African Journal of Biotechnology Vol. 6 (7), pp. 908-913, 2 April 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Physico-chemical and microbiological characteristics of honey from the sudano-guinean zone of West Cameroon

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Accepted 9 November, 2006

The physico-chemical and microbiological characteristics of honey from the sudano-guinean zone of West Cameroon were determined. The indices obtained were within their respective ranges recorded for honey in temperate countries, except for calcium content whose value was on the upper side of its reference range. A bacterial type and eight fungi species contamination were found to have contaminated the honey from the local markets. However, there were no microbes present in honey harvested from the Bee Research Farm of the University of Dschang. This indicated contamination from secondary sources, during handling and or adulterations of honey from our local markets. Honey used in this study showed medium crystallisation tendency and granulation was faster when stored under dark than light at similar conditions. The mixed floral or nectar sources, fluctuating environmental conditions and processing procedures played major roles in the physico-chemical differences observed between the local and temperate honeys.

Key words: Honey, physico-chemical properties, microbial contamination, crystallisation, West Cameroon.

INTRODUCTION

Honey, as defined by the Codex Alimentarius (1989) is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant-sucking insects living on parts of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. Honey is a complex mixture and presents very great variations in composition and characteristics due to its geographical and botanical origin (Crane, 1975, 1980; Ramirez 2000; ANONN, 2003a; 2001-2004), its main features depending on the floral origin or the nectar foraged by bees. The composition and quality of honey also depend on several environmental factors during production such as weather and humidity inside the hive, nectar conditions and treatment of honey during extraction and storage.

Bacterial spores and less often *Clostridium* sp. may be present but honey possesses antibacterial activities that do not support bacterial growth and/or production of toxins (Terence, 1996; Taormina 2001; Mundo 2002; Cooper 2002; ANONN, 2003b; 2001-2004). However, fungi have been reported to grow and ferment or spoil unprocessed honeys (ANONN, 2003a; 2003b) although proper processing and handling can control spoilage. Therefore, the microbiological quality of honey may serve as an indicator of the hygienic conditions under which the product was processed, handled and stored. The shelf-life of honey or tendency to crystallise is directly related to its glucose and water contents, origin, age and storage conditions (Bogdanov, 1993; Assil, 1991; Jimenez, 1994; Rairmez, 2000; Manikis and Thrasivoulou, 2001; Anonn,

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Abbreviations: WC, West Cameroon; CB, coloured bottles; TB, clear transparent bottle; DP, dark plastic; and WP, white translucent plastic.

2003a; 2001-2004). Crystallisation may be undesirable in liquid honey but controlled or deliberately induced crystallisation can be used to make desirable honey products such as creamed honey.

Honey has numerous uses and functional applications worldwide such as in food systems, religious and magical ceremonies as well as in human and veterinary medicine (Crane, 1980; Taormina, 2001; Sable, 1997; Cooper, 2002; ANONN, 2003b; Eileen De Mars, 2003). Although bee products are increasingly being used for various purposes in the Western Highlands of Cameroon and the socio-economic importance and techniques of beekeeping also known (Tchoumboue 2001; Mzeka, 2003), there is no information on the properties of these products from the region. This preliminary research was therefore carried out to study some physical, chemical and microbiological properties of honey produced in beekeeping areas in the sudano-guinean zone of West Cameroon (WC).

MATERIALS AND METHODS

Study area

The sudano-guinean zone of West Cameroon (WC) is located between latitude 5 – 7°N and longitude 8 – 12°E with an altitude ranging from 1400 to 2700 m above sea level. The vegetation is mainly shrubby savannah with pockets of forest galleries and is heavily modified by human activities, especially farming. The average annual temperature varies between 16 and 27°C while the relative humidity is 40 – 97%. There are two main seasons: the rainy season (late March to October) and dry season (November to early March) and the mean annual rainfall is about 2000 mm.

Sample collection

A total of forty nine WC honey samples were bought from Dschang (14), Mbouda (14) and Fongo-Tongo (15) local markets. Seven other samples were aseptically collected directly from bee hives located in the Bee Research Farm of the University of Dschang (*miel campus*) and used as the reference honey sample in this study. All samples were collected and transported in sterilised sealed bottles. Physico-chemical analyses were carried out in the Animal Nutrition laboratory while mineral analysis was done in the Soil Science laboratory of the University of Dschang. Samples for microbiological examination were transported under ice and analysed within six hours in the Animal Physiology laboratory of the University of Dschang.

