Indonesian Food Science and Technology Journal
IFSTJ : Vol : (6) No :1, December, 2022 (PP : 1-6)
ISSN : 2615-367X

INDONESIAN FOOD SCIENCE AND TECHNOLOGY JOURNAL (IFSTJ)

Journal homepage: online-journal.unja.ac.id/ifstj/issue/archive

Changes in Lactic Acid Bacteria and Quality of Gac Yoghurt Supplemented with Carbohydrate Sources during Chilled Storage

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***Abstract* — Gac yoghurt is formulated by mixing gac juice with cow milk and probiotics to form a thick yellow fluid. The research objective was to study the effect of carbohydrate sources (inulin, honey, sucrose, fruit syrup, and sucrose) on lactic acid bacteria remaining after the chilled storage for 28 days. The gac yoghurt was analyzed with physicochemical and sensory characteristics. The experimental design was a Complete Randomized Design (CRD) with eight parameters (total soluble solids, pH, lactic acid content, syneresis, texture, colour, lactic acid bacteria count, and sensory evaluation). The results showed that gac yoghurt in the presence of honey had a higher lactic acid count than other yoghurt samples throughout 28 days of storage. The lactic acid growth was observed in the first two weeks, then it was almost constant in the third week and levelled off at the end of storage. The addition of carbohydrates resulted in a lower pH than that of the control. It increased total soluble solids, titratable acidity, and syneresis effect. Gac yoghurt with inulin enrichment kept for 28 days showed a firmer texture and lighter in colour than the rest. However, sensory results showed that gac yoghurt with sucrose was the most preferred formulae based on the average hedonic scores followed with the yoghurt fermented by honey, inulin, and fruit syrup, respectively.**

***Keywords* — yoghurt; gac fruit; lactic acid bacteria; inulin; natural sweeteners**

Manuscript received June 23, 2022; revised July 31, 2022; accepted Dec 30, 2022. Available online Dec 30, 2022.

I. Introduction

Gac yoghurt is the new taste yoghurt, which is formulated as a functional food. The biofunctional yoghurt combines multi-function derived from biologically active substances, probiotic microorganisms, and micro-macro nutrients [1,2]. Yoghurt has been worldwide recognised as probiotic food because it has an alive and active bacteria to regulate the gastrointestinal system. Traditionally, yoghurt is produced from pasteurised milk fermented by a probiotic yoghurt starter. The starter ferments lactose. Then increaes lactic acid bacteria to give a sour taste and coagulate milk protein at an iso-electric point to form textural gel. The casein network increases the strength of the yoghurt gel by controlling pH, starter culture, milk quality, and fermentation process [1,3,4]. For healthy issues, the whole milk is replaced with skim milk to reduce the fat content. In the case of gac fruit yoghurt, the fruit is squeezed and the expressed juice is mixed with pasteurised milk to enrich the phytochemicals and colourants. See gac yoghurt in **Fig. 1** (a) in comparison with plain yoghurt (b).

Gac fruit (*Momordica cochinchinensis* (Lour.) Spreng.) or gac, in short, is a tropical plant in Southeast Asian countries (Thailand, Laos, Cambodia, Myanmar). The gac fruits are a nutritious source of lycopene, carotenoids, tocopherol, and fatty acids, including polyphenol compounds and flavonoids. The fruit contain carbohydrate (14.17%), total dietary fiber (1.6%), protein (2.1%), total fat (0.3%), and calcium (0.036%) [5,6]. The ripe fruit has a high potential for extraction of yellow and orange colourants applying in numerous food products such as yoghurt, sauces, food supplements and beverages [2]. The health benefits of gac fruit consumption is to control cholesterol levels, to prevent cardiovascular diseases, to improve eyesight, to combat depression, and to prevent ageing [5,6].

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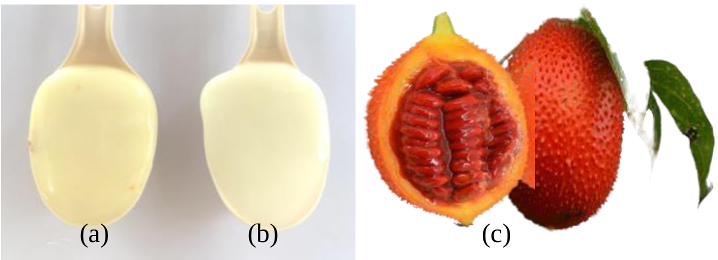

Fig. 1 gac/fruit syrup yoghurt (a), plain yoghurt (b), and gac fruits (c)

However, the edible part of gac fruit is the big seeds surrounded by thin, soft, and oily pulp see Fig.1 (c), while its skin is toxic to humans causing sickness for several days [5].

