

Effect of Drying and *in vitro* Gastrointestinal Digestion on Antioxidant Activities of Orange and Purple Fleshed Sweet Potatoes

Rungarun Sasanatayart^{1,*}, Titikan Liangpanth¹ and Fessehaye Hdremariam Desale²

Abstract

Colored sweet potato flesh is especially rich in bioactive compounds that exert antioxidant properties. Drying was applied to reduce moisture content of sweet potatoes for a safe storage. However, little is known how their antioxidant activity changes after drying and gastrointestinal digestion. In this research, orange (OFSP) and purple (PFSP) fleshed sweet potato were peeled, sliced and subsequently dried at three different conditions including 55 °C for 16 h, 70 °C for 10 h and 85 °C for 8 h to reduce moisture content below 8% (wb). Bioactive compounds in terms of total phenolic content (TPC), total carotenoid content (TCC), total anthocyanin content (TAC) and their associated antioxidant activities by Ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays were analyzed. In addition, the digesta obtained in each phase (oral, gastric and intestinal) of gastrointestinal digestion were used to determine all antioxidant activities, accordingly. Results showed drying at 70 and 85 °C tended to give negative effects since TPC and TCC of OFSP and TPC and TAC of PFSP decreased to a significant extent ($p < 0.05$) compared to those of freeze dried samples. The exception was for PFSP dried at 55 °C that showed the increased TPC and TAC corresponding to the highest antioxidant activities either by FRAP and DPPH. Based on *in vitro* gastrointestinal digestion, a slight increase or decrease in TPC, FRAP and DPPH of both sweet potato varieties was observed during gastric and intestinal phases. Results suggested that low drying temperature at 55 °C was recommended for both OFSP and PFSP since it provided high antioxidant properties which were stable through gastrointestinal digestion.

Keywords: Sweet potatoes, Hot air drying, Antioxidant activities, *in vitro* gastrointestinal digestion

¹ Program of Postharvest Technology, School of Agro-industry, Mae Fah Luang University, Thasud, Chaing Rai, Thailand

² Department of Agricultural Engineering at Hamelmalo Agricultural College, Asmara, Eritrea

* Corresponding author(s), e-mail: rungarun.s@mfu.ac.th

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1. Introduction

The presence of high moisture of 50–81% in fresh roots of sweet potato (*Ipomea batatas*) limits its storage and usage for longer period of time (FAO, 1998). To reduce losses and extend their utilization, fresh sweet potato could be dried and converted into flour (Chakraverty, 2004). Drying is based on the removal of moisture from the fresh produce which is susceptible to microbial growth (a_w of 0.9 to 1.0) to a safe a_w near 0.6 (Oliveira *et al.*, 2015). However, the qualities of food stuff are also altered by hot air drying. Among drying, freeze drying best retains food qualities in terms of appearance, color, texture, taste and nutritional qualities (Jeng *et al.*, 2015).

Sweet potatoes are rich sources of functional components and the major active compounds are phenolics and other color-related phytonutrients such as carotenoid (Tang *et al.*, 2015). The type combination and amount of pigments vary to produce skin and flesh of various colors, depending on the cultivar. Purple fleshed sweet potato contains anthocyanins, contributing to blue, red and purple colors. Orange fleshed sweet potato contains high amount of β -carotene which is a precursor of vitamin A. (Tang *et al.*, 2015; Ruttarattanamongkol *et al.*, 2016). They are effective natural food colorant and also good in radical scavenging and beneficial health effects (Nedunchezhiyan and Ray, 2010).

Many research reported that drying affected amount of bioactive compounds and their corresponding antioxidant properties of sweet potato. Drying significantly enhanced anthocyanin contents (Ruttarattanamongkol *et al.*, 2016) but decreased β -carotene content (Ruttarattanamongkol *et al.*, 2016, Yang *et al.*, 2010). However, health effects of bioactive compounds depend not only the amount consumed but also their bioavailability through human digestion.

