https://doi.org/10.17221/284/2020-CJFS

Dynamic high pressure microfluidization (DHPM): Physicochemical properties, nutritional constituents and microorganisms of yam juice

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Citation: Liu M., Wang R., Li J., Zhang L., Zhang J., Zong W., Mo W. (2021): Dynamic high pressure microfluidization (DHPM): Physicochemical properties, nutritional constituents and microorganisms of yam juice. Czech J. Food Sci., 39: 217–225.

Abstract: Dynamic high pressure microfluidization (DHPM) is considered an emerging and promising technique for the continuous production of fluid foods. This study measured the effect of DHPM on yam juice. After DHPM processing, the content of total soluble solids (TSS), turbidity, flavonoid and non-enzymatic browning was significantly decreased, with the biggest drops being 35.5, 86.2, 20.7, and 66.7%, respectively. Moreover, the average particle size was decreased from 1 944 nm to 358 nm, which showed a strong positive correlation with turbidity. The reduction coefficients and electric conductivity of *Escherichia coli*, *Saccharomyces cerevisiae* and *Lactobacillus plantarum* were increased significantly after DHPM processing. Combined with morphological analysis, DHPM processing had good bactericidal effects on *E. coli* and *S. cerevisiae*. These results provided reference values for the application of DHPM technology in the development of yam juice.

Keywords: particle size distribution; scanning electron microscopy; process; bactericidal effect

Extending the shelf-life of fruit and vegetable juices would be beneficial, and processing technology has an important role. Thermal processing is the most commonly used technique to extend the shelf-life of fruit and vegetable juices. However, it leads to irreversible loss of nutritional qualities, as well as undesirable changes in sensory properties. Minimal processing can use non-thermal technologies, such as high pressure processing (HPP), pulsed electric fields (PEF), ultrasound and high-pressure homogenisation (HPH). HPH processing inactivates microbes as a result of cavitation, shear stress, turbulence, and impingement (Patrignani and Lanciotti 2016). HPH has also been shown to inactivate or modulate the activity of enzymes that led to phase separation in fruit or vegetable juices, which can help to preserve the initial juice colour, flavour, and aromas, subsequently increase the shelf-life (Patrignani et al. 2019). Dynamic high pressure microfluidization

Supported by the Science and Technology Program of Henan Province (No. 212102110201) and the Fundamental Research Funds for the Central Non-profit Research Institution of Chinese Academy of Forestry (CAF) (No. CAFYBB2018GB001).

https://doi.org/10.17221/284/2020-CJFS

(DHPM) technology is considered an emerging and promising technique for the continuous production of fluid foods. Compared with HPH, the DHPM device has some advantages. The DHPM device can lead to much higher pressures, which increase the flow rate of fluids, and the collisions are also increased. As a result, a finer fluid product with smaller particle size and greater stability can be obtained. At present, DHPM technology has been used in the processing of kiwi fruit juice (Wang et al. 2018), peach juice (Wang et al. 2019), buckthorn juice (Abliz et al. 2021), sugarcane juice (Tarafdar et al. 2019), and others. The results show that this technique can effectively improve the sensory quality and storage stability of fruit juice.

Yams are the tubers of the Dioscoreaceae family, high in mucin protein, diosgenin, active polysaccharides, and other nutrients. Yams also have a role in regulating the stomach, lowering blood glucose and blood lipids and are antioxidants. The biological activity of yam is correlated with its nutritional contents. There are many studies on the pharmacological action of yams, but few studies focus on their processing and use (Go et al. 2015). Yam juice is a good use of yam, which would solve some of its storage problems and would be a good value-added product. This study aimed to investigate the impact of DHPM on physicochemical properties, nutritional constituents and microorganisms of yam juice to provide helpful information for the processing of yam.

MATERIAL AND METHODS

Preparation of yam juice

Huai yam (500 g) without diseases and pests was cut into small pieces with a thickness of about 2–3 cm. The pieces were soaked in a mixture of ascorbic acid (0.5%) and citric acid (0.5%) for 1 h for colour protection and then rinsed with distilled water (Wang et al. 2013). The rinsed yam and water (1 : 9) were juiced 3 times using a colloid mill with a grinding gap of 20 μm (JMS-300; Long Tong Machinery Co., Ltd., China). The mixture was centrifuged at 5 600 × g for 5 min (HC-3618R; Zhongke Zhongjia Scientific Instrument Co., Ltd., China) to get yam juice for DHPM processing and stored at 4 °C for a maximum of 3 days.