Apart from gac fruit as a source of phytochemicals and milk as a source of protein, carbohydrate sources are another factor to level up the lactic acid bacteria population, for example, sucrose enriches the bacterial growth, texture and flavour development during fermentation of gac/passion fruit yoghurt [1]. In addition, inulin (a complex sugar called fructan), honey and fruit syrup (sweet fluids with a high amount of natural sugar) have a potential effect on the microbial, physical and sensory characteristics of yoghurt [7-9].

However, scant publication on comparison of numerous carbohydrate sources on the microbial survival and gac yoghurt quality. Therefore, this research aimed to monitor the lactic acid survival during the storage gac yoghurt enriched with different carbohydrate sources (inulin, honey, fruit syrup, sucrose) and analyze the product quality in terms of chemical, physical, and sensory profiles during a chilled storage controlled for 28 days.

II. Material and Methods

A. Materials

The fresh ripe gac fruits organically grown in Chiang Mai, Thailand used as a functional ingredient. The aril was manually separated from the seed and peeled by knife. The aril was diluted with drinking water by volume (1:1), then manually blended. The gac puree was then filtered by using a filter clot. Commercial pasteurized milk, with the fat content (5%) (CP, Meji, Thailand) was purchased from convenient store. Freeze-dried starter culture of Goat Nutrition®, GN were obtained from Goat Nutrition Lid, Units B & C, Smarden Business Estate, Kent, UK. As per the supplier leaflet, 1 unit of GN culture was used for each batch (1 L of milk, reheated to fermentation temperature at 45 °C). carbohydrate sources such inulin (Sweety Keto, Chiang Mai), honey (Doi Kham, Bangkok), fruit syrup (Monin Syrup Pina Colada, Thailand) 65°Brix, and sucrose (Mitr Phol Pure Refined Sugar, Singburi Sugar Co.,Ltd., Singburi, Thailand) are used as the yoghurt starter enhancers.

The microbiological media and chemicals, De Man, Rogosa and Sharpe aga or MRS agar (Merck, USA) and peptone (Bacto, USA) for lactic acid analysis, sodium hydroxide (Merck, USA) and phenolphthalein (Labchem, USA) for acid titration were analytical grade and used without further purification. Distilled water was used as a solvent in the sample dilution and solution preparations.

B. Preparation of yoghurt starter

The pasteurized milk in water bath was heated at 90 °C for ten minutes. After that, the hot milk was rapidly cooled down to the optimum temperature of 45 °C. The probiotic GN yoghurt starter culture (5 grams) was quickly added into 250 millilitres prepared milk in a conical flask, then incubated the flask at 45 °C for 6 hours until the final pH of the mixture was 4.5. The starter culture was cooled and stored at 4 °C prior use.

C. Preparation of gac yoghurt

The gac puree was blended with commercial milk at a ratio of 1:4, then was heated at least till 50 °C, mixed with carbohydrate sources (inulin, honey, fruit syrup, or sucrose) at a constant concentration, 2.5% (w/w). The gac puree and milk without carbohydrate was used as a control. All five treatments were proceeded in a similar fermentation procedure as described earlier by using prepared starter culture.

D. Quality analysis of gac yoghurt

Microbiological analysis: Lactobacillus spp. in gac yoghurt was enumerated by using MRS agar. The sample was diluted with peptone water up to 10^{-6} dilution. The dilute sample was poured on Petridis containing MRS agar. The sample was loaded in an anaerobic jar and then incubated at 42 °C as long as 24 hours. Colonies of lactic acid bacteria on MRS agar was counted and expressed as log CFU/g sample.

