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RESEARCH

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Aberrant in physicochemical properties, functional health and medicinal grades of honeys from different sales outlets in Southwest Nigeria

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Abstract

Background: Honey is consumed for the derived benefits as sweetener, for healing wounds, skin burns, regulating blood sugar level, treatments of some ailments, etc. In Nigeria, adulteration of honey is a serious concern, and these adulterated or fake honeys have health implications. This research aims to compare and contrast the physicochemical qualities, functional health and medicinal values of honey samples from local honey parkers, supermarkets and beekeepers' sales outlets in Southwest Nigeria.

Results: The darkness of the honey from the three sources was significantly different. The honey samples from the beekeepers have ash contents values of 0.25–1.0 in line with CAC and IHC, whereas 7(46.66%) and 2(13.33%) of honey samples from the supermarkets and local handlers/parkers, respectively, deviated from this standard values. The wound healing development after 8 days of topical treatment of incision wounds with honey from beekeepers shows a good development. 93.3% of the honeys sourced from the beekeepers, 58.06 and 66.05% of the honeys sourced from the supermarkets, and the local honey markets were found to be original.

Conclusion: The originality of honey for consumers' satisfaction is dependent on the complimentary factors of physicochemical properties, functional health and medicinal values. These findings provide information for consumers' awareness on categorization of honey as original/pure, adulterated and fake and the health implications.

Keywords: Original, Fake, Adulterated, Medicinal, Physicochemical

Background

In Nigeria, various honeys are available in the market, honey produced by bees, harvested by beekeepers and packaged is original honey, this honey could also be found in the supermarkets and with the local honey parkers, and other honeys found in the market could be adulterated and fake. Honey adulteration can be direct or indirect (Blanka et al. 2015). Direct adulteration means that a substance is added directly to the honey, while indirect adulteration is feeding the honeybee with adulterating substance (Blanka et al. 2015). Indirect adulteration is peculiar to beekeepers, while direct adulteration is generally done by honey parkers. Furthermore, adulteration of honey can naturally occur when plant substances such as pyrrolizidine and alkaloids are found in it. Direct adulteration with sucrose may give a sucrose content of 30% or more (Crane 1990) which is considered unsafe for human consumption. The average concentration of sucrose varied in individual groups of honeys; however, sucrose content in pure honey should not be more than 2–3.9% (Čelechovská and Vorlova 2001). Irresponsible honey handlers engaged in honey adulteration and

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infiltrating the global market with these honeys. Identification of the honey is very challenging particularly for consumers with lack of knowledge on characteristics of pure honey.

Honey acquires its physicochemical properties through nature and worker bees' conversion of nectar to honey. Color and flavor of honey depend largely on the kind of plant species foraged by the worker bees (Akinwande et al. 2016). Several workers in Nigeria have identified some wild flowers and cultivated plants grown for agricultural and ornamental purposes and tree fruits as good sources of nectar, collected by honeybees and converted to honey by the bees (Anikwe et al. 2016). The honey produced by the bees acts as food store for the bee colony during dry seasons.

Honey, as a natural product, is made up of numerous essential components. It is a supersaturated sugar solution of two main sugars, namely glucose and fructose, with small amounts of other more complex sugars. Other substances present in pure honey include phenolic acids, flavonoids, water, vitamins, organic acids, proteins, phytochemicals and minerals which are largely responsible for the differences among individual honey types (White 1980; White and Doner 1980; Bertoncelj et al. 2007). The concentration of the sugar is frequently the characteristic of the plant species from which the nectar fluid was taken, although this concentration also varies with environmental conditions (Flodhazi 2004). Fructose (levulose) is the dominant sugar with about 38.5 g/100 g honey, glucose (dextrose) 31 g/100 g, with at least 22 other more complex sugars such as sucrose 1.5 g/100 g honey, maltose, isomaltose, erlose, kojibiose, melezitose and all others constituting 4 g /100 g honey (White and Doner 1980; Bertoncelj et al. 2007). The mineral content of honey usually varies and is recognized as an environmental indicator (Terrab et al. 2004). All the trace minerals that are essential for good health are found in honey. These mineral contents of honey are closely related to the floral type, mineral resources in the soil and environmental factors. White and Doner (1980), Terrab et al. (2004) and Bertoncelj et al. (2007) claimed 100 g of honey contain 6 mg calcium, 4 mg phosphorus, 4 mg sodium, 52 mg potassium, 0.42 mg iron, 0.22 mg zinc, 2 mg magnesium, 0.80 mg selenium, 0.04 mg copper and 0.08 mg manganese. In addition, honey also contains vitamin C (0.50 mg), vitamin B (0.02 mg), folate (2 mg), pantothenic acid (0.07 mg), niacin (0.12 mg), riboflavin (0.04 mg) per 100 g (White and Doner, 1980). Organic acids add to honeys the antibacterial properties. Gluconic acid which is the major organic acid found in honey is the product of the enzymatic glucose oxidase reaction. It increases calcium absorption and contributes very largely to the acidic properties of honey. Other organic acids in honey are acetic, formic, butyric, succinic, malic, oxalic, citric, maleic and pyroglutamic acids. As with other components in honey, the organic acid content varies according to the flowers foraged by the bees (Crane, 1975). Honey also contains protein, enzymes, dietary fiber (0.20 g per 100 g). It contains no fat and cholesterol, and typically composed of trace amount of ash (0.20 g per 100 g) (White 1975; Gome et al. 2010).

