Original article

Effects of processing and storage conditions on the stability of sweet potato (*Ipomoea batatas* L.) leaf flavonoids

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Summary   The effect of heat, high hydrostatic pressure (HHP) treatment, pH, light, temperature and simulated digestion on sweet potato leaf flavonoids (SPLF) stability was studied. Results showed heat treatment at 75 °C for 90 min or HHP treatment at 600 MPa for 30 min did not cause significant effect on SPLF. Heat treatment at 100 °C for 60 min and 90 min led to decrease in antioxidant activity by 20% and 25%, respectively, while pH 7.0 and 8.0 significantly decreased amount of SPLF by approximately 75%, decreased antioxidant activity by about 30% and 47% separately. Light treated samples recorded a decrease in SPLF by 52% and antioxidant activity by 24%. No significant effect on SPLF was observed for samples stored at −18 °C, 4 °C or room temperature. The retention of flavonoids and antioxidant activity was 45.9 ± 3.6% and 56.2 ± 2.6%, individually in SPLF after simulated digestion.

Introduction

The world sweet potato production is about 112.83 million tonnes; China accounts for 63.84% of the total production (FAOSTAT, 2017). After harvesting the roots, there is almost equal amount of sweet potato leaves (SPL) waiting to be discarded. SPL is rich in flavonoids and possesses antioxidant activity which could slow down chronic diseases, such as rheumatism, cardiovascular diseases and cancers (Chen *et al.*, 2019).

The stability of flavonoids during processing and storage is critical to factories and consumers; in order to attain the desired nutritional and functional effects, heat treatment and HHP treatment were usually employed to attain processing and bactericidal effect (Li *et al.*, 2017). Compared to traditional heat treatment, HHP treatment has less adverse effects on flavonoids during processing or preservation (Khan *et al.*, 2018).

Zeng *et al.* (2019) studied the influence of hydrothermal treatment at 60, 80 and 100 °C on total flavonoid content (TFC) and found out that TFC decreased by 3.5–5.8 % after 30 min, with a slight decrease in the antioxidant activity (<10%). Fernández-Jalao *et al.* (2019) recorded a 30% of increase in TFC for Spain apples after HHP treatment (400 MPa, for 5 min). Echeverry *et al.* (2018) revealed a decrease in TFC in *Passiflora quadrangularis* leaf extracts by 28.2% under alkali hydrolysis and 7.8% under acid hydrolysis. A decrease (>54%) in total anthocyanin content was reported when been exposed to sunlight after 3 months (Bakowska *et al.*, 2003). Further, TFC in pomegranate juice, pulp and extract was shown to decrease by 13.8%, 16.4% and 26.7%, respectively, after simulated gastric digestion (Mosele *et al.*, 2015). Industrial processed tomato sauce with higher amounts of flavonoids recorded a decrease in antioxidant activity by 60.3%, 60.5% and 75.3%, respectively, after digestion in oral, stomach and intestinal phase (Tomas *et al.*, 2017). Based on the literatures, flavonoids are sensitive to digestion, environmental and processing conditions, with the rate of degradation varying with the severity of the process.

The overall stability of SPLF during processing and storage is not well understood; thus, the purpose of this study was to elucidate the effects of different

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treatments (heat, HHP, pH, light, temperature and simulated digestion) on the stability of SPLF. The information got from this research will be important in providing the stability profile of SPLF with potential use as a food additive.

Materials and methods

Materials

The SPL (Cultivar: Yuzi No.7), which exhibited the highest amount of polyphenols content and strongest antioxidant activity, was assessed in the leaves among 40 sweet potato cultivars harvested in China, was chosen for this study based on previous work (Sun *et al.*, 2014). The leaves were provided by Chongqing Sweet Potato Engineering & Technology Research Center (Chongqing, China), transported to the laboratory by plane, washed, dried by vacuum freezer and grounded by ultra-micro pulveriser. The grounded material was sieved through the 100-mesh screen, sealed in aluminium foil bags and stored at 4 °C in the refrigerator.

Myricetrin, isoquercitrin, astragalin, quercitrin, tiliroside, quercetin, kaempferol and rhamnetin were obtained from An Apoptosis and Epigenetics Company (Houston, USA). The purity of the individual flavonoid was more than 98%. α-amylase with the activity ≥16 units mg^{-1}, pepsin with the activity ≥250 units mg^{-1}, trypsin with the activity ≥1500 units mg^{-1} and bile salt with the purity of choleic acid more than 60% were purchased from Sigma Chemical Co (St. Louis, MO, USA). 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was obtained from Solarbio Life Sciences (Beijing, China). High-performance liquid chromatograph (HPLC) grade solvents were obtained from Fisher Chemical (Beijing, China). HPLC grade water was prepared by the Molgene water purification system from Molecular (Shanghai, China). All other analytical grade reagents were purchased from Sinopharm Chemical Reagent (Beijing, China).

Preparation of sweet potato leaf flavonoids

Briefly, 50 g of SPL was extracted two times at 57 °C for 76 s using Ultrasonic-microwave assisted extractor CW-2000 (ultrasonic frequency and power was 40 kHz and 50 W, microwave frequency and power was 2450 MHz and 100 W, respectively, and microwave wavelength was 0.12 m) (Shanghai Xintuo Analytical Instruments Co., LTD, China). Thereafter, the crude ethanol extract attained above was extracted by petroleum ether and ethyl acetate to get 1.55 g SPLF with the purity of 76.10 ± 3.11 (%, DW) (Liu *et al.*, 2019). Based on the solubility of flavonoids and

antibacterial capacity of ethanol, 1.40 g SPLF was dissolved into 70 mL ethanol (approximately 5% of the total volume) and divided into 28 portions (2.5 mL for each portion) for processing and storage experiment. In addition, 0.15 g SPLF was dispersed into 15 mL of distilled water and used for simulated digestion study.

