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# Fortification of Rice Grain with Gac Aril (*Momordica conchinchinensis*) Using Vacuum Impregnation Technique

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#### Abstract

The objective of this research was to improve functional property of rice by fortification with gac aril using vacuum impregnation (VI) process. Effects of rice variety, preparation method and VI condition on gac aril fortified rice quality were investigated. Sao Hai (SH) and Khaw Dok Mali 105 (KDML 105) were prepared to achieve polished and unpolished rice. The samples were impregnated with 30% gac aril solution under VI condition (vacuum pressure 500 mmHq), 0% gac solution was used as a control. After drying, the samples were analyzed for physicochemical property i.e. texture, color L\*, a\* and b\*, total fiber, lycopene, β-carotene and total phenolic (TPC) content and antioxidant activity (DPPH assay) as well as sensory quality (9-point hedonic scale). The results showed that both varieties of unpolished rice had higher hardness and darker colour than polished samples. Unpolished SH impregnated with gac aril showed the highest content of  $\beta$ -carotene (22.10±0.83 mg/g), lycopene (8.38±0.11 µg/g), and TPC (0.24±0.03 mg GAE/g) while antioxidant activity of all gac aril fortified samples were not significantly different (p>0.05) (DPPH value ranged 1.39-1.72 mmol TE/g) and higher than control. However, sensory evaluation showed that gac fortified unpolished KDML 105 had the highest score of the overall acceptability. Based on all properties, unpolished KDML 105 was chosen for studying the suitable VI condition in further step. Unpolished KDML 105 was soaked in 30% gac aril solution under different vacuum pressures (0, 300 and 500 mmHg) and pressurizing techniques (single and pulse pressure). The dried samples were analyzed and it was found that the high vacuum pressure at 500 mmHg and pulse pressure gave the highest  $\beta$ -carotene (41.41 ± 4.53 mg/g), lycopene (25.07 ±1.74 µg/g) and TPC (0.21±0.03 mg GAE/g) content and DPPH value (2.91±0.90 mmol TE/ g), while physical properties, texture and sensory were not significantly different (p>95%). In addition, glycemic index (GI) of rice after VI process was decreased from 84.24 to 72.04. Finally, it can be concluded that non-polishing process, high vacuum pressure and pulse pressurizing are the suitable condition to prepare gac aril fortified rice. The health benefits of rice are improved with high antioxidant activity and lower GI.



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#### Keywords

Antioxidant activities, Gac aril, Rice, Vacuum impregnation.

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Increasing demand of healthy food products by consumers is of concern nowadays. There are various approaches to escalate intake of healthpromotion or disease prevention foods include dietary diversification and direct fortification of functional components into foods<sup>1</sup>. Enrichment of staple foods is one of the practice to improve the quality of the foods and provide public health benefit with minimal risk to health<sup>2</sup> and could be easily implemented due to regular consumption of staple foods by the population in sufficient amounts<sup>1</sup>.

Gac (Momordica cochinchinensis Spreng) is a tropical fruit that is extraordinary sources of carotenoids ( $\beta$ -carotene and lycopene), mainly in the red seed aril<sup>3</sup>.  $\beta$ -carotene is the dominant carotenoid with concentration as high as 35,500 µg/100 g in ripe gac fruit4. Gac aril covers 24.6% of the whole fruit<sup>5</sup>. In aril, lycopene concentration was ranged from 2.378 to 3.728 mg/g FW and those of  $\beta$ -carotene were from 0.257 to 0.379 mg/g FW<sup>6</sup>. For this reason, gac fruit has been used for food and traditional medical purpose in East and Southeast Asia7. It is commonly consumed as fresh fruit, healthy drink product or gac fruit powder as natural colorant<sup>8</sup>. Vuong and colleagues<sup>9</sup> showed that the concentration of lycopene was as high as 705  $\mu$ g/g, with an average of 408  $\mu$ g/g and as much higher than the lycopene concentration usually found in tomatoes, 25 µg/g.

Rice is most consumed staple food in Asia. Rice can be eaten alone or with another condiment/ seasonings. In Vietnam, Xoi gac or red rice is a popular dish. Xoi gac is made by mixing rice grain with fresh gac aril before steaming. It could be consumed for dietary as well as medical uses and as rice colorant due to its intense red color from its high carotenoid content. It was reported that daily consumption of Xoi gac significantly improved plasma level of retinol,  $\alpha$ - and  $\beta$ -carotene and lycopene in pre-school children after 30 days of supplementation<sup>10</sup>. Most popular Thai rice are Sao Hai (SH) and Khaw Dok Mali 105 (KDML 105). KDML is aromatic rice with low amylose content and the grains are clear and translucent<sup>11</sup>. Based on Bureau of Rice Research and Development<sup>12</sup> KDML has natural fragrant aroma depending on its age, and when cooked, rice kernels shall have a tender texture, while SH rice is relatively less glutinous but more fluffy, crumbly and do not form any sticky lumps<sup>13</sup> compared to KDML. The amylose content of SH is 27-28% and the glycemic index level is medium to low<sup>14</sup>.

