# Effect of Thermal Treatments on Antioxidant Properties of Pumpkin Flesh and Their Stability during *in-vitro* Gastrointestinal Digestion

**Titikan Liangpanth[1] and Rungarun Sasanatayart[1*]**

## Abstract

Flesh of pumpkin (*Cucurbita maxima*) is especially rich in carotenoids and phenolic compounds that exert antioxidant properties. In this study, two varieties of pumpkin, Srimuang (Thai variety with rough skin) and Kabocha (Japanese variety with smooth skin) were subjected to four different heat treatments including boiling (90 °C for 5 min), steaming (100 °C for 5 min) and hot air drying (60 °C for 16 h and 70 °C for 10 h). Uncooked samples were served as control. Results showed that rough skin pumpkin contained higher total phenolic content (TPC) and total carotenoid content (TCC) than smooth skin pumpkin and consequently, the higher antioxidant activities based on Ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Among heat treatments, Drying at 70 °C of smooth skin pumpkin showed the strongest TPC and antioxidant activity base on FRAP and DPPH. In rough skin pumpkin, drying at 70 °C showed the highest TPC corresponding to the highest FRAP values. After boiling, TCC increased in rough skin pumpkin but, reduced to the lowest extent in smooth skin pumpkin. During *in-vitro* digestion, TPC slightly increased from oral, gastric to intestinal phase in all heat-treated samples. However, the corresponding antioxidant activities based on FRAP and DPPH were found to decrease on digestion. The exception was for DPPH values of heat treated smooth skin pumpkin that remained unchanged. Results showed the higher correlation among TPC, FRAP and DPPH of samples before digestion ($r = 0.704–0.902$, $p < 0.01$) than that of samples after digestion ($r = 0.411–0.716$, $p < 0.01$). The content of bioactive compounds depended not only on their concentration in pumpkin, but also processing conditions and gastrointestinal digestion which influenced their stability and consequently, changes in antioxidant activities.

**Keywords:** Pumpkin, Phenolic compound, Carotenoids, Antioxidant activity, *in-vitro* gastrointestinal digestion

[1] Program of Postharvest Technology, School of Agro-Industry, Mae Fah Luang University, Thasud, Chiang Rai, 57100 Thailand

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

*Received: 7 August 2018/ Revised: 2 October 2018/ Accepted: 3 March 2019*

## 1. Introduction

Pumpkin (*Cucurbita maxima*) is rich in dietary fiber, vitamins, minerals and some bioactive compounds beneficial to health (Azevedo-Meleiro and Rodriguez-Amaya, 2007). Phenolics are bioactive compounds found in all plants as their secondary metabolites, providing antioxidant activities. Other major bioactive substance in pumpkin is carotenoids, which are pigments that give yellow to red color. Carotenoids have strong antioxidant capacity to scavenge free radicals because of their conjugated double bonds (Stahl and Sies, 2003). Composition of phenolics and carotenoids may also be affected by several factors such as variety, cultivar, maturation stage, geography, climate, harvesting and post-harvesting, and processing methods (Azevedo-Meleiro and Rodriguez-Amaya, 2007, Murkovic *et al*., 2002, Randhir *et al*., 2008).

Generally, pumpkin is cooked, either by boiling, steaming or roasting before consumption. Such thermal processing can greatly affect quantity and functional quality of its component (Aydin and Gocmen, 2015, Provesi *et al*., 2011). During thermal processing, plant cell wall is softened by heat and consequently, phenolic compounds, carotenoid and other bioactive compounds are easily released (Isantea and Wongajang, 2015). On the other hands, heat may cause degradation or polymerization on some bioactive compounds to a more or less active form. It has been reported that some thermal processes could increase bioactive compounds and enhance the antioxidant activity of fruit and vegetables (Dini *et al*., 2013, Ribeiro *et al*., 2015).

