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Inhibition of xanthine oxidase and uric acid in canned bamboo shoot by Yanang juice

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Abstract Effects of temperature (30, 80 and 95 °C) and time (5, 10, 15, 20, 25 and 30 min) were studied on xanthine oxidase activity and uric acid content of bamboo shoots in Yanang juice. The ratio of bamboo shoots to Yanang juice was investigated for physical and microbiological properties and uric acid content after subjection to 121 °C at F_0 5 min. Results showed that increasing temperatures resulted in decreasing uric acid content. Heating bamboo shoots in Yanang juice resulted in greater reduction of uric acid than heating in water. The inhibitory effect of heating on xanthine oxidase in terms of IC_{50} value at 95 °C was the highest (57.08%) as its IC_{50} value was the lowest (74.09 µg/mL), followed by heated samples at 80 °C (82.71 µg/mL) and 30 °C (84.34 µg/mL), respectively. Heating bamboo shoots in Yanang juice at 95 °C for 30 min was selected as the optimal preparation condition before the canning process. Ratios of bamboo shoots to Yanang juice at 1:3, 1:3.5 and 1:4 showed little effect on quality of the canned sample. Hardness of canned bamboo shoots was lower than fresh bamboo shoots. Lower viscosity and darker color of heated bamboo shoot samples were observed in Yanang juice compared to fresh samples. Total bacterial counts and flat sour bacteria were not detected in any of the products. Canned bamboo shoots undergoing high temperatures for a long time to maximize product safety contained uric acid at 4.12-8.82 mg/100 g bamboo shoots. Therefore the practical applications of the step of bamboo shoot preparation in canned processing could be applied to the food industry.

Keywords: Bamboo shoot, Canning process, Uric acid, Xanthine oxidase, Yanang juice

Introduction

Bamboo shoots are seasonal delicacies in Thailand and East-Asia regions. Bamboo shoots are one of very low calorie vegetables (Pongdee and Gatsorn, 2014). Bamboo hearts are also rich in B-complex group of vitamins such as thiamin, riboflavin, niacin, vitamin B-6 (pyridoxine), and pantothenic

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acid. Those are essential for optimum cellular enzymatic and metabolic functions (Pongdee and Gatsorn, 2014; Suwankanid *et al*., 2006). However, bamboo shoot composed of taxiphyllin, a toxic cyanogenic glycoside (Yiumhan, 2010). Cyanide alkaloids inhibit cytochrome-oxidase, an important enzyme in cellular respiration. Uric acid is another undesirable compound that is found in bamboo shoot (Aichayawanich *et al*., 2018). Halevi (2016) reported that uric acid in bamboo shoots was 29 mg/100 g of bamboo shoot.

The preparation steps of bamboo shoot is a very important step because it affects the cyanide and uric acid content. Treating bamboo shoot in salted boiling water readily degrades these toxic compounds. Aichayawanich *et al.* (2018) studied the effects of boiling temperatures (60, 70, 80, 90, and 100 °C) and shape (whole shoots, slices, and rectangular rods) on the degradation of cyanide and uric acid in bamboo shoots during the boiling process. They found that uric acid content of bamboo shoot slightly decreased when the bamboo shoot was boiled. The uric acid content was lower when boiled at high temperatures for a long time.

One of the good ways to reduce toxic inside bamboo shoot is cooked with Yanang juice (Pongdee and Gatsorn, 2014; Suwankanid *et al*., 2006). Yanang is a species of flowering plant native to mainland Southeast Asia and used particularly in the cuisines of northeast Thailand and Laos. Yanag juice is used to make the broth, primarily as a thickening agent rather than for its flavor (Pongdee and Gatsorn, 2014). This juice may be prepared from scratch, from fresh leaves. Therefore, bamboo shoot is mainly consumed cook by heating with Yanang juice. Eadmusik *et al.* (2019) studied effect of heating temperature 60, 70 and 80 °C for 15 min on xanthine oxidase inhibition in Yanang juice. They reported that xanthine oxidase activity is associated with gout incident. The xanthine oxidase inhibition in Yanang juice depends on heating temperature and time. The preparation process at 80 °C for 15 min gave the lowest activity at 72.64%. Only one research studied effect of heating bamboo shoot in Yanang juice on uric acid content (Promnuchit and Khotmee, 2011). They reported that uric acid in the bamboo shoot can be reduced by boiling the bamboo shoot in Yanang juice in high temperature for a long time.

