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Physico-chemical Properties and Antioxidant Activity of Gac Fruit Vinegar Fermented by *Acetobacter aceti* TISTR 354

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ABSTRACT

The purpose of this study was to investigate the effects of various factors on vinegar production from the gac fruit [*Momordica cochinchinensis* (Lour.) Spreng.] using *Acetobacter aceti* (TISTR 354) and to evaluate the physico-chemical properties and antioxidant activity of gac fruit vinegar. Gac fruit vinegar was produced through a two-stage fermentation process involving alcoholic fermentation by *Saccharomyces cerevisiae* and ethanol oxidation by acetic acid bacteria. The fermentation parameters included initial alcohol concentrations of 5, 7 and 9% (v/v), 10% (v/v) inoculum, pH 4.88, and 7 °Brix of total soluble solid (TSS) incubated statistically at room temperature for 15 days. At the end of the fermentation, gac fruit vinegar at different initial alcohol concentrations had acetic acid content ranging from 4.60-5.05%, pH values within 4.41-4.56, TSS content of 8.50-9.13 °Brix, and alcohol content of 3.97-5.70%. The *L**, *a** and *b** values showed lightness (*L**) shifting toward white, *a** values shifting negatively (–*a**) toward green, and *b** values shifting positively (+*b**) toward yellow. The antioxidant activity, calculated as a percentage of the radical scavenging activities, was 19.44-40.97%. Gac fruit vinegar fermented with 9% initial alcohol showed the highest acetic acid content and antioxidant activity among the vinegars included in this study. The findings of the study can be used to improve the production of fermented vinegar and promote the use of gac fruit as a functional food.

Key words: Acetic acid, *Acetobacter aceti*, antioxidant activity, gac fruit, vinegar

INTRODUCTION

Vinegar is produced by fermenting fruits in two stages. The first process is alcoholic fermentation, which converts fermentable sugars into ethanol without oxygen. This process usually involves the yeast *Saccharomyces*. The second process is acetic acid fermentation, which involves the oxidation of ethanol by *Acetobacter* in the presence of oxygen. Fermentation is an essential process for producing fruit vinegar, resulting in the production of volatile organic compounds, polyphenols and organic acids from the activities and metabolism of microorganisms. Different methods of preparing fruit juice from raw materials affect the sensory properties, bioactive compound content and quality of the final vinegar product. Fermented vinegar is commonly used to improve digestion and promote the growth of healthy gut bacteria. Therefore, it is classified as a functional food. The differences in bioactive compound concentrations, different flavors and characteristics of vinegar, acetic acid content, residual alcohol, TSS and pH depend on the raw materials used in production. Because of the popularity of natural health products, extensive studies have been conducted on the use of agricultural products, such as malt, barley, apples, grapes, cherries, berries and dates (Özen *et al.*, 2020), or even rice, palm sugar, onions, tomatoes, sugarcane, coconut, mango, mulberry, oranges, bananas, tamarind, pineapple and cashew nuts (Luzón-Quintana *et al.*, 2021).

Gac [*Momordica cochinchinensis* (Lour.) Spreng.] is a medicinal plant with high phytonutrient value. Because of the pharmaceutical properties of its pulp, peel, aril, seed, leaves and roots, it has been used in traditional medicinal applications (Do *et al.*, 2019). Gac fruit contains higher levels of beta-carotene and lycopene than carrots and tomatoes and has high levels of unsaturated fatty acids, vitamin E, polyphenols, and flavonoids (Müller-

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Maatsch *et al.*, 2017). All parts of the gac fruit, including the aril, seeds, pulp, and peel, can be used and processed into powdered and capsule oil products. Research has found that consuming the aril of the ripe gac fruit provides many benefits, including potent antioxidant, antibacterial, anti-inflammatory, anticancer, neurotropic and immunomodulatory properties (Mat *et al.*, 2024). For commercial use, gac pulp is processed into various products to facilitate consumption. The pulp of gac has good antioxidant properties because of its high bioactive compound content. Gac fruits have been used in the pharmaceutical, cosmetic and food industries in the form of concentrated gac products, powdered extracts, gac oil capsules and gac juice drinks, which are available in Vietnam, China, and Thailand (Wimalasiri *et al.*, 2020). Given the benefits of gac fruit and its potential for further processing, it is valuable to explore its use in vinegar production with acetic acid bacteria. The transformation of a local herbal plant into a variety of healthy food products increases the value of agricultural products and promotes its agricultural cultivation. In addition, fermented vinegar has many benefits for the body, including antibacterial, anti-infective, anti-oxidative, control of blood sugar levels and fat metabolism, weight loss and anticancer activities. The physiological functions of vinegar are attributed to its organic acids, polyphenols and melanoidins, which contribute to its antioxidant activity through the fermentation process and help to regulate blood glucose, lipid metabolism and weight control owing to its acetic acid content (Chen *et al.*, 2016).

