International Journal of Food Sciences (IJF)

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International Journal of Food Sciences ISSN 2789-7680 (online)



Vol. 6, Issue 3, No. 1, pp 1 - 20, 2023

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Determination of the Chemical Properties of Honey from Suba Region, Homa Bay County-Kenya

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Article History

Received 18th November 2023 Received in Revised Form 29th November 2023 Accepted 13th December 2023



How to cite in APA format:

Ondogo, L., Oyoo, D., & Bichanga, R. (2023). Determination of the Chemical Properties of Honey from Suba Region, Homa Bay County-Kenya. *International Journal of Food Sciences*, 6(3), 1–20. https://doi.org/10.47604/ijf.2240

Abstract

Purpose: The purpose of this study was to determine the chemical properties of honey produced in the Suba region, specifically in the Suba South Sub-County, Gwassi North and South divisions' four regions, Nyandiwa (Western), Tonga (Northern), Magunga (Eastern), and Nvancha (Central) of Homa Bay County, Kenya. The study focuses on assessing the chemical properties of honey such as pH, free acidity, HMF content, proline content, glucose content, fructose content, sucrose content and mineral concentrations (K,Na,Ca,Mg,Fe,Zn,and Mn) to ascertain whether they are within the acceptable limits as set by the Codex Alimentarius Commissions.

Methodology: An experimental approach was used to investigate the various chemical properties of honey sourced from the four regions of the Suba region in Homa Bay County. Codex Alimentarius Commission's defined methods were used to analyze each property. Both primary and secondary data were collected using interviews and purposive sampling techniques to collect a total of 40 honey samples. A pH meter, UV-visible spectrophotometer, and HPLC instruments were used to measure the pH, free acidity, HMF content, proline content, fructose content, glucose content, and sucrose content of the honey, respectively. The levels of minerals were measured using AAS for Na and K, while Ca, Zn, Mg, Mn, and Fe using a flame photometer. Statistical Package for Social Sciences version 21 was used to perform various statistical tests and generate graphical representations of data. The collected samples were analysed at the Kenyatta University Research Laboratory.

Findings: The findings showed that the chemical properties had the following mean values for the honey sample: $pH = 4.01\pm 0.03$, free acidity = 35.83 ± 0.45 mEq/kg, HMF = 19.95±1.76 mg/kg, proline = 629.77 ± 9.80 mg/kg, sum of glucose and fructose = $68.37\pm0.51\%$. While for the minerals, manganese was the least common and potassium the most common. The mean values result of this study show that honey from the Suba region satisfies the set standards by Codex Alimentarius Commission.

Unique Contribution to Theory, Practice and Policy: The study validates the theory of international standards in honey production, emphasizing the positive impact of aligning with global benchmarks on honey quality and market acceptance in the Suba region. It emphasizes the importance of quality certification, which contributes to consumer trust and economic growth. The research also supports the theory through sensitization and education, which increase awareness about honey quality and educate beekeepers on proper production techniques. The study advocates for economic diversification through sustainable apiculture, resulting in improvements in economic stability and financial well-being in the Suba region.

Keywords: Honey, Chemical Properties, Codex Alimentarius Commission

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INTRODUCTION

Honey is a naturally sweet substance produced by honeybees from floral nectar, living plant parts' secretions, or insect waste. Honeybees get their nectar from flowers, whereas plant-sucking insects provide trees and plants with their excretions and secretions (Cherian *et al.*, 2010). There are two types of honey, namely, honeydew honey and blossom/nectar/floral honey. Blossom honey is a type of honey formed by bees from the nectar of various flowering plants. Its flavor, color, and aroma are influenced by the specific types of flowers the bees collect nectar from. Blossom honey can vary in taste and characteristics based on the predominant floral sources in the bees' foraging area. Blossom honey is further divided into mono/unifloral and multifloral honey (Robert *et al.*, 2010). Honeydew honey is a special variety of honey collected by bees from the sugary secretions produced by insects like aphids and scale insects. These insects feed on plant sap and excrete a sweet substance known as honeydew. Bees gather this honeydew from leaves and branches, process it within their hives, and transform it into honey (Ouchmoukh *et al.*, 2007).

The type of plant from which the bee gathers nectar determines the content of the honey. In honey, carbohydrates make up about 95% of the dry matter (Bogdanov *et al.*, 2004). Sugars of various types can be found in honey. The dominant monosaccharides found in honey, constituting approximately 85% to 95% of its sugar content, are fructose and glucose (Buba, *et al.*, (2013). Honey also includes minerals, water, enzymes, proteins, minerals, vitamins, amino acids, lipids, organic acids, flavonoids, volatile molecules, phenolic acids, and chemicals that resemble carotenoids (Salim *et al.*, 2011).

Human beings use honey for both its nutritional and medicinal benefits (Bogdanov *et al.*, 2004). Honey has been demonstrated to be effective in treating a variety of ailments due to its antibacterial qualities, including ulcer wounds and digestive issues, among others (Bogdanov *et al.*, 2004). Additionally, honey is utilized as a sweetener and flavoring agent in a wide variety of meals and drinks (McCarthy, 1995).

There are different types of beehives where bees make the combs and produce honey. The common beehives used in Kenya include the Kenya Top Bar Hive (KTBH), the Traditional Log beehive, and the Langstroth beehive. The traditional log type beehives are inefficient and ineffective when extracting honey because the combs are crushed and compressed, thus destroying the combs. This reduces honey production, while the Langstroth beehive is efficient and effective because of its innovative nature of extracting honey while leaving the honeycombs for continuous production.

Honey production capacities vary significantly from one country to another. For instance, China boasts the highest production yield, generating 170,000 tonnes, whereas Argentina produces 45,500 tonnes (Oliveira *et al.*, 2015). According to WEEMA (2016), Ethiopia holds the top spot as the largest honey producer in Africa, followed by Tanzania, with Kenya ranking third. Kenya demonstrates a substantial demand for honey, occasionally resorting to imports from Tanzania when local supply falls short. In a global context, the foremost importers of honey are primarily the United States, Germany, Japan, the United Kingdom, and France (Oliveira *et al.*, 2015).

