

Physical and biochemical properties of Yanang juice mixed with longan flower-honey following high pressure processing

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Abstract

This study investigated the effects of high hydrostatic pressure (HHP) at 400-600 MPa/25 and 50°C/20 min on physicochemical qualities and bioactive compounds retention in Yanang juice mixed with 10% (v/v) longan flower-honey. The results showed that the parameter (brightness) significantly increased in pressurized juices when compared to fresh juice, while the greenness ($-a^*$) of all processed juices slightly decreased, in particular pressurized batches at 50°C. There was no significant difference in the pH values of fresh and pressurized juices. The levels of ascorbic acid and antioxidant capacity (DPPH and FRAP assays) decreased according to treatment severities, particularly the juices processed at milder-temperatures. The highest retention of total phenols and flavonoids was found in HHP treated juices at 600 MPa. This study also demonstrated that pressure could inactivate the indicator microorganisms including total plate counts, yeasts-moulds and fecal coliforms to levels conforming to the Thai Community Product Standard for Yanang juice.

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Keywords

Yanang-honey juice

High pressure processing

Physical property

Biochemical quality

Bioactive components

Introduction

Yanang (*Tiliacora triandra* (Colebr.) Diels) is an indigenous vegetable used in many cuisines of Thailand and Laos. It has been widely consumed as a health food due to high amounts of various bioactive constituents including phenols, flavonoids, alkaloids, β -carotene, ascorbic acid and minerals. Over the last decades, Yanang extract has been used as a herbal medicine for fever relief, malaria remedy, anti-inflammation, antioxidant, anticancer, antifungal and antibacterial properties (Mahidol *et al.*, 1994; Sireeratawong *et al.*, 2008; Phomkaivon and Areekul, 2009; Singthong *et al.*, 2009; Naibaho *et al.*, 2012). More recently, pasteurized Yanang juice has been developed and promoted as a recommended product of the One Tumbol One Product (OTOP) program by the Thai government. However, the conventional method of pasteurization can lead to undesirable effects on the qualities of juice, including flavor and color changes, non-enzymatic browning and degradation of certain vitamins and heat-labile compounds (Chaikham *et al.*, 2013, 2014).

The increasing consumption of plant beverages containing honey is a current global consumer trend. Honey has been used as sweetening agent instead of sugar from ancient times. Honey also serves as a good source of natural antioxidants. The

predominant antioxidative compounds found in honey are phenolic acids, flavonoids and ascorbic acid (Wang and Li, 2011; Akhmazillah *et al.*, 2013), which all contribute to its beneficial properties. The health-promoting effects of honey can be attributed to its healing, antimicrobial, antioxidant, anticancer, anti-atherogenic, immunoregulatory and anti-inflammatory properties (Fauzi *et al.*, 2011; Fernandez-Cabezudo *et al.*, 2013; Spilioti *et al.*, 2014). These properties can vary depending on the floral sources, seasonal and environmental factors (Al-Mamary *et al.*, 2002; Aljadi and Kamaruddin, 2004). For honeys in Thailand, honey produced from longan flowers showed the highest levels of total phenols and antioxidant capacity followed by wild flowers, sunflowers and litchi flowers, respectively (Sangsrichan and Wanson, 2008).

There is an increasing demand from consumers for minimally processed foods which contain the flavor, color and composition similar to the fresh raw materials. Therefore, processing methods incorporating gentle preservation is required. High hydrostatic pressure (HHP) is an alternative processing which helps to maintain color, flavor, phytochemical components and nutritional values, while also inactivating various strains of pathogenic and spoiling microorganisms in fruit and vegetable juices (Landl *et al.*, 2010; Chaikham and Apichartsrangkoon, 2012).