Honey acts as a dietary antioxidant, when honey is applied alone or in combination with conventional therapy, and it might serve as an antioxidant in the control of commonly associated oxidative stress (Gheldof et al. 2003). This ability of honey in antioxidant properties is related to the brightness of honey; therefore, the darker the honey, the higher the value of antioxidant. In the honey, the phenolic compounds are the major factor for this antioxidant activity, since the phenolic level is connected with the radical absorbance activity values of the honey. The antimicrobial activity of honey is because honey does not support the growth of yeast and bacteria (Voidarou et al. 2011). The factors responsible for this antimicrobial activity of honey are the enzymatic glucose oxidation reaction, high osmotic pressure/low water activity, low pH/acidic environment, low protein content, high carbon-to-nitrogen ratio, low redox potential due to the high level of reducing sugars, a viscosity that limits dissolved oxygen and other chemical agents/phytochemicals, such as glucose oxidase, and hydrogen peroxide (Beretta et al. 2007) The peroxidases and many chemical products with low antibacterial level were antimicrobial agents in honey, and this includes terpenes, pinocembrin, benzyl alcohol, 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid), methyl-3,5-dimethoxy-4-hydroxybenzoate (methyl syringate), 2-hydroxy-3-phenylpropionic acid, 2-hydroxybenzoic acid, 3,4,5-trimethoxybenzoic acid and 1,4-dihydroxybenzene (Obi et al. 1994).

This research investigates and compares the physicochemical qualities of different honey samples from different sales outlets and consumed in Southwest Nigeria; the effects of the honey samples on health issues (glycemic, antiseptic and healing properties) provide information for consumers' awareness on categorization of honey as original/pure, adulterated and fake.

Methods

Sampling of honeys

Forty-five freshly stocked honey samples were randomly collected from three different sales outlets, and 15 samples (each 250 g) were obtained directly from beekeepers (HB), supermarkets (HS) and the local branded honey parkers (HL). The honey samples were purchased as brands with highest demand by consumers. All the samples were placed in sterile bottles, labeled according to

sources (e.g., HL_1, HL_2, HL_3,..., HL_{15}) and kept at room temperature until when needed.

Physicochemical analyses

The samples of honey were analyzed to determine its moisture content, electrical conductivity, turbidity, pH, sugar content, ash content, color properties and hydroxymethyl furfural (HMF) level. All the experimental analyses were done for all the samples collected. The chemicals and reagents used in this experiment were of analytical grade.

Test for moisture content (Bognadov et al. 2004)

5 g of each honey samples in labeled crucibles was placed in an oven at 103 °C for 2 h. Thereafter, the dried honeys were re-weighed and the moisture contents were calculated and expressed as percentage of initial weight (5 g).

Test for electrical conductivity

Electrical conductivity of 20 g of each honey sample in 100 ml distilled water was measured using a conductivity meter (Cond 3210 WTW, Fisher Scientific, UK) set at 20 °C, and the values were determined by the cell immersed in the solution. The results were expressed in micro-Siemens per centimeter.

Test for turbidity

The transparency of 5 g of each honey sample was dissolved in 25 ml of distilled water, put into a cuvette with a path length of 1 cm. The cuvette was placed inside the turbidity meter, and the absorbance level was measured at 660 nm. The results were taken and mean ± S.D for each sample recorded.

Test for pH (Bogdanov et al. 2004)

Solution of each honey sample was prepared by dissolving 10 g in 75 ml of distilled water. The mixture was homogenized, the pH electrode was immersed in the solution, and the pH value was recorded using pH meter (Basic 20).

Test for ash content (Bogdanov 2002)

5 g of each sample was heated overnight in a muffle furnace at 550 °C until a constant mass was attained. The residue was weighed after cooling to room temperature in a desiccator. The total ash content (%) was calculated using the following equation:

$$\mathrm{WA} = \frac{\left(m^1 - m^2\right) \times 100}{m^0}$$

where WA = weight of ash; m^0 = weight of honey taken; m^1 = weight of dish + ash; and m^2 = weight of dish. Result was expressed as a percentage by weight (CAC, 2001; IHC 2009).

Test for color properties

The honey samples were further evaluated on their color differences which was based on the CIELab method, where L* (lightness), a* (degree of greenness/redness) and b* (degree of blueness/yellowness) values were measured using Colorimeter PCE-C8M 2. CIELab method, an acceptable method for measuring color of food products (Wilczynska 2014). This color system is practical because any color can be defined by a mixture of red, blue and green colors (Zsanett et al. 2021). Honey samples were put in cuvettes for measuring the color of liquids and powders, and the colorimeter was placed on the plate. The resulting L*, a* and b* values were recorded for each sample. (L* = lightness of the sample (0 = black; 99 = white), a* = redness of the sample (in + 60 direction red, in − 60 direction green) and b* = yellowness of the sample (in + 60 direction yellow, in − 60 direction blue) (Wilczynska 2014).

Test for HMF content (White 1979)

5 g of each honey sample was dissolved in 25 ml of distilled water, treated with a clarifying reagent (0.5 ml of 15% potassium ferrocyanide and 30% zinc acetate), and then, the volume was diluted to 50 ml with distilled water using a drop of ethanol to suppress foam formation. The sample was filtered with the first 10 ml discarded, and 5.0 ml of the remaining filtrate was then transferred into two test tubes. 5.0 ml of distilled water was added to one test tube and mixed. This served as the sample solution. 5.0 ml of 0.20 bisulfite solution was added to the second test tube, as the reference solution. The absorbance of the filtrate was measured using 284 nm and 336 nm wavelength. The HMF content (in mg/100 g honey) was calculated using the equation below (Bogdanov 2002):

$$\mathrm{HMF} = \frac{(A284 - A336)}{W} \times 74.87$$

A284 = absorbance at 284 nm; A336 = absorbance at 336 nm and W = weight of sample

$$\mathrm{Factor} = \frac{126 \times 100 \times 1000 \times 100}{16830 \times 1000} = 74.87$$

126 = molecular weight of HMF and 16,830 = molar absorptivity of HMF at 284 nm.

