



Physico-chemical Properties and Biological Activity of Honey from Two Districts of Tien Giang Province, Vietnam

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This study was conducted to determine the physico-chemical properties and biological activity of honey collected from the Tan Phu Dong and Cai Be districts of Tien Giang province, Vietnam. The results showed that the 19% and 20% moisture content honey from Tan Phu Dong district had viscosities of 1075 Cp and 1013.33 Cp, insoluble solid contents of 0.12% and 0.09%, acidities of 32.49 mg/1000 g and 30.49 mg/1000 g, diastase index of 4.5 Schade and 4.09 Schade, free reducing sugar content of 65% and 67%, HMF contents of 0.98 mg/100 g and 0.86 mg/100 g and antioxidant activity of 0.47 and 0.52 mg vitamin C equiv./mg dry wt, respectively. The honey from Cai Be district with 22% moisture content, viscosities, insoluble solid content, acidity, diastase index, free reducing sugar, HMF content and DPPH antioxidant activity displayed as 619 Cp, 0.119%, 46.41 mg/1000 g, 5 Schade, 60.28%, 0.38 mg/100 g and 0.45 mg vitamin C equiv./mg dry wt, respectively.

Keywords: Honey, Moisture content, Physico-chemical properties, Biological activity, Vietnam.

INTRODUCTION

Honey is a sweet and viscous product produced by several species of bees, the most famous of which is the honey bee [1]. Bees produce honey by collecting and then purifying sugar secretions from plants (mainly nectar) or from other insects, such as aphids [2]. Honey production takes place inside individual bees through the process of combining with their specific substances, dehydrating, storing and leaving in the mature honeycomb [3]. The main component of honey is carbohydrates, which make up from 95% to 97% of its dry weight. Furthermore, honey consists of major compounds, such as proteins, vitamins, amino acids, minerals and organic acids. Raw honey also consists of several natural compounds such as flavonoids, polyphenols, reducing sugar, alkaloids, glycosides, cardiac glycosides, anthraquinones and volatile compounds [4]. Monosaccharides (fructose and glucose) are the most important sugars of honey and contribute to most of the nutritional and quality effects of honey [5]. Honey is also used as a function food due to its health benefits [6]. In addition, honey is used in the treatment of eye diseases,

bronchial asthma, pharyngitis, tuberculosis, hiccups, fatigue, dizziness, hepatitis, constipation, piles, worm infections, eczema, heal ulcers and wounds [7]. The components of honey have been shown to have antioxidant, antibacterial, antiproliferative, anticancer, anti-inflammatory and antimetastatic effects [8].

Physico-chemical properties and biological activities of honey in different regions of the world is being studied extensively. Ghorab *et al.* [9] evaluated the physico-chemical properties and bioactivity of honey from Babors Kabylia region (Algeria). The results showed that 5-hydroxymethylfurfural (HMF) content of honey was 9 mg/kg, diastase activity was 11.3 Schade, water content was 18.1% and acid content was 32.4 mg acid equivalent/kg. Seraglio *et al.* [10] evaluated the physico-chemical properties of wild Croatian and Spain honey. The features of wild Croatian honey displayed moisture content of 16.1%, free acid content of 27.6 mg acid equivalent/kg, diastase index as 9 Schade. Meanwhile, the physico-chemical properties of Spain honey were determined including moisture content as 15.3%, acidity as 34.6 mg acid equivalent/kg and diastase index as 30 Schade.

In Vietnam, the Mekong Delta region, especially in Tien Giang province, has a thriving beekeeping production due to various sources of pollen from different fruit trees [11]. To the best of our knowledge, most studies have evaluated only a few physico-chemical parameters of honey in different regions of the world. In addition, the studies evaluating the quality of honey originating in Vietnam are limited (especially in the Mekong Delta region). Furthermore, honey in the Mekong Delta region has unstable quality due to the time of harvest and the constantly changing pollen source. This study aims to provide a comprehensive assessment of the physico-chemical properties and biological activity of honey from Tan Phu Dong and Cai Be districts from Tien Giang province, Vietnam. This study could help to standardize the source of honey materials in the two regions above.