In Kenya, it is estimated that the honey production capacity is around 100,000 metric tonnes per year, according to Carroll *et al.*,2013. However, the current annual production stands at only 25 metric tonnes because many highly productive areas remain untapped. If its full potential is realized, it has the potential to generate a foreign exchange income of



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approximately 15 to 20 billion dollars annually, as noted by Robert et al., (2010). Honey production in Kenya is very low due to a lack of knowledge of the honey market by the beekeepers (Robert et al., 2010). Lack of proper coordination when the honey is collected, processed, and marketed due to a lot of fragmentation is another limiting factor. This leads to the exploitation of the bee farmers by middlemen (Robert et al., 2010). The bee farmers are also slowly embracing the modern technologies of beekeeping as they continue with the traditional methods, leading to poor methods of managing the bee colonies (Otieno et al., 2021). The poverty level in Homa Bay County stands at 48%, as compared to the national poverty indicator at 45% (Kandagor et al., 2018). The Suba region in Homa Bay County is one of the most hardship-stricken areas. Residents of the Suba region in Suba South Sub-County, Gwassi North, and Gwassi South divisions of Homa Bay County have been left with no alternative but to practice fishing and subsistence farming as their main economic activities. To reduce poverty levels in Suba region, Homa Bay County, the Green Forest Social Initiative (GFSI), an initiative by a Dutch foundation in the Netherlands, was established in 2008. The long-term goal of the GFSI was to alleviate poverty by developing the economy, enhancing income through social business, and reimpositioning the environment. The main purpose of GFSI was to prompt economic accreditation by putting in place supportable income-generating opportunities for small-scale farmers and by investing in social welfare and protecting the environment. In the year 2011, the initiative sensitized and encouraged smallholder farmers to venture into other economic activities such as tree farming, beekeeping, aloe, and jatropha farming. The GFSI intervened by supporting the value chains to enhance income-generating opportunities for the farmers. Apiculture farmers in the area were encouraged and supported to embrace modern and innovative beehives, namely the Langstroth, which they were given freely and this led to increased honey production.

The main objective of this study was to determine the chemical properties of honey produced from the four regions of Gwassi North and Gwassi South divisions, namely, Nyandiwa (Western), Tonga (Northern), Magunga (Eastern), and Nyancha (Central) in Suba South Sub-County, Homa Bay County, to ascertain if they met the permitted International Honey Standards as specified by the Codex Alimentarius Commission. The Codex Alimentarius Standard for quality honey includes several physical and chemical characteristics, such as mineral content, moisture levels, hydroxymethylfurfural (HMF) content, diastase activity, free acidity, apparent quantity of sugar, and water insoluble solids content.

Statement of the Problem

Most of the inhabitants of the Suba region, specifically Suba South Sub-County in Homa Bay County, rely on fishing and subsistence farming for their livelihoods. However, recently, they have ventured into apiculture as a new economic activity to improve their economic well-being. The chemical properties of honey from this region have not been thoroughly researched or documented with the aim of describing and regulating its quality. It is essential to train local beekeepers in proper procedures for identifying, classifying, processing, and storing honey to ensure its quality meets the globally recognized Honey Quality Standards set by the Codex Alimentarius Commission. The outcomes of this study will serve to educate farmers and the public about the quality of honey from the Suba region, Suba South Sub-County, Gwassi North, and Gwassi South divisions. This effort aims to encourage more farmers to engage in beekeeping, thereby boosting the local and international honey market and increasing farmers' income, ultimately improving their standard of living.



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LITERATURE REVIEW

Theory of Floral Sources and Honey Composition

The theory suggests that the chemical composition of honey is linked to the types of flowers from which bees gather nectar. This theory, rooted in the 18th-century work of Swiss botanist François Huber, explains that variations in honey's flavor, color, aroma, and nutritional content are a result of the diverse floral sources available to bees during the nectar-collecting process. The theory is particularly relevant in the Suba Region, where understanding the botanical origins of honey is crucial for deciphering its unique chemical profile. Different floral sources in the region may result in honey with distinct chemical compositions, providing valuable insights into the quality and potential benefits of honey produced in the Suba Region. The theory aligns with established literature and has practical implications for beekeepers and researchers in the Suba Region. By exploring this theory, the study can contribute valuable knowledge to honey production practices, quality assessment, and a broader understanding of the relationship between floral sources and honey's chemical characteristics (Crane, 1990).

Chemical Properties of Honey

The pH

The acidity of honey can vary depending on several factors, including its botanical source and processing methods. However, honey is typically mildly acidic, with a pH range that falls between 3.2 and 4.5 (White, 1975). This is due to the presence and concentration of various organic acids such as gluconic acid, lactic acid, oxalic acid, formic acid, and other basic compounds; therefore, honey has a lower pH value (Al-Farsi *et al.*, (2018). The pH of honey influences its consistency, stability, and shelf life, particularly when honey is harvested and also stored (Cherian *et al.*, 2010). It acts as a trustworthy indicator of the honey's place of origin (Raweh, *et al.*, 2022). The variations in pH are due to various factors, such as the foraged plants and flowers, the salivary bees' secretions, and the raw materials' fermentation (Raweh *et al.*, 2022). According to the 2001 Codex Standards, nectar-derived honey (blossom honey) and honeydew honey contain a pH value ranging from 4.5 to 5.5. It is because nectar and honeydew honey contain a pH value ranging from 4.5. According to Ahmed and Karaman (2007), the pH range of Turkish honey is between 3.7 and 4.5.

Free Acidity

Free acidity (FA) in honey refers to the presence of acidic compounds soluble in water, which is used to monitor the fermentation process. It is influenced by nectar or bee secretions and natural organic acids like gluconic acid, formic acid, and acetic acid (Yadata, 2014). Gluconic acid is the predominant organic acid in honey, accounting for 70% to 90% of the total acidity. It can be increased during storage, fermentation, and adulteration with sugars. Honey contaminated with sugar syrup has a low acidity, while honey adulterated with invert sugar is high due to its acidic nature (Yadata, 2014). The acidity content in honey is directly proportional to the balance of naturally occurring organic acids, which changes based on floral origin and bee type (Sousa et al., 2016). During the processing and ripening of honey, the glucose oxidase enzyme that bees add produces gluconic acid, which is the main acid in honey (Machado De-Melo et al., 2018). The Codex Alimentarius (2001) suggests 50 mEq/kg as the maximum acidity permissible in honey. Spanish honey's acidity ranges from 17.6 to 39.8 mEq/kg (Terrab et al., 2004), while Pakistan honey's free acidity is 29.37 mEq/kg (Iftikhar, 2011).



