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ORIGINAL ARTICLE

Preservation of orange juice using propolis

Wenchao Yang[1,3] · Zhenhong Wu[1,2] · Zachary Y. Huang[1,2] · Xiaoqing Miao[1,3]

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Abstract Orange juice is one of the most popular and the most consumed fruit juices all over the world, especially in Europe and the chemical food preservatives, such as sodium benzoate, potassium sorbate and their mixtures, have long been used in orange juice sold on the market. Excessive consumption of these preservatives may be hazardous to human health. Propolis, composed of resins collected from plant buds and exudates and mixed with salivary gland secretions and beeswax by honey bee workers, has been used as a human medicine and natural food preservative. We hypothesis that propolis, without alcohol, can serve as an alternative and non-synthetic preservative of orange juice. In this study, the preservative effect of propolis emulsion on orange juice was determined up to 35 days. Propolis emulsion (0.02 g/mL propolis, 12 mL), emulsion control (12 mL containing Tween-80, hydrophilic phospholipid and polyethylene glycol 400), sodium benzoate (0.4 g) and potassium sorbate (0.4 g) was each added to 388, 388, 400 and 400 mL orange juice respectively. Propolis emulsion showed significant inhibition of bacteria growth and L-ascorbic acid degradation. Orange juice pH value, titratable acidity, total phenolic content, color and antioxidant capacity were effectively maintained by propolis emulsion. A control solution with all the same emulsifying agents without propolis did not show these properties. It was concluded that propolis can be used as a natural additive agent in orange juice or other fruit juices as an alternative to chemical preservatives.

Keywords Orange juice · Propolis · Color · Antioxidant capacity · L-ascorbic acid

✉ Zachary Y. Huang
bees@msu.edu

✉ Xiaoqing Miao
fjbees@126.com

[1] College of Bee Science, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, People's Republic of China

[2] Department of Entomology, Michigan State University, East Lansing, MI 48912, USA

[3] Bee Product Processing and Application Research Center of the Ministry of Education, Fuzhou 350002, Fujian, People's Republic of China

Introduction

Propolis is a hive product that honey bees (*Apis mellifera*) produce by collecting resins from plant buds and exudates and mixing them with their salivary secretions and beeswax. Propolis is composed of plant resin (50%), beeswax (30%), essential oils (10%), and pollen (5%). It contains various organic compounds (5%) such as vitamins (B1, B2, B3 and B6), benzoic acid, fatty acids, ketones, lactones, quinones, steroids, sugars and natural pigments chlorophyll and carotenoids (Christov et al. 2006). Propolis is highly variable chemically, due to the wide diversity of plant species that honey bees use as resin sources for its production, which vary with geographical locations and seasons (Sforcin et al. 2000; Sahinler and Kaftanoglu 2005; Christov et al. 2006; Mercan 2006; Wiryowidagdo et al. 2009; Valencia et al. 2012). More than 300 components of propolis, mainly phenolic compounds such as flavonoids, phenolic acids, and phenolic acid esters, have been identified (Righi et al. 2013). Propolis is thought to be used as a protective barrier and sterilizing agent inside hives or nests (Simone-Finstrom and Spivak 2010), and has recently been

found to play a key role in maintaining colony health (Popova et al. 2014; Borba et al. 2015).

Propolis is not only important for honey bees, but can also be used for improving human health. It has been used as folk medicine since ~300 BC, and is reported to possess antioxidant (Gregoris and Stevanato 2010; Kumazawa et al. 2004), anti-inflammatory (Paulino et al. 2006, 2008; McLennan et al. 2008), immunostimulating (Nassar et al. 2012; Eyng et al. 2013), antiviral (Amoros et al. 1992; El Hady and Hegazi 2002; Schnitzler et al. 2010), antibacterial and antifungal (Kujumgiev et al. 1999; Sforcin et al. 2000; Ghasem et al. 2007; Netíková et al. 2013) attributes. Furthermore, propolis also has preservative effect on food. Propolis (dissolved in alcohol) has been added to mandarin, apple, orange, and white grape juices because of its antifungal abilities (Koc et al. 2007; Silici and Kevser 2014; Luis-Villaroya et al. 2015). Therefore, propolis has been proven to effectively preserve solid (Yang 2001; Ali et al. 2010; Chen et al. 2011) and liquid foods.

