

Effect of oven and freeze drying on antioxidant activity, total phenolic and total flavonoid contents of fig (*Ficus carica* L.) leaves

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Abstract

Effect of drying processes on the antioxidant activities, total phenolic (TPC) and total flavonoid (TFC) contents of three fig (*Ficus carica* L.) leaves' cultivars namely Brown Turkey Masuri 6 (BTM 6), Masui Dauphine Jumbo (MD-J) and Taiwan Golden Fish Jumbo (TGF-J) were studied. Oven drying which was conducted at 40°C, 50°C, 60°C and freeze drying at -80°C were run for 48 hrs. Antioxidant activities were evaluated using radical-scavenging capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric-reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) assays. TPC and TFC were evaluated using Folin-Ciocalteu and aluminum chloride assays respectively. It was found that using oven drying at 40°C revealed significantly ($p < 0.05$) the highest antioxidant activities of the three fig cultivars followed by oven drying at 50°C, freeze dried and oven drying at 60°C respectively. Fresh leaves revealed the significantly lowest antioxidant activities for all antioxidant assays and for all fig cultivars. Regardless of the drying process, fig cultivar BTM 6 revealed significantly ($p < 0.05$) the highest antioxidant activities followed by TGF-J and MD-J. Positive correlations between antioxidant, TPC and TFC activities of fig leaves extracts were observed at oven drying at 40°C, oven drying at 60°C and freeze drying but not with oven drying at 50°C and fresh samples. High levels of antioxidant activities were obtained in *F. carica* L. leave samples (all cultivars), indicating that the leaves have potential as a source of natural antioxidants compounds.

1. Introduction

In the past few years, farmers in Malaysia successfully started importing and growing different fig (*Ficus carica* L.) cultivars, where the main products sold are fruits and leaves. Their leaves have been used as a tea and claimed to have medicinal properties. Previous studies of antioxidant activity of fig leaves were conducted (Trifunski and Ardelean, 2013; Ahmad *et al.*, 2013; Allahyari *et al.*, 2014), but no research has been directed on the impact of drying process on their antioxidant, total phenolic (TPC) and total flavonoid (TFC) activities.

The benefits of fig leaves are associated with the secondary metabolites made by these plants. The use of plants as an origin of antioxidants remains substantial for

the reason that they are consumed to heal illnesses (Oliveira *et al.*, 2006; Lim and Murtijaya, 2007; Hossain *et al.*, 2010). Moreover, the secondary metabolites of plant work as antioxidants have been proved to fight cardiovascular diseases, cancer and diabetes (Chan *et al.*, 2009; Shahinuzzaman *et al.*, 2019). The protective properties fighting these illnesses remain possibly implemented by the occurrence of several functional secondary metabolites, like polyphenols, vitamins and minerals (Asami *et al.* 2003; Chang *et al.*, 2006; Roy *et al.*, 2007; Sagrin and Chong, 2013). Polyphenols from various plant sources include an excessive diversity of bioactive compounds, such as flavonoids (anthocyanins, flavonols, flavanols, flavanones) and numerous classes of non-flavonoids such as (phenolic acids, stilbenes and other molecules) (Panche *et al.*, 2016; Tan *et al.*, 2018).

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Drying is mainly a process of water removing and reducing the content of moisture, intended to prevent enzymatic and microbial activities, subsequently protecting the crop for lengthening shelf life and phytochemical effectiveness. Furthermore, it also reduces the volume and weight of the crop with significant positive consequences to improve reduction of final product cost, such as storage and shipping (Calixto, 2000; Chan *et al.*, 2009; Tan *et al.*, 2013).

Nevertheless, to the best of our knowledge, there is no literature on the effect of drying of *Ficus carica* L. leaves on the antioxidant, TPC and TFC. Among the most used drying technique is oven drying which is easy to handle, available and relatively cheap to operate. While freeze drying is not as reachable as oven drying, it is assumed to be more efficient medicinally to other drying methods.

Since antioxidants are delicate to air, heat and light, an appropriate drying process procedure must be optimized for each type of plant's leaves depending on their structure physically and chemically. Therefore, the aim of this study was to evaluate the effectiveness of oven and freeze-drying processes on the TPC, TFC and antioxidant activities of selected fig leaves cultivars.