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Hydroxymethylfurfural (HMF)

According to Markowicz *et al.*, (2012), fructose metabolism produces the cyclic aldehyde known as hydroxymethylfurfural (HMF). Honey contains simple sugars, acids, and minerals that contribute to enhanced HMF synthesis. HMF concentrations are influenced by factors such as storage conditions, temperature, and the chemical properties of honey. HMF levels indicate heat exposure, which can diminish honey quality. Low HMF levels are associated with well-preserved, high-quality honey (Teshpome *et al.*, 2020). HMF analysis helps assess the freshness of honey, with increasing levels over time suggesting prolonged storage or unfavorable conditions. Fresh honey typically exhibits lower HMF values < 1 mg/kg (Zappala *et al.*, 2005). HMF also serves as a valuable tool for detecting adulteration, particularly the presence of added sugars like invert sugar. Elevated HMF levels can signal the use of such additives, safeguarding the authenticity and purity of genuine honey (Salim *et al.*, 2011).

The Codex Alimentarius Standard has a maximum limit of 60 mg/kg for HMF in honey, while the EU sets a maximum restriction of 40 mg/kg, with a higher limit of 80 mg/kg for honeys from tropical climates. A study in Kenya by Muli et al. (2007) found an average HMF content of 3.70 mg/kg to 389.36 mg/kg. HMF levels in honey from Pakistan ranged from 0.030 mg/kg to 42.896 mg/kg, far below the maximum permissible amounts of 80 mg/kg (Asif *et al.*, 2002).

Proline

Honey's proline content is utilized to assess ripeness and adulteration. Proline is an amino acid that can be found in small quantities in honey. Honey has an amino acid concentration of around 1%, with proline accounting for between 50% and 85% of the overall amino acids (Machado De-Melo et al., 2018). This is because pollen mostly contains the amino acid proline (Ball, 2007). In addition to proline, honey also contains other amino acids such as glycine, leucine, alanine, and phenylalanine. These amino acids are derived from plant products. Proline is present in tiny levels in honeybee saliva and nectar (Machado De-Melo et al., 2018). Honeybees also produce proline as a byproduct of the nectar-to-honey transformation process through enzymatic processes (Hermosin et al., 2003). The accumulation of proline is one of the indicators that the nectar has been processed and transformed into honey by bees. The internationally approved minimum proline concentration for completely matured honey is 180 mg/kg (Hermosn et al., 2003). Proline was proposed as a quality control criterion for honey in terms of sugar adulteration but also honey maturity; hence, proline readings below 180 mg/kg indicate honey that is either unripe or contaminated, whereas high levels of proline exceeding the minimum recommended limit indicate that honey is ripe, mature, and is not contaminated, signifying good quality honey (Bogdanov & Martin, 2002). Meda et al., (2005) investigated honey from Burkina Faso and found that its proline content varied between 437.82 mg/kg and 2,169.4 mg/kg, with a mean of 989.5 mg/kg. This showed that the honey was ripe. Honey from Nepal with a mean proline concentration of 100.08 mg/kg (Qamar et al., 2008) is either immature or contaminated.

Carbohydrates

Honey primarily consists of carbohydrates, with sugars accounting for the majority (95% of its dry matter content). The predominant sugars found in honey are glucose and fructose, both of which are monosaccharides. In addition to these, honey contains other sugars, albeit in smaller amounts, including disaccharides, oligosaccharides, trisaccharides, and sucrose (Persano *et al.*, 2004). Sucrose, is typically found in smaller quantities.



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Glucose and Fructose

Glucose and fructose constitute the main primary monosaccharides found in honey. They account for 80% to 85% of sugars (Buba, *et al.*, (2013). Fructose is more plentiful than glucose. Honey's physical and nutritional qualities are affected by its high fructose content (Persano *et al.*, 2004). The quantities of the two major monosaccharides, fructose and glucose, have a key role in the classification of monofloral honeys (Bogdanov *et al.*, 2004). The minimal value established by Codex for the total of fructose and sugar is 60 g/100 g, including all nectar honeys, and 45 g/100 g for honeydew honey (Codex 2001). The Codex 2001 has suggested a minimal threshold for complete reduction of sugars of 65 g/100 g. According to the results of Muli's (2007) investigation on the quality of West Pokot honey, the total reducing sugar content was 61.5%.

Sucrose

The quantity of sucrose contained in the honey samples is a crucial determinant of the honey's maturity and its adulteration with invert sugars. Sucrose is a common sugar found in many plants and is found in small quantity in honey (Bogdanov *et al.*, 2004). Due to the presence of the enzyme invertase in honey, there is relatively little sucrose in pure or natural honey because the enzyme invertase is involved in the conversion of sucrose to fructose and glucose. Overheating honey samples often denatures the enzyme invertase, leading to elevated amounts of sucrose. Sugar-fed bees are associated with concentrations of sucrose greater than 8%. Higher sucrose concentrations may also be the consequence of honey that was collected prematurely (unripe), where sucrose has not been completely converted into glucose and fructose by the enzyme invertase (Azeredo *et al.*, 2003; Teresa *et al.*, 2011). The maximum sugar content permissible for blossom honey is 5 g/100g, whereas honeydew honey should include 15% sugar. The quantities of sucrose in honeys were found to range between 1.06% and 22.68% in an Algerian study (Chefrour *et al.*, 2009).

Minerals

Honey contains various mineral substances, with potassium being the most prevalent due to its increased concentration in plant tissues (Machado De-Melo *et al.*, 2018). Other minerals found in honey include sodium, calcium, zinc, magnesium, manganese, and iron. Analyzing these elements is crucial in determining the geographical source of honey, as different areas have different levels of soil mineral elements (Salinas *et al.*, 1994). The amount of minerals in honey is determined by physiological assimilation through plants from their developing soil and surroundings or artificially through the chemical composition of artificial sources like sugar or syrup consumed by bees (González-Miret *et al.*, 2005; Van Hanen *et al.*, 2011). Honey can serve as an indicator of environmental contamination levels in the area where it is found, as honeybees can reach an area of around 50 km² and come into contact with air, land, and water (Przybylowski *et al.*, 2001). The proportions of trace elements in honey can accurately indicate the amount present over the whole studied region (Ioannidou *et al.*, 2005). Minerals also affect the color of honey, with dark honeys and honeydew honeys having the highest mineral content compared to lighter honey (Adebiyi *et al.*, 2004). Recent research by Fernandes Torrez (2005) found eleven different minerals in Spanish honeys derived from various botanical sources.