Simulated *in vitro* digestion is method imitating gastrointestinal condition that is widely used to study the gastro-intestinal behavior of food or pharmaceuticals. Simulated digestion methods typically include the oral, gastric and small intestinal phases. These models of human digestion have been used to answer the scientific questions as the digestibility and bioaccessibility (Minekus *et al*., 2014). Many research reported on changes of bioactive compounds and antioxidant properties during *in vitro* digestion on pumpkin and butternut squash (Koh and Loh, 2018) and other plant materials (Chen *et al*., 2014, Pavan *et al*., 2014, Tagliazucchi *et al*., 2010, Grijalva *et al*., 2017). Various changes in antioxidant properties were observed among studies. The changes upon chemical and physiological complication under *in vitro* digestion were still unclear and further investigation is needed for heat-treated samples.

This research aims to compare antioxidant compounds between two varieties of pumpkin and investigate change of antioxidant activity after subjected to heat treatments and during *in vitro* digestion.

## 2. Materials and Methods

### 2.1 Materials

Two varieties of pumpkin, Srimuang (Thai variety with rough skin) and Kabocha (Japanese variety with smooth skin) of commercial size and maturity were peeled and cut into uniform pieces (3*3*4 cm) and were subjected to four different heat treatments including (1) boiling at 90 °C for 5 min (2) steaming at 100 °C for 5 min (3) hot air drying at 60 °C for 16 h and (4) hot air drying at 70 °C for 10 h. Uncooked samples were served as control. Heat treatments were assigned to experimental units of three replications in a completely randomized design (CRD). All samples were subsequently freeze dried to obtain moisture content less than 10%, 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 hrs 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

Pumpkin powder was soaked in 95% hexane (95:5 of hexane:ether v/v), mixed for 10 mins using vortex mixer and centrifuged at 3000x g for 10 min . The supernatant from 3 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 (βCE) per 100 g of dry samples (Desobry *et al*., 1997).

### 2.4 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, 10mM 2,4,6 tripyridyl-s-triazine (TPTZ) and 20mM $FeCl_3$ 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 $FeSO_4 7H_2O$ solution was used to calculate the FRAP value as µmole Ferrous sulfate per 100g dry sample.

**2.5 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 0.00236g DPPH in methanol to a total volume of 100 mL). Reaction mixture was stored in dark place for 30 mins 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).

**2.6 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 5 mL pepsin (from porcine gastric mucosa, 250 U/mg, Sigma-Aldrich, St Louis, USA) in 0.02M HCl was added and maintained pH 2.00 ± 0.01. Digesta was collected at 30mins 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 ± 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's multiple range test at 95% level of confidence ($p < 0.05$). Pearson correlation coefficient was used to study the relationship among different properties ($p < 0.01$ and $p < 0.05$).

## 3. Results and Discussion

### 3.1 Varietal difference in TPC, TCC and antioxidant activity of pumpkin flesh

TPC, TCC and antioxidant properties based on FRAP and DPPH assays are shown in Table 1. Results showed that "Srimuang", Thai pumpkin variety with rough skin contained higher TPC (296.48 mg GAE/100g db) and TCC (24.13 mg βCE/100g db) than "Kabocha", Japanese variety with smooth skin that had TPC of 67.83 mg GAE/100g db and TCC of 4.29 mg βCE/100 g db. A significant higher amount of these bioactive compounds possibly resulted in a higher antioxidant activities based on FRAP (1,978.81 mmole $FeSO_4$/100 g for rough skin pumpkin and 739.90 mmole $FeSO_4$/ 100 g for smooth skin pumpkin) and DPPH (21,836.58 µmol TE/100g db for rough skin pumpkin and 16,949.61 µmol TE/100 g db for smooth skin pumpkin) ($p < 0.05$). It has been reported that pumpkins grown in different location and climate had different amount of TPC and TCC (Murkovic *et al*., 2001, Norshazila *et al*., 2014). Amount of bioactive compounds in this study was among the range earlier reported.

