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Changes and degradation kinetics of some bioactive compounds in dried Orthosiphon aristatus (Java tea) leaves during elevated temperature storage

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Abstract

BACKGROUND Orthosiphon aristatus is a traditional medicinal herb mostly used in South East Asia, and has many health benefits. The packaging types and storage temperatures were investigated in order to select the best condition to produce high bioactive compounds (BC) of two kinds of dried Orthosiphon aristatus leaves.

RESULTS Blanched leaves were vacuum packed in polypropylene (PP) and aluminum foil laminated with polyethylene terephthalate and polyethylene (PET/Al/PE) and dried in a freeze dryer (B_FD) or heat pump-assisted dehumidified dryer (B_HPD60) at 60°C prior to storage at 15°, 25° and 35°C for 6 months. Leaves in PET/Al/PE bags had higher total phenolic content (TPC), antioxidant activity (AOA) and BC than in PP bags ($p \leq 0.05$). Storage at 15 °C retained the highest TPC and AOA in PET/Al/PE bags ($p \leq 0.05$). The degradation kinetics for BC, sinensetin and eupatorin, followed first-order kinetics. Half-lives (t1/2) for BC in PET/Al/PE were higher than in PP and were the highest at 15°C for both packaging types.

CONCLUSIONS Low temperature and PET/Al/PE bags provided the highest bioactive compound retention. The dried leaves from B_HPD60 and packed in PET/Al/PE bags had higher resistant to degradation of sinensetin than B_FD in PP bags.

Keywords

eupatorin, half-lives, kinetics, Orthosiphon aristatus leaves, Java leaves, sinensetin

Disciplines

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Comments

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Changes and degradation kinetics of some bioactive compounds in dried *Orthosiphon aristatus* (Java tea) leaves during elevated temperature storage

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Abstract

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CONCLUSIONS: Low temperature and PET/Al/PE bags provided the highest bioactive compound retention. The dried leaves from B_HPD60 and packed in PET/Al/PE bags had higher resistant to degradation of sinensetin than B_FD in PP bags.

Keywords: eupatorin; half-lives; kinetics; *Orthosiphon aristatus* leaves; Java leaves; sinensetin

INTRODUCTION

Orthosiphon aristatus (Java tea) is a medicinal herb found throughout South East Asia and is shown to have many health-improving properties, for example, anti-allergic, antihypertensive, anti-inflammatory, diuretic properties. It has been used for many centuries in treating ailments of the kidney, bladder stone, urinary tract infection, liver and bladder problems, diabetes, cholesterol and blood pressure, rheumatism and gout[1]. It also relieves muscle spasms in the walls of the internal organs, making it valuable for gallbladder problems[2]. Java tea is shown to have antimicrobial properties as well; in-vitro tests with aqueous extracts of Java tea leaf showed marked inhibition of both gram-positive and gram-negative bacteria[1]. Dolečková *et al.*[3] studied the anti-proliferative and anti-angiogenic effects of flavone eupatorin and showed that it reduced the number of viable cancer cells to the same extent as the leaf extract. The ability of eupatorin to nonspecifically inhibit many protein kinases was said to be the probable cause of its cellular effects.

Commercial Java tea leaves consists of dried leaves and stem tips of *O. aristatus* harvested shortly before flowering. It contains up to 12% minerals with a high proportion of potassium (600-700 mg 100 g^{-1} fresh leaves), up to 0.7% of essential oil and approximately 0.2% lipophilic flavones. The flavones in leaves include sinensetin (SEN), flavonol glycosides, caffeic acid derivatives (mainly rosmarinic acid and 2,3-dicaffeoyl-l-

tartaric acid), inositol, phytosterols (β-sitosterol) and saponins. The dominant polyphenol components in the leaves of the Java tea herb are four polymethoxylated flavones, sinensetin, eupatorin, 3'-hydroxy-5,6,7,4'-tetramethoxyflavone and rosmarinic acid[2]. The medicinal properties of these compounds come from the prevention of many chemical reactions, for example, diuretic effect is caused by high potassium content in the leaves and the presence of inositol (and possibly saponins) and flavones, sinensetin and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone. These compounds exhibited a diuretic activity in rats after intravenous administration of 10 mg kg^{-1} body weight of Java tea extract[1].

Drying is frequently used for herb and tea preservation and is usually conducted at temperatures up to 60°C as part of processing and packaging. The effect of high temperature exposure on antioxidant compounds relates to oxidative degradation of bioactive compounds[5]; however, some compounds can resist high temperatures up to 80°C. For such compounds, high temperature short time processing conditions can be used to retain higher amounts of bioactive antioxidants than low temperature and longer times. Degradation reactions for a quality attribute depend on the nature of the attribute; factors that control the rate of these reactions (temperature, humidity, oxygen availability) can be manipulated to minimize the changes during storage[6]. Presence of oxygen (O_2) and free oxygen radicals lead to oxidative lipid cleavage or autoxidation. Oxidation rates are not enhanced by water content or water activity; they proceed at maximum rates at particular moisture contents or a_w. Moreover, oxidation reaction rates are enhanced by light, heat, moisture content, and presence of heavy metals among other factors; for many chemical reactions, a decrease in a_w to below 0.6 can significantly decrease reaction rates because most reactions become diffusion-limited[6] at lower water activities.

Literature has related packaging types and storage conditions, including oxygen and light, to degradation of bioactive components in foods. Lycopene content of dehydrated tomato powder was influenced by storage conditions including packaging material; subsequent storage of product in metalized polyethylene bag was suggested to protect it against light, oxygen and humidity[7]. Common effects of improper storage on food products are both browning and development of off-flavor. This is caused by formation of insoluble compounds from the Maillard reaction, loss of nutritional value, moisture gain and microbial growth[8].