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To date, many reports are available on the effect of HHP on the physicochemical and microbiological qualities of various products, for instance, strawberry juice (Cao *et al.*, 2012), cucumber juice (Zhao *et al.*, 2013), Chinese bayberry juice (Yu *et al.*, 2013) and mango pulp (Kaushik *et al.*, 2014). However, no information exists concerning HHP effect on Yanang-honey juice. Hence, the objective of this work was to compare fresh and pressurized Yanang juice mixed with longan flower-honey in regard to their color, pH, chlorophyll contents, ascorbic acid, phenols and antioxidant capacity, in order to determine the comparative effects of processing temperatures on the above-mentioned parameters.

Materials and Methods

Preparation of Yanang-honey juice

Yanang was freshly harvested from an orchard in Chiang Mai, Thailand. Upon arrival at the laboratory, the Yanang leaves were washed twice with running tap water and the damaged portions were removed. The washed leaves were soaked in potassium permanganate solution (300 ppm) for 30 min before being rinsed twice more with running tap water. The cleaned leaves were extracted with distilled water at a ratio of 1:1 (w/v). Subsequently, the solids were separated by centrifuging at 1,500 rpm for 15 min through a filter cloth. The filtrate was combined with 10% (v/v) longan flower-honey which was collected from a bee farm in Lamphun, Thailand.

High pressure processing

A 150-ml of fresh Yanang-honey juice was poured into a laminated bag (nylon plus polyethylene) and subjected to different pressure levels of 400, 500 and 600 MPa at 25°C and 50°C for 20 min. The high pressure vessel was a 'Food Lab' model 900 high pressure rig (Stansted Fluid Power, UK) with a pressure increase rate of 315 MPa/min. The pressure transmitting medium was a mixture of castor oil and 98% ethanol (Chemical & Lab Supplies, Thailand) at a ratio of 20:80 (v/v). All pressurized samples were kept at 4°C prior to analysis.

Color parameter and pH measurements

A colorimeter (Color Quest XE HunterLab, USA) was used to measure the color parameter, *L* (brightness), *a*^{*} (redness) and *b*^{*} (yellowness), of fresh and pressurized Yanang-honey juices. The pH values of all samples were measured using a pH meter (Sartorius PB-20, Germany).

Analysis of chlorophylls

Chlorophyll contents were determined using the method described by Lefsrud *et al.* (2007) with some modifications. A 1.5-ml of juice was mixed with 2.5 ml of tetrahydrofuran (THF; Sigma-Aldrich, Switzerland) and then centrifuged at 1,000 rpm for 5 min. The supernatant was filtered through a 0.20- μ m nylon filter (Sartorius, Germany) before injection into a Shimadzu LC-10AD HPLC (Shimadzu, Japan). The HPLC system consisted of a low-pressure pump and a photodiode array detector (SPD-M20A; Shimadzu). Chromatographic separation was performed with a C18 column (YMC-Pack ODS-AM, 5 μ m, 4.6 mm ID \times 250 mm; YMC, Japan) which was maintained at 20°C using a thermostatic compartment. The mobile phases were a mixture of A: 75% acetonitrile, 20% methanol, 4.935% hexane, 0.05% butylated hydroxytoluene (BHT) and 0.015% triethylamine (TEA) and B: 50% acetonitrile, 25% THF, 24.985% hexane and 0.015% TEA with a flow rate of 1 ml/min. The gradient system of the mobile phase commenced from 0 min (100% A) to 30 min (50% A), 32 min (0% A) and 34 min (100% A), and then maintained this state to 45 min prior to the next injection. Chlorophylls a and b from a 20- μ l injection were detected at 665 and 652 nm, respectively. Standards of chlorophylls a and b (Sigma-Aldrich) were dissolved in methanol to obtain concentrations of 25-250 and 10-200 μ g/ml respectively for the calibration curves.