2. Materials and methods

2.1 Samples collection and preparation

Fig (*F. carica* L.) leave samples were freshly collected from a fig farm (Taman Agro-Fertigasi) located in Kajang, Malaysia. There were three *F. carica* L. leaves' cultivars namely Brown Turkey Masuri 6 (BTM 6), Masui Dauphine Jumbo (MD-J) and Taiwan Golden Fish Jumbo (TGF-J). The leaves' samples were transported back to the food science laboratory in the Faculty of Science and Technology, Universiti Kebangsaan Malaysia. to be processed accordingly. The samples were divided to two groups; the first as fresh and the second were handled with two drying methods: non-thermal drying using a freeze dryer (Labconco, Benchtop Freeze Dry, USA) and thermal drying using an oven at different temperatures (40°C, 50°C and 60°C) (Memmert GmbH + CO. KG, Germany) for 48 hrs, then ground with a food grinder (Philips, China) to produce a fine powder. About 0.1 g each of *F. carica* L. leave samples were weighed and 10 mL aqueous 50% acetone (VWR International, France) was added. All extracted samples were centrifuged using Eppendorf centrifuge (5810 R, Eppendorf, Germany) for 10 mins at 2180 × g and then filtered with 0.22 µm PTFE syringe filter (Osaka Chemical, China).

2.2 Determination of moisture content

Moisture content was determined after 48 hrs of oven drying by Association of Official Analytical Chemists (AOAC) (Association of Official Analytical Chemist, 1990) methods.

2.3 Determination of total phenolic content (TPC)

The determination of TPC was conducted based on Singleton and Rossi (1965) with modification based on the method of Aminah and Permatasari (2013). The calculation of results was based on the equation generated by the gallic acid standard curve. The result was expressed as milligrams of gallic acid equivalents per 100 g of dry sample (mg GAE/100 g DW).

2.4 Determination of total flavonoid content (TFC)

TFC content was determined following the method of Benzie and Strain (1996) with modification based on the method of Bakar *et al.* (2009). The calculation of results was based on the equation generated by quercetin standard curve. Results were expressed as milligrams of quercetin equivalents (QE) per 100 g of dry sample (mg QE/100 g DW).

2.5 Antioxidant assay determination

2.5.1 Radical-scavenging activity (DPPH)

DPPH determination was carried out according to the method of Musa *et al.* (2011). The calculation of results was based on the equation generated by Trolox standard curve. Results were expressed as milligrams of Trolox equivalents per 100 g of dry sample (mg TE/100 g DW).

2.5.2 Radical-scavenging activity (ABTS)

ABTS determination was carried out according to the method of van den Berg *et al.* (1999). The calculation of results was based on the equation generated by Trolox standard curve. Results were expressed as milligrams of Trolox equivalents per 100 g of dry sample (mg TE/100 g DW).

2.5.3 Ferric reducing/ antioxidant power (FRAP)

FRAP determination was carried out according to the method of Benzie and Strain (1999) with modification based on the method of Abdullah Sani *et al.* (2018). The calculation of results was based on the equation generated by Trolox standard curve. Results were expressed as milligrams of Trolox equivalents per 100 g of dry sample (mg TE/100 g DW).

2.5.4 Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC determination was carried out according to the method of Apak *et al.* (2008). The calculation of results was based on the equation generated by Trolox standard curve. Results were expressed as milligrams of Trolox equivalents per 100 g of dry sample (mg TE/100 g DW).

2.6 Statistical analysis

Data were analyzed using MINITAB® (version 17.1.0, USA). One-way ANOVA with Fisher test at $p < 0.05$ was carried out to test significant differences between levels of treatment. Principal component analysis (PCA) was performed using XLstate software (Addinsoft, version 2016.02, France). Pearson's correlation analyses were performed to determine the relationship between antioxidant, TPC and TFC activities.

3. Results

3.1 Moisture content

The moisture content of fig leaves was determined after drying processes were completed. No significant difference ($p < 0.05$) in the moisture content was shown between the three fig cultivars and the values were 81.63%, 82.35%, 83.57% and for fig cultivars MD-J, BTM 6 and TGF-J respectively as in Table 1.