Packaging has to protect food from different undesirable influences and positively contribute to product shelf-lives. Packaging materials and conditions also affect oxidative quality degradation. Keeping quality and hygroscopic properties of dried food products, including leaves, are influenced by their water activity which is influenced by water vapor permeability of packaging materials. Polypropylene (PP) is a thermoplastic polymer used in a wide variety of applications including packaging and labeling but it has rather high oxygen and water vapor transmission rates[9,10]. PET/Al/PE, on the other hand, is an aluminum foil laminated with polyethylene terephthalate and polyethylene layers and is widely used in premium products or products that need to avoid light, oxygen and moisture contents as it has very low oxygen and water vapor transmission rates[11]. Filipović *et al.*[12] studied the effect of packaging types on shelf-lives of hard dry biscuit also called rusk, with results showing favorable preservation for PET/Al/PE. In another study, the packaging material was shown to affect the storability of leafy herbs like *Echinacea purpurea*, indicating that the freeze-dried materials sealed in polyethylene terephthalate/aluminum foil/polyethylene or nylon/polyethylene bags and stored in dark at 10–20°C and 40–60%

relative humidity retained higher levels of bioactive compounds[13]. The quality changes of dried pomegranate arils measured during storage suggested that aluminum laminated polyethylene had better protective effect than the high density polypropylene (HDPP) [14].

Hasmah *et al.*[15] studied degradation of phytochemicals from *Orthosiphon stamineus* leaves (Misai Kucing) at 5°, 10° and 25°C; their results showed that the losses of such phytochemicals were considerably much higher at ambient condition 25°C than at lower temperatures. There is no available report about effect of storage temperatures and packaging types as well as blanching and drying treatments using freeze dryer (B_FD) and heat pump-assisted dehumidified dryer (B_HPD60) at 60°C on bioactive compounds of blanched and dried *Orthosiphon aristatus* leaves which is the subject of this study. The objective of this study is to investigate the effect of drying treatments, storage temperatures, periods and packaging types on the keeping quality of previously dried Java leaf in terms of antioxidant activity and bioactive compounds.

MATERIALS AND METHODS

Materials

Matured *Orthosiphon aristatus* (OA) leaves were harvested from a local private garden in Kalasin province in Thailand. OA leaves were vacuum packaged in polyamides/ polyethylene bags before blanching at 100ºC for 75s which showed blanching adequacy in preliminary experiments against peroxidase enzyme. The packaged blanched leaves were then cooled in water at around 5-8°C and dried immediately. Blanched OA leaves (B) were dried using either a heat pump-assisted dehumidified dryer (HPD)[16] at 60°C for 16 min (B_HPD60), or a freeze dryer (FD) (Christ, DELTA 2-24 LSC, Fisher Scientific,

Loughborough, UK) (B_FD) for 18 h and stored at -20°C before storage studies as described in Klungboonkrong *et al*.[17]

Storage conditions

B_HPD60 and B_FD OA leaves (1 g) were vacuum-packaged (Henkelman, Elmherst, IL, USA) in 12 µm of polypropylene (PP) (ULINE Pleasant Prairie, WI, USA) and 7 µm of aluminum foil laminated with 12 µm polyethylene terephthalate and 70 µm linear low density polyethylene (PET/Al/PE) (ABC Packaging, Westlake, OH, USA). The leaves in PP and PET/Al/PE were stored in dark incubators set at 15ºC (MyTEMP Mini, Benchmark, NJ, USA) and 25ºC and 35ºC (Isotemp Incubator Model 304R, Fisher Scientific, USA). They were stored for six months and triplicate samples were taken out after 0, 2, 4 and 6 months. Storage chambers had an average relative humidity (RH) of 30, 13 and 11% for 15º, 25º and 35ºC chambers, respectively during the storage period. The RHs and temperatures used in this study cover the RH and temperature ranges in practice. Moisture content (MC)[18], water activity (a_w) (AquaLab 4TE, METER Group, Inc. WA, USA), total color difference (ΔE*) (CIE LAB), total phenolic content (TPC), antioxidant activity (AOA), sinensetin and eupatorin contents[19,20] were measured periodically at specified months above

Sinensetin and eupatorin degradation kinetics

Degradation kinetics for bioactive compounds (sinensetin and eupatorin) in dried OA leaves during storage were compared using zero order (Eqn (1)) and first order (Eqn (2)) reactions.

$$C = k_1 t + C_0 \quad (1)$$

$$C = C_0 \exp(k_2 t) \quad (2)$$

where C is the measured concentrations of bioactive compounds at storage times (t); C_0 is the initial value of bioactive compounds at time zero; t is the storage time (month) at a given temperature; k_1 is the zero-order kinetic constant ($month^{-1}$) and k_2 is the first- order kinetics constant ($month^{-1}$). Negative values for reaction rate constants (k-values) indicated the degradation of bioactive compounds with time and temperatures.