Gac fruit is widely recognized for its high nutritional value and can be used as medicine. The commercial use of gac fruit in health supplements is attributed to its antioxidant, antibacterial, anti-inflammatory, anticancer, neurostimulatory and immunomodulatory properties. However, gac fruit has not yet gained commercial popularity, resulting in less competition in the food market. Furthermore, its benefits may be lost during processing because the active compounds degrade under harsh conditions. Therefore, this study aimed at developing a method for processing gac fruit to improve the production of fermented vinegar and add value to healthy beverage products. This study was conducted to produce fermented vinegar from gac fruit wine at different initial alcohol concentrations and to investigate its physico-chemical properties and antioxidant activity.

MATERIALS AND METHODS

The gac fruit used in this study was obtained from farmers in Maha Sarakham Province. The samples were collected at the fully ripe fruit development stage, aged 140-150 days. The ripe gac fruits were washed, and the peel was separated from the pulp. Gac fruit juice was prepared by squeezing 10 kg of gac pulp and aril and filtering using a filter cloth. The concentrated gac fruit juice was mixed with water in a ratio of 1:1 (by volume). Gac fruit juice was determined for TSS, pH, total titratable acidity (% of citric acid) using the Association of Official Analytical Chemists, and colour values using a spectrophotometer before alcohol fermentation.

Sugar was added to gac fruit juice to adjust the TSS content to 20 °Brix, and the pH was adjusted to 5.0 using acetic acid. The juice was sterilized by adding 0.2 g/l of potassium metabisulfite and left for at least 12 h before the addition of the yeast starter.

Commercial yeast powder (*Saccharomyces cerevisiae*, LALVIN EC 1118®, LALLEMAND Inc.) was obtained from a market in Maha Sarakham Province. Alcohol was produced from gac fruit juice using 500 ml of prepared gac fruit juice in a sterilized flask. 0.25 g of yeast powder was added as a starter for alcohol fermentation. The flask was closed with cotton plugs and incubated at room temperature for 24 h. Then, 5% of the yeast starter was transferred to the remaining gac fruit juice. Alcoholic fermentation was performed under static conditions at 30°C for 15 days, measuring parameters at 3-day intervals. After fermentation, the gac fruit wine was transferred to new sterile bottles and pasteurized at 60°C for 30 min for further acetic acid fermentation.

The starter culture *Acetobacter aceti* (TISTR 354) was obtained from the Thailand Institute of Scientific and Technological Research, Thailand. The seed culture was inoculated using one loop of *A. aceti* (TISTR 354) into 5 ml of glucose yeast broth medium and incubated at 30ºC for 7 days. Subsequently, 10% of the seed culture was transferred into the prepared gac fruit juice and incubated with shaking at 150 rpm, 30 °C for 72 h.

Gac fruit wine with an initial TSS content of 7 °Brix was adjusted to an initial alcohol concentration of 5, 7 and 9% (by volume) using sterilized distilled water, and the initial pH value was 4.88. The prepared starter culture 10% (by volume) was transferred into gac fruit wine at initial alcohol concentrations of 5, 7 and 9%. Each 500 ml sample was fermented in a plastic tray (13 cm wide, 20 cm long and 7 cm deep), covered with a filtered cloth, and statistically incubated at room temperature for 15 days. Samples were collected at 5-day intervals (at 0, 5, 10, and 15 d) to evaluate the total titratable acidity by titrating 10 ml of the sample with 0.1 N NaOH using phenolphthalein as an indicator, expressed as a percentage of acetic acid. Alcohol content, TSS and pH were measured using a vinometer, hand refractometer and pH meter, respectively. At the end of fermentation, the vinegar was transferred to a sterilized bottle and pasteurized at 60°C for 30 min.