Preparation of sample solutions for processing and storage stability evaluation

Sweet potato leaf flavonoids was diluted by distilled water to obtain the control sample, heat-treated samples and HHP-treated samples. Heat-treated samples were placed in plug test tubes and HHP-treated samples were placed in high-pressure bags. SPLF was prepared at different pH solutions using phosphate buffer (pH 3.0, 5.0, 7.0, 8.0). The SPLF used to study the storage stability evaluation at −18 °C was diluted by 40% ethanol in which the freezing point was −23 °C. For other storage stability studies, the SPLF was prepared using distilled water. The final concentration of all the SPLF samples prepared was adjusted to 1 mg mL^{-1}.

Processing and storage treatment on the sample solutions

Processing treatment on SPLF was performed as explained by Cao *et al.* (2018), storage treatment on SPLF was carried out according to the method of Struck *et al.* (2016), respectively. Heat treatment on SPLF was done at 50, 75 and 100 °C for 30, 60 and 90 min in the oven to simulate enzyme inactivation, heating and boiling process. HHP treatment on SPLF was done at 200, 400 and 600 MPa for 10, 20 and 30 min at RT using high pressure equipment (HPP. L3, Huataisenmiao Bioengineering Technology Co., Ltd., Tianjin, China) to evaluate the stability of SPLF after HHP treatment comprehensively; meanwhile, industrial level conditions would be much milder, usually no more than 10 min, as such long times are not need to inactivate microorganism (main application of HHP), which might have less effect on SPLF.

Sweet potato leaf flavonoids was prepared at different pH (3.0, 5.0, 7.0, 8.0) which simulated acid, weak acid, neutral and weak alkaline environment. The samples were placed at RT away from light following the method as described by Bradwell *et al.* (2018). SPLF stored under light was placed at laboratory in the presence of natural light, while SPLF stored without light was kept away from light under the same environment (Holzwarth *et al.*, 2013). Also, SPLF was kept at −18 °C and 4 °C in the refrigerator, while other samples were kept at RT in the laboratory away from the light (Touati *et al.*, 2014).

Simulated digestion in vitro of sweet potato leaf flavonoids

Simulated digestion was carried out according to the standard static *in vitro* digestion method described by Minekus *et al.* (2014). The SPLF, 15 mL, at the concentration of 10 mg mL^{-1}, was mixed with 10.5 mL simulated salivary fluid (pH 7.0) and 1.5 mL α-amylase (1500 U mL^{-1}). Then, 75 μL $CaCl_2$ and 2.92 mL distilled water were added, and the pH adjusted to 7.0. The contents were agitated for 2 min at 37 °C. After that, 25 mL oral digestive juice was mixed with 15 mL simulated gastric fluid (pH 3.0) and 3.76 mL pepsin (25 000 U mL^{-1}). Following, 11.8 μL $CaCl_2$ and 1.63 mL distilled water were added. Next, the pH was adjusted to 3.0 and agitated continuously for 2 h at 37 °C. The gastric digestive juice (36.5 mL) was mixed with 20 mL simulated intestinal fluid (pH 7.0), 7.5 mL trypsin (500 U mL^{-1}), 5.5 mL bile salts (10 mg mL^{-1}), 85 μL $CaCl_2$ and 2.78 mL distilled water. Subsequently, the pH was adjusted to 7.0 and agitated continuously for another 2 h at 37 °C. Digestive juice (5 mL) of each step was collected, adjusted to pH 2.0, kept at 4 °C and finished analysing within 1 week.

Determination of total flavonoids, antioxidant activity and individual flavonoid

Total flavonoid content was determined by the method of Lim *et al.* (2016) with some modifications (detailed description given in the Supporting Information). The result was calculated by the standard curve of quercetin in the linear range of 0–0.05 mg mL^{-1}. TFC was expressed in milligrams of quercetin equivalent per g of dry weight (mg QUE^{-1} g^{-1} DW). The retention of TFC was calculated by the equation as follows:

$$R_1(\%) = C_1/C_0 \times 100\% \qquad (1)$$

where R_1 was the retention of TFC in the sample after processing, storage or digestion, C_1 was the TFC in the sample after processing, storage or digestion, and C_0 was the TFC in the initial sample.