Storage half-lives

Half- lives ($t_{1/2}$) for bioactive compounds storage at different temperatures were estimated from Eqn (3) for first-order reactions. Coefficient of determination (R^2) and standard errors of estimated (SEE) were used as the basis for selecting the best fit.

$$t_{1/2} = \frac{\ln 0.5}{k} \quad (3)$$

where k is the degradation rate constant ($month^{-1}$)

Activation energies calculation

The Arrhenius equation was used to evaluate the temperature dependence of sinensetin and eupatorin degradation during storage. The relationship between the degradation rate constant and the temperature is shown as[20]:

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \quad (4)$$

where k is the reaction rate constant ($month^{-1}$) at a given temperature; k_0 is reaction rate at a reference temperature and a pre-exponential constant ($month^{-1}$); E_a is the activation energies for degradation reaction (kJ mol^{-1}); R is the universal gas constant (8.3145 J mol^{-1}·K) and T the storage temperature (K).

Analytical procedures

Extraction of bioactive compounds in leaves

Bioactive compound determination in leaves was done on the extract obtained as followed[19]. For sinensetin (SEN) and eupatorin (EUP) determination, 0.2 g dried OA leaves were extracted for 4 h with 10 mL chloroform and for TPC and AOA determination, leaves were extracted with 50% aqueous methanol at 40°C in a shaker water bath. The extracts were filtered through a filter paper (Whatman No.1) and centrifuged before any assay.

HPLC determination of sinensetin (SEN) and eupatorin (EUP) content

The solvent composition and the isocratic conditions were utilized following the modified method of Akowuah *et al.*[20]. The HPLC analysis was performed with an ultra-high pressure system (Accela 1250 pump, autosampler and PDA Detector, Thermo Scientific) and a C18 column (250×4.6 mm i.d., 5 mm, Waters, USA) at 25°C. A modified mobile phase consisted of a solution of methanol: water: tetrahydrofuran at 45:50:5 v/v ratios. The flow rate was 1 mL min^{-1}; detector was at 340 nm and the injection volume was 20 µL. The SEN and EUP were identified by comparing their HPLC retention times with those of pure standards.

Total phenolic content (TPC)

TPC in leaf extracts was determined following the method of Akowuah *et al.*[19]. Two hundreds µL of extract was added with 0.2 mL of Folin–Ciocalteu reagent in a test tube and mixed thoroughly using a vortex mixer. After 4 min, 1 mL of 15% Na_2CO_3 was added and then the mixture was allowed to stand for 2 h at 30 °C. The absorbance was measured at 760 nm using a spectrophotometer (UV-160, Shimadzu, Japan). The TPC of the extracts was calculated and expressed as gallic acid equivalents per dry weight (mg GAE g^{-1} d.w.) based on the gallic acid standard curve. Each sample was measured in triplicate and averaged.

Antioxidant activity (AOA)

The AOA of OA leaf extracts was determined following the method of Akowuah *et al.*[20]. The methanolic solution (2 mL) of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM) were mixed with 200 µL of OA leaf extracts and made up with pure methanol to a final volume of 3 mL. After 60 min standing in dark cabinet, the absorbance of the mixture was measured at 517 nm against pure methanol as blank using a spectrophotometer (UV-160, Shimadzu, Japan). Free radical-scavenging activities of reference Trolox (0.05 mg mL^{-1}) were also determined using the same procedure and expressed as Trolox equivalents per gram of dry weight (mg Trolox g^{-1} d.w.). Each sample was measured in triplicate and averaged.

Statistical analyses

The measurement means were processed using nonlinear regression methods using SPSS 19.0 software for Windows (SPSS, Inc., Chicago: IL). The degree of fit of the tested

models was evaluated with the coefficient of determination (R^2) and standard error of estimate (SEE). A completely randomized 4x2x2 split-split-plot design was used for storage data analyses. Main plot unit was storage times; the sub-plot treatment factor was storage temperatures and the sub-sub plot treatment factor was packaging types. Triplicate sampling was carried out for each experiment set. Analysis of variance (ANOVA) at 95% significance level (α=0.05) and Duncan's multiple range test were employed to compare means at a 95% confidence interval.

RESULTS AND DISSCUSION

Moisture content, water activity and total color difference

Moisture content (MC), water activity (a_w) and total color difference for blanched and dried OA leaves for B_FD and B_HPD60 during storage are shown in Table 1 and 2, respectively. At the start of the storage (t=0), the freeze dried (B_FD) and heat pump-assisted dehumidified dried at 60ºC (B_HPD60) OA leaves had low moisture content of 2.8 and 8.3 % dry basis (d.b.), respectively and water activity of 0.28 and 0.53, respectively. The growth of spoilage microorganisms was potentially inhibited at low a_w values equal or below 0.6. The effects of storage times, temperatures, and packaging types on MC and a_w of B_FD and B_HPD60 OA leaves were significantly different ($p \leq 0.05$). The moisture contents of B_FD and B_HPD60 during storage for 6 months were in the range from 4.1 to 11.1 and 4.8 to 12.8 % d.b., respectively while a_w were in the range from 0.32 to 0.67 and 0.35 to 0.67, respectively for the same temperature range. The types of packaging, temperatures and storage times were reported to significantly affected ($p \leq 0.05$) moisture content of apple peel powder[22] as seen in our results.

The MC and a_w of polypropylene bags were decreased at higher storage temperatures because higher temperature (35°C) had lower RH values. For leaf samples in aluminum foil laminated with polyethylene terephthalate and polyethylene (PET/Al/PE), MC and a_w were changed very little or none during the storage period. Lower water vapor permeability for PET/Al/PE (7.68 x 10^{-12}g mm $m^{-2}day^{-1}kPa^{-1}$) compared to 2.46 x 10^{-11}g mm $m^{-2}day^{-1}kPa^{-1}$ for PP seems to be the main reason. Water vapor pressure (WVP) values have been also reported, for example, 0.25 g 100 cm^{-2} in 24h at 37.8°C and RH of 90% for PP[10] and close to zero for PET/Al/PE[23]. These reports and our results indicated that PP had higher permeability but PET/Al/PE had higher barrier properties against moisture. Oxygen transmission rate (OTR) reported in literature for PET/Al/PE 0 cm^3 m^{-2} $d^{-1}atm^{-1}$ at 23 ºC, 50% RH[11] and 149.2 cm^3 m^{-2} d^{-1} atm^{-1} at 23°C and 50% RH for PP[9] also indicated that PP allowed more oxygen to permeate into food, thus causing detrimental effects on quality including oxidation.

