

HEAVY METALS IN THAI HONEY

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Abstract

This study was to determine heavy metal contents in 57 Thai honey, samples from different botanical origins and regions of Thailand using inductively coupled plasma-optical emission spectrometry (ICP-OES). The results showed that the levels of arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb) in the honey samples were <0.006-0.075 mg/kg, <0.001-0.015 mg/kg, 0.009-0.489 mg/kg, 0.025-30.349 mg/kg and 0.005-0.215 mg/kg, respectively. The average Ni level was significantly higher than the levels of the other heavy metals in different honey sources ($p < 0.05$). The Pb concentration in the longan honey from Chiang Mai Province was the only heavy metal that was significantly higher than the longan honey from Lamphun Province ($p < 0.05$). The honey from the northern Thailand had significantly higher heavy metal contents than other regions ($p < 0.05$). The levels of heavy metals in Thai honey were all below the Thai food containing contaminants standard, as well as the EU and Codex Alimentarius standards. It was concluded that the Thai honeys are a safe food for consumption.

Keywords: Contamination, food, heavy metal, honey, Thailand

Introduction

Longan, lychee, macadamia, bittersvine and korlan honey are produced primarily in the northern Thailand, but others (sunflower, rambutan, sesame, bitter bush, coconut and mangosteen honey) are produced in different

geographical locations in Thailand (Chuleeporn *et al.*, 2016). The botanical origin of honey can be wild or from the garden or from the orchard. The properties and composition of honey depend on season,

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region, sensorial qualities, nectar plant source, chemical and microbiological characteristics, prophylactic properties, adulteration and toxicant contamination (Rekha *et al.*, 1997).

Several authors reported that honey can be used as a biological indicator for the environment in a bee swarm area (Przybyłowski and Wilczyńska, 2001; Porrini *et al.*, 2003; Stefan *et al.*, 2007; Paulius *et al.*, 2010). Moreover, bee products can be contaminated with different types of chemicals used in orchards, as well as those heavy metals in the exhaust from industrial plants and transportation vehicles. Heavy metal contamination of food is one of the most important aspects of food quality assurance (Berdanier *et al.*, 2007). However, these elements can be toxic to human when ingested at high doses (Demirbas, 2008). Generally, the contamination of heavy metal in honey does not present a health hazard. In Thailand, various types of honey are produced and consumed; however, reports comparing the levels of heavy metal contents various honeys from Thailand are very limited. Thus, the aim of this research was to determine the concentration of heavy metal contents (As, Cd, Cr, Ni, Pb) in honeys obtained from different botanical origins and regions of honey production in Thailand.

Materials and Methods

Sample Collection

Honey samples from different regions were obtained from the Department of Agricultural Extension in the Ministry of Agriculture and Cooperatives and the beekeepers of Thailand. Fifty seven honey samples were produced in rural areas, except for bitter bush, bittervine and korlan honey, which were produced in the mountains by apiculture producers. In the south, rambutan (n=3), coconut (n=3), and para rubber tree (n=3), were produced. In the east, mangosteen (n=3), In the central region, sunflower (n=2), sesame (n=3) were produced, while in the northeastern region, bitter bush (n=2), sesame (n=2), and red gum (n=3) were obtained. The

northern regions produced, longan (n=22), lychee (n=4), korlan (n=3), macadamia (n=2), and bittervine (n=2). Upon collection, these samples were stored in plastic tubes with a screw cap, and were immediately transferred to -10°C storage before analysis.

All chemicals were of analytical reagent grade. Double-deionized water obtained from a MilliQ water purification system (Millipore) was used in all dilutions. A 1,000 mg/L multi-element standard (ICP standard) of As, Cd, Cr, Ni and Pb was obtained from Merck (Germany), and was diluted to the appropriate concentration. Concentrated nitric acid (69%), hydrogen peroxide (30%) and hydrochloric acid (37%) were purchased from QRec[®], New Zealand.

Sample Preparation

Honey samples (5 g) were dried in a porcelain crucible at 100°C. The samples were later heated to 550°C for 4 h (adapted from the 920, 180 method of the AOAC, 2000a). Then, 5 mL of hydrochloric acid was added and diluted to 10 mL with distilled water (dilution ratio 1:2). Triplicate samples were analysed.