Chemical analysis: A pH meter (Mettler Toledo, USA) was used to record pH of a diluted yoghurt sample with distilled water (1:1). Total soluble solids of the samples were tested by a digital refractometer (Atago, Japan) at 4 °C. Titratable acidity expressed as percentage of lactic acid was carried out by the AOAC942.15 [10]. The dilute sample (gac 1 ml yoghurt: 1 ml distilled water) was titrated against 0.1 N NaOH using phenolphthalein as an indicator). Yoghurt sample was monitored for chemical parameters during storage for 1,7,14, and 28 days at 4 °C. The results were expressed as an average±standard deviation (SD) of five measurements.

Physical analysis: Syneresis of the gac yoghurts stored for 28 days at 4 °C was tested by a centrifugation in 2,400 round per minute at 4 °C for ten minutes. The syneresis was calculated as weight of the water supernatant divided by weight of initial sample, and later multiplied by 100. CIE (L*, a*, b*) colour space of all samples was measured by using a hand-held colourimeter (Minolta Konika C-400, Japan). Compression testing of gac yoghurt in a cap was investigated by using a texture analyser (TA.XT plus, Stable Micro System, UK). The compression was observed dipping at 4 °C. The experiments were carried out using a cone probe, Perspex, 45°. The sample cup was static at the base while the moving arm attached to the probe were went down to the lower direction. The low-test speed was set at 0.5 mm/s. The typical compression results showed a relationship between force (N) and time (second). The firmness is calculated as the peak force of the compression as seen in **Fig.2**. The consistency was the total area under the firmness. Cohesiveness of the yoghurt mixture was read from the negative peak. The results were expressed as an average±SD of five measurements.

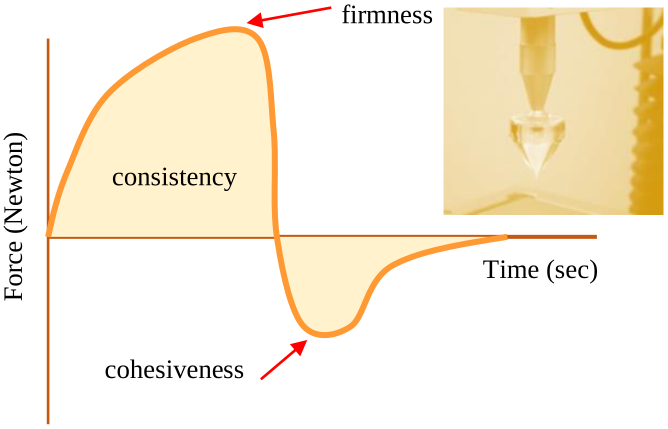

Fig. 2 Texture parameters for yoghurt compression test

Sensory evaluation: Sensory evaluation was employed to compare the gac fruit yoghurt preference by using 5-points hedonic scale. The four treatments with carbohydrate sources addition and control were served in a small cup at 4 °C for 14 days, then evaluated by 30 untrained students. According to the hedonic scale, score 1 corresponded with "dislike extremely" while score 5 corresponded with "like extremely". The Panelists evaluated the appearance, colour, flavour, and texture, respectively. Finally, the overall liking of each yoghurt was concluded.

E. Statistical analysis

The data from the quality analysis of gac yoghurt were presented as the mean value±SD. The CRD was selected to compare the five treatments. The results of total soluble solids, pH, titratable acidity, syneresis, colour (L*, a*, b*), texture profiles (firmness, consistency, cohesiveness), sensory characteristic (appearance, colour, flavour, taste, texture, and overall liking) were evaluated using analysis of variance (ANOVA). Tukey HSD test was used to compare the differences between the four carbohydrate sources (inulin, honey, fruit syrup, and sucrose) with control. The statistical analysis was performed using statistic programme, Statistix 10, USA.

III. Result and Discussion

A. The growth lactic acid bacteria during 28-day storage of gac yoghurt at 4 °C.

Lactic acid bacteria are a broad heterogeneous group of food microorganisms found in traditionally fermented foods. *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, and *Bifido bifidum* are common lactic acid bacteria supply for yoghurt starter culture [3,4]. *Lactobacillus acidophilus* is normal flora that plays a beneficial role in the human gastrointestinal tract. Lactic acid bacteria grown in MRS agar by direct incubation at 42 °C for as long as 24 hours under anaerobic condition shows the milky white, elevated, moist, circular colonies (**Fig. 3**). Our lactic acid starter had a log 9 CFU/g sample, which was higher than the Thai Food Standard of application of probiotics in food products (more than log 6 CFU/g) [11]. Lactic acid bacteria can ferment the lactose in cow milk to lactic acid at an optimum pH of 4.5 and temperature of 10 °C. Change in lactic acid bacteria during chilled storage gac yoghurt was significantly observed in control, where the initial bacterial count was increased from log 8.75 CFU/g to 9.25 CFU/g within 14 days, followed by an almost constant bacterial population in the third week, and dropped to 8.85 CFU/g at the end of experiment as shown in **Fig.4**.