**Table 1**. Bioactive compounds and antioxidant activity of uncooked and thermal-treated pumpkin pulp

| Pumpkin varieties | Thermal treatments | Total carotenoid content mg βCE/100g db | Total phenol content mg GAE/100 g db samples | FRAP mmole $FeSO_4$/100 g db samples | DPPH µmol TE/100 g db samples |
|---|---|---|---|---|---|
| Srimuang | Control | 24.13 ± 0.29$^{g}$ | 296.48 ± 1.43$^{c}$ | 1,978.81 ± 25.44$^{g}$ | 21,836.58 ± 23.09$^{f}$ |
| (Thai variety | Boiling | 51.82 ± 0.27$^{a}$ | 267.84 ± 2.54$^{d}$ | 2,614.73 ± 16.13$^{d}$ | 22,078.16 ± 30.53$^{e}$ |
| with rough skin) | Steaming | 35.32 ± 0.28$^{c}$ | 248.53 ± 1.23$^{de}$ | 2,263.63 ± 50.33$^{e}$ | 20,214.57 ± 28.12$^{i}$ |
| | Drying 60 °C | 27.59 ± 0.75$^{f}$ | 455.49 ± 0.81$^{b}$ | 2,081.51 ± 20.78$^{f}$ | 23,187.39 ± 19.17$^{c}$ |
| | Drying 70 °C | 39.72 ± 0.97$^{b}$ | 469.76 ± 1.10$^{b}$ | 3,166.62 ± 30.43$^{c}$ | 21,027.75 ± 17.82$^{h}$ |
| Kabocha | Control | 42.86 ± 0.53$^{j}$ | 67.83 ± 1.33$^{g}$ | 739.90 ± 45.01$^{j}$ | 16,949.61 ± 64.38$^{j}$ |
| (Japanese | Boiling | 10.24 ± 0.83$^{i}$ | 122.89 ± 7.48$^{f}$ | 1,426.19 ± 37.20$^{i}$ | 22,729.20 ± 40.13$^{d}$ |
| variety with | Steaming | 15.13 ± 0.95$^{h}$ | 230.23 ± 3.64$^{e}$ | 1,812.83 ± 38.77$^{h}$ | 21,590.43 ± 55.39$^{g}$ |
| smooth skin | Drying 60 °C | 29.88 ± 0.26$^{e}$ | 460.51 ± 5.89$^{b}$ | 3,199.96 ± 42.63$^{b}$ | 24,321.24 ± 64.46$^{b}$ |
| | Drying 70 °C | 32.76 ± 0.27$^{d}$ | 617.23 ± 40.80$^{a}$ | 4,440.44 ± 56.85$^{a}$ | 24,963.25 ± 21.16$^{a}$ |

**Note:** Data were expressed as mean ± standard deviation.

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

### 3.2 Effect of thermal process on TPC, TCC and antioxidant activity of pumpkin flesh

After thermal treatments (boiling, steaming and drying), TPC and TCC apparently increased in both pumpkin varieties (Table 1). The exceptions were boiling and steaming of rough skin pumpkin in which TPC slightly decreased and boiling of smooth skin pumpkin in which TCC apparently decreased. Among heat treatments, drying at 70 °C increased TPC, TCC, FRAP and DPPH values to the greatest extent in smooth skin pumpkin with respective values of TPC 617.23 mg GAE/100 g db, TCC 32.76 mg βCE/100 g db, FRAP 4,440.44 mmole $FeSO_4$/100 g db and DPPH 24,963.25 µmol TE/100 g db. In rough skin pumpkin, the greatest TCC (51.82 mg βCE/100 g db) was obtained after boiling but the greatest TPC (469.76 mg GAE/100 g db) was obtained from drying at 70 °C corresponding to the highest FRAP value of 3,166.62 mmole FeSO4/100 g db. However, DPPH value was noted from drying at 60 °C (51,817.06 mg βCE/100 g db). Overall, thermal treatments (boiling, steaming and drying) showed the positive effect on antioxidant properties compared with uncooked pumpkin. Drying at 70 °C tended to enhanced TPC, TCC, FRAP and DPPH values to the greatest extent in smooth skin pumpkin, except for DPPH in rough skin pumpkin.

