Microbiological quality of artisanal honey sold in informal markets: A case study in Accra, Ghana

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ABSTRACT

An increase in consumer consciousness for health has contributed to an increase in honey consumption in Ghana. In Accra, artisanal honey is often retailed unprocessed and packaged in recycled plastic bottles, yet consumers show confidence in its safety. There are however no data to support such confidence in unprocessed artisanal honey sold in informal markets. Bacteriological studies conducted in other parts of the world have reported the presence of hygiene indicators such as aerobic bacteria and coliforms, fungi, and bacterial spores of public health importance. The aim of the study was to assess intrinsic properties and microbial quality of artisanal honey in Accra. Thirty honey samples were purchased from five informal markets in Accra. Random sampling was used to select two retailers from each of the five markets. The intrinsic parameters, pH and moisture content, were measured for each sample and correlated with concentrations of yeast and mould, total aerobic bacteria, coliforms and aerobic spore-forming bacteria. The results showed that the pH and moisture of the samples ranged from 3.77-5.40 and 15.38%-19.71% respectively. These were within the reference limits of 3.5-5.5 for pH and <20% for moisture. Coliforms, yeasts, moulds, aerobic spore-forming bacteria and aerobic viable bacteria in artisanal honey exceeded the 2 log CFU/gm limit recommended by the International Commission for Microbiological Specification of Foods. There was no correlation between their intrinsic properties and microbial quality, indicating that the presence of microorganisms was a result of recent contamination, likely from poor handling and storage practices. Although the physical properties did not favour the growth and survival of microbes, their presence in excess of the recommended limit suggests unhygienic handling and storage. Training of processors and retailers on good hygienic practices will control the introduction of contaminants into artisanal honey.

Keywords: Artisanal honey, quality, safety, intrinsic properties

Introduction

Honey is an amber yellow or dark yellowish glutinous fluid made by bees. It is defined as a natural food produced by honey bees from the nectar of plants, secretions of living parts of plants or excretions of plant sucking insects on the "living parts of plants" (Codex Alimentarius Commission, 2001). Honey is made up of sugars, mainly glucose, fructose and sucrose, and other minor components including water, proteins, fats, vitamins, minerals and antimicrobial agents (Buba *et al.*, 2013). Honey is highly stable. This is largely due to its intrinsic properties such as low pH, high solute content, low water activity and the presence of natural antimicrobial compounds. Some intrinsic properties such as pH and moisture content are monitored as indicators of commercial quality of honey (Thrasyvoulou *et al.*, 2018). Globally, honey is used as a sweetener and as an ingredient in bakery, confectionary, breakfast cereals, dairy, dressing, sauces, ice cream, spreads and snack bars (Bellik and Iguouaada, 2010). However, in Ghana, the uses of honey are limited. Honey is normally consumed in its unprocessed state mostly as spreads. It is also used as an ingredient in pharmaceuticals and traditional medicinal preparations, and to a lesser extent

in confectionaries, cosmetics and baking (Wagner *et al.*, 2008).

In Ghana, most of the honey commercially available are artisanal. Artisanal honeys are traditionally produced raw, unprocessed, unpasteurized and unhomogenized. On the local market, they are commonly available in recycled water and soft drink polyethylene terephthalate (PET) bottles, and in other recycled packaging materials such as beer bottles and oil gallons (Akangaamkum *et al.*, 2010). These honey are characteristically dark and viscous as it is assumed that the light and thin ones are diluted and of inferior quality (Akangaamkum *et al.*, 2010).

Currently, quality control for artisanal honey production in Ghana is rare. Due to the high sugar concentration, low moisture content and additional presence of antimicrobial agents such as methylgloxal and peptide bee defensin-1, honey is not a suitable medium for microbial growth or long-term survival of vegetative cells (Sinacori *et al.,* 2014; Stanway, 2013; Iurlina and Fritz, 2005). Nonetheless, honey is not microbiologically sterile. Low concentrations (< 2log cfu/g to 4log cfu/g) of total bacteria including yeast and mould and bacterial spores have been reported in honey (ICMSF, 2011; Snowdon and Cliver, 1996). These microorganisms are suggested to be contaminants from air, dust, the honeybee's digestive system and handlers (Olaitan *et al.*, 2007). The presence of these microorganisms has implications for the commercial and sanitary quality of honey. Of particular concern is the presence of pathogens like *Clostridium botulinum* and *Bacillus cereus* spores, and other general foodborne pathogens, such as pathogenic *E. coli, Salmonella* and *Staphylococcus aureus,* that cause serious foodborne illnesses such as infantile botulism, emetic food poisoning and gastroenteritis respectively (Hartheway, 1993; Snowdon and Cliver, 1996). Such organisms have traditionally been known to contaminate honey through postharvest activities (Snowdon and Cliver, 1996).