DHPM processing

Dynamic high pressure microfluidization (DHPM, FPG12800E-F; Stansted Fluid Power Ltd., the United Kingdom) with a flow rate of 40–50 mL min^{-1} and a single maximum pressure of 350 MPa was used. Yam juice was processed using the DHPM with pressures at 80, 120, and 160 MPa (single-stage) with 2 and 4 cycles. The homogenisation temperature was maintained at 25 °C. Homogeneous samples (400 mL) of each process were cooled rapidly in an ice-water bath and stored at 4° C until analysis, a maximum of 3 days.

Physicochemical properties

Total soluble solids (TSS) were measured using a GR15 automatic refractometer (Zhuoguang Instrument Technology Co., Ltd., China). Titratable acidity (TA) was determined by titration. Reducing sugar was determined using the dinitrosalicylic acid method (DNS) described by Choi et al. (2016). Turbidity was measured using the method of Leite et al. (2016). Non-enzymatic browning was determined based on the method of Gao et al. (2015). The colour of yam juice was measured using an automatic colour difference meter (Kangguang Optical Instrument Co., China) using the method of Gao et al. (2015). Particle size distribution (PSD) was determined using the software with the instrument using an automatic laser particle analyser (Malvern Zetasizer Nano-ZS90; Malvern, United Kingdom), and the average particle size and PSD were obtained.

Nutritive constituents

Total phenol contents (TPC) of yam juice were determined using the Folin-Ciocalteu method described by Gao et al. (2015). Total flavonoid content (TFC) was measured using rutin (R) to generate the standard curve. DPPH radical-scavenging ability was determined using a method described by Aadil et al. (2013).

Microorganisms

Escherichia coli, Saccharomyces cerevisiae and Lactobacillus plantarum cell enumeration. The freeze-dried cultures of *Escherichia coli* (GIMT 1.163), *Saccharomyces cerevisiae* (GIM 2.167) and *Lactobacillus plantarum* (GIM 1.140) were obtained from the Guangdong Culture Collection Center (Guangzhou, Guangdong Province, China). Activated strains were diluted with 0.85% sterile physiological saline. Each strain (1 mL) was inoculated into 100 mL of sterile yam juice (121 °C for 15 min). The inoculated yam juice was preheated to ~20–60 °C. After the treatment of 80, 120, and 160 MPa (single-stage) with 2 and 4 cycles, the treated yam juice flowed into sterile bottles and was then used for microbiological and conductivity measurements. Microbial growth was measured using the previously described media, and colonies were counted using a colony counter according to the method of Fauzi et al. (2017).

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Conductivity

The conductivity of yam juice was measured using a conductivity meter (DDSJ-308F; Shanghai Vidian Science Instrument Co., China). Results were expressed as μs cm^{-1}.

Scanning electron microscopy (SEM)

The samples were prepared for scanning electron microscopy (SEM) using the method proposed by Patrignani et al. (2016). Samples (1–2 drops) were put onto a cover glass and dried in an oven at 70 °C (BGZ-140; Shanghai Boxun Industry and Commerce Co., Ltd., China), and then covered with gold at 10 nm for 150 s using ion sputtering. The microstructure of the strains was observed with SEM at 20 kV and a magnification of 2 000–15 000 (DDSJ-308F; Shanghai Vidian Science Instrument Co., China).

Statistical analyses

Univariate analyses of variance (ANOVA) and least significant differences (LSD) were done using JMP Pro 12 to measure. A difference was considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Influence of DHPM on the physicochemical properties of yam juice

TSS, TA and reducing sugars. After DHPM processing, a significant difference was observed in the TSS and reducing sugar content of yam juice, with the biggest drop being 35.5% and 10.2%, respectively (Table 1). With the pressure and frequency increased, TSS decreased from 0.93 Brix to 0.60 Brix. The reducing sugars showed a significant variation with different pressures and frequencies. The maximum reduction in reducing sugars was 120 MPa, 2 cycles (431 μg mL^{-1}) compared to the control (480 μg mL^{-1}). However, there was no significant difference in titratable acid after DHPM processing, which was consistent with the effects of HPP on red grapefruit juice (Gao et al. 2015) and ultra-HPH of apple juice (Suarez-Jacobo et al. 2011).

