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Statistical approach to reveal propolis as a potential biopreservative for fruit juices

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ABSTRACT

This study was carried out to reveal the feasibility of propolis as a biopreservative for fruit juices. A statistical approach was used to analyze the previously published experimental data. Different pasteurization techniques including the use of propolis, thermal and non-thermal methods were considered in data collection. First order degradation kinetic model was used to determine the degradation rate of fruit juices based on the quality attributes such as ascorbic acid content (30 datasets), total plate count (35 datasets), pH (30 datasets) and antioxidant capacity (16 datasets). Propolis was found to inhibit the growth of bacteria effectively with the negative rate constant (-44,874.66 CFU/g/day), whereas the kinetic constants of other techniques were in positive values ranged from 7.75–9992.17 CFU/g/day. Propolis was also the most effective method to preserve the antioxidant capacity of juices with the lowest degradation rate (-0.0033 mg/g/day). Factor analysis revealed that the remarkable property of propolis was mainly contributed by its phenolics, and partly attributed to its flavonoids. The antibacterial property of propolis was more effective to inhibit the growth of gram positive bacteria (Staphylococcus aureus) than gram negative bacteria (Escherichia coli). A weak correlation between antioxidant and antibacterial properties of propolis was also observed.

1. Introduction

Pasteurization is the common preservative technique applied in food industries in order to improve the stability of fruit juices. Pasteurization can be classified as thermal and non-thermal pasteurization. Thermal pasteurization techniques include High-Temperature Short-Time (HTST), High-Temperature Long-Time (HTLT), Moderate-Temperature Short-Time (MTST) and moderate-temperature long-time (MTLT). While, non-thermal pasteurization techniques are Pulsed Electric Field (PEF), High Pressure Processing (HPP), Ultraviolet (UV), Ultrasound (US) and microwave. Although pasteurization prolongs storage duration, it may accelerate the quality degradation of fruit juices, specifically the thermal techniques (Margean et al., 2020; Aguayo et al, 2017; Rabie et al. 2015).

Recently, propolis is likely to be one of the good choices as a natural preservative in food industries. Yang et al. (2017) explained that propolis could be better to serve as an alternative chemical preservative of fruit juice. Propolis is a natural product collected by bees from plant resins. It is known as bee glue which can be used to repair bee hives by closing any hive cracks. Propolis is also used as a shielding barrier against foreign intruders such as lizards, snakes and ants by bees. It has been a popular traditional folk remedy since centuries because of its antioxidant, anti-microbial, anti-inflammatory and cytotoxicity activities (Miguel and Antunes, 2011). This phenomenon explains the rapid development of propolis as alternative medicines, dietary supplements and cosmetic products in the market recently.

Propolis contains more than 300 components such as polyphenols, phenolic aldehydes, amino acids, steroids and terpenoids depending upon geographical origin (Anjum et al., 2019). Propolis from Asia mainly contains phenolic acids and flavonoids, while propolis from Brazil contains terpenoids and prenylated derivatives of p-coumaric acids. Different compounds lead to different antioxidant capacity of propolis. The components have the ability to scavenge radicals to promote antioxidation, and to exhibit bacteriostatic and bactericidal properties of propolis (Yoshimasu et al., 2018; Bonvehí et al., 1994). Previous researchers reported that the biological property was attributed to the complex combination and association of compounds in propolis. They had analyzed more than 600 bacterial strains against propolis from different countries. They found that propolis showed greater activity against gram positive bacteria than gram negative bacteria (Tukmechi et al, 2010; Przybyłek and Karpiński, 2019; Petruzzi et al., 2020)

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The most commonly used models to evaluate antioxidant activity are DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and ABTS assays. DPPH assay generates stable free radicals, while ABTS assay creates radical cations which carry positive charges (Gunawardena, 2019). The assays can be performed rapidly at a wide range of pH values (Ratnavathi and Komala, 2016). The inhibition of DPPH radicals follows both SET (single electron transfer) and HAT (hydrogen atom transfer) mechanisms (Dorsey and Jones, 2017). However, ABTS follows the mechanism of HAT. Therefore, DPPH assay alone is unable to differentiate between the two mechanisms (Prior et al., 2005). Xie and Schaich (2014) reported that HAT occurred more slowly than the rate of SET.