3.2 Effect of drying on the total phenolic content (TPC)

The TPC of the three fig leaves' cultivars were affected by drying processes. As in Table 1, oven drying at 40°C showed the highest TPC for the three cultivars, where BTM 6 revealed significantly ($p < 0.05$) the highest activity (1,125 mg GAE/100 g DW), followed by TGF-J (1,056 mg GAE/100 g DW) and MD-J (993 mg GAE/100 g DW). Significantly there were no differences among freeze drying and oven drying at (50°C and 60°C) results of the three fig cultivars, where the activities were ranging from 591 to 655 mg GAE/100 g DW. In contrast, fresh leaves showed significantly ($p < 0.05$) the lowest TPC and ranged between 187 to 240 mg GAE/100 g DW.

3.3 Effect of drying on the total flavonoid content (TFC)

The TFC of the three fig leaves' cultivars were affected by drying process. As in Table 1, fig cultivar BTM 6 showed significantly ($p < 0.05$) the highest activity (8,277 mg QE/100 g DW) with oven drying at 40°C, followed by fig cultivars TGF-J (7,055 mg QE/100 g DW). There were no significant differences between oven drying of fig cultivar MD-J (4,888 mg QE/100 g DW) at 40°C and oven drying of fig cultivar BTM 6 (4,500 mg QE/100 g DW) at 50°C. There were no significant differences between freeze drying of fig cultivar BTM 6 (3,389 mg QE/100 g DW) and oven drying of fig cultivar TGF-J (4,888 mg QE/100 g DW) at 50°C. There were no significant differences between

Table 1. Effect of drying process on the antioxidant activity of fig leaves by total phenolic content (TPC)^A, total flavonoid content (TFC)^B, radical-scavenging activity (DPPH)^C, ABTS^D, ferric-reducing antioxidant power (FRAP)^E, cupric reducing antioxidant capacity (CUPRAC)^F

Fig cultivar	Drying process	Moisture content	TPC	TFC	DPPH	ABTS	FRAP	CUPRAC
BTM 6	40°C	82.35%	1,125±95 ^a	8,277±385 ^a	1,740±68 ^a	2,917±108 ^a	2,015±36 ^a	3,893±162 ^a
	50°C		645±35 ^{def}	4,500±289 ^c	581±36 ^f	1,848±127 ^c	943±22 ^d	1,984±49 ^d
	60°C		655±27 ^{de}	3,166±289 ^{de}	651±89 ^c	1,865±288 ^c	708±28 ^c	1,800±62 ^c
	Freeze dried		655±12 ^{de}	3,389±347 ^d	704±38 ^{de}	1,737±8 ^{cd}	959±32 ^d	1,954±24 ^d
	Fresh		187±14 ^g	1,388±96 ^g	168±32 ⁱ	526±134 ^f	134±15 ⁱ	434±18 ⁱ
MD-J	40°C	81.63%	993±33 ^c	4,888±255 ^c	1,479±49 ^c	2,292±187 ^b	1,661±26 ^c	3,011±176 ^c
	50°C		666±24 ^d	2,889±255 ^{ef}	674±36 ^{de}	1,523±220 ^{de}	647±31 ^{fg}	1,738±61 ^e
	60°C		605±22 ^{ef}	1,555±192 ^g	502±41 ^g	1,548±105 ^{de}	622±4 ^g	1,417±68 ^g
	Freeze dried		625±15 ^{def}	2,889±254 ^{ef}	663±28 ^{de}	1,703±121 ^{cd}	693±23 ^e	1,724±47 ^e
	Fresh		216±28 ^g	1,222±152 ^g	298±12 ^h	606±265 ^f	241±21 ^h	634±16 ^h
TGF-J	40°C	83.57%	1,056±24 ^b	7,055±536 ^b	1,640±15 ^b	2,362±237 ^b	1,746±32 ^b	3,473±61 ^b
	50°C		613±22 ^{def}	3,389±245 ^d	729±28 ^d	1,484±91 ^{de}	632±9 ^g	1,552±45 ^{fg}
	60°C		626±41 ^{def}	1,666±167 ^g	681±17 ^{de}	1,537±146 ^{de}	699±18 ^e	1,782±145 ^e
	Freeze dried		591±44 ^f	2,610±96 ^f	703±22 ^{de}	1,387±101 ^e	685±10 ^{ef}	1,681±6 ^{ef}
	Fresh		240±22 ^g	444±36 ^h	303±17 ^h	509±133 ^f	272±4 ^h	523±12 ^{hi}