Colour values were measured using a spectrophotometer (Hunter Lab Color Flex EZ, VA, USA). The colour evaluation of gac fruit vinegar was performed using the CIELAB scale with the colour space values *L** (lightness, 0-100), *a** (+red/–green), and *b** (+yellow/–blue). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to estimate antioxidant activity. Briefly, the DPPH free radical scavenging activity was determined using 1 ml of the sample solution and 3 ml of 0.1 mM DPPH solution added to a test tube, and the mixture was homogenized and incubated for 30 min in the dark at room temperature. Absorbance was measured using a UV spectrophotometer at a wavelength of 514 nm. The percentage of DPPH radical scavenging activity was calculated as follows:

$$\% \text{ Radical scavenging} = \frac{A_{514} \text{ of control} - A_{514} \text{ of sample}}{A_{514} \text{ of control}} \times 100$$

Where, A_{514} was the absorbance measured at 514 nm.

All experiments were performed in triplicate, and data were presented as means ± standard deviation (SD). The obtained data were statistically analyzed using the SPSS statistical software (SPSS Inc., Chicago, IL, USA) using one-way analysis of variance, followed by Duncan's multiple comparison tests. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

This study investigated the effect of the initial alcohol content on vinegar production from gac fruit juice fermented by *A. aceti* TISTR 354. The physico-chemical properties of unfermented gac fruit juice are shown in Table 1. The changes in pH, TSS, and alcohol content of gac fruit wine during 15 days of alcohol fermentation are given in Fig. 1; as fermentation proceeded, alcohol content increased, TSS decreased and pH slightly differed. Gac fruit wine with TSS of 7 °Brix, initial alcohol concentration adjusted to 5, 7 and 9% (by volume), inoculum size of 10% (by volume), and pH of 4.88 was fermented at room temperature under stationary conditions. After 15 days of fermentation, the TSS increased slightly throughout the fermentation period for all vinegar samples with different initial

Table 1. Gac fruit juice physico-chemical properties

Parameters	Gac fruit juice
TSS (°Brix)	10.00±0.00
pH	5.33±0.00
Total titratable acidity (% of citric acid)	1.50±0.00
Colour values	
*L**	25.78±0.14
*a**	40.99±0.17
*b**	32.90±0.23

Values are means ± standard deviation (n = 3).

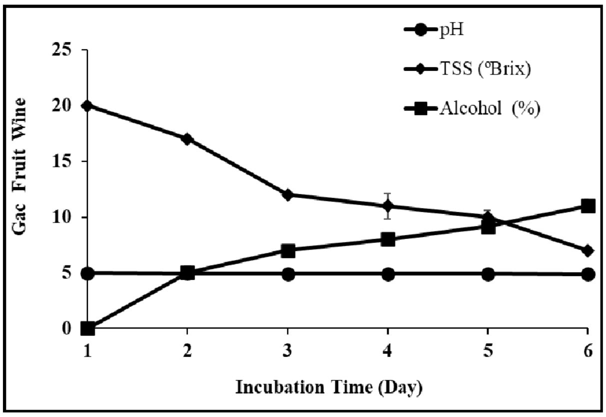

Fig. 1. Changes in chemical properties of gac fruit wine during 15 days of alcoholic fermentation.

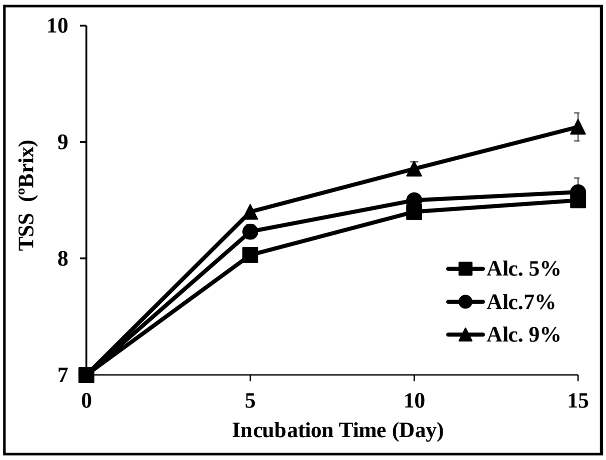

Fig. 2. Changes in TSS (ºBrix) of gac fruit vinegar fermentation during 15 days at different initial alcohol concentrations.

alcohol contents (Fig. 2). Soluble solids (sugar content) such as glucose, fructose and sucrose were measured as the TSS in the sample. In addition to sugar, organic acids such as citric acid, malic acid, tartaric acid, amino acids and vitamin C are soluble in water. The measured value is the sum of the concentrations of all sugars and organic acids that are soluble in the sample. The TSS content of vinegar is due to other components present in trace amounts, such as organic acids and free amino acids (Kang *et al.*, 2020). The TSS content and the pH value are essential for assessing the quality of vinegar, which is included as a product characteristic.