The turbidity and non-enzymatic browning. Turbidity and non-enzymatic browning can reflect the stability and shelf-life of fruit and vegetable juices. Table 1 shows turbidity and non-enzymatic browning of yam juice before and after DHPM processing. It showed that the turbidity was significantly different from the control. With the increase in pressure, the absorbance decreased and reached a minimum at 160 MPa, 4 cycles (0.09). These results were consistent with the reports of HPH on tomato juice and orange juice, which reported a decrease in turbidity (Leite et al. 2016). Non-enzymatic browning showed significant variation and trended down from 0.51 to 0.17 after DHPM processing. This phenomenon was consistent with the results of the study by Tamaoka et al. (1991), which indicated that the increased pressure inhibits the generation of free radical of melanins and then restrains the non-enzymatic browning reaction.

Colour. The impact of DHPM processing on yam juice colour parameters L^*, a^*, and b^* is summarised in Table 1. The DHPM processed yam juice had a higher L^* value compared with the control. The L^* value was significantly increased by 18.1% at 160 MPa, 4 cycles, which suggested increased brightness. These results were consistent with the study of HPH of banana juice (Calligaris et al. 2012). Compared with the control, the maximum of a^* and b^* value was at 80 MPa, 2 cycles, with 4.29 and 18.8, respectively. Overall, according to the evaluation index of Carreño et al. (2010), the value of ΔE gradually increased from 4.63 (well visible) to 11.5 (great), which suggested that the colour of yam juice was significantly changed after DHPM processing.

Particle size. Figure 1A shows that the conductivity decreased significantly after DHPM processing. As the pressure increased, the average particle size decreased from 1 944 nm to 358 nm. However, at the same pressure, no significant differences were observed between 2 and 4 cycles, which were consistent with the study of HPH of tomato and strawberry juices (Karacam et al. 2015). On the basis of Stokes' law, the sedimentation rate of particles is proportional to the size; thus stability would be increased. Moreover, transmittance was reduced significantly after HPH processing leading to a more visually opaque juice (Carreño et al. 2010).

The PSD of DHPM processed yam juice is shown in Figure 1B. The polydispersions PSD of untreated yam juice was distributed from 190 nm to 6 440 nm with two peaks at 1 480 nm and 5 560 nm. The PSD of yam juice was not changed significantly at 80 MPa, 2 cycles compared to the control. As the pressure and frequency increased, yam juice had significantly different PSD ranging from 51 nm to 1 720 nm with two peaks at ~90 nm and 710 nm. These results were consistent with the study of the homogenisation of orange and pineapple juices (Sentandreu et al. 2011). Size reduction is presumably due to the higher impact collisions (Torres and Velazquez 2005).

The influence of DHPM on nutritive constituents of yam juice

As shown in Table 2, when the pressure was < 120 MPa, the total phenol content was not changed

Czech Journal of Food Sciences, 39, 2021 (3): 217–225

https://doi.org/10.17221/284/2020-CJFS

Table 1. Effect of DHPM processing on physicochemical properties of yam juice (mean ± SD; n = 3)

Pressure (MPa)	Cycles	TSS (Brix)	Reducing sugar (μg mL^{-1})	TA (%)	Turbidity	Non-enzymatic browning	Colour			
							L^*	a^*	b^*	ΔE^*
Control	–	0.93 ± 0.05^{a}	480 ± 1^{a}	0.41 ± 0.04^{a}	0.65± 0.030^{a}	0.51 ± 0.001^{a}	55.1 ± 0.40^{g}	4.10 ± 0.20^{b}	15.3 ± 0.3^{c}	–
120	4	0.63 ± 0.05^{d}	444 ± 1^{e}	0.37 ± 0.01^{ab}	0.30± 0.001^{c}	0.32 ± 0.000^{c}	60.2 ± 0.05^{e}	4.28 ± 0.04^{a}	17.6 ± 0.1^{b}	5.64
	2	0.73 ± 0.06^{b}	431 ± 1^{f}	0.37 ± 0.04^{ab}	0.23± 0.003^{d}	0.25 ± 0.001^{d}	62.4 ± 0.20^{d}	3.20 ± 0.02^{c}	16.9 ± 0.4^{b}	7.50
	4	0.67 ± 0.05^{bc}	461 ± 1^{c}	0.38 ± 0.02^{ab}	0.18 ± 0.00^{e}	0.23 ± 0.001^{e}	63.7 ± 0.30^{c}	2.20 ± 0.04^{d}	16.0 ± 1.0^{c}	8.83
160	2	0.63 ± 0.06^{bcd}	469 ± 1^{b}	0.36 ± 0.03^{b}	0.12± 0.010^{f}	0.17 ± 0.004^{g}	64.8 ± 0.10^{b}	1.40 ± 0.10^{e}	12.0 ± 0.2^{d}	10.60
	4	0.60 ± 0.01^{cd}	480 ± 1^{a}	0.38 ± 0.03^{ab}	0.09± 0.003^{g}	0.18 ± 0.003^{f}	65.1 ± 0.10^{a}	0.85 ± 0.05^{f}	10.6 ± 0.1^{e}	11.50