In vitro digestion models are widely used to study the structural changes, digestibility, and release of food components under simulated gastrointestinal conditions, consisting with oral, gastric and small intestinal phases (Hur *et al.*, 2010, Minekus *et al.*, 2014). Many research reported on changes of bioactive compounds and antioxidant during *in vitro* digestion of various fruits and vegetables (Chen *et al.*, 2014, Tagliazucchi *et al.*, 2010, Grijalva *et al.*, 2017). Bioactive compounds and antioxidant activities of food could be either decrease (Bouayed *et al.*, 2011) or increase (Chen *et al.*, 2014, Tagliazucchi *et al.*, 2010). Changes in antioxidant activity during digestion were correlated to the type and concentration of bioactive compounds as well as to the pH.

There has been a rapid increase in reporting of data on the bioactive compounds and antioxidant capacity of sweet potato (Ahmed *et al.*, 2010, Ruttarattanamongkol *et al.*, 2016, Yang *et al.*, 2010). However, studies detailing the stability of antioxidant properties after *in-vitro*

digestion in sweet potato are limited. To provide physiological relevant data, this study investigates antioxidant properties of two varieties of sweet potato, orange fleshed (OFSP) and purple fleshed (PFSP) dried at varying temperatures before and during *in vitro* gastrointestinal digestion.

2. Materials and Methods

2.1 Materials

Two varieties of sweet potato roots, orange fleshed (OFSP) and purple fleshed (PFSP) sweet potatoes were washed with tap water, peeled, and subsequently sliced into uniform 3mm thickness and dipped in to a solution of 0.4 g/L potassium metabisulfite for 2 min to prevent enzymatic browning. After draining, sweet potato slices were spread on perforated trays to obtain single layer samples and dried using hot air dryer at three different conditions; (1) 55 °C for 16 h (2) 70 °C for 10 h and (3) 85 °C for 8 h to final moisture content less than 8% (w/w). Fresh sweet potato was freeze dried and served as control. All samples were ground and sieved through a 80-mesh screen to obtain fine powder.

2.2 Phenolic extraction and determination

Based on method of Tang *et al.*, (2015) with slight modification, pumpkin powder was soaked in acidic 70% acetone (70:29.5:0.5 acetone:water:acetic acid v/v/v) and continuously shaken for 3 h and left in the dark for 12 h. The mixture was centrifuged at 3000x g for 10 min and supernatant was collected for the assay of total phenolic content (TPC) and antioxidant activities by Ferric reducing antioxidant power assay (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. TPC was determined using the Folin–Ciocalteu method. One ml of diluted sample extract was transferred into tubes containing 5.0 mL of 10% v/v Folin-Ciocalteu's reagent. After that, 4.0 mL of 7.5% w/v sodium carbonate solution was added. TPC was determined spectrophotometrically at 765 nm and expressed as mg Gallic acid equivalents (GAE) per 100 g of dry samples.

2.3 Carotenoid extraction and determination

Sweet potato powder was soaked in 95% hexane (95:5 of hexane:ether v/v), mixed for 5 min using vortex mixer and centrifuged at 3000x g for 10 min. The supernatant from 2 times extraction was collected for the assay of the carotenoids. Total carotenoids content (TCC) was determined spectrophotometrically at 454 nm and expressed as mg β -carotene equivalents (β CE) per 100 g of dry samples (Desobry *et al.*, 1997).

2.4 Anthocyanin extraction and determination

Sweet potato powder was soaked in mixed solvent containing 85:15 of ethanol:HCl, centrifuged at 3000x g for 5 min and supernatant was collected for the assay of the carotenoids. Total anthocyanin content (TAC) was determined spectrophotometrically at 520 and 700 nm and expressed as mg cyaniding-3-glucoside (cy-3-glu) equivalents (mgCyE) per 100 g of dry samples (Ruttarattanamongkol *et al.*, 2015).

2.5 Ferric reducing antioxidant power assay (FRAP)

The method of Benzie and Strain (1999) was used to determine Ferric reducing power of samples. The FRAP reagent was prepared freshly by mixing 300mM acetate buffer (pH 3.6), 40 mM HCl, 10 mM 2,4,6 tripyridyl-s-triazine (TPTZ) and 20mM FeCl₃ in the ratio 10:1:1 respectively. Exact amount of 400µl sample extract was then mixed with 2.6ml FRAP reagent solution, incubated at 37 °C for 30 min and absorbance was measured at 595nm against blank. A calibration standard curve of FeSO₄7H₂O solution was used to calculate the FRAP value as µmole Ferrous sulfate per 100 g dry sample.