EXPERIMENTAL

Longan honey derived from Tan Phu Dong district, Tien Giang province was collected from June to November with an initial moisture content of 19% and 20%. Multi-flowered honey from the Cai Be district was collected from June to September with moisture content of 22%. The chemicals in this study were supplied from Xilong (China) including potassium permanganate (KMnO_4 , 99%), copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 99%), potassium tartrate tetrahydrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$, 99%), potassium ferrocyanide ($\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$, 99%), zinc acetate ($\text{Zn}(\text{CH}_3\text{COO}) \cdot 2\text{H}_2\text{O}$, 99%), potassium hydroxide (KOH, 99%), hydrochloric acid (HCl, 38%), sodium hydroxide (NaOH, 99%), potassium iodide (KI, 99%), iodine (I_2 , 99.8%), sulfuric acid (H_2SO_4 , 98%), sodium acetate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, 99%), phenolphthalein, 3,5-dinitrosalicylic acid (DNS, 98%), sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 99%) and sodium chloride (NaCl, 99%). 2,2-Diphenyl-1-picrylhydrazyl (DPPH, 95%) used for antioxidant activity determination was purchased from Sigma-Aldrich (USA).

Evaluation of quality of honey: Honey in Tan Phu Dong and Cai Be districts was harvested from the farms, filtered by filter bags and put into a heat pump dryer (with temperatures of hot and cold chambers of 45 °C and 18 °C, respectively) to reduce the moisture content to a preserving effect. After drying, the products was stored in tanks and determined for the physico-chemical properties including viscosity, moisture content, diastase activity, acidity, hydroxy methyl furfural content (HMF), free reducing sugar content, insoluble solids content and antioxidant activity.

Determination of moisture content and viscosity: The moisture content was determined using a honey specialized refractometer (BR5892, OEM-Taiwan). The viscosity of honey was measured using a Brookfield viscometer (DVEELVTJO, USA).

Determination of insoluble solids: The content of insoluble solids was carried out according to the method of Almeida *et al.* [12] with some modifications. First, 20 g of honey was dissolved in 100 mL of distilled water and heated up to 80 °C. Then, the solution was filtered using Whatman filter paper No. 1. The residue on the filter paper was washed with hot water (100 °C) to remove residual sugar and weighed. The content of insoluble solids was calculated using eqn. 1:

$$X = \frac{P_1 - P_2}{m} \times 100 \quad (1)$$

where P_1 : weight of filter paper and the residue after drying (g); P_2 : weight of filter paper before drying (g); m : weight of honey sample (g).

Determination of acidity: Determination of acid content was carried out using the method of Popek *et al.* [13]. In brief, 10 g of honey was dissolved in 75 mL of distilled water. The solution was titrated with NaOH of 0.05 M until the pH value of 8.5. In control sample, 85 mL of distilled water was titrated similarly [13]. The acidity value was calculated using eqn. 2:

$$Z = \left(\frac{a - b}{m} \times 50 \right) \quad (2)$$

where a : volume NaOH solution to titrate the sample (mL); b : volume of NaOH solution to titrate the blank (mL); m : the mass of sample (g).

Determination of diastase activity: The diastase activity of honey was performed according to the method of Saka *et al.* [14] with some modifications. In short, 5 mL of starch solution was diluted in 10 mL distilled water and incubated at 40 °C for 15 min using a temperature bath. After incubation, 1 mL of solution was added to 10 mL of iodine solution (0.007 N) and the absorption coefficient was determined at the wavelength of 660 nm. The absorption coefficient of the solution was in the range of 0.76 ± 0.02 .

Honey (5 g) was dissolved in 15 mL of water, 2.5 mL of acetate buffer and 1.5 mL of NaCl solution. The solution was diluted to 25 mL with distilled water. Then, 10 mL of the sample solution was mixed with 5 mL of standardized starch solution and incubated at 40 °C for 15 min using a water bath. Every 5 min, 1 mL of sample solution was mixed with 10 mL of iodine solution (0.007 N) and the absorption coefficient was determined at the wavelength of 660 nm. The time (min) at which the absorbance of the solution at a value of 0.235 or less was recorded. The diastase activity of the sample (Scade) was calculated according to the following formula (eqn. 3):

$$\text{Diastase} = \frac{300}{t} \quad (3)$$

where t : time at which the absorbance value reaches 0.235 or less.