As mentioned above, there are many works studyon toxic in bamboo shoot. However, no information is available on the effect of Yanang juice in uric acid content of bamboo shoot during canned processing.

The research objectice was to minimize uric acid during production of canned bamboo shoot in Yanang juice that affected from processing steps i.e., cutting, heat treatment and sterilization for the change of uric acid and xanthine oxidase activity.

Materials and methods

Sample preparation

Fresh bamboo shoots (*Thyrsostachys siamensis*) were purchased from a local market in Prachinburi. The bamboo shoot was washed, peeled, and cut with dimensions of 0.5×0.5×3 cm. A rod of bamboo shoot was immediately soaked in tap water for 5 min.

Yanang leafs (*Tiliacora triandra*) were purchased from a local market in Prachinburi. The ratio of Yanang leaves to water was 1:9 and then sample were mixed at 17,000 rpm for 1 min by blender (Buono-17778P, ROC, Taiwan). Removal of suspended solids from liquid by filtration through 2 layer-muslin cloth. The Yanang juice was heated and stirred continuously until sample temperature reached 70 ℃ for 1 min.

Heat treatment

A rod of bamboo shoot was exposed to air for 5, 10, 15, 20, 25 and 30 min at room temperature (~30 °C). The bamboo shoot containg the highest uric acid was used to study effect of heat treatment. Bamboo shoot samples were boiled in water or Yanang juice at 30, 80 and 95 °C for 5, 10, 15, 20, 25 and 30 min using a heating bath. The ratio of bamboo shoot to liquid was equal to 1:4 (w/v). After boiling, the bamboo shoot samples were packed in plastic bags and immediately cooled for 10 min. Uric acid and xanthine oxidase inhibition were evaluated.

Retort processing

The optimal condition from the heat treatment was selected before retort processing. Approximately 500 g of sample (bamboo shoots and Yanang juice) at ratios of 1:3, 1:3.5 and 1:4 (w/v) were poured into a can 3-7/16 inches in diameter and 4-9/16 inches in height (307×409). The samples were filled with Yanang juice, maintaining a headspace of 5 mm. The cans were fixed with thermocouple glands (Ellab, Denmark) and a thermocouple probe (Ellab, Denmark) was inserted. The cans were fitted with a thermocouple at the cold point. The cans were exhausted in steam for 10 min and immediately double seamed. The sealed cans were loaded inside a horizontal still retort (Patkol, Thailand) and processed at temperatures of 121 ± 1 ℃ under pressure of 15 psi to achieve F_0 value of 5 min for low acid food. Time-temperature data were collected during heat processing using an Ellab data recorder (CTF 9008, Ellab,

Denmark). After heating, the cans were cooled for 20 min in running cool water and the data logger was recorded.

Uric acid content analysis

High performance liquid chromatography (HPLC) can be used to determine uric acid content in fresh and heated bamboo shoot samples, the methods described by Zuo *et al*. (2015) were applied. 100 g of samples were extracted using 150 mL methanol for 5 h. Then, the extracted solution was filtered and evaporated at a temperature of 56 °C under pressure of 306 Mbar until 6 mL remained. Then the uric acid content of the extracted solution was analyzed using HPLC Model 600E, Waters, USA. Chromatographic separation was conducted by using an Atlantis C18 column (150 × 4.6 mm, 5 µm). The flow rate was 1.2 mL/min with UV detection.

