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Some Physiochemical Properties of Acacia Honey from Different Altitudes of the Asir Region in Southern Saudi Arabia

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Abstract

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The physiochemical properties of six Acacia honey samples taken from three different altitudes in the Asir region were determined. The means of all the studied parameters were within the international standards and were comparable to previous studies (0.11 ± 0.08% for ash, 10.93 ± 1.97% for water, 3.56 ± 0.19 for pH, 38.63 ± 17.17 meq/kg for acidity, 659.51 ± 324.98 μS/cm for conductivity, and 1.45 ± 0.02 for specific gravity). Comparison of the mean values of the parameters at the different altitudes revealed gradual increases with increasing altitude, except for ash and specific gravity. Significant differences were seen in ash and water percentages, acidity, and conductivity. While the mean values of the studied physiochemical properties of the Acacia honey samples were within the ranges of international standards, the honey produced at high altitudes exhibited variable physiochemical properties.

Keywords: acidity; climate changes; conductivity; water percentage

The Asir region is located in the middle of the south-western part of Saudi Arabia; altitudes in the region vary from zero-level elevation (sea level) up to 3015 m a.s.l. (Saudi Geological Survey 2012). Beekeeping is one of the major professions in the Asir region, and Acacia honey is the major honey that is produced across all altitudes.

Acacia honey is consumed for its nutritive and medicinal properties (Abeshu & Geleta 2016), and comes in two colours, pale yellow and dark yellow. This pilot study was conducted to obtain data regarding the physiochemical properties of the Acacia honey in the Asir region, as no studies on this topic have yet been published.

According to Codex Alimentarius standards (Codex Alimentarius 1981) and the United States National Honey Board reference guide (National Honey Board 2005), ash in honey should not constitute more than 0.6%, moisture or water percentage not more than 20%, pH range should be between 3.4 and 6.1, conductivity less than 800 μS/cm, acidity concentration should be less than 50 meq/kg. The specific gravity is water content-dependant; however, at 15% moisture the specific gravity would be 1.435 and at 18% moisture it would be 1.4171.

It has been reported that the altitude and climate affect the the physiochemical properties and quality of honey (Popov-Raljić *et al.* 2015; Nayik *et al.* 2016).

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The aim of this pilot, descriptive, case-control study was to investigate the effect of altitude on some of the physiochemical proprieties of Acacia honey in the Asir region of Saudi Arabia. The parameters investigated were the percentages of ash and water, pH, acidity, conductivity and specific gravity. The botanical origin of the honey samples was confirmed by pollen analysis.

MATERIALS AND METHODS

Samples. Six Acacia honey samples were collected from three bee farms at different altitudes in the Asir region. Two samples were from Alsouda with an altitude of 2200 m a.s.l., two from Rigal Almaa at 1200 m altitude, and two from Mahayil asir at 1000 m a.s.l. Some of the ecological characteristics are summarised in Table 1. The three altitudes were chosen according to the location of the bee farms. All the samples were collected directly from hives on the bee farms. The samples were filtered and the filtrated honey was kept at room temperature (17–25°C) for two weeks before analysis.

Pollen analysis. Pollen analysis was carried out for the purpose of confirming the origin of the honey samples, i.e., that they were Acacia. The morphological appearance of the pollens of the Acacia species and the honey samples was determined according to the Louveaux method for pollen analysis (Louveaux *et al.* 1978). The pollens of the honey were photographed and compared to the pollens of three *Acacia* species: *Acacia tortilis* (Samar), *Acacia ehrenbergiana* (Salam), and *Acacia arabica*. These three *Acacia* species were selected because they were in their blooming periods at the time of sampling.

Ash percentage. Five grams of honey were burned at 550°C in a furnace oven. The percentage of ash was calculated using (ash weight/honey weight) × 100 (Liberato & Morais 2013).

Moisture percentage. Five grams of each honey sample were heated for six hours at 100°C in an oven to evaporate the water from the honey samples. The water percentage was calculated as follows (Khalil *et al.* 2001):

Humidity percentage = [weight of water (weight of honey before heating – weight of honey after heating)/weight of honey before heating] × 100

pH value. Five grams of honey sample were dissolved in 37.5 ml distilled water in a 250-ml beaker and the pH was determined using a pH meter (Kinati *et al.* 2011).

