

Effect of steam-cooking on (poly)phenolic compounds in purple yam and purple sweet potato tubers

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Abstract

This study investigated the influences of steam-cooking on (poly)phenolic compounds and colors in purple yam (*Dioscorea alata* L.) and purple sweet potato (*Ipomoea batatas*) tubers cooked at the household level. Steam-cooking did not significantly change the contents of total phenolic compounds, flavonoids, anthocyanins and antioxidant capacity measured by ABTS radical scavenging assay in purple yam ($P \geq 0.05$), but lowered the antioxidant capacity measured by Crocin assay ($P < 0.05$). The liquid chromatograph–mass spectrometer–Ion Trap–Time of Flight (LCMS-IT-TOF) indicated the degradation of high MW alatanin B to lower MW alatanins due to the loss of glucose units. Purple sweet potato, however, responded to steam-cooking differently from purple yam ($P < 0.05$). Steam-cooking drastically increased total phenolic compounds, flavonoids, anthocyanins, antioxidant capacities, and retained the vivid reddish-purple color of cooked purple sweet potato ($P < 0.05$). The stability of polyphenolic compounds in starchy tubers against steam-cooking at the household level was, in part, due to the different contents of indigenous phenolic compounds in the raw tubers.

1. Introduction

Tuber crops are an essential carbohydrate source in tropical and subtropical countries. Sweet potato (*Ipomoea batatas*) is the second-largest tuber production in the world, followed by yam (*Dioscorea alata* L.) (Lebot, 2020). Numerous studies have shown that yam and sweet potatoes are a good source of essential nutrients and bioactive compounds (Kano *et al.*, 2005; Christina and Rifa'i, 2014; Lebot, 2020). Isoprenoids and (poly)phenolic compounds in tuber crops have received much attention during recent decades for their potential effects on human health, particularly in neurodegenerative diseases, inflammation, cancer, cardiovascular health, type 2 diabetes, obesity, and interactions with gut microbiota (Cory *et al.*, 2018) due to their antioxidant capacities (Cornago *et al.*, 2011).

At the cellular level, phenolic and polyphenolic compounds stored in the vacuole. During cooking, the interactions among these bioactive compounds and other nutrients, particularly protein, could affect their bioavailability. Therefore, it is essential to understand the effect of cooking, even at typical culinary preparation, on the changes of (poly)phenolic

compounds in order to preserve the contents or prevent the loss of these compounds.

Anthocyanins in purple yam and purple sweet potato can be classified into non-acylated, mono-acylated, and di-acylated with *p*-hydroxybenzoic, ferulic, or caffeic acid as acyl group. Cyanidin-3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside and peonidin 3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside were two dominant di-acylated anthocyanins in purple sweet potato (Truong *et al.*, 2010; de Aguiar Cipriano *et al.*, 2015; Hong and Koh, 2016; Chen *et al.*, 2017). While, predominant anthocyanin in purple yam is cyanidin 3-(6-sinapoylgentiobioside) (Moriya *et al.*, 2015). Although the aglycone in anthocyanins of both plants is cyanidin nucleus, the glycosides and acyl groups vary considerably. The anthocyanins from different plants could give different responses to external factors such as pH, heat, light, and O₂. Tsukui (1988) reported that the purified anthocyanins from *ube* yam were the most heat-stable anthocyanin compared to the anthocyanins from purple corn, purple sweet potato, red cabbage, and grape.

Nonetheless, the responses of anthocyanins to heat in gelatinized starchy matrices could be different and varied

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considerably depending on plant compositions. Steed and Truong (2008) reported that steaming of purple sweet potato did not change the total phenolic content but decreased monomeric anthocyanin in the methanol (MeOH) extract, reported as cyanidin 3-glucoside (C3G) from 269 to 220.34 mg C3G/100 g dry basis (d.b.). However, Chen *et al.* (2017) reported that steaming increased the anthocyanin content in the MeOH extract of purple sweet potato from 455.08 to 503.63 mg C3G/100 g d.b. Besides, steaming of purple yam increased phenolic contents from 85.36 to 167.22 mg GAE/100 g d.b. and raised anthocyanin level from 36.09 to 57.28 mg C3G/100 g d.b. as reported by Imanningsih *et al.* (2013).