Determination of xanthine oxidase inhibition

Xanthine oxidase activity was assayed by reacting the enzyme with xanthine under aerobic condition according to the method described by Umamaheswari *et al*. (2007) with slight modifications. Briefly, the reaction mixture contained 100 µL of sample solution (in 1% DMSO), 300 µL of 50 mM potassium phosphate buffer with pH = 7.5 at temperature of 25 ℃, 100 µL of xanthine oxidase enzyme solution by preparing a solution contained 0.1-0.2 unit/mL of xanthine oxidase in cold water and then adding 100 µL of distilled water. An aliquot of 200 µL of 0.15 mM xanthine solution was added after the reaction mixture was incubated at 37 ℃ for 15 min. After 20 min, 200 µL of 0.5 M HCl was added to stop the reaction. Uric acid formation was monitored at 290 nm using a spectrophotometer. The IC_{50} value for xanthine oxidase activity at each heating temperature for 30 min was determined and estimated based on linear regressions of profit-transformed values of inhibition (%).

Color, viscosity and texture measurement

The color of sterilized Yanang juice was measured in a Hunter colorimeter (Hunter Lab, Model Colorflex 45/0, Virginia) Reflection spectra were registered and Hunter Lab color parameters for 10 ° vision angle and D65 illuminant were calculated. 15 mL of Yanang juice was placed in a Petri dish and at least two readings of each sample were measured. The total color change (ΔE) was calculated as:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$
$$\Delta L^* = L^* - L_0\,,\ \Delta a^* = a^* - a_0 \text{ and } \Delta b^* = b^* - b_0$$

where L_0, a_0, b_0 are the color values of fresh Yanang juice.

Apparent viscosity of the sterilized Yanang juice was determined at 25 ℃ using a Brookfield viscometer, Model LVDV-II+ Pro (Brookfield Laboratories, Massachusetts) at 100 rpm (spindle #0).

Hardness was measured using a texture analyzer (Brookfield Texture Analyzer, Model no. CT3, Germany) equipped with a blade probe.

All determinations of physical properties were made in at least triplicate and results were expressed as mean values.

Microbiological analysis

Total plate count (TPC) and yeast and mold numbers in each the sterilized sample were determined using the pour plate technique. Viable microbial numbers were enumerated by pour plating onto PCA incubated at 37 °C for 48 h for total bacteria and onto PDA incubated at 30 °C for 72 h for yeasts and molds. Flat sour bacteria were also investigated. All experiments were repeated three times and average values were reported.

Statistical analysis

All experiments were carried out in triplicate (n=3) for each sample and mean average values with standard error (±SD) were calculated. Significant differences between means were determined using the statistical package for the social sciences (SPSS). Significance of differences was defined at $p \leq 0.05$.

Results

Effect of bamboo shoots exposed to air

The changes of uric acid content in bamboo shoots during exposure to air before subjection to heat treatment is shown in Table1. Uric acid in fresh bamboo shoots after cutting was 161.43 ± 1.53 mg/100 g of bamboo shoot. Uric acid content increased with time on exposure to air. Highest value of uric acid content (244.73 ± 1.82 mg/100 g of bamboo shoots) was recorded in the sample exposed to air for 30 min. After this period, uric acid content remained constant with the reaction at equilibrium (data not shown).

Table 1. Uric acid content after sliced bamboo shoots were exposed to air

Time (min)	Uric acid content (mg/100 g bamboo shoots)
0	161.43 ± 1.53^{f}
5	169.30 ± 2.29^{e}
10	168.63 ± 1.82^{e}
15	185.67 ± 2.28^{cd}
20	189.45 ± 1.81^{c}
25	217.03 ± 2.28^{b}
30	244.73 ± 1.82^{a}

Note: In each row, abcdef superscripts represent significant differences ($p \leq 0.05$).

The results showed that uric acid content increased with increasing time. The linear changes of uric acid content during expose to air were observed. The empirical correlation was proposed to represent the relationship between the change of uric acid and time as follows:

$$Y = 0.0116t + 104.33; \quad R^2 = 0.9339 \qquad (2)$$

Where Y is concentation of uric acid (mg/100 g bamboo shoots), t is time (min). A good correlation was found with the $R^2 = 0.9339$, indicating that the loss of uric acid content during exposed to air significantly affected the time. Therefore, bamboo shoots exposed to air for 30 min were selected to study the effects of heat treatment. Samples were subjected to heating at 30, 80 and 95 °C in either water or Yanang juice.