2.6 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH free radical scavenging activity was determined following method of Molyneux (2004) with some modifications. Sample extract (50 µL) was mixed with 1950 µL of freshly prepared DPPH 60 mM solution (prepared by dissolving 0.00236 g DPPH in methanol to a total volume of 100 mL). Sample was stored in dark place for 30 min and absorbance was measured at 517 nm against blank. The results were expressed as the equivalent content of Trolox (µmole TE/100 g dry sample).

2.7 Simulated *In-vitro* gastrointestinal digestion

In vitro digestibility was determined according to method of Sopade and Gidley (2009) with some modifications. For 0 digestion time (oral phase), 0.25 g sample were mixed with 1 mL artificial saliva (porcine pancreatin α - amylase 250 U/mL (Sigma-Aldrich, St Louis, USA) while, pH was maintained approximately 6.0. Sample was continuous digested in two phases of digestion, gastric and intestinal phase under controlled temperature of 37 °C in reciprocating water bath with a shake speed of 200 strokes/min. In gastric digestion, simulated gastric fluid containing 5ml pepsin (from porcine gastric mucosa, 250 U/mg, Sigma-Aldrich, St Louis, USA) in 0.02 M HCl was added and maintained pH 2.00 $\pm$ 0.01. Digesta was collected at 30 mins representing gastric phase and added into tubes containing 3 ml ethanol to inactivate enzyme activities. After 30 mins of digestion, intestinal digestion stage was started by neutralizing pH with 5 mL of 0.02 M NaOH followed by adding 5 mL simulated intestinal fluid containing pancreatin (from porcine pancreas 4USP, Sigma-Aldrich, and amylogucosidase (from *Aspergillus Niger*, 3300 units/mL, Megazyme,) and maintained pH 6.80 $\pm$ 0.01. Digesta

collected at 120 min represented intestinal phase. All digesta collected were then analyzed for TPC and antioxidant activities following 2.2, 2.4 and 2.5.

2.7 Statistical analysis

SPSS for windows version 20 (IBM SPSS statistics) was used to analyze one way statistical analysis of variance (ANOVA). The comparison of means was done using Duncan method at 95% level of confidence. Pearson correlation coefficient was used to study the relationship among different properties.

3. Results and Discussion

3.1 Varietal difference in TPC, TCC, TAC and antioxidant activity of sweet potato flesh

In this study, total phenolic content (TPC) and total anthocyanin (TAC) which was subgroup of TPC, and total carotenoid content (TCC) were investigated. Phenolic substances, the organic compounds with a presence of at least one aromatic ring hydroxyl-substituted are secondary metabolites of plants, with various functions, such as protection against pathogens and predators, mechanical support, attraction of pollinating animals, and prevention of ultraviolet radiation (Nedunchezhiyan and Ray, 2010). The total phenolic substances in the potatoes in current study were determined calorimetrically using Folin–Ciocalteu phenol reagent (Tang *et al.*, 2015). The amount of anthocyanin (TAC) present in purple fleshed sweet potato (PFSP) was determined by measuring the color change in absorbance at two different pH values, based on a reversible structural transformation of anthocyanins as a function of pH (Ruttarattanamongkol *et al.*, 2015). Total carotenoids content (TCC) present in orange fleshed sweet potato (OFSP) is based on a C40 tetraterpenoid structure with a centrally located, extended conjugated double-bond system (Murkovic *et al.*, 2002).

TPC, TCC and TAC of freeze dried samples (control) are shown in Table 1. A slightly higher TPC of OFSP (362.76 mgGAE/100 g db) than PFSP (330.91 mgGAE/100 g db) was observed. TCC measured in OFSP was 79.99 mg β CE/100 g db and TAC, a subgroup of TPC measured in PFSP was 8.04 mgCyE/100 g db. Tang *et al.* (2015) compared phenolics, carotenoids and antioxidative capacities among sweet potatoes of five colored varieties; white, yellow, orange, light purple and deep purple. The higher anthocyanin contents and antioxidant capacities were detected in purple sweet potato species, while higher carotenoid contents were detected in yellow and orange ones. The amount of TPC, TCC and TAC of this study were comparable to those reported in literature.

