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# APPLICATION OF *Litsea cubeba* TO IMPROVE SHELF-LIFE OF FRESH-CUT 'PACKHAM'S TRIUMPH' PEARS

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*Original scientific paper*

## Summary

Plant-derived natural products can be of interest as a source of alternatives to improve the shelf-life and the safety of food. *Litsea cubeba* has recently received much attention due to multiple functions as antibacterial, antifungal, insecticidal, antioxidant and anticancer agent. The application of *Litsea cubeba* essential oil (LC) in this investigation is one of the first experiments made in order to improve shelf-life of minimally processed fruits. The aim of this work was to investigate the effect of LC at different concentrations (50, 100 and 250 ppm), on shelf-life of fresh-cut pears 'Packham's Triumph' variety. Colour of fresh-cut pears as well as, polyphenol content, antioxidant activity and flesh firmness of untreated and treated samples, were determined. Analysis were carried out immediately following oil treatments, and on 1, 7, 14 day of storage at 2 °C. Treatment with 50 ppm of LC was the most effective treatment to maintain colour of fresh-cut pears during 14 day of storage. Phenolic compounds and antioxidant activity in samples treated with solutions of LC remain stable during 14 day of storage. The lowest loss of firmness was detected in fresh-pears treated with 250 ppm LC. Essential oil of *Litsea cubeba* has promising application as a treatment of fresh-cut pears.

*Keywords:* pears, *Litsea cubeba*, fresh-cut, shelf-life

## Introduction

Fresh-cut fruit products is large and rapidly growing segment in the food service and retail markets, so far, and further growth can be anticipated (Pilizota and Sapers, 2004; Rojas-Graü et al., 2011; Sapers and Miller, 1998; Soliva-Fortuny and Martín-Belloso, 2003; Wang and Ryser, 2014). Minimal processing damages fruit tissue, which in turn limits the shelf-life of products. During the preparatory steps of minimal processing, the natural protection of fruit is generally removed, and hence, it becomes highly susceptible to microbial spoilage (Martín-Belloso et al., 2006). In addition, cross-contamination may occur during cutting and shredding operations because sanitation may not have been carried out properly (Oms-Oliu et al., 2010). Oxidation is the second most important cause of food deterioration after that induced by microbiological contamination. The main oxidative reactions are enzymatic browning. So, all phenomena (cutting, loss of firmness, etc.) lead to the starting of browning reactions which induce losses or changes of flavour, odour and nutritional value (Toivonen and Brummell, 2008).

Phenolic compounds have the ability to prevent the oxidation of low-density lipoprotein (LDL) due to their antioxidant properties, attributable to the free radical-scavenging properties of their constituent hydroxyl groups. The inhibition of LDL oxidation has been associated with a lower incidence of coronary diseases. Among the several classes of plant phenolics four have been reported in pear fruits: phenolic acids, flavonols, flavan-3-ols and anthocyanins. Polyphenols have been widely studied in relation to their chemistry, and the changes in their content during postharvest life have been extensively reviewed (Sánchez et al., 2003).

In recent years, the interest in natural antimicrobial compounds has increased, and numerous studies on the shelf-life extension of fresh-cut fruits and vegetables with a wide range of natural compounds have been reported (Ayala-Zavala et al., 2009; Du et al., 2009; Gradvol et al., 2015; Lanciotti et al., 2004). Plants and plant-derived natural products can be of the interest as a source of alternatives to improve the shelf-life and the safety of food (Oms-Oliu et al., 2010). Essential oils and oil compounds have been previously evaluated for their ability to protect food against many microorganisms, including some pathogens (Lanciotti et al., 2004). Antimicrobial activity of essential oils is associated with the terpenoids, organic sulphur compounds, aldehydes and alcohols, among others. Well-known terpenoids include citral, menthol, camphor, geraniol, eugenol, menthol and cinnamaldehyde (Ayala-Zavala et al., 2009; Rojas-Graü et al., 2011). Their main limitation is due to the strong odours and tastes that may transfer to the product.