Results were expressed in mg/kg (IHC 2009).

Tests of the functional health and medicinal grades of the honeys

Description of the animal models used

80 Albino mice (50 ♂30♀) (weight = 22.0 ± 0.1 g)) from the Animal house, Biology Department, Federal University of Technology, Akure, were used for the tests. The mice were housed in clean plastic cages with mice pelletized food and water provided ad libitum. All rules applying to animal care and safety were observed. The animals were handled for 5 min daily, 2 weeks before the experiment to habituate them to handling, presence of investigator and experimental environment. Fifty-two mice (34 ♂**18**♀) were selected, and the mice were grouped into five, groups A to C of 16 mice each and groups D to E, 2 mice each. The randomization was performed such that the male-to-female ratio is 2:1 and average body weights of the animals in each group were comparable. Eight honey samples each were randomly selected from the 15 samples each of HL, HS and HB ($n = 8 \times 3 = 24$), each of the selected honey samples represents a treatment in its sample category, and it was used for 2 mice in its group. All the tests were carried out between 8.00 am and 8.00 pm.

Effect of honey on body weight

At the end of the two weeks of acclimatization, the weights of the mice were taken using a weighing scale and measurements were recorded daily. In groups A, B and C, the experimental groups were fed with different honey samples. Feeding treatment was done using 2.0 g of the food with oral cannula. Groups D and E (control groups) were fed with distilled water and golden syrup, respectively. The mice were treated once daily, and changes in weights were recorded (Table 1).

Effect of honey on glucose level

The fed mice were placed in a restrainer, the tails of the mice were disinfected with 70% (v/v) ethanol, and blood was collected from the tails by nipping with a sterile scissors. The first drop of blood was discarded, and the second drop was analyzed using a test strip and glucometer device (ACCU-CHEK Active, Roche, Germany). Glucose level was measured at 8am, and the reading was recorded. Foods were removed immediately through overnight from the cage, and the mice were fasted for 24 h. Thereafter, their weights were measured again. Blood samples were collected through the tails after the fasting hours and the glucose level were recorded as fasting blood glucose level (FBGL). Again, the mice were treated with different food samples (Table 2). After 24 h, blood samples were collected using the tail nipping method, a drop of blood was placed on the test strip inserted into the glucometer and blood glucose measurement was recorded (BGL). The blood glucose levels were expressed in milligrams per deciliter (mg/dl).

Effect of honey on wound healing

The 5 groups of 52 albino mice were retained, each mouse about 8 weeks old, substitutions were made to have healthy male/female mice of nearly equal weight

Table 1 Turbidity and color properties of the honey samples

Properties/Group	HB	HS	HL	*t*-Value	*p*-Value
Color L*	36.02 ± 8.20^{b}	31.98 ± 5.17^{ab}	28.54 ± 7.37^{a}	− 21.20	0.038
a*	16.65 ± 11.06^{b}	22.74 ± 6.01^{b}	22.05 ± 2.55^{b}	− 18.47	0.058
b*	31.35 ± 0.36^{a}	33.71 ± 3.14^{b}	32.56 ± 10.71^{c}	− 21.71	0.000
Turbidity values (NTU)	57.78 ± 55.41^{b}	14.62 ± 11.13^{a}	42.11 ± 43.73^{ab}	− 4.80	0.08

Values followed with the same letters (a, b and c) in column are not significantly different ($p > 0.05$) values with different letters are significantly different ($p < 0.05$)

Table 2 Blood glucose level (BGL) before and after treatments in mice

Animal group	Treatments	RBGL	FBGL	1 h	3 h	6 h	9 h	12 h
A ($n = 15$)	HL	129.20 ± 26.81^{a}	86.20 ± 21.99^{ab}	101.20 ± 22.97^{a}	108.60 ± 8.99^{c}	111.80 ± 7.50^{d}	114.00 ± 8.46^{d}	114.00 ± 8.46^{d}
B ($n = 15$)	HS	124.00 ± 12.90^{a}	82.00 ± 11.05^{b}	101.20 ± 27.71^{a}	108.20 ± 11.17^{b}	109.20 ± 10.71^{b}	111.60 ± 10.21^{b}	111.60 ± 10.21^{b}
C ($n = 15$)	HB	99.60 ± 21.16^{a}	79.20 ± 14.58^{a}	101.60 ± 12.24^{a}	72.00 ± 7.65^{a}	71.60 ± 7.47^{a}	70.20 ± 3.96^{a}	70.20 ± 3.96^{a}
D ($n = 3$)	Dist. Water	111.20 ± 27.71^{a}	83.40 ± 11.10^{ab}	79.20 ± 26.81^{a}	77.60 ± 5.90^{c}	67.40 ± 6.12^{c}	65.40 ± 6.12^{c}	65.40 ± 6.12^{c}

RBGL (Random blood glucose level)-Blood glucose level taken after feeding the mice with equal weight pelletized mice feed for 5 days;

FBGL (Fasting blood glucose level)-Blood glucose level taken after 24 h from daytime to overnight fasting

Values followed with the same letters (a, b and c) in column are not significantly different ($p > 0.05$), values with different letters are significantly different ($p < 0.05$) (Tukey's HSD test at $p = 0.05$)