Heavy Metal Determination

The high-temperature dry oxidation (HTDO) method adapted from the 985,01 method by AOAC (2000b) and sample preparation according to the 920, 180 by method AOAC (2000a) were used in this study. For each sample, the concentration of each heavy metal was determined using inductively coupled plasma-optical emission spectrometry (ICP-OES; model Optima 8300). Measured wavelengths required to detect As, Cd, Cr, Ni and Pb were 188.979, 226.502, 267.716, 231.604 and 220.353nm, respectively. The standard curves for heavy metal determinations were constructed using a series of standard ICP multi-element MERCK[®] IV solutions. The detection limit values of each metal were 0.006, 0.001, 0.001, 0.001, and 0.005 mg/kg for As, Cd, Cr, Ni and Pb, respectively. The percentages of recovery ranged from 96-100 for the studied elements, determining as acceptable (United States Environmental Protection Agency, 1994).

Table 1. Concentrations (mg/kg) of trace elements from honey in the five tested regions of Thailand

Region	As	Cd	Cr	Ni	Pb
Northern (33)	UD-0.075	UD-0.015	0.009-0.489	0.049-30.349	0.005-0.215
Southern (9)	UD-0.02	0.001-0.004	0.015-0.026	0.025-0.208	0.011-0.05
Eastern (3)	UD-0.012	UD-0.004	0.017-0.344	0.038-0.252	0.003-0.04
Central (5)	UD-0.011	UD-0.001	0.014-0.027	0.033-1.283	0.011-0.033
Northeastern (7)	UD-0.013	UD-0.001	0.015-0.027	0.098-0.368	0.007-0.03

Note: UD = Undetected (heavy metal assay reporting levels: As $\geq$ 0.006, Cd $\geq$ 0.001 mg/kg)

Statistical Analysis

All data were calculated to determine the minimum, maximum values as well as median values of three determinations, then analysed using the Mann-Whitney U test's statistic value and Kruskal-Wallis test's statistic value, and followed by multiple comparison tests with equal variances assumed (Duncan method). A probability of 0.05 or less was considered as a significant difference. Data were analyzed using IBM SPSS software Version 16.

Results

The heavy metal concentrations in 57 honey samples from the five tested regions of Thailand are shown in Table 1. Ni was the most abundant element presented in all the honey samples, while Cd was present at the lowest amount

The highest As level (0.075 mg/kg) was found in longan honey, followed by coconut (0.02 mg/kg), and para rubber honey (0.017 mg/kg), respectively. The median As concentration was 0.014 mg/kg. As levels in the honey samples were lower than 0.006 to 0.075 mg/kg.

The highest Cd level was 0.015 mg/kg in longan honey, followed by rambutan (0.004 mg/kg), and korlan honey (0.003 mg/kg), respectively. The median value of Cd was 0.002 mg/kg. Cd levels in the honey samples were <0.001 to 0.015 mg/kg.

The highest Cr level (0.489 mg/kg) was found in longan honey, followed by macadamia (0.214 mg/kg), and sesame honey (0.027 mg/kg), respectively. The average Cr concentration was 0.048 mg/kg. Cr level in the samples ranged from 0.009-0.489 mg/kg.

The highest Ni level (30.35 mg/kg) was found in longan honey, followed by sunflower (1.283 mg/kg), and macadamia honey (0.836 mg/kg), respectively. The mean Ni concentration was 1.059 mg/kg. Ni level in the samples ranged from 0.025-30.349 mg/kg.

The highest Pb level (0.215 mg/kg) was found in longan honey, followed by rambutan (0.05 mg/kg), and para rubber honey (0.049 mg/kg), respectively. The mean Pb concentration was 0.029 mg/kg. Pb level in the samples ranged from 0.005-0.215 mg/kg.