Effects of heat treatment

The evolution of uric acid content in bamboo shoots during heating is shown in Figure 1. Initial uric acid content of fresh bamboo shoots after exposure to air for 30 min was 244.73 ± 1.82 mg/100 g bamboo shoots. Uric acid content in the samples decreased rapidly during the early period of heating. After that, the uric acid content decreased at a much slower rate until reaching equilibrium. Higher heating temperatures resulted in faster decrease in uric acid content as expected. However, decrease in the rate of uric acid at 95 ℃ was slightly higher than at 80 ℃. Heating bamboo shoots in Yanang juice (Figure 1B) led to faster decrease rates than heating in water (Figure 1A) for all heating temperatures. Yanang juice was effective in reducing uric acid content in bamboo shoots and the results of xanthine oxidase inhibition (%) in Yanang juice is shown in Figure 2.

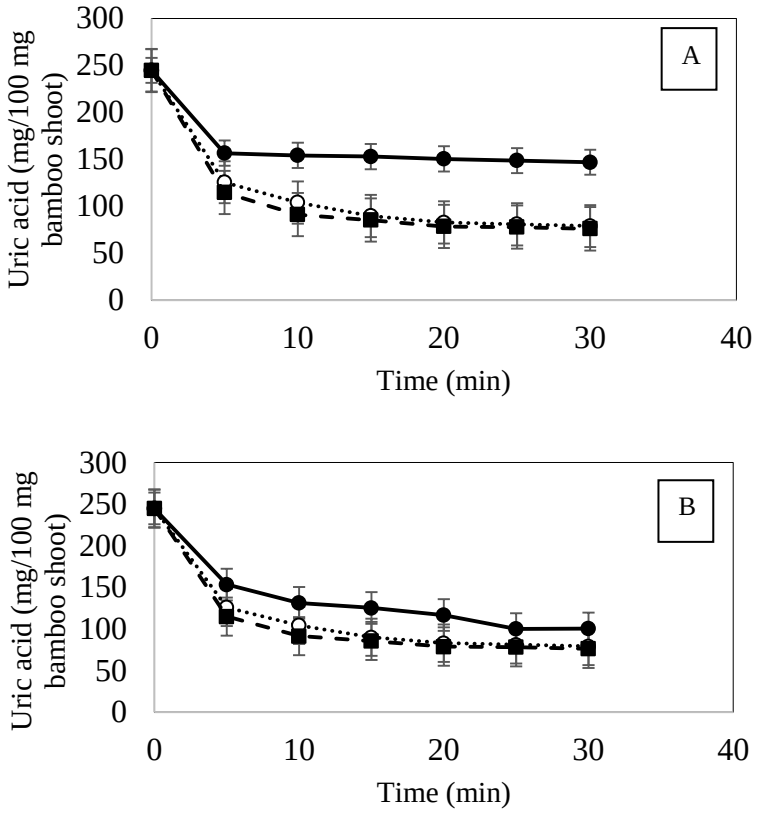

Figure 1. Uric acid content in bamboo shoots during heating at 30 ℃ (●), 80 ℃ (○) and 95 ℃ (■) in water (A) and Yanang juice (B)

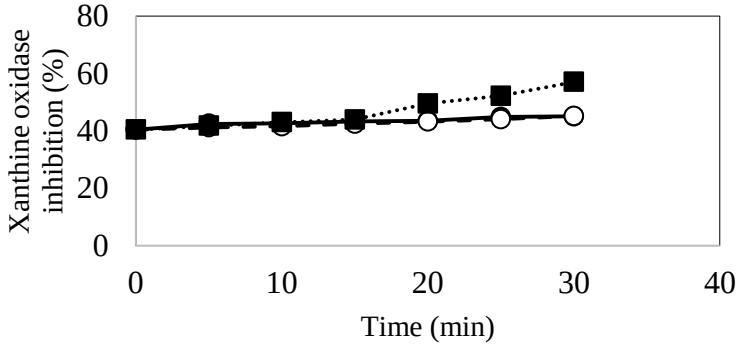

Figure 2. Xanthine oxidase inhibition (%) in Yanang juice during heating at 30 ℃ (●), 80 ℃ (○) and 95 ℃ (■)