The antioxidant activity of SPLF was evaluated by the method of Li *et al.* (2012) with some modifications. Equal volume of ABTS (7.4 mM) and $K_2S_2O_8$ (2.6 mM) were
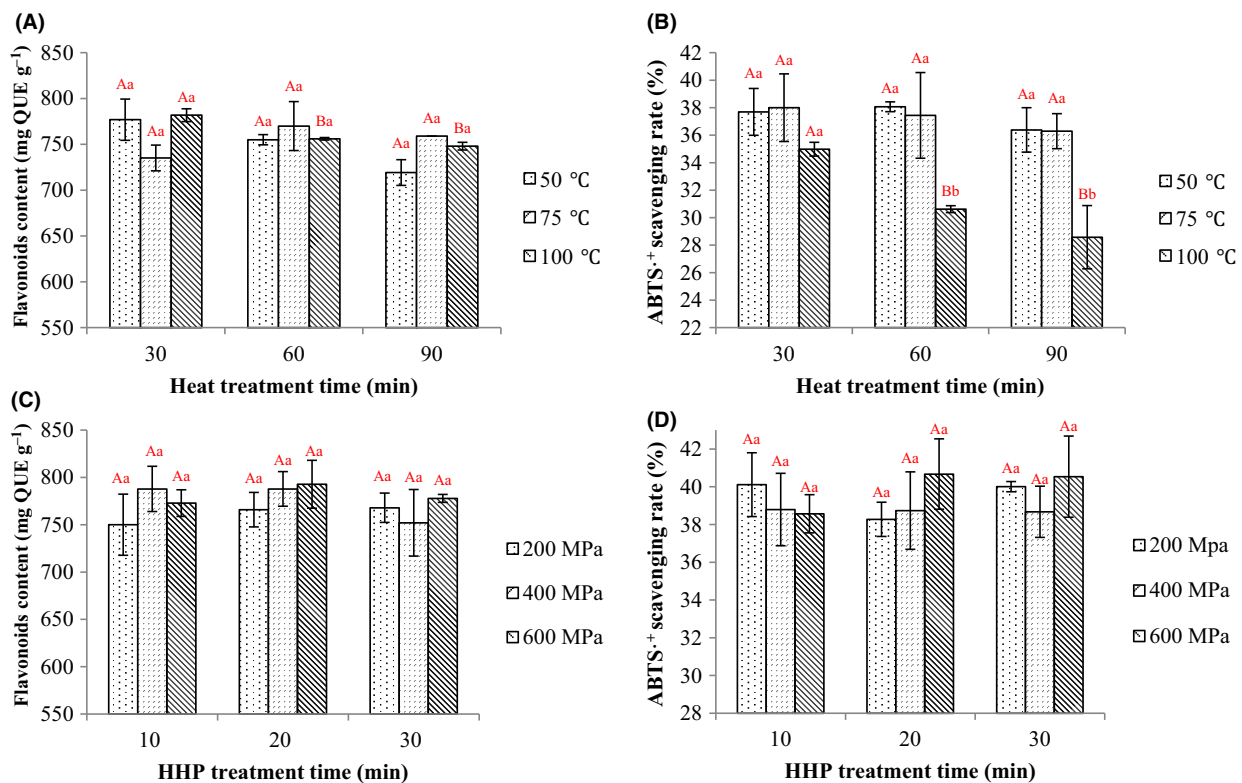
Figure 1 Processing stability of SPLF (A: flavonoids content (mg QUE^{-1} g^{-1}) after heat treatment; B: ABTS$^+$ scavenging rate (%) after heat treatment; C: flavonoids content (mg QUE^{-1} g^{-1}) after HHP treatment; D: ABTS$^+$ scavenging rate (%) after HHP treatment). Different letters (A, B) represent values were significant difference ($P < 0.05$) at the same temperature or pressure and (a, b) represent values were significant difference ($P < 0.05$) at the same treatment time. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1 The content of individual flavonoid (mg/g, DW) after heat treatment

Individual flavonoid	Untreated	50 °C			75 °C			100 °C		
		30 min	60 min	90 min	30 min	60 min	90 min	30 min	60 min	90 min
Myricetrin	6.51 ± 0.02[a]	6.73 ± 0.02[a]	6.64 ± 0.01[a]	6.67 ± 0.03[a]	6.62 ± 0.01[a]	6.65 ± 0.02[a]	6.53 ± 0.03[a]	6.69 ± 0.01[a]	6.68 ± 0.02[a]	6.84 ± 0.02[a]
Isoquercitrin	62.44 ± 0.13[a]	62.85 ± 0.07[a]	62.37 ± 0.14[a]	62.84 ± 0.11[a]	62.90 ± 0.09[a]	62.51 ± 0.22[a]	62.91 ± 0.16[a]	62.90 ± 0.08[a]	62.71 ± 0.12[a]	62.88 ± 0.15[a]
Astragalin	480.40 ± 1.71[a]	478.31 ± 1.93[a]	478.11 ± 2.34[a]	476.72 ± 3.72[a]	477.10 ± 2.55[a]	476.77 ± 3.22[a]	475.25 ± 3.93[a]	474.40 ± 2.75[a]	472.39 ± 2.61[a]	471.35 ± 2.30[a]
Quercitrin	96.07 ± 0.29[a]	95.34 ± 0.27[a]	95.11 ± 0.21[a]	95.64 ± 0.25[a]	95.67 ± 0.20[a]	95.04 ± 0.23[a]	95.19 ± 0.17[a]	94.91 ± 0.22[a]	95.22 ± 0.31[a]	95.30 ± 0.32[a]
Tiliroside	18.83 ± 0.04[a]	18.05 ± 0.03[ab]	17.88 ± 0.05[ab]	16.31 ± 0.06[c]	16.77 ± 0.02[c]	15.75 ± 0.08[cd]	16.07 ± 0.02[cd]	15.21 ± 0.07[d]	15.24 ± 0.03[d]	14.66 ± 0.04[e]
Quercetin	12.64 ± 0.04[a]	12.42 ± 0.04[a]	12.55 ± 0.03[a]	11.71 ± 0.06[ab]	11.56 ± 0.03[b]	11.44 ± 0.06[b]	11.28 ± 0.05[b]	11.46 ± 0.05[b]	11.28 ± 0.03[bc]	11.15 ± 0.03[c]
Kaempferol	6.03 ± 0.02[a]	6.05 ± 0.03[a]	5.91 ± 0.01[a]	5.93 ± 0.02[a]	5.93 ± 0.02[a]	5.97 ± 0.02[a]	6.06 ± 0.03[a]	5.95 ± 0.02[a]	5.98 ± 0.02[a]	5.95 ± 0.03[a]
Rhamnetin	4.31 ± 0.02[a]	4.35 ± 0.02[a]	4.27 ± 0.01[a]	4.33 ± 0.02[a]	4.36 ± 0.02[a]	4.39 ± 0.01[a]	4.33 ± 0.01[a]	4.28 ± 0.02[a]	4.31 ± 0.02[a]	4.35 ± 0.01[a]

Different letters (a–e) meant values were significantly different ($P < 0.05$).