Vacuum impregnation (VI) method is a method to enhance fortification of compounds from the solution into food structure under pressure difference. At primary phase of VI, vacuum condition removes water and gas from the cells following with the filling of intracellular capillaries with the desired solutes when return to atmospheric pressure at the secondary phase<sup>15</sup>. VI can be applied in various food systems to extend shelf life, improve organoleptic quality and microbial safety<sup>16</sup>, enrich nutritional/functional ingredients<sup>17</sup>, reduce the freezing damage<sup>18</sup>, reduce oxidative reaction<sup>19</sup> and reduce browning reaction<sup>20</sup>. From an engineering point of view, VI renders the fast process, low energy cost and the external solution may be reused many times<sup>19</sup>.

The objective of this research was to study the suitable process to produce healthy rice by fortification of gac aril into rice under VI condition. The influence of rice variety and rice preparation process as well as VI condition on physicochemical and sensory property of rice were evaluated.

#### Materials and Methods Raw Material

SH paddy was kindly provided by Jek Choei company, Saraburi. KDML 105 was purchased from local rice producer in Khon Kaen, Thailand. The paddy was grown in the same crop of the same year.

#### Chemical

Potassium chloride, pepsin from porcine gastrin mucosa, maleic acid,  $\alpha$ -amylase, potassium, glacial acetic acid, sodium acetate, pure potato starch, amyloglucosidase, pancreatin, ethanol, sodium hydroxide, potassium iodide, 2,2-diphenyl-1-picrylhydrazyl, trolox, hydrochloric acid, methanol, butylated hydroxytoluene, gallic acid anhydrate, Folin-Ciocalteu reagent, and sodium carbonate were purchased from Sigma Aldrich (Arklow, Ireland). Acetone and methanol were obtained from Merck (Singapore). Glucose GOD-POD kit was purchased from Megazyme (Bray, Ireland). Diethyl ether and n-hexane was obtained from RCI Labscan (Bangkok, Thailand), whereas tris(hydroxymethyl) aminomethane was bought from Thermo Fischer Scientific (Waltham, Massachusetts, USA).

#### **Preparation of Rice**

The paddy was sorted to remove fleas and insects, then weighed as rough rice mass. Each variety of rice was milled by a miller (Mitsubishi Electric type SC-KR, Thailand) to obtain brown rice and further polished to produce white rice. Polishedand unpolished rice were dried at 45°C for 12 hr or until the moisture content was less than 14%. Rice samples were stored in PVC vacuum plastic bag at room temperature prior to analysis.

#### **Preparation of Gac Aril Solution**

Gac fruit has been reported for its high carotenoid content. Among peel, pulp and aril of Thai gac, aril was noted for the highest lycopene and  $\beta$ -carotene<sup>21</sup>. Therefore, gac aril was chosen to fortify in the rice grain to improve the health benefits of the staple food. Gac at fully ripe stage, which has been shown to possess the maximum antioxidant content<sup>5,21</sup>, as indicated by red color and soft texture was purchased from local farm in Chiang Rai. The seeds and aril were removed from fruits and stored at -20°C. The frozen samples were thawed at 5°C prior to the aril collected. Distilled water was used to prepare gac aril solution. The solution was homogenized and used immediately to prevent changes of quality.

#### Vacuum Impregnation Equipment

The VI equipment comprises vacuum container and vacuum pump. Gac aril solution was added into the vacuum container. The rice grain was submerged sufficiently within the gac aril solution, ratio of rice to gac aril solution was 1:2. The container was tightly closed prior to the air inside was eliminated by vacuum pump. The pressure was controlled according to the desired level. Single pressure was conducted by constantly applying vacuum pressure for 60 min (t1) following with atmospheric pressure for 60 min (t2). Pulse pressure was carried out by controlling shorter length of t1 (15 min) and t2 (15 min) for four cycles until total time was 120 min.

#### Effects of Rice Variety and Preparation Process on Quality of Gac Aril Fortified Rice

The purpose of study was to determine the effect of rice variety and the preparation process on physicochemical and sensory property of rice grain after impregnation with 30% gac aril solution. SH and KDML 105 were employed and the processing applied was polishing and non-polishing. The rice samples were impregnated with solution at different aril concentration (0 and 30%) under single VI condition of 500 mmHg vacuum pressure where t1 and t2 was equally 1 hr. After impregnation, the rice grain was subjected to drying by a tray dryer at temperature of 45°C for 12 hr or until moisture content was <14%. The fortified rice quality was further determined.

### Study of Suitable VI Condition for Gac Fortified Rice Production

Optimum VI condition was investigated. Vacuum pressure (0, 300 and 500 mmHg) and single/pulse processes were applied during VI in the 30% gac aril solution whereas rice without VI treatment was served as control. The final products were analyzed for physicochemical and sensory quality.

#### Physical Properties Analysis Color Determination

Color of samples was determined according to International Commision on Illumination using HunterLab (ColorQuest XE, USA)<sup>22</sup>. Lightness, redness and yellowness were measured as L\*, a\* and b\* value, respectively.

#### Hardness and Stickiness Determination

Texture analyzer (TA-XT2i, USA) was used to determine hardness and stickiness of cooked rice. Based on Leelayuthsoontorn and Thiparat<sup>23</sup>, cooked rice sample was prepared and weighed (15 g), then placed inside the test cylinder of 6 cm diameter and pressed with 100 g weight for 30 seconds before conducting the actual test. A spherical plate plunger of 35 mm diameter was employed. Pre-test speed, test speed and post-test speed of plunger were 1.0, 1.0 and 10 mm/s, respectively. Target value (10 mm) and trigger load (0.5 g) were altered. Compression distance was set at 50% strain. Hardness was indicated by the maximum compressive force during

extrusion (g) and stickiness was area under the curve (g).