Results showed mean±SD in triplicate. ^{a-i} Values in each column marked by the same letter are not significantly different at $p > 0.05$. ^A Milligrams of gallic acid equivalent (GAE) per 100 g of dry weight (DW). ^B Milligrams of quercetin equivalent (QE) per 100 g of dry weight (DW). ^{C,D,E,F} Milligrams of Trolox equivalent (TE) per 100 g dry weight (DW)

oven drying at 60°C and fresh leaves' samples.

3.4 Effect of drying on the antioxidant activities

3.4.1 Effect of drying on radical-scavenging activity (DPPH)

The radical-scavenging activity by DPPH assay of three fig leaves' cultivars was affected by drying processes (Table 1). The oven drying at 40°C showing the highest activity were fig cultivar BTM 6 revealing significantly ($p < 0.05$) the highest activity (1,740 mg TE/100 g DW), followed by fig cultivars TGF-J (1,641 mg TE/100 g DW) and MD-J (1,479 mg TE/100 g DW) respectively. There were no significant ($p < 0.05$) differences between freeze drying for all the three cultivars tested. Fresh samples revealed significantly ($p < 0.05$) the lowest activity and ranged between 303 to 168 mg TE/100 g DW.

3.4.2 Effect of drying on radical-scavenging activity (ABTS)

The radical-scavenging activity by ABTS assay of three fig leaves' cultivars was affected by the drying process (Table 1). Oven drying at 40°C revealed the highest antioxidant activity was fig cultivar BTM 6 revealed significantly ($p < 0.05$) the highest antioxidant activity (2,917 mg TE/100 g DW) followed by fig cultivars TGF Jumbo (2,362 mg TE/100 g DW) and MD Jumbo (2,293 mg TE/100 g DW).

3.4.3 Effect of drying on ferric reducing/ antioxidant power (FRAP)

The antioxidant activity by FRAP assay of the three fig leaves' cultivars was affected by drying process (Table 1). Fig cultivar BTM 6 showed significantly ($p < 0.05$) the highest activity (2,014 mg/100 g DW) followed by fig cultivars TGF-J (1,746 mg TE/100 g DW) and MD-J (1,661 mg TE/100 g DW) respectively. Fig cultivar BTM 6 showed no significant difference between freeze drying (959 mg TE/100 g DW) and oven drying at 50°C (943 mg TE/100 g DW). There were no significant differences between oven drying at 60°C for both fig cultivars BTM 6 (708 mg TE/100 g DW) and TGF-J (699 mg TE/100 g DW) and freeze drying for both fig cultivars MD-J (693 mg TE/100 g DW) and TGF-J (685 mg TE/100 g DW). Fresh fig leaves for the three fig cultivars showed less significant difference.

3.4.4 Effect of drying on cupric reducing antioxidant capacity (CUPRAC)

The antioxidant activity by CUPRAC assay of the three fig leaves' cultivars was affected by drying processes (Table 1). At 40°C fig cultivar BTM 6 showed significantly ($p < 0.05$) the highest activity (3,893 mg

TE/100 g DW), followed by fig cultivars TGF-J (3,473 mg TE/100 g DW) and MD-J (3,011 mg TE/100 g DW) respectively. Fig cultivar BTM 6 revealed no significant differences between freeze drying (1,984 mg TE/100 g DW) and oven drying at 50°C (1,954 mg TE/100 g DW). There were no significant differences between oven drying at 60°C for both the fig leaves' cultivars BTM 6 (1,800 mg TE/100 g DW) and TGF-J (1,782 mg TE/100 g DW), oven drying for fig cultivar MD-J at 50°C (1,738 mg TE/100 g DW) and freeze drying for both fig leaves' cultivars MD-J (1,724 mg TE/100 g DW) and TGF-J (1,681 mg TE/100 g DW).