HB (selected honey samples from beekeepers); HS (selected honey samples from supermarkets); and HL (selected honey samples from local honey parkers

(approximately 25–30 g), and the mice were kept in clean plastic cages under usual experimental conditions of temperature 23 ± 2 °C. Sterile scissor and blade wounds were made on the mice according to Jimi et al. (2017). The longitudinal wound on the dorsal side measured 1 cm. The wounds in all groups were treated, every day for two weeks. Treatments involved 8 honey samples randomly selected from each of HL, HS and HB (total $n = 24$). In groups A, B and C, wound surfaces were treated topically with selected honey samples in HL, HS and HB, respectively; group D mice were not treated and wounds of E were treated with sheep oil. All treatments (1 g) (Table 2) were applied topically on the wounds using a sterile cotton swab, and the wounds sizes were measured (± cm) at wound edge, contraction and time taken to heal (7th and 14th day). During the period, the mice were fed on commercial pellet and water ad libitum (Table 3).

Categorization of the honeys

Categorization of the honeys was based on the level of aberrations from the standard physicochemical parameters using Codex Alimentarium Commission (CAC) or International Honey Commission (IHC) standards and the results obtained from the functional health and medical values.

Statistical analyses

Data were analyzed using SPSS v 11 and Microsoft Excel (2010). Values were compared using Student's t test, and differences in the values were considered statistically significant when $p < 0.05$. Honey physicochemical properties and other parameters were discussed and compared with the standard legislation of CAC and IHC.

Results

Physicochemical properties

Moisture content and pH

The moisture content of the honey within and between the groups was significantly different ($p < 0.05$). Moisture content values that fall below and above the CAC standard range of 16.4–20.0% were recorded for 7(46.67%) and 5(33.33%) samples from the local honey parkers (HL) and supermarkets (HS), respectively (Table 4). The average moisture content for honey sampled from the beekeepers (HB) and supermarkets was significantly related. There was no significant difference in values within and between the group of the honey samples from the three sources. The pH values of all the honey samples are within 3.2–4.5 (CAC). The honey samples from the beekeepers have ash contents values of 0.25–1.0 in line with CAC and IHC, whereas 7 (46.66%) and 2 (13.33%) (Table 4) of honey samples from the supermarkets and local handlers/parkers, respectively, deviated from this standard values.

Electrical conductivity and Hydroxymethylfurfural

Electrical conductivity, which expresses the relationship in the concentration of mineral and organic acids, showed no significant differences in values within and between groups of honeys from the three retailing sources.

The values of electrical conductivity in the investigated honey samples were between the Codex standard value of 0.09–1.99mS/cm. However, 4 (26.7%) and 3 (20%) (Table 4) honey samples obtained from the supermarkets and local honey parkers, respectively, deviated from this standard range. However, average values within and between the three groups of honey samples were not significantly different ($p > 0.05$). Honey, because it is rich in glucose and fructose, contains HMF. However, in 6 (40%) and 4 (26.66%) honey samples retailed by the beekeepers and the supermarkets HMF occurs at very low concentrations, below the Codex standard range of 40.0–80.0 mg/kg number.

Turbidity and color

The turbidity values of all the honeys within and between the groups were not significantly different ($t = -4.80$, $P = 0.08$, $p > 0.05$). All the honeys have low turbidity (Table 1). Color of the honey was measured using the

Table 3 Wound healing after topical treatment of the incision surface

Animal Group	Treatment	Initial wound cut Size (cm)	Wound cut size after 4 days	Wound cut size after 8 days
A ($n = 15$)	HL	1.00	0.77 ± 0.15^{b}	0.67 ± 0.17^{b}
B ($n = 15$)	HS	1.00	0.63 ± 0.12^{c}	0.49 ± 0.10^{c}
C ($n = 15$)	HB	1.00	0.43 ± 0.06^{a}	0.30 ± 0.10^{a}
D ($n = 3$) (-)	No treatment	1.00	0.81 ± 0.00^{b}	0.77 ± 0.31^{b}
E ($n = 3$) (+)	Sheep oil	1.00	0.77 ± 0.06^{b}	0.67 ± 0.06^{b}

Values followed with the same letters (a, b and c) in column are not significantly different ($p > 0.05$), values with different letters are significantly different ($p < 0.05$) (Tukey's HSD test at $p = 0.05$)

Table 4 Categorization of the honeys based on the values obtained from physicochemical properties, functional health and medicinal grades

		Physicochemical parameter evaluation				Treatments		Blood glucose level		Wound healing	
Honey Sample (*n* = 15)	**Values compared to CAC/IHC**	**Moisture content No (%)**	**Ash content No (%)**	**Electrical Conductivity No (%)**	**HMF No (%)**	**Honey sample (*n* = 8)**	**Numb. of mice treated**	**Compared to FBGL**	**After 12 h No (%)**	**Closure of wound size**	**After 8 days No (%)**
HB	High[A]	0 (0)	0 (0)	0 (0)	0 (0)	HB	*n* = 16	Fall below[P]	15 (94%)	Closed up[P]	14 (93.8%)
	Low[F]	0 (0)	0 (0)	0 (0)	6 (40)			Rise above[F]	1 (6.25%)	Little close up[A]	1 (6.25%)
	Standard[P]	15 (100)	15 (100)	15 (100)	9 (60)			Same level[A]	0 (0%)	No improvement[F]	0 (0%)
HS	High[A]	2 (13.3)	0 (0)	0 (0)	0 (0)	HS	*n* = 16	Fall below[P]	5 (31.3%)	Closed up[P]	7 (43.8%)
	Low[F]	3 (20)	7 (46.67)	3 (20)	4 (26.6)			Rise above[F]	11 (69%)	Little close up[A]	4 (25.0%)
	Standard[P]	10 (66.67)	8 (53.3)	12 (80)	11 (73.3)			Same level[A]	0 (0%)	No improvement[F]	4 (25.0%)
HL	High[A]	3 (20)	0 (0)	0 (0)	0 (0)	HL	*n* = 16	Fall below[P]	4 (25.0%)	Closed up[P]	5 (31.3%)
	Low[F]	4 (26.6)	2 (13.3)	0 (0)	0 (0)			Rise above[F]	12 (75%)	Little close up[A]	4 (25.0%)
	Standard[P]	8 (53.3)	13 (86.67)	15 (100)	15 (100)			Same level[A]	1 (6.25%)	No improvement[F]	6 (37.5%)