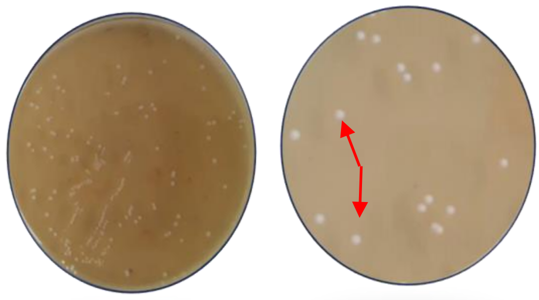

Fig. 3 Growth of lactic acid bacteria in MRS agar

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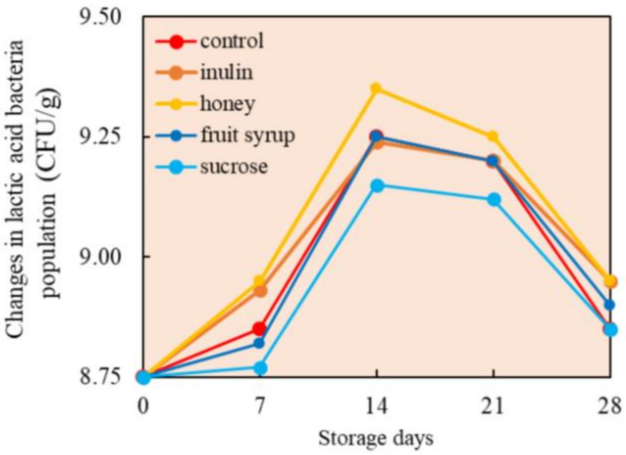

Fig. **4** Effects of carbohydrate sources at 2.5% (w/w) on lactic acid bacteria population during 28-day storage of gac yoghurt

According to Fig. 4, the reduction of lactic acid bacteria after 14-day storage might be caused by excessive lactic acid production and toxicity of hydrogen peroxide to bacterial cells [7]. Gac yoghurt with fruit syrup resulted in a higher bacterial count of 8.95 CFU/g at the final stage than in the control. Sugar such as sucrose, and fructose in fruit syrup might have an osmotic stress effect to reduce available water for the survival of the starter culture [1]. In the case of gac yoghurt added with sucrose 2.5 (w/w), the high osmotic pressure could prolong the initial 7-day fermentation process. Conversely, inulin and honey enhanced the microbial survival in the first week at 8.93 log CFU/g and 8.95 log CFU/g, respectively. In the fourth week, both carbohydrate sources enhanced the bacteria growth to the highest population of lactic acid bacteria at log 9 CFU/g. It was possible that inulin was a prebiotic to stimulate the growth of *Lactobacillus acidophilus* [12] and similar to honey was activated the viable count of Bifidobacterium [13].

B. Quality of gac yoghurt with carbohydrate addition

According to **Table 1**, the total soluble solids of gac yoghurt after fermentation was 8.2 °Brix and remained stable throughout 28-days storage in a refrigerator. Various carbohydrate additions in the yoghurt increased total soluble solids up to approximately 10 °Brix due to the sugar component solubilised in the hydro phase of the yoghurt. There was an insignificant change in the total soluble solids of all gac yoghurts with carbohydrate addition during chilled storage.