Heavy Metals in Longan Honey From Chiang Mai and Lamphun Provinces

Chiang Mai and Lamphun provinces are the major cities producing longan honey in Thailand. Results in Table 2 found that the levels of As, Cd, Cr, Ni and Pb from Lamphun province were lower than 0.006-0.011 mg/kg, lower than 0.001-0.015 mg/kg, 0.01-0.029 mg/kg, 0.252-1.001 mg/kg and 0.005-0.23 mg/kg, respectively. The results showed that the levels of As, Cd, Cr, Ni and Pb from Chiang Mai province were lower than 0.006-0.075 mg/kg, lower than 0.001-0.003 mg/kg, lower than 0.001-0.489 mg/kg, 0.27-30.349 mg/kg and 0.005-0.215 mg/kg, respectively. The highest level of heavy metal was Ni from Chiang Mai province (30.35 mg/kg).

Discussion

The median As concentration was 0.014 mg/kg, which was below the maximum allowed level (0.20 mg/kg) for the Thai honey (Ministry of Public Health, 2000). A similar result of As level was obtained from Croatian honey (0.019 mg/kg; Bilandžić *et al.*, 2011).

Table 2. Median concentrations (mg/kg) of heavy metals in longan honey from Chiang Mai and Lamphun Provinces

Province	As	Cd	Cr	Ni	Pb
Lamphun (11)	0.0070	0.0020	0.0160	0.4465	0.0133
Chiang Mai (11)	0.0135	0.0020	0.0210	0.5130	0.0290
<i>p</i> -value	0.466	0.325	0.392	0.975	0.028*

*Significant difference at the 0.05 probability level.

However, it was higher than the As level from Siena country, Italy (0.007 mg/kg; Pisani *et al.*, 2008), Thailand (0.002 mg/kg; Wetwitayaklung *et al.*, 2017) and the republic of Latvia (0.001-0.003 mg/kg; Vincevica-Gaile *et al.*, 2012), but was lower than the As level in honey from New Zealand (0.08 mg/kg; Vanhanen *et al.*, 2011).

The median value of Cd was 0.002 mg/kg. The Cd level did not exceed the standard for the allowed level (0.20 mg/kg) (FAO/WHO, 1984). The observed Cd level was lower than that observed in several reports including from New Zealand (0.149 mg/kg; Vanhanen *et al.*, 2011), Chile (0.01-0.05 mg/kg; Fredes and Montenegro, 2006), Thailand (0.025 mg/kg; Wetwitayaklung *et al.*, 2017), and Switzerland (Not detected-0.026 mg/kg; Stefan *et al.*, 2007). Similar results were presented in studies from Croatia (0.001 mg/kg; Bilandžić *et al.*, 2011), Lithuanian (0.004-0.016 mg/kg; Matusevicius *et al.*, 2010), Siena County of Italy (0.003 mg/kg; Pisani *et al.*, 2008), Turkey (0.009-0.018 mg/kg; Tuzen *et al.*, 2007), Poland (0.015 mg/kg; Przybyłowski and Wilczyńska, 2001) and the USA (0.001 mg/kg; U.S. Food and Drug Administration, 2014).

The average Cr concentration was 0.048 mg/kg. The WHO considered that supplementation of chromium should not exceed 0.250 mg/day (European Food Safety Authority, 2010). The obtained Cr level in Thai honey was lower than the levels found in the honeys from Chile (0.03-1.92mg/kg; Fredes and Montenegro, 2006 and Thailand (0.121 mg/kg; Wetwitayaklung *et al.*, 2017) but was higher than the honey from Switzerland (0.005-0.037mg/kg; Stefan *et al.*, 2007) and Turkey (0.002-0.037 mg/kg; Tuzen *et al.*, 2007).

The mean Ni concentration was 1.059 mg/kg. The level of Ni in Thai honey was higher than that in the honey from Chile (0.01-1.48 mg/kg; Fredes and Montenegro, 2006), New Zealand (0.02-0.65 mg/kg; Vanhanen *et al.*, 2011), Switzerland (Undetected-1.966 mg/kg; Stefan *et al.*, 2007) , Thailand (0.013-0.513 mg/kg; Wetwitayaklung *et al.*, 2017) and the USA (0.001-0.058 mg/kg; (U.S. Food and Drug Administration, 2014). However, a similar result was found in the honey from Turkey (2.6-29.9 mg/kg; Tuzen *et al.*, 2007). No reference was available on levels of nickel in the honeys from other countries or Thailand. The average Ni level was significantly higher than the other heavy metals. The statistical analysis results using the Kruskal Wallis test showed a significant difference only in the Ni from all Thai honeys ($p < 0.05$).