Color values measured as total color difference (ΔE*) (Table 1 and 2) indicated overall color difference for dried OA leaves before and after storage in both packages. ΔE* for stored B_FD and B_HPD60 leaves were ranged from 0.62 to 10.96 and 0.86 to 16.58, respectively during 6 months. Storage temperatures, times and packaging types affected total color difference significantly ($p \leq 0.05$). Low temperatures and PET/Al/PE storage resulted in less total color difference whereas total color difference was increased with increasing storage time ($p \leq 0.05$) at all storage temperatures in this study. Lesser oxygen and water vapor permeation, for example, in PET/Al/PE bags and lower temperatures could prevent many factors that influenced the color change including non-enzymatic browning, and lipid oxidation.

Total phenolic content and antioxidant activity

The TPC and AOA in B_FD and B_HPD60 OA leaves stored at 15º, 25 º and 35ºC for 6 months are summarized in Table 1 and 2, respectively. Both TPC and AOA were influenced by storage times, temperatures and packaging types for both B_FD and B_HPD60 leaves ($p \leq 0.05$). The initial values of TPC for B_FD and B_HPD60 were 16.22 and 14.51 mg GAE g^{-1} d.w., respectively whereas the initial values of AOA for B_FD and B_HPD60 were 27.19 and 25.16 mg Trolox g^{-1} d.w., respectively. The TPC and AOA were decreased with increased storage times and temperatures. These results were similar to Korus[24] who reported that TPC and AOA in kale leaves were decreased during storage time for 12 months at two storage temperatures (10º and 20ºC). For both B_FD and B_HPD60 leaves, the highest TPC and AOA retention were seen at 15 ºC for the same storage period and package type. In addition, TPC and AOA retention in vacuum packaged leaves in PET/Al/PE were higher than in PP for the same conditions and storage times. The highest retention was at 15ºC for both B_FD and B_HPD60 leaves. Many factors affect the antioxidant activity of a food product during storage, such as antioxidant concentration, material, temperature, pH, processing treatment and storage time[25]. Henríquez *et al.*[22] reported that TPC was decreased during the storage period in high density polyethylene and metalized films of high barrier packaging materials. Hasmah *et al.*[15] reported that the TPC of *Orthosiphon stamineus* at one week of storage at low temperature (5° and 10°C) were ranged from 623.4 to 640.4 mg GAE 100 g^{-1} d.w.

The loss of TPC in OA leaves vacuum packed in PP bags during storage was influenced by oxidation as indicated by higher levels of water and oxygen permeation from

the environment into food compared to PET/Al/PE films. In fact, a high level of moisture accelerates the degradation of the TPC[26]. The decrease in polyphenols is also correlated to their capacity to take part in the formation of Maillard reaction products[27].

Content of bioactive compounds during storage

Sinensetin and eupatorin in B_FD and B_HPD60 dried OA leaves at different storage times, temperatures and packaging materials are presented in Table 3. The sinensetin values at day 0 (control) for B_FD and B_HPD60 were 0.2556 and 0.2630 mg g^{-1} d.w., respectively whereas the eupatorin values at day 0 (control) for B_FD and B_HPD60 were 0.166 and 0.1752 mg g^{-1} d.w., respectively. For B_FD, in PET/Al/PE packaging at 15º, 25 º and 35ºC, sinensetin contents were lost by 34, 40, and 43% from initial amount of 0.2556 mg g^{-1} d.w. at 15º, 25º and 35ºC storage, respectively. The corresponding sinensetin contents losses for PP packaging were 37, 40, and 44% from original contents. Eupatorin contents for B_FD leaves in PET/Al/PE packaging at 15º, 25º and 35ºC were lost by 67, 74, and 80% from the initial amount of 0.1658 mg g^{-1} d.w.; the corresponding loss for PP packaging were 73, 76 and 80%, respectively. The losses of these bioactive compounds were also found in B_HPD60 dried leaves for both types of packaging. For PET/Al/PE packaging at 15º, 25º and 35ºC, sinensetin contents was lost by 42, 47 and 51% while for PP packaging, they were 47, 52 and 56% from initial amount of 0.2630 mg g^{-1} d.w., respectively. Eupatorin losses for B_HPD60 at 15º, 25º and 35ºC were 67, 74 and 79% for PET/Al/PE packaging and 73, 78 and 79% for PP packaging from initial amount of 0.1752 mg g^{-1} d.w., respectively.

Storage times, temperatures and packaging types affected sinensetin and eupatorin retention significantly ($p \leq 0.05$). These bioactive compounds were decreased with increasing storage times because of the oxidation effects as discussed earlier. For both B_FD and B_HPD60, the highest sinensetin and eupatorin contents were found at 15ºC for the same storage period. Predictably, OA leaves in PET/Al/PE had higher sinensetin and eupatorin contents than PP at the same storage conditions (time, temperature). Sinensetin and eupatorin retetion after 6 months at 15ºC for B_FD OA leaves in PET/Al/PE were higher (17% and 61%, respectively) than leaves in PP stored at 35ºC, respectively. Sinensetin and eupatorin retention for B_HPD60 leaves after 6 month in PET/Al/PE and at 15ºC were higher (31% and 61%, respectively) than leaves in PP stored at 35ºC, respectively.

