International Journal of Sciences

Research Article Volume 6 – August 2017 (08)

Composition of Sugars in Honey Produced in the South-South and South-West Regions of Nigeria

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Abstract: Six samples of honey from two states in Nigeria, Cross River and Oyo were investigated. (Igoli in Ogoja, farm bred and wild species from Obudu, Agoi Ibami, Eruwa and Iseyin) were analysed for their sugar components, Hydroxymethyl furfural (HMF), free acidity and electrical conductivity. Honey is composed of various sugars but the dominant sugars are two monosaccharide, fructose and glucose which are also reducing sugars. Honey is used for various purposes including medicinal, cosmetics, in the food industry, bakery and confectionary. The sugar contents were analysed using the HPLC with refractive Index (RI). The European Commission procedures were used to analyse HMF and electrical conductivity, while free acidity was analysed by AOAC methods (1999). The results of the analysis showed that although fructose and glucose were predominant in all the samples, only two samples, Igoli in Ogoja and Obudu farm bred met the Codex Alimentarius standard for fructose and glucose at 60g/100g. Fructose values (g/100g) reported for Igoli in Ogoja, Obudu farm, Agoi Ibami, Obudu wild, Eruwa and Iseyin were 34.92 ± 0.12, 36.45 ± 0.14, 30.50 ± 0.06, 28.45 ± 0.09, 33.40 ± 0.17, and 30.45 ± 0.09 respectively. Sucrose in all the honey samples met the Codex Alimentarius standard of < 5g/100g limit. Fructose/glucose ratio for all the samples was greater than 1 which indicated slower crystallization of all the honey samples. Values of free acidity ranged from 11.57 ± 0.07 to 18.60 ± 0.10meq/kg, which were lower than the 50meq/kg limit set by the Council of the European Union showing the absence of undesirable fermentation. HMF concentration in the samples all met the International optimum value of 40mg/kg reported by European Union and 80mg/kg for honey from the tropics. Electrical conductivity of the samples varied from 0.41 ± 0.01 to 0.63 ± 0.00mS/cm. The results showed that the honey samples sold locally in Nigeria meet the required international standards for honey.

Keywords: Sugars, Hydroxymethyl Furfural, Free Acidity, Electrical Conductivity

Introduction:

Honey (Apis mellofera) is a sweet, thick, supersaturated sugar solution produced by honey bees from plant nectars, plant secretion and excretions of plant-suckling insects of the living parts of plants (Codex Alimentation, 2001). It is one of the known natural sources of sweetness and energy for man. Honey is composed mainly of disaccharides which contain two monosaccharaides, glucose and fructose, with a percentage of water and other group of substances (Kamal and Kleen; 2010). Small quantities of other sugars are also present, in the form of other disaccharides, trisaccharides and oligosaccharides which are formed during the ripening and storage effects of bee enzymes and acids of honey (Ball, 2007). Chemical compositions of honey differ depending on the plant species on which the bees forage, the climatic conditions and other factors (Buba *et al*., 2013). The very concentrated solution of several sugars produce the characteristic physical properties of honey like high viscosity, high density, graduation tendencies, tendency to absorb water from the atmosphere and immunity from some types of spoilage (White and Doner, 1980). Honey has been used as food and for medicinal purposes since stone age. It's medicinal properties as remedy for burns, scars, wound healing and ulcers have been established. It is also used as a sweetening agent for children's drugs, treatment of sore throat, cough and hay fever (Adenekan *et al*., 2012; Aloisi, 2010; Fasasi, 2010). The wax from bees is used in food processing industries as additives, making of chewing gums and in cosmetic industries for making lip balm, lip gloss, hand creams, moisturizers and many others (Ezekiel *et al*., 2013; Dike and Onwuka, 2016).

Honey has been reported to have an inhibitory effect on around 60 species of bacteria, some species of

Published at: http://www.ijsciences.com/pub/issue/2017-08/
DOI: 10.18483/ijSci.1410; Online ISSN: 2305-3925; Print ISSN: 2410-4477