Orange juice (OJ) is one of the most popular and the most consumed fruit juices. OJ contains bioactive compounds such as carotenoids, flavonoids, and vitamin C (Sanchez-Moreno et al. 2003). OJ has been shown to scavenge for free radicals (Sanchez-Moreno et al. 2003) and reduce oxidative DNA damage in blood cells (Guarnieri et al. 2007). The plasma concentrations of markers for inflammation and oxidative stress are improved after OJ consumption (Ghanim et al. 2010).

Parameters of OJ quality include microbiological stability, color, pH, titratable acidity, antioxidant capacity and contents of soluble solids, Vitamin C and total phenolics. Colors can be evaluated by a spectrophotometer (Lee and Coates 2003) or a colorimeter (Wibowo et al. 2015). pH, titratable acidity and soluble solids of OJ change due to microorganism reproduction (Wibowo et al. 2015). Total phenolics and vitamin C are the main components responsible for antioxidant properties, with vitamin C contributing about 65–100% of the antioxidant potential of OJ (Gardner et al. 2000).

Preservation of OJ and other foods can be achieved by low temperature, but only for a short period of time. To prolong their shelf life, chemical food preservatives, such as sodium benzoate, potassium sorbate and their mixtures, have long been used in OJ (Li et al. 1989). However, excessive consumption of these chemical preservatives may be hazardous to human health (Ward 1997).

The objective of the present study is to determine whether propolis, without alcohol, can serve as an alternative and non-synthetic preservative of OJ. We compared the protective effects among propolis, sodium benzoate and potassium sorbate on the microbiological stability and several quality-related parameters of OJ stored at 25 °C, including color, pH, titratable acidity, concentrations of soluble solids, vitamin C and total phenolics, and antioxidant capacity.

Materials and methods

These experiments were conducted between Sept and Nov, 2015 in Fuzhou, China.

Ethanol extraction of propolis

Crude poplar propolis (5 g, provided by Fujian Shenfeng Technology & Development Co., Ltd.; China) was dissolved in 50 mL 80% ethanol solution. Ultrasound (frequency: 40 kHz) sonication was used to help propolis dissolve for 20 min. After this it was incubated at 60 °C for 3 h and then kept at room temperature (RT, 25–30°C) for 24 h. Ethanol was partially removed by a rotary evaporator (RE52CS-2, Shanghai Yarong Biochemical Instrument Factory, China) in a vacuum (0.098 MPa) at 50 °C. Beeswax solidified on the surface by storing propolis extract overnight at 4 °C, after which the beeswax was removed. The propolis extract was placed in a vacuum drying oven at 55 °C for 2 h until no weight loss to completely remove the ethanol. This ethanol extract of propolis (EEP) was used for further experiments.

Determination of total flavonoid concentrations and flavonoids in propolis

Total flavonoid concentrations in EEP were measured using a spectrophotometer at 510 nm, and calculated from a standard curve made of rutin (Sinopharm, China). Briefly, various volumes (0, 2, 4, 6, 8 mL) of 0.2 mg/mL rutin aqueous solution were placed in a 50 mL volumetric flask. Then, 2 mL 5% (W/V) sodium nitrite (Sinopharm, China) and 2 mL 10% (W/V) aluminum nitrate (Sinopharm, China) were added to the flask, shaken and incubated for 6 min. RO water was added to reach a total of 50 mL after 20 mL 1.075 mol/L sodium hydroxide aqueous solution was added. This solution was shaken and placed for 15 min before their absorbance were determined. Propolis solution (10.28 mg/mL, 95% ethanol) was diluted 25 times by 95% ethanol for determination. The diluted propolis solution (4 mL) was placed in a 50 mL volumetric flask and prepared the same way as described above before absorption was determined.

The concentrations of 7 main types of flavonoids in EEP were determined by HPLC. Briefly, standard rutin, quercetin, kaempferol, galangin, apigenin, chrysin and luteolin solutions were used for standard curves using a HPLC system (LC-10A, UV detector). The mobile phase was

0.4% phosphoric acid: methanol = 45: 55 at a flow rate of 1.0 mL/min. The 20 μL standard and sample solutions were injected after filtered by a 0.22 μm filter. The detection lasted for 75 min at 270 nm wavelength. The retention times of standard rutin, quercetin, kaempferol, galangin, apigenin, chrysin and luteolin were 5.746, 11.917, 14.312, 19.425, 21.677, 48.868 and 57.961 min, respectively.