In recent times, there has been an increase in the consumption of honey both locally and worldwide due to its nutritious and therapeutic properties. Most honey producers and consumers in Ghana prefer the unprocessed honey because it is presumed to be natural and safe. Since the production of artisanal honey is not regulated in Ghana, the microbiological safety of artisanal honey can be underestimated, especially because production and packaging are done under uncontrolled conditions which offer opportunities for microbial contamination. This case study therefore aims to provide baseline information on the assessment of safety and quality of artisanal honey sold in some informal Ghanaian markets in Accra.

Materials and Methods

Sampling

This study was conducted using 30 samples of artisanal honey collected from 5 different local markets in Accra. These markets were selected based on their proximity to University of Ghana main campus and how busy they are. Also, random sampling was used to obtain three samples each from two different sale points in each market. The samples were transported at ambient temperature to the microbiology laboratory and stored in a cool dry place until analysis was done the following day. The study used Kaneshie, Madina, Malam, Makola and Agbogbloshie markets in Accra. The markets were coded MD1 to MD5 in no particular order. The purchase points were tagged as distributor 1 and distributor 2.

Moisture and pH Determination

The percent moisture of the honey was determined in triplicate using the refractive index and the Wedmore conversion table. The refractive index of the honey was measured using the Carl Zeiss Abbe Refractometer (Germany). The refractive index measured was corrected for a standard temperature of 20°C by adding or subtracting 0.00023 per every degree Celsius increase or decrease. The corrected index was converted to percent moisture using the Wedmore table.

The pH of the honey was determined in triplicates. A 10% (w/v) of honey was prepared in de-ionized water and the pH was determined using the Oakton Water Proof Hand-Held pH/ Conductivity/ Temperature meter (Eutech Instruments, Singapore).

Microbiological Analysis

Ten grams of each of the honey samples were homogenized in 90 ml of sterile 0.1% buffered peptone water in a stomacher. These served as 10^{-1} dilution. Additional serial dilutions were subsequently prepared with sterile 0.1% buffered peptone water. Serial dilutions were pour plated in duplicate with Standard Plate Count Agar (PCA, Park Scientific) for mesophilic bacteria, Malt Extract Agar (MEA, Merk) for yeast and mould, Eosin Methylene Blue Agar (EMB, Oxoid) for coliforms and Nutrient Agar (NA, Park Scientific) for aerobic bacterial spores. Honey samples cultured on Nutrient Agar were heated at 85 ˚C for 15 min to activate bacterial spores prior to inoculation. The inoculated PCA, EMB and NA plates were incubated at 37 ˚C for 24 hours prior to enumeration. Inoculated MEA plates were incubated at 25 ˚C for 5 days prior to enumeration.

Statistical Analysis

Colony counts for total mesophilic bacteria, coliforms, yeast and mould and aerobic spores were subjected to one-way ANOVA with a p-value of <0.05 using Stats Graphic Centurion Version 1.5. Least squares were used to determine statistical differences in bacterial counts, pH and moisture, and between honey distributers from different markets. Pearson Correlation was used to draw a relationship between the pH, moisture and the various counts.

Results and Discussions

Moisture and pH of honey

The moisture content and pH of honey samples is presented in Table 1. The moisture content of these samples ranged between 15.38±0.25 and 19.71±0.04 which meets the Codex Alimentarius standard of ≤ 20% for moisture in honey (Codex Alimentarius, 2001). The varying moisture content of the honey samples may have been a result of the different bee-hive handling practices, the type of harvesting and extraction methods applied by the producers, the harvesting season, the degree of maturity reached in the hive and some other environmental factors (Feas *et al.,* 2010). The low moisture content of all honey samples tested suggests that these market samples do not support microbial growth. Microbial growth in honey will cause quality deterioration through sugar fermentation (Tornuk *et al.,* 2013).