#### Chemical Properties Analysis

### Rice Extraction (for Determination of DPPH and TPC Content)

The extraction method was modified from Jun and colleagues<sup>24</sup>. One g of rice powder was mixed with methanol (ratio of rice powder to methanol was 1:3 w/v) at  $60^{\circ}$ C for 20 min. The methanol was choosen due to its polarity. The supernatant was separated from the residue by centrifuge at 10,000 rpm for 10 min.

#### **Glycemic Index Determination**

The method was introduced by Goni and colleagues<sup>25</sup>. Rice was cooked, then underwent freeze drying prior to grinding. Rice powder (50 mg) was mixed with 10 ml HCl-KCl pH 1.5 buffer. The mixture was homogenized for 2 min and 0.2 ml pepsin solution, 1 mg pepsin in 10 ml HCI-KCI buffer pH 1.5, were added following with incubation at 40°C for 60 min in shaking water bath. TRIS-Maleate buffer, pH 6.92 (25 ml) was included into the mixture. Starch hydrolysis was initiated by adding 5 ml (2.6 UI)  $\alpha$ -amylase in TRIS-maleate buffer and placed in shaking water bath at 37°C. An aliquot sample (1 ml) was taken every 30 min during 0-3 hr and heated for 5 min at 100°C to inactivate the enzyme. To the sample, 3 ml sodium acetate buffer (0.4 M, pH 4.75) was incorporated. Amyloglucosidase (60 µl) was used to hydrolyze digested starch into glucose after 45 min at 60°C. Duplicated aliquots of 0.5 ml were incubated with glucose GOD-PAD kit. The color reaction was measured in a UV/VIS spectrophotometer at the wavelength 505 nm. Digestion rate was expressed as the percentage of glucose in each sample (mg glucose/100 mg) at each time interval. Hydrolysis curve was built and the area below the hydrolysis curves (AHC) was calculated. A hydrolysis index (HI) was calculated by comparison of AHC of each sample and the AHC of a reference food (white bread). GI was then estimated in relation to HI value.

#### **Amylose Content Determination**

The amylose content determination was conducted according to the method of Juliano<sup>26</sup>. Pure potato starch was used as a standard. To 1 ml of 95% ethanol and 9.0 ml of 1 M NaOH, 40 mg of

pure potato starch was added. The mixture was shaken and boiled over water bath for 10 min. The concentration of amylose ranged 0-1 mg/ml were made. The absorbance of the standard was read at 620 nm and a standard graph was plotted. Rice powder sample (100 mg) was mixed with 1 ml of 95% ethanol and 9.0 ml of 1 M sodium hydroxide and left overnight. To the sample, 100 ml distilled water was added. Volume of 5 ml of sample was mixed with 1 ml of 1 M acetic acid and 2 ml of iodide solution and made up volume to 100 ml by distilled water. The mixture was stirred and allowed to stand for 20 min. The absorbance value was read with the spectrophotometer at wavelength 620 nm.

#### **DPPH Scavenging Activity Determination**

The DPPH assay was conducted to determine reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) concentraion according to the method of Molyneux<sup>27</sup> using trolox as standard. Rice extract (1.95 ml) was added with 60  $\mu$ M DPPH solution and left in the dark for 30 min at room temperature. Absorbance of the samples was measured at 517 nm using methanol as blank. The radical scavenging activity was expressed as mmol trolox equivalent (TE)/g dry weight samples.

#### **Insoluble Dietary Fiber Analysis**

Insoluble dietary fiber determination was conducted according to AOAC<sup>28</sup>. One gram of defatted dried samples was added to 20 ml of sodium phosphate buffer pH 6.0 and mixed. To the mixture, 0.1 ml of  $\alpha$ -amylase was included and covered with alumunium foil. The sample was then heated up in a shaking water bath at temperature of 100°C for 15 min. After cooling down, the pH was adjusted to 1.5 with HCl solution. The mixture was added with 1 ml pepsin, incubated in a shaking water bath at 40°C for 60 min and then the pH was adjusted to 6.8 with NaOH solution. 100 mg of pancreatin was mixed and re-incubated at 40°C for 60 min prior to pH adjusted to 4.5 with HCl solution. The mixture was filtered and washed with 2x 10 ml of distilled water. The residue in crucible was washed with 2 x 10 ml ethanol and 90% acetone, then dried at a temperature of 105°C overnight and weighed. The sample was put in muffle furnace at 550°C for 5 hr. The final weight was recorded and % insoluble dietary fiber was calculated.

 $\beta$ -Carotene content analysis was conducted according to the method described previously<sup>29</sup>. Rice powder (3 g) was extracted with 10 ml of the mixture of n-hexane and diethyl ether (9.5:0.5) prior to centrifuge at 15,000 rpm for 5 min. Absorbance was read at 454 nm.  $\beta$ -Carotene was calculated and reported as mg/100g.

