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Changes in lychee (*Litchi chinensis* Sonn.) texture and volatile compounds due to ultra-high pressure processing

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Abstract

The purpose of this study was to determine the changes of textural properties and flavor profiles of lychee after high pressure processing. It was found that the firmness values of pressurized lychee decreased with increasing pressure levels, temperatures and processing times, in particular lychee pressurized at 600 MPa and 60°C for 20 min. Considering the confocal scanning laser microscopic (CSLM) images, the microstructure of lychee treated under extreme conditions (600 MPa/60°C/20 min) was more severely affected than samples treated at 200 MPa and 20°C for 10 min. GC-MS chromatograms elucidated that ethanol and ethyl acetate were the major volatiles in fresh and pressurized samples. Pressurization at 200 MPa could maintain ester volatiles (freshness or fruity aromas) present in lychee better than more extreme processing level.

Introduction

Lychee (*Litchi chinensis* Sonn.) is an economically important tropical and subtropical fruit in many countries, i.e. Thailand, China, India, Viet Nam and Myanmar. Its pulp consists of a transparent-white, juicy aril, surrounding a large brown seed and covered with a reddish gristly skin. The unique flavor of lychee fruit is usually described as honey, rose-floral and citrus-fruity (Mahattanatawee *et al*., 2007; Wu *et al*., 2009). In addition, it is an excellent source of phytochemical components including ascorbic acid and polyphenols such as anthocyanins and other flavonoids (Menzel, 2002; Zhang *et al*., 2013; Kingwatee *et al*., 2015). Although, lychee is consumed principally as fresh fruit, attempts are being made to produce sterilized fleshes. Sterilization is one of the most popular methods used to preserve this product, but this processing usually degrades natural color and flavor, and induces to loss of nutrients and phytochemical constituents (Apichartsrangkoon *et al*., 2013).

Ultra-high pressure processing, a novel non-thermal technology, constitutes a promising alternative to substitute the conventional treatments such as blanching, pasteurization and sterilization (Chaikham and Prangthip, 2015; Denoya *et al*., 2015). It has been discovered to conserve the bioactive compounds, color and flavor, and inhibit some disagreeable microorganisms and enzymes of various fruit- and vegetable-based products (Chaikham and Apichartsrangkoon, 2012; Keenan *et al*., 2012; Apichartsrangkoon *et al*., 2014; Chaikham *et al*., 2014). Chaikham and Apichartsrangkoon (2012) revealed that pressurization could be an alternative technique to obtain a longan juice with high retention of bioactive compounds (i.e. polyphenols and ascorbic acid) and antioxidant capacity (DPPH assay), and low residual polyphenol oxidase (PPO) and peroxidase (POD) enzymes as well as microbial counts. Moreover, the reports of Apichartsrangkoon *et al.* (2009, 2013) illustrated that high pressure processing better retained the most desirable aroma volatiles and texture properties in pennywort juice (400 MPa/<30°C/20 min) and green chili paste (300-600 MPa/30°C/20 min) respectively, as compared with thermal processing. Quaglia *et al.* (1996) showed that pressurizing of green peas between 400 and 900 MPa for 5-10 min at 20°C did not significantly affect the texture. On the other hand, Rastogi *et al*. (2008) found that pressure above 200 MPa was able to induce a significant increase in the hardness of carrots.

However, the influence of high pressure processing on the alternation of lychee texture and aroma volatile profiles has not been reported to date.

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Thus, this study intended to compare the textural properties of fresh and pressurized lychee with various pressure conditions. The volatile compounds of the samples were also identified by gas chromatography-mass spectrometry (GC-MS) combined with solid-phase micro-extraction (SPME).

Methods and Materials

High pressure processing

Lychee fruits (cv. *Hong Huay*) were freshly harvested in their growing season during the months of April and May at an orchard in Chiang Mai, Thailand. After peeling and stone removal, ~50 g of the flesh were vacuum-packed into polyethylene bags (Siampack, Bangkok, Thailand) and then processed with different pressure levels of 200, 400 or 600 MPa at 20 or 60°C for 10 or 20 min, using a Stansted "Food-Lab" model 900 high pressure rig (Stansted Fluid Power Ltd., Stansted, UK). The rate of pressure increase was approximately 320 MPa/min. The pressure transmitting medium was a mixture of castor oil and 98% ethanol (Chemical and Lab Supplies, Bangkok, Thailand) at a ratio of 20:80 (v/v).