METHODOLOGY

The study investigated the chemical properties of honey from four Suba regions in Homa Bay County to determine if they meet international standards set by the Codex Alimentarius Commissions. Before collecting samples, beekeeping farms were identified and interviewed to



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determine harvesting time, processing, and storage. Forty honey samples were collected from the four regions using purposive sampling, with ten samples obtained directly from local farmers in December 2013. During harvesting, frames with honey ready for harvesting were retrieved, uncapped, and placed in a honey extractor. The frames were then spun to separate the honey from the honeycomb. The harvested and extracted samples were stored in 500 ml airtight plastic bottles in a refrigerator for analysis.

Instrumentation and Apparatus

The main instruments that were used include; a UV-Visible Spectrometer (CECIL CE 2041 2000 series) for HMF and proline determination. HPLC (Shimadzu LC 20 AT using a gradient elution pump) was used for the determination of sugar content. A flame photometer (Sherwood 410) was used in determining the mineral levels of K and Na. An atomic absorption spectrophotometer (Bulk Model Scientific 210VGP-USA) was used in the determination of mineral levels of Ca, Fe, Zn, Mg, and Mn. The apparatus that was used is the pH meter Mettler Toledo Delta 320) for pH and free acidity determination.

Experimental Design

Determinations of various parameters, including pH, free acidity, HMF content, proline content, sucrose content, fructose content, sucrose content and mineral content of the collected honey samples from the Suba region, Suba South Sub-County, Gwassi North, and Gwassi South divisions in Homa Bay County, were done. Each determination was conducted in triplicate. Data obtained for the various parameters determined from the four regions was compared to Codex and EU standards using descriptive statistics. A comparison of each parameter for each of the four regions for recognition of the existence of similarities or differences in the honeys was performed using analysis of variance (ANOVA) with the aid of the computer statistical package SPSS.

Preparation of Standards

Method validation was done by using the recovery method. For analysis of mineral levels, commercially available standards of 1000 ppm for K, Ca, Na, Mg, Fe, Zn, and Mn were purchased. 100-ppm stock solution was made by taking 1 ml of each commercial standard and diluting it to 100 ml. For sugars, 2.000 g of fructose, 1.500 g of glucose, and 0.250 g of sucrose were dissolved in 40 ml of distilled water, then added to a 100 ml graduated flask containing 25 ml of methanol and topped to the mark with distilled water. Chemicals and reagents of analytical grade were used.

Experimental Procedures

Determination of pH and Free Acidity (AOAC, 1990)

In this study, the pH was calibrated using standard buffer solutions with known pH values, typically at pH 3 and 9, whereas for the free acidity determination, the potentiometric titration method (AOAC, 1990) was utilized. On a magnetic stirrer, 10 g of each honey sample were dissolved in 75 ml of carbon dioxide-free distilled water in a 250 ml beaker. While stirring the solution on a magnetic stirrer, the pH electrodes were immersed in the solution, and the pH was then determined. For the determination of the free acidity of the same solution, a titration with a 0.1M sodium hydroxide (NaOH) solution using a phenolphthalein indicator was carried out until the pH reached 8.30. The measurements were taken three times for each sample to ensure accuracy. The mean value for NaOH used in the titration was calculated and used to determine the free acidity using the formula in equation 1. The free acidity of honey, which is the content



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of total free acids, is quantified in milliequivalents or millimoles of acid per kilogram of honey, and this is calculated by multiplying the volume of 0.1 M NaOH used by 10. The result is reported with two decimal places, where acidity is represented as 10 times the volume (V) of 0.1 M NaOH in 10 g of honey. *Free Acidity (milliequivalents) = Volume (ml) of* 0.1 *M NaOH* × 10 [1]

Determination of Hydroxymethylfurfural (HMF) (Bogdanov, 2009)

The HMF was calculated using a spectrophotometric method that was developed based on the work of Bogdanov, 2009.15 g Potassium hexacyanoferrate (II),K₄Fe (CN) ₆. $3H_2O$ was dissolved in distilled water, and the solution was made up to 100 ml in preparation of Carrez solution I. The Carrez solution II was prepared by dissolving 30 g of zinc acetate, Zn (CH₃COO)₂.2H₂O, in distilled water, and the solution was made up to 100 ml. A sodium bisulphite solution was obtained by dissolving 0.20 g of sodium hydrogen sulphite, NaHSO₃ in distilled water, and the solution was made into 100 ml.

A 5 g of the honey sample were dissolved in 25 ml of distilled water, and the resulting solution was quantitatively transferred into a 50 ml volumetric flask. Then, 0.5 ml of Carrez solution I and 0.5 ml of Carrez solution II were added, and the mixture was topped up to the mark. The resulting solution was filtered using filter paper, with the first 10 ml of the filtrate being discarded. Aliquots of 5 ml were put into test tubes; to the test tube containing the sample solution, 5 ml of water was added, whereas to the test tube containing the reference solution, 5 ml of NaHSO₃ solution was added. Within one hour, the absorbance of the sample solution was measured against the absorbance of the reference solution at 284 nm and 336 nm in 10 mm quartz cells. The HMF was then quantitatively calculated using equation [2] proposed by the International Honey Commission (IHC). *HMF in* $\frac{mg}{kg} = (A_{284} - A_{336}) \times 149.7 \times 5$

[2]

Where; A_{284} = absorbance at 284nm;	A_{336} = absorbance at 336nm
149.7 = 126 *1000* 1000 /16830* 10* 5;	5 = theoretical nominal sample weight

Determination of Proline

The technique developed by Ough (1991) was used in determining the amount of proline present in the honey samples. A purple-colored complex is formed when proline and ninhydrin react (Bhagavan, N. V. 2002). When 2-propanol is added, the extinction of the sample and reference solution at a wavelength near maximum is obtained. Proline content is then determined from the ratio. In preparation for the 3% ninhydrin solution, 3 g of ninhydrin was dissolved in ethylene glycol monomethyl ether and made up to 100 ml. A proline stock solution was made by dissolving 40 g of proline in distilled water and making it up to 50 ml. The stock solution (1 ml) was then made up to 25 ml using distilled water to give the proline reference solution. The 50% 2-propanol was prepared by mixing equal quantities of 2-propanol and distilled water. In accordance with the procedure, 5.0 g of each honey sample was dissolved in a volume of 50 ml of distilled water, then quantitatively transferred to a 100 ml volumetric flask and topped up to the mark using distilled water. In three separate test tubes, 0.5 ml of the sample solution, a standard solution for proline, and water were put in. Then 1 ml of the solution containing both formic acid and 1 ml of ninhydrin solution was added to each tube, caped, and shaken vigorously for 15 minutes using a Kotterman water bath shaker. They were then placed in a water bath heated to 70°C for 10 minutes. After that, 5 ml of a 2-propanol-water solution was added to each tube, and they were immediately caped. The mixture was then put in a 1-cm



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cell, and absorbance readings at 510 nm were recorded after 45 minutes. The proline content was then calculated using the equation [3] below.