Emulsion of propolis

Emulsifiers, composed of 0.8 g Tween-80 (Zhengzhou Jinrun Additives Co., Ltd.; China) and 1.2 g hydrophilic phospholipid (Shanghai Daoqin Biological Science and Technology Co., Ltd.; China), were added to 40 mL distilled water. While this mixture was dispersed at a speed of 10,000 rpm (GF-1 disperser, Jintan Ronghua Instrument Manufacture CO. Ltd.; China), 5 mL 20% (w/v) propolis in polyethylene glycol 400 (PEG 400) was added. Distilled water was added to adjust the volume of propolis emulsifier solution to 50 mL. Then dispersing of propolis emulsifier solution was operated in a 100 mL beaker for 20 min until the final emulsion was formed. Thus the concentration of propolis in emulsion was 0.02 g/mL.

A control emulsion was made in the same way but contained no propolis. The 50 mL control emulsion contained 0.8 g Tween-80, 1.2 g hydrophilic phospholipid and 5 mL PEG 400.

Orange juice preparation

Navel orange (Geelong Citrus Packers Pty, Ltd, Australia; Purchased in Yonghui supermarket in Fuzhou, China) was processed using a clean and disinfected fruit juicer (WF-A2000, Zhejiang Yongkang Tiange Electric Appliance Co., Ltd.; China). The prepared fresh OJ was filtered with silk cloth (first 80 then 200 mesh) and pasteurized at a moderate temperature (80 °C) for 20 min. Propolis emulsion (12 mL), emulsion control (12 mL), sodium benzoate (0.4 g) and potassium sorbate (0.4 g) were each added to 388, 388, 400 and 400 mL OJ respectively. The additive concentrations were propolis at 0.6 mg/mL (+ emulsion agents at 0.03 mL/L OJ), emulsion control at 0.03 mL emulsion agents/L OJ, sodium benzoate at 1 mg/mL and potassium sorbate at 1 mg/mL. The four OJ treatments were kept in the same incubator (LHH-250GP, Shanghai Yiheng Scientific Instruments Co., Ltd.; China) at 25 °C for 35 days, in total darkness. OJ was separately stored in 50 mL glass bottles (30 mL OJ/bottle). One bottle of OJ was sampled once a week (20 mL each time) to determine the bacillus number, chemical components and parameters for 5 weeks. The experiment was replicated 3 times.

The number of *Bacillus* spore determination during storage

Serial dilutions (10 x) of 10 mL OJ samples were prepared with sterilized ultra-pure water (WP-UP-LH-20, Sichuan Walter Instrument Co., Ltd.; China). *Bacillus* number was studied by total aerobic plate counts using plate count agar (BR, Sinopharm; China). Each dilution was plated (in 90 mm PCA plates) in triplicate and incubated at 30 °C for 3 days.

Color, pH and titratable acidity measurements

All measurements were carried out at room temperature (20–25 °C). The color of OJ was measured with a Colorimeter & Color Difference Meter (WCS-S, Shanghai Instrument Physical Optics Instrument Co., Ltd.; China). Equipment was set up with the following parameters: illuminant D65, illuminant sample area Φ20, 10° observer angle and wide viewing field. CIE $L*$ (lightness), CIE $a*$ (greenness to redness) and CIE $b*$ (blueness to yellowness) were determined.

pH was measured using a pH-meter (PHS-3C, Shanghai INESA Scientific Instrument Co., Ltd.; China), which was first calibrated using buffers of pH 6.86 and 9.18 at RT.

Titratable acidity was measured by the amount (mL) of 0.1 M NaOH required to obtain pH 8.1 in a 120 mL solution (10 mL OJ with 110 mL distilled water).

L-ascorbic acid analysis

L-ascorbic acid content in OJ was determined by HPLC (Elez-Martinez and Martin-Belloso 2007). A sample of 5 mL OJ was mixed with 10 mL of a solution containing 10% (w/v) metaphosphoric acid (BR, Sinopharm; China) and 0.5% 2,3-dimercapto-1-propanol (Sigma Aldrich, China). The homogenate was centrifuged at 15,300×*g* at 4 °C for 15 min (5804R Eppendorf, Eppendorf Ltd.; German). The 0.25 mL vacuum-filtered (0.53 mm filter) supernatant was diluted to 10 mL with a solution containing 10% (w/v) metaphosphoric acid and 0.5% 2,3-dimercapto-1-propanol. Thus the OJ has been diluted 120 times (3 × 40). An aliquot of 20 μL sample, after being filtered with a Millipore 0.45 μm membrane, was injected into HPLC system (Shimadzu, 20AT; Japan) with a WondaSil-C18 column (250 mm × 4.6 mm, 5 μm). Mobile phase was 0.05% formic acid aqueous solution. The flow rate was 0.8 mL/min at RT. Detection was monitored with UV detector (SPD-M20A, Shimadzu; Japan) at 254 nm. The retention time of L-ascorbic acid in OJ was 5.88 min. A standard curve was constructedwith L-ascorbic acid (98.7%, Supelco, USA) at concentrations of 0, 0.004, 0.008, 0.01, and 0.08 mg/mL.

