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Lipoxygenase activity and browning formation of lychee in syrup after high pressure and canning processes

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Abstract

This study aimed to evaluate the effect of high pressure (200, 400 and 600 MPa at 25°C for 10 and 20 min) and canning (100°C for 18 min) processes on lipoxygenase (LOX) activity and browning formation of lychee in syrup. Results showed that the pH profile of LOX activity in lychee was bell-shaped with a maximum activity around pH 4.0. After pressurization, the activities of LOX in lychee flesh apparently diminished with the increasing the pressure levels and processing times, in particular at a pressure of 600 MPa for 10 and 20 min the decreases in activity were 86% and 89%, respectively. For lychee in syrup, LOX was completely inactivated in canned sample, whereas the residual activities in pressurized batches as treated at 200-600 MPa for 20 min were ranged of 18-88%. In addition, pressurized samples depicted much lower browning index than that canned product, since heated lychee extract resulted in the pink color formation with a maximum absorption at 544 nm.

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Introduction

Lychee (*Litchi chinensis* Sonn.) is one of the major fruits grown commercially in Northern region of Thailand including Chiang Mai, Chiang Rai and Lamphun provinces. The fruit is liked for its characteristic sweet-acidic taste, unique aroma and high nutritional values (Phunchaisri and Apichartsrangkoon, 2005; Qiao *et al*., 2012). Availability of fresh fruit is limited because of its short shelf-life. The most common process to preserve lychee flesh is canning or sterilization; however, natural sensory and nutritional properties of the fruit cannot be maintained by the method (Apichartsrangkoon *et al*., 2009, 2013a, 2013b; Chaikham *et al*., 2014). Currently, consumers are demanding fresh, healthful and flavorful foods with long shelf-life. Therefore, high pressure processing which is an alternative non-thermal technology is preferred to use, since it can minimally impair the nutritional and quality parameters (Cao *et al*., 2011; Barba *et al*., 2013). Patras *et al.* (2009) pressurized strawberry purée at 400-600 MPa and 25°C for 15 min and found that ascorbic acid in each product was more than 91% retention. Terefe *et al.* (2009) reported that polyphenol contents in strawberry were unaffected after pressurization at 300-600 MPa and 20°C for 2-10 min. Moreover, Apichartsrangkoon *et al.* (2012) illustrated that pressurization at 400 MPa and < 30°C for 20 min could noticeably retain more ascorbic acid, total polyphenols and antioxidant capacity (FRAP assay) in pennywort juice than that of sterilization (121°C for 4 min).

Lipoxygenase (LOX) is of particular importance in food science and technology because of its essential fatty acids (i.e. linoleic, linolenic and arachidonic acids) destruction resulting in off-flavors and off-odors developments in the products (Apichartsrangkoon *et al*., 2013b). Therefore, the influence of pressurization and canning process on the activity of lychee LOX is interesting to study. Ludikhuyze and Hendrickx (2001) revealed that the effects of pressurization on enzymes can be either reversible or irreversible and inactivation relates to conformational changes in the protein structure. The effects depended on the type of enzyme, substrate, pressure, temperature and processing time. Enzymatic reactions may be stimulated or inactivated by pressure depending on whether the volume change associated with the reaction is positive or negative. Pressure-induced changes in the catalytic rate may be due to changes in the enzyme-substrate interaction,

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changes in the reaction mechanism, the effect on a particular rate-limiting step or the overall catalytic rate. Apichartsrangkoon *et al.* (2013b) found that the residual LOX activities in pressurized green-chili pastes at 100-600 MPa and 50°C for 20 min were retained around 21.13-60.29%, while in pasteurized (90°C for 3-5 min) and sterilized (121°C for 4 min) batches, the activities were completely inhibited. Therefore, this work was intended to find out the effects of pressurization (200, 400 and 600 MPa at 25°C for 10 and 20 min) and canning process (100°C for 18 min) on LOX activity and browning index of lychee in syrup. Pink discoloration in heated product was also determined.