#### Lycopene Content Determination

Determination of lycopene content was carried out according to the established method by Fish and co-researchers<sup>30</sup>. Sample (0.6 g) was added to 5 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) and vortexed. Five ml of 95% USP grade ethanol and 10 ml of hexane were added sequentially. The mixture was centrifuged at 1,500 rpm at 5°C for 15 min. After shaking, 3 ml of distilled water was added and continue centrifuged for an additional 5 min at 1,500 rpm. The mixture was left at room temperature for 5 min. Absorbance of the upper, hexane, layer was measured at 503 nm with n-hexane as the blank.

#### **Total Phenolic Content Determination**

Total phenolic content (TPC) of extracts was determined by Folin Ciocalteu method as described by Materska and Perucksa<sup>31</sup>. Briefly, 1 ml of sample extract was mixed with 5 ml of fresh Folin-Ciocalteu reagent and left to stand at room temperature for 5 min. Four ml of 7.5% (w/v) sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) was added to mixture and allowed to completely react for 60 min at room temperature in dark condition. The absorbance of blue color was read with spectrophotometer at 765 nm. The phenolic content was expressed as mg of gallic acid equivalent (GAE)/100g FW.

#### **Sensory Evaluation**

Sensory evaluation was conducted by a total of 30 untrained panelists. Sensory attributes evaluated were color, odor, taste, texture and overall acceptance by the 9-point hedonic scale where 9 point category scale was labeled as '1=dislike extremly 5= neither like nor dislike, and 9= like extremely<sup>32</sup>.

#### **Statistical Analysis**

Statistical analysis was carried out according to SPSS 22.0 software program. Analysis of variance was performed by the General Linear Model. Duncan's multiple range tests were used to determine significant differences between the means. Mean differences were considered significant at the  $P \le 0.05$  level.

#### **Results and Discussion**

#### Effect of Rice Variety and Processing on Physical Quality of Rice Products

Different rice variety and preparation process affected the hardness and stickiness of rice significantly. Table 1 shows that SH variety had higher value of hardness and low value of stickiness compared to KDML 105. Okonogi and colleagues reported that amylose content of SH was  $21.8 \pm 0.3\%$  whereas that of KDML 105 was 17.5 ± 0.5%14. Based on González and co-researchers, rice with higher amylose and long chain amylopectin tended to have hard cooking properties<sup>33</sup>. The addition of gac aril solution decreased the hardness and increased the stickiness value. The decreasing value of hardness could happen due to increasing of porosity of rice. Yu et al., showed that the texture of brown rice could be improved when structure of rice was altered and allowed easier water penetration into the rice grain during cooking<sup>34</sup>. It is thought that VI process removed the air from structure of rice<sup>15</sup>. As a result, the porosity of the rice grain structure might increase, hence softer texture is obtained.

Because of the remaining of brown husk, unpolished rice had dark, more intense of red and yellow color, as indicated by low L\* and high in a\* and b\* values, respectively, than polished rice (Table 1). Moreover, it was observed that the addition of gac aril increased the redness and yellowness, but decreased the lightness of the rice. Absorption of natural red color of gac aril, due to high amount of  $\beta$ -carotene and lycopene, was thought to be the reason of these results. According to Rahman and colleagues, L\*, a\*, and b\* values of gac aril were 26.62±1.10, 36.35±1.24, and 27.62±2.63 respectively<sup>35</sup>. Comparison of different varieties, aromatic rice KDML 105 tended to have darker color than SH. It was thought to be due to the lower amylose content in KDML 105 compared to SH as previously reported<sup>36</sup>. Study in Egyptian rice varieties, the cooked grain whiteness was adversely correlated with the amylose content where the higher amylose content showed darker color.

Rice variety	Processing	Gac (%)	Hardness (g)	Stickiness (g)	L*	a*	b*
SH	Unpolished	0	$61.98 \pm 0.76^{a}$	$-4.80 \pm 0.76^{a}$	67.24 ± 0.91°	2.71 ± 0.41 <sup>d</sup>	15.68±0.89 <sup>d</sup>
SH	Polished	0	54.57 ± 0.58 <sup>b</sup>	$-7.28 \pm 0.58^{b}$	$70.28 \pm 0.73^{a}$	$1.37 \pm 0.48^{\text{ef}}$	12.72±1.59 <sup>f</sup>
KDML	Unpolished	0	$8.41 \pm 0.58^{d}$	$-9.89 \pm 0.59^{\circ}$	$62.26 \pm 0.34^{d}$	2.21 ± 0.25 <sup>de</sup>	14.12±0.58 <sup>e</sup>
KDML	Polished	0	$8.35 \pm 0.93^{d}$	-9.89 ± 0.93°	$68.06 \pm 0.44^{b}$	$0.90 \pm 0.15^{f}$	11.48±1.27 <sup>f</sup>
SH	Unpolished	30	55.76 ± 0.42 <sup>b</sup>	$-3.73 \pm 0.42^{a}$	53.74 ± 0.42 <sup>e</sup>	11.55 ± 1.37 <sup>b</sup>	19.81±0.57℃
SH	Polished	30	40.49 ± 0.28°	$-3.97 \pm 0.25^{a}$	$62.25 \pm 0.65^{d}$	10.35 ± 0.59°	24.64±0.51ª
KDML	Unpolished	30	$8.29 \pm 0.57^{d}$	-8.23 ± 0.59b	51.24 ± 0.23 <sup>f</sup>	10.99 ± 1.74 <sup>bc</sup>	19.21±1.43°
KDML	Polished	30	$8.29 \pm 0.59^{d}$	$-8.59 \pm 0.59^{bc}$	$51.41 \pm 0.27^{f}$	$13.94 \pm 0.40^{a}$	22.47±0.74 <sup>b</sup>

Table 1: Effect of rice variety and processing on physical properties of products

Different letters indicate significant differences at the same column (p≤0.05).