Texture analysis

The firmness of samples was measured with a penetration test using a TA-XT Plus texture analyser (Stable Micro Systems Ltd., Surrey, UK). The lychee flesh was divided longitudinally into two equal segments. The firmness of each segment was tested with a cylindrical aluminium probe 4 mm in diameter fixed to a 5 kg load cell. The area under the curve up to the maximum compression of the first bite is the work done by the machine and was used as an index of firmness (g.mm).

Confocal scanning laser microscopy (CSLM)

Samples which preserved in 70% ethanol were sliced perpendicular to the long axis of the tuber from surface to core using a mechanically guided razor blade. Rectangular specimens (10-13 × 7-8 × 2-3 mm) were placed directly on a glass slide, and washed with phosphate buffer saline (PBS, pH 7.3: Oxoid, Hampshire, UK) to remove the ethanol. The specimens were then soaked in a 0.01% tetraethylrhodamine B fluorescent dye solution (Sigma, Munich, Germany) for 20 min. Excessive dye was removed by washing with PBS. The specific protein-dye conjugate was excited at 568 nm by the laser light source, highlighting the cellular structure. Each sample was viewed using a Leica scanning laser microscope (Leica Microsystems, Wetzlar, Germany).

Determination of volatile compounds

Samples were cut by scissors and then mixed with deionized water at a ratio of 1:1 (w/w) before being grinded and blended in a mortar for 3 min. Twenty grams of homogenate were poured into a 40 ml chromatography glass vial with a Teflon-coated septum (Qmx Laboratories Limited, Dunmow, UK). The volatile components forming the headspace gas were then extracted at 30°C for 45 min by SPME fiber, using a 75 µm thickness of carboxen-polydimethylsiloxane (PDMS) fiber (Supelco, PA, USA). Extracted compounds were then analyzed by a GC-MS (HP5972 MSD with HP5890 GC, Agilent, CA, USA), using a fused silica capillary column Cpsil 8 liquid phase (60 m × 0.25 µm ID, 0.25 mm in film thickness). The volatile compounds were desorbed from the SPME fiber by heating at 250°C, with an initial oven temperature 40°C (2 min) rising to 250°C at 20°C/min (10 min). 1,2-Dichlorobenzene (Aldrich Chemical, Gillingham, UK) as an internal standard (0.1 µl of a solution in ethanol, 130.6 µg/ml) was injected into GC-MS prior to the injection of samples. The carrier gas was helium at 1 ml/min linear velocity. The injector, transfer line and ion source were maintained at 250, 280 and 170°C, respectively. The mass spectrometer scanned from m/z 28.5 to m/z 400 at a scanning rate of 3.88 scans/sec, and the instrument was operated at an ionization voltage of 70 eV.

The GC-MS was calibrated daily by running 0.1 µl of a 100 ppm standard mixture of n-alkanes (C_5-C_{25}). Qualities of volatile compounds were identified by comparing their linear retention indices (LRI) to mass spectra of the bibliographic data of known compounds from the NIST/EPA/NIH mass spectral database (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the laboratory database (Adams, 1995, 2001). Approximate quantities of each compound were determined by comparison of their areas to the integrated peaks of the total ion chromatogram. These were calculated based on peak area with that of the 1,2-dichlorobenzene as the internal standard. The chromatograms of total volatile compounds in fresh and pressurized lychee are shown in Figure 1.

Determination of lipoxygenase (LOX) activity

In brief, 10 g of samples were homogenized with 20 ml of 100 mM phosphate buffer (pH 6.5: Sigma, MO, USA) containing 1 mM EDTA (BDH, UK) plus 0.1% (w/v) Triton X-100 (Sigma, MO, USA) using a bender and then filtered through Whatman® paper