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fungi and viruses Human Metabolomic Databases (2009). It has antioxidant, anti-inflammatory and anti-microbial properties (Alvarez-Suarez et al., 2014). Its antioxidant capacity is due to its wide range of compounds which include peptides, organic acids, phenolic enzymes and Maillard reaction products (Eteraf-Oskouei and Najafi, 2013). Honey is consumed for its high nutritive value and effects on human health. Among the sugars in honey include sucrose, turanose, nigerose, maltose, kejibiose, with traces of trehalose, isomaltose and raffinose (Cantarelli *et al.*, 2008). Kejibiose is a reducing sugar which was accidentally discovered by scientists in Germany. Nigerose is an unfermented sugar obtained by partial hydrolysis of nigeran. It is a product of the caramelization of glucose (Human Metabolomic Database. 2009). Fructose and glucose are the dominant sugars in honey (Chaker *et al*., 2016; El-Sahaimy *et al*., 2015; Fasasi, 2012; Kamal and Klein, 2010; White and Doner, 1980). Hydroxymethyl furfural (HMF), another component of honey, indicates freshness of honey and good conservation. It is a product of the decomposition of fructose and it is found in trace amounts in honey (Buba *et al*., 2013; Fasasi, 2012; Canterelli *et al*., 2008). HMF of honey can be increased by various factors including storage temperature, heat, moisture content and acidity (Buba *et al*., 2013). The Codex Alimentarius Commission recommended the optimal value for HMF in honey as 40mg/kg for the European Union and 80mg/kg for the tropics (Bogdanov, 2009). Acidity of honey is directly related to its floral sources and in combination with its electrical conductivity, can affect its quality and classification (Yadata, 2014). Electrical conductivity is the indication of ionizable acids and compounds in aqueous solution (Chaker, 2016). Bee-keeping have been the way of life of the people of Cross River and Oyo States of Nigeria, although it had not been on a commercial scale until recently when honey became an alternative for table sugar and also a possible foreign exchange commodity.

This study determines the various sugar components, HMF, free acidity and electrical conductivity of honey from the northern and central senatorial districts of Cross River State and central senatorial district of Oyo State in Nigeria.

Materials and Methods

The honey samples were purchased from farmers from Igoli in Ogoja, Obudu (farm bred and wild), Agoi Ibami, Eruwa and Iseyin, stored in a dark cupboard at room temperature until needed for the analysis.

Analysis of sugars.

The method Bogdanon and Baumann (1988) determines fructose, glucose, sucrose, furanose and maltose in honey, for which precision data were required. It can also be used for the quantification of other saccharides such as melezitose, erlose, isomaltose, raffinose and others as described by Bogdanon and Baumann (1988). Sugar content was determined by High Performance Liquid Chromatography (HPLC) with Refractive Index(RI) detector and analytical steel column in amine modified silica gel of 5-7micrometer particle size and 250x4.6mm diameter. A measured amount (5g) of honey was dissolved in 40ml of water, 25ml methanol was pipetted into a 100ml volumetric flask and the honey solution transferred into the flask. This was then filled to the mark with water. Thereafter, 2.0g of fructose, 1.5g of glucose, 0.25g of sucrose and 0.15g each of turanose, maltose and all other sugars analysed were used as standards. The solution was poured through a membrane filter and collected in sample vials (Bognadov *et al*., 1997; Bogdanov and Baumann, 1988). All sugars were purchased from Merck (Switzerland) except turanose and erlose (Senn Chemicals, Dielsdorf, Switzerland).

Hydromethyl furfural (HMF) analysis

HMF was determined according to the European Commission method of analysis (Bogdanov *et al*., 1997). Twenty grams (20g) of the sample was dissolved in 100ml milli-Q water, filtered through a 0.22mL nylon filter into an HPLC vial, capped and injected with 20microlitre loop into the HPLC. HMF concentration in the honey was calculated by comparing the peak area to that of the standards taking the solution into account (Bogdanon *et al*., 1997). There is a linear relationship between the concentration and the peak area and the results were expressed in mg/kg honey.

Free Acidity and Electrical Conductivity analyses

Free acidity was determined by titrimetric method. The addition of 0.10N sodium hydroxide (NaOH) was stopped at pH 8.3 (AOAC, 1999). While electrical conductivity was measured on 20% (g/g by dry weight) honey solution in carbon dioxide free deionised distilled water at 20^{o}C Bognadov *et al*., (1997) and expressed in millisiemens per cm (mS/cm).