This effect could be attributed to the breakdown of food matrices by heat treatments and loosen the bioactive compounds-binding fibers, leading to bioactive compounds release. After heat treatment, the bioavailability of the active components could either increase as a result of depolymerization into the more active form (Aydin and Gocmen, 2015) or decrease as a result of degradation (Ribeiro *et al*., 2015). Aydin and Gocmen, (2015) reported that oven-drying of pumpkin led to higher phenolic contents and antioxidant capacity. This could possibly contribute to production of redox-active secondary metabolites such as Maillard reaction, influencing the amount of bioactive compounds (Dini *et al*., 2013). Dini *et al*. (2013) reported that various heat-treatments including boiling, steaming, microwaving, cooking, grilling and frying markedly increased TPC and antioxidant properties of pumpkin pulp. Other study showed that TCC in pumpkin increased after boiling and steaming (Ribeiro *et al*., 2015). To this study, the increase in TCC on boiling was observed only in rough skin pumpkin. In smooth skin pumpkin, TCC apparently decreased. Azizah et al. (2009) had also showed that beta-carotene increased 2 to 4 times after boiling and stir frying pumpkin for 2, 4 and 6 min whilst, some losses of TPC was found. However, the free radical scavenging activity of cooked pumpkins was still high.

Overall, the increase in TPC and TCC of heat-treated pumpkin corresponded to the significant increase in antioxidant activities by FRAP and DPPH (Table 2). Results showed the high correlation between TPC and FRAP (r=0.716, $p < 0.01$) and between TPC and DPPH (r=0.902, $p < 0.01$). This was greater than that of TCC and FRAP (r=0.361, $p < 0.05$) and TCC

and DPPH (r=0.672, $p < 0.01$). TPC measurement by Folin–Ciocalteu method was based on reducing capacity and this correlated better with FRAP and DPPH than the measured TCC. Antioxidant activities of bioactive compounds depended not only on their concentration in pumpkin, but also processing or cooking conditions.

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

| | TPC | TCC | FRAP | DPPH |
|---|---|---|---|---|
| **TPC** | 1 | | | |
| **TCC** | 0.557** | 1 | | |
| **FRAP** | 0.716** | 0.361* | 1 | |
| **DPPH** | 0.902** | 0.672** | 0.704** | 1 |

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

### 3.3 Effect of *in-vitro* digestibility on TPC and antioxidant activity of pumpkin flesh

Based on the greater correlations of TPC over TCC on corresponding antioxidant activities, only TPC were investigated under simulated *in-vitro* gastrointestinal digestion. Figure 1 and 2 show TPC and antioxidant activities based on FRAP and DPPH of uncooked and heat-treated pumpkin during *in-vitro* digestion of rough and smooth skin pumpkin, respectively. Overall, TPC of all samples gradually increased along digestion from oral phase (0 min) to gastric phase (30 min) and intestinal phase (120 min). Samples dried at 70 °C apparently showed the highest TPC during digestion in both varieties. However, the more apparent effect was observed in smooth skin pumpkin than rough skin pumpkin. Other heat treatments (drying at 60 °C, boiling and steaming) showed the lesser effects. The result was similar with the previous research that TPC increased from gastric to intestinal phase in different varieties of apple (Bouayed *et al*., 2011). The increase in TPC under *in-vitro* digestion could be due to digestive enzymes that broke chemical bonds between phenolics and proteins, enhancing the release of unbound phenolics (Ti *et al*., 2015).