Titratable acidity contents of organic acids in gac yoghurt, for example, lactic acid and citric acid were observed at the initial storage period and slightly increased afterwards. There was a significant upward trend of titratable acidity content of gac/honey yoghurt during the chilled storage period. It can be explained that honey has glucose and fructose that is supply monosaccharides for lactic acid fermentation. Honey has a higher content of essential minerals and vitamins, which might enhance the growth of yoghurt culture. A previous study reported a symbiotic growth of lactic acid bacteria in honey [13]. Inulin is complex fructose used as a prebiotic of lactic acid bacteria [7]. The results of titratable acidity content in Table 1 were a main factor to manipulate the lower lactic acid bacteria population explained in **Fig. 4**. However, the addition of honey or inulin was a possible carbohydrate of choice to boost the lactic acid bacteria resulting in 3.2% the final bacteria survival in comparison with the control. In general, an increase in titratable acidity content of gac yoghurt was a diverse trend with the pH values. The pH results show the adjusted pH values of the gac yoghurt fermentation was 4.5, then dropped to 4.3 after 14 days. The pH of gac yoghurt samples in this study was in an optimum condition between 3.5 and 6.5 [3].

TABLE 1
CHANGES IN TOTAL SOLUBLE SOLIDS, PH, TITRATABLE ACIDITY, AND SYNERESIS OF GAC YOGHURT WITH DIFFERENT CARBOHYDRATE SOURCES (2.5 %W/W) DURING 28-DAY UNDER CHILLED STORAGE AT 4 °C

Carbohydrate Source	**Day**		
	0	**14**	**28**
Total soluble solids (ºBrix)			
Control	8.2±0.1b	8.3±0.2b	8.3±0.2b
Inulin	10.7±0.2a	10.6±0.1a	10.7±0.1a
Honey	10.5±0.1a	10.8±0.2a	10.7±0.1a
Sucrose	10.6±0.2a	10.7±0.1a	10.3±0.2a
fruit syrup	10.4±0.1a	10.6±0.1a	10.3±0.2a
Titratable acid content (%)			
Control	0.36±0.02aC	0.39±0.02aB	0.41±0.02bA
Inulin	0.32±0.01bC	0.38±0.01bB	0.42±0.01aA
Honey	0.37±0.00aC	0.41±0.02aB	0.45±0.01aA
Sucrose	0.34±0.01aC	0.39±0.01aB	0.42±0.02bA
fruit syrup	0.32±0.01bC	0.37±0.02bB	0.41±0.01bA
pH			
Control	4.48±0.02bB	4.35±0.03aA	4.36±0.02aA
Inulin	4.42±0.03aB	4.32±0.01bA	4.30±0.01bA
Honey	4.45±0.02cB	4.28±0.02cA	4.27±0.03cA
Sucrose	4.42±0.01cB	4.43±0.02aB	4.33±0.01aA
fruit syrup	4.47±0.02cB	4.26±0.01cB	4.31±0.02bA
Syneresis (%)			
Control	61.27±0.25aA	68.03±0.50bB	67.97±0.35aB
Inulin	62.67±0.61aA	67.67±0.61aB	71.40±0.46bC
Honey	72.10±0.36cA	72.43±0.40dA	73.57±0.55cB
Sucrose	71.60±0.46cA	71.37±0.57cA	76.60±0.42dB
fruit syrup	67.87±0.53bA	71.97±0.52cB	72.93±0.51cB

*a-e following the mean value (n=5) of each column suggested a significant difference between treatments at $p<0.05$.
*A-C following the mean value (n=5) of each row suggested a significant difference between refrigerated storage days at $p<0.05$.

The percentage of syneresis of gac yoghurt added with carbohydrate sources increased clearly during the storage periods. It was observed that the more pronounce syneresis effect on all samples with carbohydrates than in control. The syneresis will affect the water content of yoghurt, the loss of nutrients, and the risk of product deterioration. Dissolved sugar may interfere protein gel matrix by reducing hydrogen bonds between protein and water molecules, thus accelerating the syneresis in the yoghurt.

Firmness is the maximum force applied to yoghurt. As shown in the textural profile in **Table 2**, gac yoghurt with 2.5 (w/w) inulin was more firm than other formulations. Literature suggested that long-chain inulin forms a complex network with a casein protein structure [13]. For yoghurt consistency or the work to deform the yoghurt gel, the results showed that gac yoghurt mixed with honey, fruit syrup, and inulin developed a more consistent cream than the rest. That probably contributed to the more bacterial viability in gac yoghurt in those carbohydrates' sources produced exopolysaccharides to bind with the milk protein network [1].