Physico-chemical analyses

The pH was measured using a pH meter (Gonnet, 1977); density calculated using a mass to volume ratio (James, 1980) while viscosity was calculated using the rate of drop of a sphere of known weight and dimensions through the honey sample (Roger, 1951). The moisture content was obtained by drying the grated pulp in a hot air oven at 105°C until a constant weight was attained and the ash content by incinerating dried samples in a muffle furnace at 550°C for four hours (AOAC, 1984). The mineral contents were analysed using the atomic absorption and spectrophotometer method as described by Pauwels (1992). The glucose content was estimated using the thiosulphate titration method described by Gonnet (1977).

Microbiological analyses

The standard plate count method was used for culturing and isolating the different micro-organisms. MacConkey Agar was used as the medium for bacteria culture while Potato Dextrose Agar was used for growing fungi (Marshal 1987; Smith and Agnes, 1983).
Bacteria colonies resulting from the first culture after incubation at 35°C for 48 h were transferred to fresh media, streaked and incubated again. After successive transfers, the resulting pure isolates were Gram stained and identified based on the colour, size and shape (Carbonnelle 1987).

For fungi, incubation was at room temperature (20 – 22°C) for 4 days. The resulting mycelia were extracted, streaked and grown successively on Malt Extract Agar until pure cultures were obtained. Identification was based on the colour of the mycelium and colour and shape of the fungus (Barnett and Barry, 1972).

Rate of crystallisation

Nine honey samples collected directly from bee hives in Mbouda (viscosity 156.05 Ns/m^2; pH 5.05; density 1.42 g/cm^3; water content 16.93%DM; Ash 1.22%DM; glucose 33.3%DM and glucose/water ratio 1.97) were used for this aspect of the study. Honey (0.40 kg) was poured into 4 different air tight storage containers: coloured (CB) and clear transparent (TB) bottles, dark (DP) and white translucent (WP) plastics. Two samples of each container type were stored under the following conditions: constant light at 21 – 22°C, constant darkness at 21 – 22°C and constant light at 24°C. These samples were stored undisturbed and observed every 30 days for a period of 150 days for possible crystal formation; air tight closure was maintained after each examination. If the honey was completely granulated it was considered to have experienced complete crystallisation (+), if granulation was only partly then it was partial crystallisation (±) and no crystallisation (-) if it remained liquid at the time of observation.

RESULTS AND DISCUSSION

Physical and chemical properties of WC honey

Table 1 shows the physico-chemical indices of WC honey. Although the deviation from the mean viscosity value in this study was wide, the mean pH, density, water content, glucose content and ash values were within the reference ranges reported for European and American honeys (Crane, 1975; 1980; Manikis and Thrasivoulou, 2001; ANONN, 2003a; 2003b; 2001-2004). Honey's viscosity is dependent upon its water content, temperature and floral source (Bogdanov, 1993; Rairmez, 2000). The annual temperature range in the Western Highlands of Cameroon is 16 – 27°C and water content of WC honey is 16.49 – 18.00% but the exact floral sources of WC honey are not fixed due to a mixture of vegetation in the region. WC honey is therefore polyfloral in origin but its viscosity could be considered as normal since the values (48 – 163 Ns/m^2) obtained here are similar to those for monofloral and polyfloral honeys recorded in other parts of the world (Anonn, 2003a). The

Table 1. Physical and chemical properties of West Cameroon honey.