Materials and Methods

Preparation of lychee flesh and syrup

Lychee (*Litchi chinensis* Sonn.) fruit was purchased from a commercial orchard in Chiang Mai, Thailand. After peeling and seed removal, the flesh was soaked in a mixture solution of 1% (w/v) calcium chloride (Fisher, UK) and 0.1% (w/v) citric acid (BDH, UK) for 15 min, subsequently washed 3 times in deionized water prior to processing. Syrup was a mixture of 300 g sucrose, 1.3 g citric acid and 700 g deionized water.

High pressure processing

Fifty grams of lychee flesh with and without 100 ml syrup addition were vacuum-packed into polyethylene bags (Cryovac Ltd., UK). Afterwards, each bag was then processed at pressure levels of 200, 400 and 600 MPa at 25°C for 10 and 20 min in a Stansted "Food-Lab" model 900 high pressure rig (Stansted Fluid Power Ltd., UK). The rate of pressure increase was about 310 MPa/min. The pressure transmitting medium was a mixture of castor oil and 98% ethanol (Chemical & Lab Supplies, Thailand) at a ratio of 20:80 (v/v).

Canning process

Canned lychee was produced following the commercial process of the Royal Agriculture Company Ltd, Chiang Mai, Thailand (Phunchaisri and Apichartsrangkoon, 2005). One hundred grams of lychee were filled into a tin can and 200 ml syrup was then added. The filled cans were exhausted in steam for 10 min prior to sealing and sterilization in boiling water for 18 min. Finally, the products were cooled down to ~ 25-30°C before analysis.

Determination of LOX activity

Ten grams of fresh and processed lychee fleshes were homogenized at 4°C with 20 ml of 100 mM phosphate buffer (pH 6.5: Sigma, USA) containing 1 mM EDTA (BDH, UK) and 0.1% (w/v) Triton X-100 (Sigma, USA) (Smith et al., 1997). The homogenates were centrifuged at 20,000×*g* for 40 min at 5°C and filtered through Whatman® paper No. 41. The filtrates were used for the enzyme assay. A 0.1-ml aliquot of crude enzyme extract was added to 2.4 ml of 0.2 M citrate-phosphate buffer (working linoleic acid substrate: Sigma, USA) containing 1.25×10^{-4} M linoleic acid (Sigma, USA) and 0.01% tween 20 (Sigma, USA) and subsequently its absorbance at a λmax 234 nm was followed for at least 5 min with a Perkin Elmer UV/VIS Spectrophotometer Lambda 20 (Waltham, USA)

The pH optimum for LOX was determined using the mixture solution of working linoleic acid substrate which adjusted the pH from 2 to 10 with 3 N HCl or 3 N NaOH and assayed as described above.

Determination of browning index

A Color Quest XE colorimeter (Hunter Lab, Reston, VA) was used to measure the CIE color parameters (L, a^* and b^*) of fresh and processed samples. Browning index (BI) was calculated from the following equations (Ferrari *et al*., 2010);

$$BI = [100(x - 0.31)]/0.172, \text{ where } x = (a^* + 1.75L)/(5.645L + a^* - 3.012b^*).$$

Determination of pink discoloration in heated sample

Anthocyanidins were generated from the leucoanthocyanidins of lychee by heating with n-butanol (Merck, Germany) containing 5% HCl over a water bath for 10 min. After cooling, the colored butanolic layer was centrifuged and filtered through a 0.45-µm nylon membrane (Vertical, Thailand). The absorbance of the filtrate was then measured by a spectrophotometer from the wavelength of 400 to 700 nm. The spectral data were used to estimate the anthocyanidins which have maximum absorption at 500-550 nm (Musingo and Wang, 2005).

Statistical analysis

All data were the means of triplicate determinations with individual duplication (n = 6). The analysis of variance (ANOVA) was performed using SPSS version 11.5 (SPSS Inc., USA). The determination of significant differences among treatment means was done by Duncan's Multiple Range Tests ($P < 0.05$).