$^{a–g}$Data with different superscript letters within columns are significantly different (P < 0.05); means were separated using least significant differences (LSD) $P \leq 0.05$; DHPM – dynamic high pressure microfluidization; TSS – total soluble solids; TA – titratable acidity; SD – standard deviation

Table 2. Effect of DHPM processing on nutritional constituents of yam juice (mean ± SD; n = 3)

Pressure (MPa)	Cycles	Total phenols (μg mL^{-1})	Flavonoid (μg mL^{-1})	DPPH radical-scavenging (%)
Control	–	100 ± 2.0^{a}	27.0 ± 0.4^{a}	78.0 ± 1.0^{c}
80	2	102 ± 1.0^{a}	24.7 ± 0.5^{b}	83.0 ± 2.0^{ab}
	4	102 ± 0.3^{a}	25.0 ± 1.0^{b}	85.0 ± 4.0^{a}
120	2	100 ± 1.0^{a}	25.0 ± 1.0^{b}	80.0 ± 1.0^{bc}
	4	97 ± 1.0^{b}	25.0 ± 1.0^{b}	72.0 ± 2.0^{d}
160	2	97 ± 2.0^{b}	27.0 ± 1.0^{a}	70.8 ± 0.4^{d}
	4	92 ± 2.0^{c}	21.0 ± 0.1^{c}	71.0 ± 1.0^{d}

$^{a–d}$Data with different superscript letters within columns are significantly different (P < 0.05); means were separated using least significant differences (LSD) $P \leq 0.05$; DHPM – dynamic high pressure microfluidization; DPPH – 2,2-diphenyl-1-picrylhydrazyl; SD – standard deviation

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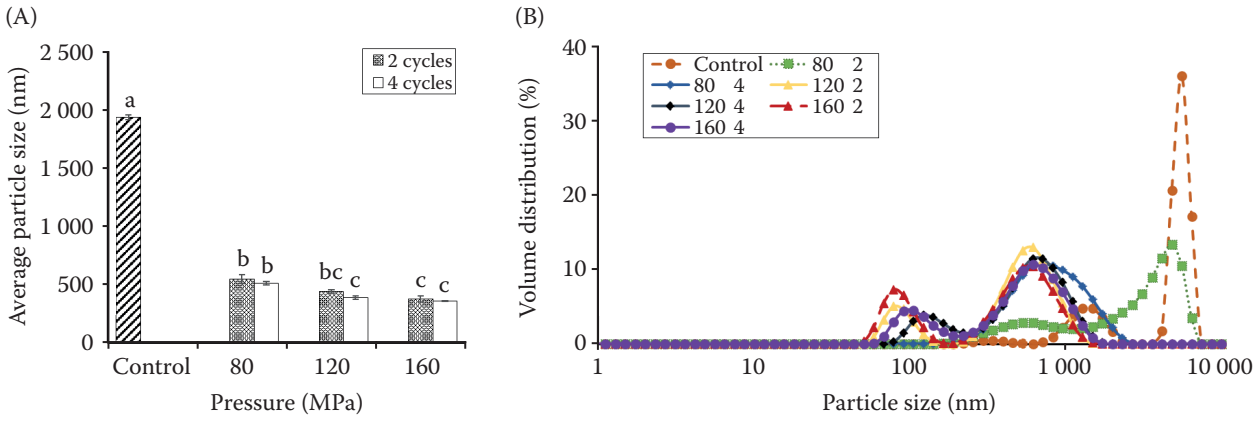