Table 2 The content of individual flavonoid (mg/g, DW) after HHP treatment

Individual flavonoid	Untreated	200 MPa			400 MPa			600 MPa		
		10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
Myricetrin	6.53 ± 0.02[a]	6.60 ± 0.03[a]	6.51 ± 0.03[a]	6.55 ± 0.01[a]	6.64 ± 0.04[a]	6.57 ± 0.03[a]	6.53 ± 0.02[a]	6.59 ± 0.03[a]	6.56 ± 0.03[a]	6.48 ± 0.02[a]
Isoquercitrin	62.41 ± 0.13[a]	62.55 ± 0.17[a]	62.30 ± 0.13[a]	62.53 ± 0.12[a]	61.61 ± 0.13[a]	61.09 ± 0.11[a]	61.72 ± 0.15[a]	61.77 ± 0.18[a]	61.50 ± 0.14[a]	61.47 ± 0.13[a]
Astragalin	480.42 ± 1.75[a]	474.20 ± 3.51[a]	477.72 ± 2.99[a]	479.03 ± 2.44[a]	479.11 ± 2.10[a]	472.30 ± 3.13[a]	479.41 ± 2.57[a]	470.33 ± 2.20[a]	470.56 ± 1.96[a]	475.12 ± 3.37[a]
Quercitrin	96.04 ± 0.29[a]	95.88 ± 0.19[a]	95.65 ± 0.20[a]	96.08 ± 0.16[a]	96.05 ± 0.24[a]	95.57 ± 0.26[a]	95.64 ± 0.22[a]	95.48 ± 0.17[a]	95.63 ± 0.24[a]	95.80 ± 0.21[a]
Tiliroside	18.88 ± 0.04[a]	18.50 ± 0.05[a]	18.04 ± 0.04[a]	18.75 ± 0.03[a]	18.13 ± 0.03[a]	18.26 ± 0.04[a]	18.38 ± 0.06[a]	18.51 ± 0.05[a]	18.73 ± 0.03[a]	18.26 ± 0.04[a]
Quercetin	12.64 ± 0.04[a]	12.43 ± 0.03[a]	12.47 ± 0.01[a]	12.70 ± 0.02[a]	12.66 ± 0.03[a]	12.44 ± 0.02[a]	12.03 ± 0.02[ab]	12.56 ± 0.03[a]	12.35 ± 0.03[a]	12.12 ± 0.01[ab]
Kaempferol	6.03 ± 0.02[a]	6.01 ± 0.02[a]	5.95 ± 0.01[a]	5.97 ± 0.03[a]	5.92 ± 0.02[a]	5.92 ± 0.03[a]	6.00 ± 0.02[a]	5.99 ± 0.03[a]	5.97 ± 0.01[a]	5.94 ± 0.02[a]
Rhamnetin	4.31 ± 0.02[a]	4.33 ± 0.02[a]	4.25 ± 0.01[a]	4.28 ± 0.01[a]	4.20 ± 0.02[a]	4.22 ± 0.02[a]	4.36 ± 0.01[a]	4.29 ± 0.01[a]	4.20 ± 0.01[a]	4.24 ± 0.02[a]

Letters (a, ab) meant values were not significantly different ($P < 0.05$).

mixed thoroughly and kept in the dark for 24 h at 4 °C to produce ABTS•+ and then adjusted the absorbance of ABTS•+ to 0.70 ± 0.02 at 734 nm by distilled water, obtained the working solution. Then, 2.0 mL ABTS•+ working solution reacted with 1.0 mL sample solution in which flavonoids concentration was adjusted to 5 μg mL^{-1}, incubated for 6 min and detected the absorbance at 734 nm, and the retention of antioxidant activity was calculated by the equation as follows:

$$R_2(\%) = A_1/A_0 \times 100\% \qquad (2)$$

where R_2 was the retention of ABTS$^+$ scavenging rate in the sample after processing, storage or digestion, A_1 was the ABTS$^+$ scavenging rate in the sample after processing, storage or digestion, and A_0 was the ABTS$^+$ scavenging rate in the initial sample.

Quantitative analysis of individual flavonoid was performed by HPLC (Shimadzu LC-20A) using the C$_{18}$ column (4.6 × 150 mm, 5 μm, Inertsil ODS-SP, Shimadzu), according to the method of Xi *et al.* (2015) with some modification (detailed description given in the Supporting Information). Standard curves

obtained in our previous studies (Liu *et al.*, 2019) were used to calculate the content of individual flavonoid.

Data analysis

All the experiments were carried out in triplicate, and data were expressed as mean ± SD. Statistic difference among diversely treated samples was calculated through one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The IBM SPSS 24.0 (Chicago, USA) software was used, and statistical difference considered significant when $P < 0.05$.