Diffusion methods such as disc diffusion and agar well diffusion are also commonly used to examine the antibacterial activity of propolis. Agar well diffusion method could provide high sensitivity results (Valgas et al, 2007; Ismail et al, 2016). This technique is not influenced by electric field caused by positive charges and negative charges of impregnated samples in discs. Unlike agar diffusion method, cationic substances may have resistance to diffuse into the medium (Valgas et al, 2007).

A multivariate statistical approach was used to analyze the huge and complex dataset collected from literature review. The change of quality attributes such as ascorbic acid, total plate count, pH and antioxidant activity in fruit juices during storage were analyzed, regardless pasteurization techniques. The statistical analyses including preliminary analysis such as Kaiser-Meyer-Olkin measure and Barlett's sphericity for sampling adequacy. Factor analysis includes component analysis, correlation matrix and total variance explained were used to classify the correlation of propolis, phenolics and flavonoids in relation with biological activities such as antioxidant and antibacterial properties. This is the first study relating the role of propolis in preserving quality attributes of fruit juices using a statistical approach. This multivariate analysis revealed the feasibility of propolis as a natural preservative, especially preserving the antioxidant capacity of fruit juices.

2. Materials and Methods

2.1. Data Collection

Data were collected from previously published journals from the database of Web of Science, PubMed, Science Direct, PLOS ONE and HINDAWI from 2005–2019. The keywords of data collection were "juice", "pasteurization", "preservative", "antioxidant", "antibacterial", "antimicrobial", radical scavenging activity" and "propolis". The keywords were used in the literature search individually and in combination. The collected data included different types of fruit juices ranged from orange, apple, pineapple, pomegranate, tamarind, soursop and Physalis peruviana L. Juices. The fruit juices were pasteurized using different techniques including HTLT (high temperature long time), HTST (high temperature short time, < 1 min), MTLT (medium temperature long time), MTST (medium temperature short time), HPP (high pressure processing), PEF (pulse electric field), UV (ultraviolet) and US (ultrasound). The change of four important quality attributes such as ascorbic acid content (30 datasets), total plate count (35 datasets), pH (30 datasets) and antioxidant activity (16 datasets) were examined upon storage from day to months. The number of datasets on the change of quality attributes treated by different pasteurization techniques is presented in Table S1.

2.2. Standardization of Unit Measurement

All data were standardized into same unit to obtain data uniformity. The unit of ascorbic acid content, total plate count and antioxidant capacity were standardized into mg/g juice, CFU/g juice and mg/g juice, respectively. The time frame of the data was converted to day.

2.3. First Order Degradation Kinetics

The quality degradation of pasteurized fruit juices was determined using first order kinetic model. The kinetic constants, k of juice quality attributes such as ascorbic acid, total plate count, pH and antioxidant capacity were determined (Equation 1) and then used to calculate the half life as explained in Equation 2.

$$C_t = C_o \exp(kt) \quad (1)$$

where C_t indicates the value of quality attributes at time t, C_o indicates the initial value of the quality attributes, and k is the rate constant (1/day).

Half-life value ($t_{1/2}$) is the required time to reduce the amount of quality attributes to be half from its initial value.

$$t_{1/2} = Ln(2)/k \quad (2)$$

2.4. Factor analysis

A multivariate statistical analysis was carried out using IBM SPSS Statistics 22 (SPSS Inc., USA). Factor analysis was used to reduce the large and multidimensional data variables without compromising data information. Preliminary analysis such as Kaiser-Meyer-Olkin Measure and Barlett's Sphericity were carried out to determine the adequacy of sample size. The number of key components was extracted in accordance to the Kaiser criterion with the eigenvalue larger than one. The function of principal components was selected to extract uncorrelated linear combination of variables, and giving factors with maximum amount of explained variance. Varimax which is an orthogonal rotation method was used to rotate the factors to better fit the data. Correlation matrix were used to investigate the correlation of propolis (10–15 datasets), total phenolics (10–12 datasets) and flavonoids (9–15 datasets) with its biological activities, namely antioxidant (DPPH or 2,2-diphenyl-1-picrylhydrazyl and ABTS or 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and antibacterial (S. aureus and E. coli) capacities. The number of datasets on the concentration of propolis, and its phenolics and flavonoids in relation with the biological activities is presented in Table S2.