Results

TABLE 1: Comparison of different forms of sugars obtained from CRS and Oyo States

Sugars g/100g	Igoli Ogoja (CRS)	Obudu farm (CRS)	Agoi Ibami (CRS)	Obudu wild (CRS)	Eruwa (Oyo State)	Iseyin (Oyo State)
Fructose	34.92	36.45	30.50	28.45	33.40	30.45
	±0.12	±0.14	±0.06	±0.09	±0.17	±0.09
Glucose	27.50	29.30	23.07	20.20	24.13	22.60
	±0.17	±0.06	±0.03	±0.06	1.08	±0.06
Sucrose	0.73	0.70	1.20	±0.73	1.07	1.20
	±0.03	±0.00	±0.06	±0.03	±0.09	±0.06
Turanose	2.40	2.38	2.12	1.87	2.20	2.38
	±0.06	±0.09	±0.11	±0.03	±0.06	±0.04
Nigerose	2.07	2.13	1.80	1.73	2.10	2.21
	±0.13	±0.03	±0.00	±0.03	±0.06	±0.00
Maltose	1.50	1.70	1.33	1.33	1.73	1.63
	±0.01	±0.00	±0.03	±0.07	±0.03	±0.03
Kojibiose	1.00	1.03	0.80	0.33	0.87	1.17
	±0.10	±0.07	±0.00	±0.03	±0.03	±0.07
Tretialose	0.43	0.60	0.53	0.60	0.43	0.60
	±0.07	±0.00	±0.07	±0.06	±0.07	±0.00
Isomaltose	0.30	0.47	0.40	0.43	0.70	0.50
	±0.00	±0.07	±0.00	±0.07	±0.00	±0.00
Erlose	0.47	0.43	0.37	0.37	0.23	0.10
	±0.03	±0.03	±0.03	±0.03	±0.07	±0.00
Melezitose	0.27	0.30	0.30	0.23	0.43	0.60
	±0.03	±0.00	±0.00	±0.03	0.17	±0.06
Raffinose	0.30	0.40	0.47	0.37	0.67	0.70
	±0.00	±0.00	±0.03	±0.03	±0.03	±0.00
Gentiobiose	0.43	0.33	0.43	0.30	0.60	0.47
	±0.03	±0.07	±0.07	0.10	±0.00	±0.03
Melibiose	0.00	0.00	0.00	0.00	0.20	0.40
	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00
Maltotriose	0.20	0.13	0.17	0.23	0.53	0.37
	±0.00	±0.03	±0.03	±0.07	±0.07	±0.07
Fruct/Glu Ratio	1.27	1.24	1.32	1.41	1.39	1.35
	±0.01	±0.01	±0.00	±0.00	±0.07	±0.01
Fruct + Glu.	62.42	65.75	53.57	48.55	57.53	53.05
	±0.29	±0.09	±0.03	±0.08	0.94	±0.03
Total Sugars	72.66	77.11	64.72	58.59	70.86	67.00
	±1.00	±0.30	±0.40	±0.14	±0.46	±0.46

Values are expressed as mean
Similar symbols have homogenous mean values ±SEM, n = 3.

TABLE 2: Comparison of physical properties of sugars obtained from Cross River and Oyo Staes

	Igoli Ogoja (CRS)	Obudu farm (CRS)	Agoi Ibami (CRS)	Obudu wild (CRS)	Eruwa (Oyo State)	Iseyin (Oyo State)
HMF mg/kg	15.37	18.90	21.87	12.77	16.07	62.60
	±0.18	±0.01	±0.38	±0.29	±0.15	±0.71
Free acidity meq/kg	12.83 ±0.24	11.57 ±0.07	12.87 ±0.18	11.77 ±0.18	18.60 ±0.10	18.20 ±0.51
Elect. Cond. mS/cm	0.41 ±0.01	0.45 ±0.01	0.42 ±0.01	0.45 ±0.01	0.61 ±0.01	0.63 ±0.00

Values are expressed as mean
Similar symbols have homogenous mean values ±SEM, n = 3.