Table 1: Mean pH and moisture contents of the honey from informal markets in Accra

Source (Markets)	Distributors	pH	Moisture
MD1	1	$4.53\pm0.04^{d, e}$	19.71 ± 0.04^{g}
	2	$4.10\pm0.01^{b, c}$	$18.24\pm0.04^{e, f}$
MD2	1	5.40 ± 0.11^{g}	$15.91\pm0.04^{a, b}$
	2	3.77 ± 0.40^{a}	$16.86\pm1.37^{b, c}$
MD3	1	$4.30\pm0.06^{c, d}$	$19.16\pm0.38^{f, g}$
	2	4.66 ± 0.21^{e}	$17.11\pm0.38^{c, d}$
MD4	1	$4.35\pm0.03^{c, d}$	15.38 ± 0.25^{a}
	2	5.06 ± 0.03^{f}	$17.27\pm0.35^{c, d, e}$
MD5	1	4.17 ± 0.25^{c}	$18.04\pm1.24^{d, e}$
	2	$3.84\pm0.21^{a, b}$	$19.31\pm0.56^{f, g}$
Overall P-value		0.000	0.000

All the values with the different superscript letters within the same column are statistically different at a p-value of <0.05

The pH of the honey samples ranged between 3.77 and 5.40. A similar pH range of 3.58-5.12 was found by Kacaniova *et al.* (2007) and Mahindru (2007) who reported honey pH values between 3.5 and 5.5. The pH of honey is influenced by the presence of organic acids, most importantly gluconic acid and its mineral content (Kacaniova *et al.,* 2007). While a high concentration of organic acids in honey reduces its pH, a high concentration of minerals increases the pH. Rehman *et al.* (2008) suggested that honey with pH above 5 is of low quality and purity, as adulterated honey has higher pH than pure honey which ranges between 3.2 and 4.5. In this study, honey from MD2 distributor 1exceeded this range (pH 5.40±0.11). which may influence its overall quality.

There was no correlation between the moisture content and pH in the honey samples tested (Table 2). However, in a similar study conducted by Ananias *et al.* (2013), there was a positive correlation between moisture and pH, which they attributed to the high activity of the glucose oxidase enzyme responsible for acid production as a result of the high moisture content in their honey samples.

Table 2: Pearson correlation co-efficient between the various plate counts, moisture content and pH in the honey samples

	Correlation Co-efficient				
	pH	Viable Cells	Coliforms	Yeast & Moulds	ASFB
Moisture	-0.388 (p-value=0.268)	-0.398 (p-value=0.255)	-0.396 (p-value=0.258)	-0.674 (p-value=0.033*)	-0.332 (p-value=0.349)
pH	1.000	0.249 (p-value=0.488)	-0.136 (p-value=0.704)	0.803 (p-value=0.005*)	-0.810 (p-value=0.810)

*Significant difference at a p-value <0.05
ASFB – Aerobic Spore-forming Bacteria

Microbiological Analysis

During sampling, it was observed that about 70% of the honey purchased in the various markets were packaged in used or recycled mineral water and soft drink plastic bottles. Others were in new plastic bottles. Akangaamkum *et al.* (2010) made the same observation in their study. The recycled bottles commonly used as packaging materials for honey could be a significant source of contamination (Sherwani *et al.,* 2013) as there were no controls for handling, storage and sanitization prior to use. It was also observed that the new bottles meant for packaging were kept under uncontrolled conditions, thus exposing them to dust and possibly pest infestation. The mean mesophilic counts in honey samples ranged from 2.96±0.57 to 3.98±0.31 log cfu/g with the highest from MD2 distributor 1 and the lowest from MD5 distributor 1 (Table 3). This result corroborates that of 2.00 to 3.96 log cfu/g reported by Tornuk *et al.* (2013). The high numbers of viable bacteria may be associated with a recent contamination from a secondary source (Kacaniova *et al.,* 2007). The aerobic bacteria count of honey is known to be influenced by the type of honey, the age of the sample and the harvesting season (Wanjai *et al.,* 2011). It also depends on the sanitary condition of the processes used to obtain the final product.

The coliform counts were between 3.67±0.86 to 4.82±0.35 log cfu/g with distributor 1 from MD2 and distributor 1 from MD5 having the highest and lowest count respectively. Their presence in honey is known to be an indicator of unsanitary conditions during production and handling of honey, and has implications on commercial quality.

Table 3: The mean counts of the honey samples sourced from informal markets in Accra