Figure 1. Average particle size and particle size distribution (PSD) of yam juice after dynamic high pressure microfluidization (DHPM) processing; (A) average particle size, (B) particle size distribution (PSD)

Data are expressed as mean ± SD (n = 3); means were separated using LSD $P \leq 0.05$; SD – standard deviation; LSD – least significant differences

significantly, but as the pressure reached 160 MPa, 4 cycles, the total phenol content was minimised at 91.5 μg mL^{-1}, which was a decrease of 8.5% compared with the control. Moreover, the content of flavonoid decreased after DHPM processing. Compared with the control, the highest decrease in flavonoid was 13.6% at 160 MPa, 4 cycles. These results were inconsistent with Betoret et al. (2009), who observed no significant effect of high pressure on the flavonoid of citrus juices. The effect of high pressure on the flavonoid can be associated with changes in the activity of enzymes involved in their synthesis (Ortega et al. 2013). Additionally, the DPPH radical-scavenging of yam juice was increased 9.02% at 80 MPa, 4 cycles, compared to the control. As the pressure increased, the DPPH radical-scavenging of yam juice decreased, with the biggest drop of 9.3% at 80 MPa, 4 cycles, which were consistent with the reports of high-pressure effects on apple, strawberry, and blackberry juices (Suarez-Jacobo et al. 2011).

Correlation analyses between physicochemical properties and nutritional constituents. The correlation between physicochemical properties and nutritional constituents of yam juice is shown in Table 3. The results suggested that DPPH radical-scavenging

Table 3. Correlation analysis between physicochemical properties and nutritional constituents of yam juice

Index	Reducing sugar	Total phenols	DPPH radical-scavenging	Flavonoid	TA	Turbidity	Non-enzymatic browning	TSS	Average particle size
Reducing sugar	1.000	–	–	–	–	–	–	–	–
Total phenols	–0.586**	1.000	–	–	–	–	–	–	–
DPPH radical-scavenging	–0.291	0.551**	1.000	–	–	–	–	–	–
Flavonoid	–0.090	0.602**	0.078	1.000	–	–	–	–	–
TA	0.202	0.194	0.351	0.083	1.000	–	–	–	–
Turbidity	0.112	0.542*	0.415	0.495*	0.481*	1.000	–	–	–
Non-enzymatic browning	0.076	0.576**	0.475*	0.438*	0.513*	0.990**	1.000	–	–
TSS	0.226	0.205	0.007	0.521*	0.446*	0.7128**	0.669**	1.000	–
Average particle size	0.414	0.266	0.191	0.463*	0.496*	0.932**	0.896**	0.777**	1.000

*, **Correlation is significant at $P < 0.05$, and $P < 0.01$, respectively; DPPH – 2,2-diphenyl-1-picrylhydrazyl; TA – titratable acidity; TSS – total soluble solids

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was positively correlated with total phenol content (r = 0.551), but the correlation coefficient was lower than the reports for fresh fruit juices, especially grape juice, with r = 0.908 and r = 0.957, respectively (Wern et al. 2016). These results may be because the antioxidant activities of fruit juices cannot be entirely predict-