Determination of antioxidant activity (DPPH): The method of determining the antioxidant activity of the samples was carried out according to the study of Kim *et al.* [15]. The formulation of a vitamin C calibration curve: 0.01 g of vitamin C was dissolved in 80% methanol and made up to 100 mL (concentration of vitamin C of 100 $\mu\text{g}/\text{mL}$). A vitamin C concentration range was prepared with concentrations of 20, 40, 60, 80 and 100 $\mu\text{g}/\text{mL}$. Each tube was added to 0.2 mL of DPPH solution (3.94 mg DPPH in 100 mL of methanol). The absorbance of solution was determined at the wavelength of 517 nm.

Then, in next step, 1 g of honey dissolved in 10 mL of water was added to 0.1 mL of sample solution containing 2 mL of DPPH solution. The mixture was shaken and incubated at 25 °C for 30 min in dark conditions. Finally, the sample was

measured for the absorbance (A) at the wavelength of 517 nm [15]. The antioxidant activity of the sample was calculated as mg equivalent of vitamin C per 100 g of dry matter as follows (eqn. 4):

$$\text{DPPH} = \frac{(y - b) \times V \times df \times 100}{a \times m (100\% - \text{moisture}\%) \times 1000} \quad (4)$$

where y: the OD value of the analyzed sample, a and b: the coefficients in the ascorbic acid standard curve equation (0-100 $\mu\text{g}/\text{mL}$), V: the volume of extract (mL), df: dilution factor, m: mass of the sample (g), 100/1000: conversion factor from $\mu\text{g}/\text{g}$ to $\text{mg}/100\text{g}$.

Determination of hydroxy methylfurfural (HMF) content:

Determining the antioxidant activity of the samples was carried out accordingly to the study of Blasa *et al.* [16]. First, 5 g of honey was dissolved with 0.5 mL of Carrer I solution (15 g $\text{K}_3\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ diluted to 100 mL) and 0.5 mL of Carrer II solution (30 g $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ dilute to 100 mL) made up to 50 mL with distilled water and then filtered. The sample tube containing 5 mL of distilled water added with 5 mL of filtered solution and and the control tube was added with 5 mL of NaHSO_3 solution. After that, the two tubes were well shaken and measured at the wavelengths of 284 nm and 336 nm [16]. The HMF content (mg/kg honey) was calculated according to eqn. 5:

$$X = (A_{284} - A_{336}) \times \frac{126}{16830} \times \frac{1000}{10} \times \frac{1000}{5} \quad (5)$$

where A_{284} : the difference between the absorbance values of the test sample and the control sample at the wavelength of 284 nm; A_{336} : the difference between the absorbance values of the test sample and the control sample at the wavelength of 336 nm; 126: molecular mass of HMF; 16830: molecular absorption coefficient of HMF (L/mol cm); 1000: coefficient for converting mg to g; 10: mL-to-L conversion factor in the test; 1000: g-to-kg conversion factor.

Determination of free reducing sugars content:

Determination of reducing sugar content was carried out according to the method of Saxena *et al.* [17]. Firstly, a glucose solution calibration curve was established by diluting glucose with water at concentrations of 0.1; 0.2; 0.3; 0.4; and 0.5% (w/v). Each glucose solution was added with 3 mL of DNS (1%) and 1 mL of $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (40%). The control tubes were prepared by adding 3 mL of DNS (1%) and 1 mL of $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (40%). All test tubes were heated at 95 °C for 15 min. After cooling, the test tubes were measured at the wavelength of 575 nm. For the honey sample, 0.1 g of honey was dissolved in 100 mL of distilled water. Then, 3 mL of the sample solution was added with 3 mL of DNS (1%) and 1 mL of $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (40%) and 0.05 mL of phenol reagent. All the test tubes were heated at 95 °C for 15 min. After cooling, the test tubes were determined at the wavelength of 575 nm. The reducing sugar content of the sample (mg/g honey) was determined from the standard curve of the glucose solutions.

Statistical analysis: Each experiment was repeated three times. The data were processed on Microsoft excel 2016 and Statgraphics Centurion XV 9 (Statgraphics Technologies, Inc., USA). Analysis of variance (ANOVA) and the least significant difference (LSD) were performed to compare the mean at 0.05 level.