Results and discussion

Stability of sweet potato leaf flavonoids after heat treatment

As shown in Fig. 1A,B, no significant difference was reported for the flavonoids content and antioxidant activity in SPLF for heat-treated samples at 50 and 75 °C for 30, 60 even 90 min. However, at the
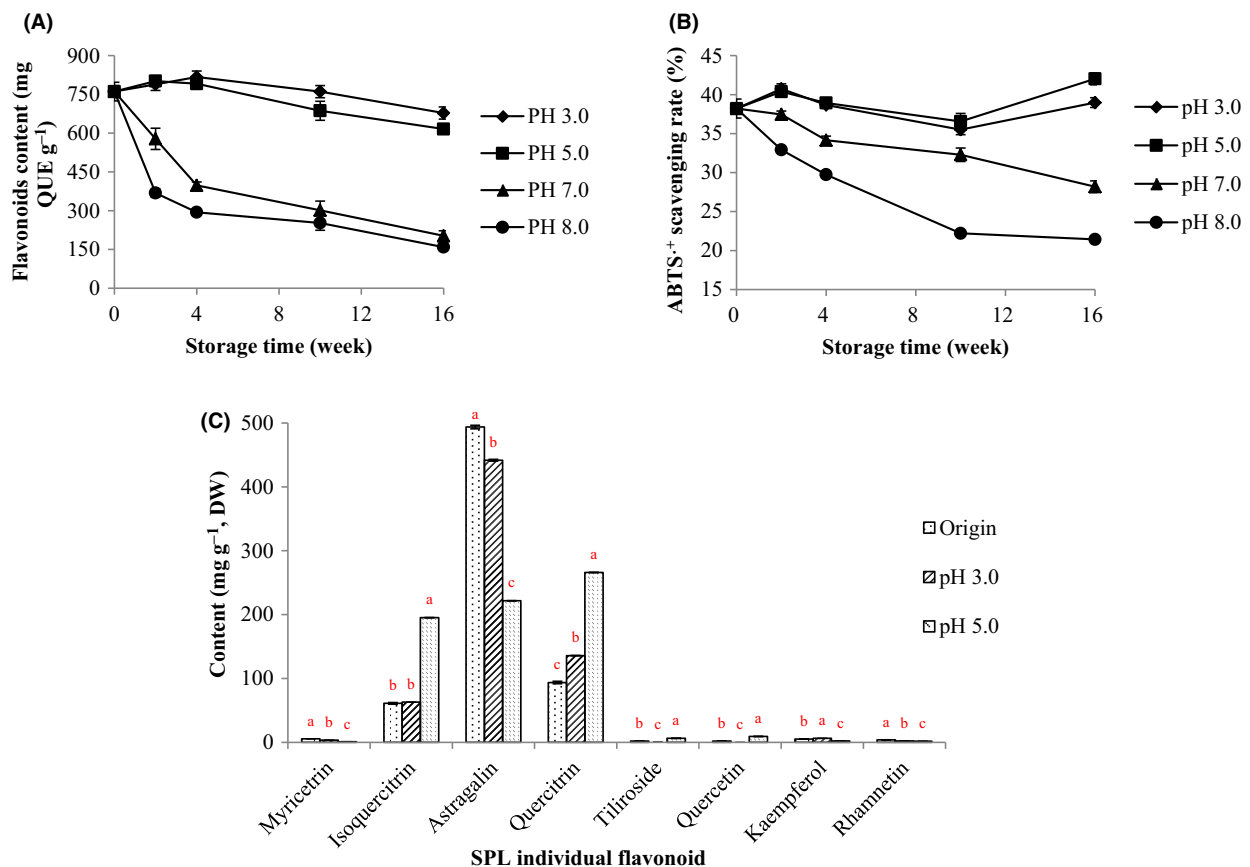
Figure 2 Influence of pH on the storage stability of SPLF (A: flavonoids content during 16 weeks; B: ABTS·+ scavenging rate during 16 weeks; C: the content of individual flavonoid after 16 weeks). Different letters (a-c) represent values were significant difference for the same individual flavonoid stored at different pH ($P < 0.05$). [Colour figure can be viewed at wileyonlinelibrary.com]