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*Litsea cubeba* Pers. is an evergreen plant which has an intensely lemon-like, fresh and sweet odour. *Litsea cubeba* essential oil (LC) and its main compound citral (a mixture of geranial and neral) have potential in inhibiting the growth of bacteria as well as fungi (Yang et al., 2010; Wang and Liu, 2010). Citral is known to be antitumoral and antifungal, and widely used flavouring agent which is employed in numerous food, industrial, and household products. Oil from *Litsea cubeba* competes to a limited extent with lemongrass, another citral-rich oil, in fragrance applications (Coppen, 1995). Raybaudi-Massilia et al. (2008) indicated that lemongrass oil and its main active compound (citral) mixed into an alginate-based coating faster inhibited *E. coli* O157:H7 (increased their antimicrobial effect and extended the microbiological shelf-life of 'Fuji' apples) than cinnamon and clove oils or their active compounds (cinnamaldehyde and eugenol).

The application of *Litsea cubeba* essential oil in this investigation is one of the first experiments made in order to improve shelf-life of minimally processed fruits. The aim of this work was to investigate the effect of *L. cubeba* oil (LC), at different concentrations (50, 100 and 250 ppm) on shelf-life of fresh-cut pears 'Packham's Triumph' variety.

## Materials and methods

*Materials*

Hydrochloric acid, potassium chloride, sodium acetate, sodium hypochlorite, methanol, hydrogen peroxide and Folin-Ciocalteu reagent were purchased from Kemika (Zagreb, Croatia) while DPPH (2,2-diphenyl-2-picrylhydrazyl) was obtained from Sigma-Aldrich (Steinheim, Germany). 'Packham's Triumph' pears were obtained from a commercial orchard in Slavonia County (Croatia) and stored at 2 °C in air for few days, before they were used for trials. Solutions of *Litsea cubeba* essential oil (China Aroma Chemical Co., Ltd. Hangzhou, Zhejiang, P.R. China) in concentration of 50, 100 and 250 ppm were prepared in 10% Tween 80, 95% ethanol and sterile distilled water.

Pears were held at room temperatures for cca 1 hr before cutting and further treatment. Pear fruit firmness was determined by measuring the force required for an 8 mm probe to penetrate into the fruit, with the skin removed, using a fruit pressure tester (McCormick, Yakima, WA, USA). Pears selected for treatment had a flesh firmness of 57-75 N.

*Fresh-cut processing*

Before experiment pears were washed in 3% hydrogen peroxide for 2 min and rinsed with sterile distilled water. To avoid contamination during sample preparation and treatment, all equipment in contact with pears were sanitised by immersion in 1000 ppm $Cl_2$ (adjusted to pH 6.5 with citric acid). Pears were cut into eight wedges with a wedge that removes a 22-mm-dia core. The wedges from individual pears were immersed into the LC solution for 1 min immediately after cutting, removed with a plastic colander and gently dried by rolling on four layers of absorbent tissue to remove excess liquid from the surface. Fruit wedges dipped for 1 min in sterile water, as well as wedges without any treatment was considered as controls. Immediately following treatment with *L. cubeba* oil at different concentrations (50, 100 and 250 ppm), or dipping in sterile water and dewatering, sets of 8 wedges were stored in high density polyethylene (HDPE) bags. Treatments were carried out in triplicate. Samples were stored at 2 °C for up to 14 days. Analysis of untreated and treated fresh-cut pear samples were carried out immediately following oil treatments, at day 1, 7 and 14 at 2 °C storage.

*Sample evaluation*

The colour of pear wedges was evaluated with a Minolta CR-300 tristimulus chromameter (Minolta Camera Co., Japan) using the standard white reflector plate. Results were expressed as L* (lightness), a* (redness), b* (yellowness), C* (intensity of colour) and h° values (hue angle, actual colour), immediately after treatments ("0" time), and during storage at day 1, 7 and 14, using the averaging mode with 8 replications. Based on the measured data, the calculation of effectiveness of LC solutions was performed by equations (Sapers and Douglas, 1987):

$$\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2} \quad (1)$$

$$\% \text{ inhibition} = (\Delta L\text{control} - \Delta L\text{treated})/\Delta L\text{control} \cdot 100 \quad (2)$$

$\Delta L\text{control} - \Delta L$ of control sample dipped in sterile water

Influence of sources of variation (different concentrations of *L. cubeba* oil) on the colour parameters (L*, C* and h°) of fresh-cut pears was examined by ANOVA, followed by post-hoc Bonferroni test ($p<0.05$); XL stat ver. 2009 3.02, Addinsoft, Inc. Brooklyn, New York, USA. Total phenol content (PC) was determined using the Folin-Ciocalteu colorimetric method described by Ough

and Amerine (1988). The measurements were performed in triplicates for each sample and the average value was interpolated on a gallic acid calibration curve and expressed as g of gallic acid equivalents (g GAE) per L of the sample. Antioxidant activity (AA) of the fresh-cut pears was determined by DPPH assays and expressed in mmol trolox equivalents (TE)/100 mL of the sample (Arnao et al., 2001). Measurements were done in triplicates. Firmness of fresh-cut pear wedges was measured at day "0", 1, 7 and 14 using a texture analyser (TA.XT 2, Stable Micro Systems, UK) fitted with a 2 mm diameter probe. The penetration depth was 5 mm and the cross-head speed was 1.5 mm $s^{-1}$.