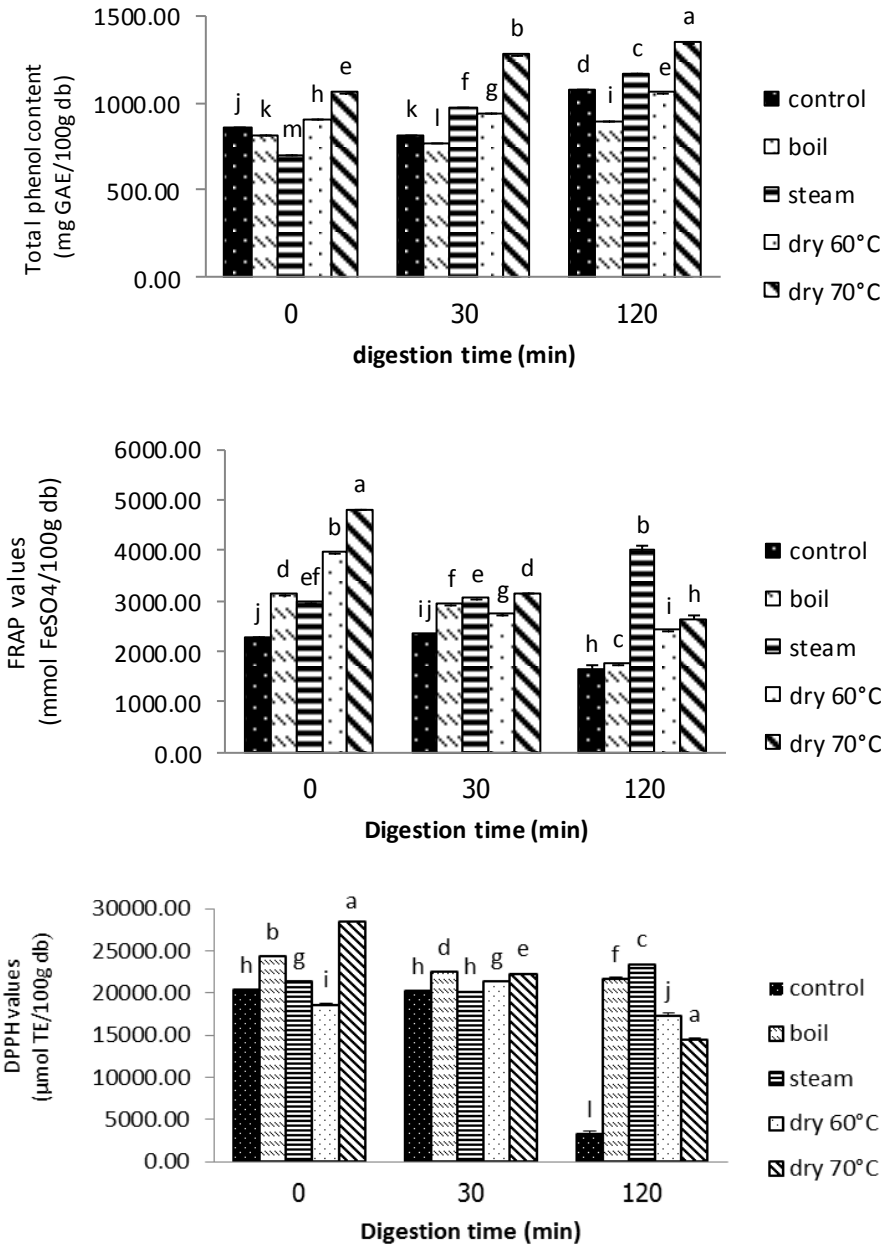


**Figure 1** TPC and antioxidant properties by FRAP and DPPH assays of Srimuang (Thai variety with rough skin) during digestion time of 0 min (oral phase), 30 min (gastric phase), and 120 min (intestinal phase). The bars with different superscripts were significantly different ($p < 0.05$).