The L-ascorbic acid contents in treated OJ were calculated by comparing to a standard curve using curve area, and multiplied by 120 (the dilution factor).

Total phenolics determination

Total phenolics concentration in OJ was determined by the Folin–Ciocalteu method. Methanol (9 mL, 80%) and OJ (1 mL) were mixed and incubated for 30 min at RT. After centrifugation at 5000×*g* for 10 min, 3.5 mL supernatant was used for determination of total phenolics concentration (Gardner et al. 2000). Pyrogallic acid (10 mg/mL) at 100, 300, 500 and 1000 μL, each added with ddH_2O to 1 mL, were prepared for a standard curve with the reagent blank of distilled deionized water (ddH_2O). An aliquot (1 mL) of 80% methanol extracts from OJ and standard solution were added 9 mL distilled water, respectively. Folin–Ciocalteu phenol reagent (BR, Sinopharm; China) (0.5 mL) was added to the mixture and shaken vigorously. After 5 min, 5 mL of 5% Na_2CO_3 (AR, Sinopharm; China) solution was added to the mixture. The solution was immediately diluted to 25 mL with distilled water and mixed thoroughly, keeping in dark for 60 min before measurement. The absorbance was measured at 750 nm with an UV–VIS spectrophotometer (T6, Beijing Purkinje General Instrument Co., Ltd.; China). Total phenolic content of sample was expressed as mg/L of gallic acid equivalent (GAE), multiplied by 10 (dilution factor for the extract).

Antioxidant capacity measurement during storage

Antioxidant capacity of OJ was determined through the evaluation of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. OJ samples (1.5 mL) were centrifuged at 6000×*g* at 4 °C for 15 min (5804R Eppendorf; German). After that 10 μL supernatant and 90 μL RO water were mixed with 3.9 mL methanol (AR, Sinopharm; China) containing DPPH (0.025 g/L) (Tokyo Chemical Industry Co. Ltd.; Japan). The mixture of OJ and DPPH solution was shaken vigorously (MS 3 Digital Vortex, IKA; USA) for 1 min and kept in dark for 30 min at RT. Absorption of these treated OJ was measured with a spectrophotometer (T6, China) at 515 nm (A1), with methanol as a reagent blank. Absorption value of radical DPPH without OJ, which was also kept in dark for 30 min at RT, was designated as A0. Results were expressed as percentage of inhibition of the radical DPPH $(1-A1/A0) * 100$.

Statistical analyses

General Linear Model with the number of bacteria spores, pH, color data, titratable acidities, soluble solids contents, L-ascorbic acid, total phenolics and antioxidant capacities as dependent variables and type of additives as the independent variable, time of measurement was treated as a repeated measure variable (after validated by Mauchly's Sphericity Test). Analysis were done using SPSS 13.0 for Windows (SPSS Inc. 1989-2004, NC, USA).

Results and discussion

Components of propolis

Total flavonoids content in propolis was 22.8 ± 0.73% and consistent with results for propolis collected in Taiwan, China (Chang et al. 2002), northern Croatia (Kosalec et al. 2004), Argentina (Moreno et al. 2000) and Brazilian red propolis (Alencar et al. 2007). But our results were higher than that of Kumazawa et al. (2004), which may be due to different determination methods used.

The concentrations of individual flavonoids of rutin, quercetin, kaempferol, galangin, apigenin, chrysin and luteolin in EEP were 4.19 ± 0.61, 0. 59 ± 0.08, 14.68 ± 0.92, 5.29 ± 0.77, 1.99 ± 0.55, 67.14 ± 3.62 and 41.19 ± 2.17 mg/g, respectively. The rutin content observed was equal to that in a sample collected from Brazil but less than other samples (Bonvehí and Coll 1994). The quercetin content observed to be the same as previous report for propolis from China while chrysin content is close to those in propolis from Argentina and Chile (Kumazawa et al. 2004). Kaempferol content was more, but galangin and apigenin were less than earlier results (Kumazawa et al. 2004). The galangin content was same as that of Markham et al. (1996). Luteolin content was between the content in propolis samples from different regions in Anatalia (Erdogan et al. 2011). The difference between our determination and previous results may be due to propolis originating from different plant species, due to differences in locations and seasons (Sforcin et al. 2000; Sahinler and Kaftanoglu 2005; Christov et al. 2006; Mercan 2006; Wiryowidagdo et al. 2009;Valencia et al. 2012).