RESULTS AND DISCUSSION

Viscosity: The experiment was conducted to determine the viscosity of honey collected in Tan Phu Dong district with moisture contents of 19% (TPD 19%) and 20% (TPD 20%) and in Cai Be district with moisture content of 22% (CB 22%), as shown in Fig. 1. The results of ANOVA analysis showed that moisture content significantly ($p < 0.05$) affected the viscosity of honey. The LSD classification test showed a clear difference between the viscosity values at the three moisture contents. This could be explained that moisture content increases, the transfer rate of molecules in honey decreases, leading to a decrease in solution viscosity [18]. It can be seen that the honeys from Tan Phu Dong district with reduced moisture contents (19% and 20%) have higher viscosities of 1075 cP and 1013.33 cP. The higher moisture content honey (CB 22%) has a lower viscosity of 619 cP. Results of Mossel *et al.* [19] showed that the viscosity values of farmed honey (17.5% moisture content) was 8000 cP, heather honey (18.7% moisture content) was 11250 cP and camellia honey (19.2% moisture content) was 11250 cP. The viscosity of honey samples in this study was lower than that in the previous studies, which can be explained by weather conditions, soil conditions and pollen sources. Moreover, honey processing methods also affect product quality.

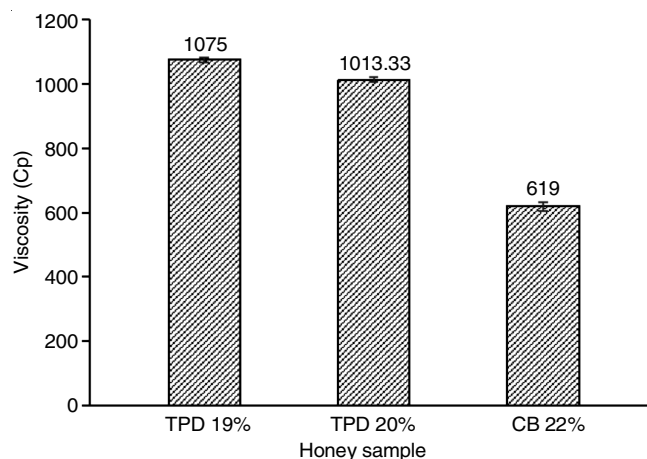


Fig. 1. Levels of viscosity in honey collected from Tan Phu Dong (TPD) and Cai Be (CB) districts of Vietnam

Total insoluble solids in different types of honey: Fig. 2 shows the water insoluble solids content of the CB 22%, TPD 19% and TPD 20% honey samples. As what shown in Fig. 2, the TPD 19% and TPD 20% honey samples have insoluble solid contents of 0.12% and 0.09%, respectively; meanwhile, the CB 22% sample has an insoluble solids content of 0.119%. ANOVA analysis also showed that water content of the honey insignificantly ($p > 0.05$) affected on total dissolved solids. The total dissolved solids content did not seem to be related to the water content of the honey. This can be explained by the fact that the total dissolved solids content is affected by bee-keeping, harvesting and product processing. Indeed, the poor processing of honey could lead to the contamination of dust and pollen, which contributes to an increase in the content of insoluble solids in the product [20].

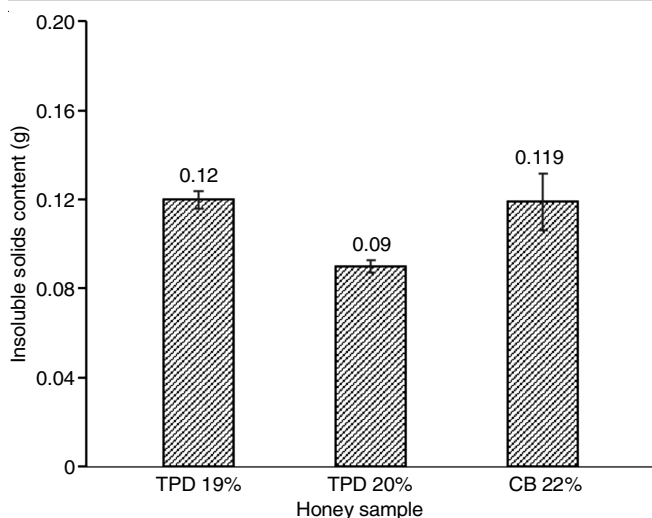


Fig. 2. Levels of Insoluble solids contents in honey collected from Tan Phu Dong (TPD) and Cai Be (CB) districts of Vietnam