Changes of xanthine oxidase inhibition (%) in Yanang juice during heating are shown in Figure 2. At 15 min, heating temperature and time did not have a significant effect on xanthine oxidase inhibition of Yanang juice. After 15 min, effect of heating temperature at 95 °C was more pronounced, while heating samples at 30 and 80 °C showed a slight increase in xanthine oxidase inhibition (%) toward the end of the heating period.

The inhibitory effects of heating temperature on xanthine oxidase in terms of IC_{50} values are shown in Figure 3. Xanthine oxidase is a form of xanthine oxidoreductase, a type of enzyme that plays a key role in the induction of hyperuricemia and raising superoxide radical level in the blood. Xanthine oxidase inhibition (%) of the heated sample at 95 °C was the highest, corresponding to the lowest IC_{50} value (74.09 µg/mL) followed by heated samples at 80 °C (82.71 µg/mL) and 30 °C (84.34 µg/mL), respectively.

Consequently, the project was continued to study the potential of producing canned bamboo shoot in Yanange juice by retorting at 121 °C under pressure of 15 psi to obtain F_0 value 5 min. Heating bamboo shoots in Yanang juice at 95 °C for 30 min (lowest uric acid content, highest xanthine oxidase inhibition and lowest IC_{50}) was selected as the condition to study the effect of the ratio of bamboo shoots to Yanang juice.

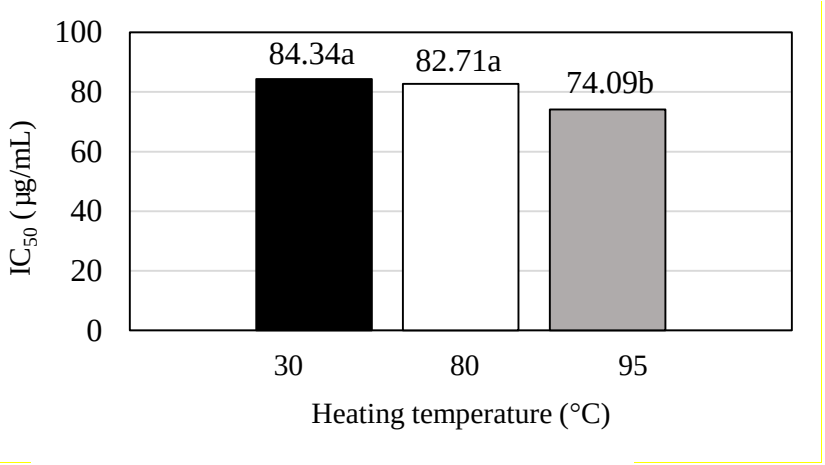

Figure 3. Half maximal inhibitory concentration (IC_{50}) of Yanang juice on xanthine oxidase at different heating temperatures

The temperature profiles and evolution of F_0 of the samples are shown in Figure 4. The sterilization process was divided into three steps as come-up time period, process time period (heating) and cooling period. Thermocouples were placed at the cold point inside the cans with the slowest heating point. Initial temperature of the samples before retorting was approximately 45 ℃. The come-up time period of retorting was 20 min. Sample temperature increased

continuously as heating time increased and approached the set target value (sterilization temperature 121 ℃). After the heating process, the cans were cooled by spraying with water for 30 min.

Sterilized conditions of the canned samples (bamboo shoots: Yanang juice) at different ratios (1:3, 1:3.5 and 1:4) are given in Table 2. All samples were subjected to the same conditions. The ratio of bamboo shoots to Yanang juice led to no significant heat penetration in samples during canned processing.