Bacteria spores numbers in different treatments

There were significant differences in the number of bacteria spores in OJ treated with different additives (Fig. 1; $F_{3,\ 8} = 124.15$, $p < 0.001$). The number of bacteria spores in OJ increased significantly with storage time for all treatments ($F_{5,\ 40} = 220.89$, $p < 0.001$). Interactions between storage time and additives were significant ($F_{15,\ 40} = 4.13$, $p < 0.01$), suggesting different additives inhibited bacteria differentially over time. Propolis had a significantly stronger inhibition effect on microbial growth in OJ compared to the emulsion control. These results were consistent with previous studies. Antifungal effect of

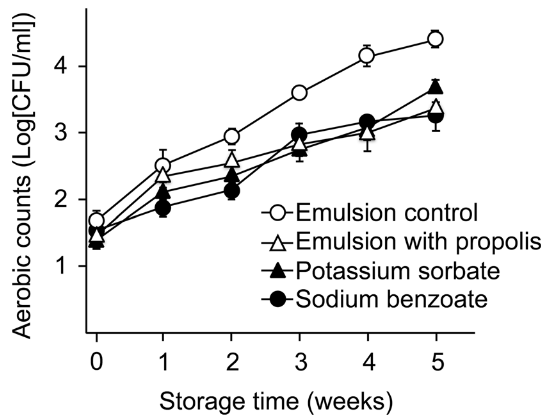

Fig. 1 Number of bacteria spores (mean ± SE) of orange juice after addition of propolis emulsion (0.6 mg/mL), emulsion (0.03 mL/L emulsion agents), sodium benzoate (1 mg/mL) and potassium sorbate (1 mg/mL). Each data point was based on three experiments

Turkish propolis on four different fruit juices (apple, orange, white grape and mandarin) was determined, but only pH, titratable acidity and Brix values were determined and ethanol was used as a solvent (Koc et al. 2007). Antimicrobial effect of propolis against bacteria was due to *p*-coumaric acid, 3-(4-hydroxy-3-(oxo-butenyl)-phenylacrylic acid, 3,5-diprenyl-4-hydroxycinnamic acid and 2,2-dimethyl-6-carboxyethenyl- 2H-1-benzopyran (Salomao et al. 2008). Potassium sorbate and sodium benzoate are widely used chemical preservatives. They have been shown to inactivate yeasts in fresh fruits, inhibit *Staphylococcus aureus Cl0* and *Bacillus cereus B7* (López et al. 2006) and reduce viability of spores of *Bacillus cereus* and *B. stearothermophilus* (Oloyede and Scholefield 1994). However, excessive consumption of these preservatives is hazardous to human health (Ward 1997).

Color, pH and titratable acidity measurement of OJ

There were significant differences among treatments in lightness of the treated OJ (Fig. 2a; $F_{3,\ 8} = 169.3$, $p < 0.0001$) and in greenness to redness (Fig. 2b; $F_{3,\ 8} = 8.04$, $p = 0.02$) but not in blueness to yellowness (Fig. 2c; $F_{3,\ 8} = 0.71$, $p = 0.57$). Interactions between the storage time and additives were not significant except for lightness ($F_{15,\ 40} = 2.56$, $p = 0.009$). Lightness of OJ treated with propolis decreased slowly, whereas lightness of other treated OJ decreased dramatically. The greenness to redness of OJ treated with propolis decreased steadily but the decrease of other treatments was more drastic. OJ color is mainly due to carotenoids, which include carotenes and xanthophyll (Meléndez-Martínez et al. 2009; Fernández-Vázquez et al. 2011). Color degradation of OJ is a complex reaction, which is due to fading of natural carotenoid components and development of pigments

