ผลของอุณหภูมิที่มีต่อปริมาณสารพฤกษเคมีและกิจกรรมการต้านอนุมูลอิสระของน้ำฟักข้าว

Effect of Thermal Treatment on Phytochemical Content and Antioxidant Activity of Gac Juice

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บทคัดย่อ

ปัจจุบันฟักข้าวได้รับความสนใจอย่างมากเนื่องจากในผลฟักข้าวมีองค์ประกอบทางพฤกษเคมีที่มีประโยชน์และมีความสามารถในการต้านอนุมูลอิสระ โดยพบมากในส่วนของเนื้อหุ้มเมล็ดของผลฟักข้าว ดังนั้นจึงมีการนำฟักข้าวมาพัฒนาเป็นผลิตภัณฑ์ออกวางจำหน่ายอย่างแพร่หลายในท้องตลาดซึ่งหนึ่งในนั้นคือ น้ำฟักข้าว แต่อย่างไรก็ตาม กระบวนการให้ความร้อนในระหว่างการผลิตเพื่อฆ่าเชื้อจุลินทรีย์ในน้ำผลไม้อาจทำลายสารประกอบทางชีวเคมีบางตัวที่เป็นองค์ประกอบในน้ำผลไม้ได้ ดังนั้นงานวิจัยนี้จึงมีจุดประสงค์ เพื่อศึกษาผลของอุณหภูมิในระหว่างกระบวนการผลิตน้ำผลไม้ต่อการเปลี่ยนแปลงสารเคมีซึ่งเป็นองค์ประกอบหลักของน้ำผลไม้ เช่น บีต้าแคโรทีน ไลโคปีน สารประกอบฟลาโวนอยด์ทั้งหมด และสารประกอบฟีนอลิกทั้งหมด รวมถึงกิจกรรมการต้านอนุมูลอิสระของน้ำฟักข้าวซึ่งเตรียมได้จากเยื่อหุ้มเมล็ด การหาปริมาณของบีต้าแคโรทีน และไลโคปีน ทำได้โดยการวัดค่าการดูดกลืนแสงที่ความยาวคลื่น 450 และ 470 นาโนเมตร ตามลำดับ การหาปริมาณฟลาโวนอยด์ทั้งหมดทำการตรวจสอบโดยใช้การวัดการดูดกลืนแสงจากสีที่เปลี่ยนไปในการเกิดปฏิกิริยาเคมี การหาปริมาณฟีนอลิกทั้งหมด โดยการใช้วิธี Folin–Ciocalteu และการวิเคราะห์หากิจกรรมการต้านอนุมูลอิสระของ น้ำฟักข้าวทำได้โดยการใช้วิธี DPPH assay และ FRAP assay ผลการทดลองแสดงให้เห็นว่าปริมาณของสารพฤกษเคมีที่เป็นองค์ประกอบหลักของน้ำฟักข้าวมีปริมาณเพิ่มขึ้นอย่างมีนัยสำคัญเมื่อทำการเพิ่มอุณหภูมิจาก 60 องศาเซลเซียส ถึง 80 องศาเซลเซียส แต่อย่างไรก็ตาม การให้ความร้อนที่มีอุณหภูมิสูงกว่า 80 องศาเซลเซียสจะส่งผลให้สารพฤกษเคมีที่เป็นองค์ประกอบและกิจกรรมการต้านอนุมูลอิสระลดลง ดังนั้นอุณหภูมิที่เหมาะสมสำหรับการฆ่าเชื้อจุลินทรีย์ในน้ำฟักข้าวโดยยังคงปริมาณของสารประกอบพฤกษเคมีและกิจกรรมการต้านอนุมูลอิสระได้สูงสุดคือ 80 องศาเซลเซียส

คำสำคัญ : *Momordica cochinchinensis* Spreng กิจกรรมการต้านอนุมูลอิสระ ไลโคปีน บีต้าแคโรทีน ฟลาโวนอยด์