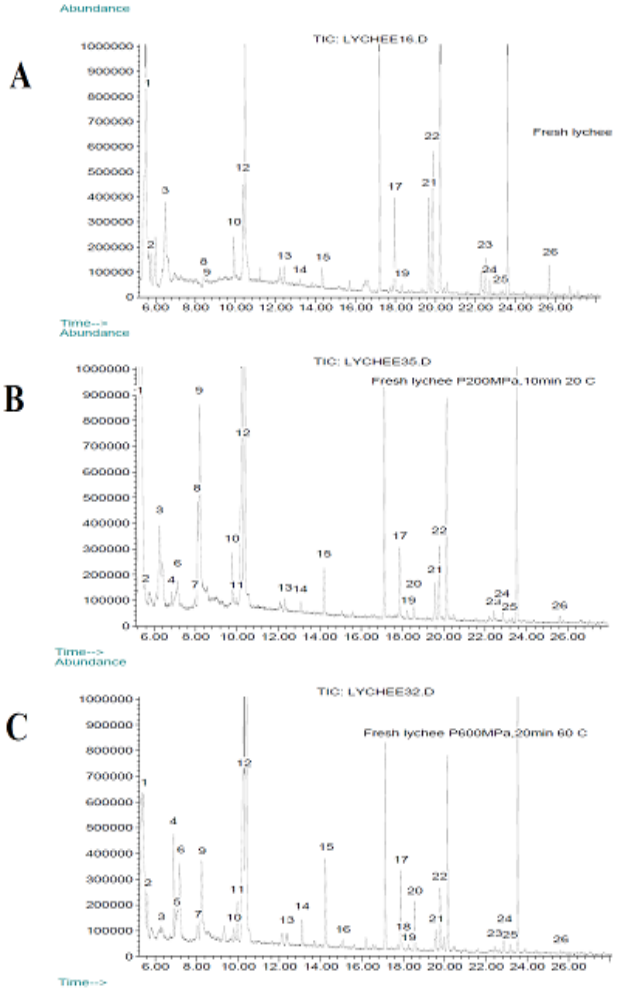

Figure 1. GC-MS chromatograms of (A) fresh lychee, (B) pressurized lychee at 200 MPa/20°C/10 min, and (C) pressurized lychee at 600 MPa/60°C/20 min; 1= ethyl acetate, 2 = 2- methyl-3-buten-2-ol, 3 = 2-methyl butanal, 4 = heptane, 5 = 2-ethyl furan, 6 = pentanal, 7 = 3-hydroxy-2-butanone, 8 = 3-methyl-3-buten-1-ol, 9 = 3-methyl-1-butanol, 10 = (*E*)-2-pentenal, 11 = 1-octene, 12 = hexanal, 13 = (*E*)-2-hexenal, 14 = 3-methylbutyl acetate, 15 = heptanal, 16 = methyl hexanoate, 17 = *β*-myrcene, 18 = 2-pentyl furan, 19 = ethyl hexanoate, 20 = octanal, 21 = *ρ*-cymene, 22 = limonene, 23 = *ρ*-cymene, 24 = nonanal, 25 = *cis*-rose oxide and 26 = menthol.

No. 4. A 0.1-ml filtrate was well mixed with 2.4 ml of 0.2 M citrate-phosphate buffer (Sigma, MO, USA) containing 1.25×10^{-4} M linoleic acid (Sigma, MO, USA) and 0.01% tween 20 (Sigma, MO, USA). The absorbance of the mixed solution was recorded every 1 min for 5 min using a UV-Vis spectrophotometer (Perkin Elmer series Lambda 35, MA, USA). One unit of enzymatic activity was defined as an increase of 0.1 unit of absorbance per min at 420 nm (Smith *et al*., 1997).

Statistical data analysis

All data were the means of six replications. The analysis of variance (ANOVA) was carried out using a statistical program. The determination of significant differences among treatment means was done by Duncan's multiple range tests (P≤0.05).

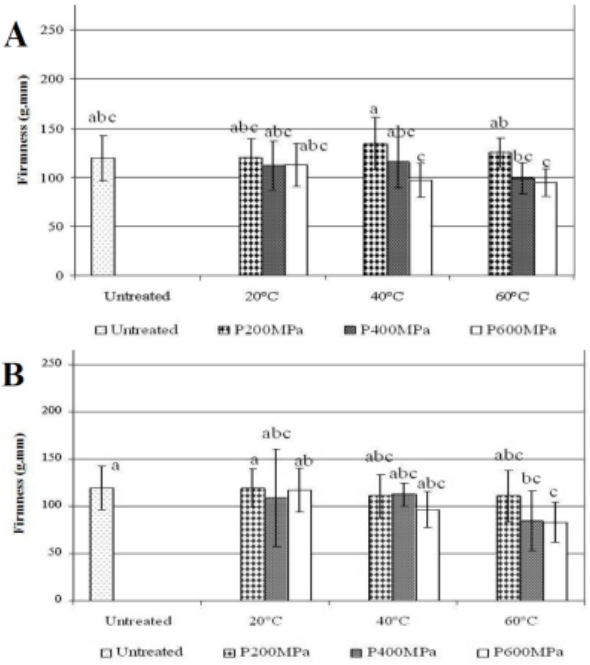

Figure 2. Combined effects of high pressure and temperature for (A) 10 and (B) 20 min on the firmness of lychee. Bars with different superscript were significantly different (P≤0.05).