The objective of this study was to further explore the influences of steam-cooking at the household level typically used to gelatinize tuber starch for direct consumption on the changes in (poly)phenolic compounds, including anthocyanins, as well as the antioxidant capacities and colors in purple yam and purple sweet potato. The alterations of these bioactive compounds may affect their availabilities in the cooked starchy tubers readily for consumption.

2. Materials and methods

2.1 Sample preparation

Purple sweet potato of unknown cultivar was purchased from a local market Talad Thai, Pathum Thani province, Thailand. The purple yam accession KKFCRC (Khon Kaen Field Crop Research Center, Department of Agriculture, Ministry of Agriculture and Co-operatives, Thailand) was supplied by the farmers in Phra Nakhon Si Ayutthaya province, Thailand. The sweet potato and yam tubers were cleaned with tap water and sliced to 5 x 3 x 1 cm, ground using a coconut shredder, and dried in a hot air oven at 40°C for 24 hrs to reduce moisture to 10-11%. The dried sample was ground and sieved through a 100-mesh screen, designated as flour from raw tubers. Purple yam tubers with 8 cm in diameter and 22 cm in length were cut into half and steam-cooked in a home-cooker on a gas stove for 60 mins to gelatinize yam starch to the level of direct consumption. Purple sweet potato tubers with a diameter of 5 cm and a length of 13 cm were cut into half and steam-cooked in a home-cooker for 30 mins. The different cooking regimes for purple yam and purple sweet potato tubers were to follow a common household cooking practice for ingestion. Prolonging the cooking time for sweet potato resulted in the soggy texture and the loss of purple pigment to the drips. After the cooked sample cooled down to room temperature, it was ground and dried to 10-11% moisture content in an oven at 40°C for 24 hrs. All

samples were kept in a sealed plastic bag placed inside the sealed aluminum foil at -18°C before analysis.

2.2 Evaluation

Approximately, 1 g of sample was added with 10 mL of 50% ethanol (EtOH) acidified with 0.1% HCl to pH 2. The mixture was stirred at 50°C for 60 mins in a water-bath shaker (PR-100, Eyela, Bunkyo-ku, Tokyo, Japan) and centrifuged at 3400 × g for 10 mins using an Ohaus benchtop centrifuge (Frontier™5000, Ohaus, Melbourne, Victoria, Australia). The supernatant was filtered through a Whatman no. 1 filter paper. The filtrate was evaporated in a vacuum evaporator centrifuge (CentriVap Benchtop Centrifugal Vacuum Concentrator, Labconco, Kansas City, Missouri, USA) at 40°C. The extract was re-diluted with 5 mL of 50% acidified EtOH, filtered through the Whatman syringe filter 0.45 µm pore size (GE Healthcare UK Limited, Little Chalfont, Buckinghamshire, UK) for analyses as follows:

2.2.1 Total phenolic content

The extract (0.1 mL) was added with 0.1 mL of Folin-Ciocalteu reagent. The mixture was allowed to react for 6 mins. Then, it was added with 1 mL of 7% NaCO₃ and 1 mL of distilled water (Dewanto *et al.*, 2002). After 90 mins of reaction, the absorbance was measured at 760 nm using a Genesys 10 UV spectrophotometer (Thermo Fischer Scientific, Waltham, Massachusetts, USA). The phenolic content was calculated using a standard curve of gallic acid and expressed as mg gallic acid equivalent (GAE) in 100 g of flour on a dried weight basis (mg GAE/100 g flour).

2.2.2 Total flavonoid content

The extract of 0.25 mL was mixed with 1.25 mL of distilled water and 0.075 mL of 5% NaNO₂ solution and allowed to react for 5 mins. The mixture was added with 0.15 mL of 10% aluminum chloride solution; the reaction was allowed to proceed for 6 mins, followed by the addition of 0.5 mL 1 M NaOH. Distilled water was added to bring the final volume to 3 mL (Yang *et al.*, 2004). The absorbance was measured at 510 nm using a Genesys 10 UV spectrophotometer. The flavonoid content was determined using a catechin standard curve and expressed as mg catechin equivalent (CE) in 100 g of flour on a dried weight basis (mg CE/100 g flour).