Determination of bioactive components

Ascorbic acid

Ascorbic acid was determined following the method of Chaikham and Apichartsrangkoon (2012) using a HPLC. A single run isocratic mode used 0.1 M acetic acid in deionized water as a mobile phase with a flow rate of 1.5 ml/min at 25°C. A 20- μ l sample was injected into the column. The peak area of each component was determined and converted to concentration. *L*-ascorbic acid (Sigma, USA) was dissolved in methanol to obtain concentrations of 10-300 μ g/ml for the calibration curve.

Total phenols

Total phenols were determined following the modified method as described by Chaikham and Apichartsrangkoon (2012). Two milliliters of samples were mixed with 8 ml of 100% cooled ethanol (Chemical & Lab Supplies) for 10 min at 25°C using a rotary mixture and then centrifuged at 3,000 rpm and 4°C for 10 min. A 0.5-ml of supernatant was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent

(Sigma) and allowed to react for 5 min. Subsequently, 2 ml saturated sodium carbonate solution (Ajax, Australia) was added to the mixture and held for 2 h at ambient temperature. The apparent blue complex was determined using a UV-Vis spectrophotometer at 765 nm (Lambda Bio-20, Perkin Elmer, USA). Total phenols were expressed as mg gallic acid equivalent per 100 ml sample (mg GAE/100 ml).

Total flavonoids

Total flavonoids were determined according to the method of Šarić *et al.* (2012) with some modifications. All samples were centrifuged at 3,000 rpm and 4°C for 10 min. Four milliliters of supernatant were mixed for 5 min with a mixture solution of 4 ml deionized water and 2 ml sodium nitrite (Sigma-Aldrich). Afterward, 2 ml of 10% (w/v) aluminium chloride (Sigma-Aldrich) were transferred into the mixture and stirred for 6 min before adding 12 ml of 1M sodium hydroxide (Merck, Germany). The solution was shaken and the absorbance was measured at 510 nm. Total flavonoids were expressed as mg quercetin equivalent per 100 ml sample (mg QE/100 ml).

Determination of antioxidant capacity

DPPH assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined following Tangkanaku *et al.* (2006) with some modifications. Two milliliters of the sample were mixed with 8 ml methanol for 10 min and then centrifuged at 3,000 rpm for 10 min. Consequently, 3.5 ml of supernatant were mixed with 0.5 ml of 1.5 µM DPPH radical (Fluka, Switzerland) methanol solution. The mixture was shaken and allowed to stand for 30 min at room temperature. Absorbance of the solution was measured at 517 nm. A control was prepared using 3.5 ml methanol. Percentage inhibition of DPPH radical was calculated:

$$\% \text{DPPH radical inhibition} = \left[1 - \left(\frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \right] \times 100$$

FRAP assay

Ferric reducing antioxidant power (FRAP) of all samples was assessed following the modified method of Benzie and Stain (1996). Two milliliters of sample were mixed with 8 ml deionized water for 10 min and centrifuged at 3,000 rpm for 10 min. Each supernatant was mixed with 3 ml FRAP reagent (a mixture of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6-tripyridyls-triazine solution and 20 mM FeCl₃·6H₂O solution at a ratio of 10:1:1) and

incubated at 37°C for 30 min before measuring the absorbance at 593 nm. FRAP value was expressed as mmol Fe (II) per milliliters of the sample (mM FeSO₄/ml).

Microbiological assessments

The assessments of total plate counts, yeasts-moulds and fecal coliforms in fresh and HHP treated samples were carried out following the Bacteriological Analytical Manual (BAM) method of US Food and Drug Administration (2001).

Statistical analysis

All data were the means of six replications. Analysis of variance (ANOVA) was carried out using a SPSS software version 11.5, and determination of significant differences among treatment means was done by Duncan's multiple range tests ($P \leq 0.05$).