The pH of vinegar fermented from gac fruit juice gradually decreased under all experimental conditions (Fig. 3). The

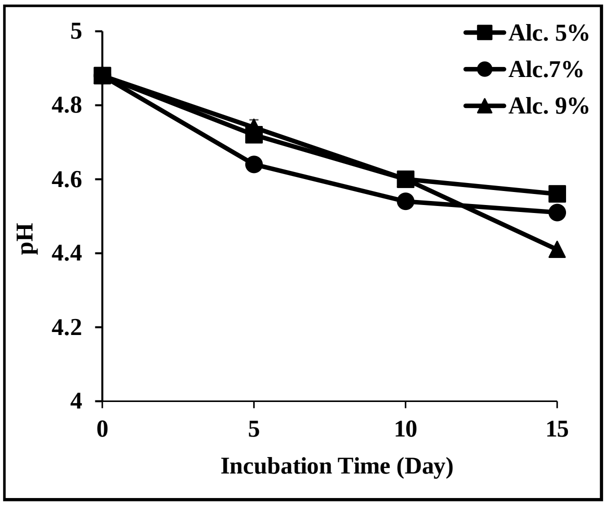

Fig. 3. Changes in pH of gac fruit vinegar for 15 days at different initial alcohol concentrations.

decreased pH value was due to the increase in acetic acid and other organic acids during fermentation.

The alcohol content of gac fruit vinegar decreased under all experimental conditions (Fig. 4). The decrease in alcohol content occurred in parallel with the increase in acetic acid concentration.

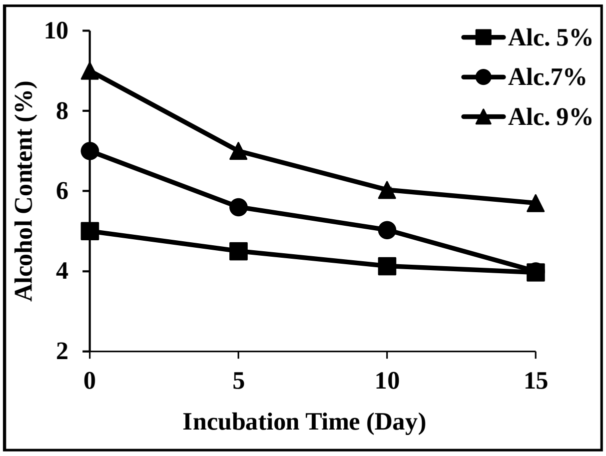

Fig. 4. Changes in alcohol content (%) of gac fruit vinegar for 15 days at different initial alcohol concentrations.

Titratable acidity measures the total acid concentration in the gac fruit vinegar and is expressed as acetic acid. Gac fruit vinegar at different initial alcohol concentrations increased acetic acid content throughout the fermentation period (Fig. 5). The increase in the acidity of gac fruit vinegar during acetic acid fermentation indicates the metabolic activity of acetic acid bacteria, which oxidize

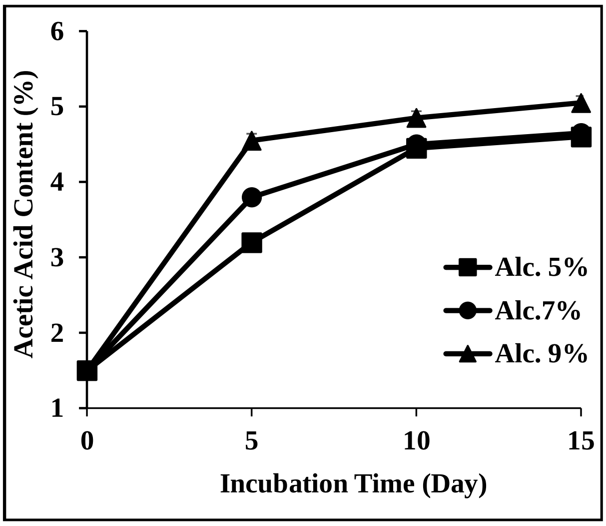

Fig. 5. Changes in acetic acid content (%) of gac fruit vinegar for 15 days at different initial alcohol concentrations.