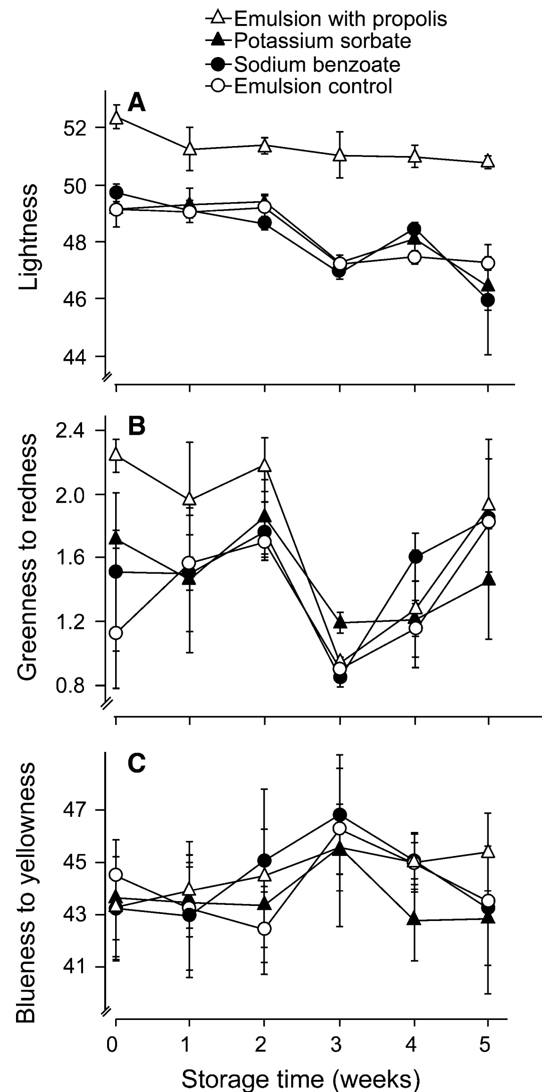

Fig. 2 The CIELab (L*, a* and b*) parameters (mean ± SE) of orange juice after addition of propolis emulsion (0.6 mg/mL), emulsion (no propolis) (0.03 mL/L), sodium benzoate (1 mg/mL) and potassium sorbate (1 mg/mL). Each data point was based on three experiments

produced by enzymatic and/or non-enzymatic reactions (Timberlake 1989). In non propolis groups, the lightness decreased during storage, which is consistent with a previous study (Wibowo et al. 2015). This decrease of lightness is caused by the instability and isomerization reactions of ζ-carotene (Wibowo et al. 2015; Khoo et al. 2011). The L* value (lightness) of OJ treated with propolis changed from 52.37 ± 0.4 CIELAB units in the first day to 50.78 ± 0.19 CIELAB units in the last day. But L* values fluctuated during storage, this was also shown in a previous study (Wibowo et al. 2015). We deduced that propolis improved the stability of carotenes in OJ. Propolis is a suitable natural additive for better preserving lightness of

OJ in comparison to synthetic preservatives, potassium sorbate and sodium benzoate.

Changes of OJ pH during storage at 25 °C for 35 days are shown in Fig. 3a. The pH of treated OJ were significantly different and changed with storage time ($F_{5,\ 40} = 3.82$, $p = 0.009$). There were significant differences in pH among treated OJ ($F_{3,\ 8} = 26.17$, $p = 0.001$). Interactions between treatment and storage time were also significant ($F_{15,\ 40} = 3.14$, $p = 0.004$). pH of OJ treated with emulsion (no propolis) and sodium benzoate decreased 0.11 after 5 weeks of storage. There were no significant differences in pH of OJ treated with propolis among the 5 weeks' storage time except the 3rd (3.81 ± 0.01) and the 5th week (3.91 ± 0.15). Changes in titratable acidities of OJ during storage at 25 °C for 35 days are shown in Fig. 3b. Titratable acidities of treated OJ changed significantly with storage time ($F_{5,\ 40} = 9.59$, $p < 0.0001$). There were no significant differences in titratable acidities among treated OJ ($F_{3,\ 8} = 2.87$, $p = 0.13$). Interactions between treatments and storage time were not significant in titratable acidities ($F_{15,\ 40} = 1.82$, $p = 0.08$). There was no significant change in titratable acidities of OJ treated with propolis up to 5 weeks of storage ($p > 0.05$). A dramatic decrease in titratable acidity was observed in OJ treated with potassium sorbate from the 4th to 5th week. These changes may be caused by the spoilage of OJ and/or microbial reproduction (Yeom et al. 2000). Sodium benzoate did not do better than propolis for maintaining pH and titratable acidity of OJ. Propolis can be used to maintain pH and titratable acidity in OJ as a substitute to potassium sorbate and sodium benzoate.