The mean Pb concentration was 0.029 mg/kg. All of the tested types of honey had Pb concentrations lower than the maximum allowed contaminant level (0.5 mg/kg) (Ministry of Public Health, 2000). Our result for Pb was similar to the levels reported from Turkey (0.008-0.105 mg/kg; Tuzen *et al.*, 2007), Switzerland (0.003-0.329 mg/kg; Stefan *et al.*, 2007), but were higher than the honey from Finland (0.004-0.018 mg/kg; Fakhhimzadeh and Lodenius, 2000), Turkey (0.017-0.032 mg/kg; Tuzen and Soylak, 2005, Taiwan and mainland China (0.007–0.029 mg/kg; Wang *et al.*, 2011), and the USA (0.001-0.018 mg/kg; U.S. Food and Drug Administration, 2014). However, this studied honey showed Pb concentration less than the levels reported 0.215 mg/kg (Wetwitayaklung *et al.*, 2017).

Furthermore, honey from the northern part of Thailand had higher concentrations of heavy metal contamination than the other regions. Statistical analyses of the Mann Whitney test showed a significant difference only in the Pb component from all longan honey samples ($p < 0.05$). Longan honey from Chiang Mai province had a median Pb concentration higher than that from Lamphun province. The differences observed in the minimum-maximum element values are presented in Table 1.

Our results and the reports by Darfour *et al.* (2015) and Stefan *et al.* (2007) showed that the highest heavy metal content in the honey was Ni followed by Cr, Pb, As and Cd, respectively. The content of metals in previous study of Thai honey of Wetwitayaklung *et al.* (2017), ranking from high to low, were Ni, Pb, Cr, Cd, and As. Comparisons of the different monofloral honeys contained differences in amount of metals (Porrini *et al.*, 2003; Vincevica-Gaile *et al.*, 2012; Wetwitayaklung *et al.*, 2017). In this study, honey from different regions and different monofloral honeys of Thailand showed differences heavy metal contents. Contamination of metals was carried to a hive of bees from their journeys gathering nectar and pollen (Porrini *et al.*, 2003; Vincevica-Gaile *et al.*, 2012; Wetwitayaklung *et al.*, 2017).

Honey can be contaminated by heavy metal via two factors: (1) an external sources, such as pollen, dust, air, soil, fertilizers, agro-chemicals and nectar (Stefan *et al.*, 2007; Somporn, 2010), and (2) an internal sources, such as the equipment used during honey processing and the conservation phases. Generally, the primary source of the contamination is difficult to control. In contrast, the secondary sources of contamination can be controlled by good manufacturing practices (Pisani *et al.*, 2008; Wang *et al.*, 2010). However, equipment materials used during the harvesting, processing and storage of honey can be corrosive due to the acidity of the honey (Fredes and Montenegro, 2006; Tuzen *et al.*, 2007; Vincevica-Gaile *et al.*, 2012), possibly causing an increase in the heavy metal concentrations.

Conclusions

All the tested honeys, in this study were of good quality because the heavy metal levels were below the Thai standard and Codex Alimentarius standards permitted limit. Although, it was obvious that two longan honey samples showed relatively high level of Cr (0.489 and 0.292 mg/kg). Almost all trace element concentrations of honey samples was very low, and the average daily consumption of honey (about 20 g) contains only 1% of the Recommended Dietary Allowance (RDA) values of essential elements. The RDA for Cr is 40 µg/day for adults. Thus, elements are minor components of honey that are safety for the human health for the daily intake.

As the levels of heavy metals in Thai honey samples were all below the Thai and the EU and Codex Alimentarius standards, the Thai honeys can be consumed and exported as safe food for human consumption. The contamination can arise from beekeeping practices or from the environment. Therefore, it is crucially important to monitoring analyze the residual heavy metals in honey for food safety and a biological indicator of environmental pollution.

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