temperature of 100 °C for 60 and 90 min, a significant decrease in the flavonoids content and antioxidant activity was observed. The result showed that the heat treatment stability of SPLF was excellent at temperatures not higher than 75 °C.
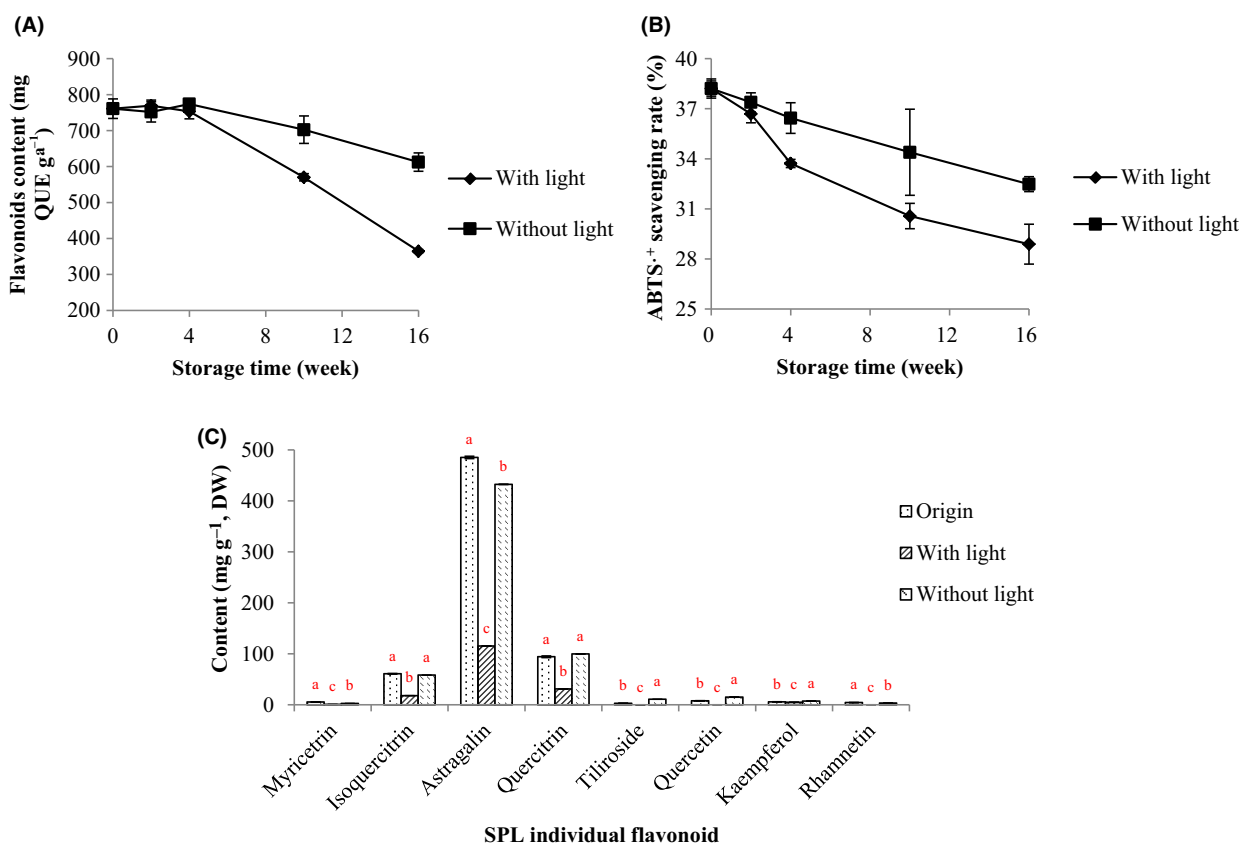
The stability of individual flavonoid under heat treatment is shown in Table 1. It was observed that most of individual flavonoid was stable except tiliroside and quercetin. However, in a different study, the content of myricetin and quercetin in bilberry spreads increased after vacuum-heated at 77 °C or homemade boiling. This could be ascribed to the difference in food structure or food state, such as jams exhibited higher solid content and lower water activity which might resist the heat shock to a certain extent when compared with juices (Može Bornšek *et al.*, 2015).

Stability of sweet potato leaf flavonoids after high hydrostatic pressure treatment

Stability of SPLF at different HHP treatment is shown in Fig. 1C,D. There was no significant difference in flavonoids content or antioxidant activity as affected by HHP treatment. Our results were in good agreement with Patras *et al.* (2009) that the anthocyanin content in strawberry and blackberry had no significant difference after treated at 400, 500 and 600 MPa for 15 min at RT (≈20 °C). At the same time, no significant effect was reported for their antioxidant activity. Not to mention that the intensity of industrial level conditions, which would be much milder (usually <10 min), might have less effect on SPLF.

The stability of individual flavonoid under HHP treatment is shown in Table 2. Results had no significant difference after HHP treatment indicating that the small molecule compounds could endure HHP and their antioxidant activities would be well kept. This result was similar to Jiménez-Aguilar *et al* (2015) who observed that the content of quercetin in prickly pears beverage under 400 MPa for 16 min had no statistical difference compared with nontreated beverage. In the same way, no statistical difference was reported in their study for isorhamnetin.

Figure 3 Influence of light on the storage stability of SPLF (A: flavonoids content during 16 weeks; B: ABTS[+] scavenging rate during 16 weeks; C: the content of individual flavonoid after 16 weeks). Different letters (a-c) represent values were significant difference for the same individual flavonoid stored at different light environments ($P < 0.05$). [Colour figure can be viewed at wileyonlinelibrary.com]

Influence of pH on the stability of sweet potato leaf flavonoids

Storage of SPLF for 16 weeks resulted in decrease tendency at pH 3.0, 5.0, 7.0 and 8.0, especially for samples at pH 7.0 and 8.0. Figure 2A showed that TPC did not decrease significantly at pH 3.0 or 5.0 after 4 weeks of storage. The retention of flavonoids, after 16 weeks of storage, was $89.08 \pm 1.53\%$ (pH 3.0) and $81.95 \pm 0.69\%$ (pH 5.0). In the meantime, the retention of flavonoids was $26.62 \pm 2.56\%$ for pH 7.0 and $20.90 \pm 1.73\%$ for pH 8.0. The results showed that SPLF was quite sensitive to pH. Storage of SPLF in acid environment is encouraged.

The antioxidant activity of SPLF gradually declined in neutral and alkaline environment. As depicted in Fig. 2B, after 16 weeks of storage, retention of antioxidant activity in SPLF fell to $73.77 \pm 5.08\%$ and $56.09 \pm 1.95\%$ at pH 7.0 and 8.0, but increased by 2.45% and 9.73% at pH 3.0 and 5.0, revealing that SPLF was quite stable in acid environment.

As observed in Fig. 2C, isoquercitrin and quercitrin were more stable at pH 5, and tiliroside and quercetin were completely destroyed at pH 3.0 after 16 weeks of storage. It should be pointed out that all individual flavonoids were almost completely destroyed within a week in pH 7.0 or 8.0, especially in pH 8.0, because the structure of flavonoids in alkaline environment underwent the opening of ring C, resulting in two fragments of ring A and ring B being produced.