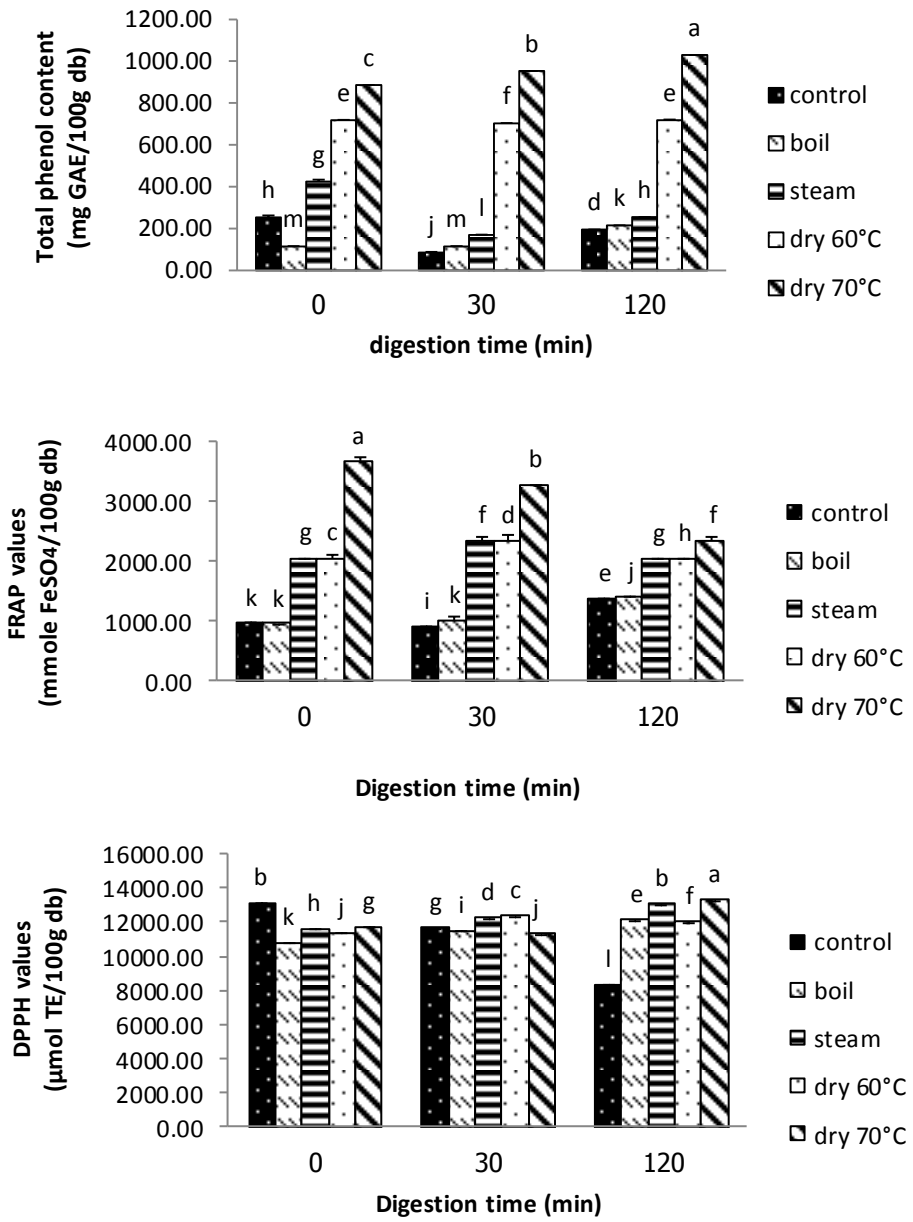


**Figure 2** TPC and antioxidant properties by FRAP and DPPH assays of Kabocha (Japanese variety with smooth skin) during digestion time of 0 min (oral phase), 30 min (gastric phase), and 120 min (intestinal phase). The bars with different superscripts were significantly different *($p < 0.05$)*.

It was expected that FRAP and DPPH values increased under *in-vitro* digestion. However, results showed a slight decrease in FRAP and DPPH of all heat treated samples during oral, gastric and intestinal digestion. The exception was for DPPH of smooth skin pumpkin that remained unchanged ($p \geq 0.05$). It was also noted that the apparent reduction of DPPH on digestion was found in uncooked sample ($p < 0.05$). It was clearly seen that even antioxidant activities of heat treated pumpkin reduced during digestion but, the values were higher than uncooked sample. Puangkam *et al*. (2017) reported that TPC, FRAP and DPPH

values of Thai ciruciferous vegetables significantly decreased after intestinal digestion due to the presence of various types of phenolic compounds and each of which has different stability in acid-base conditions. Polyphenols had sensitivity to alkaline condition in intestine where structural changes by enzyme lead to chemical property alteration.