Honey samples	**% Original (P)**	**% Adulterated (A)**	**% Fake (F)**
HB	91.3	8.7	0
HS	58.06	6.39	34.55
HL	66.05	8.54	25.4

Values under parameters with superscript F were considered too extreme and classified as fake, and values under parameters with superscript A were considered moderate and classified as adulterated, while values under parameters with superscript P were considered standard and as original/pure

Original honeys are pure, and adulterated honeys are made poor in quality by addition of some inferior substances, while fake honeys are not genuine

HB (selected honey samples from beekeepers); HS (selected honey samples from supermarkets); and HL (selected honey samples from local honey parkers

CIE (1986) L*a*b* Color Scale, where color is displayed in three-dimensional system, and the **L*** vertical coordinate values range from 0 (black) to 100 (for white); on the scale, honey from the beekeepers is darker in color (36.02 ± 8.20) compared to honeys from the supermarkets and local retailers which are 31.98 ± 5.17 and 28.54 ± 7.37, respectively. The darkness of the honey from the three sources was significantly different ($t = -21.20$, $P = 0.038$, $p < 0.05$) (Table 1). On the scale, a* is the horizontal coordinate which ranges from − 80 (green) to + 80 (red); all the honey samples moved toward red. The redness was not significantly different ($t = -18.47$, $P = 0.058$, $p > 0.05$) (Table 1). b* is the horizontal coordinate the values of which range from − 80 (blue) to + 80 (yellow). The yellow range of the honey samples was significantly different ($t = -21.71$, $P = 0.000$, $p < 0.05$) (Table 1).

Body weight before and after feeding the mice for 2 weeks

The mean weights of the mice increased from 28.17 ± 1.68 to 28.93 ± 2.05 and 27.57 ± 1.19 to 29.07 ± 1.40 when fed with distilled water and golden syrup, respectively, and there were no significant differences ($p > 0.05$) in the weight increase within the group. The mean weight increased from 27.50 ± 2.76 to 28.53 ± 3.67 and 26.77 ± 0.68 to 26.10 ± 0.96 was recorded when fed with some honeys sampled from local honey parkers and supermarkets respectively, and there were significant differences in weight increase ($p < 0.05$) within the groups. For the honeys obtained from the beekeepers, the mean weights decreased from 28.50 ± 0.50 to 27.87 ± 0.50. The change in weight when fed with this honey was not statistically different ($p > 0.05$).

Blood glucose level before treatment

The mice blood glucose level (BGL) and their fasting blood glucose level (FBGL) measured for all the groups in the same weight range were positively correlated ($r = 0.734$), although there was significant low BGL and FBGL for the mice in group C (Table 2).

Blood glucose level hours after treatment

In the groups of mice fed with honey samples from HL and HS, there was sustained high BGL after 3 h compared to the FBGL, and the rise in the glucose levels in the two groups was significantly different ($p < 0.05$) (Table 2). The mice with mean value of FBGL = 86.20 ± 21.89 mg/dl when fed with HL recorded mean value of 101.20 ± 22.97 mg/dl after 1 h of feeding, and it rose to 114.00 ± 8.46 mg/dl after 12 h without further feeding (Table 2, Fig. 1). This is significantly different from the response observed when fed with HS; the mice with mean value of FBGL = FBGL = 82.00 ± 11.05 mg/dl recorded mean value of 101.20 ± 22.71 mg/dl in 1 h after feeding, and it rose to 111.60 ± 10.21 mg/dl after 12 h (Table 4, Fig. 1) with further feeding stopped.

Mice fed with honey samples from HB did not give sustained high blood glucose level after 3 h (Fig. 1); instead, there was a significant drop in the blood glucose levels as compared to the fasting blood glucose level ($p < 0.05$). The mean value of FBGL = 79.20 ± 14.58 mg/dl in the mice; the blood sugar level rose sharply to mean value of 101.60 ± 12.24 mg/dl an hour after feeding with honey samples from HB, and it sharply decreased below the FBGL to 72.00 ± 7.65 mg/dl after 3 h and 70.20 ± 3.96 mg/dl after 12 h without further feeding.

In the negative control group (distilled water), the average BGL in the mice decreased sharply and significantly after 1 h and remained relatively low compared to the levels obtained with mice fed with the honeys. The mice have mean FBGL = 83.40 ± 11.10 mg/dl, and at the end of the 12 h, BGL decreased to 63.20 ± 3.96 mg/dl, whereas, in the positive control (golden sugar syrup), when the mice with mean FBGL = 86.00 ± 20.92 mg/dl were fed with the dose of the syrup, there was sharp rise in the BGL after 1 h (124.00 ± 12.90 mg/dl) to 12 h (116.60 ± 9.79 mg/dl) and remained significantly higher ($p < 0.05$) than the levels obtained with mice fed with the honeys (Fig. 1).