The loss of phytochemical compounds in similar types of plants during storage have been reported, for example, degradation of phytochemicals in *Orthosiphon stamineus* were considerably higher at 25ºC than low temperature storage (5° and 10°C)[15]. They reported total phenolic contents, total flavonoid and rosmarinic acid losses by 19, 25 and 27% from initial amount of 587.9 mg GAE 100 g^{-1} d.w., 697.8 mg catechin equivalents (CE) 100 g^{-1} d.w. and 229.7 mg 100 g^{-1} d.w., respectively. Ferriera and Rodriguez-Amaya[28] reported that lycopene embedded in corn starch carrier matrix was diminished by 86% after 10 days in dark and transparent polyethylene film bags whereas β-carotene loss was at 27% after 20 days in the dark. In addition, the β-carotene and lycopene of lyophilized guava wrapped in transparent polyethylene film was lost by 63 and 65%, respectively after 20 day in the dark at ambient temperature.

Sinensetin and eupatorin degradation kinetics during OA leaf storage, half-lives and activation energies

First-order kinetics was a better fit for the degradation of bioactive compounds, sinensetin and eupatorin in dried OA leaves with higher coefficients of determination (R^2) and lower standard errors of estimated (SEE) compared to zero-order so only estimated first-order kinetics parameters are presented in Table 4. The R^2 of the first-order kinetics for B_FD and B_HPD60 were ranged from 0.8244 to 0.9968 and 0.8243 to 0.9940, respectively which indicated very good fit whereas the R^2 of the zero-order kinetics for B_FD and B_HPD60 were ranged from 0.7522 to 0.9961 and 0.7279 to 0.9903, respectively (data not shown). Results indicated that the first order degradation rates were dependent on storage temperatures. The degradation of bioactive compounds in food matrices that have low water content, such as cereal grains and derived products, was shown to follow a first-order degradation reaction[29]. First-order kinetics was also the most common and the best studied in the destruction of pigments during processing and storage[30], lipid oxidation and development of rancidity, microbial growth, vitamin losses in dried foods and loss of protein quality[31]. In the first-order reaction, the rate of a chemical change is directly proportional to the concentrations of the reacting substance so it is evident that the rates of the process must fall off as the reaction proceeds for the reactants are being continuously consumed[32]. The reaction rates for dried OA leaves bioactive compounds also indicated similar trend.

Table 5 shows estimated half-lives ($t_{1/2}$) and activation energies (E_a) of sinensetin and eupatorin degradations in B_FD and B_HPD60 OA leaves vacuum packed in PET/Al/PE and PP bags. The $t_{1/2}$ for sinensetin and eupatorin of B_FD and B_HPD60 OA

leaves in PET/Al/PE were longer than PP at the same temperatures because of less oxygen and water vapor diffusion. The longest $t_{1/2}$ was for sinensetin and eupatorin in B_FD and B_HPD60 OA leaves at 15ºC storage for both types of packaging. Compared with the same temperature and packaging, sinensetin had longer half-life than eupatorin which indicates faster eupatorin degradation in the leaves.

Activation energies (E_a) is the minimum energy need to initiate a chemical reaction, for examples, bioactive compounds degradation reaction. For B_FD OA leaves, the E_a for sinensetin degradation at 15˚ to 35˚C storage in PET/Al/PE and PP were 79.95 and 47.27 kJ mol^{-1}, respectively. The respective values for eupatorin in B_FD OA leaves in PET/Al/PE and PP were 96.98 and 51.60 kJ mol^{-1}, respectively. In B_HPD60 OA leaves, the E_a for sinensetin in PET/Al/PE and PP were 105.92 and 72.25 kJ mol^{-1}, respectively for 15 to 35˚C storage. For eupatorin, they were 96.42 and 51.49 kJ mol^{-1}, respectively. Comparing E_a of sinensetin between B_FD and B_HPD60, it is seen that B_HPD60 had higher E_a than B_FD which mean that the dried leaves from B_HPD60 had higher resistance to degradation compared to B_FD. Perhaps the structure of B_FD leaves was porous after freeze drying with higher surface area exposed and lower water activity (0.2752) giving way to lesser E_a and faster degradation of bioactive compound. It was reported that lower water activity caused higher oxidation reactions in dried products, such as dried apple and spice[33]. Oxygen transmission rate and water vapor permeation in PP bags were higher than in PET/Al/PE bags potentially leading to increased oxidation and lower E_a.

Bechoff *et al.*[34] reported that the E_a for β-carotene degradation in dried sweet potato chips during storage at 10° to 40°C was 64.2 kJ mol^{-1}. Sharma and Maguer[35] reported that the E_a of lycopene degradation in tomato pulp solids stored at -20°, 5° and 25°C were in the

range from 19.87 to 27.74 kJ mol^{-1}. Lutein degradation E_a in freeze-dried sweet corn during storage under vacuum and dark condition was 32.40 kJ mol^{-1} which was higher than in air and dark storage condition (28.64 kJ mol^{-1})[30]. E_a for sinensetin and eupatorin degradation in OA leaves seem to be higher than bioactive compounds reported above providing with more stable storage environment in indicated packages.