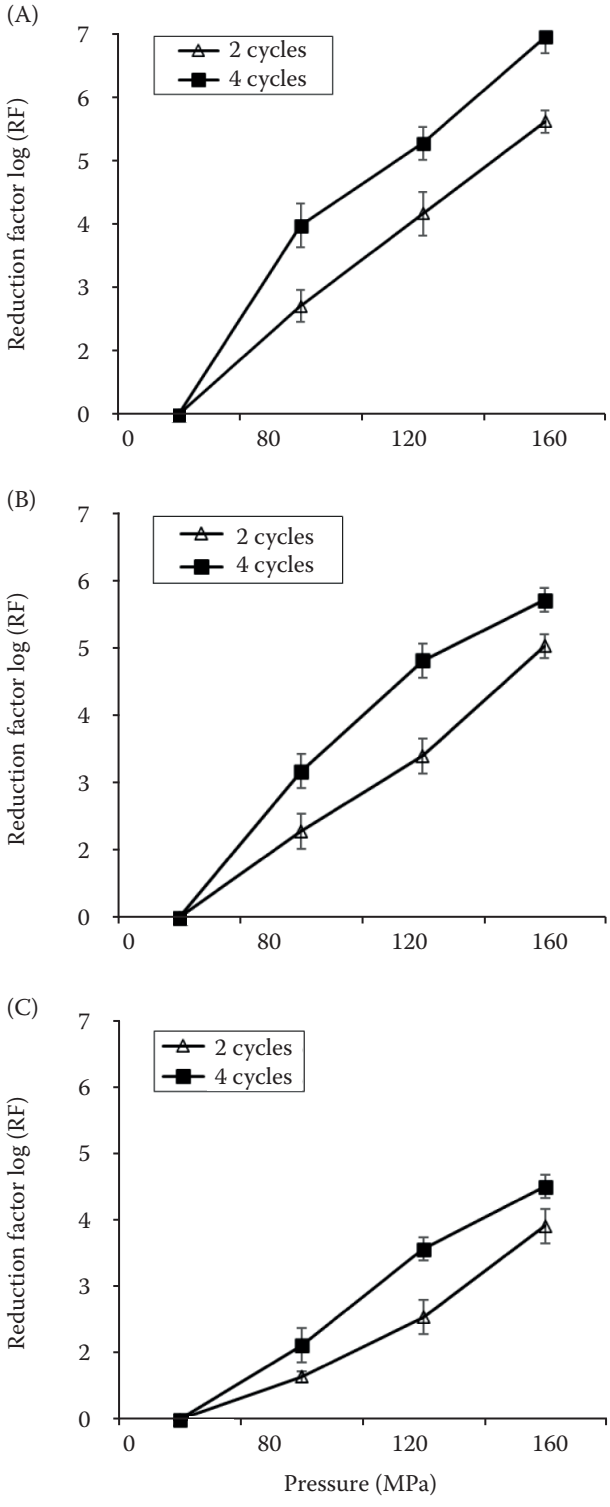

Figure 2. Cells enumeration of (A) *E. coli*, (B) *S. cerevisiae* and (C) *L. plantarum* after DHPM processing