Wound healing

For the healing development after 8 days of topical treatment of incision wounds, there was an average reduction of 0.30 ± 0.10 cm in 1.00 cm incision wound for the mice in category C treated with honeys sourced from the beekeepers (HB). This is significantly different ($p < 0.05$) from the mean value reduction (= 0.67 ± 0.17 cm) in wound size obtained from the mice in category A group treated with honeys sourced from the local parkers market (HL) and in wound size obtained from the mice in category B groups with mean value reduction (= 0.49 ± 0.10 cm) treated with honeys sourced from the local supermarkets (Table 3).

There was no significant difference, 8 days after treatment, in the incisions wound size in the mice in groups A, D and E. Average reduction of 0.67 ± 0.17 cm in the incision wound was obtained in group A (treated with honeys sourced from HL); 0.77 ± 0.31 cm incision wound size reduction was obtained in mice group D with no treatment, and 0.67 ± 0.06 wound size reduction was obtained in mice group E with wounds treated with sheep oil; these values were not significantly different from each other ($p > 0.05$) (Table 3). The progress of wound healing was monitored regularly to determine

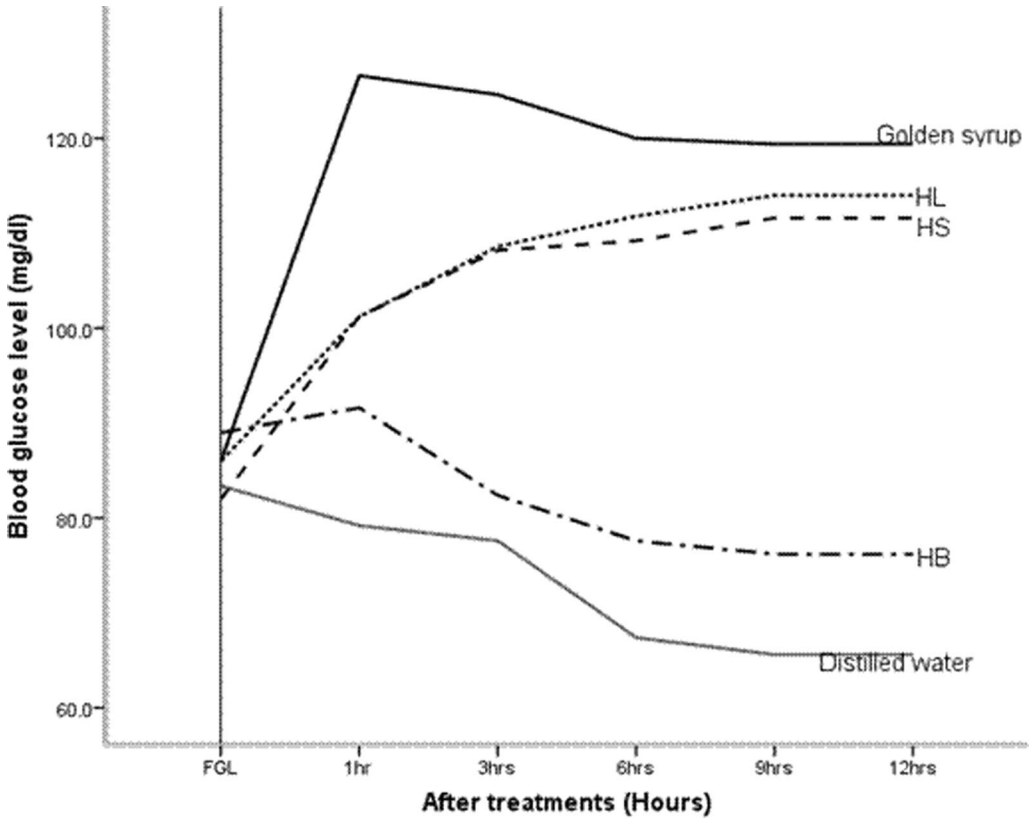

Fig. 1 Changes in blood glucose level in mice treated with selected samples of honeys from different sources. HB: Honey from beekeepers with none adulterated. HS: Honey from supermarkets with some adulterated. HL: Honey from local parkers with some adulterated and fake.

the effectiveness of the honey samples in wound dressing and the time taken for the wounds to heal.

Categorization of the honeys

Honey samples were categorized according to the levels of aberrations from the physicochemical parameters compared to the standard proposed by the CAC or IHC and the functional health and medicinal values. Therefore, the honey samples were tagged as original, fake and adulterated. The original honeys are pure, and adulterated are made poor in quality by adding some inferior substances, while fake honeys are not genuine. 93.3% of the honeys sourced from the beekeepers (HB), 58.06 and 66.05% of the honeys sourced from the supermarkets (HS), and the local honey markets respectively, were original (Table 4); the samples have standard range of physicochemical properties (CAC or IHC), maximum medicinal values and no health implications; 6.39% and 8.54% of the honeys sourced from the supermarkets (HS) and local markets respectively, were adulterated, while 34.55 and 25.4% of the honeys from the supermarkets (HS) and local honey markets (HL) respectively, were faked (Table 4). There are a lot of aberrations in the physicochemical properties of the fake honeys compared to CAC and IHC values, and the samples have no functional health and medicinal values.

Discussion

Physicochemical properties

A. mellifera honey is influenced by the flowers foraged by the bees (Anikwe et al. 2016; processing techniques, storage and climatic conditions (Akinwande and Adeuya 2021), geological region of production and environmental factors (Gobessa et al. 2012).