2.6. Statistical analysis

The quality degradation of juice attributes treated with different pasteurization techniques were analyzed using a Tukey-Kramer post-hoc test after one-way analysis of variance (ANOVA) for multiple pairwise comparison at 95% confident level.

3. Results and discussion

3.1. Effect of pasteurization techniques on fruit juice stability

The quality attributes of fruit juices are degrading upon storage. In order to minimize the degradation rate, fruit juices are always pasteurized prior to marketing. Different pasteurization techniques include thermal and non-thermal processing have been developed and applied to prolong the shelf life while preserving juice quality. In the present study, previous published data were collected and analyzed using first order degradation kinetics. The results showed the degradation of ascorbic acid, increment of total plate count, depletion of antioxidant capacity and change of pH upon storage. The kinetic constants of the quality attributes are presented in Fig. 1. Fig. 1(c) shows that the pH of fruit juices may increase or decrease depending upon juice types. The total plate count of fruit juices was increased as the storage time progressed. Fruit juices may contain sugar and other nutrients to promote the growth of microorganisms. However, the kinetic constant of juices treated by propolis was found to be in negative value. Its large negative kinetic constant explains the effectiveness of propolis to inhibit the bacterial growth compared to other pasteurization techniques. Similarly,

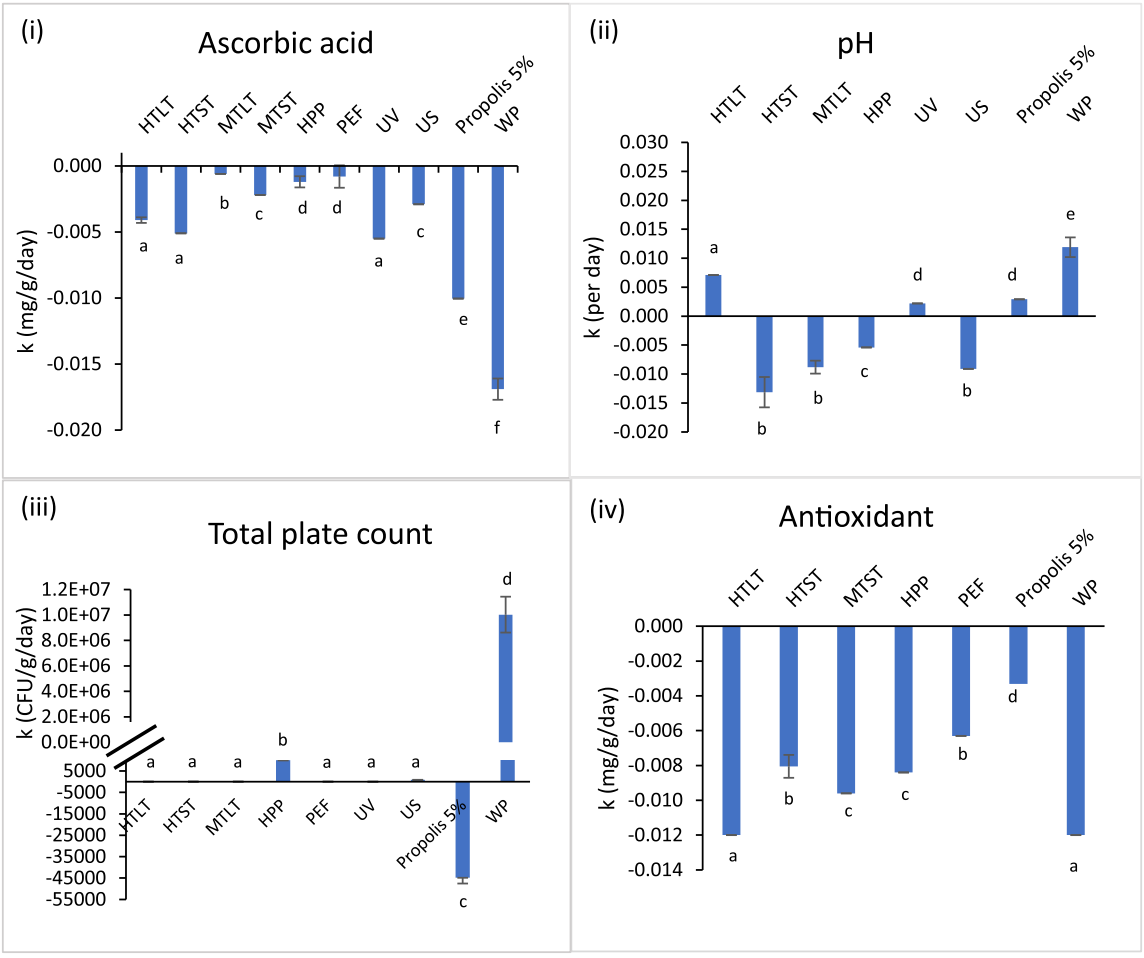

Fig. 1. First order degradation kinetic constant (k) of (i) ascorbic acid, (ii) pH, (iii) total plate count and (iv) antioxidant capacity for fruit juices. HTLT, high temperature long time; HTST, high temperature short time; MTLT, medium temperature long time; HPP, high pressure processing; PEF, pulse electric field; UV, ultraviolet; US, ultrasound; WP, without pasteurization. The Tukey-Kramer post-hoc test after one-way ANOVA was used to do multiple pairwise comparison. The groups with similar letter explains that the pasteurization techniques are not significantly different.

propolis showed its capability to prevent the degradation of antioxidant capacity of fruit juice with the lowest kinetic constant (Fig. 1(d)). However, propolis was unable to compete with other pasteurization techniques to protect ascorbic acid from degradation. Medium temperature (65–75 ˚C) and non-thermal pasteurization were found to be better in preventing degradation kinetics of ascorbic acid (Fig. 1(a)). The kinetic constants were then used to determine the half-lives of juices after pasteurized with different techniques (Fig. 2). Propolis was found to have the highest half-life of antioxidant degradation. Therefore, propolis was likely to be a good biopasteurization technique to preserve antioxidant and to inhibit the bacterial growth in fruit juices.