Results and Discussion

Table 1 shows that *L* parameter (brightness) of Yanang-honey juice significantly increased ($P \leq 0.05$) after pressurization, as compared to the control (fresh juice). At the same pressure level, HHP treated samples at 50°C showed a significantly higher *L* value than those treated at 25°C ($P \leq 0.05$). In addition, it was found that the brightness of pressurized samples increased with rising pressure levels. Previously, Chaikham and Apichartsrangkoon (2012) have reported that longan juice pressurized at 500 MPa/25°C/20 min had a higher value of *L* parameter when comparison with a fresh batch. Jannok *et al.* (2010) pressurized pennywort juice at 400-600 MPa/30-50°C/20 min and reported that *L* parameters in all HHP treated samples increased with the temperature, however, pressure levels had no influence on this parameter. On the contrary, Krebbers *et al.* (2002) illustrated that after pressurization at 500 MPa for 1 min the brightness value of green bean was lower than that fresh sample.

The *-a** parameter (greenness) of fresh and pressurized juices are also presented in Table 1. The results revealed that the greenness values of pressurized juices at 25 and 50°C were 7.59-7.66 and 7.41-7.48, respectively. HHP treatment at 50°C had a significant effect ($P \leq 0.05$) on *-a** parameter of Yanang-honey juice more than pressurization at 25°C. Although the samples pressurized at 25°C showed a slight decrease in the *-a** parameter, no significant difference was observed between the fresh and pressurized juices ($P > 0.05$). At the same temperature, pressure levels had no effect on this parameter. It was interesting to note that HHP

Table 1. Color parameters and pH of fresh and pressurized Yanang-honey juices

Samples	Color parameters			pH value
	<i>L</i> (lightness)	<i>-a*</i> (greenness)	<i>+b*</i> (yellowish)	
Fresh juice	19.33±0.12 ^e	7.67±0.03 ^a	22.72±0.05 ^d	5.40±0.01 ^a
400 MPa/25°C	19.45±0.04 ^e	7.66±0.08 ^a	23.51±0.10 ^c	5.40±0.01 ^a
500 MPa/25°C	20.62±0.09 ^c	7.62±0.04 ^a	24.27±0.08 ^b	5.42±0.03 ^a
600 MPa/25°C	22.63±0.05 ^b	7.59±0.09 ^a	24.14±0.12 ^b	5.41±0.01 ^a
400 MPa/50°C	20.10±0.05 ^d	7.48±0.02 ^b	24.32±0.07 ^b	5.43±0.02 ^a
500 MPa/50°C	22.51±0.15 ^b	7.41±0.05 ^b	25.16±0.11 ^a	5.41±0.01 ^a
600 MPa/50°C	25.51±0.04 ^a	7.42±0.06 ^b	24.22±0.06 ^b	5.40±0.02 ^a

Means in the same column followed by the same letters are not significantly different ($P>0.05$). Means were the determination of six replications ($n = 6$)

treatment at 400-600 MPa at 25°C resulted in limited green color change of Yanang-honey juice. This might be caused by cell disruption during HHP treatment resulting in the leakage of chlorophyll into the intercellular space yielding a more intense bright green color in the sample (Oey *et al.*, 2008). Similar results were reported by Krebbers *et al.* (2002) with green beans after HHP treatment at 500 MPa/ambient temperature/1 min. However, the green color of the samples visibly shifted to the red zone ($+a^*$) after pressurization at 1,000 MPa/75°C/80 s. Besides *L* and a^* parameters, the yellowness ($+b^*$ parameter) of all pressurized juices was significantly increased ($P\leq 0.05$) after HHP treatment when compared to the control, in particular the juice pressurized at 500 MPa/50°C (Table 1). An analogous result was reported by Chaikham *et al.* (2014), which found that the $+b^*$ parameter of a herbal-plant infusion (a mixture of pennywort, dried longan and purple rice extracts) increased after pressurization at 400-500 MPa/25°C/15-30 min. Overall, these indicated that HHP could better retain the greenness, but milder-temperature degraded the green chlorophyll to green-yellowish pheophytin (Apichartsrangkoon *et al.*, 2013), as displayed in Figure 1 and Table 2.