Results and Discussion

Firmness

Firmness is the most common quality indicator used in evaluating the texture of fruits and vegetables. It is principally determined the tissue structure, cell wall thickness and strength (Toivonen and Brummell, 2008; Zhang *et al*., 2015). In this study, our results showed that some high pressure treatments at high temperature (60°C) had a significant effect (P≤0.05) on firmness of lychee samples (Figure 2). Although the lychee pressurized at 600 MPa for 10 min at temperatures of 40 and 60°C showed the lowest firmness values, no significant difference (P>0.05) was found between the fresh and pressurized samples (Figure 2A). Similarly, lychee pressurized at 200-600 MPa/20-40°C/20 min and 200 MPa/60°C/20 min also displayed no significant difference (P>0.05) in firmness values as compared to the untreated batch. These results are due to the preservation of pectin, which plays an important role in the texture maintenance of fruits and vegetables (Tangwongchai *et al*., 2000). Increasing the temperature to 60°C was associated with a decrease in firmness, particularly in samples pressurized at 400 and 600 MPa for 20 min which displayed significantly lower values

than the control (P≤0.05). It was interesting to note that increasing temperature over the course of time caused a decrease in firmness. The detrimental effects appeared to a greater extent at more extreme conditions (600 MPa/60°C/20 min). Greve *et al.* (1994) explained that simultaneous heat and pressure can induce depolymerization of the pectin substances, which results in a high degree of softening during the processing of fruits and vegetables. Tangwongchai *et al*. (2000) found that the textural damage of whole cherry tomatoes increased with increasing pressures (0-400 MPa), the tomatoes appeared softer with some evidence of free water. In overall, we found that lower and higher levels of pressure, temperature and time showed more effects on firmness of samples. Therefore, lychee pressurized at 200 MPa/20°C/10 min and 600 MPa/60°C/20 min were chosen for investigation the microstructure and flavor profiles compared to control (fresh lychee).

Microstructure

The internal structural images of fresh and pressurized lychee at 200 MPa/20°C/10 min and at 600 MPa/60°C/20 min are depicted in Figure 3. The image of fresh lychee displays turgid and anisotropic cell structures (Figure 3A). After pressurization at 200 MPa/20°C/10 min, the lychee flesh contained large amount of extracellular water, and displayed thin and weakened cell walls. Most cells lost their turgidity, causing the physical changes in cell structure. The outer layer of cells is extremely wrinkled, though some of them maintain their structure. The inner cells are largely collapsed and densely packed with irregular shapes (Figures 3B). For lychee treated at 600 MPa/60°C/20 min, the irregular inner structure of cells is similar to those treated at 200 MPa, with a more collapsed and densely packed appearance than the other samples. The innermost layer of the cells indicates more severe histological damage than for the others (Figure 3C). Overall, cells in pressurized lychee exhibited smaller than the untreated samples. Similar result was reported by Zhang *et al*. (2015) with pressurized asparagus lettuce at pressures 100-300 MPa. This finding concurred with Ludikhuyze and Hendrickx (2001), who demonstrated that at low pressure (100 MPa), the slight loss of firmness in pear and celery was caused by the compaction of cellular structures. Severe texture changes occurred at higher pressures (greater than 200 MPa) because of the cellular membranes rupturing and the consequent loss of turgor pressure. Knorr (1995) reported that pressures up to 350 MPa can be applied to plant systems without any major destruction on texture and structure. The high pressure changes cell permeability

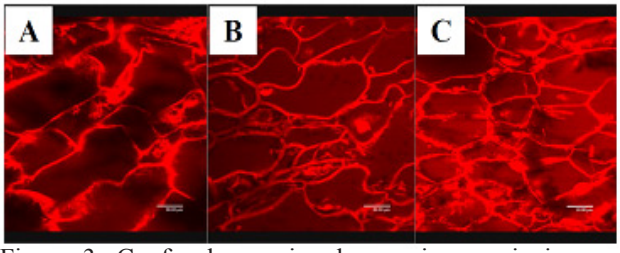

Figure 3. Confocal scanning laser microscopic images of (A) fresh lychee, (B) pressurized lychee at 200 MPa/20°C/10 min, and (C) pressurized lychee at 600 MPa/60°C/20 min

and enables the movement of water out of the cell.