## Results and discussion

The aim of this work was to investigate application of essential oil from *Litsea cubeba* (LC) to improve shelf-life of minimally processed pears. Trials carried out in our laboratory indicated that LC essential oil at used concentrations has no impact to the sensorial properties of 'Packham's Triumph' pears (evaluated by means three panelists).

*Effect Litsea cubeba on colour*

Colour is a critical quality property of fresh-cut fruit such as pear, apple, banana, etc. since cutting operations lead to enzymatic browning. The colour of pear wedges was evaluated with a tristimulus chromameter using the standard white reflector plate. Results were expressed as L* (lightness), a* (redness), b* (yellowness), C* (intensity of colour) and h° values (hue angle, actual colour), immediately after treatments ("0" time), and during storage at day 1, 7 and 14.

The L* value is a useful indicator of darkening during storage, either resulting from increasing pigment concentrations or from oxidative browning reactions (Sapers and Douglas, 1987). These reactions result from polyphenol oxidase (PPO) catalysed oxidation of phenolic compounds to *o*-quinones which subsequently polymerise to form dark-coloured pigments. Table 1 shows the changes in surface pear colour, with and without treatment and stored at 2 °C, as given by lightness (L*), chroma (C*) and hue angle (h°). The value of chroma C* (the saturation index) is proportional to its intensity. Dipping solutions and storage time had a significant effect ($p<0.05$) on the lightness of fresh-cut pears in comparison with control samples (untreated and samples treated with sterile water). L* values of control samples decreased rapidly during the 24 hours (after day 1) which shows the similar trend as was found by Sapers and Miller (1998), Rocha and Morais (2003). L* values for pear wedges treated with 50 and 100 ppm LC oil were slightly reduced during first 24 hours of storage and were significantly different from the control samples except ones treated with 250 ppm LC, only. However, after 14 days of storage, L* values of LC treated samples were similar, and significantly different from control (untreated and treated with water) samples. C* values for control samples increased quickly after 24 hours, while changes in C* value were less significant in samples treated with LC. Dipping solutions and storage time also significantly ($p<0.05$) affected the hue angle (h° values). Pears treated with LC oil maintained lower reduction in hue angle than those of controls throughout 14 days. Also, according to Oms-Oliu et al. (2006) decrease in h° values along time (28 days) was more pronounced in control samples (pear wedges dipped in distilled water) than those treated with N-acetyl-L-cysteine (0 to 3%) or reduced glutathione (0 to 3%). The pear sample treated with 50 ppm LC showed the smallest change in L*, C*, as well as in h° values.

**Table 1.** Influence of sources of variation (different concentrations of *L. cubeba* oil) on the colour parameters (L*, C* and h°) of fresh-cut pears examined by ANOVA, followed by post-hoc Bonferroni test