TABLE 2
TEXTURE PROFILE OF GAC YOGHURT WITH DIFFERENT CARBOHYDRATE SOURCES (2.5 %W/W) STORED FOR 28 DAYS AT 4 °C

Carbohydrate Source	**Texture parameter**		
	Firmness (N)	**Consistency (N.s)**	**Cohesiveness (N)**
Control	0.186±0.001b	2.020±0.001d	0.018±0.002d
Inulin	0.194±0.002a	2.044±0.002c	0.021±0.001c
Honey	0.185±0.002b	2.198±0.004a	0.026±0.003a
Sucrose	0.180±0.001c	2.019±0.003d	0.021±0.001c
fruit syrup	0.175±0.003c	2.056±0.001b	0.023±0.001b

*a-d following the mean value (n=5) of each column suggested a significant difference between treatments at $p<0.05$.

As seen in **Table 3**, The lightness of gac/fruit syrup yoghurt was the lowest because of dark brown pigments in fruit syrup and Maillard reaction during the milk pasteurization [14]. Thus, the redness and yellowness of the sample was the highest values resulting in a shade of orange (see Fig. 1(a)).

TABLE 3
COLOUR PROFILE OF GAC YOGHURT WITH DIFFERENT CARBOHYDRATE SOURCES (2.5 %W/W) STORED FOR 28 DAYS AT 4 °C

Carbohydrate Source	**Colour parameter**		
	L*	**a***	**b***
Control	70.63±0.44c	6.76±0.09b	30.32±0.07b
Inulin	73.62±0.03a	5.94±0.08b	28.17±0.04b
Honey	71.78±0.05b	6.31±0.18b	29.61±0.14b
Sucrose	70.91±0.05b	6.02±0.33b	28.02±0.81b
fruit syrup	68.49±0.08d	7.25±0.56a	32.63±0.14a

*a-d following the mean value (n=5) of each column suggested a significant difference between treatments at $p<0.05$

The results of sensory evaluation of prepared yoghurt samples (**Table 4**) revealed that there were no significant differences in appearance score among all treatments except the sample fermented with fruit syrup which had a slightly lower appearance score. For colour, panellists could not differentiate the shade of orange and the average colour score of all samples was 7 (like) (data not show here). In addition, the panellists mostly preferred the flavour and sweet taste of gac yoghurt using sucrose as a fermentation stimulant and gel consistency. Literature mentioned that acetaldehyde produced by lactic acid bacteria (*Lactobacillus bulgaricus*) is the main compound found in yoghurt [15]. Generally, the panellists highly accepted the gac yoghurt made with sucrose addition.

TABLE 4
SENSORY PROFILE OF GAC YOGHURT WITH DIFFERENT CARBOHYDRATE SOURCES (2.5 %W/W) STORED FOR 28 DAYS AT 4 °C

Carbohydrate source	**Sensory attributes**		
	appearance	**texture**	**overall liking**
control	7.7±1.6a	5.9±1.7c	6.2±1.5c
inulin	7.2±1.2a	6.5±1.6b	6.5±1.2b
honey	7.2±1.8a	6.5±1.4b	6.7±1.3b
sucrose	7.4±1.7a	7.2±1.4a	7.7±1.3a
fruit syrup	6.7±1.5b	5.8±1.3c	6.2±1.2c

*a-c following the mean value (n=5) of each column suggested a significant difference between treatments at $p<0.05$.

IV. CONCLUSION

Since the chilled storage for 28 days affected the survival rate of the pure strain of lactic acid bacteria in gac yoghurt. The lactic acid counts of all yoghurts supplemented with inulin, honey, fruit syrup, and sucrose achieved the Thailand food standard. The longer storage time increased titratable acidity and syneresis. At the end of chilled storage, gac/inulin yoghurt resulted in the highest texture characteristics (firmness, consistency, and cohesiveness) and lightness. The sensory profile showed that the gac yoghurt with carbohydrate sources had a higher hedonic score on flavour, texture, and overall acceptability. Therefore, inulin and honey as a natural carbohydrate source of choice assisted the lactic bacterial survival, texture and organoleptic properties of functional gac yoghurt in this study.

ACKNOWLEDGMENTS

We would thank, Chiang Mai Sustainable Agricultural Federation for organic gac fruits supply.

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