Conductivity. Five grams of honey were dissolved in 50 ml of deionized water (10%) and conductivity was measured using a conductometer (Desissa 2014).

Acidity. Five grams of honey were weighed and poured into a conical flask containing 37.5 ml distilled water. The honey was titrated against a 0.1 N NaOH solution and phenolphthalein was used as an indicator. The end point was taken when the colour changed from colourless to pink. The acidity was calculated as follows (The Association of Public Analysts 1992):

Acidity (meq/kg) = (titration volume × 0.1 × 1000)/ weight of honey (kg)

Specific gravity. The specific gravity of each honey sample was determined by comparison with water. Water was weighed into a 10-ml volumetric flask and each honey sample was weighed into the same flask. The specific gravity of honey was calculated as the weight of honey/weight of water.

Statistical analysis. ANOVA with the Least Significant Difference test (LSD) was used for the analysis of the obtained results in SPSS v20 software.

Table 1. Climate and the *Acacia* species found in the areas used for honey sampling

Altitude (m)	Yearly mean temperature (°C)	Humidity (%)	Rain fall (mm)	Dominant flora
1000	28–36	not available	summer rains, 300	*Acacia ehrenbergiana* (Salam); *Acacia tortilis* (Samar)
1200	28–36	not available	summer rains, 300	*Acacia seyal* (Seyala); *Acacia assak* (Dahiana)
2200	18–20	30–85	all the year, 200–500	*Acaia arabica* (Talha)

The information in the table was obtained from the official internet site of the government of Asir (http://www.ars.gov.sa/AsirRegion/AsirInLines/Pages/default.aspx), the Saudi General Authority of Meteorology and Environmental Protection (http://www.pme.gov.sa/en/pages/default.aspx) and from oral consultation with bee keepers; the *Acacia* species information was obtained from the beekeepers

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RESULTS AND DISCUSSION

Honeys from all three altitudes was found to contain Acacia pollens, confirming their botanical origin. The appearance of the pollens in the honey deteriorated with increasing altitude (Figure 1).

The mean values of all the studied parameters were within Codex Alimentarius standards of those of the US National Honey Board (Table 2). All values were within the standard ranges except for those for acidity and conductivity; these deviating values are due to differences in samples taken from the altitude of 2200 m (Table 3).

The mean values for moisture (water percentage), pH, acidity and conductivity increased with increasing altitude (Figures 2 and 3). The ash percentage was increased at 1200 m altitude and decreased at 2200 m altitude, and the ash percentage at an altitude of 2200 m (0.13%) was far greater than its value at 1000 m (0.04%). We can conclude that ash percentage generally pattern increased with increasing altitude (Figure 4). The specific gravity followed a different pattern; mean values decreased with increasing altitude (Figure 5).

When the mean values of the parameters at the three different altitudes were compared using the ANOVA test, the only significant increase in the parameters was seen in water percentage (between