| Day | Colour parameter | Sample/treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control untreated | Control treated with water | 50 ppm LC | 100 ppm LC | 250 ppm LC |
| "0" | L | $81.28\pm0.73^{a}$ | $79.48\pm0.67^{ab}$ | $78.38\pm2.00^{b}$ | $79.07\pm1.34^{b}$ | $78.75\pm0.85^{b}$ |
| | C | $14.91\pm1.63^{b}$ | $16.78\pm1.11^{bc}$ | $20.27\pm1.50^{a}$ | $18.22\pm1.97^{ab}$ | $18.30\pm1.51^{ab}$ |
| | h° | $103.28\pm2.14^{a}$ | $100.34\pm1.13^{b}$ | $90.91\pm2.04^{d}$ | $97.78\pm1.69^{b}$ | $94.33\pm1.65^{c}$ |
| 1 | L | $70.48\pm0.83^{bc}$ | $68.39\pm1.89^{c}$ | $74.60\pm1.79^{a}$ | $74.09\pm1.97^{a}$ | $71.21\pm1.02^{b}$ |
| | C | $26.04\pm1.79^{a}$ | $26.52\pm2.29^{a}$ | $23.98\pm1.99^{ab}$ | $22.69\pm2.13^{b}$ | $24.89\pm1.76^{ab}$ |
| | h° | $87.09\pm1.90^{a}$ | $84.09\pm2.63^{b}$ | $84.01\pm1.09^{b}$ | $86.70\pm1.93^{ab}$ | $85.04\pm1.15^{ab}$ |
| 7 | L | $70.46\pm1.01^{b}$ | $67.65\pm2.29^{c}$ | $72.55\pm1.15^{ab}$ | $73.86\pm1.61^{a}$ | $71.08\pm1.93^{b}$ |
| | C | $26.45\pm1.61^{ab}$ | $27.16\pm2.11^{a}$ | $25.53\pm1.43^{ab}$ | $24.24\pm1.17^{b}$ | $25.06\pm1.15^{ab}$ |
| | h° | $85.58\pm1.98^{ab}$ | $82.03\pm1.52^{c}$ | $83.98\pm1.14^{bc}$ | $86.69\pm0.78^{a}$ | $84.90\pm1.56^{ab}$ |
| 14 | L | $65.03\pm2.15^{b}$ | $65.91\pm2.02^{b}$ | $70.97\pm1.97^{a}$ | $71.69\pm1.29^{a}$ | $70.77\pm1.21^{a}$ |
| | C | $27.43\pm2.15^{a}$ | $27.05\pm2.41^{a}$ | $25.56\pm1.30^{a}$ | $24.63\pm1.46^{a}$ | $25.36\pm1.70^{a}$ |
| | h° | $80.49\pm1.05^{b}$ | $81.91\pm1.97^{ab}$ | $82.83\pm1.55^{a}$ | $83.61\pm1.89^{a}$ | $82.33\pm1.76^{ab}$ |

*data with the same letter in the same column are not significantly different ($p<0.05$)

Changes in total colour difference (ΔE) of pear wedges, as influenced by each treatment during storage, are shown in Fig. 1. Samples dipped in sterile water had the highest total colour difference (i.e. underwent similar colour difference to control untreated sample) except at the end of storage (14$^{th}$ day). The results showed that ΔE of pear wedges treated with LC oil at the lowest concentration (50 ppm) was the most effective treatment to prevent cut surface browning of pear wedges during 14 day storage. Besides ΔE, calculation of effectiveness of LC solutions was performed by % inhibition, according to L* parameter of colour. Treatments with 50 and 100 ppm LC oil were more effective than the treatment with 250 ppm LC (Fig. 2).