CONCLUSIONS

The bioactive compounds, such as sinensetin and eupatorin, TPC and AOA of the dried OA leaves were monitored during storage at temperatures 15°, 25° and 35°C and were losses at high temperatures and packed in PP bags. The dried OA leaves (B_HPD60) vacuum packed in the PET/Al/PE bags and stored at low temperature provided the highest bioactive compound retention. First-order kinetics suitably expressed the degradation reactions in the dried leaves in both packages. The $t_{1/2}$ of bioactive compounds of the dried OA leaves and vacuum packed in PET/AL/PE bags were higher than PP bags at the same temperature. The dried leaves from B_HPD60 and packed in PET/Al/PE bags had higher resistant to degradation of sinensetin than B_FD and packed in PP bags.

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Table 1. Moisture content, water activity, total color difference, total phenolics content and antioxidant activity during storage of blanched and freeze dried OA leaves

Packaging	Temperature (°C)	Time (months)	Moisture content (% d.b.)	Water activity (a_w)	Total color difference (ΔE^*)	Total phenolic contents (mg GAE g^{-1} d.w.)	Antioxidant activity (mg Trolox g^{-1} d.w.)
	Control	0	2.82 ± 0.35^{l}	0.2752 ± 0.010^{k}	0.00 ± 0.00^{r}	16.22 ± 0.24^{a}	27.19 ± 0.39^{a}
PET/AL/PE Vacuum Packed	15	2	4.07 ± 0.20^{h}	0.3606 ± 0.002^{f}	0.62 ± 0.15^{q}	13.84 ± 0.20^{b}	24.84 ± 0.23^{b}
		4	4.38 ± 0.17^{g}	0.3669 ± 0.009^{e}	1.14 ± 0.20^{p}	13.03 ± 0.17^{d}	22.76 ± 0.13^{f}
		6	4.62 ± 0.11^{f}	0.3591 ± 0.005^{f}	2.72 ± 0.14^{k}	12.71 ± 0.26^{f}	20.99 ± 0.11^{k}
	25	2	4.28 ± 0.13^{g}	0.3317 ± 0.003^{j}	1.32 ± 0.13^{o}	12.75 ± 1.04^{ef}	23.53 ± 0.24^{d}
		4	4.30 ± 0.19^{g}	0.3428 ± 0.005^{hi}	2.11 ± 0.12^{n}	12.29 ± 0.14^{i}	21.89 ± 0.22^{h}
		6	4.33 ± 0.24^{g}	0.3454 ± 0.002^{ghi}	3.16 ± 0.28^{j}	12.26 ± 0.23^{i}	19.66 ± 0.09^{m}
	35	2	4.07 ± 0.27^{h}	0.3230 ± 0.013^{k}	1.31 ± 0.65^{o}	12.56 ± 0.22^{g}	22.81 ± 0.11^{f}
		4	4.31 ± 0.14^{g}	0.3485 ± 0.001^{g}	2.54 ± 0.22^{l}	12.21 ± 0.16^{i}	21.39 ± 0.14^{j}
		6	4.27 ± 0.23^{g}	0.3465 ± 0.006^{gh}	3.81 ± 0.14^{i}	11.52 ± 0.19^{l}	19.39 ± 0.20^{n}
PP Vacuum Packed	15	2	10.75 ± 0.22^{b}	0.6714 ± 0.005^{a}	2.29 ± 0.13^{m}	13.18 ± 0.17^{c}	24.05 ± 0.21^{c}
		4	11.08 ± 0.19^{a}	0.6556 ± 0.009^{b}	6.42 ± 0.27^{d}	12.46 ± 0.23^{h}	22.15 ± 0.17^{g}
		6	11.08 ± 0.18^{a}	0.6563 ± 0.004^{b}	7.19 ± 0.24^{c}	12.26 ± 0.17^{i}	20.10 ± 0.08^{l}
	25	2	5.76 ± 0.28^{d}	0.4616 ± 0.015^{c}	4.13 ± 0.14^{h}	12.81 ± 0.13^{e}	22.97 ± 0.15^{e}
		4	5.89 ± 0.12^{c}	0.4482 ± 0.007^{d}	6.06 ± 0.19^{f}	12.25 ± 0.29^{i}	21.45 ± 0.19^{j}
		6	5.74 ± 0.14^{d}	0.4517 ± 0.002^{d}	7.46 ± 0.47^{b}	12.01 ± 0.12^{k}	19.46 ± 0.16^{n}
	35	2	4.73 ± 0.28^{ef}	0.3416 ± 0.014^{i}	4.69 ± 0.20^{g}	12.10 ± 0.21^{j}	21.65 ± 0.29^{i}
		4	4.70 ± 0.19^{ef}	0.3311 ± 0.012^{j}	6.19 ± 0.27^{e}	11.16 ± 0.24^{m}	21.34 ± 0.14^{j}
		6	4.79 ± 0.14^{e}	0.3446 ± 0.006^{ghi}	10.96 ± 0.36^{a}	9.99 ± 0.13^{n}	16.66 ± 0.22^{o}

Values in a column not followed by the same superscript letter are significantly different ($p \leq 0.05$).