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Abstract

Recently, gac (*Momordica cochinchinensis Spreng*) has attracted more interest due to their beneficial phytochemical components and antioxidant activity in the aril part of the fruit. Consequently, a number of new products from gac have been launched to the global market. One of those is gac juice. However, thermal treatment introduced to the juice manufacturing as a method to pasteurize juices may deteriorate some of the biochemical compounds in the juice. Therefore, the aim of this work was to investigate the effect of thermal processing on the change of the main bioactive compounds (beta-carotene, lycopene, total flavonoid and total phenolic acid) and the antioxidant properties of gac juice made from its aril. The quantification of beta-carotene and lycopene were carried out by using spectrophotometer measuring at 450 nm and 470 nm, respectively. The total flavonoid content was determined using the colorimetric method. Total phenolic acid content was accessed by Folin–Ciocalteu assay. The antioxidant capacity of gac juice was analyzed by DPPH free radical scavenging assay and Ferric reducing antioxidant power (FRAP) assay. The result revealed that the quantity of the main phytochemical compounds significantly increased when the temperature was raised from 60 to 80ºC. However, raising temperature above 80ºC resulted in decreasing of the phytochemical compounds and antioxidant activity in gac juice. To conclude, the optimum temperature to retain high amount of the phytochemical compound and antioxidant capacity of gac juice was at 80ºC.

Keywords : *Momordica cochinchinensis* Spreng, antioxidant activity, lycopene, beta-carotene, flavonoid content

Introduction

Gac (*Momordica cochinchinensis* Spreng) belongs to the Cucurbitaceae family, is mostly cultivated in Southeast Asia. Gac fruit exhibits a number of health beneficial effects with bioactive compounds such as fatty acid, carotenoid and polyphenolics (Aoki *et al*., 2002). The phytochemical compounds in gac fruit have shown the antioxidant activities and some biological properties such as reducing the risk of certain type of several cancers such as prostate, digestive-tract cancers and lung cancer, activation of a proper development of the cell membranes and promoting a healthy vision (Goula & Adamopoulos, 2005; Kubola & Siriamornpun, 2011). To gain such health benefits from gac fruit, the functional drink made from aril part of gac fruit has been emerged due to the need for convenience of consumer. Usually thermal treatment is applied to prolong a shelf life of product. However, thermal treatment can destroy some of antioxidants resulting in having less nutritional value than fresh juice preparation (Lo Scalzo *et al*., 2004) Therefore, the objective of this study was to evaluate the effect of thermal treatment on the nutritional quantity of gac juice by determining the total phenolics content, total flavonoids content, lycopene contents, beta-carotene contents and antioxidant activity of fresh gac juice and thermal-treated gac juice prepared from its aril part.

Materials and Methods

Gac juice preparation and thermal treatment

Fresh gac fruits were purchased from a local market in Nakornpathom Province, Thailand. The red aril was scooped out from the half cut gac fruit. Then the seeds were removed from aril part. Gac paste was homogenized and diluted into 20% v/v by distilled water in order to get the concentration of gac juice sold in the market. Five different thermal treatments were conducted: cooked at 60ºC for 2 min, 70ºC for 2 min, 80ºC for 2 min, 90ºC for 2 min and 100ºC for 2 min. Unboiled gac juice was used as control. All samples were filtered and kept at -20ºC for further analyses

Determination of total phenolic content (TPC)

The amount of total phenolic content (TPC) was determined using Folin–Ciocalteu method (Abu Bakar *et al*., 2009). Briefly, 0.25 ml of gac juice were taken into a test tube. To this solution, 1.25 ml of Folin–Ciocalteu reagent (10x dilution) were added and the tube was shaken thoroughly. After 5 min, 0.2 ml of sodium carbonate solution (7.5% w/v) were added and the mixture was incubated at 50ºC for 5 min with intermittent shaking. Absorbance was measured at 760 nm using spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in µg per ml sample.

Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) was determined using the colourimetric method (Abu Bakar *et al*., 2009). Briefly, 100 µl of sample were mixed with 450 µl of distilled water in a test tube followed by addition of 30 µl of 5% $NaNO_2$ solution. After 6 min, 60 µl of a 10% $AlCl_3.6H_2O$ solution were added and allowed to stand for another 5 min before 200 µl of 1 M NaOH were added. The mixture was mixed well using a vortex. The absorbance was measured immediately at 510 nm using a spectrophotometer. Results were expressed as µg rutin equivalents in 1 ml of sample solution (µg RE/ml sample).

Determination of beta-carotene and lycopene

The samples were extracted using the method reported previously with some modifications (Olives Barba *et al*., 2006). A sample (5 ml) was mixed with 100 ml of extraction solvent containing hexane/ acetone/ ethanol: 50:25:25 (v/v/v). The mixture was magnetically stirred during 10 min. Then 15 ml of distilled water were added. The upper layer was collected and placed in a round-bottomed flask. The solvent was removed by using rotary evaporator. Hexane was added to the sample. The absorbance was measured at 450 and 470 nm for beta-carotene and lycopene determination, respectively. The quantities of beta-carotene and lycopene were

calculated using extinction coefficient of 2560 $M^{-1}cm^{-1}$ for beta-carotene and 3450 $M^{-1}cm^{-1}$ for lycopene (Hart & Scott, 1995).

DPPH free radical scavenging assay

Free radical scavenging activity of extracts was assessed using the procedure reported earlier with a slight modification (Gulluce *et al*., 2007). Briefly, 50 µl of sample with five different concentrations (0.1, 0.3, 0.5, 0.7 and 1% v/v) were mixed with 150 µl of a 0.2 mM methanolic solution of DPPH. The mixtures were incubated in the dark condition at room temperature for 30 min. Then, the absorbance at 517 nm was recorded. The DPPH radical scavenging activity was calculated according to the following equation and IC_{50} was expressed as the concentration of 50% of DPPH radical scavenging activity.

$$\%\text{Scavenging activity} = \left(\frac{A_C - A_S}{A_C}\right) \times 100 \quad ...(1)$$

Where A_C is the absorbance of the control and A_S is the absorbance of the sample juice.

Ferric reducing antioxidant power (FRAP) assay

The total reducing capacity of gac juice was determined using FRAP assay (Benzie & Strain, 1996). The FRAP reagent was initially prepared including 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was warmed at 37ºC prior using. 20 µl of gac juice were mixed with 180 µl of the FRAP solution and incubated for 4 min. The absorbance was then recorded at 593 nm using a spectrophotometer. The FRAP values were calculated by standard curves prepared with known concentrations of $FeSO_4$ and expressed as µmol $FeSO_4$/ml sample.

Statistical analysis

Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS software. All experiments were carried out in triplicate. A significant difference was considered at the level of $p<0.05$.

Results and Discussion

Determination of total phenolics content (TPC) and total flavonoid content (TFC)

Phenolic compounds play important roles in the antioxidant activities and free radical scavenging capacities (Govindarajan *et al*., 2007). TPC in gac juice was determined by the Folin-Ciocalteu assay. According to Figure 1, the highest TPC was obtained from the juice prepared