Table 2. Sterilized condition of canned samples at F_0 = 5 min

Ratio of (bamboo shoots: Yanang juice)	Sterilization temperature (℃)	Come up time (min)	Process time (min)	Cooling time (min)
1:3	121	20	25	30
1:3.5	121	20	25	30
1:4	121	20	25	30

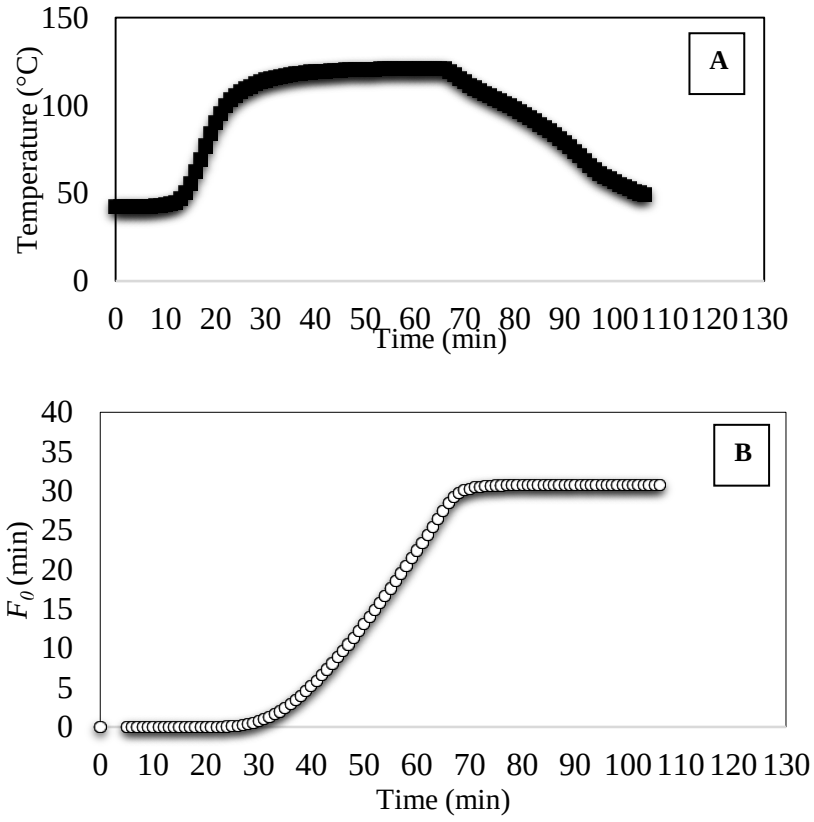

Figure 4. Temperature profile (A) and F_0 (B) at cold point of canned bamboo shoots in Yanang juice during sterilization at 121 ℃

Effect of retort processing

Physical and microbiological properties and uric acid content of samples after the canning process are given in Table 3. Heating in the retort presented little effect on the color of samples, with the ratio of bamboo shoots to Yanang juice of canned samples showing no significant differences. Microbiological testing did not detect flat sour bacteria in the canned samples after sterilization.

Table 3. Properties of canned bamboo shoot in Yanang juice after sterilization at 121 °C, 15 psi, F_0 = 5 min

The ratio of bamboo shoot : Yanang juice	$\triangle E$ of Yanang juice^{ns}	Viscosity of Yanang juice (cP)	Hardness of bamboo shoot (N)	Total bacteria (CFU/g)	Flat sour bacte-ria (CFU/ g)	Uric acid content (mg/100 g bamboo shoot)
Fresh	-	375.08 ± 0.18^{a}	6205.36 ± 138.09^{a}	2.67 × 10^{3}	-	161.43 ± 1.53^{a}
1:3	2.59 ± 0.21	264.08 ± 0.17^{b}	4212.05 ± 283.05^{c}	ND	ND	8.82 ± 0.91^{b}
1:3.5	2.02 ± 0.49	202.00 ± 0.14^{d}	5150.94 ± 81.67^{b}	ND	ND	6.79 ± 0.53^{b}
1:4	2.67 ± 0.01	214.00 ± 0.13^{c}	5326.93 ± 344.59^{b}	ND	ND	4.12 ± 0.53^{c}

Fresh Yanang juice L^* = 15.88 ± 0.13, a^* = -1.75 ± 0.06, b^* = 0.85 ± 0.62;
ND = Not detected;
In each column, abcd superscripts represent significant differences ($p \leq 0.05$). ns represent not significant differences.