To sum up, the microscopic appearance revealed that changes in lychee treated at 600 MPa/60°C/20 min were more extensive than in the groups treated at 200 MPa/20°C/10 min. The 600 MPa/60°C/20 min group lost turgor pressure and increased cell permeability as a consequence of high pressure affecting the cell structure. This resulted in the cell looking wrinkled and collapsed, with the overall tissue somewhat irregular.

Flavor characterization and LOX activity

In this study, approximately 38 volatiles including 9 aldehydes, 6 alcohols, 4 esters, 13 hydrocarbons, 4 ketones and 2 miscellaneous compounds were identified. The selected volatile compounds which were detected in fresh and pressurized lychee are shown in Table 1. It was found that fresh lychee contained the largest contents of ethanol and ethyl acetate, whereas the most abundant volatiles in pressurized samples were ethanol, ethyl acetate and hexanal. Wu *et al.* (2009) identified 43 volatile components of nine lychee cultivars from Southern China and found high concentrations of ethyl acetate (38.3-364.4 μg/L) and ethanol (571.8-2,680.3 μg/L) in all the samples. Lychee pressurized at 200 MPa and 20°C for 10 min had a higher retention of ethanol and ethyl acetate when compared to extreme level (600 MPa/60°C/20 min). After pressurization under both conditions, the quantities of hexanal significantly increased (P≤0.05), but an apparent reduction of (*E*)-2-butenal was observed. A significant increase of hexanal (grassy odor) in high pressure treated samples at 200 MPa/20°C/10 min (~7 times) and at 600 MPa/60°C/20 min (~11 times) was detected, as compared to fresh batch. Similar outcome was reported by Poretta *et al.* (1995) who found that hexanal content in tomato juice increased significantly after high pressure treatment at 500 MPa for 3 min. Lea *et al.* (2001) revealed that pressurization could enhance the formation of hexanal via lipid oxidation or the enzymatic reaction of LOX on linoleic acid, resulting in off-flavor and

Table 1. Predominant volatile compounds in fresh and pressurized lychees

Volatile compounds (ng/kg sample)*	LRI**	Fresh lychee	Pressurized lychee at 200 MPa/20°C/10 min	Pressurized lychee at 600 MPa/60°C/20 min
ethanol	< 600	302.09 ± 19.58^{a}	280.61 ± 21.13^{a}	231.05 ± 5.24^{b}
ethyl acetate	607	59.71 ± 4.50^{a}	53.47 ± 5.44^{a}	18.83 ± 1.18^{b}
2-methyl-3-buten-2-ol ^{ns}	618	1.79 ± 0.22	1.51 ± 0.08	1.74 ± 0.38
(*E*)-2-butenal	651	3.11 ± 0.53^{a}	1.53 ± 0.51^{b}	1.34 ± 0.45^{b}
2-methyl butanal	661	3.86 ± 0.31^{b}	12.08 ±1.30^{a}	3.77 ± 0.14^{b}
3-methyl-3-buten-1-ol	734	3.14 ± 0.45^{a}	4.81 ± 1.02^{a}	nf ^{b}
3-methyl butanol	739	4.75 ± 0.30^{b}	9.55 ± 0.96^{a}	5.94 ± 1.17^{b}
hexanal	804	4.46 ± 3.20^{c}	29.77 ±2.54^{b}	48.53 ± 5.89^{a}
heptanal ^{ns}	904	2.65 ± 0.64	1.96 ±0.13	2.66 ± 0.61
limonene	1033	4.33 ± 0.31^{a}	4.86 ± 0.91^{a}	2.67 ± 0.15^{b}

Components followed by different letters within each row are significantly different (P≤0.05). nf = not found, ns = non-significant.

* Approximate quantities (ng) in headspace for 10 g sample is estimated by comparison with 100 ng of 1,2-dichlorobenzene as an internal standard.