DHPM – dynamic high pressure microfluidization

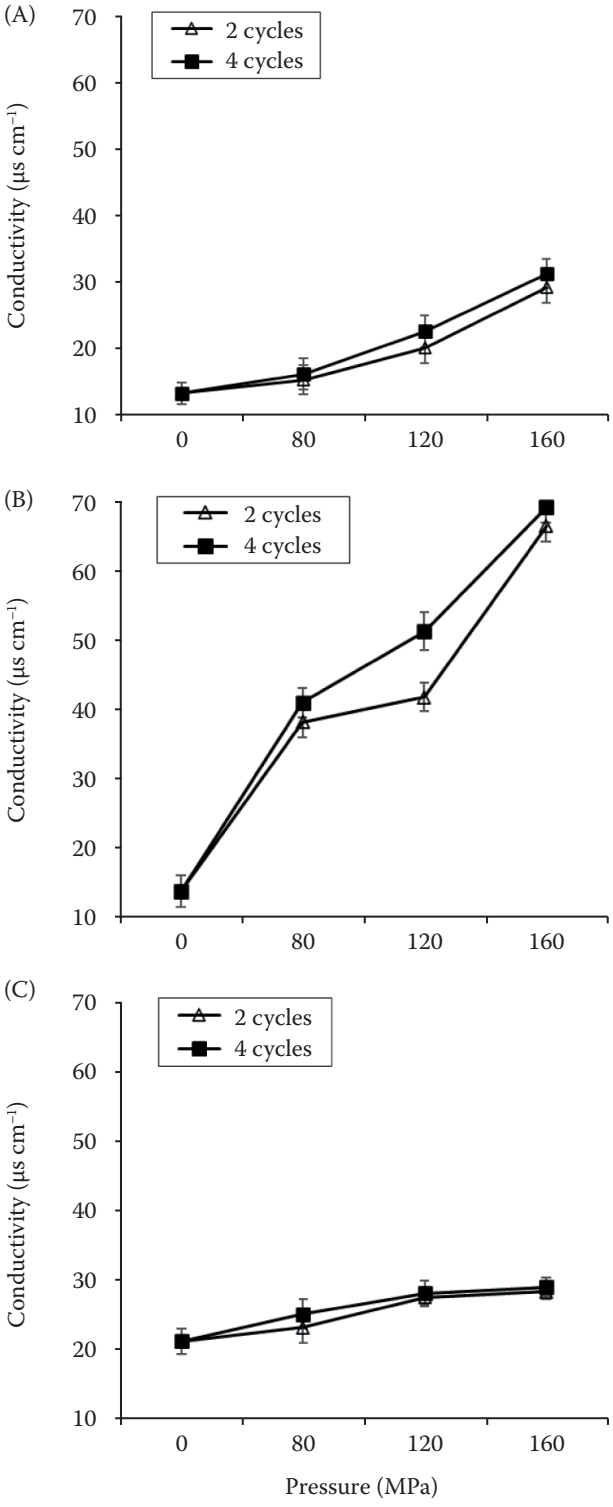

Figure 3. Conductivity of (A) *E. coli*, (B) *S. cerevisiae* and (C) *L. plantarum* solution after DHPM processing

DHPM – dynamic high pressure microfluidization

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ed on the basis of their phenol content, as vitamin C and carotenoids in the juices also partially contribute to antioxidant functions (Velázquez-Estrada et al. 2013). However, the flavonoid content was not proportional to DPPH radical scavenging, which was consistent with the study of Sharma et al. (2015). Moreover, there was a strong positive correlation between the average particle size and turbidity (r = 0.932). Additionally, non-enzymatic browning showed a significant correlation with turbidity (r = 0.991) and average particle size (r = 0.899), and a negative correlation was observed between total phenols and reducing sugars (r = –0.587).

The effect of DHPM processing on inactivation of *E. coli, S. cerevisiae* and *L. plantarum*

Cell enumeration. It can be concluded from Figure 2 that DHPM processing had a significant effect on the reduction coefficients of *E. coli*, *S. cerevisiae* and *L. plantarum.* A higher pressure led to a larger reduction coefficient. The reduction coefficients of *E. coli*, *S. cerevisiae* and *L. plantarum* solution at 160 MPa for 4 cycles were 6.96, 5.5, and 4.1, respectively. As the pressure increased, the microbes decreased, which may be because the pressure damaged the microbial cells. The results showed that the effect of DHPM processing on *E. coli* was slightly greater than that of *S. cerevisiae* and *L. plantarum.*

Conductivity. Figure 3 shows the influence of DHPM processing on the conductivity of *E. coli, S. cerevisiae and L. plantarum* culture. As the pressure increased, the conductivity of the culture increased, and it reached 31.2, 69.2, and 29.0 μs cm^{-1} at 160 MPa for 4 cycles, respectively. Meanwhile, with the same pressure, the conductivity of *E. coli, S. cerevisiae* and *L. plantarum* culture at 4 cycles was a little higher than 2 cycles. The reason for the increase in conductivity may be that the pressure was higher, and the destruction was stronger, which can lead to the loss of the semi-permeable cell membranes releasing cell fluids into the overall solution (Ebrahimi and Alam 2016).

Microstructure. SEM was used to observe the cell surfaces (Figure 4). Before DHPM processing, the morphology of *E. coli, S. cerevisiae* and *L. plantarum* was