Uric acid contents of canned bamboo shoots ranged from 4.12 ± 0.53 to 8.82 ± 0.91 mg/ 100 g bamboo shoots. Results indicated that uric acid content in fresh bamboo shoots was reduced by 20-fold in the canned samples. The uric acid content of bamboo shoots slightly decreased when the sample ratio of bamboo shoots to Yanang juice increased. Lowest uric acid content was found in the sample with ratio of bamboo shoots to Yanang juice at 1: 4 (4.12 ± 0.53 mg/ 100 g bamboo shoots).

Discussion

Uric acid is a product of the metabolic breakdown of purine nucleotides and is a normal component of urine. High blood concentrations of uric acid can lead to gout. The reactions catalyzed on purines are:

$$\text{Hypoxanthine} + H_2O + O_2 \longrightarrow \text{Xanthine} + H_2O_2 \quad (3)$$

and

$$\text{Xanthine} + H_2O + O_2 \longrightarrow \text{Uric acid} + H_2O_2 \quad (4)$$

The cutting process ruptured the bamboo shoot cells. During the exposure of sliced bamboo shoots to air for 30 min, xanthine oxidase enzymes released oxygen that produced reactive oxygen species and catalyzed the oxidation of xanthine to uric acid as stated by Tang *et al*. (2014). Therefore, uric acid increased linearly with increased time exposure to air. No research has studied the effect of time exposure to air of sliced bamboo shoots on uric acid content. Aichayawanich *et al.* (2018) reported uric acid content in fresh bamboo shoots at 247.8 mg/100 g of bamboo shoots.

Fresh bamboo shoots are often heated in water before being used in other ways. This reduces the bitter taste and destroys natural toxins (Satya *et al*., 2010). The influence of each heat treatment affected the concentration of uric acid differently. Soaking bamboo shoots in solution for 5 min sharply reduced uric acid content. The reason for this might be that soaking bamboo shoots in liquid solution (water and Yanang juice) prevented sample expose to oxygen. After that, heat treatment at high temperatures for a long time resulted in reduction of uric acid content. This finding concurred with Aichayawanich *et al*. (2018) who reported significant changes in uric acid content of bamboo shoots after cooking. The evolution rate of uric acid constantly increased with increasing temperature. Soaking bamboo shoots in either water or Yanang juice for 15 min showed no significant difference in reduction of uric acid content. This was because the sample presented temperature stability during heating and heat treatment was not sufficient to reduce uric acid in all treatments.

Considerable decrease of uric acid was recorded in bamboo shoots heated in Yanang juice, with higher reduction than in water at 30 ℃. Reduction in uric acid by heat treatment was caused by the inhibition of xanthine oxidase in Yanang juice. Slicing the bamboo shoots before heat treatment caused a significant increase in uric acid content because tissue disruption during slicing led to release of xanthine oxidase which produced hypoxanthine (Tang *et al*., 2014). This project monitored xanthine oxidase inhibition, which disrupted synthesis of uric acid from purine contained in para-hydroxy benzoic acid and minecoside. Xanthine oxidase prevents the breakdown of hydrogen peroxide (H_2O_2). Soaking bamboo shoots in Yanang juice resulted in an increase in xanthine oxidase inhibition. Yanang juice contains high amounts of various bioactive constituents including phenols, flavonoids, alkaloids, β-carotene, ascorbic acid and minerals. Heat treatment reduces uric acid content because the enzymes are destroyed by heat. Increased temperatures caused the enzymes to work more quickly but when the temperature was too high the enzymes stopped working. Most enzymes become denatured at very high temperatures (Adams, 2007). Similar results were found by Promnuchit and Khotmee (2011) who reported that uric acid content could be

reduced by heating the sliced bamboo shoots in Yanang juice at a high temperature for a long soaking time.

