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Effects of conventional and ultrasound treatments on physicochemical properties and antioxidant capacity of floral honeys from Northern Thailand

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ABSTRACT

Three different honey samples viz. longan flower, lychee flower and wildflower honeys were collected from Northern Thailand and subjected to conventional thermal (90 °C/5 min) and ultrasound (40% and 80% amplitudes/20 kHz/30 min) treatments. The physicochemical characterizations such as color parameters, browning index (BI), pH, viscosity, diastase activity, 5-hydroxymethylfurfural (HMF) content and antioxidative properties affected by processing treatments were determined. Our results illustrated that both thermal and ultrasonic processes sufficiently eliminated the indicator microbes to the complied limits of the Thai Agricultural Standard (TAS 8003-2013). Based on the results, the color, BI, pH, diastase activity and HMF content of ultrasonicated honeys were minimally altered. Ultrasonication yielded higher quality of antioxidant compounds and properties as compared to the conventional thermal technique. Additionally, ultrasound treatment at 40% amplitude noticeably improved the levels of total phenols, total flavonoids and antioxidant capacity (FRAP assay). Thus, ultrasonication, especially with low amplification, is an alternative preservation technique for maintaining the quality of honey samples.

1. Introduction

Honey is a viscous-sweet food traditionally consumed by people around the world. It comprises of monosaccharides, bioactive constituents and antioxidants, i.e. phenolic acids, flavonoids, anthocyanins, carotenoids and vitamins (Akhmazillah, Farid & Silva, 2013; Erejuwa, Sulaiman & Ab Wahab, 2012; Kowalski, 2013). Bogdanov, Jurendic, Sieber, and Gallmann (2008) demonstrated that honeys possessed antioxidant, antimutagenic, antitumor, antimicrobial, antiviral, antiparasitic and anti-inflammatory activities. However, color, flavor and composition as well as the levels of health-promoting phytochemicals and antioxidant capacity in honey samples principally depend on the flower sources, geographical regions, weathers and honeybee types. Besides, preservation methods, packaging conditions and storage time also affect the properties of honey (da Silva, Gauche, Gonzaga, Costa & Fett, 2016). Earlier, Sangsrichan and Wanson (2008) illustrated that freshly harvested longan flower honey from bee farms in Northern Thailand had significantly higher levels of total phenols and antioxidant capacity than in wildflower, sunflower and lychee flower honeys, respectively.

In the last decade, floral honey industries in the Northern part of Thailand produced a wide range of honey varieties for both domestic and export markets. During the processing, honey is generally heated above 70 °C to eliminate spoilage bacteria, facilitate packing and procrastinate crystallization (Fauzi, Farid & Silva, 2014; Fauzi and Farid, 2015). Nevertheless, this conventional method usually damages the transparency of such honey due to brown turbidity, and also results in alterations in undesired flavor and detriment of phytochemical compounds and antioxidant properties (Chaikham and Prangthip, 2015; Fauzi et al., 2014). Fauzi and Farid (2015) stated that after thermal process, the decrease of antibacterial activity and accumulation of 5-hydroxymethylfurfural (HMF), a brown pigment compound, in honey were observed. In fact, darkening of honey is related to the increase of HMF content which affects the acceptance of consumer. Additionally, Akhmazillah et al. (2013) found that total phenols in manuka honey apparently improved after high pressure processing (a novel non-thermal technique).

To preserve honey qualities and properties, there is great interest to develop innovative non- or mild-thermal technique such as ultrasonic processing in order to inactivate the spoilage

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microorganisms and undesirable enzymes present in the products, without destroying or minimal impairing the sensorial attributes, color, flavor, nutritional values and antioxidant activities (Chaikham and Prangthip, 2015; Tiwari, Patras, Brunton, Cullen & O'Donnell, 2010; Zinoviadou et al., 2015). Currently, there has been little research conducted on the effects of ultrasonic processing on microbiological and physicochemical qualities in floral honeys. However, in recent years various researchers have studied the effect of ultrasonication on the qualities of several fruit juices including pear, apple, grape and orange juices (Abid et al., 2013; Gabriel, 2012; Saeeduddin et al., 2015; Walkling-Ribeiro, Noci, Cronin, Lyng & Morgan, 2009). Overall, they summarized that ultrasonic treatments have eliminated the indicator microbes and improved or preserved the nutrients, bioactive compounds, antioxidant properties and sensory attributes of each fruit juice. Therefore, this novel method may be an alternative processing to conserve the beneficial properties of honeys more effectively than conventional method.

This study was aimed to investigate the alterations of physicochemical properties, bioactive components and antioxidant capacity in longan flower, lychee flower and wildflower honeys by using ultrasound treatment (40% and 80% amplitude/20 kHz/30 min) and compare with conventional thermal processing (90 °C/5 min).