** LRI is Linear Retention Index.

Table 2. A summary of total volatile classes in fresh and pressurized lychees

Total volatiles (ng/10 g sample)*	Fresh lychee	Pressurized lychee at 200 MPa/20°C/10 min	Pressurized lychee at 600 MPa/60°C/20 min
Aldehydes	14.31 ± 2.48^{c}	51.54 ± 6.17^{b}	61.51 ± 3.76^{a}
Alcohols	312.45 ± 15.64^{a}	297.14 ± 30.55^{a}	239.13 ± 26.33^{b}
Esters	60.10 ± 3.61^{a}	54.37 ± 8.16^{a}	20.42 ± 7.45^{b}
Hydrocarbons	18.28 ± 1.59^{a}	14.76 ± 2.50^{b}	10.30 ± 2.08^{c}
Ketones ^{ns}	2.12 ± 0.45	2.22 ± 0.62	1.79 ± 0.34
Miscellaneous	nf ^{c}	0.92 ± 0.17^{b}	1.72 ± 0.20^{a}

Components followed by different letters within each row are significantly different (P≤0.05). nf = not found, ns = non-significant.

* Approximate quantities (ng) in headspace for 10 g sample is estimated by comparison with 100 ng of 1,2-dichlorobenzene as an internal standard.

off-odor developments in foods (Apichartsrangkoon *et al.*, 2013). In this present study, the residual LOX activities in pressurized lychee samples were 80.84±2.45% and 20.68±3.70% for pressurization at 200 MPa/20°C/10 min and 600 MPa/60°C/20 min, respectively. These findings suggested that high pressure may break the cell, releasing LOX to form a considerable amount of hexanal, which then affects the flavor of lychee. Some volatiles including 2-methyl butanal, 3-methyl butanol and limonene were significantly increased (P≤0.05) or relatively stable (P>0.05) after being pressure treated at 200 MPa/20°C/10 min and at 600 MPa/60°C/20 min. Concentrations of 2-methyl-3-buten-2-ol, 3-methyl-3-buten-1-ol and heptanal in fresh and pressurized samples were similar, though extreme pressurization which showed no 3-methyl-3-buten-1-ol content in the sample. This result suggested that 3-methyl-3-buten-1-ol was more heat or/and pressure sensitive than other selected components.

Total volatile compounds in fresh and pressurized lychee are displayed in Table 2. Amongst the quantified compounds, the predominant volatiles found in both fresh and pressurized samples were the alcohol group, which accounted for ~70.60-82.90% of the total volatiles. In this experiment, pressurization with both conditions had no effect on ketones. High pressure processing at low temperature (20°C) affected flavor volatiles the most, with a trend to greater retention of alcohols, esters and hydrocarbons compared to pressurization at moderate temperature (60°C). For other groups, aldehydes and miscellaneous compounds (furans) showed the highest contents in lychee pressure-treated at 600 MPa and 60°C. The results from this study suggested that pressurization at 200 MPa/20°C/10 min could maintain the fresh, fruity aroma (ester class) of lychee better than the process at 600 MPa/60°C/20 min. Wongfhun *et al.* (2010) stated that pressure and temperature are important parameters in determining the amount aroma volatile compounds in a fruit. The lowest results of some aroma volatiles in samples treated at 60°C might be caused by thermal degradation. Carelli *et al.* (1991) and Jouquand *et al*. (2004) reported that aroma compounds bind to macromolecules through hydrophobic interactions. This effect was enhanced by thermal treatment by escalating the temperature from 25 to 65°C, leading to the lower levels of some volatiles in the products.

Conclusions

The results from this study suggest that the firmness values of lychee after pressurization

decreased with increasing the pressure levels, temperatures and holding times. These findings were confirmed by CSLM images, which revealed that the microstructures of lychee treated with pressure 600 MPa and 60°C for 20 min were extensively changed from the samples treated at 200 MPa and 20°C for 10 min and the control. The GC-MS analysis displayed that ethanol and ethyl acetate were the most predominant volatiles, which were detected in all fresh and pressurized lychee. Pressurization at 200 MPa and 20°C for 10 min could preserve the ester volatiles (freshness or fruity aromas) of lychee more effectively than the extreme condition. Although sterilization is normally applied to extend shelf-life of lychee in syrup, but this method damages the desired characteristics of the products. Thus, ultra-high pressure could be an alternative lychee in syrup processing method to obtain lychee with ordinary texture and flavor, and low LOX activity. However, the influence of pressurization on other quality attributes and consumer acceptability of lychee in syrup should be investigated in the future research work.

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