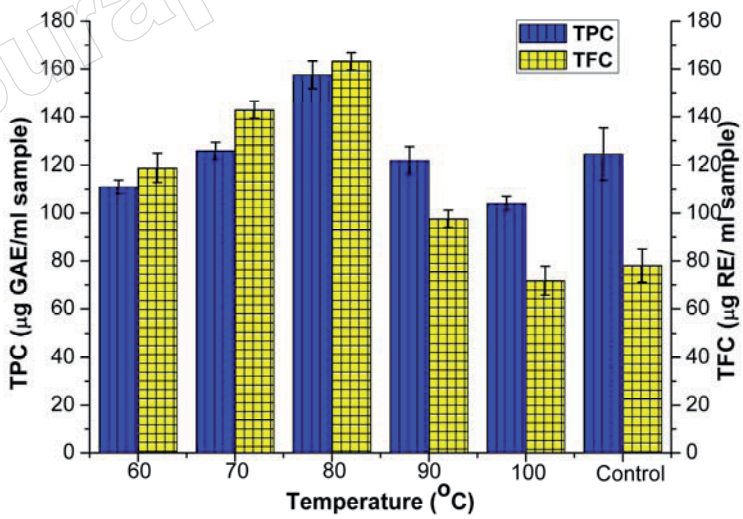

Figure 1 Effect of thermal processing on total phenolic content (TPC) and total flavonoid content (TFC) in gac juice (mean±SD, n = 9).

at 80ºC for 2 min with 124.41±10.88 µg GAE/ml sample, while the lowest value of 104.06±3.02 µg GAE/ml sample was achieved at 100ºC for 2 min.

TFC was examined in comparison to standard rutin. The result demonstrated that the juice prepared at 80ºC for 2 min again showed the highest TFC with 163.20±3.71 µg RE/ml sample, while sample heated at 100ºC revealed the lowest TFC value at 71.75±6.05 µg RE/ml sample. Moreover, TPC and TFC value gradually increased when the temperature raised to 80 ºC then the values declined with increased temperature to 100ºC.

The result suggested that the heat treatment could increase TPC and TFC by liberating covalently bound phenolic and flavonoid compounds from the tissue. However, some of phenolics and flavonoids may degrade at high temperature resulting in decreasing of TPC and TFC (Gazzani *et al*., 1998).

Determination of beta-carotene and lycopene content

Beta-carotene and lycopene are compounds called carotenoids. Carotenoids can act as antioxidant and scavenger for free radicals. It may also reduce the oxidative damage to DNA and inhibit the oxidation of LDL cholesterol (Bohm *et al*., 2003).

The quantity of beta-carotene increased from 0.75±0.01 µmol/ml sample to 0.99±0.02 µmol/ml sample (Figure 2) as well as that of lycopene raised from 0.53±0.01 µmol/ml sample to 0.73±0.02 µmol/ml sample when the temperature increased from 60ºC to 80ºC ($p<0.05$). However, significant loss of beta-carotene content and lycopene content was found when the temperature increased above 80ºC.

Determination of antioxidant activity

DPPH radical scavenging activity

The principle of the DPPH assay is that the antioxidants react with the stable free radical that is α,α-diphenyl-β-picrylhydrazyl (violet) and convert it to α,α-diphenyl-β-picrylhydrazine (yellow). The degree of discoloration indicates the scavenging potential of the antioxidant sample (Abdille *et al*., 2005). The result of DPPH radical scavenging activity of the gac juice was expressed as IC_{50} value in Figure 3. In addition, less IC_{50} value generally presents a high scavenging potential of sample. The result revealed that IC_{50} value of gac juice slowly decreased from 1.12±0.17 ml sample to 1.03±0.09 ml sample when the temperature increased from 60ºC to 70ºC. Interestingly, the lowest IC_{50} values, implying