3.2. Relationship pf propolis and its biological property

Kaiser-Meyer-Olkin Measure showed to have values ranged from 0.599 to 0.705 for 3 datasets, namely propolis concentration, phenolic and flavonoid contents as presented in Table S3. The values indicated that a factor analysis is suitable for the datasets. Beaumont (2012) stated that the value below 0.5 is considered to be miserable, whereas Tabachnick & Fidell (1996) explained a value above 0.6 was the acceptable for factor analysis. The suitability of datasets was further confirmed based on the significance level ($p < 0.05$) of probability in the Bartlett's test of sphericity (Pallant, 2013; Field, 2000).

There was only a factor with the initial eigenvalue more than one and accounted for 56.6% of the variability in the dataset of propolis concentration (Table S4). The total number of initial eigenvalue more than one was increased to two for both datasets of phenolics and flavonoids in the subsequent analysis. The first two components of the datasets accounted for 78.9 and 72.2% of the variability, respectively. The total phenolics and flavonoids could explain better the antioxidant and antibacterial activities of propolis with higher total variance. In particular, the phenolic content could explain the data slightly better than the flavonoid content in propolis.

Table S5 is the correlation matrix of propolis concentration with its antioxidant and antibacterial activities in a positive manner. It means higher concentration of propolis would contribute to higher antioxidant and antibacterial activities. Table S5 also shows stronger correlation between propolis and antioxidant activities than the correlation of propolis and antibacterial activities. The strong correlation can be seen from higher coefficient of determination around 0.7 for both DPPH and ABTS assays. Kurek-Górecka et al. (2013) mentioned that propolis is a natural antioxidant substance rich in phenolic acids and flavonoids. However, the coefficient of determination between propolis and antibacterial activities is only 0.530 for S. aureus and 0.283 for E. coli. Possibly, propolis inhibited the bacterial growth by increasing membrane permeability, changing membrane potential, decreasing bacterial mobility and reducing energy production of bacteria (Cushnie and Lamb, 2019; Przybyłek and Karpiński, 2019). The inhibitory action of propolis was stronger against the growth of S. aureus than E. coli. The finding is in line with the findings of Al-Ani et al (2018), who reported the sen-