Table 2. Moisture content, water activity, total color difference, total phenolics content and antioxidant activity during storage of blanched and heat pump-assisted dehumidified dried OA leaves

Packaging	Temperature (°C)	Time (months)	Moisture content (% d.b.)	Water activity (a_w)	Total color difference (ΔE*)	Total phenolic contents (mg GAE g^{-1} d.w.)	Antioxidant activity (mg Trolox g^{-1} d.w.)
	Control	0	8.29 ± 0.25^{i}	0.5279 ± 0.054^{j}	0.00 ± 0.00^{q}	14.51 ± 0.28^{a}	25.16 ± 0.51^{a}
PET/AL/PE Vacuum Packed	15	2	9.37 ± 0.29^{ef}	0.5570 ± 0.010^{d}	0.86 ± 0.35^{p}	12.75 ± 0.33^{b}	21.54 ± 0.19^{b}
		4	9.42 ± 0.24^{de}	0.5498 ± 0.006^{e}	2.24 ± 0.19^{l}	11.24 ± 0.25^{f}	19.60 ± 0.20^{f}
		6	9.30 ± 0.23^{f}	0.5491 ± 0.001^{ef}	4.61 ± 0.18^{j}	9.89 ± 0.10^{j}	15.57 ± 0.11^{j}
	25	2	9.49 ± 0.27^{d}	0.5559 ± 0.004^{d}	0.93 ± 0.18^{o}	12.51 ± 0.09^{c}	21.28 ± 0.22^{c}
		4	9.28 ± 0.24^{f}	0.5356 ± 0.009^{h}	4.95 ± 0.35^{i}	10.96 ± 0.23^{g}	17.21 ± 0.23^{h}
		6	9.15 ± 0.28^{g}	0.5418 ± 0.002^{g}	6.38 ± 0.13^{h}	9.77 ± 0.21^{j}	14.87 ± 0.14^{l}
	35	2	9.29 ± 0.19^{f}	0.5469 ± 0.005^{f}	1.91 ± 0.13^{m}	12.27 ± 0.21^{d}	20.70 ± 0.24^{e}
		4	9.30 ± 0.20^{f}	0.5315 ± 0.001^{i}	4.94 ± 0.09^{i}	10.10 ± 0.08^{i}	16.66 ± 0.12^{i}
		6	9.04 ± 0.14^{h}	0.5344 ± 0.008^{h}	8.60 ± 0.20^{d}	9.51 ± 0.09^{k}	14.37 ± 0.18^{m}
PP Vacuum Packed	15	2	11.82 ± 0.04^{c}	0.6551 ± 0.005^{c}	1.74 ± 0.09^{n}	12.59 ± 0.15^{c}	21.28 ± 0.19^{c}
		4	11.99 ± 0.16^{b}	0.6679 ± 0.005^{a}	3.77 ± 0.22^{k}	11.00 ± 0.09^{g}	18.66 ± 0.16^{g}
		6	12.83 ± 0.17^{a}	0.6623 ± 0.002^{b}	7.00 ± 0.23^{f}	9.79 ± 0.36^{j}	15.19 ± 0.14^{k}
	25	2	6.25 ± 0.16^{j}	0.4504 ± 0.009^{l}	3.81 ± 0.36^{k}	12.35 ± 0.04^{d}	21.04 ± 0.15^{d}
		4	5.99 ± 0.02^{k}	0.4483 ± 0.006^{l}	6.63 ± 0.20^{g}	10.71 ± 0.14^{h}	16.69 ± 0.17^{i}
		6	6.27 ± 0.26^{j}	0.4695 ± 0.003^{k}	8.82 ± 0.14^{c}	9.61 ± 0.13^{k}	14.40 ± 0.19^{m}
	35	2	4.91 ± 0.10^{l}	0.3583 ± 0.009^{m}	7.09 ± 0.69^{e}	11.59 ± 0.13^{e}	20.61 ± 0.14^{e}
		4	4.82 ± 0.18^{l}	0.3470 ± 0.005^{o}	12.50 ± 0.37^{b}	10.08 ± 0.21^{i}	14.85 ± 0.26^{l}
		6	4.88 ± 0.27^{l}	0.3522 ± 0.008^{n}	16.58 ± 0.09^{a}	8.83 ± 0.30^{l}	12.84 ± 0.18^{n}

Values in a column not followed by the same superscript letter are significantly different ($p \leq 0.05$).

Table 3. Sinensetin and Eupatorin of blanched and dried OA leaves using freeze-dryer and heat pump-assisted dehumidified dryer during storage