			Count (log cfu/g)			
Source (Markets)	Distributors	Packaging	Mesophilic count (±SD)	Coliforms (±SD)	Yeast & Moulds (±SD)	ASFB (±SD)
MD1	1	Fresh bottles	$3.14\pm0.17^{a, b}$	3.67 ± 0.86^{a}	$3.56\pm0.24^{a, b}$	$3.22\pm0.21^{b, c}$
	2	Recycled bottles	$3.80\pm0.29^{c, d}$	3.71 ± 0.36^{a}	$3.84\pm0.38^{b, c, d}$	$3.46\pm0.29^{b, c}$
MD2	1	Fresh bottles	3.98 ± 0.31^{d}	$4.08\pm0.21^{a, b, c}$	4.13 ± 0.41^{d}	$3.33\pm0.27^{b, c}$
	2	Fresh bottles	$3.75\pm0.41^{c, d}$	$4.07\pm0.41^{a, b, c}$	$3.62\pm0.06^{a, b, c}$	$3.25\pm0.28^{b, c}$
MD3	1	Recycled bottles	$3.78\pm0.03^{c, d}$	$3.79\pm0.28^{a, b}$	$3.66\pm0.32^{a, b, c, d}$	2.59 ± 0.36^{a}
	2	Recycled bottles	$3.43\pm0.11^{a, b, c}$	$3.95\pm0.06^{a, b}$	$4.07\pm0.24^{c, d}$	$3.17\pm0.24^{b, c}$
MD4	1	Recycled bottles	$3.47\pm0.24^{b, c}$	$4.68\pm0.03^{c, d}$	$3.86\pm0.12^{b, c, d}$	$3.54\pm0.14^{c, d}$
	2	Recycled bottles	$3.44\pm0.19^{a, b, c}$	$4.20\pm0.07^{a, b, c, d}$	$4.07\pm0.09^{c, d}$	$3.00\pm0.11^{a, b, c}$
MD5	1	Recycled bottles	2.96 ± 0.57^{a}	4.82 ± 0.35^{d}	$3.66\pm0.44^{a, b, c, d}$	4.10 ± 0.13^{d}
	2	Recycled bottles	$3.24\pm0.17^{a, b}$	$4.40\pm0.51^{b, c, d}$	3.54 ± 0.27^{a}	$2.97\pm0.77^{a, b}$
Overall P-value			0.006	0.019	0.035	0.003

Values with the same subscript letters in the same column are statistically the same since there is no significant difference between them at a p-value <0.05; ASFB-Aerobic Spore-Forming Bacteria

The yeast and mould counts also ranged from 3.54±0.27 to 4.13±0.41 log cfu/g with the lowest and highest value from MD5 distributor 2 and MD2 distributor 1, respectively. Also, all the honey samples' counts were above 2 log cfu/g, the maximum level of yeast and mould count allowed for trade in the Southern America Common Market (MERCOSUR) (Ananias *et al.,* 1996). According to Ananias *et al.* (1996), high yeast and mould counts in honey are normally related to contamination from post-harvest activities such as handling, the environment and the equipment used during processing. Studies have also shown that high moisture levels (above 19%) in honey, coupled with low pH and high storage temperatures, increase the risk of yeast and mould growth in the honey, as their presence can cause fermentation spoilage in the honey. Yeast and mould count is therefore an indicator of the sanitary or commercial quality of honey (Giraldo *et al.,* 2013). This finding further suggests that the samples used in this study were at risk of spoilage.

In honey, aerobic bacterial spores are predominant and can be detected in bee larvae (Farris *et al.,* 1986). Farris *et al.* (1986) also stated that the presence of these spores in honey is often attributed to the unsanitary conditions under which they are processed and other secondary sources of contamination. The aerobic spore-forming bacteria count ranged from 2.59±0.36 to 4.10±0.13log cfu/g with the highest and lowest count coming from MD3-distributor 1 and MD5-distributor 1, respectively (Table 3).

In general, the results showed that distributor 1 from MD1 had the least microbial load as the packaging material used was new. It may also be attributed to some good hygienic practices adopted during harvesting, extraction and processing. However, the honey sample from MD2-distributor 1 had the highest colony counts and their honey was also packaged in new plastic bottles. Nonetheless, the storage conditions of these plastic bottles may have rendered them non-sterile and increased the risk of contamination as they were left in the open and mixed with some of the other retail products sold. This may have been the cause of the high counts since microbes present in honey are normally from secondary contamination sources, in this case the handling and packaging.

Lastly, this study showed a negative correlation between moisture and yeast and mould counts, and a positive

correlation between pH and the yeast and mould counts (Table 2). This means that the yeast and mould counts were affected by pH and moisture in one way or the other. There were also no correlations between moisture, pH and the other microbial counts. Ananias *et al.* (2013) suggest that in order to get accurate and precise information on this relationship, determination should be done over some period of time.

Conclusion

All honey samples studied had moisture content < 20% which is the standard set by Codex Alimentarius Commission. Also, all the values obtained for pH fell within the reference range of 3.5 to 5.5. These indicate that the honey is of good physico-chemical quality.

In contrast to the physio-chemical quality, all the microbial counts were higher than the standard of 2.0 log cfu/g, suggesting low commercial quality.

It is recommended that beekeepers, honey producers and honey distributors should be trained on the total quality of honey, most importantly the effect of their processing, handling activities and packaging on the microbial quality of their product and its implications for shelf life stability and consumer health.

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