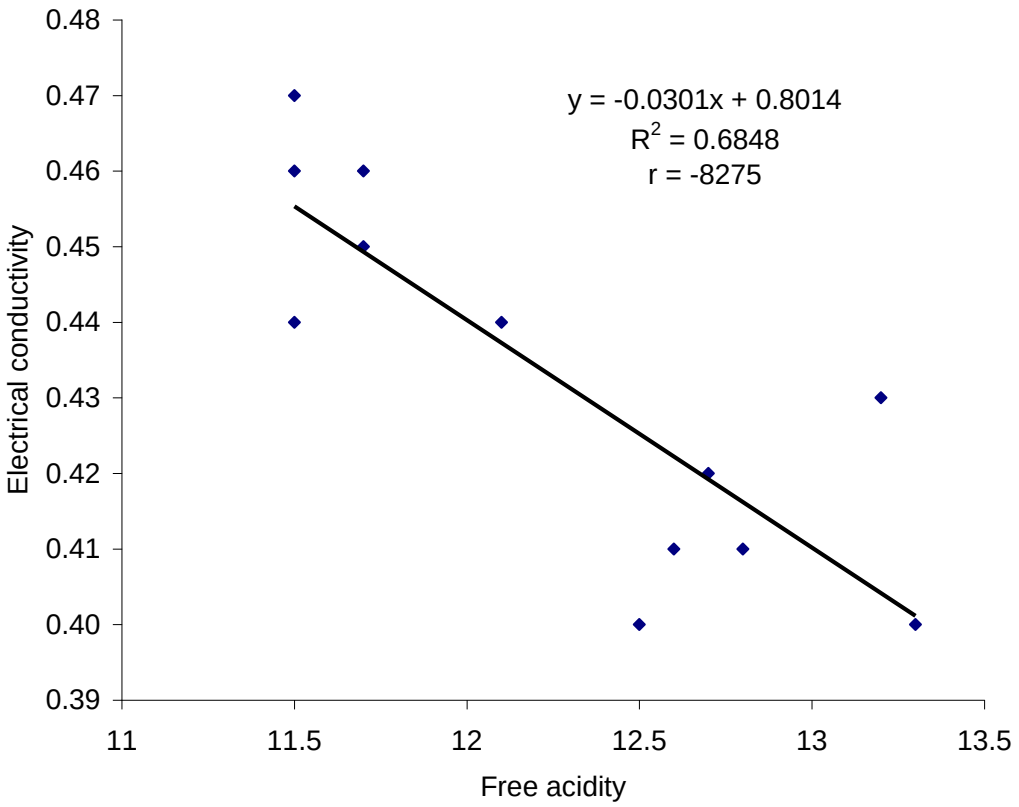

Figure 1: Relationship between free acidity and electrical conductivity of honey harvested in Cross River State.

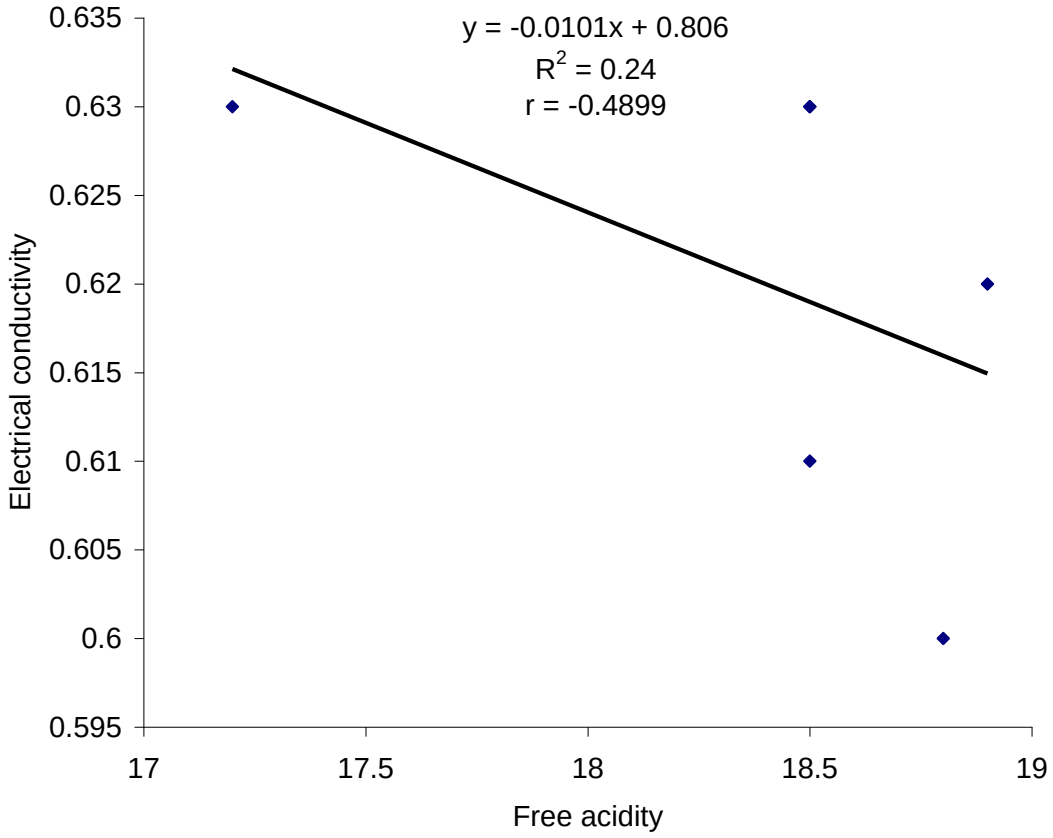

Figure 2: Relationship between free acidity and electrical conductivity of honey harvested in Oyo State.