There was no significant difference in the pH values of fresh and pressurized juices ($P>0.05$), indicating that HPP had no effect on this quality (Table 1). Similar observations were previously observed in HHP treated longan juice and herbal-plant infusion (Chaikham and Apichartsrangkoon, 2012; Chaikham *et al.*, 2014).

Chlorophyll is a green compound found in the leaves and green stems of Yanang leaves. As shown in Table 2, the amounts of chlorophyll a, chlorophyll b and total chlorophylls in Yanang-honey juice significantly decreased ($P\leq 0.05$) after pressurization at 400-600 MPa, except in the juices processed at 25°C chlorophyll b and total chlorophylls were parallel with the control ($P>0.05$). It was worth noting that chlorophylls a and b had different stabilities towards pressure and temperature. Butz

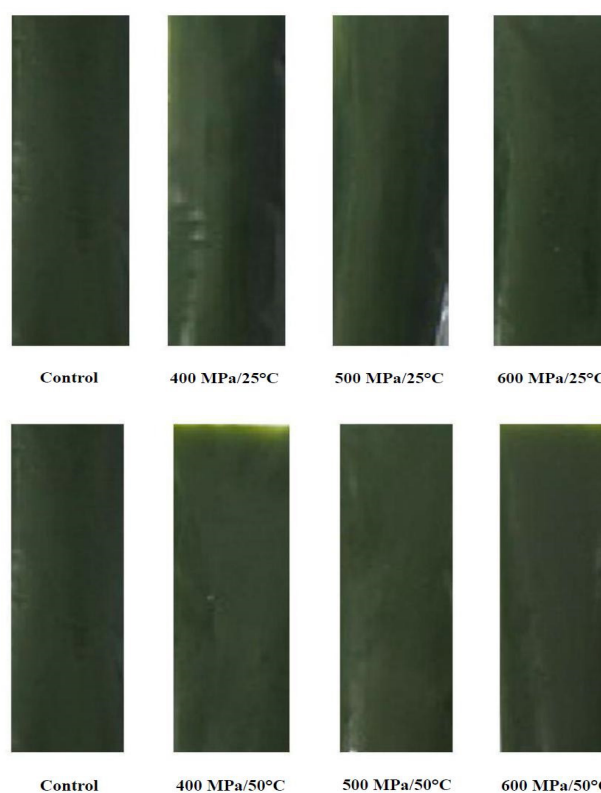


Figure 1. Appearance of fresh and pressurized Yanang-honey juices

et al. (2002) stated that at ambient temperature (~25°C) chlorophylls *a* and *b* exhibited extreme pressure stability but at temperatures higher than 50°C, HHP treatment affected their stability. Van Loey *et al.* (1998) discovered a significant reduction in the chlorophyll content of broccoli juice after pressurization at 200-800 MPa and 50-120°C. They also found that pressurization at >50°C the degradation rate of chlorophyll *b* is higher than that of chlorophyll *a*. Similar findings were reported by Matser *et al.* (2004) with pressurized green beans and spinach (700 MPa/90°C/1 min).

Ascorbic acid levels were significantly declined ($P\leq 0.05$) by pressurization when compared with fresh juice. Pressurization at 50°C resulted in a marked reduction of ascorbic acid compared to samples treated

Table 2. Chlorophyll contents in fresh and pressurized Yanang-honey juices

Samples	Chlorophyll <i>a</i> (ppm)	Chlorophyll <i>b</i> (ppm)	Total chlorophylls (ppm)
Fresh juice	93.07±2.23 ^a	42.07±3.66 ^a	135.14±5.57 ^a
400 MPa/25°C	87.03±1.87 ^b	39.61±0.55 ^a	126.64±2.20 ^a
500 MPa/25°C	85.53±2.86 ^b	41.58±3.83 ^a	127.12±4.54 ^a
600 MPa/25°C	89.62±2.87 ^b	40.66±2.45 ^a	129.28±4.28 ^a
400 MPa/50°C	80.81±1.14 ^c	34.41±2.67 ^c	114.22±2.84 ^c
500 MPa/50°C	80.59±2.71 ^c	37.10±0.96 ^b	117.69±1.89 ^c
600 MPa/50°C	85.04±1.22 ^b	32.48±3.48 ^b	117.52±4.36 ^c