Packaging	Temperature (°C)	Time (months)	Freeze-dryer		Heat pump-assisted dehumidified dryer	
			Sinensetin (mg g^{-1} d.w.)	Eupatorin (mg g^{-1} d.w.)	Sinensetin (mg g^{-1} d.w.)	Eupatorin (mg g^{-1} d.w.)
	Control	0	0.26 ± 0.02^{a}	0.17 ± 0.01^{a}	0.26 ± 0.01^{a}	0.18 ± 0.00^{a}
PET/AL/PE Vacuum Packed	15	2	0.23 ± 0.03^{b}	0.12 ± 0.02^{b}	0.20 ± 0.05^{b}	0.13 ± 0.02^{b}
		4	0.19 ± 0.03^{cd}	0.09 ± 0.02^{e}	0.19 ± 0.03^{c}	0.10 ± 0.02^{e}
		6	0.17 ± 0.01^{ghi}	0.06 ± 0.01^{i}	0.15 ± 0.02^{e}	0.06 ± 0.01^{j}
	25	2	0.20 ± 0.02^{c}	0.10 ± 0.01^{c}	0.17 ± 0.01^{d}	0.11 ± 0.01^{c}
		4	0.17 ± 0.03^{fgh}	0.08 ± 0.01^{g}	0.15 ± 0.03^{e}	0.08 ± 0.01^{g}
		6	0.15 ± 0.03^{jkl}	0.04 ± 0.02^{j}	0.14 ± 0.03^{gh}	0.05 ± 0.03^{k}
	35	2	0.18 ± 0.04^{ef}	0.10 ± 0.02^{e}	0.15 ± 0.03^{ef}	0.10 ± 0.02^{e}
		4	0.16 ± 0.02^{ij}	0.07 ± 0.01^{h}	0.13 ± 0.01^{hi}	0.07 ± 0.01^{h}
		6	0.15 ± 0.02^{kl}	0.03 ± 0.01^{kl}	0.13 ± 0.04^{ij}	0.04 ± 0.01^{m}
PP Vacuum Packed	15	2	0.19 ± 0.02^{de}	0.11 ± 0.01^{c}	0.17 ± 0.01^{d}	0.11 ± 0.01^{c}
		4	0.16 ± 0.03^{ij}	0.08 ± 0.01^{f}	0.16 ± 0.02^{e}	0.09 ± 0.01^{f}
		6	0.16 ± 0.01^{ij}	0.05 ± 0.02^{j}	0.14 ± 0.04^{g}	0.05 ± 0.02^{k}
	25	2	0.18 ± 0.01^{fg}	0.10 ± 0.02^{d}	0.15 ± 0.03^{e}	0.10 ± 0.02^{d}
		4	0.15 ± 0.02^{jk}	0.07 ± 0.01^{g}	0.14 ± 0.01^{g}	0.08 ± 0.01^{g}
		6	0.15 ± 0.03^{jkl}	0.04 ± 0.02^{k}	0.13 ± 0.01^{j}	0.04 ± 0.02^{l}
	35	2	0.17 ± 0.01^{hi}	0.09 ± 0.01^{e}	0.15 ± 0.01^{fg}	0.10 ± 0.01^{e}
		4	0.15 ± 0.02^{kl}	0.07 ± 0.02^{h}	0.13 ± 0.01^{ij}	0.07 ± 0.02^{i}
		6	0.14 ± 0.02^{l}	0.03 ± 0.01^{k}	0.12 ± 0.03^{k}	0.04 ± 0.01^{m}

Values in a column not followed by the same superscript letter are significantly different ($p \leq 0.05$).

Table 4. Estimated first-order degradation kinetics parameters for bioactive compounds in dried OA leaves (B_FD and B_HPD60)

Bioactive compounds	Packaging		First-order of B_FD [$C=C_0 \exp(k_2 t)$]				First-order of B_HPD60 [$C=C_0 \exp(k_2 t)$]			
			C_0 ($\mu g\ g^{-1}$ d.w.)	k_2 ($month^{-1}$)	R^2	SEE ($\mu g\ g^{-1}$ d.w.)	C_0 ($\mu g\ g^{-1}$ d.w.)	k_2 ($month^{-1}$)	R^2	SEE ($\mu g\ g^{-1}$ d.w.)
Sinensetin (µg/g d.b.)	PET/AL/PE Vacuum Packed	15°C	0.2570	-0.0700	0.9968	0.0004	0.2577	-0.0883	0.9641	0.0014
		25°C	0.2496	-0.0876	0.9690	0.0013	0.2471	-0.1189	0.8514	0.0036
		35°C	0.2442	-0.1011	0.9069	0.0025	0.2449	-0.1432	0.8243	0.0044
	PP Vacuum Packed	15°C	0.2439	-0.0865	0.8715	0.0026	0.2474	-0.1149	0.8536	0.0035
		25°C	0.2419	-0.0976	0.8517	0.0031	0.2468	-0.1406	0.8602	0.0039
		35°C	0.2394	-0.1079	0.8244	0.0036	0.2464	-0.1603	0.8590	0.0042
Eupatorin (µg/g d.b.)	PET/AL/PE Vacuum Packed	15°C	0.1665	-0.1587	0.9943	0.0006	0.1760	-0.1591	0.9940	0.0006
		25°C	0.1646	-0.2114	0.9920	0.0008	0.1739	-0.2114	0.9920	0.0008
		35°C	0.1645	-0.2474	0.9885	0.0010	0.1738	-0.2474	0.9886	0.0010
	PP Vacuum Packed	15°C	0.1647	-0.1991	0.9807	0.0012	0.1741	-0.1992	0.9805	0.0012
		25°C	0.1645	-0.2294	0.9856	0.0011	0.1738	-0.2294	0.9856	0.0011
		35°C	0.1642	-0.2530	0.9919	0.0008	0.1736	-0.2530	0.9918	0.0009

Table 5. Half-lives ($t_{1/2}$) and activation energies (E_a) for sinensetin and eupatorin degradations in dried OA leaves (B_FD and B_HPD60)

Packaging	Temperature (°C)	$t_{1/2}$ = 0.693/k (month)				$k=k_0 \exp(E_a/RT)$							
						k_0 (1/month)				E_a (kJ/mol)			
		sinensetin		eupatorin		sinensetin		eupatorin		sinensetin		eupatorin	
		B_FD	B_HPD60	B_FD	B_HPD60	B_FD	B_HPD60	B_FD	B_HPD60	B_FD	B_HPD60	B_FD	B_HPD60
PET/AL/PE Vacuum Packed	15	9.90	7.85	4.37	4.35	0.1315	0.2033	0.3424	0.3416	79.95	105.92	96.98	96.42
	25	7.91	5.83	3.28	3.28								
	35	6.85	4.84	2.80	2.80								
PP Vacuum Packed	15	8.01	6.03	3.48	3.48	0.1253	0.2031	0.2990	0.2989	47.27	72.25	51.60	51.49
	25	7.10	4.93	3.02	3.02								
	35	6.42	4.32	2.74	2.74								