3.5 Correlation analysis

The correlations between antioxidant, TPC and TFC activities with regard to the different drying processes were studied (Table 2). Drying fig leaves at 40°C showed high positive correlation between all antioxidant, TPC and TFC assays. DPPH and TFC (1.000) revealed significantly ($p < 0.05$) the highest correlation followed by CUPRAC with TPC (0.999). ABTS with TFC (0.836) revealed the least positive correlation. While drying fig leaves at 50°C revealed a mix of correlations ranging from high negative to high positive correlation. TPC and TFC revealed low negative correlation (-0.187), while DPPH and TPC (-0.480) revealed a medium negative correlation. On the other hand, ABTS, FRAP and CUPRAC with TPC showed low to medium positive correlation ranging from 0.215 to 0.533 respectively. ABTS and FRAP with TFC showed high positive correlation (0.919 and 0.939) respectively. DPPH revealed a strong negative correlation with ABTS, FRAP and CUPRAC ranging from -0.960 to -0.998 respectively. The highest positive correlation ($p < 0.05$) were between ABTS and FRAP assays (0.998). Drying fig leaves at 60°C revealed medium to high positive correlations between TPC, TFC and antioxidant activities in contrary with drying at 50°C, and the FRAP with CUPRAC assays showed significantly ($p < 0.05$) the highest positive correlation (0.999). Drying fig leaves with a freeze dryer showed a mixture of correlations ranging from medium, negative to high positive correlation. TPC with TFC, ABTS, CUPRAC and FRAP revealed high positive correlation ranging from 0.979 to 0.859. While TPC and DPPH (-0.012) revealed a low negative correlation. TFC and DPPH (0.190) revealed low positive correlation while TFC with ABTS, FRAP and CUPRAC revealed high positive correlation ranging from 0.825 to 0.977. DPPH and ABTS (-0.398) revealed a medium negative correlation while DPPH with FRAP and CUPRAC showed medium positive correlation at 0.502 and 0.395 respectively. The highest correlation with freeze drying was showed between FRAP and CUPRAC assays at 0.993. TPC, TFC and antioxidant

Table 2. Pearson's correlation coefficients between TPC, TFC and antioxidant activities^x under influence of different drying process (n = 3)^y

Drying process	Correlation coefficient (r)	TPC	TFC	DPPH	ABTS	FRAP
40°C	TFC	0.983				
	DPPH	0.987	1.000*			
	ABTS	0.922	0.836	0.848		
	FRAP	0.965	0.901	0.911	0.991	
	CUPRAC	0.999*	0.991	0.994	0.901	0.95
50°C	TFC	-0.187				
	DPPH	-0.48	-0.772			
	ABTS	0.215	0.919	-0.96		
	FRAP	0.161	0.939	-0.943	0.998*	
	CUPRAC	0.533	0.731	-0.998*	0.941	0.921
60°C	TFC	0.932				
	DPPH	0.717	0.414			
	ABTS	0.894	0.996	0.329		
	FRAP	0.869	0.629	0.968	0.556	
	CUPRAC	0.841	0.587	0.98	0.51	0.999*
Freeze-dried	TFC	0.979				
	DPPH	-0.012	0.19			
	ABTS	0.922	0.825	-0.398		
	FRAP	0.859	0.945	0.502	0.594	
	CUPRAC	0.914	0.977	0.395	0.685	0.993
Fresh	TFC	-0.909				
	DPPH	0.914	-0.661			
	ABTS	-0.253	-0.173	-0.624		
	FRAP	0.974	-0.79	0.982	-0.467	
	CUPRAC	0.507	-0.102	0.814	-0.962	0.69

^x Total phenolic content (TPC), total flavonoid content (TFC), radical-scavenging activity (DPPH), radical-scavenging activity (ABTS), ferric-reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC), ^y Replication, * Significant level at p<0.05.

activities of fresh fig leaves showed negative correlations except for TPC with DPPH (0.914), FRAP (0.974) and CUPRAC (0.507), DPPH with FRAP (0.982) and CUPRAC (0.814) and FRAP with CUPRAC (0.690). Negative correlations were shown between TPC with TFC (-0.909) and ABTS with CUPRAC (-0.962).