SH = Sao Hai and KDML= Khaw Dok Mali 105

#### Effect of Rice Variety and Processing on Chemical Property of Rice Products

Rice variety and the processing led to a significant difference of insoluble dietary fiber. Without the addition of gac aril solution, unpolished rice had higher insoluble dietary fiber compared to the polished rice (Table 2). Abdul-Hamid and Yuan illustrated that rice bran contained high insoluble dietary fiber (24.99 ± 0.43 %) and soluble dietary fiber  $(2.25 \pm 0.13 \%)^{37}$ . According to previous studies, rice bran has been reported to encompass high amount of dietary fiber and being added for healhty snacks and breakfast<sup>38-39</sup>. However, polishing could eliminate the bran and consequently reduce dietary fiber of the rice. Insoluble dietary fiber was recorded to be higher in SH than KDML 105. This is thought to be due to the higher amylose content in SH than KDML 105<sup>40</sup>. Total dietary fiber in sample of four indica rice cultivars in Yangzhou, China positively correlated with the amylose content<sup>41</sup>. Improved dietary fiber composition was found in rice containing high resistant and less digestible starch. The transgenic rice line generated from the cultivar 'Te-qing' showed higher total dietary fiber than the other cultivars which have lower amylose content.

Prior to addition of gac aril it was noted that the insoluble dietary fiber decreased in unpolished rice and increased in the case of polished rice. According to Nagarani and co-researchers, total dietary fiber in gac fruit (about 1.1 %) is comparatively lower than rice husk<sup>42</sup>. Therefore, substitution of gac aril led to a decrease in dietary fiber content in unpolished

rice. However, polished rice contained much lower insoluble fiber than unpolished rice. Its dietary fiber was increased by the addition of gac aril (Table 2).

It was observed that all samples without gac fortification had very low lycopene and  $\beta$ -carotene content. Recently, there was an attempt to increase carotenoid content in Golden rice and the amount was increased up to approximately 0.8 µg/g<sup>43</sup>. Since lycopene and  $\beta$ -carotene are major phytochemicals reported in gac fruit, lycopene and  $\beta$ -carotene content of rice significantly increased by the addition of gac aril as expected (p≤0.05). Unpolished SH with the addition of gac aril solution have the highest amount of lycopene and  $\beta$ -carotene, followed by polished- and unpolished KDML and polished SH respectively.

It has been reported that total TPC content of the bran of Thai rice is different and it is correlated to their color where white, red and black rice bran extracts contain TPC in the range of 0.8931-0.9884, 1.0103-1.0494 and 1.0810-1.2239 mg GAE mg/ g, respectively<sup>44</sup>. Therefore, it is not surprising that polished rice had significantly lower TPC than unpolished rice in the current study (Table 2). Zhou and colleagues reported that, in the rice, the phenolic compounds are localized mainly in the external layers of the grain<sup>45</sup>. Addition of gac aril slightly increased TPC content in rice. Based on Kubola and Siriamornpun, gac aril contains substantial amount of TPC being  $4.29 \pm 0.15$  mg GAE/g<sup>46</sup>. TPC content in brown KDML 105 varied from

0.6 – 1.3 mg GAE/g whereas that in polished samples were ranged 0.5 – 0.7 mg GAE/g. As far as we know, there was no information about TPC value in SH rice, but TPC content of long grain rice has been reported at similar value (0.32 mg GAE/g)<sup>47</sup>. In fact, correlation of antioxidant content and DPPH value was reported. It was expected that

unpolished rice showed greater antioxidant activity than the polished samples and fortification of gac aril significantly increased DPPH value. Based on Chantarangsee<sup>48</sup>, DPPH value in gac aril was 2.05 mg TE/g which is considerably higher than that in KDML 105 (0.01 mmol TE / g)<sup>49</sup>.