Results and Discussion

pH optimum for lychee LOX

The pH optimum of lychee LOX was 4.0 with linoleic acid as substrate. The results showed that, on increasing the pH from 4.0 to 5.0, a sharp drop in enzyme activity was observed. In addition, this enzyme displayed very low activity at pH < 4.0. At pH below 2.0 and at pH above 5.0, enzyme had no activity. This result was in agreement with the maximum value found by Nielsen *et al.* (2004) and Hammer (1993) for LOX from leeks and tomato, respectively. On the other hand, López-Nicolás *et al.* (2001), Szymanowska *et al*. (2009) and Yoshie-Stark and Wäsche (2004) found that the pH profiles of LOX activity from eggplant, pear seeds and lupins were bell-shaped with the maximum around 7.0, 5.5 and 6.8, respectively. Liagre *et al*. (1996) and Szymanowska *et al.* (2009) revealed that optimum pH for LOX activity depended on plant species, varieties, climates, cultivations and store conditions.

Effect of processing on LOX activity

Residual LOX can cause chlorophyll destruction and off-flavor development in fruits and vegetable products. Figure 1 exhibits that pressure treatment led to a reduction of the enzyme activity in lychee flesh for both times of 10 and 20 min. A decrease in LOX activity of 86% was observed after being treated at 600 MPa for 10 min and only 11% of the original activity remained when treated at the longer time (20 min). This agrees with several investigations on green bean (Indrawati *et al*., 2000), peas (Indrawati *et al*., 2001) and soybean (Ludikhuyze *et al.*, 1998), which reported that pressure inactivation of LOX occurred between 400 and 600 MPa at ambient temperature.

LOX was completely inactivated in canned sample, whereas the residual activities of pressurized batches that were treated at 200-600 MPa for 20 min were ranged of 18-88% (Figure 2). The results also illustrated that after pressurization the activities of LOX in the samples apparently diminished ($P < 0.05$) with an increasing the pressure levels. However, when lychee pressurized with syrup the effects were less marked due to the baroprotective effect of the syrup as has been found for peroxidase (POD) and polyphenol oxidase (PPO) in a study of Phunchaisri and Apichartsrangkoon (2005). Thus, it was interesting to note that LOX is more sensitive to pressure than POD and PPO. In this study, a decrease in LOX activity of 82% was observed after being treated at 600 MPa for 20 min, whereas the decreases in POD and PPO activities at the same condition were 17% and 43%, respectively (Phunchaisri and Apichartsrangkoon, 2005). Seyderhelm *et al.* (1996) stated that it was possible to rank the enzymes in buffer according to their pressure induced inactivation in the following order: LOX, lactoperoxidase, pectin methylesterase, lipase, phosphatase, catalase, PPO and POD.

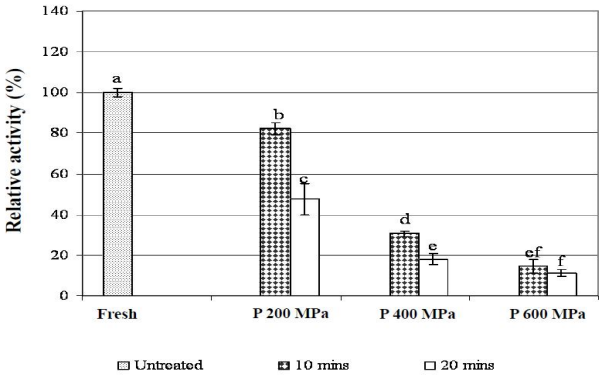

Figure 1. Relative activity of LOX in (▭) fresh and pressurized lychees at 200-600 MPa for (▦) 10 and (□) 20 min