Diastase index of different types of honey: This experiment was conducted to determine the diastase index of honey raised in Tan Phu Dong district with 2 moisture contents of 19% (TPD 19%) and 20% (TPD 20%) and Cai Be honey with 22% moisture content (CB 22%) (Fig. 3). ANOVA analysis showed that moisture content did not affect on the diastase activity index, with $p > 0.05$. Diastase is a naturally enzyme in honey, which breaks down at high temperatures. The diastase value was used to indicate the age of honey and whether the honey had been exposed to heat. An increasing in the diastase index leads to better quality of honey [21]. Two honey samples TPD 19% and TPD 20% have a diastase activity index of 4.5 (Schade) and 4.09 (Scade), respectively. Meanwhile, the CB 22% honey sample had a diastase activity index of 5 (Scade). The diastase index showed small variation between the honey samples. According to a research of Tkáč *et al.* [22], longan flower honey from 3 regions Poland, Italy and Turkey had a diastase index of 22.6 Shades, 21.8 Shades and 16.6 Shades, respectively [22]. Comparing the above results with three samples of honey in this study, it could be observed that the

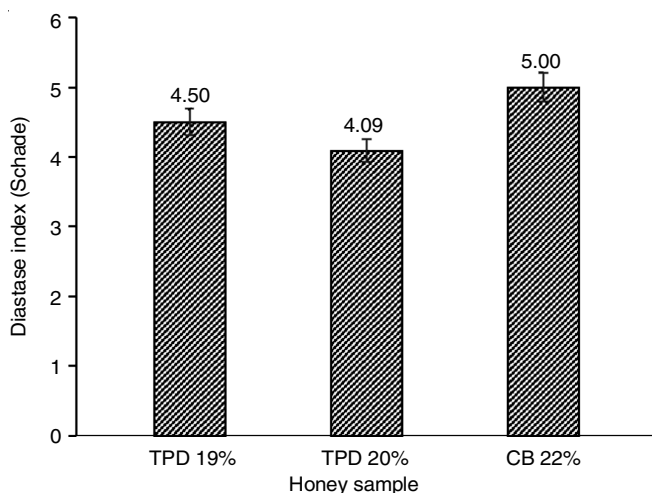


Fig. 3. Diastase activity index of honey collected from Tan Phu Dong (TPD) and Cai Be (CB) districts of Vietnam

diastase index of Tan Phu Dong and Cai Be honey was much lower. The difference could be explained by ages of honey, weather factors and honey processing [21].

Antioxidant activity (DPPH) of different types of honey:

Fig. 4 shows the antioxidant activity (mg vitamin C equivalent/mg dry wt) of the CB 22%, TPD 19% and TPD 20% honey samples. In this study, moisture content was not to be related to the antioxidant activity of honey. The antioxidant activity of honey was due to the presence of bioactive compounds including polyphenols, flavonoids, *etc.* [23]. Furthermore, the antioxidant activity of honey also depends on pollen source, weather conditions and honey processing process. A poor preliminary processing could affect the content of bioactive substances in the sample [24]. Based on Fig. 4, two honey samples TPD 19% and TPD 20% had antioxidant activity of 0.47 mg ascorbic acid equiv./g dry matter and 0.52 mg ascorbic acid equiv./g dry matter, respectively. Meanwhile, the CB 22% sample had an antioxidant activity of 0.45 mg ascorbic acid equiv./g dry matter. The obtained results were similar to previous studies [24].

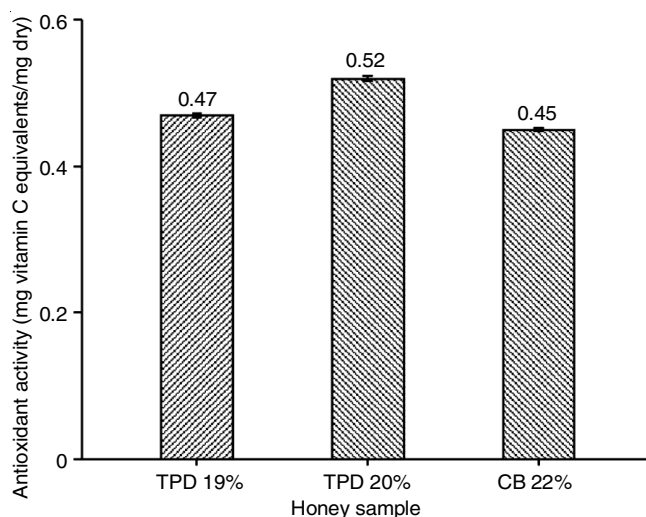


Fig. 4. Antioxidant activity data of honey collected from Tan Phu Dong (TPD) and Cai Be (CB) districts of Vietnam