Means in the same column followed by the same letters are not significantly different ($P>0.05$). Means were the determination of six replications ($n = 6$)

Table 3. Bioactive components and antioxidant capacity of fresh and pressurized Yanang-honey juices

Samples	Ascorbic acid (mg/100 ml)	Total phenols (mg GAE/100 ml)	Total flavonoids (mg QE/100 ml)	DPPH inhibition (%)	FRAP value (mM FeSO ₄ /ml)
Fresh juice	48.28±1.92 ^a	120.26±2.16 ^c	58.26±1.56 ^b	36.88±1.34 ^a	21.54±0.33 ^a
400 MPa/25°C	42.01±1.06 ^b	121.68±1.48 ^c	57.31±1.60 ^b	32.93±2.36 ^b	19.90±0.53 ^c
500 MPa/25°C	39.42±2.58 ^{bc}	123.24±2.92 ^{bc}	54.98±3.23 ^b	33.09±1.91 ^b	20.62±0.17 ^b
600 MPa/25°C	38.53±1.70 ^c	127.80±0.43 ^a	64.80±2.61 ^a	30.14±1.71 ^c	18.84±0.13 ^d
400 MPa/50°C	33.50±2.08 ^d	124.86±1.19 ^b	55.69±2.17 ^b	32.75±0.28 ^b	18.31±0.37 ^d
500 MPa/50°C	32.70±1.82 ^d	126.12±3.23 ^{ab}	58.12±2.04 ^b	30.53±1.09 ^c	18.74±0.25 ^d
600 MPa/50°C	29.99±1.40 ^a	130.89±3.87 ^a	62.03±2.70 ^a	31.16±2.14 ^{bc}	18.51±0.87 ^d

Means in the same column followed by the same letters are not significantly different ($P>0.05$). GAE = gallic acid equivalent and QE = quercetin equivalent. Means were the determination of six replications ($n = 6$)

at 25°C. After HHP treatments, the concentration of ascorbic acid significantly diminished ($P<0.05$) when the pressure levels increased (Table 3). Ascorbic acid degradation could be induced by enzyme activation during pressurization. Nagy (1980) revealed two of the main reasons for ascorbic acid degradation, namely oxidative reactions by enzymes such as cytochrome oxidase, ascorbic acid oxidase, polyphenol oxidase and peroxidase available in the juice; and aerobic and anaerobic non-enzymatic reactions. High pressure affects the secondary, tertiary and quaternary structures of proteins; such conformational changes can enhance enzyme activity by uncovering active sites and consequently facilitate catalytic conversion. Patras *et al.* (2009) found that the ascorbic acid content in strawberry puree was reduced by around 19% after being treated at 400-600 MPa/20°C/15 min. Similar findings were observed by Barba *et al.* (2010) and Landl *et al.* (2010) with vegetable beverages and apple purees, respectively.

Total phenols and flavonoids levels were enhanced after being treated with various pressure levels. In particular, at a pressure of 600 MPa both of these compounds showed a significant increase ($P<0.05$). At the same pressure levels, temperature had no effect on these compounds (Table 3). Cao *et al.* (2011) found that total phenols in strawberry pulp were unchanged after pressurization at 400-600 MPa for 5-25 min, while these components were

apparently improved in blueberry (Barba *et al.*, 2013) and pomegranate (Varela-Santos *et al.*, 2012) juices when high pressure did. Of note was that phenols and flavonoids appeared to be pressure tolerant than ascorbic acid. Corrales *et al.* (2008) stated that the increase of phenol contents in the products might be due to the rupture of plate tissues by the increased pressure.