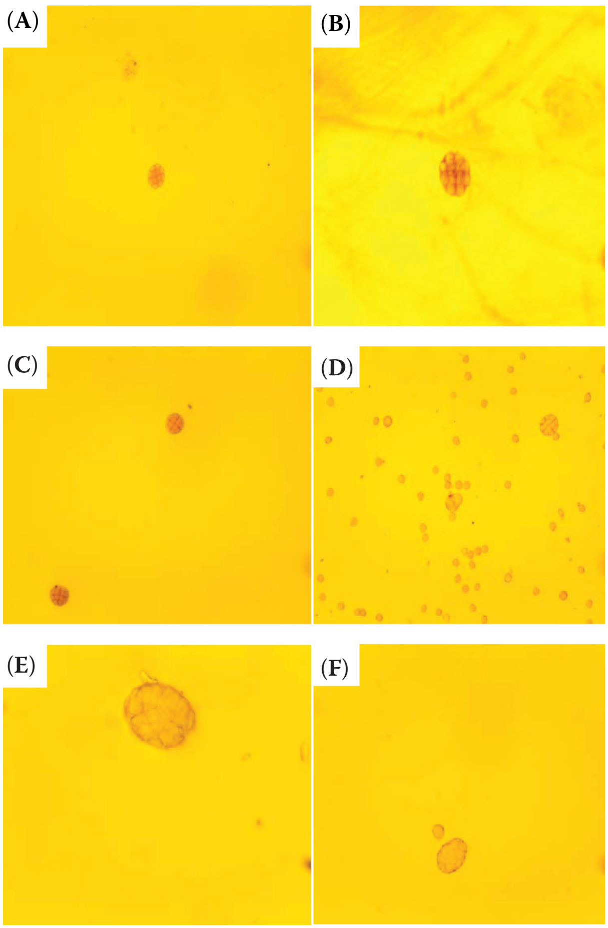

Figure 1. Pollen analysis of three *Acacia* species and the honey from the three altitudes: (**A**) pollen of *Acacia tortilis* (Samar); (**B**) pollen of *Acacia ehrenbergiana* (Salam); and (**C**) *Acacia arabica* pollen. There were slight differences between the three pollens. The photos in (**D**), (**E**), and (**F**) are of pollens in the honeys from altitudes of 1000, 1200 and 2200 m, respectively. It is clear that pollen appearance in the honey deteriorates with increasing altitude

Table 2. The ranges, means and standard deviations of the studied Acacia honey parameters

	Parameter	Range	Mean	SD
1	ash (%)	0.02–0.2	0.11	0.08
2	water (%)	8.8–13.85	10.93	1.97
3	pH	3.32–3.77	3.56	0.19
4	acidity (meq/kg)	19.8–65	38.63	17.17
5	conductivity (µS/cm)	363.75–1207	659.51	324.98
6	specific gravity	1.42–1.46	1.45	0.02

The upper limits of acidity and conductivity ranges exceeded the standards of the Codex Alimentarius and the US National Honey Board; SD – standard deviation

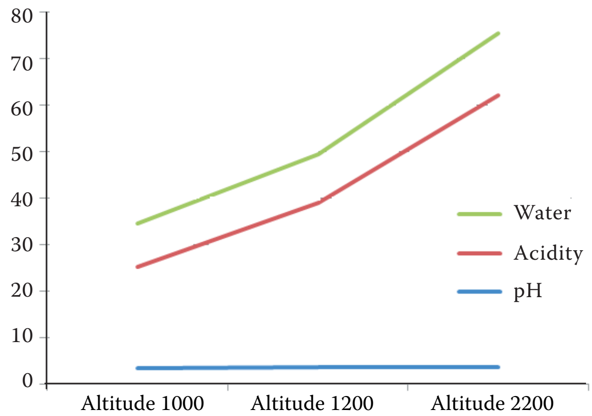

Figure 2. Effects of altitude on the water percentage, pH, and acidity of Acacia honey from the Asir region. The water percentage, pH, and acidity increased with increasing altitude. The increase in the water percentage may be due to the increase in environmental humidity at higher altitudes. The increase in the pH and equivalent acidity of the honey may be due to the effects of high-altitude characteristics on the bees and pollens. High altitude is characterised by hypoxia, low barometric pressure, low temperatures, and high levels of X-radiation

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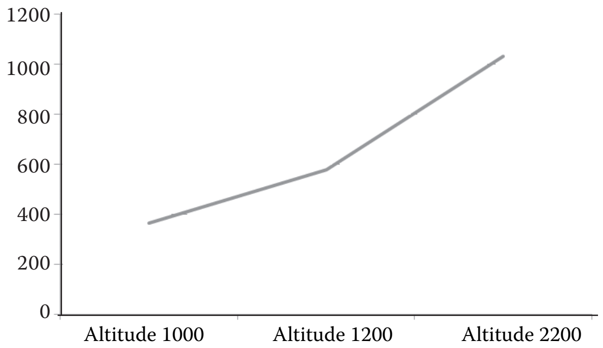

Figure 3. Effect of altitude on the conductivity of Acacia honey from the Asir region. Conductivity increased with increasing altitude. The increase in conductivity may be caused by the climatic conditions at high altitudes

altitudes of 2200 and 1200 m, and between 2200 and 1000 m, $P = 0.012$ and 0.006, respectively), in pH values between altitudes of 2200 m and 1000 m ($P = 0.039$), in acidity between altitudes of 2200 m and 1200 m and between 2200 and 1000 m ($P = 0.03$ and 0.009, respectively) and in conductivity between altitudes of 2200 m and 1000 m ($P = 0.019$). The remaining differences between the mean values were not significant (Table 4).