2.2.3 Monomeric anthocyanin

Monomeric anthocyanin in the extract was determined using a pH-differential method described by Lee *et al.* (2005). Two buffer systems: 0.025 M potassium chloride buffer, pH 1.0 and 0.4 M sodium acetate buffer, pH 4.5, were used. The absorbance was

read at 510 nm and 700 nm using a Genesys 10 UV spectrophotometer. Total monomeric anthocyanin content was calculated and reported as mg cyanidin-3-glucoside in 100 g of flour on a dried weight basis (mg C3G/100 g flour).

2.2.4 Antioxidant capacities

2.2.4.1 Trolox equivalent antioxidant capacity (TEAC) assay

The ABTS radical scavenging assay was based on electron transfer (ET) mechanism (Re *et al.*, 1999; Huang *et al.*, 2005). The ABTS^{•+} was generated by mixing 5 mL of 7 mM ABTS with 88 μ L of 140 mM K₂S₂O₈ in the dark and held for 24 hrs at room temperature before use. The ABTS^{•+} stock solution was diluted to get an absorbance of 0.700 at 734 nm in phosphate buffer saline (PBS) assessed by a Genesys 10 UV spectrophotometer. A volume of 5 μ L of diluted sample was mixed with 1 mL of ABTS^{•+} radical solution. The decolorization of the ABTS^{•+} at 734 nm after 4 mins at 30°C using PBS as a control was evaluated by measuring the absorbance at 734 nm using a Genesys 10 UV spectrophotometer. The ET antioxidant capacity of the extract was reported as μ mol Trolox equivalent (TE)/g flour on a dried weight basis.

2.2.4.2 Crocin assay

The Crocin bleaching assay (CBA) described by Tubaro *et al.* (1998) and Di Majo *et al.* (2008) was used to evaluate the antioxidant activity of the extract based on the hydrogen atom transfer (HAT) mechanism (Huang *et al.*, 2005). The assay was based on the comparison of Crocin bleaching rates in the absence and presence of the antioxidant. The standard antioxidant Trolox (0.1 – 1 mM) was used to quantify the EtOH extract's antioxidant capacity determined from the plot between relative bleaching rate and Trolox concentration. The extract's antioxidant capacity was expressed as μ mol Trolox equivalent (TE)/g flour on a dried weight basis.

2.2.4.3 Identification of anthocyanin of purple yam by liquid chromatography-mass spectrophotometry

Anthocyanins in the EtOH extract from flour prepared from a raw yam tuber and steam-cooked tuber were characterized by LCMS-IT-TOF (SHIMADZU, Chiyoda-ku, Tokyo, Japan) coupled with Prevail™ C18 column (5 μ m, 150 \times 4.6 mm) as described previously (Srivichai and Hongprabhas, 2020). Mass spectrometry parameters (MS) were operated using a capillary voltage of 4.5 kV, interface temperature 200°C, heat block temperature 200°C, and N₂ gas flow of 1.5 L/min. The instrument was operated in a positive ion mode, scanning from *m/z* 100 to 2500, collision gas (argon) pressure at

50%, and collision energy at 50%.

2.3 Statistical analysis

The experiment was carried out using a completely block randomized design with two independent replications. The data were analyzed by analysis of variance (ANOVA) at a 95% significance level, followed by Duncan's multiple range tests (DMRT) for post hoc analysis using SPSS software version 12 (SPSS Inc., USA).

3. Results and discussion

The KKFCRC purple yam flesh (Figure 1) had a vivid purple color, although the purple pigment did not distribute evenly within the whole tuber. The purple pigment retained after drying the raw tubers at 40°C for 24 hrs (Figure 1C), suggesting that yam purple pigment in raw tuber was quite stable to drying in a hot air oven at 40°C for 24 hrs.