Proline in
$$\frac{mg}{kg} = \frac{E_S}{E_a} \times \frac{E_1}{E_2} \times 80$$
[3]

Where: E_s = Absorbance of sample solution; E_a = Absorbance of proline standard

 $E_1 = mg$ of proline for standard solution; $E_2 = weight$ of honey in grams; 80 = Constant

Determination of Glucose, Fructose and Sucrose

Honey sugar analysis was conducted using high-pressure liquid chromatography (HPLC). This method, originally described by Bogdanov and Baumann in 1988, involves filtering the solution and subsequently determining sugar content using HPLC with refractive index (RI) detection. Peaks were recognized by their specific retention times, and quantification is accomplished using the external standard method based on peak areas or peak heights. 5.0 g of honey was dissolved in 40 ml of distilled water in a beaker, and 25 ml of HPLC-grade methanol was added. The mixture was then quantitatively transferred to a 100 ml volumetric flask and filled to the mark with distilled water. After that, the solution was then filtered, and the resulting solution was collected in sample vials using a syringe and pre-mounted filter and analyzed for fructose, glucose, and sucrose. HPLC separation was carried out on an analytical column Shodex Asahipak® NH2P-50 4E packed with amino-modified silica gel, using a mobile phase consisting of acetonitrile and water in a ratio of 78:22 (v/v). The flow rate was set at 1.4 ml/min, and a 0.45 µm pore size membrane filter was employed for filtering aqueous solutions. The column and detector were maintained at a temperature of 30°C. A 5µl injection volume for standards and 2µl for sample was used, and the analytical column, made of stainless steel, had a diameter of 4.6 mm and a length of 250 mm. It contained amine-modified silica gel with particle sizes ranging from 5 to 7 µm. The analysis time was 45 minutes per sample, and prior to injection, solvents and diluents were filtered through 0.45 µm corona filters.

Determination of Minerals

Digestion of the samples was done by taking 2 g of the honey sample and dissolved it in 10 ml of concentrated HNO₃, then evaporated at 120°C to almost complete dryness. Then 10 ml of concentrated HNO₃ was added, the solution cooled, transferred to a 50 ml volumetric flask, and then made up to the mark with distilled water. The determination of K and Na was done by using flame photometry, while that of Mg, Ca, Fe, Mn, and Zn was done by using atomic absorption spectroscopy. The flame photometer and AAS were optimized by calibration using distilled water, which produced an absorbance of 0.000 and a standard for each element of known concentration. This was done to ensure that the instruments were properly working during the analysis of the mineral elements. The flame photometer linearity was done using solutions of concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, 60 ppm, 80 ppm, and 100 ppm for Na⁺, K⁺, and Ca⁺. The linearity of the AAS was done using solutions of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm for Mg⁺, Fe⁺, Zn⁺, and Mn⁺. The absorbance against the concentration of standards was plotted, and a liner graph was obtained from which a linear equation y = mx + c was determined from the calibration curve.

Experimental Data Analysis

The study utilized SPSS version 21 for statistical tests and data visualization. The mean, standard deviation, and range of each parameter were calculated to understand the central



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tendency and variability of the data. An ANOVA was used to compare honey properties across four regions, determining significant differences using a p-value of 0.05. The overall mean values were compared with the Codex Alimentarius Commission standards.

RESULTS AND DISCUSSION

The pH

The mean pH values and the range obtained for the four regions are represented in Table 1 below.

Table 1: Mean and Range of pH	Values of Honey from Four Different Regions of Gwassi
North and Gwassi South	

	рН		
Sampling site	Mean ± SE	Min	Max
Central(Nyancha)(n=10)	4.32±0.02	4.07	4.51
Western(Nyandiwa)(n=10)	4.28±0.03	4.01	4.53
Northern(Tonga) (n=10)	3.72±0.04	3.36	3.97
Eastern(Magunga) (n=10)	3.73±0.04	3.28	3.98
Total (n= 40)	4.01±0.03	3.28	4.53

The mean pH values of honey from the Central (Nyancha), Western (Nyandiwa), Northern (Tonga), and Eastern (Magunga) regions were found to be 4.32 ± 0.02 , 4.28 ± 0.03 , 3.72 ± 0.04 , and 3.73 ± 0.04 , respectively. The mean results obtained indicate that this honey is acidic. The mean pH values for the investigated regions were all below the permitted pH limit of maximum 4.5 recommended for blossom or floral honey (Codex Alimentarius 2001). This implies that most of the honey samples from the four regions were blossom or floral honey. The pH values of honey from the Central (Nyancha) region varied from 4.07 to 4.51, with a mean of 4.32. The mean pH value of 4.32 ± 0.02 indicates that most of the honey samples from the Central (Nyancha) region were floral honey. The maximum pH range in this region was 4.51, which indicates the presence of honeydew honey. The pH values from Western (Nyandiwa) ranged from 4.01 to 4.53 with a mean of 4.28 ± 0.03 , which clearly indicates that the majority of the honey from Western (Nyandiwa) is blossom or floral honey. The maximum range was 4.53, an indicator of a honeydew honey sample. The pH values in the Northern (Tonga) and Eastern (Magunga) regions were below 4.5, which depicts a floral or blossom type of honey. The overall mean pH values for honey from the four regions were found to be 4.01 ± 0.03 , similar to studies done by Terrab et al., (2004), whose pH levels of several Spanish honeys ranged from 2.55 to 4.79, with a mean of 4.2. The variations in the mean pH values are due to differences in mineral elements in honey dew and blossom/floral honey (Buba et al., 2013). The p-value for the pH values between the means of the four regions was p < 0.001, indicating that there was a significant difference between the pH values. Variations in pH levels can be attributed to discrepancies in the mineral content of nectar or honeydew sources in the vicinity of the hives that honeybees visit for foraging (Seeley, 1995). The acidity of honey is determined by organic acids like gluconic acid, oxalic acid, lactic acid, and lactone, and alterations in their concentrations influence pH levels (Nanda et al., 2003). This may also be applicable to honey originating from the Suba region.