Ash percentage. Our obtained results revealed the range, mean and standard deviation of the ash percentage for the six samples to be 0.02–0.2, 0.11, and 0.08%, respectively (Table 2). This result is within the Codex Alimentarius standards and the US National Honey Board reference guidelines (Codex Alimentarius 1981; National Honey Board 2005). However, in some Bangladeshi honey samples the mean ash percentage was 0.36 ± 0.08% (Asaduzzaman *et al.* 2015). Buba and her colleagues studied 18 honey samples from Nigeria and found that the ash percentage was 0.42 ± 0.009%, which is higher than our findings (Buba *et al.* 2013). Our results

Table 3. The ranges, means and standard deviations of the studied Acacia honey parameters in samples taken from the three different altitudes

Parameter	Altitude (m)	Range	Mean	Standard deviation
Ash (%)	1000	0.02–0.05	0.04	0.02
	1200	0.13–0.2	0.17	0.05
	2200	0.06–0.2	0.13	0.99
Water (%)	1000	8.8–9.7	9.25	0.64
	1200	10–10.4	10.2	0.28
	2200	12.85–13.85	13.35	0.71
pH	1000	3.32–3.33	3.33	0.01
	1200	3.53–3.75	3.64	0.01
	2200	3.63–3.77	3.7	0.16
Acidity (meq/kg)	1000	19.8–24	21.9	2.97
	1200	33–38	35.5	3.54
	2200	52–65	58.5	9.19
Conductivity (μS/cm)	1000	363.75–366.77	365.26	2.14
	1200	544.7–615.3	580	49.92
	2200	859.55–1207	1033.28	245.68
Specific gravity	1000	1.46	1.46	0.000
	1200	1.44–1.46	1.45	0.014
	2200	1.42–1.44	1.43	0.014

The ranges and the means of all the parameters increased with increasing altitude, except for the ash percentage and the specific gravity

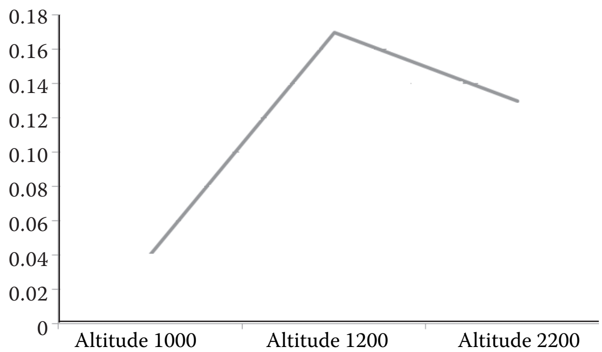

Figure 4. Effect of altitude on the ash percentage of Acacia honey from the Asir region. The ash percentage increased at an altitude of 1200 m and decreased at 2200 m a.s.l. However, the ash percentage at an altitude of 2200 m was still far greater than the ash percentage at 1000 m a.s.l.

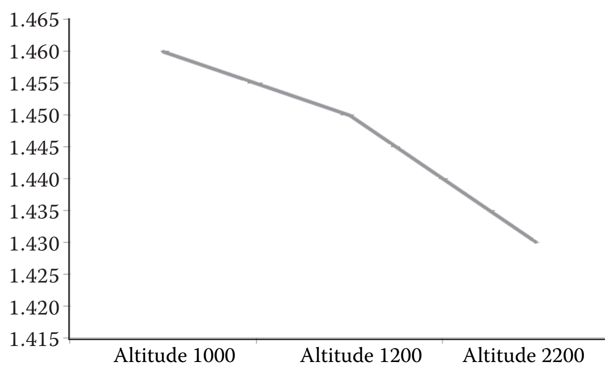

Figure 5. Effect of altitude on the specific gravity of Acacia honey from the Asir region. The specific gravity decreased with increasing altitude. The decrease in the specific gravity of the honey is acceptable since there was an increase in honey moisture

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with respect to ash percentage are to some extent similar to the results obtained by Cantarelli *et al.* (2008) who reported range, mean, and STD values of 0.06–0.21, 0.11, and 0.04%, respectively, for ash percentage.