One method alone is not sufficient for accurate testing of antioxidant capacity, as many factors influence this characteristic. Thus, it is indispensable to execute multiple assessments. Antioxidant capacity of fresh and pressurized Yanang-honey juices was assessed using DPPH and FRAP tests. The data in Table 3 showed that the decrease in antioxidant capacity of HHP treated Yanang-honey juices was associated with the reduction of ascorbic acid. Sánchez-Moreno *et al.* (2005) stated that antioxidant capacity of the products is related to the composition and concentration of antioxidative constituents such as ascorbic acid, phenols, carotenoids, anthocyanins or flavonoids. When pressurized at extreme level, the juice showed a markedly lower ($P<0.05$) antioxidant capacity than other processed juices (Table 3). Comparable findings were illustrated by Indrawati *et al.* (2004) and Dede *et al.* (2007) with carrot and tomato juices.

Table 4 displays the microbiological qualities of fresh and HHP treated Yanang-honey juices. It was

Table 4. Microbiological qualities of fresh and pressurized Yanang-honey juices

Samples	Total plate counts (CFU/ml)	Yeasts & moulds (CFU/ml)	Fecal coliforms (CFU/ml)
Fresh juice	4.52±0.84 ^a ×10 ³	1.23±0.35 ^a ×10 ²	6.70±1.80 ^a ×10
400 MPa/25°C	8.60±2.30 ^b ×10	nd ^b	nd ^b
500 MPa/25°C	nd ^c	nd ^b	nd ^b
600 MPa/25°C	nd ^c	nd ^b	nd ^b
400 MPa/50°C	nd ^c	nd ^b	nd ^b
500 MPa/50°C	nd ^c	nd ^b	nd ^b
600 MPa/50°C	nd ^c	nd ^b	nd ^b

Means in the same column followed by the same letters are not significantly different ($P>0.05$). Means were the determination of six replications ($n = 6$). nd = not detected

found that total plate counts, yeasts-moulds and fecal coliforms in pressurized Yanang-honey juices were practically eliminated ($P\leq 0.05$), though there were some traces of microbes detected at 400 MPa/25°C. However, all pressurized juices had microbiological qualities that complied with the Thai Community Product Standard (TCPS No. 1443/2009) for Yanang juice (Thai Industrial Standard Institute, 2009), which established that Yanang juice should have total plate counts below 10⁴ CFU/ml, fecal coliforms below 2.2 MPN/100 ml and no detectable yeasts-moulds. Thus, it was suitable to use HHP as a replacement for conventional thermal processing, since this study showed that pressure could inactivate the indicator microorganisms to a satisfactory level. For others products, Lavinias *et al.* (2008) found that aerobic mesophilic bacteria, yeasts, filamentous fungi and *Escherichia coli* in cashew apple juice were completely inactivated after being treated at 350-400 MPa for 3-7 min. Landl *et al.* (2010) reported that total aerobic mesophilic microorganisms and yeasts-moulds in pressurized apple purée (400-600 MPa/20°C/5 min) were below 50 CFU/g.

Conclusion

The results demonstrated that the *L* parameter (brightness) significantly increased in pressurized drinks when compared to the fresh juice. The greenness of all processed juices was slightly decreased, in particular pressurized batches at 50°C. There was no significant difference in the pH values of fresh and pressurized juices. The amount of ascorbic acid and antioxidant capacity declined according to treatment severities, particularly the juice processed at 50°C. On the other hand, the concentrations of total phenols and total flavonoids in the juices after pressurization were slightly higher than in the fresh sample, with the highest amounts of these components present in pressurized juices at 600 MPa. The microbiological assessments illustrated that total plate counts, yeasts-moulds and fecal coliforms in all pressurized juices

were effectively eliminated.

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