Free Acidity

The free acidity mean and range values obtained for the four regions are represented in Table 2 below.



Table 2: Mean and Range of Free Acidity of Honey from Four Different Regions of Gwassi North and Gwassi South

	Free Acidity (mEq/kg)		
Sampling site	(Mean ± SE)	Min	Max
Central(Nyancha)(n=10)	31.14±0.41	28.00	36.20
Western(Nyandiwa)(n=10)	32.42±0.70	27.40	38.40
Northern(Tonga) (n=10)	40.30±0.46	37.40	44.80
Eastern (Magunga) (n=10)	39.45±0.46	37.50	44.85
Total (n=40)	35.83±0.45	27.40	44.85

The mean free acidity values of honey from the Central (Nyancha), Western (Nyandiwa), Northern (Tonga), and Eastern (Magunga) regions were found to be 31.14 ± 0.41 mEq/kg, 32.42 ± 0.70 mEq/kg, 40.30 ± 0.46 mEq/kg, and 39.45 ± 0.46 mEq/kg, respectively. The mean free acidity values for the investigated regions were within the maximum permitted free acidity limit of 40 mEq/kg. However, 13% of the total honey samples, specifically from the Northern and Eastern regions, had a higher free acidity above the permitted limit of 40 mEq/kg. The higher levels of free acidity in these samples could be a result of increased gluconic acid due to the action of the glucose oxidase enzyme on glucose. The highest mean free acidity of 40.30 ± 0.46 mEq/kg was from Northern (Tonga) region honey, which had a variation of 37.40–44.80 mEq/kg, whereas the least mean value of 31.14 ± 0.41 mEq/kg with a range of 28.00-36.20mEq/kg was found in the Central (Nyancha) region. The total mean free acidity from the four Suba regions was found to be 35.83 ± 0.45 mEq/kg; this mean value is favorably comparable with Spanish honey, which recorded a range of 17.6–39.8 mEq/kg with a mean of 34.5 mEq/kg (Terrab et al., 2004). The p-value for the free acidity values between the mean values of the four Suba regions was p < 0.001, indicating that there was a significant difference between the free acidity values. The differences in free acidity between the four regions, just like the pH of the honey, may also be attributed to the botanical variations of the plants the honeybees forage on. Various plants have different acid levels in their nectarines, resulting in variation in free acidity in the four regions.

Hydroxymethylfurfural (HMF) Content

The HMF mean and range values obtained from the four regions are represented in Table 3 below.

	HMF (mg/kg)		
Sampling site	Mean ± SE	Min	Max
Central(Nyancha)(n=10)	6.52±1.19	0.44	23.65
Western(Nyandiwa)(n=10)	11.01 ± 1.45	2.39	31.58
Northern(Tonga) (n=10)	17.57±1.99	2.84	44.01
Eastern(Magunga), (n=10)	44.69±3.65	23.80	90.71
Total (n=40)	19.95±1.76	0.44	90.71

 Table 3: Mean and Range of Hydroxymethylfurfural mg/kg (HMF) of Honey from Four

 Different Regions of Gwassi North and Gwassi South

The HMF mean values of the honey samples for Nyancha (Central), Nyandiwa (Western), Tonga (Northern), and Magunga (Eastern) regions were $6.52 \pm 1.19 \text{ mg/kg}$, $11.01 \pm 1.45 \text{ mg/kg}$, $17.57 \pm 1.99 \text{ mg/kg}$, and $44.69 \pm 3.65 \text{ mg/kg}$, respectively. The mean HMF values from all four regions met both the maximum limit of 40 mg/kg of the Codex Alimentarius Commission standard (2001) and the maximum limit of 80 mg/kg of the European Union standard (2002). This implies that all the honey harvested from the four regions was fresh and had not been



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overheated during the extraction process. There was no significant difference between the Central (Nyancha) region honey samples with a mean of 6.52 ± 1.19 mg/kg and the Western (Nyandiwa) region honey samples with a mean of 11.01 ± 1.45 mg/kg. However, there was a significant difference between the Northern (Tonga) region with a mean value of 17.57 ± 1.99 mg/kg and the Eastern (Magunga) region with a mean of 44.69 ± 3.65 mg/kg. This could be attributed to variations in temperature, harvesting practices, and storage conditions of the honey samples (see Table 3 above). However, Eastern (Magunga) region honey samples had a maximum value of 90.71 mg/kg obtained from sample E₃₇ which was above the 40 mg/kg and 80 mg/kg permitted limits. This could be a result of the honey sample being subjected to high temperatures, which leads to the formation of high levels of HMF. The honey samples that are overheated result in increased HMF, as reported by Teshpome et al., 2020. The overall mean HMF value for the four regions was 19.95 ± 1.76 mg/kg, similar to the findings of Terrab and colleagues (2002), who reported Moroccan honey having a mean value of 19.20 mg/kg. The pvalue for the HMF values between the mean values of the four Suba regions was p < 0.001, indicating that there was a significant difference between the HMF values. The difference in HMF levels in the honey samples from the four regions could be a result of changes in climate, harvesting methods, extraction, and storage conditions.

Proline

Table 4 below represents the mean, maximum, and minimum values of proline content from the four regions.

Table 4: Mean and Range of Proline (mg/kg)) of Honey from Four Differe	ent Regions of
Gwassi North and Gwassi South		

	Proline (mg/kg)		
Sampling Site	Mean ± SE	Min	Max
Central(Nyancha)(n=10)	666.57±19.49	589.80	759.40
Western(Nyandiwa)(n=10)	624.76±22.75	477.60	816.70
Northern (Tonga) (n=10)	695.11±18.53	482.30	802.30
Eastern(Magunga) (n=10)	532.63±9.25	444.10	608.90
Total (n=40)	629.77±9.80	444.10	816.70