Table 1. Bioactive compounds and antioxidant properties of sweet potato flour obtained from different drying temperature

Drying Temperatures	TPC (mgGAE/100g db)	TCC ($\mu\text{g } \beta\text{-carotene}/100\text{g db}$)	TAC (mgCyE/100g db)	FRAP (mmolFeSO ₄ /100g db)	DPPH ($\mu\text{molTE}/100\text{g db}$)
Freeze dried OFSP	362.76 $\pm$ 2.58 ^b	-	7998.53 $\pm$ 167.71 ^a	4269.03 $\pm$ 103.58 ^d	6423.43 $\pm$ 22.47 ^{cd}
55 °C	315.03 $\pm$ 0.67 ^{cd}	-	4576.57 $\pm$ 116.63 ^b	3821.09 $\pm$ 21.89 ^e	6372.64 $\pm$ 81.00 ^{cd}
70 °C	301.50 $\pm$ 0.22 ^d	-	1387.45 $\pm$ 87.95 ^c	3989.75 $\pm$ 156.06 ^{de}	6201.98 $\pm$ 134.05 ^d
85 °C	293.46 $\pm$ 16.91 ^d	-	1024.77 $\pm$ 49.64 ^d	3756.96 $\pm$ 110.95 ^e	6193.86 $\pm$ 38.24 ^d
Freeze dried PFSP	330.91 $\pm$ 21.69 ^c	8.04 $\pm$ 0.11 ^b	-	4977.28 $\pm$ 150.50 ^c	6468.75 $\pm$ 145.81 ^c
55 °C	408.90 $\pm$ 8.68 ^a	16.89 $\pm$ 0.26 ^a	-	8810.41 $\pm$ 76.36 ^a	7467.86 $\pm$ 103.59 ^a
70 °C	306.63 $\pm$ 7.59 ^{cd}	6.04 $\pm$ 0.95 ^c	-	5057.55 $\pm$ 55.25 ^c	6819.28 $\pm$ 27.77 ^b
85 °C	320.80 $\pm$ 11.90 ^{cd}	4.93 $\pm$ 0.15 ^c	-	5575.21 $\pm$ 198.55 ^b	6788.98 $\pm$ 150.06 ^b

Note: Values were expressed as mean $\pm$ standard deviation.

TPC, TCC and TAC represent total phenolic content, total carotenoid content and total anthocyanin content respectively.

In a column, values with different subscripts were significantly different ($p < 0.05$).

Phenolic compounds contributed largely to radical scavenging performance. As the predominant pigment and functional phenolics in PFSP, anthocyanins are the naturally strong free-radical scavengers. In OFSP, carotenoids have strong antioxidant capacity to scavenge free radicals because of their conjugated double bonds. These antioxidant activities were measured by two methods in this study. Based on Ferric reducing antioxidant power (FRAP) assay, with the presence of reductant or antioxidants under low pH, ferric tripyridyltriazine (Fe (III)-TPTZ) is reduced to the ferrous tripyridyltriazine (Fe (II)-TPTZ) that has an intensive blue color detected at the wavelength of 593 nm (Benzie and Strain, 1999). The anti-oxidative efficiency of components was also indicated by the degree of discoloration of the violet solution containing 2,2-diphenyl-1-picrylhydrazyl (DPPH) which is a free radical (Molyneux, 2004). Results showed that the associated antioxidant activity by FRAP and DPPH were high in PFSP compared to OFSP (Table 1). The respective values of FRAP in PFSP and OFSP were 4,977.28 and 4,269.03 mmol FeSO₄/100 g db. That of DPPH in PFSP and OFSP were 6,423.43 and 6,468.75 μ mole TE/100 g db, respectively.