Hydroxy methylfurfural content of honey (HMF): Fig. 5 shows the hydroxyl methyl furfural (HMF) content of the CB 22%, TPD 19% and TPD 20% honey samples. HMF is a substance, which is formed from fructose during storage and increased very quickly when heating. Therefore, the HMF content was used to assess the freshness and quality of honey product [25]. According to Fig. 5, the HMF content tends to decrease when the moisture content in honey increased. Two honey samples TPD 19% and TPD 20% have HMF contents of 0.98 mg/100 g and 0.85 mg/100 g, respectively; meanwhile, the CB 22% honey sample had an HMF content of 0.38 mg/100 g. The results of ANOVA analysis also showed that moisture content significantly ($p < 0.05$) affected the HMF content of honey. The LSD classification test showed a clear difference between the HMF content values of the three moistures. One possible explanation for this is that the concentration of HMF in honey was found to be more diluted when the moisture level was higher. Additionally, the preliminary processing of

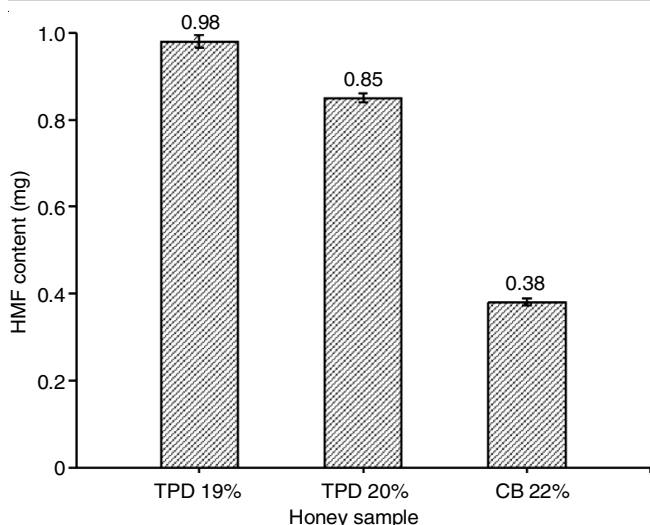


Fig. 5. Levels of hydroxy methylfurfural (HMF) content of honey collected from Tan Phu Dong (TPD) and Cai Be (CB) districts of Vietnam

honey by drying methods also contributed to an increase in the HMF content of the sample [26]. The results in this study were different compared to previous publications on HMF content in honey. According to Shapla *et al.* [27], the HMF content in raw Indian honey was 0.015-0.17 mg/100 g and raw Turkish honey was 0-0.115 mg/100 g [27]. Compared with the results of three honey samples in this study, HMF content of honey samples from Tien Giang was clearly higher. The explanation for this phenomenon is due to the effects of storage time, weather, climate and soil conditions [28]. However, the HMF index of honey in this study was still lower when compared with the Europe standard (AOAC) of less or equal to 4 mg/100 g [29].

Reducing sugar content of different types of honey:

Fig. 6 shows the reduced sugar content of honey samples at Cai Be with a moisture content of the CB 22%, TPD 19% and TPD 20% honey samples. The TPD 19%, TPD 20% and CB22% honey had reducing sugar contents of 67%, 65%, 60.28%, respectively. The results of ANOVA analysis showed that moisture content significantly ($p < 0.05$) affected the reducing sugar content of honey. The LSD classification test showed a clear