The mean proline content values of the honey samples from the Central (Nyancha), Western (Nyandiwa), Northern (Tonga), and Eastern (Magunga) regions were 666.57 ± 19.49 mg/kg, 624.76 ± 22.75 mg/kg, 695.11 ± 18.53 mg/kg, and 532.63 ± 9.25 mg/kg, respectively. The mean proline content values from the four regions were within the accepted minimum value of 180 mg/kg. This shows that all the honey samples from the four regions were fully ripened by the time of harvesting. The proline content of honey from the Central (Nyancha) region varied from 589.80 to 759.40 mg/kg, while the one from the Western (Nyandiwa) region varied from 477.60 to 816.70 mg/kg. In the Northern (Tonga) and Eastern (Magunga) regions, proline content varied from 482.30 to 802.30 mg/kg and 444.10 to 608.90 mg/kg, respectively. Turkish honey samples reported a range of 404.2-881.7 mg/kg, almost similar to values obtained from the Western (Nyandiwa) region with a range of 477.60-816.70 mg/kg (Beykaya, 2021). The honey samples with the lowest proline content value were from the Eastern (Magunga) region with a proline content of 444.10 mg/kg, while the sample with the highest proline content value was from the Western (Nyandiwa) region with 816.70 mg/kg of proline content. This indicates that honey from the Western (Nyandiwa) region matured and ripened better than from the other three regions. The overall mean value of proline content in honey from the four regions was 629.77 ± 9.80 mg/kg, which is above the acceptable limit of minimum 180 mg/kg honey



(Codex Alimentarius Commission, 2001). Confirmation that honey from the four regions had ripened well and was fully matured by the time of harvest. The p-value for proline content values between the mean values of the four Suba regions, p<0.001, indicating that there was a significant difference between the proline values. This can be attributed to the different levels of maturity reached by the honey.

Sugars

Fructose, glucose, and sucrose mean levels and range obtained from the four regions are represented in Table 5 below.

Table 5. Mean of Sugars of Honey Samples from the Four Different Regions of	f Gwassi
North and Gwassi South	

Sugar Content (g/100g)					
Sample Site	Fructose %	Glucose (%)	Sum of F &G	Sucrose (%)	F:G ratio
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean± SE
Central(Nyancha)(n=10)	39.32±0.71	28.10±0.66	67.42±0.76	2.10±0.12	1.43±0.06
Western(Nyandiwa)(n=10)	39.68±0.67	25.81±0.66	65.49±0.88	2.40±0.26	1.57±0.06
Northern(Tonga) (n=10)	43.10±0.41	29.30±0.81	72.40±1.08	3.32 ± 0.08	1.50±0.03
Eastern (Magunga)(n=10)	43.28±0.55	24.60±0.57	67.88±0.89	2.93±0.18	1.79±0.05
Min	33.24	19.25	60.12	0.15	1.06
Max	50.70	39.70	86.47	4.67	2.45
Total (n=40)	41.39±0.34	26.98±0.38	68.37±0.51	2.70±0.09	1.57±0.03

The study analyzed honey samples from the Central, Western, Northern, and Eastern regions of Nigeria. The mean fructose content was $39.32\% \pm 0.71\%$, $39.68\% \pm 0.67\%$, $43.10\% \pm 0.41\%$, and $43.28\% \pm 0.55\%$, respectively. The mean glucose content was $28.10\% \pm 0.66\%$, $25.81\% \pm 0.66\%$, $29.305 \pm 0.81\%$, and $24.60\% \pm 0.57\%$. The fructose levels were higher than the glucose levels, indicating the natural feeding of honeybees. Balancing fructose and glucose in honey can lead to slow or faster crystallization. According to Khalil et al. (2012), the results are within the expected range. The proportions of fructose and glucose in honey can vary depending on the honey's source or type. The results confirm that fructose and glucose are the main sugars in honey samples. In honey samples from all four regions, the average amount of glucose and fructose was over 60%. In the study area as a whole, the average amount of fructose and glucose was over 60%. This means that all of the honey. Crystallization occurs due to the formation of monohydrate glucose crystals, which can vary in terms of number, shape, size, and quality depending on the composition of honey and its storage conditions. The fructose-to-glucose ratio is a standard measure of honey quality with regard to crystallization.

The average values of sucrose from Central (Nyancha), Western (Nyandiwa), Northern (Tonga), and Eastern (Magunga) were $2.10\% \pm 0.12\%$, $2.40\% \pm 0.26\%$, $3.32\% \pm 0.08\%$, and $2.93\% \pm 0.18\%$, respectively. The differences in sucrose levels between the four regions could be attributed to nectar sources and the handling of honey. Handling honey can influence sucrose levels through factors such as incomplete ripening during harvesting, the removal of enzymes during filtration, enzymatic changes due to excessive heating, crystallization during storage, and potential adulteration (Eshete *et al.*, 2019). Maintaining the natural composition and quality of honey requires diligent and responsible processing practices. All the honey samples in this study had a level of sucrose below 5%, which is the maximum prescribed limit according to Codex and EU standards. This shows that the honey from the four regions had attained maturity levels and was well ripened, indicating that most of the sucrose had been converted to fructose



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and glucose. The lower values of sucrose also indicate that there was no adulteration of the honey. In an Algerian study of North-East honey sucrose, 1.06%–22.68% was detected (Chefrour *et al.*, 2009), similar to the findings of this study. The p-value for the sucrose content values between the mean values of the four regions was p<0.001, an indication that there was a significant difference between the sucrose values. This could be a result of the variation in the maturity of the honey from the four regions. Sucrose levels depict levels of honey maturity; thus, higher values of sucrose are an indication of honey that has not matured well, where sucrose has not been converted to fructose and glucose.

Minerals

Table 6 below represents the mean, maximum, and minimum values of mineral content from the four regions.

Table 6: Mean Mineral Elements Content of Honey Samples from Four Different Regions of Gwassi North and Gwassi South

Mean ± SD Mineral Elements Concentration in (ppm).							
Sampling site	K	Na	Ca	Mg	Zn	Fe	Mn
Central (Nyancha)(n=10)	71.50±1.57	23.17±0.24	2.55±0.19	6.30±0.32	0.24±0.02	0.24±0.02	0.03±0.00
Western(Nyandiwa)(n=10)	66.96±0.89	23.40±0.19	2.92±0.15	$7.90{\pm}0.22$	0.18±0.03	$0.40{\pm}0.02$	0.07 ± 0.01
Northern(Tonga) (n=10)	69.43±1.42	23.03±0.21	$1.90{\pm}0.18$	7.87±9.32	0.38±0.16	0.23±0.12	0.07 ± 0.01
Eastern (Magunga) (n=10)	69.74±1.35	23.13±0.25	2.87 ± 0.07	7.98±10.20	$0.16{\pm}0.02$	0.26±0.03	0.06 ± 0.00
Min	60.20	21.00	0.60	2.06	0.02	0.05	0.01
Max	94.40	26.00	4.68	10.20	2.87	0.66	0.19
Total(n =40)	69.41±0.67	23.18±0.11	2.56 ± 0.09	7.54±0.17	$0.24{\pm}0.04$	0.28 ± 0.01	0.06 ± 0.00