Influence of light on the stability of sweet potato leaf flavonoids

The retention of flavonoids was stable in first 4 weeks, and there was not significant difference between samples exposed to light or not, then showed a descending tendency, as was observed in Fig. 3A. The difference in flavonoids retention became more pronounced as storage time increased. The retention of flavonoids after 16 weeks of storage was $80.46 \pm 3.35\%$ for samples kept away from light. However, a significant lower value was reported for samples exposed to light ($47.93 \pm 0.60\%$). Our findings clearly showed that SPLF was quite sensitive to light with detrimental effects after long time exposure. In the same way,
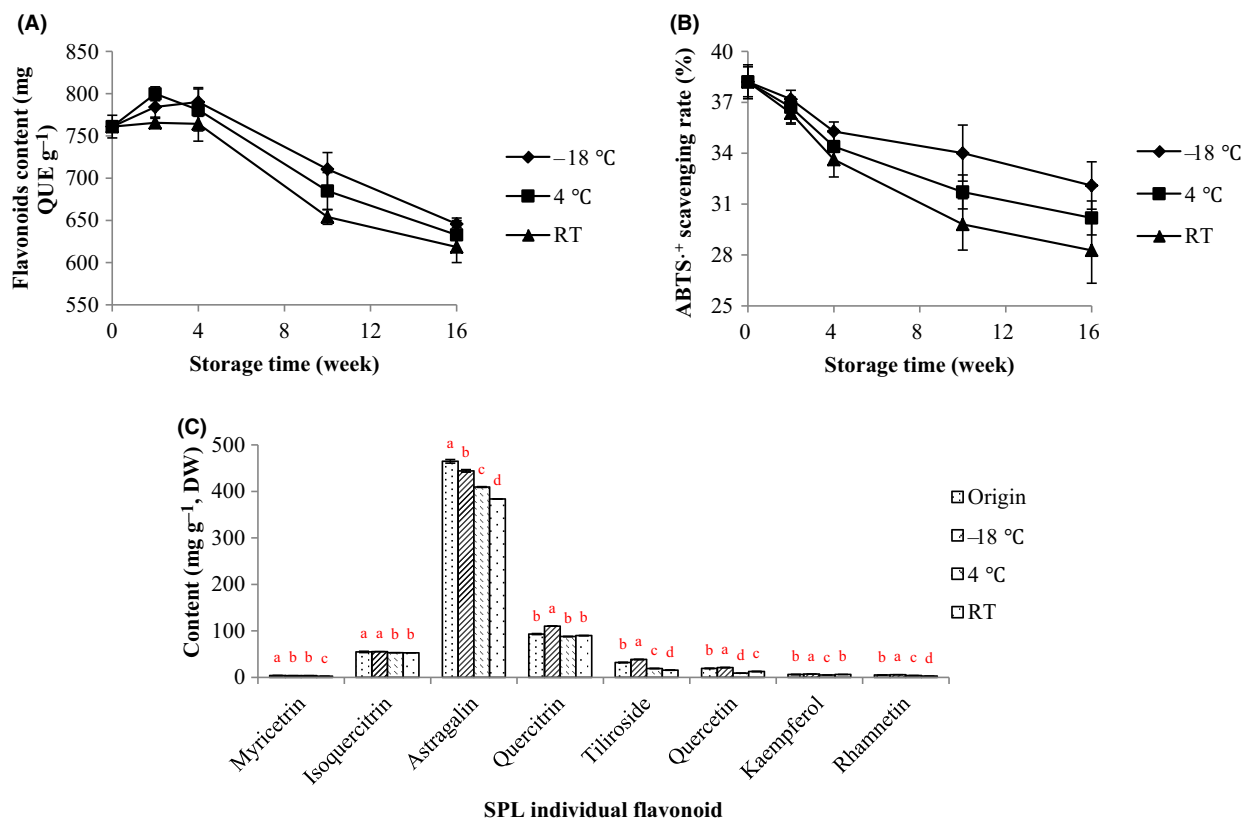
Figure 4 Influence of temperature on the storage stability of SPLF (A: flavonoids content during 16 weeks; B: $ABTS^+$ scavenging rate during 16 weeks; C: the content of individual flavonoid after 16 weeks). Different letters (a-d) represent values were significant difference for the same individual flavonoid stored at different temperature ($P < 0.05$). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 3 The stability of SPLF after simulated digestion

	Oral digestion			Gastric digestion			Intestinal digestion		
	SPLF	GIN	PRO	SPLF	GIN	PRO	SPLF	GIN	PRO
Retention of flavonoids (%)	75.41 ± 2.56[b]	78.01 ± 1.63[b]	88.57 ± 2.57[a]	54.95 ± 2.56[b]	59.12 ± 3.47[b]	73.69 ± 4.82[a]	45.99 ± 3.57[a]	27.54 ± 0.32[b]	8.43 ± 2.27[c]
Retention of antioxidant activity (%)	75.89 ± 5.18[a]	81.57 ± 0.96[a]	75.95 ± 1.94[a]	65.00 ± 4.37[a]	53.62 ± 2.19[b]	46.35 ± 2.43[c]	56.17 ± 2.59[a]	34.46 ± 0.73[b]	28.20 ± 1.46[c]

Different letters (a-c) meant values were significantly different ($p < 0.05$).

Rabadán, Álvarez-Ortí, Pardo & Alvarruiz (2018) reported that total polyphenols content of almond, walnut and pistachio was significantly lower under light storage at RT compared dark storage.

The antioxidant activity of SPLF showed a continuous decreasing tendency. The retention of antioxidant activity after 16 weeks of storage was 75.60 ± 3.13% in SPLF under light storage and 85.24 ± 1.17% under dark storage (Fig. 3B).