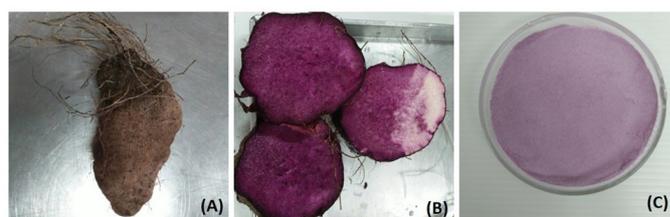


Figure 1. The appearance of (A) KKFCRC yam tuber, (B) yam flesh, and (C) yam flour made from a raw tuber

The contents of phenolic and (poly)phenolic compounds, namely flavonoids and anthocyanins, in EtOH extract of purple yam shown in Figure 2A suggested that steam-cooking did not affect the contents of these compounds ($P \geq 0.05$). This result was in agreement with Cornago *et al.* (2011), who reported that heating did not affect (poly)phenolic compounds in purple yam. However, Figures 2B and 2C illustrate slightly different profiles of anthocyanins in raw and steamed-cooked purple yam. The major anthocyanins in KKFCRC yam in Thailand have been reported earlier as the alatanin B (peak 5) and alatanin C (peak 6) isomers, which had molecular ion precursor [M]⁺ (*m/z*) at 1347 and 817, respectively (Srivichai and Hongprabhas, 2020). Both alatanins were also predominant in the Philippine *ube* yam (Yoshida *et al.*, 1991).

Both raw and steam-cooked yam contained anthocyanins identified as summarized in Table 1. Although steam-cooking did not affect total monomeric anthocyanin, it changed the anthocyanin profile quantitatively evaluated as the area under the curve (AUC). The AUC of alatanin C (peak 6), the major anthocyanin in raw KKFCRC tubers and steam-cooked

Table 1. Effect of steam-cooking at household level on anthocyanin profiles of EtOH extracts from flour prepared from the raw and cooked yam tubers

Peak number	Retention time (min)	Tentative identification*	Molecule structure	Molecular ion precursor [M] ⁺ (m/z)	Anthocyanin unit	Sugar unit(s)	Acyl unit (s)	The ratio of AUC	
								Raw tuber	Steam-cooked tuber
3	40.28	Cyanidin 3-gentiobioside	C ₂₇ H ₃₁ O ₁₆	611	Cyanidin	2 glucose	-	0.046 ^b	0.270 ^a
4	41.36	Alatanin E	C ₄₄ H ₅₁ O ₂₅	979	Cyanidin	3 glucose	1 sinapoyl	0.395 ^b	0.594 ^a
5	41.74	Alatanin B	C ₆₁ H ₇₁ O ₃₄	1347	Cyanidin	4 glucose	2 sinapoyl	0.682 ^a	0.525 ^b
6	42.84	Alatanin C	C ₃₈ H ₄₁ O ₂₀	817	Cyanidin	2 glucose	1 sinapoyl	1.000 ^a	1.000 ^a
7	43.43	Alatanin G	C ₃₇ H ₃₉ O ₁₉	787	Cyanidin	2 glucose	1 feruloyl	0.172 ^a	0.176 ^a
8	43.63	Alatanin D	C ₅₅ H ₆₁ O ₂₉	1185	Cyanidin	3 glucose	2 sinapoyl	0.100 ^b	0.175 ^a
9	44.44	Alatanin F	C ₃₉ H ₄₃ O ₂₀	831	Peonidin	2 glucose	1 sinapoyl	0.064 ^a	0.045 ^a

Different letters in the same row indicate significant differences based on paired t-test ($P < 0.05$).

* Tentative identification for KKFCRC purple yam was previously reported by Srivichai and Hongsprabhas (2020)