Moisture as a physicochemical property is related to the climatic conditions and degree of maturity of honey, flower sources, beekeeping manipulations. Codex Alimentarium set moisture content limits at 20%. The moisture contents of *Apis cerana* honey is 16.6%, *Apis dorsata* honey had the lowest 10.03%, *Apis florea* honey is 11.7% and *Apis mellifera* honey is 14.38%, (Krishnasree, and Ukkuru 2017). The average moisture contents of the honey samples are in the accepted range of moisture contents for *Apis mellifera* honeys, from other parts of Africa, e.g., Ethiopian honeys (Admassu and Tura 2021). Aberrations from the standard moisture contents of the

honey could be attributed to the presence of adulterants (Alemu and Dachasa 2019) from dilutions with water or other substances. It could also be due to harvesting of uncapped honey (Thrasyvoulou et al. 2018), unsuitable storage containers, storage places, humidity of the air surrounding the beehives (Majewska et al. 2019; Fechner et al. 2016) plants source and climatic conditions (Akinwande et al. 2016; Gobessa et al. 2012). Unripe honey is subjected to fermentation and spoilage due to the presence of several yeast species (Thrasyvoulou et al. 2018).

Electrical conductivity is a good indicator of adulteration in honey from its original form; the honeys found with aberrant electrical conductivities among the samples from local honey parkers and supermarkets could be adulterated based on this indicator (Crane 1975). Electrical conductivity has direct correlation with physicochemical properties like ash content, pH, acidity, minerals, proteins and other substances in the honey (Bogdanov et al. 2004).

The pH values of honey samples reported by many authors range between 3.0 and 5.5 (CAC, IHC) depending on the botanical source, mandibular substances added to the nectar may influence the pH of the honey, a process that begins with the transport of nectar to the hive in the honey vesicle (Evangelista-Rodrigues et al. 2005). Low pH values of honey make it acidic, and the acidic nature contributes to the broad-spectrum antimicrobial properties and the wound healing by causing oxygen release from hemoglobin, to inhibit the growth of microorganisms, and it also extends the honey shelf life (Krishnasree and Ukkuru 2017). Where the pH value is aberrant from the standard, it facilitates fermentation, and or a sign of adulteration (Frías and Hardisson 1992; Vidal and Fregosi 1984).

HMF is found in trace amounts in fresh honeys, but during heat processing and/or due to aging. HMF of some of the honeys from the beekeepers falls below recommended values by CAC and IHC (< 40 mg/kg), and this concurred with some rainforest honey, home brand and mallee honey from Australian reported with low HMF concentrations which range between 2.2 and 34.0 mg/kg (Fallico et al. 2004). High HMF content of some honeys from the supermarkets and local honey parkers indicates poor storage conditions and overheating or addition of heated substances. Khalil et al. (2010) claimed high HMF indicates low purity and freshness of honey. Processing of Tanzania honey is responsible for its high HMF (Boussaid et al. 2014).

HMF values of some honey samples from supermarkets and local honey parkers in this study were consistent with the values reported by Nayik and Nanda (2015) in Kashmir honeys and Khalil et al. (2010) in Malaysian Tualang honey that have been stored for more than one year.

Turbidity is the measures of the amount of light that is scattered by the presence of suspended particles; in the honey, low turbidity indicates sufficient clarification and a high quality and can also be taken as an indicator of honey granulation. Turbidity is influenced by temperature and moisture content. Turbidity of the most honey samples values ranges from beekeepers < supermarkets < local parkers. Similar observations were made by Lawal and Adekalu (2009) in the study of original and adulterated honeys in Nigeria.

The ash content indicates the richness of honey in mineral content. Ash contents vary depending on the botanical origin, region, bee species and type of manipulation. The minerals in trace amounts in honey are calcium, magnesium, iron, copper, cadmium and zinc in the form of sulfate (SO_4^{2-}) and chloride (Cl^-) (Gome et al. 2010). These minerals influence the color of honey and in higher concentrations in dark honey than light-colored honey (Finola et al. 2007). The low ash contents obtained from all the honey samples did not distinguish or categorized them as adulterated or fake. *Apis mellifera* (Am) honey and *Trigona* (Ti) honey had similar levels of ash content of 0.16% (White, 1975; Gome et al. 2010) which agreed with the ash contents of some of the honeys evaluated from the beekeepers' outlets.

The color of honey is an indication of its various components such as polyphenols, minerals and pollen (Gomes et al. 2010), floral sources (Sanz et al. 2005). The color evaluation of all the honey samples appeared differently in the characteristics of color range from light yellow, through to amber and dark reddish amber to a nearly black color (Bertoncelj et al. 2007). The monofloral honey is semi-transparent, golden amber with thick consistency, poly floral honey appeared as semi-transparent, light amber with light consistency and forest honey appeared as opaque, dark amber with thin consistency. The dark color of honeys from the beekeepers' outlets signifies high phenolic compounds, and according to Estevinho et al. (2008), it suggests higher antibacterial activity. Color of honey in the region is an instant evaluation of consumer preference. However, it relates to factors such as the proportion of fructose and glucose present, nitrogen content and the instability of fructose in an acid solution (Bath and Singh 1999).

Functional health and medical grades of the honey

Mice are nocturnal animals and consume two-thirds of their total food intake during the night. Fasting was initiated by 8 am in the morning all through the night until 8 am the following day to accurately evaluate the fasting blood glucose level. Honey samples that significantly reduced blood glucose level lower than the fasting blood glucose level, some hours after the consumption, were

the original honeys, mostly sourced from the beekeepers' outlets and few from the supermarkets and local parkers outlets. Yapucu and Eser (2007) claimed original honey controls glycemia.