Table 2. Colour parameters of gac fruit vinegar fermentation for 15 days at different initial alcohol concentrations

Colour values	Incubation time (Day)	Alc. 5%	Alc. 7%	Alc. 9%
*L**	0	9.68^{a}±0.12	6.58^{b}±0.34	6.58^{b}±0.40
	5	9.89^{a}±1.50	5.43^{b}±0.25	5.44^{b}±0.27
	10	7.38^{a}±0.15	4.98^{b}±0.15	4.72^{b}±0.30
	15	7.36^{a}±0.45	5.37^{b}±0.45	4.86^{b}±0.16
*a**	0	-0.30^{a}±0.09	-0.25^{a}±0.01	-0.23^{a}±0.03
	5	-0.51^{a}±0.05	-0.38^{b}±0.04	-0.37^{b}±0.06
	10	-0.95^{a}±0.13	-0.73^{b}±0.04	-0.67^{b}±0.09
	15	-1.22^{a}±0.08	-0.79^{b}±0.05	-0.74^{b}±0.05
*b**	0	2.80^{a}±0.17	0.31^{b}±0.02	0.26^{b}±0.13
	5	2.50^{a}±0.20	0.44^{b}±0.01	0.43^{b}±0.07
	10	2.17^{a}±0.13	0.84^{b}±0.07	0.87^{b}±0.18
	15	2.19^{a}±0.12	0.88^{b}±0.04	0.98^{b}±0.07

Data are presented as the mean of three replicates±standard error. Different superscripts indicate significant differences ($P<0.05$) according to Duncan's multiple-range test.

ethanol to acetic acid under aerobic conditions. Therefore, alcohol is a carbon source for acetic acid bacteria, and the increase in total acidity observed during fermentation is due to the accumulation of organic acids, primarily acetic acid, produced by acetic acid bacteria, which are the main microorganisms involved in this fermentation. The colour values of gac fruit vinegar at different initial alcohol concentrations showed a decrease in brightness (*L**) throughout the fermentation period. The *a** value was negative, indicating a green colour, and the *b** value was positive, indicating a yellow colour (Table 2).

The antioxidant activity results showed that gac fruit vinegar had the highest antioxidant activity at 9% initial alcohol concentration (Fig. 6). Fermented gac fruit juice exhibited higher antioxidant activity than unfermented gac fruit juice. In addition, a previous study documented that the antioxidant activities of DPPH and FRAP showed a significant relationship with the *L** and *b** values of the colour parameters of wood vinegar; samples with darker colours and lower yellow colour values tended to have higher antioxidant activities (Petchpoung *et al.*, 2020). Furthermore, vinegar with a high phenolic content tends to have lower lightness/ darkness values but a higher concentration of red pigments. The differences in the physicochemical properties of fruit vinegar can mainly be attributed to the raw materials and technologies used in production. Fruit vinegar is an essential source of bioactive compounds, indicating the high potential of fruit vinegar as an antioxidant and antimicrobial agent that can be used as a functional food ingredient (Sengun *et al.*, 2019).

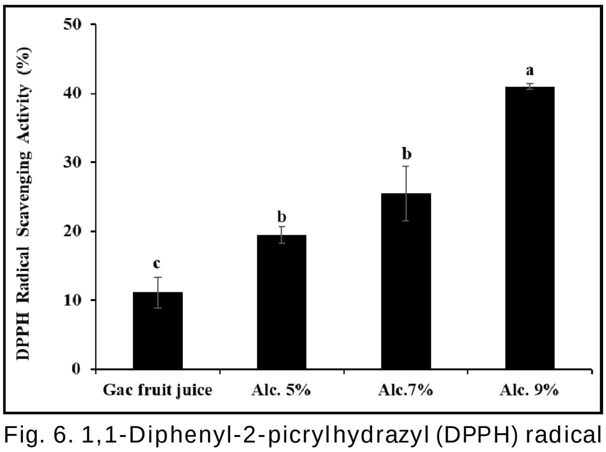

Fig. 6. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of gac fruit juice and gac fruit vinegar fermentation during 15 days at different initial alcohol concentrations. Different letters above the error bar indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

CONCLUSION

In the present study, vinegar was produced from gac fruit juice by *A. aceti* TISTR 354 using different initial alcohol concentrations of 5, 7 and 9% (by volume) and fermented at room temperature for 15 days under static conditions. The results showed that the alcohol content tended to decrease, while the acetic acid content tended to increase with incubation time. At the end of the fermentation, all samples showed pH values ranging from 4.4-4.5, TSS content of 8.50-9.13 ºBrix, and *L** values decreased in brightness. The *a** value shifted negatively (toward green), while the *b** values shifted positively (toward yellow). Among the vinegars produced, gac fruit vinegar with an initial alcohol concentration of 9% had the highest acetic acid content and antioxidant activity.

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