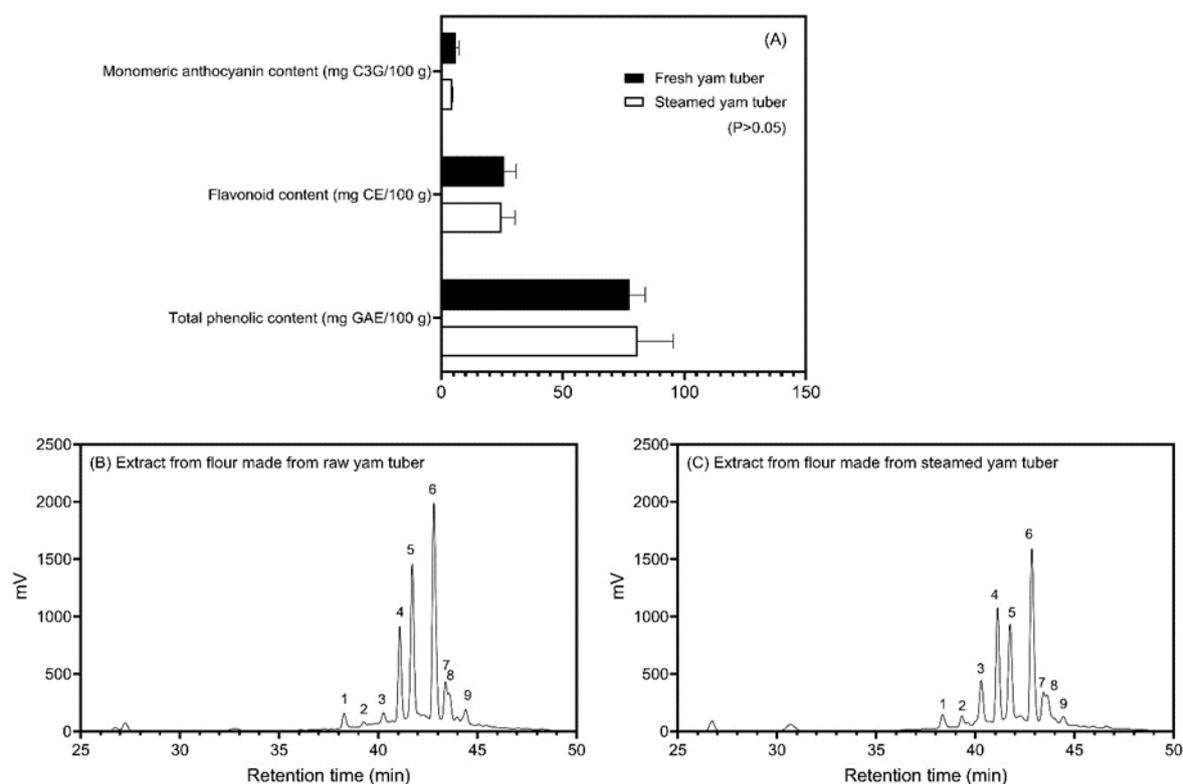


Figure 2. (A) The content of (poly)phenolic compounds in raw and steam-cooked purple yam in EtOH extract, (B) LC-MS/MS chromatograms of raw purple yam and (C) of steam-cooked purple yam. Peak numbers refer to Table 1

ones, was used as the reference anthocyanin for comparison within the same treatment, i.e., raw or steam-cooked yam tuber. The relative ratio of each isomer (compared to alatanin C) was subjected to a paired *t*-test to assess the influence of steam-cooking on the isomers compared to the raw one. Table 1 shows that alatanin B (peak 5), which had the highest MW, decreased, while the smaller MW cyanidin-3 gentiobioside (peak 3), alatanin E (peak 4), and alatanin D (peak 8) increased after steam-cooking. The relative increase in smaller MW anthocyanins in KKFCRC yam tuber may be due to the loss of glucose units, which agreed with the report on alatanin B degradation to cyanidin-3 gentiobioside after

steam-cooking investigated by Sadilova *et al.* (2006).

However, the influences of steam-cooking on the antioxidant capacities of purple sweet potato were different from purple yam (Figure 3). EtOH extract from raw purple sweet potato had a higher antioxidant capacity measured by TEAC assay than raw purple yam (Figure 3A). Steam-cooking drastically increased the antioxidant capacity of purple sweet potato based on ET mechanism ($P < 0.05$) but did not affect the ET-based antioxidant capacity of purple yam ($P \geq 0.05$). Crocin assay, which determined antioxidant capacity based on HAT mechanism, indicated that steam-cooking did not affect the antioxidant capacity of purple sweet potato but

lowered that of purple yam ($P < 0.05$).