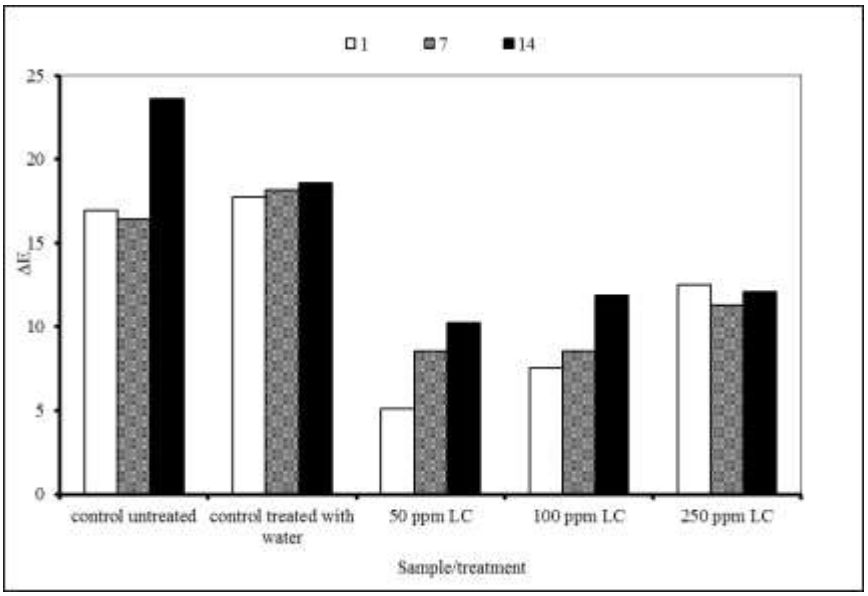


**Fig. 1.** Effect of LC on total colour change (ΔE) of pear wedges during storage 14 days at 2 °C

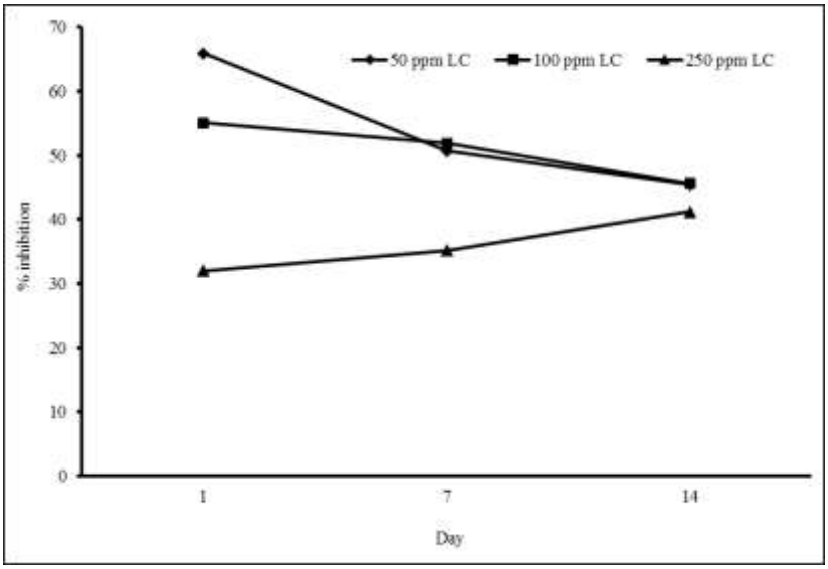


**Fig. 2.** Effect of LC on colour of pear wedges during storage 14 days at 2 °C (% inhibition according to L parameter of colour)

*Effect Litsea cubeba on total phenolic content and antioxidant activity*

On the first day of measurement, the total phenol values of pear samples varied around 2 g/L GAE, which is in accordance with the investigations of Sánchez et al. (2003) and Nedić Tiban et al. (2011). During refrigerated storage, polyphenol content in control samples slightly increased. It is possible that during pear storage, some compounds are formed that react with Folin-Ciocalteu reagent and significantly enhance the phenolic content (Piljac-Žegarac et al., 2009). Phenolic compounds in samples treated with solutions of LC oil remain stable during 14 day of storage at 2 °C (Fig. 3). The evolution of DPPH radical scavenging capacity in pear wedges with time is shown in Fig. 4. The initial radical scavenging capacities of pear sample treated with water slightly decreased during storage (tissue breakdown, which produced a dark and uneven water-logged appearance). Antioxidant activity during 7 days storage decreases in all other samples, followed by increase at the end of storage period (14 day of storage). According to Pinelo et al. (2004) the increase in the antioxidant activity may be explained by the strong tendency of polyphenols to undergo polymerization reactions, whereby the resulting oligomers possess larger areas available for charge delocalization. When the degree of polymerization exceeds a critical value, the increased molecular complexity and steric hindrance reduce the availability of hydroxyl groups in reaction with DPPH radicals, which causes a decrease in the antiradical capacity.