3.6 Principal component analysis (PCA)

The PCA of fig leaves achieved by various drying process is shown in Figure 1. Fig leaves' cultivars showed the same trend. For fig cultivar BTM 6 leaves, the PC1 vs. PC2 biplot accounted for 99.21% of the total variance (PC1 = 97.46%, PC2 = 1.75%). According to Figure 1 (A), fresh leaves grouped on the left of the graph, freeze dried and oven dried leaves at 50°C and 60°C were grouped in the middle of the graph. While oven drying at 40°C clustered on the right side of this figure. This distribution revealed that different drying processes present significant differences (p<0.05) in the antioxidant, TPC and TFC activities. Oven drying at 40°C grouped together with antioxidant, TPC and TFC assays. Based on grouping, TPC and ABTS were formed

into one group, similarly, with TFC, DPPH and FRAP, while CUPRAC stands alone. As for fig cultivar TGF-J leaves, the PC1 vs PC2 biplot accounted for 94.75% of total variance (PC1 = 94.74%, PC2 = 3.99%) (Figure 1 (B)) and for fig cultivar MD-J leaves, the PC1 vs. PC2 biplot accounted for 97.09% of total variance (PC1 = 97.09%, PC2 = 2.01%) (Figure 1 (C)).

4. Discussion

The stabilization of plant leaves by drying comprises fluctuation in the plant matter which could alter the combinations of chemical structure in the dry matter and the extractability (Mediani *et al.*, 2014; Pham *et al.*, 2015). Hence, in this study the antioxidant, TPC and TFC activities of fig leaves dried with various drying processes were assessed. Higher oven drying temperature showed reduction in the activities. Moreover, the reduction of antioxidant levels in the fig leaves correlated with the TPC and TFC activities (Table 2), an indication that the reduction of antioxidant activities in fig leaves with oven drying at 50°C and