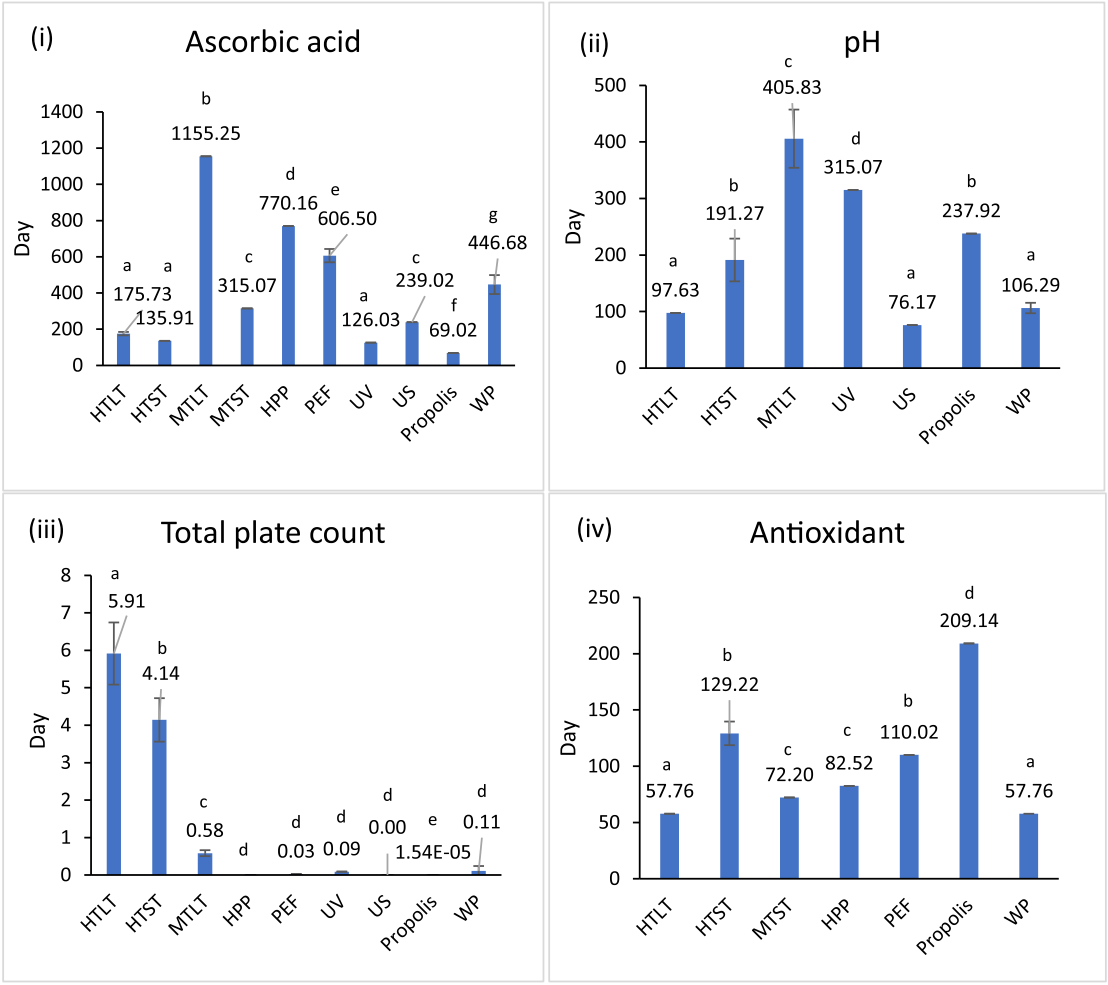

Fig. 2. Half-lives of fruit juices treated with various pasteurization techniques based on the degradation of (i) ascorbic acid, (ii) pH, (iii) total plate count and (iv) antioxidant capacity. HTLT, high temperature long time; HTST, high temperature short time; MTLT, medium temperature long time; HPP, high pressure processing; PEF, pulse electric field; UV, ultraviolet; US, ultrasound; WP, without pasteurization. The Tukey-Kramer post-hoc test after one-way ANOVA was used to do multiple pairwise comparison. The groups with similar letter explains that the pasteurization techniques are not significantly different.

sitivity of gram positive bacteria than gram-negative bacteria against propolis concentration. E. coli is a gram negative bacterium with multilayered cell structure which would restrict the diffusion of bioactive compounds into bacterial cells (de Freitas Araujo et al, 2012). The resistance of gram negative bacteria could be also due to the presence of efflux pumps which prevented the intracellular entry of propolis constituents (Petruzzi et al, 2020). Anyhow, Table S5 also reveals that there is a weak relationship of antioxidant and antibacterial properties with low coefficient of determination (0.195–0.315). Probably, the bioactive compounds that are responsible to scavenge radicals may not inhibit the bacterial growth.