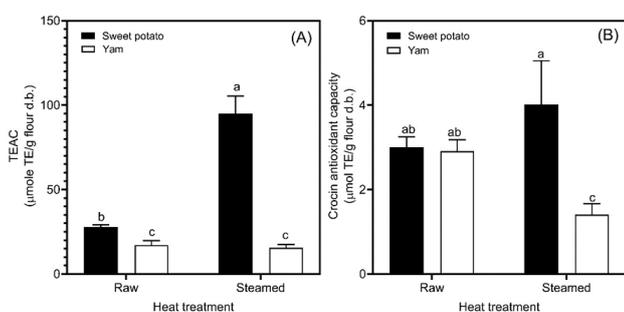


Figure 3. Effect of steam-cooking at household level on antioxidant capacities of purple sweet potato and purple yam: (A) electron transfer mechanism measured by TEAC (ABTS radical scavenging capacity); (B) hydrogen atom transfer mechanism measured by Crocin assay. Bars represent standard deviation

The flour prepared from raw purple sweet potato showed brown color, while the flour made from steam-cooked purple sweet potato showed reddish-purple color (Figure 4). Moreover, steam-cooking increased the contents of total phenolic compounds, flavonoids, and anthocyanins in purple sweet potato (Table 2). Total monomeric anthocyanin and flavonoid contents increased around 13 folds and 5 folds, respectively, while the content of phenolic compounds in steam-cooked sweet potato increased around 4 folds after steam-cooking. Steam-cooking of purple sweet potato tubers not only gelatinized starch but also inactivated the polyphenol oxidase (PPO), thus reducing the PPO browning reactions (Nevara *et al.*, 2018). Steam-cooking of purple sweet potato even at the household level for 30 min could increase the availability of the (poly)phenolic compounds readily for consumption.

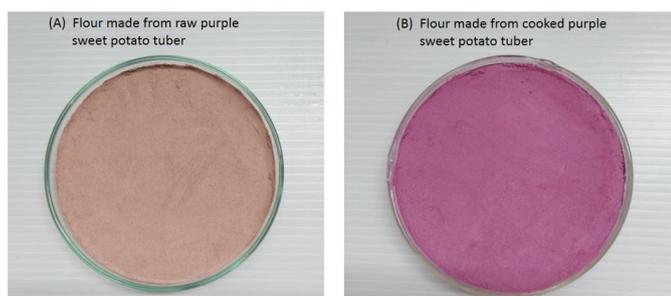


Figure 4. Effect of steam-cooking at household level on the color of purple sweet potato flour from (A) a raw tuber; (B) from a steam-cooked tuber

Phenolic compounds and flavonoids are substrates

Table 2. Effect of steam-cooking at household level on phenolic and polyphenolic compounds in purple sweet potato flour

Polyphenolic constituent	Raw sweet potato tuber	Steam-cooked sweet potato tuber
Total phenolic content (mg GAE/100 g, d.b.)	176.11±24.48 ^b	691.07±46.89 ^a
Total flavonoid content (mg CE/100 g, d.b.)	86.28±14.59 ^b	463.10±29.24 ^a
Total monomeric anthocyanin (mg C3G/100 g, d.b.)	4.54±1.34 ^b	62.81±4.11 ^a

Values are expressed as mean± s.d., n = 2 independent trials. Values with different superscript in the same row indicate significant differences ($P < 0.05$)

for PPO during the enzymatic browning reaction, in the presence of O_2 (Belitz *et al.*, 2009). Raw purple yam and purple sweet potato tubers showed a similar amount of monomeric anthocyanin, i.e., around 4 mg C3G/100 g ($P \geq 0.05$). However, purple sweet potato tubers contained much higher phenolic and flavonoid contents than the KKFCRC yam tubers ($P < 0.05$). The raw purple sweet potato could undergo enzymatic browning during the drying process at 40°C for 24 h to a greater extent than did the purple yam, generating *o*-quinone that polymerized to brown pigment melanin in raw purple sweet potato flour (Figure 4). In contrast, the raw purple yam flour retained the purple color (Figure 1).

In summary, steam-cooking at the household level could help to retain (poly)phenolic compounds in purple yam while making them more available for consumption in purple sweet potato. Nonetheless, the different responses to heat and O_2 of (poly)phenolic compounds are, in part, governed by the contents of indigenous phenolic compounds and flavonoids prone to PPO activities. The insights obtained from this study could be used in tailoring the biosynthesis of phenolic compounds and anthocyanins in the colored tuberous plant breeding program in the future.

Conflict of interest

The authors declare no conflict of interest.

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