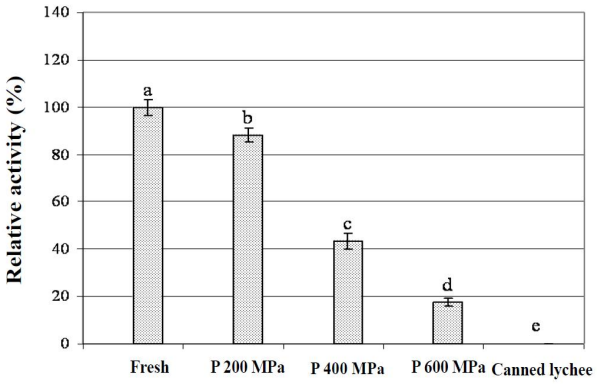

Figure 2. Relative activity of LOX in fresh, pressurized (20 min) and canned lychees in syrup

Effect of processing on browning index

Browning is the most important issue reflecting the quality of many food products. The intensity of the color could be expected by BI. The results showed that pressurized (200-600 MPa for 20 min) and untreated products displayed much lower BI than that of canned product. No significantly difference in BI was found in both pressurized and unprocessed batches (Figure 3), indicating that these products had the greatest color quality. Similar results were reported by Chaikham and Apichartsrangkoon (2012), who illustrated that longan juice pressurized at 500 MPa and 25°C for 30 min received a significantly lower in BI than pasteurized juice (90°C for 2 min). High BI in canned lychee could mainly be due to the influence of non-enzymatic Maillard condensation (Chaikham *et al*., 2014), since PPO which is the main influence on enzymatic discoloration in lychee was completely inactivated after sterilization (Phunchaisri and Apichartsrangkoon, 2005). Moreover, pink pigment

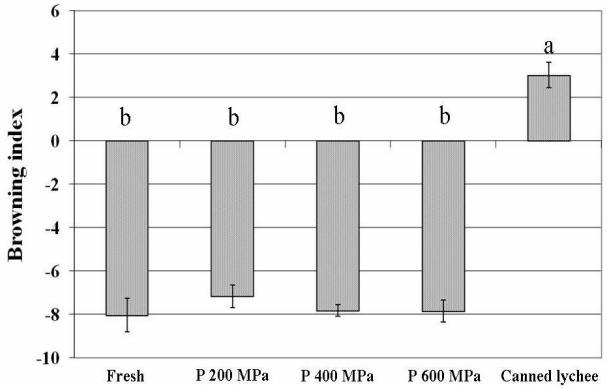

Figure 3. Browning index (BI) of fresh, pressurized and canned lychees in syrup

in the fruits could be generated by the conversion of leucoanthocyanins to anthocyanidins under thermal processing (Manay and Shadaksharaswamy, 2005).

Pink discoloration in heated lychee

As shown in Figure 4a, extraction of fresh lychee with n-butanol containing 5% HCl did not change the original color and displayed no peak at 544 nm. Heating the acidified extract in boiling water for 10 min resulted in a darkening of the solution within 5 min and a deep burgundy color was then developed within 10 min. The color change was also associated with an increase in absorbance at 544 nm (Figure 4b). Leucoanthocyanidins and anthocyanidins are water soluble compounds found in lychee. The presence of anthocyanidins is indicated in plants by the red color and it has a maximum absorption at 500-550 nm (Musingo and Wang, 2005). Hence, the red color development and increase in absorbance at 544 nm in butanolic extract of lychee after heating with acid demonstrated the presence of anthocyanidins in lychee. It was worth noting that the increase of BI in canned lychee (Figure 3) might be due to the conversion of colorless leucoanthocyanidins into anthocyanidins. Besides lychee, the pink discoloration has been found in other canned fruits and vegetables such as apples, pears, bananas, guavas, gooseberries and peaches (Adams and Brown, 2007). The pathway of pink pigment formation in canned lychees has been postulated by Wu and Chen (1999), who revealed that the pink discoloration in canned lychee may result from hydrolysis of condensed tannin into catechin and leucoanthocyanins. The leucoanthocyanins are further degraded into anthocyanins when the product is heated under acidic conditions. The pathway of pink pigment formation in canned lychee is shown in Figure 5. Phenolic compounds can be enzymatically converted to leucoanthocyanidins and these in turn can be enzymatically converted to a different colorless intermediate compound that changes into a red-