Effect of *in-vitro* digestion on antioxidant properties varied among research. Either increase or decrease in antioxidant activities of plant material was reported. Chen *et al* (2017) reported that antioxidant activity of sweet potato leaves increased after intestinal digestion similar to Pavan *et al*. (2014) who reported that antioxidant activity of araticum and jackfruit increased after digestion. Other report by Tagliazucchi *et al*. (2010) who investigated TPC and antioxidant activities in grape showed that they increased during gastric digestion and decreased when transition to intestinal digestion. Grijalva *et al*. (2017) reported the reduction in phenol, flavonoid and antioxidant capacity in oregano during digestion. It well established that among different type of plants and condition studied, bioactive compound were influenced by gastrointestinal digestion and corresponded to various changes in antioxidant activities.

Under simulated gastrointestinal digestion, the correlations among TPC and antioxidant activities by FRAP and DPPH were lower that before digestion (Table 3). Results showed the high correlation between TPC and DPPH (r=0.716, $p < 0.01$) than TPC and FRAP (r=0.411, $p < 0.01$).

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

| | TPC | FRAP | DPPH |
|---|---|---|---|
| **TPC** | 1 | | |
| **FRAP** | 0.411* | 1 | |
| **DPPH** | 0.716** | 0.547* | 1 |

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

## 4. Conclusion

Results showed that pumpkin variety with rough skin contained higher TPC, TCC, FRAP and DPPH than pumpkin variety with smooth skin. The thermal treatments including drying, boiling and steaming showed the positive effects on antioxidant properties of two pumpkin varieties when compared with uncooked pumpkin. Drying at 70 °C of smooth skin pumpkin showed the strongest TPC and antioxidant activity base on FRAP and DPPH. In rough skin pumpkin, drying at 70 °C showed the highest TPC corresponding to the highest FRAP values. However, bioavailability of these bioactive compounds could be greatly

influenced by gastro-intestinal digestion, resulting in a lower bioavailability with respect to their original content.

**5. Acknowledgement**

The authors would like to thank Mae Fah Luang University for financial support.

**References**

Aydin, E. and Gocmen, D. 2015. The influences of drying method and metabisulfite pre-treatment on the color, functional properties and phenolic acids contents and bioaccessibility of pumpkin flour. LWT - Food Science and Technology. 60: 385–392.

Azevedo-Meleiro, C.H. and Rodriguez-Amaya, D.B. 2007. Qualitative and quantitative differences in carotenoid composition among *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita pepo*. Journal of Agricultural and Food Chemistry. 55(10): 4027–33.

Azizah, A.H., Wee, K.C., Azizah,O. and Azizah, M. 2009. Effect of boiling and stir frying on total phenolics, carotenoids and radical scavenging activity of pumpkin (*Cucurbita moschata*) International Food Research Journal. 16: 45–51.

Benzie, I.F.F. and Strain, J.J. 1999. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry. 239: 70–76.

Bouayed, J., Hoffmann, L. and Bohn, T. 2011. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. Food chemistry.128: 14–21.

Chen, G.L., Chen, G.S., Zhao, Y., Li, J.L.X.C. and Gao, Q.Y. 2014. Total phenolic contents of 33 fruits and their antioxidant capacities before and after *in vitro* digestion. Industrial Crops and Products. 57: 150–157.

Chen, Y., Fu, Z.F, Tu, Z.C., Wang, H., Zhang, L., Xie, X. and Liu, G. 2017. Influence of *in vitro* gastrointestinal digestion on the bioavailability and antioxidant activity of polyphenols from *Ipomoea batatas* leaves. International Journal of Food Science and Technology. 1–7.

Desobry, S.A., Netto, F.M., and Labuza, T.P., 1997. Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. Journal of Food Science. 62(6): 1158–1162.

Dini, I., Tenore Carlo, G. and Dini A. 2013. Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. LWT - Food Science and Technology. 53: 382–385.