During thermal processing (retorting), heat transfers by conduction and convection (Potter and Hotchkiss, 1998). In conduction, heat moves from one particle to another in more or less straight lines as presented in bamboo shoots. While convection heating is much more rapid, the temperature of Yanang juice showed heats up more rapidly than the pieces of bamboo shoot (Awuah and Ramaswamy, 2007). The ratio of bamboo shoots to Yanang juice exhibited the same pattern of temperature profile to reach F_0 = 5 min. Different ratios showed no significant differences in process time. A thermocouple probe was inserted at the cold point of the can, which was always Yanang juice. Heat transfer occurred by convection. The bamboo shoots prevented liquid flow during heating, resulting in no effect on process time.

During canning, quality changes may occur when bamboo shoots are subjected to high temperatures for a long time, such as the non-enzymatic browning reaction and nutritional degradation. The sterilization process led to low L^* (lightness), with high a^* (redness) and b^* (yellowness) values as the dark green color of Yanang juice when compared with fresh juice. This was due to the heat stability of chlorophyll in Yanang juice (Suwankanid *et al*., 2006; Phungamngen *et al*., 2016). Canned bamboo shoots were softer than fresh bamboo shoots with decreased hardness value. High temperatures during sterilization also affected the viscosity of Yanang juice. Microbiological testing results were lower than the required standard for low acid food products (Japan National Standard, 2004) and flat sour bacteria were not detected after sterilization. However, all canned products still recorded small concentrations of uric acid. The sterilization process could not completely eliminate uric acid in the product. Therefore, preparation steps during slicing bamboo shoots and exposure to air (O_2) must be well designed to ensure the safety of the final product.

Overall, it could be concluded that appropriate preparation step in coupled with appropriate heating condition should be well designed in order to ensure the safety of a canned bamboo shoot product as regular retorting procedure (based on the desired F_0 = 5 min.) might not eliminate all uric acid in a canned product if the initial uric acid is too high. Good Manufacturing Practice (GMP) and good preparation step of sliced bamboo shoot (not expose to O_2 for long time) for should be implemented to reduce the uric acid concentration. From this knowledge could be applied in the food industry.

It is concluded that the effects of heating temperature at 30, 80, 95 ℃ and time for 5, 10, 15, 20, 25, 30 min on uric acid content in bamboo shoot and xanthine oxidase activity in Yanang juice were investigated. Sliced bamboo

shoot expose to air for 30 min then it was heating in water either Yanang juice. The results showed that high temperature (95 ℃) could reduce uric acid in bamboo shoot better than 80 ℃ and 30 ℃, respectively. In all cases the uric acid content decreased with an increase in heating time. Soaking bamboo shoot in Yanang juice showed small effect on uric acid content especially, in the case of high temperature (80 and 95 °C). This observation consistent with the change in xanthine oxidase activity. The xanthine oxidase inhibition (%) of heated sample at 95 °C for 30 min was the highest value while its IC_{50} value was lowest. The preparation step (heating bamboo shoot in Yanang juice at 95 ℃ for 30 min) was selected condition to study the quality of canned sample with different ratio of bamboo shoot to Yanang juice (1:3, 1:3.5 and 1:4). Quality changes of canned bamboo shoots in Yanang juice were also investigated. Canned sample presented lower viscosity and darker color of than the fresh Yanang juice. Lower hardness of canned bamboo shoot was observed. All microbiology tested was less than standard. Different ratio of bamboo shoots to Yanang juice showed little effect on uric acid content. Higher volume of Yanang juice in the container led to lower uric acid content contained in canned product. From the results obtained the contributions to knowledge was applied to the production of canned bamboo shoot in Yanang juice for export.

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