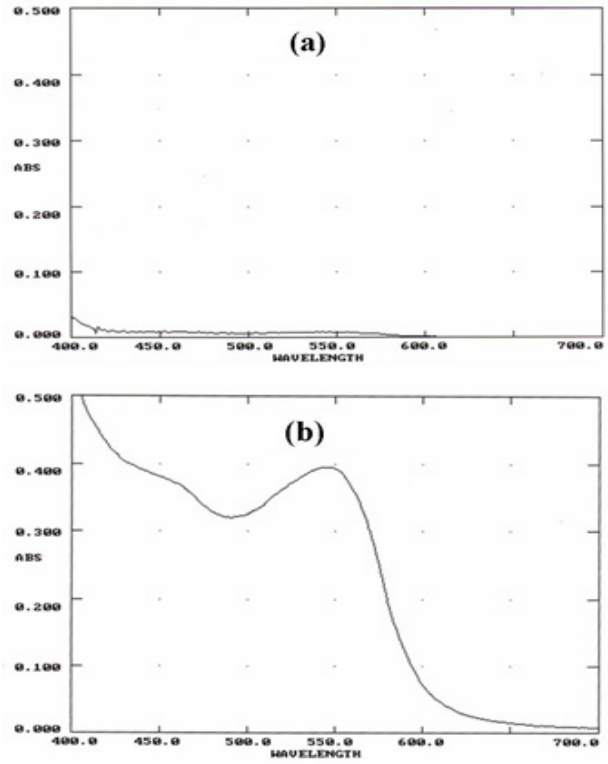

Figure 4. Absorbance of alcoholic extracts of (a) fresh and (b) heated lychees in acidic solution measured by a spectrophotometer with the wavelength of 400 to 700 nm

colored compound during canning and storage. Iron and tin are apparently involved in pigment formation either as co-pigments or catalysts. Moreover, they successfully isolated flavanone-3-hydroxylase and dihydroquercetin-4-reductase from lychee flesh and concluded that these two enzymes play a key role in the biosynthesis of leucoanthocyanins. They proposed a pathway for the pink discoloration during canning as follows: after peeling and pitting, the flavonones in mature lychee flesh are first converted into eriodictyol-containing compounds, then hydrolyzed by flavanone-3-hydroxylase to dihydroquercetin-containing compounds which are further reduced to leucocyanidin-containing compounds by dihydroquercetin-4-reductase; when the lychee is heated, the leucocyanidin-containing compounds are converted into cyanidin-containing colored compounds (Wu and Chen, 1999).

Conclusion

The pH profile of LOX activity in lychee was bell-shaped with a maximum activity around pH 4.0. Pressure treatments at 200-600 MPa for 10 and 20 min led to a reduction in activity of LOX in flesh lychee with and without syrup addition; in particular, at pressure 600 MPa the decreases in activity were

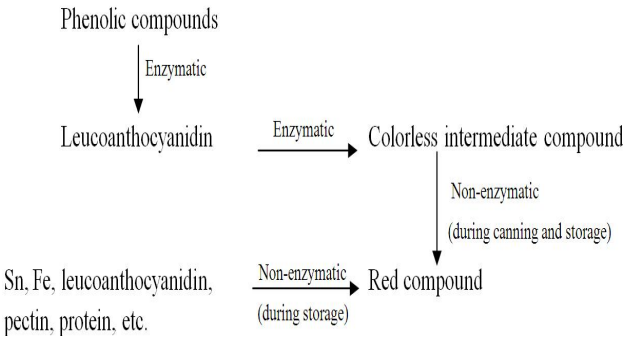

Figure 5. Possible pathway of pink compound formation in canned lychee (adapted from Hwang and Cheng, 1986)

ranged of 82-89%. LOX was completely inactivated in canned sample. Additionally, pressurized samples displayed much lower BI than that of canned product. The heated lychee extract resulted in the red color formation with a maximum absorption at 544 nm.

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