Rice variety	Processing	Gac (%)	Insoluble fiber(%)	Lycopene (µg/g)	$\beta$ -Carotene (mg/g)	TPC (mg GAE/g)	DPPH (mmol TE/g)
SH	Unpolished	0	$4.52 \pm 0.71^{a}$	0.05±0.02 <sup>e</sup>	0.49±0.14°	0.18±0.02 <sup>ab</sup>	1.04±0.47 <sup>bc</sup>
SH	Polished	0	0.72 ± 0.48°	0.02±0.02 <sup>e</sup>	0.4227±0.11°	$0.06 \pm 0.00^{d}$	0.36±0.20 <sup>d</sup>
KDML	Unpolished	0	$2.60 \pm 0.09^{abc}$	0.22±0.04 <sup>e</sup>	0.47±0.32 <sup>e</sup>	0.16±0.02 <sup>bc</sup>	0.77±0.13 <sup>bc</sup>
KDML	Polished	0	1.72 ± 0.45°	0.01±0.02e	0.32±0.17 <sup>e</sup>	0.06±0.01 <sup>d</sup>	0.40±0.19 <sup>d</sup>
SH	Unpolished	30	$3.93 \pm 1.24^{ab}$	8.38±0.11ª	22.10±0.83ª	0.24±0.03ª	1.39±0.01 <sup>ab</sup>
SH	Polished	30	1.95 ± 1.11 <sup>bc</sup>	3.64±0.60 <sup>d</sup>	13.65±1.83d	0.11±0.00°	1.66±0.09 <sup>a</sup>
KDML	Unpolished	30	1.61 ± 0.51°	5.88±0.47°	17.29±1.12°	0.19±0.00 <sup>ab</sup>	1.62±0.11ª
KDML	Polished	30	$1.84 \pm 1.37^{bc}$	7.04±0.91 <sup>b</sup>	20.27±0.14 <sup>b</sup>	0.18±0.04 <sup>b</sup>	1.72±0.04ª

Table 2: Effect of rice variet	y and processing on chem	ical property of rice products
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Different letters indicate significant differences (P≤0.05) at the same column.

SH = Sao Hai, KDML = Khaw Dok Mali 105, TPC = total phenolic content, DPPH = 2,2-diphenyl-1-picryl-hydrazyl

#### Effect of Rice Variety and Processing on Sensory Property of Rice Products

Table 3 shows sensory scores of the samples. All samples had similar scores for armoa attribute whereas gac aril fortification tended to improve the acceptability scores of appearance, color and texture of rice but not significantly different in all samples. However, it is noteworthy to point out that higher scores in appearance, texture and overall acceptability were observed in unpolished KDML 105 containing gac aril. In SH, the texture acceptability was slightly higher in polished rice compared to brown rice in both with and without gac aril. In case of KDML 105, texture acceptability of polished cooked rice was slightly lower than unpolished rice. From the hardness value (Table 1), it can be seen that the harder texture was not preferable by consumer as indicated from low score in texture in those samples.

Based on chemical properties as affected by different rice variety and processing, gac aril fortification in unpolished SH was considered to be the best condition with the slightly higher in lycopene,  $\beta$ -carotene and TPC content. However, sensory evaluation showed that unpolished SH had lower

score than that in unpolished KDML 105 with gac aril. Therefore, unpolished KDML 105 was chosen to study the VI condition in the next step.

### Effects of VI Condition on Physical Property of Gac Aril Fortified Rice

According to previous study, pulsed pressure application in osmotic dehydration process increased the pickling rate of salted egg compared to traditional processing<sup>50</sup>. It was thought that pulse pressure may enhance absorption of gac aril into rice grain during VI process. Table 4 shows that hardness of rice was slightly decreased at the highest vacuum applied and pulse pressure treatment. When single and pulse pressure was considered, it was noted that pulse pressure led to a lower hardness of the product. Del Valle and his colleagues reported in various fruits, apple, banana and peach, that apparent porosity increased as the absolute pressure of the vacuum pulse decreased, probably as a result of tissue damage or deformation-relaxation phenomena<sup>51</sup>. As the deformation and relaxation phenomena happens more frequently on pulse pressure, the structure will be more porous and caused a decrease of the hardness. On the other hand, stickiness was not significantly different (p>0.05). However, higher vacuum pressure and the pulsing technique applied slightly increased stickiness of rice (Table 4). Similar results were observed in case of color. Increasing of vacuum pressure in combination with pulse pressure reduced the lightness of rice as indicated by L\* value, but a\* and b\*, on the other hand, increased which infers that the rice became more red and yellow.

Rice variety	•	Gac (%)	Appearance	Color	Texture	Aroma	Taste	Overall
SH	Unpolished	0	4.93 ± 2.21⁵	4.87 ± 1.98 <sup>b</sup>	4.23 ± 1.89°	5.07 ± 2.18ª	4.63 ± 2.19 <sup>b</sup>	4.60 ± 1.92 <sup>b</sup>
SH	Polished	0	$5.37 \pm 1.81^{ab}$	$5.60 \pm 1.83^{ab}$	$5.83 \pm 1.60^{ab}$	$6.17 \pm 1.56^{a}$	$6.30 \pm 1.49^{a}$	$6.00 \pm 1.55^{a}$
KDML	Unpolished	0	$5.53 \pm 1.87^{ab}$	$6.17 \pm 1.60^{ab}$	$5.70 \pm 1.99^{ab}$	$6.20 \pm 1.58^{a}$	$6.23 \pm 1.50^{a}$	6.30 ± 1.21ª
KDML	Polished	0	$6.07 \pm 1.64^{ab}$	$6.13 \pm 1.61^{ab}$	$5.67 \pm 1.47^{ab}$	$5.93 \pm 1.66^{a}$	$5.43 \pm 1.43^{ab}$	$5.80 \pm 1.40^{ab}$
SH	Unpolished	30	$5.00 \pm 1.74^{ab}$	5.17 ± 1.98 <sup>♭</sup>	$4.90 \pm 1.88^{bc}$	$5.43 \pm 1.98^{a}$	$5.27 \pm 2.03^{ab}$	$5.40 \pm 2.03^{ab}$
SH	Polished	30	$5.73 \pm 1.78^{ab}$	$6.07 \pm 1.46^{ab}$	$5.43 \pm 1.48^{abc}$	$5.70 \pm 1.62^{a}$	$5.77 \pm 1.70^{ab}$	$5.83 \pm 1.70^{ab}$
KDML	Unpolished	30	$6.37 \pm 1.67^{a}$	$6.57 \pm 1.38^{a}$	$6.53 \pm 1.43^{a}$	$6.10 \pm 1.94^{a}$	$6.10 \pm 2.01^{a}$	$6.57 \pm 1.61^{a}$
KDML	Polished	30	$6.17 \pm 1.68^{ab}$	$6.63 \pm 1.25^{a}$	$6.10\pm2.16^{ab}$	$5.60 \pm 1.69^{a}$	$5.77 \pm 2.08^{ab}$	$6.07 \pm 1.64^{a}$