Myricetrin, isoquercitrin, astragalin, quercitrin and kaempferol were sensitive to light resulting in extra loss of 36%, 66%, 65%, 72% and 40%, respectively, while tiliroside, quercetin and rhamnetin were completely destroyed, as observed in Fig. 3C. Similar findings were reported by Bakowska *et al.* (2003) on the loss of anthocyanin-3-glucoside stored in the dark 3 months.

Influence of storage temperature on the stability of sweet potato leaf flavonoids

As shown in Fig. 4A, the retention of flavonoids was stable in first 4 weeks and then decreased tendency was observed for the remaining period of storage. The retention of flavonoids dropped to 84.86 ± 0.53%, 83.16 ± 2.62% and 81.28 ± 2.42% for samples stored at −18 °C, 4 °C and RT away from light, respectively. It can be deduced that SPLF was not temperature sensitive with more than 80% TPC preserved as revealed in this study, which was similar to the findings of Bradwell *et al.* (2018) who reported that the TFC in sorghum phenolic extracts had no significant difference at 4 °C and 22 °C during the first 6 weeks of storage. However, a decrease in the flavonoid content was reported after weeks of storage.

The antioxidant activity of SPLF was shown in Fig. 4B. A sharp decrease in the antioxidant activity was observed in first 4 weeks before slowing down for the rest of the time. The decrease in the antioxidant activity could be attributed to increase in temperature with storage time achieving final retentions of 84.23 ± 3.66%, 79.86 ± 0.78% and 74.02 ± 5.08%,

for −18 °C, 4 °C and RT, respectively. Even so, nearly 75% of antioxidant activity could still be preserved after 16 weeks of storage at RT without any refrigeration equipment. Our results were consistent with Tsai *et al.* (2004) who observed that the antioxidant activity in new mulberry wine decreased to 80.94% after a year's storage at 20 °C.

As observed in Fig. 4C, the individual flavonoid showed different stabilities after 16 weeks. Isoquercitrin and quercitrin had no significant difference at 4 °C or RT, and quite unstable when compared with at −18 °C. The stability of astragalin, tiliroside and rhamnetin decreased significantly with increase in temperature. Our findings were similar to Teleszko *et al.* (2016) who illustrated that lower temperature could limit the degradation process generally. On the other hand, Häkkinen *et al.* (2000) observed that storage at −18 °C for 3 to 9 months decreased myricetin and kaempferol more than quercetin.

Retention of sweet potato leaf flavonoids after simulated digestion

Retention of SPLF after simulated digestion is shown in Table 3. Ginkgo biloba extract (GIN) and propolis flavone (PRO) were taken as the control. TFC in SPLF, GIN and PRO was 761.0 ± 31.1, 457.7 ± 33.4 and 568.5 ± 27.8 mg g^{-1}, respectively. After simulated digestion, the retention of flavonoids decreased to 45.9 ± 3.6%, 27.5 ± 0.3% and 8.4 ± 2.3% in SPLF, GIN and PRO, respectively. Our results were similar to Gunathilake *et al.* (2018) who found that the retention of flavonoids in *Centella asiatica*, *Gymnema lactiferum*, *Sesbania grandiflora* and *Passiflora edulis* after gastrointestinal digestion was 55%, 48%, 42% and 40%, respectively. Meanwhile, the retention of antioxidant activity after simulated digestion in SPLF, GIN and PRO was 56.2 ± 2.6%, 34.5 ± 0.7% and 28.2 ± 1.5%, respectively. Our results were supported by Spínola *et al.* (2018) who reported the retention of antioxidant activity in *Rumex maderensis* as 51.8%, 60.0% and 50.7%, in leaves, flowers and stems, respectively. Compared to the

control samples (GIN and PRO), SPLF had higher stability during simulated digestion.

Conclusion

The results of our study showed that the stability of SPLF after heat and HHP treatment was high. More specifically, heat treatment at 75 °C for 90 min or HHP treatment at 600 MPa for 30 min did not cause any significant destruction. The stability of SPLF under acid environment was good (able to preserve 50% of TFC and 35% of antioxidant activity after 16 weeks of storage) compared with neutral or alkaline environment. Likewise, SPLF kept away from light exhibited better retention of TFC (30%) and antioxidant activity (10%) after 16 weeks of storage. No significant difference in SPLF was observed for the samples stored at −18 °C, 4 °C and RT. Interestingly, more than 80% of TFC and 75% of antioxidant activity was preserved under these conditions (−18 °C, 4 °C and RT) after 16 weeks. Overall, the retention of SPLF was high compared to GIN and PRO, after simulated digestion, retaining $45.9 \pm 3.6\%$ of TFC and $56.2 \pm 2.6\%$ of antioxidant activity.

Acknowledgments

Conflict of interest

The authors declare no competing financial interest.

Ethical approval

Ethics approval was not required for this research.

Data availability statement

Research data are not shared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The HPLC chromatography of SPLF (1 mg mL^{-1}) (Peak 1: Myricetrin, retention time (RT) 6.10 min; Peak 2: Isoquercitrin, RT 7.10 min; Peak 3: Astragalin, RT 7.65 min; Peak 4: Quercitrin, RT 8.21 min; Peak 5: Tiliroside, RT 10.62 min; Peak 6: Quercetin, RT 10.97 min; Peak 7: Kaempferol, RT 12.75 min; Peak 8: Rhamnetin, RT 13.88 min).

Figure S2. The HPLC chromatography of individual flavonoid (The concentration of individual flavonoid was 50 μg mL^{-1} except astragalin, the latter was 100 μg mL^{-1}).