Discussion

Honey has been used in a variety of ways for centuries. Nutritionally, honey is recently used as an alternative to sucrose as sugar and it is also widely used for industrial purposes. It also serves as a foreign exchange earning where it is widely produced.

Table 1 shows the results of the sugar analysis of the honey samples from the two states. The results obtained confirmed that fructose and glucose are the dominant sugars in honey (White and Doner, 1980). Fructose (g/100g) in all the samples ranged from 28.45± 0.09 in Obudu wild honey to 36.45± 0.14 in Obudu farm. It has been reported that honey is rich in reducing sugars (Chaker *et al*., 2016; El-Sohaimy *et al*., 2015; Santos et al., 2014; Aloisi, 2012; Fasasi, 2012; Amir *et al*., 2010 and Caterelli *et al*., 2008) but low in other sugar components. There is no standard limit to the individual sugars in honey. Honey sample from Obudu farm has the highest fructose and glucose values. There was no significant difference between the fructose and glucose values in the two samples from Oyo state but the values vary significantly in the four samples from Cross River state. Obudu farm also has the highest sum of all sugars in all the samples. The sucrose content of all the samples met the required standard of not more than 5g/100g (European Council 2002). The sucrose content of the samples vary between 0.70 ± 0.00 to 1.20 ± 0.06g/100g. The fructose/glucose (F/G) ratio were 1.27 ± 0.01, 1.24 ± 0.01, 1.32 ± 0.00, 1.41 ± 0.00, 1.39 ± 0.07 and 1.35 ± 0.01 for Igoli in Ogoja, Obudu farm, Agoi Ibami, Obudu wild, Eruwa and Iseyin respectively. F/G ratio is an indication of honey quality. It indicates the ability of honey to crystallize since glucose is less soluble in water than fructose (Amir *et al*., 2010). Honey crystallization is slower when the ratio is more than 1.0 (Draiaia *et al*., 2015) and faster when it is less than 1.0 (Amir *et al*., 2010). The F/G ratios in all the honey samples were more than 1.0, which indicates slower crystallization. Crystallization also depends on storage temperature. Obudu farm has the lowest F/G ratio at 1.24 but there

was no significant difference between the ratios in all the samples studied.

HMF is a representation of the freshness of honey (Cantarell *et al.*, 2008). The HMF value for all the samples ranged from 12.77 ± 0.29mg/kg to 62.60 ± 0.71mg/kg. Obudu wild honey recorded the lowest mean value of 12.77 ± 0.29mg/kg and Iseyin has the highest of 62.60 ± 0.71mg/kg which is still within the international standard for the tropics (80mg/kg) Codex Alimentations (2001). The results obtained for HMF in this study were significantly higher than those reported by Aloisi (2010), Fasasi (2010) and Canterelli *et al.* (2008). The European Council has set a limit of less than 15mg/kg HMF concentration for any honey labeled as virgin. This value can increase as honey undergoes heat treatment to reduce viscosity and prevent crystallization and to facilitate filling (Subramanian *et al.*, 2014). These results indicate that the honey samples in this study meet the standard for virgin honey as indicated by European Council (2002).

Acidity of honey is due to the presence of organic acids and contributes to its characteristic tastes. Values of free acidity (meq/kg) in this study ranged from 11.57 ± 0.07 to 18.60 ± 0.10. The values were lower than the allowed limits of 50 meq/kg (The Council of the European Union, 2002), showing the absence of undesirable fermentation (Yadata, 2014; Eteraf-Oskouei and Najafi, 2013; Adenekan *et al.*, 2010; Aloisi, 2010; Garcia *et al.*, 2001).
Electrical conductivity (mS/cm) of the honey samples in this study ranged from 0.41 ± 0.01 to 0.63 ± 0.00. The samples from Oyo state had higher value for electrical conductivity than those from Cross River state. The results were within the range of values reported by Chaker *et al.* (2016) and Santos *et al.* (2014). There was a negative correlation between the electrical conductivity and free acidity in all the samples as shown in figures 1 and 2.

Conclusion

The sum of fructose and glucose in (Obudu wild) honey presumes that it can be used by diabetic patients as the value is below the 60g/100g recommended by Codex Alimentarius standard. Also, the fructose/glucose ratio for all the samples was more than 1 which indicates slower crystallization. There may be a need for Nigeria to set country specific values for honeys produced in the country as the range in the samples studied, varied widely. Honeys from Igoli and Obudu were within the international standards set by both European Council and Codex Alimentation. Sucrose content for all the honey samples were well within the European Council standard of not more than 5g/100g. Total acidity and HMF values showed good indicators of the freshness of the honey samples.

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