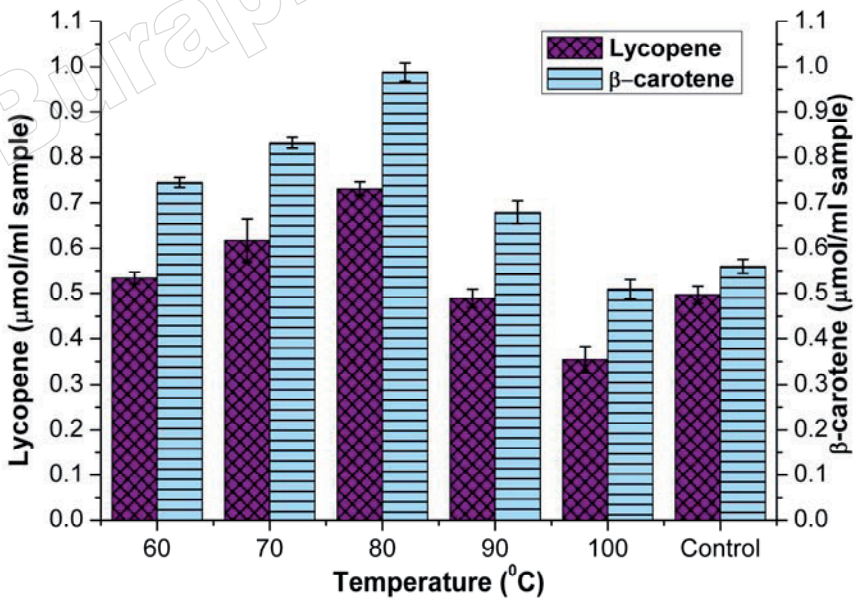

Figure 2 Effect of thermal processing on beta-carotene and lycopene in gac juice (mean±SD, n = 9).

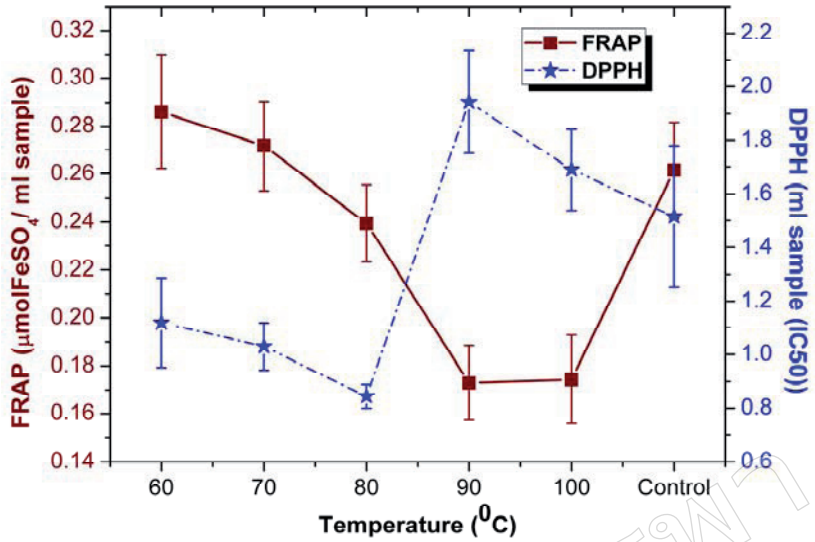

Figure 3 Effect of thermal processing on DPPH radical scavenging activity and reducing power in gac juice (mean±SD, n = 9).

the highest antioxidant activity, was 0.84±0.04 ml sample at 80ºC. Increased temperature up to 100ºC drastically resulted in increasing IC_{50} value. This result was correlated to TPC, TFC, beta-carotene content and lycopene content.

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power assay (FRAP assay) is a method to determine the reducing power of antioxidant sample from the reduction of Fe^{3+} to Fe^{2+} resulting in the change of color from yellow to blue. This blue solution i.e. Fe^{2+}-TPTZ complex, absorbed the visible light at 593 nm. It is worthy to note that the reducing power capacity of compound may serve as a significant indicator of its potential antioxidant activity. The juice heated at 60ºC revealed the highest reducing power with 0.29±0.02 μmol $FeSO_4$/ml sample (Figure 3). Surprisingly, significant loss of FRAP activity was observed when the temperature increased ($p<0.05$). This result may be due to the loss of some antioxidants having the reducing power as major antioxidant activities.

Conclusion

The juice heated at 80ºC for 2 min demonstrated the highest TPC, TFC, beta-carotene content, lycopene content and DPPH radical scavenging activity. Hence, the optimum temperature for thermal processing of gac juice that can retain the maximum phytochemical compounds and antioxidant activity was 80ºC. Further information would still need to be investigated such as microbial and sensory properties to determine if this pasteurization condition (80ºC, 2 min) would appropriate for gac juice preparation.

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