recovering carotenoids and antioxidant capacity from Gac peel using response surface methodology. *International Journal of Food Science & Technology, 52*(4), 972–980.

Cooperstone, J. L., Ralston, R. A., Riedl, K. M., Haufe, T. C., Schweiggert, R. M., King, S. A., ... Schwartz, S. J. (2015). Enhanced bioavailability of lycopene when consumed as cis-isomers from tangerine compared to red tomato juice, a randomized, cross-over clinical trial. *Molecular Nutrition & Food Research, 59*(4), 658–669.

Ejaz, U., Ahmed, A., & Sohail, M. (2018). Statistical optimization of immobilization of yeast cells on corncob for pectinase production. *Biocatalysis and Agricultural Biotechnology, 14*, 450–456.

Hernández-Almanza, A., Montañez-Sáenz, J., Martínez-Ávila, C., Rodríguez-Herrera, R., & Aguilar, C. N. (2014). Carotenoid production by Rhodotorula glutinis YB-252 in solid-state fermentation. *Food Bioscience, 7*, 31–36.

Ilahy, R., Hdider, C., Lenucci, M. S., Tlili, I., & Dalessandro, G. (2011). Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. *Journal of Food Composition and Analysis, 24*(4–5), 588–595.

Kubo, M. T. K., Augusto, P. E. D., & Cristianini, M. (2013). Effect of high pressure homogenization (hph) on the physical stability of tomato juice. *Food Research International, 51*(1), 170–179.

Kwaw, E., Ma, Y., Tchabo, W., Apaliya, M. T., Wu, M., Sackey, A. S., ... Tahir, H. E. (2018). Effect of lactobacillus strains on phenolic profile, color attributes and antioxidant activities of lactic-acid-fermented mulberry juice. *Food Chemistry, 250*, 148–154.

Ladole, M. R., Nair, R. R., Bhutada, Y. D., Amritkar, V. D., & Pandit, A. B. (2018). Synergistic effect of ultrasonication and co-immobilized enzymes on tomato peels for lycopene extraction. *Ultrasonics Sonochemistry, 48*, 453–462.

Liang, X., Ma, C., Yan, X., Liu, X., & Liu, F. (2019). Advances in research on bioactivity, stability, metabolism and delivery systems of lycopene. *Trends in Food Science & Technology, 93*, 185–196.

Liu, J., Bi, J., Liu, X., Zhang, B., Wu, X., Wellala, C. K. D., & Zhang, B. (2019). Effects of high pressure homogenization and addition of oil on the carotenoid bioaccessibility of carrot juice. *Food & Function, 10*(1), 458–468.

Lu, P., Wang, R., Zhu, C., Fu, X., Wang, S., Grierson, D., & Xu, C. (2019). Microscopic analyses of fruit cell plastid development in loquat (Eriobotrya japonica) during fruit ripening. *Molecules, 24*(3), 448.

Mapelli-Brahm, P., Barba, F. J., Remize, F., Garcia, C., Fessard, A., MousaviKhaneghah, A., ... Meléndez-Martínez, A. J. (2020). The impact of fermentation processes on the production, retention and bioavailability of carotenoids: An overview. *Trends in Food Science & Technology, 2020*(99), 389–401.

Müller, L., Caris-Veyrat, C., Lowe, G., & Böhm, V. (2016). Lycopene and its antioxidant role in the prevention of cardiovascular diseases—A critical review. *Critical Reviews in Food Science and Nutrition, 56*(11), 1868–1879.

Nisha, P., Singhal, R. S., & Pandit, A. B. (2011). Kinetic modelling of colour degradation in tomato puree (*Lycopersicon esculentum* L.). *Food and Bioprocess Technology, 4*(5),

781–787.
Porretta, S. (2019). Traditional tomato products and the need for innovation. In S. Porretta (Ed.). *Tomato chemistry, industrial processing and product development* (pp. 304–329). London: Royal Society of Chemistry.
Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012). Nanoemulsion delivery systems: Influence of carrier oil on β-carotene bioaccessibility. *Food Chemistry, 135*(3), 1440–1447.
Rojas, M. L., Alvim, I. D., & Augusto, P. E. D. (2019). Incorporation of microencapsulated hydrophilic and lipophilic nutrients into foods by using ultrasound as a pre-treatment for drying: A prospective study. *Ultrasonics Sonochemistry, 54*, 153–161.
Rojas, M. L., Leite, T. S., Cristianini, M., Alvim, I. D., & Augusto, P. E. (2016). Peach juice processed by the ultrasound technology: Changes in its microstructure improve its physical properties and stability. *Food Research International, 82*, 22–33.
Salehi, B., Sharifi-Rad, R., Sharopov, F., Namiesnik, J., Roointan, A., Kamle, M., ... Sharifi-Rad, J. (2019). Beneficial effects and potential risks of tomatoes consumption for human health: An Overview. *Nutrition, 62*, 201–208.
Saricaoglu, F. T., Atalar, I., Yilmaz, V. A., Odabas, H. I., & Gul, O. (2019). Application of multi pass high pressure homogenization to improve stability, physical and bioactive properties of rosehip (Rosa canina L.) nectar. *Food Chemistry, 282*, 67–75.
Septembre-Malaterre, A., Remize, F., & Poucheret, P. (2018). Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. *Food Research International, 104*, 86–99.
Williams, D. L., Schückel, J., Vivier, M. A., Buffetto, F., & Zietsman, A. J. (2019). Grape pomace fermentation and cell wall degradation by Kluyveromyces marxianus Y885. *Biochemical Engineering Journal, 150*(15), 107282.
Zhou, S., Sun, W., Bei, M. R., Zahi, Q. Yuan, & Liang, H. (2018). Preparation and antimicrobial activity of oregano essential oil Pickering emulsion stabilized by cellulose nanocrystals. *International Journal of Biological Macromolecules, 112*, 7–13.