Table 3: Effect of rice variety and processing on sensory quality of the products

Different letters indicate significant differences in the same column (P $\leq$ 0.05). SH = Sao Hai and KDML = Khaw Dok Mali 105

Table 4: Effect of VI condition on physical property of gac aril fortified rice

Vacuum pressure (mmHg)	VI condition	Hardness (g)	Stickiness (g)	L*	a*	b*
0 300	Single	8.40 ± 0.76ª 8.35 ± 0.58ª	$-8.70 \pm 0.76$ <sup>ns</sup> $-8.23 \pm 0.58$ <sup>ns</sup>	48.86 ± 0.81ª 49.41 ± 0.29ª	9.95 ± 0.81⁵ 11.12 ± 0.48ª	14.84 ±1.25 <sup>d</sup> 17.47 ± 0.58°
500 300 500	Pulse	8.17 ± 0.59ª 8.35 ± 0.93ª 7.58 ± 0.42 <sup>b</sup>	$-9.18 \pm 0.59$ ns $-8.41 \pm 0.93$ ns $-9.05 \pm 0.42$ ns	49.17 ± 0.73 <sup>a</sup> 47.30 ± 0.94 <sup>b</sup> 45.88 ± 0.75 <sup>c</sup>	$\begin{array}{l} 11.02 \pm 0.84^{a} \\ 11.09 \pm 0.71^{a} \\ 11.68 \pm 0.50^{a} \end{array}$	19.12 ±1.08 <sup>b</sup> 19.87 ±1.40 <sup>ab</sup> 21 <sup>1</sup> .23 ±1.01 <sup>a</sup>

Different letters indicate significant differences (P≤0.05) at the same column.

ns = Non-significant difference

## Effects of VI Condition on Chemical Property of Gac Aril Fortified Rice

Lycopene and  $\beta$ -carotene values increased at higher vacuum pressure applied (Table 5). At 500 mmHg, rice samples had the highest  $\beta$ -carotene and lycopene value whereas control or 0 mmHg had the lowest values. Similar results were observed in TPC and also DPPH value. Application of different vacuum pressure and pulse pressure caused an increase in DPPH values, however, no significant difference was noted among treatment (p>0.05). Higher vacuum pressure has been well established for increasing impregnation behavior. It has been described by Phianmongkhol and Wirjantoro that sucrose uptake, as indicated by total soluble solid, increased with the higher vacuum pressure levels in half-ripen and ripen mango impregnated with sucrose solution<sup>52</sup>. The higher vacuum pressure led to more of the sucrose solution occupied the pore of mango tissues. Mújica-Paz and co-researchers stated that solute gain of jalapeño pepper which was impregnated with brine containing sodium chloride and acetic acid was different between with and without vacuum pulse<sup>53</sup>. Once the pickling solution has almost filled the intercellular pores, solute transport through cell walls occurs by diffusion mechanisms driven by a concentration gradient. In addition, the application of vacuum pulse induced quicker infiltration of the pickling solution to the inner void of whole jalapeño pepper, leading to the establishing of a concentration gradient in the external and internal sides of the pepper tissue, which would contribute to the solute impregnation of the pepper matrix<sup>53</sup>. Likewise, applying vacuum impregnation at high vacuum pressure of 500 mmHg in combination with pulse technique could increase impregnation of aril into rice grain, therefore, increased antioxidant content as well as activity of rice product.

VI condition	Lycopene (µg/g)	β–carotene (mg/g)	TPC (mg GAE/g)	DPPH (mmol TE/ g)
	19.45 ± 0.73°	31.53 ± 0.95°	0.11 ± 0.03 <sup>b</sup>	$1.83 \pm 0.38^{ns}$
Single	19.93 ± 1.37 <sup>bc</sup>	33.74 ± 1.96 <sup>bc</sup>	$0.16 \pm 0.01^{ab}$	$2.12 \pm 0.17^{ns}$
	$22.40 \pm 0.35^{ab}$	$39.26 \pm 1.33^{ab}$	$0.19 \pm 0.01^{a}$	$2.78 \pm 0.42^{\text{ns}}$
Pulse	$23.21 \pm 0.35^{a}$	$38.09 \pm 1.95^{abc}$	$0.19 \pm 0.02^{a}$	$2.77 \pm 0.42^{ns}$ $2.91 \pm 0.90^{ns}$
	Single	( $\mu$ g/g) 19.45 ± 0.73° Single 19.93 ± 1.37 <sup>bc</sup> 22.40 ± 0.35 <sup>ab</sup>	$\begin{array}{c} (\mu g/g) & (mg/g) \\ \\ 19.45 \pm 0.73^{\circ} & 31.53 \pm 0.95^{\circ} \\ 19.93 \pm 1.37^{bc} & 33.74 \pm 1.96^{bc} \\ 22.40 \pm 0.35^{ab} & 39.26 \pm 1.33^{ab} \\ \\ \\ \text{Pulse} & 23.21 \pm 0.35^{a} & 38.09 \pm 1.95^{abc} \end{array}$	$\begin{array}{c} \mbox{(}\mu g/g) \mbox{(}m g/g)$