Moisture percentage. The mean moisture percentage of our samples was 10.93 ± 1.97% (Table 2), which is also within the standard Codex Alimentarius range and that of the US National Honey Board. Our results are comparable with those of Cantarelli who studied 38 honey samples from central Argentina and found the range, mean and STD of water percentage to be 14.28–18.6, 16.24, and 0.19%, respectively. Khalil *et al.* (2001) studied five uni-floral honey samples from Bangladesh and found that the water percentage ranged from 13.3% to 14.8%. Liberato *et al.* (2013) examined 22 samples of honey of different botanical origins from Ceará State in Nothern Brazil and found that the water percentage range was 13.63–20.4%; however, their values, although within the Codex Alimentarius standards, exceed the upper limit of our registered range.

pH. The range, mean and STD of the pH of the honey samples in this study were 3.32–3.77, 3.56, and 0.19, respectively. These results are within the range of the US National Honey Board, which is 3.4–6.1. Our results are comparable to those of Kinati *et al.* (2011) who reported a pH range and mean of 3.45–4.18 and 3.81, respectively. However, in Pakistan, the pH values of 24 honey samples exhibited a different pH range (3.24–6.5), which deviates slightly from the range recommended by the US National Honey Board (Iftikhar *et al.* 2014).

Acidity. The acidity range of our six Acacia honey samples was 19.8–65 meq/kg and the mean was 38.63 ± 17.17 meq/kg. The upper limit of this range is greater than the Codex Alimentarius standards and the reference guide of the US National Honey Board. In a study conducted in the United Arab Emirates (UAE), 16 honey samples from arid regions were analysed for different physiochemical properties. The UAE study recorded a range of acidity of 10.88–40.69 meq/kg and a mean value of 29.96 meq/kg (Habib *et al.* 2014). In another study, the range of free acidity in seven different honey samples from Anatolia was 12–32 meq/kg and the mean value was 21.7 ± 7 meq/kg (Yilmaz & Küfrevioglu 2003).

Conductivity. The range of conductivity for the six Acacia honey samples exhibited a higher upper limit than the Codex Alimentarius and National Honey Board standards: 1207 μS/cm compared to not more than 800 μS/cm. The mean value was 659.51 μS/cm and the standard deviation was very high (324.98 μS/cm) due to the large differences in conductivity between the 2200 m altitude samples and the samples from other altitudes (Table 2). However, similar to our results, Aloisi (2010) found that the range of conductivity in 62 Argentinean honey samples was 0.14–1.08 mS/cm which is equal to 140–1080 μS/cm. Similarly, Ahmed *et al.* (2014) found that one sample of honey from Algeria exhibited a conductivity of 1057 μS/cm. Another study compared four honey samples from Saudi Arabia, Egypt, Yemen, and Kashmir and reported that the honeys from Egypt and Yemen had high conductivity (4.18 ± 0.05 and 1.98 ± 0.03 μS/cm, respectively) (El Sohaimy *et al.* 2015). The above-mentioned studies concerning honeys from Argentina, Algeria, Egypt, and Yemen reported, similarly to this study, that honey can exhibit conductivity which exceeds 0.8 μS/cm. High conductivity values reflect high mineral content, which is a positive nutritional property of these honeys leading to their prescription as a source of minerals for diseases associated with mineral deficiency. Our results are similar to the results of Popov-Raljić *et al.* (2015) who stated that the concentration of certain minerals in Acacia honey increases with increasing altitude. High-altitude Indian Acacia honey showed low mineral content compared with pine honeydew and multi-floral honey (Nayik *et al.* 2016).