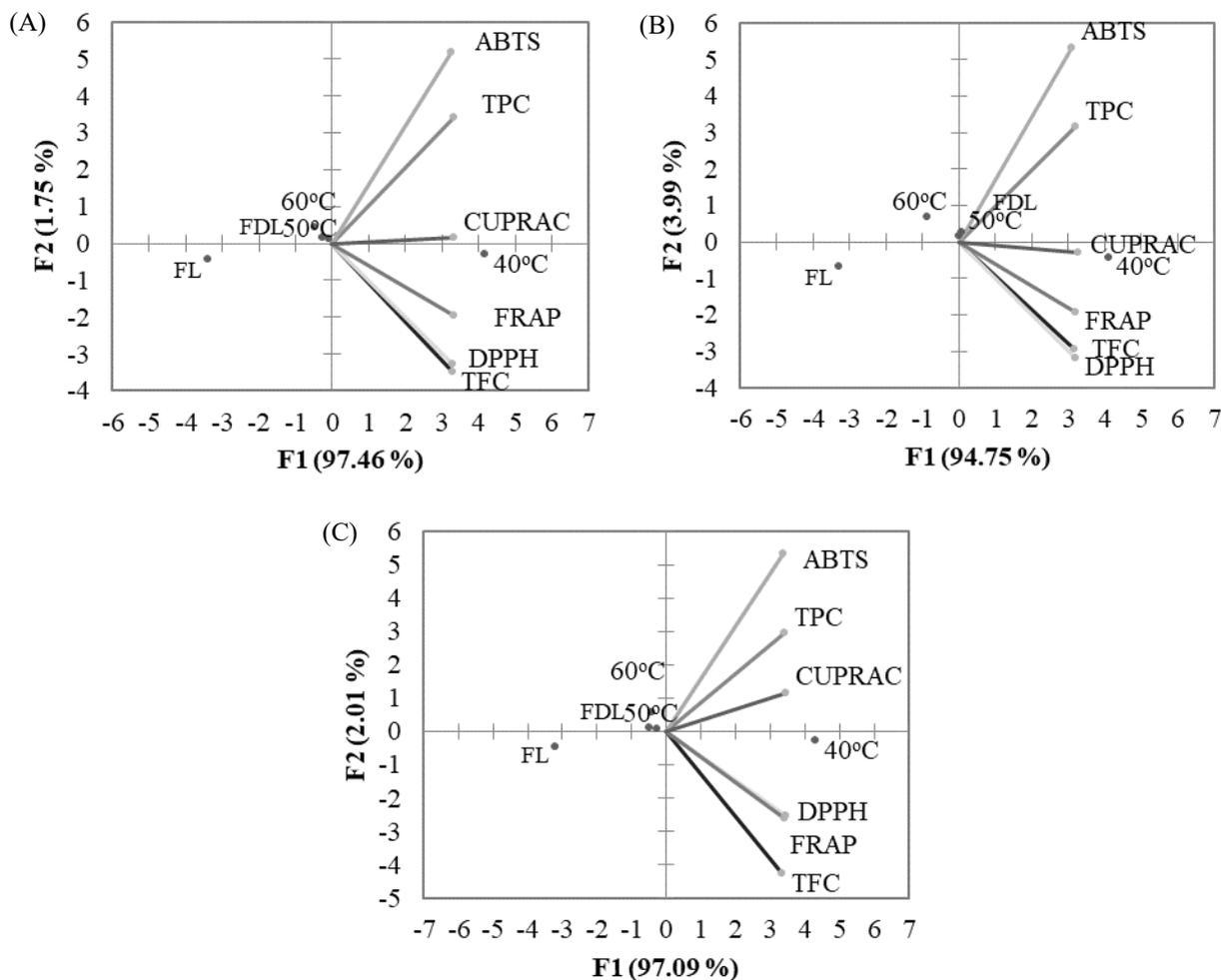


Figure 1. Principal component analysis on the effect of drying process of three fig (*Ficus carica* L.) leave cultivars (A) Brown Turkey Masuri 6 (BTM 6), (B) Masui Dauphine Jumbo (MD-J) and (C) Taiwan Golden Fish Jumbo (TGF-J) based on antioxidant activities (DPPH, ABTS, FRAP and CUPRAC), total phenolic (TPC) and total flavonoid (TFC) contents
FL: Fresh leaves; FDL: freeze dried leaves. F1 = Factor 1; F2 = Factor 2

above are due to deterioration of the phenolic and flavonoid compounds. Consequently, drying process is crucial in the production of fig leaves which preserve their antioxidant activities and levels of polyphenolic compounds. Larrauri *et al.* (1998) suggested that thermal degradation was the main reason of significant decline of antioxidant activity with drying at high temperatures up to 100°C, which agrees with the current findings. Katsube *et al.* (2003) for the purpose of drying of mulberry leaves, temperatures between 40 – 110°C were used. Wiriya *et al.* (2009) dried chilies at temperatures between 50 – 70°C and Rodríguez *et al.* (2016) dried *Aristotelia* berries at 40 – 80°C. According to the above-mentioned references, when drying temperatures used lower than 60°C, this involves a lengthier drying period especially with air drying, causing a reduction in phenolic content in the presence of oxygen which causes oxidation. While using temperatures at 60°C and above lower the phenolic content because of thermal degradation. Comparing other researchers' results with the current findings using lower temperature at 40°C for 48 hrs in the oven confirmed that the drying method and temperature is chosen were crucial and should be studied

for each plant types and parts.

It suggests that the drying process affects the antioxidant activities, TPC and TFC. Thermal drying at 40°C showed the highest correlation among TPC, TFC and antioxidant activities, while thermal drying at 50°C showed the least positive or negative correlations. Thermal drying at 60°C and freeze drying showed a similar trend, while fresh fig leaves showed mostly negative correlations especially between TPC and TFC.

The strong negative relationship proposed that different drying processes might affect the behaviour of the antioxidant activities, TPC and TPC. The negative correlation found between ABTS and DPPH has been reported previously, revealing that some of the secondary metabolites with ABTS activity may not show radical scavenging capacity by DPPH assay (Wang *et al.*, 1998; Thoo *et al.*, 2010).

5. Conclusion

It was found that oven drying at 40°C showed the highest antioxidant activities, TPC and TFC. In order to

improve the drying processes, the temperature of drying has to be as low as possible without reducing the fig product quality.

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