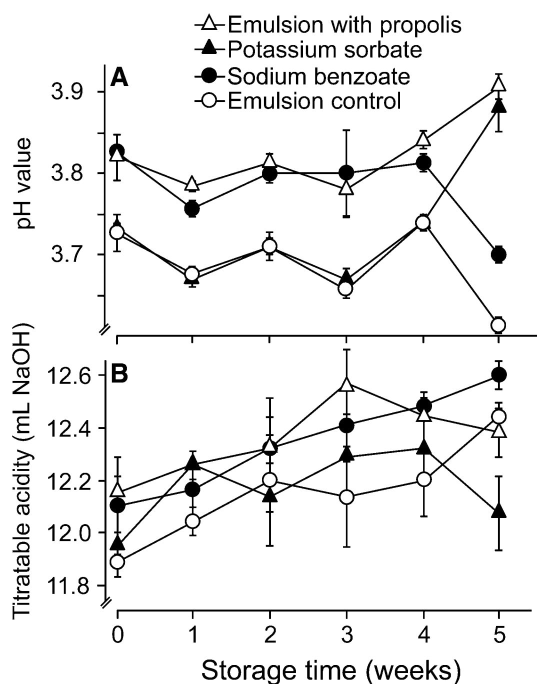

Fig. 3 pH and titratable acidity (mean ± SE) of orange juice after addition of propolis emulsion (0.6 mg/mL), emulsion (no propolis) (0.03 mL/L), sodium benzoate (1 mg/mL) and potassium sorbate (1 mg/mL). Each data point was based on three experiments

L-ascorbic acid analysis

L-ascorbic acid contents of treated OJ and their changes during storage at 25 °C for 35 days are shown in Fig. 4. There were no significant difference in L-ascorbic acid contents among treatments ($F_{3,\ 8} = 1.3$, $p = 0.34$). L-ascorbic acid contents in different treatments changed with the storage time ($F_{5,\ 40} = 5.28$, $p = 0.001$). There were no significant interactions between treatments and storage time ($F_{15,\ 40} = 1.86$, $p = 0.06$). The L-ascorbic acid contents decreased during 5 weeks storage time (0 week vs. 5th week, $p = 0.036$). The decrease of L-ascorbic acid contents in OJ is consistent with a previous study (Sarkar et al. 2014). The degradation of L-ascorbic acid during storage were described as a first-order kinetic model and is affected by storage time, temperature and dissolved oxygen (Remini et al. 2015). In our study, the degradation rate of L-ascorbic acid in OJ treated with emulsion (no propolis), sodium benzoate, potassium sorbate and propolis emulsion treatments separately are 35.03, 16.83, 14.82 and 21.91% after 5 weeks storage. In another study, 7% vitamin C remained in untreated blood orange juice after storage for 28 days at 20 °C (Remini et al. 2015), in contrast with 73.9% remaining in emulsion (no propolis) treated OJ in this study. This difference may be caused by the different species of orange used in our study. Based on the effect on L-ascorbic acid preservation, propolis is a proper alternative of potassium sorbate in OJ.

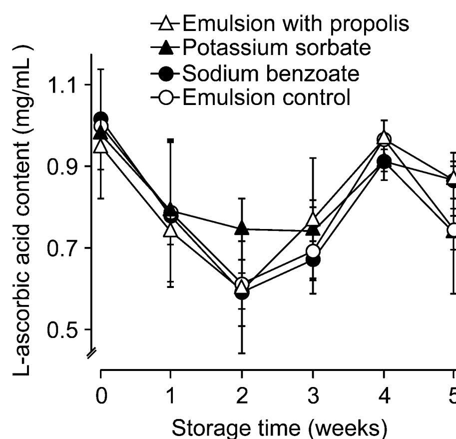

Fig. 4 L-ascorbic acid content (mean ± SE) in orange juice after addition of propolis emulsion (0.6 mg/mL), emulsion (no propolis) (0.03 mL/L), sodium benzoate (1 mg/mL) and potassium sorbate (1 mg/mL). Each data point was based on three experiments