3.2 Effect of drying on TPC, TCC, TAC and antioxidant activity of sweet potato flesh

Overall, drying showed negative effects on bioactive compound and antioxidant activities of OFSP and PFSP with the exception of drying PFSP at 55 °C (Table 1). Considering the corresponding value of TPC of freeze dried OFSP as reference (362.76 mgGAE/100 g db), after drying at different drying temperatures (55, 70 and 85 °C), TPC tended to decrease approximate 13–19%. It was expected for the greater reduction in TPC with increasing drying temperatures. However, there was no significant difference among drying temperatures ($p > 0.05$). In PFSP, TPC apparently increased from 330.91mgGAE/100 g db to the greatest value of 408.9 mgGAE/100g db (approx. 24%) after drying at 55 °C. Similarly to TPC, TAC which was the sub-category of TPC in PFSP increased substantially after drying at 55 °C (16.89 mgCyE/100 g db) which was about two times of freeze dried samples (8.04 mgCyE/100 g db). However, a significant reduction of TPC and TAC upon drying at 70 and 85 °C was observed around 3–7% and 25–39% of reference values.

The increase in the amount of TPC and TAC as a result of drying can be explained by breakdown of cell wall and release of bounded phenolic compounds (Gumusay *et al.*, 2015). This also contributed to the higher extractability of other bioactive compounds from plant samples (Kamiloglu *et al.*, 2015). Similar results of increased phenolic content during hot air drying of sweet potatoes compared to freeze dried samples were also reported by Que *et al.* (2008) and Yang *et al.* (2010).

The greater losses of TCC over TPC and TAC were observed in OFSP after drying (Table 1). TCC steeply decreased ($p < 0.05$) with increasing drying temperatures of 55 °C (43%), 70 °C (83%) and 85 °C (87%). During drying, loss of carotenoid content may occur by oxidation process (Bechoff *et al.*, 2009). Loss of TCC after heat treatment has been also reported elsewhere (Ahmed *et al.*, 2010, Ruttarattanamongkol *et al.*, 2015; Tang *et al.*, 2015).

Antioxidant capacities of sweet potato extracts as affected by drying temperatures are also presented in Table 1. Overall, associated FRAP and DPPH values changed in regarding to the amount of bioactive compounds in samples. The reduction in antioxidant activity as a result of drying is related to the degradation of biologically active compounds at high temperatures, due to chemical, enzymatic or thermal decomposition (Nicoli *et al.*, 1999). The positive correlations between TPC of all samples and their associated FRAP ($r = 0.795$, $p < 0.01$) and DPPH ($r = 0.733$, $p < 0.01$) were observe (Table 2). In OFSP, the apparent reduction in FRAP (approx. 6–12%) and DPPH (approx. 1–4%) decreased as a result of decreasing TPC and TCC. A significant correlations between TCC and FRAP ($r = 0.704$, $p < 0.05$) and between TCC and DPPH ($r = 0.837$, $p < 0.01$) were observed. The less contribution of carotenoid than phenolic compounds to antioxidant activities was also reported by Yang *et al.* (2010).

Table 2. Pearson correlation coefficients of bioactive compounds and antioxidant activity before *in-vitro* gastrointestinal digestion

	TPC	TCC (OFSP)	TAC (PFSP)	FRAP	DPPH
TPC	1				
TCC (OFSP)	NA	1			
TAC (PFSP)	NA	NA	1		
FRAP	0.795**	0.704*	0.929**	1	
DPPH	0.733**	0.837**	0.801*	0.950**	1

Note: *Correlation was significant at $p < 0.05$, **Correlation was significant at $p < 0.01$, NA = no analysis

In PFSP dried at 55 °C, as expected, antioxidant capacities increased corresponding to the increase in TPC and TAC. FRAP value increased substantially to 77% whereas, DPPH increased to 15.45%. It has been suggested that the high antioxidant activity in fruits and vegetables after drying might be related to the fact that partially oxidized polyphenols have greater antioxidant activity than nonoxidized polyphenols (Nora *et al.*, 2014). In addition, an increase in antioxidant capacity after drying may be related to the Maillard reaction products,

which can be formed as a consequence of heat treatment, which generally exhibit strong antioxidant properties (Kamiloglu and Capanoglu, 2014).