Further investigation was carried out on the correlation of propolis, specifically its phenolics and flavonoids in relation with antioxidant and antibacterial properties. Table S6 explains the correlation matrix of total phenolic content in propolis and its biological properties. There is a negative relationship of total phenolic content and antioxidant activities expressed in IC50. IC50 is the effective concentration of phenolic compounds required to inhibit 50% radical scavenging activities in the antioxidant assays. Therefore, the lower IC50 explains the better scavenging activities of samples. Similarly, negative correlation was also reported by Daraghmeh and Imtara (2020). The phenolic compounds in propolis were capable to scavenge radical cations ($ABTS^{\bullet+}$) effectively with high coefficient of determination (0.818), but only moderate correlation with free radicals. The phenolic compounds could have higher affinity against radical cations due to the presence of hydroxyl substituents in the aromatic skeleton (Kefalas et al, 2003). Hence, the functional groups of phenolics play an important role to quench radicals. Phenolic compounds with two hydroxyl groups linked to the aromatic ring at the ortho position more strongly quench $ABTS^{\bullet+}$ radical (Mathew et al, 2015). Hagerman et al (1998) stated that high molecular weight of phenolic compounds was able to quench more $ABTS^{\bullet+}$. One of the commonly found high molecular weight phenolic compounds in propolis is proanthocyanidins or condensed tannins (Mayworm et al., 2014). Propolis from countries such as Argentina, Japan and Greece also demonstrated strong correlation between total phenolic content and radical scavenging activity (Isla et al, 2009; Hamasaka et al, 2004). The total phenolic content in propolis also correlates negatively with MIC. MIC is the minimum inhibitory concentration to inhibit the growth of bacteria. The lower MIC indicates the better of inhibitory action of propolis against the bacterial growth. Phenolic compounds could also contribute to the antibacterial properties of propolis based on the increment of coefficient of determination. In particular, the stronger inhibition against the growth of S. aureus was observed.

In comparison with the correlation of flavonoids and antioxidant activities, the coefficients of determination become slightly lower. The reduction of coefficient of determination explains that the radical scavenging activities were mainly contributed by phenolics and partly attributed to flavonoids in propolis. Flavonoid which is one of the sub-groups of

phenolics contains two phenyl rings connected by a a heterocyclic 3 carbons ring. Cao et al, 2020 reported that glycosylation of hydroxyl groups in flavonoids could weaken the antioxidant activity. Flavonoids also show higher affinity towards radical cations. Kamal et al, (2015) highlighted that flavonoid could be a good radical scavenger or terminator because of the presence of hydroxyl substitutes attached to the aromatic rings. The observation explained that the number of hydroxyl groups attached to flavonoids was lower than the number of hydroxyl groups from phenolic compounds which composed of polyphenols and phenolic acids in propolis. Therefore, the coefficient of determination for flavonoids and antioxidant capacity (-0.593 to -0.719, Table S7) was slightly lower than that value of total phenolics and antioxidant capacity (-0.646 to -0.818, Table S6). Besides, flavonoids in propolis also correlated moderately with the antibacterial property. Again, the antioxidant and antibacterial properties of propolis have a weak relationship.

4. Conclusion

As a conclusion, propolis showed to have stronger scavenging ability against radical cations which was observed from the ABTS assay. Therefore, propolis is likely to follow electron transfer mechanism to exhibit antioxidant property which was mainly contributed by phenolics. A greater inhibition against S. aureus (gram positive bacterium) than E. coli (gram negative bacterium) was also observed for propolis. A weak correlation of antioxidant and antibacterial activities of propolis was observed because the biological activities were most probably contributed by different compounds in propolis. Antioxidative propolis was mainly contributed by phenolics, but phenolics and flavonoids were just partly attributed to antibacterial property of propolis. Propolis could be a biopasteurization agent to preserve antioxidant fruit juice while inhibiting the bacterial growth.

Declaration of Competing Interest

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. “Related” means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2021.100051.

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