However, the majority of published papers have registered values within the Codex Alimentarius standards and US National Honey Board reference guidelines (Desissa 2014; Iftikhar *et al.* 2014).

Specific gravity. Our Acacia honey had a specific gravity range of 1.42–1.46 and mean of 1.45 ± 0.02. This value is comparable to the reference guidelines of the US National Honey Board (1.41–1.435). However, Khalil (2001) found that the specific gravity of five uni-floral honey samples ranged between 13.33–13.36.

Results from different altitudes. The results for the ash and water percentages, pH and specific gravity from the three altitudes were within the Codex Alimentarius standards and the reference guidelines of the US National Honey Board. Acidity and conductivity at an altitude of 2200 m were greater than the standards: acidity range was 52–65 meq/kg and the conductivity range was 859.55–1207 μS/cm (Table 3).

Comparison of the means of the parameters from the three different altitudes revealed that the mean

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Table 4. Comparison of the mean values of the studied parameters using the ANOVA-LSD test

Altitude (m)	Ash		Water		pH		Acidity		Conductivity		Specific gravity	
	1000	1200	1000	1200	1000	1200	1000	1200	1000	1200	1000	1200
1200	0.035		0.196		0.06		0.106		0.235		0.45	
2200	0.24	0.628	0.006	0.012	0.039	0.613	0.009	0.03	0.019	0.052	0.081	0.182

Significant differences between the means of the parameters at the different altitudes were observed for ash percentage, water percentage, pH, acidity, and conductivity

water percentage, pH, acidity, and conductivity increased with increasing altitude (Figures 2 and 3). The ash percentage means increased at an altitude of 1200 m and decreased at 2200 m, while remaining far greater than the value at 1000 m (Figure 4). The specific gravity was decreased from 1000 m altitude to 2200 m altitude; this decrease in the specific gravity may be due to the increased water percentage (Figure 5). In sum, our findings suggest an effect of altitude on the different physiochemical properties of acacia honey.

Significance of the altitude effect. To investigate the effect of altitude on the different studied parameters, the ANOVA test with LSD was used to compare between the means of the six parameters at the three different altitudes. Mean ash percentage at an altitude of 1200 m was significantly increased when compared to the mean at 1000 m altitude (P = 0.035) (Table 4). The mean moisture percentage of Acacia honey at 2200 m altitude was significantly increased compared to the mean moisture of Acacia honey at 1200 m altitude (P = 0.012) and to the mean of Acacia honey moisture at 1000 m altitude (P = 0.006) (Table 3). The mean pH of Acacia honey at an altitude of 2200 m was significantly increased when compared to the mean pH value of the Acacia honey at an altitude of 1000 m (P = 0.039) (Table 4). The mean acidity of the Acacia honey at an altitude of 2200 m was significantly greater than the mean acidity of Acacia honey at the altitudes of 1200 m (P = 0.03) and of 1000 m (P = 0.009) (Table 4). The mean conductivity of Acacia honey at an altitude of 2200 m was significantly increased when compared to the mean conductivity value of the Acacia honey at an altitude of 1000 m (P = 0.019) (Table 4). Variations in the physiochemical properties of honey are due to seasonal variations, botanical origin and environmental factors (Aloisi 2010; Shahnawaz *et al*. 2013; El Sohaimy *et al*. 2015). High altitude is characterised by hypoxia, hypobaria, high humidity (moisture), and high levels of X-radiation (Milledge 2007); these factors may have contributed to the variations in the physiochemical properties of the Acacia honey from the Asir region.

CONCLUSIONS

(1) The mean values of ash, water, pH, acidity, conductivity, and specific gravity of Acacia honey from the Asir region are within the ranges of the Codex Alimentarius standards and the reference guidelines of the US National Honey Board.

(2) Altitude has a significant positive effect on the ash, moisture, pH, acidity, and conductivity of Acacia honey from the Asir region in southern Saudi Arabia.

(3) The upper acidity and conductivity limits of the Acacia honey from an altitude of 2200 m exceeded the standards of the Codex Alimentarius and the US National Honey Board.

Future studies should replicate these experiments on a large scale with larger numbers of samples, altitudes, and parameters.

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