However, the reduction in TPC and TAC in PFSP samples dried at 70 and 85 °C resulted in a reduction in antioxidant activities. The apparent reduction in FRAP (approx. 2–12%) and DPPH (approx. 5%) was observed. In PFSP, TAC correlated well with FRAP ($r = 0.929$, $p < 0.01$) and DPPH ($r = 0.801$, $p < 0.01$). Results indicated that the changes in antioxidant activities as a result of drying were due to the retention of bioactive compounds upon drying.

3.3 Effect of *in-vitro* digestibility on TPC and antioxidant activity of sweet potato flesh

From the practical point of view, this experiment was based on the water extraction which is most suitable for human consumption. Figure 1 and 2 show TPC and antioxidant activities based on FRAP and DPPH of freeze dried (control) and hot air dried OFSP and PFSP during *in-vitro* digestion, respectively. TPC and antioxidant activities were unstable throughout the *in-vitro* digestion process. The instability of TPC, FRAP and DPPH values has been reported elsewhere (Bouayed *et al.*, 2011; Chen *et al.*, 2014; Grijalva *et al.*, 2017; Gunathilake *et al.*, 2018).

Freeze dried sample showed the greatest TPC compared to all hot air dried samples. Overall, a slight change in TPC of OFSP during digestion was observed compared to those from PFSP that show the greater change. Both varieties showed similar observation in that TPC decreased from oral phase (0 min) to gastric phase (30 min), and subsequently increased during intestinal phase (120 min). The exceptions were for freeze dried OFSP in which TPC gradually increased and conversely, TPC of 85 °C dried PFSP gradually declined. It could be observed that 55 °C dried PFSP with the highest TPC, FRAP and DPPH before digestion could maintain its antioxidant stability through oral, gastric and intestinal digestion. In other drying temperatures, either increase or decrease in corresponding antioxidant activities was observed along gastrointestinal digestion.

In previous research, various changes in bioactive compound and antioxidant activities during *in-vitro* digestion were found. The increase in TPC and antioxidant activities from gastric to intestinal phase was reported by Bouayed *et al.*, (2011). The increase in TPC and antioxidant activities during gastric phase and subsequently declined during intestinal phase was observed by Chen *et al.*, (2014). The increase in TPC on digestion could be due to digestive enzymes that hydrolyzed chemical bonds between phenolics and proteins, enhancing the release of unbound phenolics. The transition from gastric to intestinal phase, contributing to the elevated pH induces structural changes in phenolic compounds and their corresponding antioxidant capacities (Ryan and Prescott. 2010; Tagliazucchi *et al.*, 2010). To this study, the

variation in TPC and antioxidant activities among OFSP and PFSP dried at different temperatures may be related to the occurrence of different individual of phenolics structure.