Honey contains enzymes such as amylase and sucrase and large amount of unbound fructose compared to glucose, and the amylase enzyme converts starch and dextrin into simple sugars, while sucrase converts sucrose into fructose and glucose. Fructose content is higher than glucose in the mixture. It is the fructose that limits the rise in blood glucose level. Fructose is absorbed into the hepatic portal system and almost entirely taken to the liver and converted to stored glycogen for use when needed. Therefore, fructose activates glycogen synthetase and inhibits phosphorylase which is involved in glycogen breakdown, and therefore, the presence of fructose contributed to the reduction in glucose output. The little or no changes in the mice weight when fed with little quantity of the honeys sourced from HB are due to lower production of fat because the glucose is broken down and not converted to fats (Obun et al. 2011), compared to some honeys sourced from supermarkets and local honey parkers.

Fructose does not raise the insulin levels, because it exerts a little direct stimulatory effect on insulin secretion, and insulin deters its uptake into tissue but rather facilitates the glucose metabolism and utilization. However, the glucose and sucrose in the honey still cause a little rise in blood sugar but not significant. Hence, the observed moderate rise in BGL 3 h after feeding and a recorded fall below the FBGL 12 h after, in the mice fed with honeys from beekeepers' outlets. This observation corroborates Erejuwa (2014) assertion that original honeys have low glycemic index. Conversely, the shoot up in the blood glucose levels immediately and some hours after consumption proved some of the honeys particularly some samples sourced from supermarkets and local honey parkers were adulterated or fake.

Wound incisions with conventional treatment using sheep oil have shown significantly reduced rates of healing compared to where no treatment was applied, Similarly, wound incisions treated with honeys selected among the samples from beekeepers have shown significantly rates of healing This is because original honey sterilizes wounds, accelerates healing process (Van den Berg et al. 2008; Samarghandian et al. 2017), promotes early granulation and reduces infections, induces leukocytes to release cytokines that initiates the tissue repair cascades and activates immune response to infection (Yaghoobi et al. 2013) and produces broad-spectrum antimicrobial activity to several bacteria causing septicemia (Lusby et al. 2005). Therefore, original honey acts as a drug and an ointment. The honey has a high viscosity which helps to provide a protective barrier to prevent infection (Lusby et al. 2005). Contrarily, some honey samples among the local honey parkers and supermarkets used to treat some wounds show little or no reduction in wound size. This observation sufficed because the honey samples have some prevailing factors working either singularly or synergistically against the healing process, and Mandal and Mandal (2011) and Israili (2014) claimed the factors are moisture contents, H_2O_2, phenolic compounds, wound pH, pH of honey and osmotic pressure exerted by the honey. Pure and original honey can promote the control and treatment of acute wounds, mild-to-moderate superficial and partial thickness burns (Simon et al. 2009) and the healing of infected wounds where conventional therapy, i.e., antibiotics and antiseptics, failed (Ahmed et al 2003). French et al. (2005) claimed Manuka honey, and Agbagwa and Frank-Peterside (2010) claimed forest honeys from SW Nigeria were reported good for the treatment of wounds.

Honey adulteration

Visual observation and layman tests are not reliable for the assessment of the purity of honey (Subrahmanyam 2007). The standard physicochemical properties of the honeys, its healing properties, glycemic index are very good criteria for originality, and similar observation was made by Subrahmanyam (2007) for pure and original honeys. However, some of the honeys obtained from the supermarkets and local honey parkers show aberrations in their physicochemical characteristics, low efficacy in the treatments of wounds and poor glycemic index. The possibility is that some of the honeys are adulterated or fake: adulterated because some substances were added intentionally or unintentionally, directly or indirectly (Blanka et al. 2015; Alemu and Dechasa, 2019). Direct addition of sucrose syrups that are produced from sugar beet, high-fructose corn syrup (HFCS), maltose syrup or industrial sugar (glucose and fructose) is major adulterants (Crane 1990; Blanka et al. 2015). Some of the honeys could be products of caramel or sugarcane.

Conclusions

In Nigeria, adulteration of honey is a serious concern. The honeys have unexpected effects on health without the consumers' knowledge. Adulterated and fake honeys are commonly found in sales outlets owned by local honey parkers and supermarkets. These honeys show aberrant physicochemical properties, poor functional health and medicinal grades. However, honeys sourced directly from beekeepers, or those supplied through beekeepers to local parkers and supermarkets with tamperproof are pure and original. They exhibit standard physicochemical

properties and promote functional health like low or no addition to body weight, low glycemic index and medicinal values as been potent in healing wounds. This study offered awareness on health implications in consumption of adulterated honey and useful information about honey markets in Southwest Nigeria.

Abbreviations

IHC: International Honey Commission; CAC: Codex Alimentarium Commission; FBLG: Fasting blood glucose level; BGL: Blood glucose level; HB: Honey sourced from the beekeepers; HS: Honey sourced from the supermarkets; HL: Honey sourced from the local markets.

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Author contributions

KLA conceived and designed the study, organized and analyzed the data and wrote the manuscript. AJO conducted the research and collected the data. AKL and AJO have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Permission for experimentation with small laboratory animals is allowed on campus in the Department of Biology, Federal University of Technology, Akure, Nigeria, with rules on ethical standard observed. The experimental mice were humanely handled and in strict compliance with the Institutional and International Guidelines on the Use and Handling of Small Experimental Animals.

Consent for publication

Not applicable.

Competing interests

There are no financial or non-financial competing interests between the two authors.

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