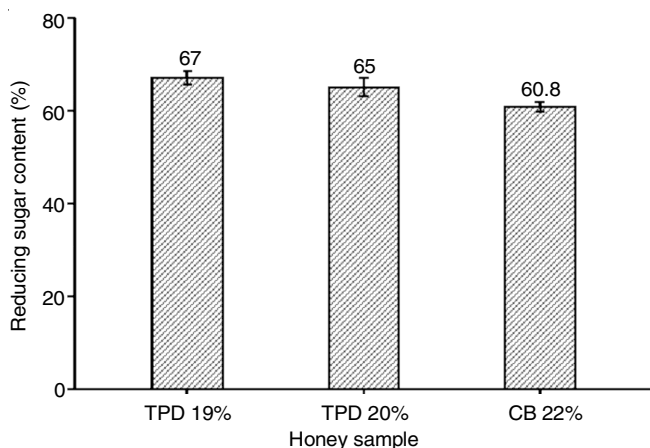


Fig. 6. Level of reducing sugar content of honey collected from Tan Phu Dong (TPD) and Cai Be (CB) districts of Vietnam

difference between the reducing sugar content values at the three moisture contents. An increasing of the moisture content led to a reduced reducing sugar content in product. This can be explained that the concentration of sugar would become more diluted at higher moisture content [30]. In addition, the preliminary processing of honey by drying methods would also increase the sugar content in the sample. According to Afshari *et al.* [31], the obtained free reducing sugar content ranged from 65.01 to 76.61%. In this study, the reduced sugar content was in accordance with the international standard published by AOAC (greater than 60%) [29].

Acidity of different types of honey: This experiment was conducted to determine the acidity content of honey raised from Tan Phu Dong district with 2 moisture contents of 19% (TPD 19%), moisture content of 20% (TPD 20%) and Cai Be honey with moisture content of 22% (CB 22%), as shown in Fig. 7. It was observed that the TPD 19% and TPD 20% had acidity contents of 32.49 mg/1000 g and 30.49 mg/1000 g, respectively. Meanwhile, the CB 22% sample had an acid content of 46.41 mg/1000 g. The acidity content of honey found in this study was lower than that in previous studies. Albu *et al.* [20] showed the acid contents of Serbian acacia honey and Slovak linden honey were 7.8 to 29.6 mL acid equiv./kg and 21.6 mL acid equiv./kg, respectively [20]. The reasons could be due to weather conditions, soil and climate leading to the differences in acidity between the honey samples. In addition, the process of harvesting and storing honey also causes a change in the preservative acid content (storage under different conditions and tools), which affects the change in acidity. Indeed, when the acid content is high, fermentation obviously occurs, adversely affecting the product [32]. However, the acid content of the honey samples being studied was still within the allowable limit according to Europe standards of less or equal to 50 mL acid equiv./k [29].

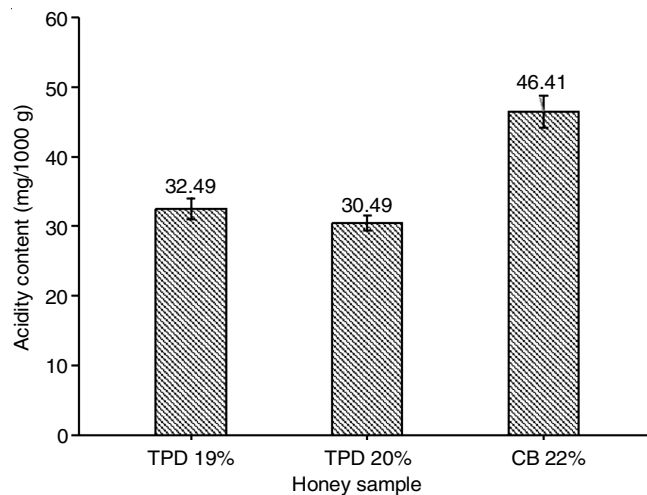


Fig. 7. Levels of acidity of honey collected from Tan Phu Dong (TPD) and Cai Be (CB) districts of Vietnam

Conclusion

Honey collected from Tan Phu Dong district significantly ($p < 0.05$) had higher viscosity, hydroxyl methyl furfural (HMF) and reducing sugar contents and lower acidity than the honey

from Cai Be district. The HMF content tends to decrease when the moisture content in the honey increased. The increase in moisture content led to a reduced viscosity and reducing sugar content of the product whereas its increase in acidity may be due to fermentation. Analysis results showed insignificant difference of insoluble solid content, diastase activity index and anti-oxidant scavenging capacity between the honey samples from different origins. Ages of honey, weather condition, beekeeping and harvesting activities and product processing process may be the factors affecting these quality indicators of honey.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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