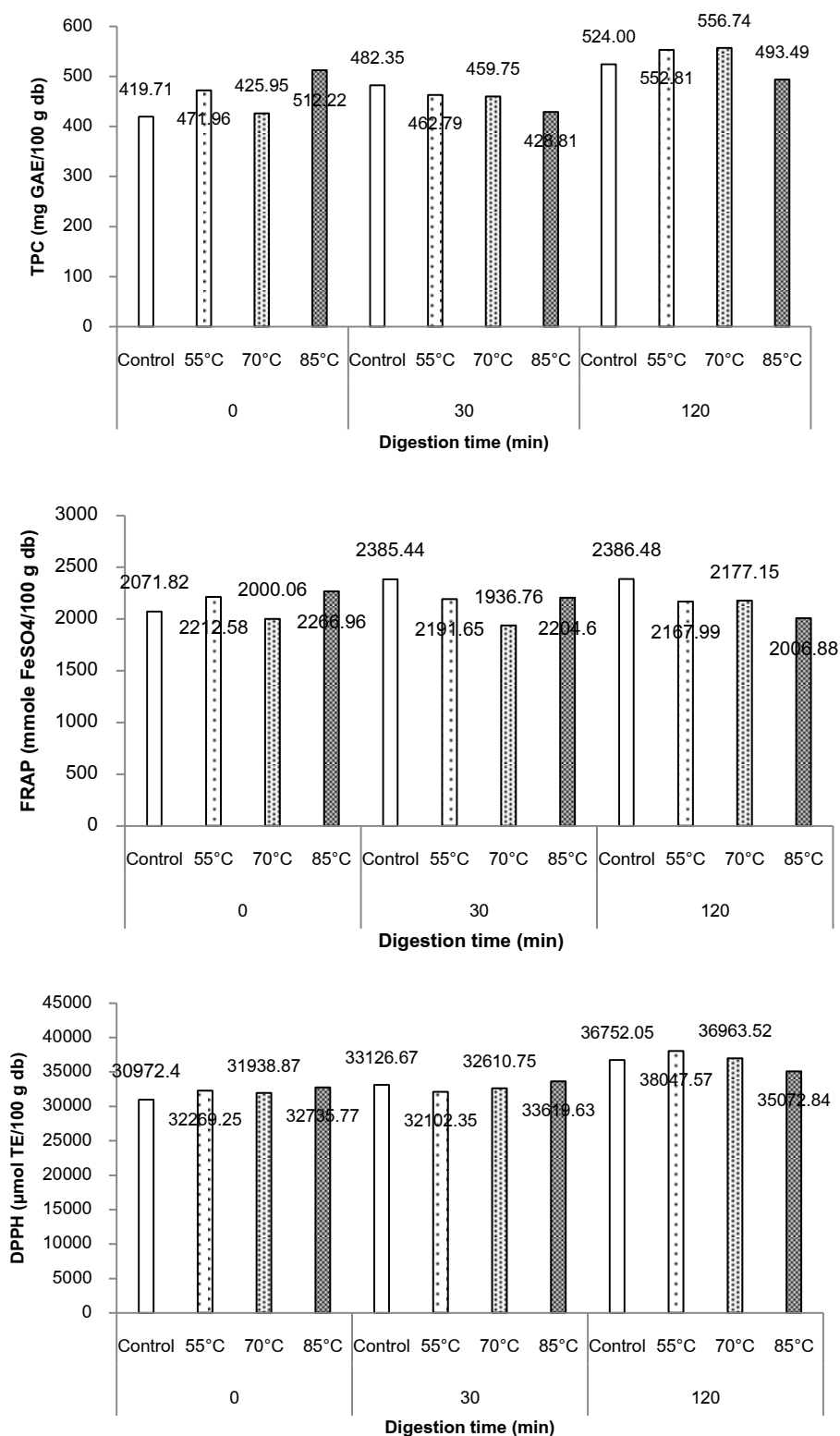


Figure 1 TPC and antioxidant properties by FRAP and DPPH assays of OFSP during digestion time of 0 min (oral phase), 30 min (gastric phase), and 120 min (intestinal phase).