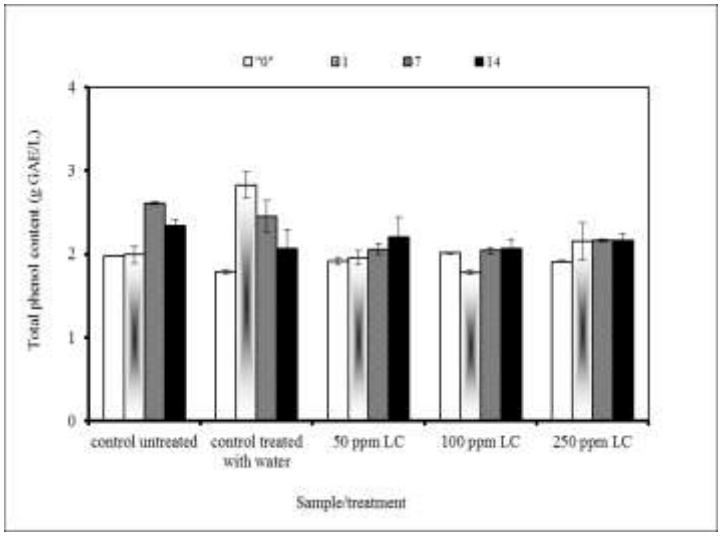


**Fig. 3.** Total phenol content (PC) of untreated and treated fresh-cut pears during storage 14 days at 2 °C

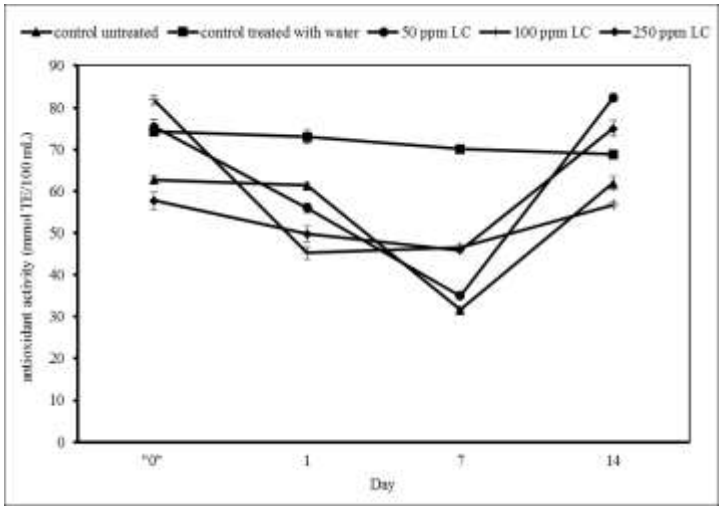


**Fig. 4.** Antioxidant activity (AA) of untreated and treated fresh-cut pears during storage 14 days at 2 °C

*Effect Litsea cubeba treatments on flesh firmness*

Flesh softening is also one of major problems connected with the extension of shelf-life of minimally processed products, since enzymes causing degradation of cell walls are not inhibited. No negative effect of dipping in LC solution treatments to flesh firmness was observed. The lowest loss of firmness was detected in fresh-pears treated with 250 ppm LC, contrary to control samples (Fig. 5). No significant loss of firmness was observed in all investigated samples (examined by ANOVA, followed by post-hoc Bonferroni test, $p<0.05$).

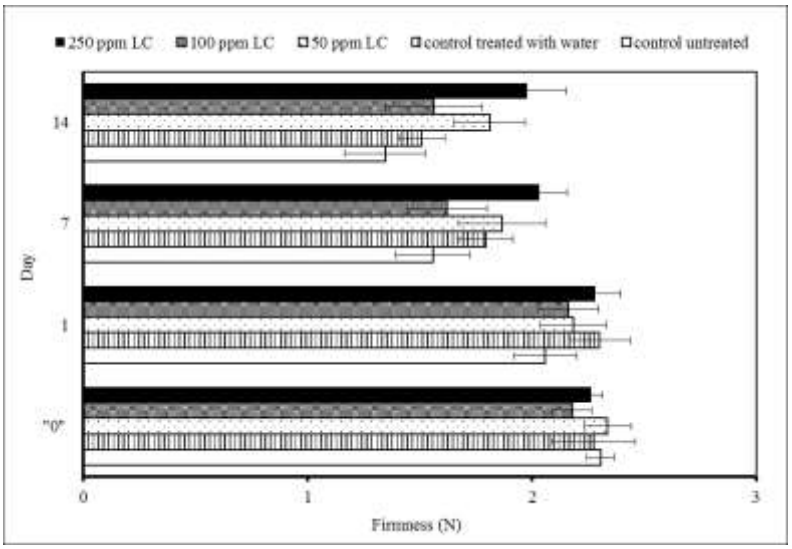


**Fig. 5.** Influence of different concentrations of LC on firmness (N) of fresh-cut pears during storage 14 days at 2 °C

## Conclusions

Essential oil of *Litsea cubeba* has promising application as a treatment of fresh-cut pears. Treatment with 50 ppm of LC oil was the most effective treatment to prevent cut surface browning during 14 day of storage. Compared to controls, LC oil at highest concentration (250 ppm) showed the greatest maintenance of firmness. Since browning and susceptibility to bruising are among the factors that limit the storage life of pears, cultivars with high level of compounds with an antioxidant capacity are recommended. Further investigations will be focused on microbial population of fresh-cut pears and effect *Litsea cubeba* on microorganisms.

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