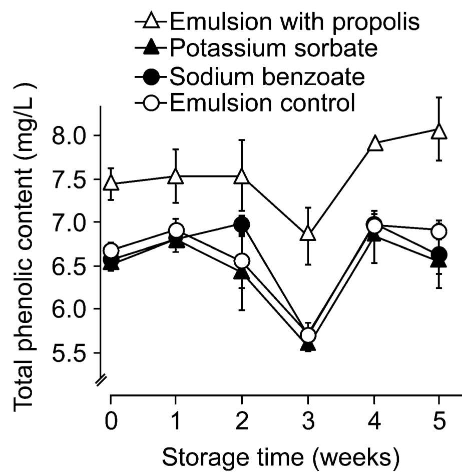

Fig. 5 Total phenolic content (mean ± SE) of orange juice after addition of propolis emulsion (0.6 mg/mL), emulsion (no propolis) (0.03 mL/L), sodium benzoate (1 mg/mL) and potassium sorbate (1 mg/mL). Each data point was based on three experiments

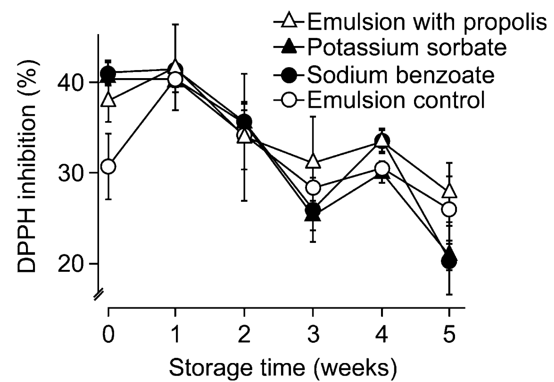

Fig. 6 Antioxidant capacity (mean ± SE) of orange juice after addition of propolis emulsion (0.6 mg/mL), emulsion (no propolis) (0.03 mL/L), sodium benzoate (1 mg/mL) and potassium sorbate (1 mg/mL). Each data point was based on three experiments

Total phenolics

There were significant differences in total phenolics contents among OJ treated with different preservatives (Fig. 5; $F_{3,\ 8} = 29.7$, $p < 0.0001$). Total phenolics contents also changed significantly with storage time ($F_{5,\ 40} = 19.83$, $p < 0.0001$). There were no significant interactions between treatments and storage time. There are 24 phenolic components in OJ (Stinco et al. 2015). The total phenolic contents in OJ treated with propolis was 1.0 mg/mL more than that of other treatments in the first day (Fig. 5). This was thought to be due to phenolic substance in propolis (Valenzuela-Barra et al. 2015).This difference increased to 1.5 mg/mL in the 5th week. These results show that propolis can remarkably protect the phenolic components in OJ.

Antioxidant capacity

There were no significant differences in antioxidant capacity among treated OJ (Fig. 6; $F_{3,\ 8} = 2.13$, $p = 0.185$). The antioxidant capacity of OJ decreased with storage time ($F_{5,\ 40} = 52.46$, $p < 0.0001$). There were significant interactions between treatments and storage time ($F_{15,\ 40} = 2.35$, $p = 0.019$). The antioxidant capacity of OJ is related to the amount and composition of bioactive compounds such as vitamin C, carotenoids or flavanones. Total vitamin C is the major factor for the antioxidant potential of OJ (Sanchez-Moreno et al. 2003; Gardner et al. 2000; Stinco et al. 2015; Zulueta et al. 2007), followed by flavonoid, carotenoids and vitamin A (Zulueta et al. 2007; Stinco et al. 2015). OJ treated with propolis has a strong antioxidant activity due to its high amount of antioxidative polyphenol and flavonoid compounds, such as caffeic acid, kaempferol, ferulic acid and phenethyl caffeate in propolis (Fabris et al. 2013; Valenzuela-Barra et al. 2015). Propolis can effectively maintain the antioxidant capacity of OJ similar to synthetic preservatives.

Conclusion

In conclusion, this study studied using propolis emulsion as an alternative, non-synthetic preservative of OJ. Propolis effectively protected the lightness, pH, titratable acidity, antioxidant capacity, total phenolics content and L-ascorbic acid. These results suggest that propolis can improve the quality of OJ compared with other chemical preservatives. Propolis, which is non-toxic and has a no-effect dose at 1400 mg/kg body weight/day for mice (Burdock 1998), can be used as a natural preservative and an alternative to synthetic preservatives for OJ and other fruit juices.

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Author's contribution WY designed the study, analyzed the data and drafted the manuscript, ZW provided the samples, XM provided funding support. ZH analyzed the data and revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

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