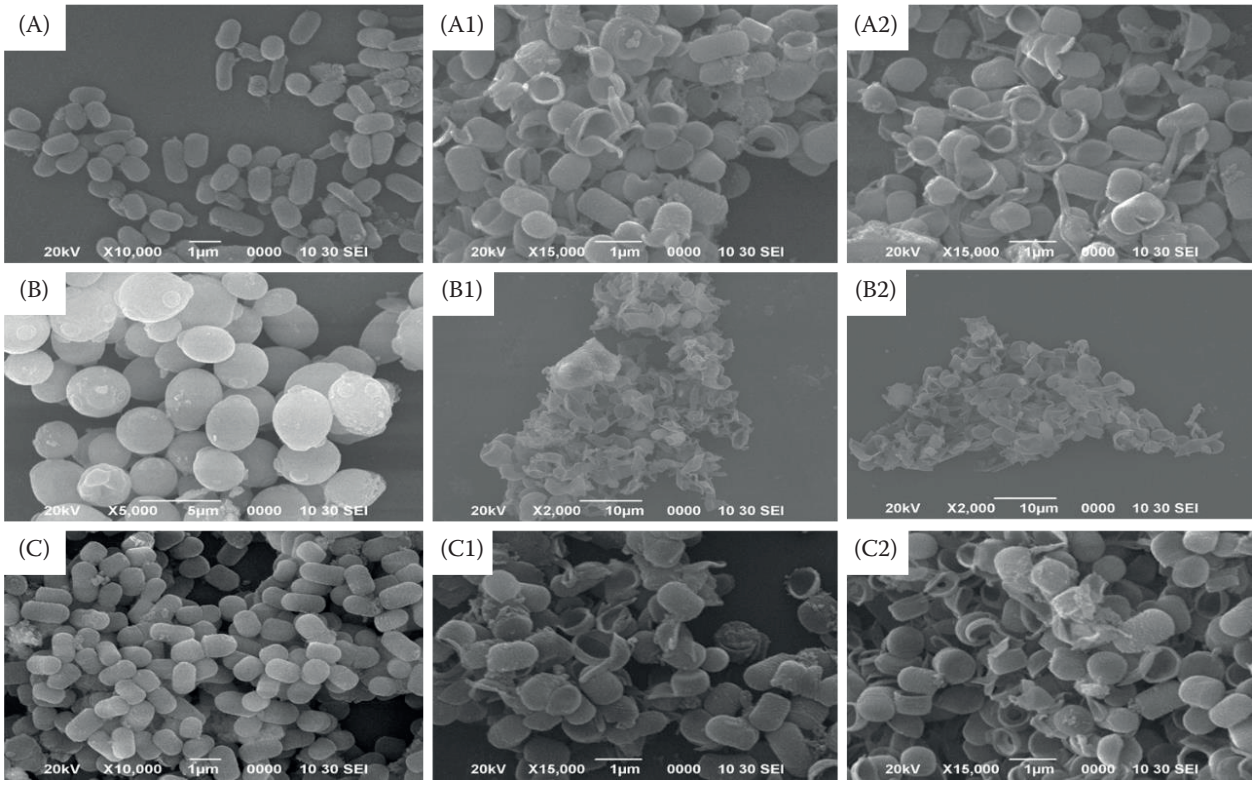

Figure 4. The morphology change of *E. coli, S. cerevisiae* and *L. plantarum* after DHPM processing (A, A1 and A2 as *E. coli* morphology before treatment, 160 MPa for 2 cycles and 4 cycles, respectively; B, B1 and B2 as *S. cerevisiae* morphology before treatment, 160 MPa for 2 cycles and 4 cycles, respectively; C, C1 and C2 as *L. plantarum* morphology before treatment, 160 MPa for 2 cycles and 4 cycles, respectively)

DHPM – dynamic high pressure microfluidization

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smooth and evenly distributed (Figures 4A, B, C). After 160 MPa treatment, a portion of them was damaged. There was no significant difference in cell membrane between 2 and 4 cycles at 160 MPa (Figures 4A1 and A2, B1 and B2, C1 and C2). Compared with *E. coli* and *L. plantarum, S. cerevisiae* was the most damaged after DHPM processing, suggesting that *S. cerevisiae* was more sensitive to high pressure.

CONCLUSION

After DHPM processing, the results showed that the content of TSS, turbidity, flavonoid and non-enzymatic browning was decreased, with the biggest drops at 35.5, 86.2, 20.7, and 66.7%, respectively. Moreover, the value of ΔE increased from 4.63 (well visible) to 11.5 (great), which indicated a significant change of yam juice colour after DHPM processing. The average particle size decreased from 1 944 nm to 358 nm, and as the pressure and frequency increased, yam juice had significant differences in PSD ranging from 51 nm to 1 720 nm. Additionally, there was a strong positive correlation between the average particle size and turbidity (r = 0.932), and non-enzymatic browning showed a significant correlation with turbidity (r = 0.991) and average particle size (r = 0.899). The reduction coefficients of *E. coli*, *S. cerevisiae* and *L. plantarum* solution were 6.96, 5.5, and 4.1 at 160 MPa for 4 cycles, respectively, and the conductivities were 31.2, 69.2, and 29.0 μs cm^{-1}, respectively. Combined with morphological analysis, DHPM processing had a good bactericidal effect on *E. coli* and *S. cerevisiae*. The results will hopefully provide important reference data for the application of DHPM with yam juice.

Acknowledgement. We would like to thank Professor Joe Regenstein at Cornell University for his assistance with editing.

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Accepted: November 30, 2020
Received: June 7, 2021