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Submission date: 28-Feb-2020 02:00PM (UTC+0700)
Submission ID: 1265834069
File name: Publikasi_ANRES_-_Imas_Arumsari.pdf (374.53K)
Word count: 1576
Character count: 9054

Effects of different cooking methods on the bioaccessibility of polyphenols and antioxidant activity of sweet leaf (*Sauropus androgynus*)

Imas Arumsari[a], Sirichai Adisakwattana[b,c], Sathaporn Ngamukote[b,c], Kittana Mäkynen[b,c,*]

[a] Graduate Program in Food and Nutrition, Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[b] Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[c] Research Group of Herbal Medicine for Prevention and Therapeutic of Metabolic Diseases, Chulalongkorn University, Bangkok 10330, Thailand

Keywords: Antioxidant, Bioaccessibility, Cooking methods, Polyphenols, *Sauropus androgynous*

*Corresponding author.
E-mail address: kittana.m@chula.ac.th (K. Mäkynen).

Abstract

The effects of different cooking methods (boiling, microwaves and raw) on the bioaccessibility of polyphenols and the antioxidant activity of sweet leaf (*Sauropus androgynous*) were investigated during *in vitro* simulated gastrointestinal digest Microwave cooking of sweet leaf can significantly retain more polyphenols and antioxidant capacity (ferric reducing antioxidant power and 2,2-diphenyl-2-picrylhydrazyl radical scavenging activity) after gastrointestinal digestion compared to raw leaves. The findings suggested that cooking sweet leaf with thermal processing such as microwaves or boiling could enhance the bioaccessibility of polyphenols during digestion which may provide better health benefits compared to raw leaves. This information may be useful to optimize culinary aspects of sweet leaf for disease prevention.

Introduction

Phenolic compounds have shown many desirable health benefits and play important roles in the prevention of chronic diseases (Liu, 2003). Nevertheless, dietary factors such as the interaction with the food matrix and differences in cooking methods may affect polyphenol bioavailability during digestion (D'Archivio et al., 2010; Bohn, 2014)

Sauropus androgynus (known as katuk/pakwan ban/cekur manis/sweet leaf) is a nutritious and palatable green leafy vegetable that is grown widely in Southeast Asia. It is rich in polyphenols and has demonstrated high antioxidant capacity (Andarwulan et al., 2012). Sweet leaf is edible as raw fresh leaves in salad or as cooked leaves using heat. However, no studies have been reported on how the different cooking methods affect the amounts of polyphenols and antioxidant activity of sweet leaf. Therefore, the aim of this study was to investigate the effects of different cooking methods (boiling and microwaves, compared to raw leaves) on the bioaccessibility of polyphenols and the antioxidant capacity of sweet leaf during *in vitro* simulated gastrointestinal digestion.

Materials and Methods

Sample preparation and cooking processes

Fresh sweet leaf (*Sauropus androgynus*) was purchased from a farmer in Nakhon Pathom, Thailand and the plant was identified by a botanist from the Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. The leaves were cleaned and homogenized using a kitchen blender before cooking. Raw(control) leaves of homogenized fresh sweet leaf were blanketed under nitrogen and kept as the raw sample. The boiled sample involved cooking the homogenized sweet leaf in boiling distilled water (1:1, weight per volume, w/v) for 5 mins. The microwaved sample was prepared by mixing homogenized sweet leaf with distilled water (1:1, w/v) and cooking in a microwave oven at 800 W for 90 s. All samples were blanketed with nitrogen and stored at -20°C until analyses.

In vitro simulated gastrointestinal digestion

The homogenized sweet leaf samples were digested according to the *in vitro* simulated gastrointestinal digestion procedure adapted from Pasukamonset et al. (2016). Briefly, 1 g of homogenized sweet leaf sample was incubated at 37°C in a shaking water bath for 1 hr with 3 mL porcine pepsin solution (40 mg/mL in 0.1 N HCl), at pH 2.0±0.1 to initiate the gastric phase. Then, the small intestinal phase was started by increasing the pH to 4.5 before the addition of amyloglucosidase solution (120 mg/mL). After 30 min at 37°C with shaking, the pH was increased to 5.3 before the addition of 9 mL of small intestinal enzyme solution containing pancreatin (3 mg/mL) and bile acid (12 mg/mL) in 100 mM $NaHCO_3$. The final volume was increased to 20 mL with bile salts solution and the pH was adjusted to 7.2±0.1, and then incubated with shaking at 37°C for 2 hr. The supernatant (aqueous fraction) was collected after centrifugation of digesta (12,000 rpm, 5°C for 1 hr), filtered through a 0.22 µm nylon filter and stored at -20°C for further analyses.