Different letters indicate significant differences (P≤0.05) in the same column.

ns = Non-significant difference

TPC = total phenolic content, DPPH = 2,2-diphenyl-1-picryl-hydrazyl

Effects of VI Condition on GI, Amylose Content and Sensory Property of Gac Aril Fortified Rice The effect of vacuum pressure on GI and amylose content are shown in Table 6. Unpolished KDML 105 could be categorized as low amylose rice as in general, amylose content in low amylose rice ranges from 10-20%<sup>26</sup>. Addition of gac aril under pulsed vacuum condition slightly elevated amylose content and hence lower in GI value of samples. It was believed that the soaking during VI process influences changes of amylose structure and may lead to formation of lipid complex and, therefore, apparent amylose content increased. This might be due to soaking enhances action of lipase enzymes in rice and leads to amylose-lipid complexes which normally are classified as resistant starch, formed during the subsequent drying. Moreover, during drying, amylopectin retrogradation increases and enhances more crystalline amylose-lipid complexes formation. The regular consumption of foods containing amylose-lipid complexes has been shown to reduce blood glucose levels in humans and the proliferation of colon cancer in rats<sup>54</sup>. The complex structure of amylose prevents digestion and lower GI.

Considering sensory property of samples, it was shown that all treatments were acceptable and no significant difference in appearance, color, aroma, texture, taste and overall acceptability of rice treated with gac aril at different VI condition (Table 7).

Table 6: Effect of VI condition on GI and amylose content of gac aril fortified rice

Vacuum pressure (mmHg)	VI condition	Glycemic index	Amylose content (%)
0		$84.24 \pm 2.59^{a}$	10.92 ± 1.78 <sup>b</sup>
300	Single	68.38 ± 1.73°	$13.85 \pm 0.63^{a}$
500	-	77.53 ± 1.73 <sup>b</sup>	$11.83 \pm 0.96^{ab}$
300	Pulse	76.62 ± 0.43 <sup>b</sup>	$14.49 \pm 0.68^{a}$
500		72.04 ± 0.86°	13.73 ± 0.11ª

Different letters indicate significant differences (P≤0.05) in the same column.

Vacuur pressu (mmHg		Appearance	Color	Aroma	Texture	Taste	Overall
0		6.10±1.35 <sup>ns</sup>	6.30±1.54 <sup>ns</sup>	6.00±1.60 <sup>ns</sup>	5.87±1.89 <sup>ns</sup>	5.70±1.66 <sup>ns</sup>	6.17±1.44 <sup>ns</sup>
300	Single	5.87±1.61 <sup>ns</sup>	6.50±1.08 <sup>ns</sup>	5.93±1.55 <sup>ns</sup>	5.53±1.60 <sup>ns</sup>	5.67±1.47 <sup>ns</sup>	5.80±1.40 <sup>ns</sup>
500		6.10±1.69 <sup>ns</sup>	6.43±1.55 <sup>ns</sup>	6.20±1.47 <sup>ns</sup>	5.60±1.99 <sup>ns</sup>	5.93±1.4 <sup>ns</sup>	6.03±1.45 <sup>ns</sup>
300	Pulse	5.97±1.69 <sup>ns</sup>	5.67±1.78 <sup>ns</sup>	5.90±1.77 <sup>ns</sup>	5.90±1.47 <sup>ns</sup>	5.70±1.69 <sup>ns</sup>	6.00±1.50 <sup>ns</sup>
500		6.03±1.38 <sup>ns</sup>	6.20±1.32 <sup>ns</sup>	5.70±1.90 <sup>ns</sup>	5.83±1.88 <sup>ns</sup>	5.40±1.81 <sup>ns</sup>	5.73±1.55 <sup>ns</sup>

Table 7: Effect of VI condition on sensory property of gac aril fortified rice

ns = Non-significant difference

#### Conclusions

Rice variety and processing affected the uptake of gac aril solution into rice grain. Fortification of gac aril in unpolished SH gave the highest antioxidant content but there was no significant effect in DPPH values whereas unpolished KDML 105 had the highest sensory acceptability. Study of the optimum VI condition showed that higher vacuum pressure allowed significantly higher impregnation of aril and hence more intense in red color and higher in antioxidant compounds. Moreover, it was evidenced that pulsed pressure increased aril impregnation. VI process also decreased glycemic index significantly. Finally, fortification of unpolished KDML 105 by gac aril using VI process at 500 mmHg pulsed pressure could increase health benefits of white rice by improvement of chemical property ( $\beta$ -carotene, lycopene, DPPH and TPC) and low GI value.

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