2. Materials and methods

2.1. Conventional thermal processing

Different types of honeys including longan flower, lychee flower and wildflower honeys were freshly collected from bee farms in Lamphun and Chiang Mai provinces of Northern Thailand. Accordingly, different floral honeys (200 g) were separately filled into the retort pouches (Siampack, Bangkok, Thailand) and then immersed in a thermostatic water bath until an inside temperature reached 90 ± 5 °C, monitoring by the thermocouple probes. After heating for 5 min, all samples were continuously submerged in cooled water for 10 min prior to analysis.

2.2. Ultrasound processing

Briefly, 200 g of honey were poured into a 150-mL glass bottle and then sonicated using a high intensity ultra-sonic processor (VCX 130 PB 130 W, Sonics & Materials Inc., Newtown, CT) with a power of 130 W. The ultra-sonic probe was inserted into the honeys to half the depth of the sample which produced a frequency of 20 kHz. The honeys with the initial temperature of 25.09 ± 2.15 °C were treated at amplitude levels of 40% and 80% for 30 min and the final temperatures of the products were 52.63 ± 2.12 and 75.09 ± 3.38 °C, respectively. Afterward, treated samples were immediately placed in cooled water for 10 min before analysis.

2.3. Standard plate counting

According to the limitations of the Thai Agricultural Standard for honey (TAS 8003-2013: National Bureau of Agricultural Commodity and Food Standards, 2013), the indicator microorganisms including *Salmonella* spp., *Staphylococcus aureus*, total plate counts and yeasts-molds in newly harvested and treated honeys were enumerated following the Bacteriological Analytical Manual (US Food and Drug Administration, 2001).

2.4. Measurements of color parameters and pH value

A colorimeter (model Color Quest XE, Hunter Lab, Reston, VA) was used to measure the color parameters including L^* (0 black, 100 white), a^* ($-a^*$ greenness, $+a^*$ redness) and b^* ($-b^*$ blueness, $+b^*$ yellowness) of all the honey samples. Accordingly, a^* and b^* values were used to calculate the browning index [$BI=(100(x-0.31))/0.172$, where $x = (a^*+1.75L^*)/(5.645L^*+a^*-3.012b^*)$] (Ferrari, Maresca & Ciccarone, 2010).

To determine the pH value, 5 g honey were well mixed with 15 mL deionized water in 50 mL beaker and then measured using a pH meter (model PB-20, Sartorius, Germany).

2.5. Measurement of dynamic viscosity

Dynamic viscosity of the samples was measured using a control stress AR 2000 rheometer (TA Instruments, Inc., New Castle, DE) combined with commercial computer software (Rheology Advantage Analysis software Version 4.1). A concentric cylinder geometry (stator inner radius 15 mm, rotor outer radius 14 mm, cylinder immersed height 42 mm, gap 5920 μm) was used. A 19.6-mL honey was poured into the stationary cup and allowed to equilibrate to 25 ± 2 °C, which controlled by a circulating water system. Viscosity was calculated from the average of five points of the flow curves obtained in the shear rate range between 1 and 10 s^{-1}.

2.6. Measurement of diastase activity

The diastase activity (DN) of untreated, heated and ultrasonicated honeys was determined by using a UV–vis spectrophotometer (Perkin Elmer UVWINLAB, Perkin Elmer, Waltham, MA), according to the procedure of Bogdanov, Martin, and Lullmann (1997) with some modifications. An insoluble blue dyed cross-linked type of starch was used as the substrate. The substrate solution was hydrolyzed by diastase enzyme in honey at 40 °C for 1 h to produce blue water-soluble fragments and detected at λ_{max} 620 nm. The absorbance (A_{620}) of the solution was used to calculate the diastase activity of honey sample [DN, Units/g$=35.2\times(A_{620}-0.46)$].

2.7. Determination of 5-hydroxymethylfurfural content

5-Hydroxymethylfurfural (HMF) was determined using a High-Performance Liquid Chromatography (HPLC, Shimadzu LC-10CE, Shimadzu, Kyoto, Japan) as described by Chaikham, Kreungngern, and Apichartsrangkoon (2013). Ten grams of honey were stirred with 40 mL deionized water for 20 min and subsequently filtered through a 0.20-μm nylon filter (Vertical, Bangkok, Thailand) before injection. The HPLC system consisted of a low-pressure pump and a photodiode array detector (SPD-M20A; Shimadzu) adjusted to a λ_{max} 280 nm. Chromatographic separation was performed with a C18 column (YMC-Pack ODS-AM, 5 μm, 4.6 mm ID × 250 mm; YMC, Kyoto, Japan). The isocratic system used a mixture of 18% (v/v) acetonitrile (Merck, Munich, Germany) and 82% (v/v) mixed acid solution (a mixture of 2 mL acetic acid and 0.2 mL phosphoric acid in 997.8 mL deionized water), as a mobile phase with a flow rate of 1 mL/min at 35 °C. A 20-μL filtrate was injected into the column. Standard HMF (Sigma–Aldrich, St. Louis, MO) was dissolved in acetonitrile to obtain the concentrations of 2–10 mg/L for the calibration curve. The peak area of each component was determined and converted to concentration.