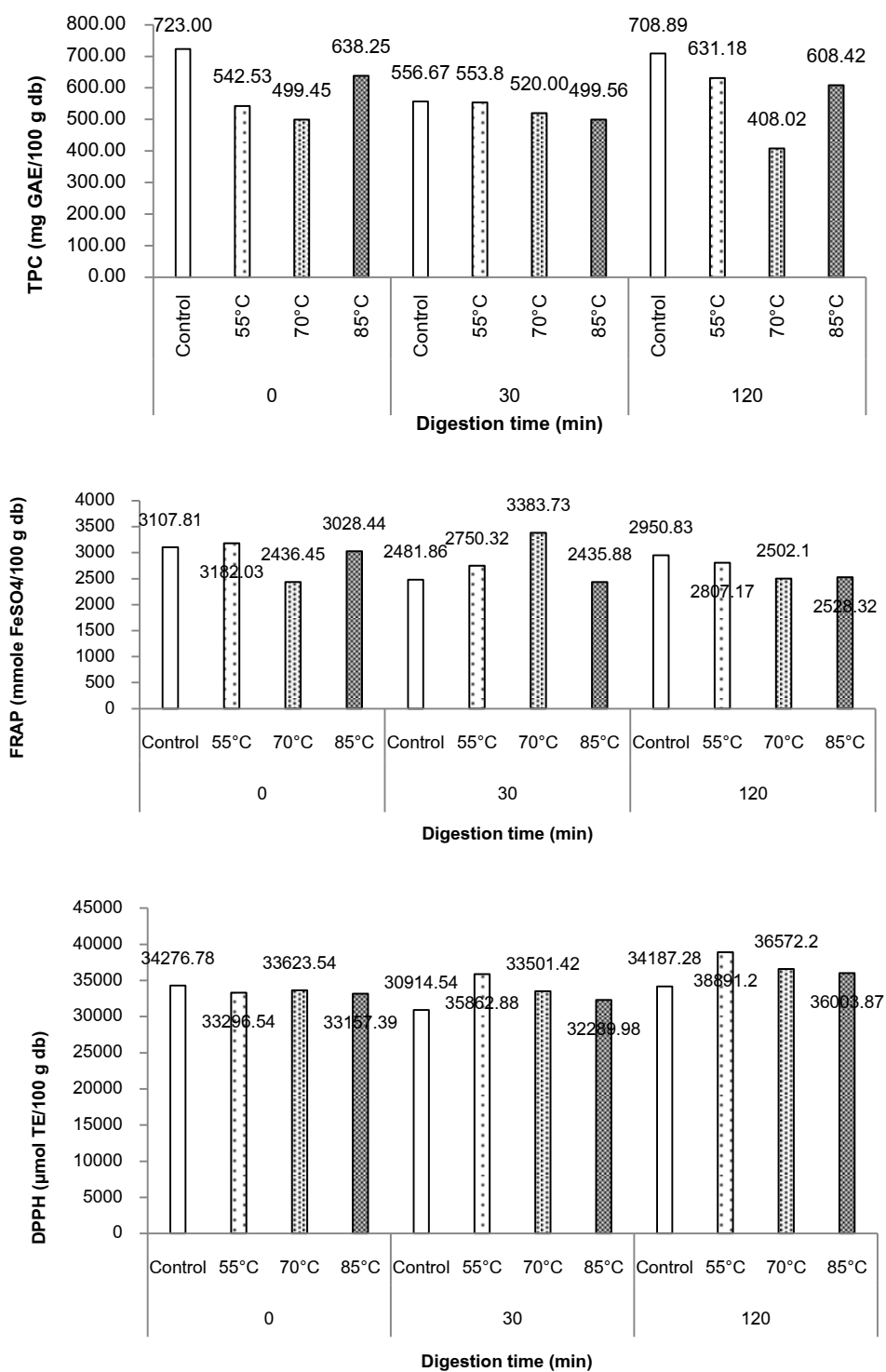


Figure 2 TPC and antioxidant properties by FRAP and DPPH assays of PFSP during digestion time of 0 min (oral phase), 30 min (gastric phase), and 120 min (intestinal phase).

Changes in TPC of OFSP and PFSP corresponded to the change in antioxidant activities based on FRAP and DPPH (Figure 1 and 2). The significantly positive correlation between TPC and FRAP and DPPH values are shown in Table 2 and 3. Interestingly, the significant correlation was observed between TPC and FRAP ($r = 0.777$, $p < 0.01$), but not between TPC and DPPH ($r = 0.164$, $p > 0.05$). Furthermore, there was no significant correlation between FRAP and DPPH values ($r = 0.132$, $p > 0.05$). The correlation between bioactive compounds and the antioxidant activities as a result of drying temperature was found less significant after digestion than before digestion.

Table 3. Pearson correlation coefficients of bioactive compounds and antioxidant activity after *in-vitro* gastrointestinal digestion

	TPC	FRAP	DPPH
TPC	1		
FRAP	0.777**	1	
DPPH	0.164	0.132	1

Note: *Correlation was significant at $p < 0.05$, **Correlation was significant at $p < 0.01$

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4. Conclusion

Results showed different effects of drying temperature on antioxidant properties of two colored sweet potatoes containing different major type of bioactive compounds. Drying at 70 and 85 °C tended to give negative effects since TPC and TCC of OFSP and TPC and TAC of PFSP apparently decreased. Only drying at 55 °C was recommended since it could enhance TPC and TAC of PFSP to the greatest extent and maintained TPC and TCC of OFSP closed to that of freeze dried sample. Overall, based on *in vitro* gastro-intestinal digestion, TPC and its corresponding antioxidant could be altered to some extent. However, drying at 55 °C could maintain the stability of TPC and its antioxidant properties through digestion states.

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