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu assay as described by Chusak et al. (2014). The results were expressed as milligrams of gallic acid equivalent per gram of sample.

Determination of DPPH radical scavenging activity

2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined as described by Chusak et al. (2014). The results were expressed as mg ascorbic acid/g sample.

Determination of Ferric Reducing Antioxidant Power

The ferric reducing antioxidant power (FRAP) was determined as described by Chusak et al. (2014). Results were expressed as micromoles of $FeSO_4$ equivalent per gram of sample.

Statistical analysis

Each experiment provided three independent replicates. All data were presented as mean ± SE. Statistical analysis was performed using the SPSS 16.0 software (SPSS Inc. Chicago, IL, USA). Differences among groups were determined using one-way analysis of variance followed by multiple comparisons. Differences between before and after digestion within the same cooking method were determined using Student's t-test. Statistically significant differences were considered at $p < 0.05$.

Results and Discussion

As shown in Table 1, with all cooking methods, the total phenolic compounds of sweet leaf after gastrointestinal digestion released more than that before digestion. This finding was similar to the study of Tagliazucchi et al. (2010) which demonstrated that incubation with pancreatic solution increased the release of flavonoids.

Table 1 Effect of different cooking methods on total polyphenols and antioxidant activity before and after *in vitro* simulated gastrointestinal digestion*

Cooking method	Total phenolic content (mg GAE/g)		FRAP (mmol Fe(II)/g)		DPPH (mg ascorbic acid/g)	
	Before	After	Before	After	Before	After
Raw (control)	23.73 ± 0.79^{a}	$34.38 \pm 0.90^{a,\dagger}$	1.61 ± 0.07^{a}	$0.78 \pm 0.02^{a,\dagger}$	16.96 ± 0.55^{a}	$13.65 \pm 0.25^{a,\dagger}$
Boiling	16.54 ± 0.45^{b}	$37.82 \pm 2.39^{b,\dagger}$	1.48 ± 0.12^{a}	$1.24 \pm 0.02^{b,\dagger}$	19.54 ± 1.11^{a}	$13.22 \pm 0.89^{a,\dagger}$
Microwaves	26.48 ± 0.80^{c}	$37.27 \pm 2.47^{b,\dagger}$	2.32 ± 0.13^{b}	$1.60 \pm 0.04^{c,\dagger}$	27.83 ± 0.57^{b}	$21.67 \pm 2.47^{b,\dagger}$

DPPH = 2,2-diphenyl-2-picrylhydrazyl; FRAP = ferric reducing antioxidant power. GAE = gallic acid equivalent
* Homogenized sweet leaf samples were analyzed before digestion and after gastric phase for 1 hr and small intestinal phase for 2.5 hr at 37°C.
Data are presented as mean ± SE (n= 3)
[a,b] Different letters indicate significant differences ($p < 0.05$) among different cooking methods
[†] Significant differences ($p < 0.05$) compared with before digestion in the same cooking method

Interestingly, after 1 hr incubation in gastric enzymes (pH 2.0±0.1) and 2 hr incubation in intestinal enzyme (pH 7.2±0.1), cooking sweet leaf using a microwave oven or boiling retained more polyphenols and antioxidant activity after gastrointestinal digestion. This result was in line with Kaulmann et al. (2016) who explained that thermal processing has a positive effect on the bioaccessibility of polyphenols. Heat treatment increases the risk of polyphenol degradation during food preparation, but on the other hand, it disrupts cell walls and facilitates polyphenol release during digestion via modification of the bioactive compound from glycoside to aglycone which is more biologically active (Bohn, 2014; Kaulmann et al., 2016).

The findings in this study suggested that cooking sweet leaf using microwaves or boiling could enhance the bioaccessibility of phytochemical compounds during digestion which may provide better health benefits compared to raw leaves.

Acknowledgements

A Scholarship provided by the Graduate School, Chulalongkorn University, Bangkok, Thailand to commemorate the 72nd anniversary of his Majesty King Bhumibol Adulyadej is gratefully acknowledged. This research was supported by a Grant for International Research Integration: Chula Research Scholar and Grant for Join Funding, Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University.

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