Grijalva, E.P.G., Escalante M.A.A., Le´on-F´elix, J. and Heredia, J.B. 2017. Effect of in vitro digestion on the total antioxidant capacity and phenolic content of 3 species of oregano (*Hedeoma patens*, *Lippia graveolens*, *Lippia palmeri*). Journal of Food Science. 1–8.

Hur, S.J., Lim, B.O., Decker, A. and McClements, D.J. 2010. *In vitro* human digestion models for food applications. Food Chemistry. 125(1): 1–12.

Isantea, O. and Wongajang, K. 2015. The Investigation of the extraction solvent system of phenolics and flavonoids rich extracts and antioxidant activity from tagetes erecta flower. National Research Council of Thailand (NRCY) Pibulsongkram Rajabhat University. 205–214.

Koh, S.H. and Loh, S.P. 2018. *In vitro* bioaccessibility of β-carotene in pumpkin and butternut squash subjected to different cooking methods. International Food Research Journal. 25(1): 188–195.

Minekus, M., Alminger, M., Alvito, P., Balance and Brodkorb, A. 2014. A standardised static *in vitro* digestion method suitable for food – an international consensus. Food and Function. 5(6): 1113–24.

Molyneux, P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity Songklanakarin Journal of Science and Technology. 26: 211–219.

Murkovic. M., Mulleder, U. and Neunteufl, H. 2002. Carotenoid content in different varieties of pumpkins. Journal of Food Composition and Analysis. 15(6): 633–638.

Norshazila, S., Irwandi, J., Othman, R., and Yumi zuhanis, H.H. 2014. Carotenoid content in different locality of pumpkin (*Cucurbita moschata*) in malaysia. Pharmacy and Pharmaceutical Sciences. 6(3): 29–32.

Pavan, V., Soriano Sancho, R.A. and Pastore, G.M. 2014. The effect of *in vitro* digestion on the antioxidant activity of fruit extracts (*Carica papaya*, *Artocarpus heterophillus* and *Annona marcgravii*). Food Science and Technology. 59: 1247–1251.

Provesi, J.G., Dias, C.O. and Amante, E.R. 2011. Changes in carotenoids during processing and storage of pumpkin puree. Food Chemistry. 128(1): 195–202.

Puangkam, K., Muanghorm, W. and Konsue, N. 2017. Stability of bioactive compounds and antioxidant activity of Thai cruciferous vegetables during *in vitro* digestion. Current Research Nutrition Food Science. 5(2): 100–108.

Randhir, R., Kwon, I.Y and Shetty, K. 2008. Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. Innovation Food Science and Emerging Technologies. 9(3): 355–364.

Ribeiro, E.M.G. Chitchumroonchokchai, C., de Carvalho, L.M., de Moura, F.F., de Carvalho, J. V. and Failla, M. L. 2015. Effect of style of home cooking on retention and bioaccessibility of pro-vitamin A carotenoids in biofortified pumpkin (*Cucurbita moschata* Duch.) Genotypes, Food Research International. 77(3): 623–626.

Sopade, P.A and Gidley, M.J. 2009. A rapid *in-vitro* digestibility assay based on glucometry for investigating kinetics of starch digestion. Starke/Strach. 61:245

Stahl, W. and Sies, H. 2003. Antioxidant activity of carotenoids. Molecular Aspects of Medicine. 24(6): 345–51

Tagliazucchi, D., Verzelloni, E., Bertolini, D. and Conte, A. 2010. *In vitro* bio-accessibility and antioxidant activity of grape polyphenols. Food Chemistry. 120: 599–606.

Tang, Y., Cai, W., and Xu, B. 2015. Profiles of phenolics, carotenoids and antioxidative capacities of thermal processed white, yellow, orange and purple sweet potatoes grown in Guilin, China, Food Science and Human Wellness. 4: 123–132.

Ti, H., Zhang, R., Li, Q., Wei, Z. and Zhang, M. 2015. Effects of cooking and *in vitro* digestion of rice on phenolic profiles and antioxidant activity. Food Research International 76: 813–820.