In this study, a total of seven elements were quantified: K, Ca, Mn, Na, Mg, Fe, and Zn (Table 6). Potassium, quantitatively, was the most abundant mineral found in honey samples, with an average of 71.50 ± 1.57 ppm from the Central (Nyancha) region. The Western (Nyandiwa) region had a mean of 66.96 ± 0.89 ppm, and the Northern (Tonga) region had an average of 69.43 ± 1.42 ppm. The Eastern (Magunga) region had an average of 69.74 ± 1.35 ppm. The Central (Nyancha) region had the highest potassium content mean of 71.50 ± 1.57 ppm, whereas the Northern (Tonga) region had the least mean value of 69.43 ± 1.42 ppm.

The high levels of potassium are attributed to the levels of K in the plant tissues. Because of its rapid release from nectar sources, potassium (K) constitutes the predominant element, accounting for nearly 80% of the overall composition (Mărgăoan et al., 2021). Similarly, Chua et al., (2012) found potassium to be the most abundant element in honeys from Malaysia with a range of 69.3ppm to 78. 6ppm. The elements Na and Mg were the next most common, followed by Ca, Zn, Fe, and Mn (see Table 6 above). The levels of potassium, sodium, magnesium, and zinc did not vary between the four regions (one-way ANOVA, p > 0.05). The levels of calcium, iron, and manganese differed significantly between the regions (p < 0.001, $\alpha = 0.05$). The mean concentration of calcium was significantly lower in honey samples from the Northern (Tonga) region $(1.90 \pm 0.18 \text{ ppm})$ as compared to the Central (Nyancha) region $(2.55 \pm 0.19 \text{ ppm})$, the Western (Nyandiwa) region $(2.92 \pm 0.15 \text{ ppm})$, and the Eastern (Magunga) region (2.87 ± 0.07 ppm). The mean concentration of iron in honey samples from the Western (Nyandiwa) $(0.40 \pm 0.02 \text{ ppm})$ region was significantly higher as compared to honey samples from the Central (Nyancha) $(0.24 \pm 0.02 \text{ ppm})$, Northern (Tonga) $(0.23 \pm 0.12 \text{ ppm})$ ppm), and Eastern (Magunga) $(0.26 \pm 0.03 \text{ ppm})$ regions. Honey samples from the Central (Nyancha) region $(0.03 \pm 0.00$ ppm) recorded significantly lower levels of manganese as compared to the Western (Nyandiwa) region $(0.07 \pm 0.01 \text{ppm})$, the Northern (Tonga) region



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 $(0.07 \pm 0.01$ ppm), and the Eastern (Magunga) region $(0.06 \pm 0.00$ ppm). The p-values of K, Na, Mg, and Zn were 0.692, 0.122, 0.462, and 0.243, respectively; therefore, there was no significant difference between the levels of K, Na, Mg, and Zn in the four regions. The p-values of Ca, Fe, and Mn were p < 0.001 for the three elements; hence, there was a significant difference between their values in the four regions. The variation in the elemental composition of the honey from the four regions can be attributed to differences in soil composition, plant type, season, and environmental conditions (Mărgăoan *et al.*, 2021).

CONCLUSION AND RECOMMENDATIONS

Conclusion

Based on the chemical properties of the honey from the four regions, the results obtained indicate that: The overall mean pH values of honey samples from the four regions were pH 4 \pm 0.03, which was within the Codex standard limit of maximum pH 5 (Codex Alimentarius, 2001). The honey is acidic due to its low pH values. The mean pH value differed significantly across the four regions in the study area (p < 0.001). This could be attributed to differences in nectar in the surroundings of the hives, upon which the honeybees forage. The measured free acidity conformed to the EU standard of < 40 mEq/kg, as the overall mean obtained was 35.83 ± 0.45 mEq/kg. There was a significant variation in the levels of free acidity across the region, and this could be due to variations in the botanical origin of the honey. In the analysis of the samples for HMF, the four regions met both the 80 mg/kg Codex Standard and the 40 mg/kg EU standard. The overall HMF mean obtained was 19.95 ± 1.76 mg/kg. Therefore, it can be concluded that most of the honey under study was fresh, had undergone little or no heating, or had not been stored for long after harvesting. The overall mean proline content value was 629.77 ± 9.80 mg/kg, which was within the acceptable international standards of a minimum of 180 mg/kg. This implies that all the honey samples from the four regions were fully mature and well ripened by the time of harvest and were also free from sugar adulteration. The overall mean sum of glucose and fructose in honey samples from the four regions of the study area was $68.37\% \pm 0.51\%$. This value is above the permitted minimum Codex limit of 60% for floral honey. This indicates that honey from this region is a predominantly floral type of honey. The honey samples in this study had an overall mean sucrose level of 2.70 $\% \pm 0.09\%$, which is below 5%, which is the maximum prescribed limit according to Codex and EU standards typical for good quality honey. This indicates that the honey was mature and was free from sugar adulteration. There was variation in the elemental composition of the honey from the four regions, and this can be attributed to differences in soil composition, plant type, season, and environmental conditions. Mineral analysis showed that potassium was the most abundant, while manganese was the least abundant, in the honey samples. The concentration increased in the order Mn< Zn < Fe <Ca< Mg < Na <K. Based on the results of this study, it can be concluded that the Suba region honey is acidic, fresh, mature, well ripened, and unadulterated, with high levels of fructose and glucose. The chemical properties of honey from the Suba region, Suba South Sub-County, Gwassi North, and Gwassi South divisions, namely; Central (Nyancha), Western (Nyandiwa), Northern (Tonga), and Eastern (Magunga) from Homa Bay County, are within the permitted International Honey Standards. Therefore, this honey is of good quality, and it qualifies for commercialization both in the local and international markets.

Recommendations

The beekeepers in Suba region, Suba South Sub-County, Gwassi North and Gwassi South divisions, namely; Central (Nyancha), Western (Nyandiwa), Northern (Tonga), and Eastern



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(Magunga) in Homa Bay County, should be sensitized to the potential market for their honey both locally and internationally.



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