Parameter	Current study	Mean or Range and Reference
Physico-chemical properties		
Viscosity (Ns/m^2)	94.70 ± 62.50	69.0**** (ANONN, 2003a)
	[83.3 ± 26.0]	138 – 48*** (ANONN, 2001 – 2004)
		189.6 – 68.4** (ANONN, 2001 – 2004)
Density (g/cm^3)	1.45 ± 0.03	1.4350 – 1.4171* (Crane, 1975; 1980;
	[1.45 ± 0.03]	ANONN, 2003a; 2001-2004)
pH	4.38 ± 0.22	3.9 {3.4 – 6.1} (ANONN, 2003a; 2001-2004)
	[5.16 ± 0.12]	
Water content (%DM)	16.49 ± 1.19	17.168 {13.4 – 26.6} (Crane, 1975; 1980)
	[18.00 ± 1.20]	17.2 {13.4 – 22.9} (ANONN, 2001-2004)
Glucose content (%DM)	31.64 ± 8.37	31.3 {22.0 – 40.7} (ANONN, 2001-2004)
	[35.30 ± 1.62]	32.789 {20.4 – 44.4}(Crane, 1975; 1980)
		30.31 {22.89 – 40.75} (ANONN, 2003a)
Glucose/Water content ratio	1.99 ± 0.26	
	[1.88 ± 0.05]	
Ash (%DM)	0.36 ± 0.31	0.20 (ANONN, 2003a; 2003b)
	[0.66 ± 0.36]	0.169 {0.020 – 1.028} (ANONN, 2001-2004)
		0.020 – 1.030 (Crane, 1975; 1980)
Minerals (mg/100 g honey)		
Calcium	22.68 ± 18.44	4.8 (ANONN, 2003a)
		6.0 (ANONN, 2003b)
		4.0 – 30.0 (Crane, 1975; 1980)
Magnesium	0.92 ± 0.34	5.0 (ANONN, 2003a)
		2.0 (ANONN, 2003b)
		0.7 – 13.0 (Crane, 1975; 1980)
Potassium	20.02 ± 8.89	50.0 (ANONN, 2003a)
		52.0 (ANONN, 2003b)
		10.0 – 470 (Crane, 1975; 1980)
Sodium	0.62 ± 0.19	2.85 (ANONN, 2003a)
		4.0 (ANONN, 2003b)
		0.6 – 40 (Crane, 1975; 1980)
Manganese	0.03 ± 0.02	2.0 (ANONN, 2003a)
		0.08 (ANONN, 2003b)
		0.2 – 10.0 (Crane, 1975; 1980)
Copper	0.02 ± 0.01	0.05 (ANONN, 2003a)
		0.04 (ANONN, 2003b)
		0.01 – 0.1 (Crane, 1975; 1980)
Phosphorus	1.80 ± 0.33	0.15 (ANONN, 2003a)
		4.0 (ANONN, 2003b)
		2.0 – 60.0 (Crane, 1975; 1980)

****: Water content 17.1% and at temperature of 25°C.
***: Water content range of 15.5 – 18.2%.
**: Temperature range of 20.6 – 29.0°C.
*: Water content range of 15.0 – 18.0% and at temperature of 20°C.
[ ]: Values for *miel campus*; the reference honey in this study.
{ }: Range of values.

viscosity of the reference honey used in this study is less than that of the other WC honey samples but similar to those of monofloral honeys elsewhere. Although few samples were obtained from campus, the standard deviations of all parameters were still smaller than those for others due to less variability of vegetation.

The calcium content of WC honey was on the upper limit of the reference range for honeys produced in temperate countries. However, the values for magnesium, potassium, sodium, copper and phosphorus were on the lower side of their respective reference ranges (Table 1) (Crane, 1975; 1980; Anonn, 2003a; 2003b; 2001-2004). On the other hand manganese content for WC honey was far below the recorded standard range. Minerals are usually present in very small quantities in honeys; potassium being the most abundant and dark honeys being the richest in mineral content (Anonn, 2001-2004). Although the WC honey samples also contained many minerals in trace quantities, calcium and potassium were both exceedingly more abundant with calcium being highest. The colour of the honey samples used in this study varied from extra light amber to amber. This agrees with earlier findings (ANONN, 2003a) stating that the colour of honey is related to its mineral content and characteristic of its floral or nectar source.

Table 2. Microbiological contamination of West Cameroon honey.

Microbes	Frequency (%) (n=49)*
Fungi	
Candida sp.	24.5
Aspergillus sp.	18.4
Geotrichum sp.	18.4
Rhizopus sp.	10.2
Curvularia sp.	6.1
Libertella sp.	2.0
Papularia sp.	2.0
Trichoderma sp.	2.0
Bacteria	
Bacillus sp.	34.7

*: No microbes were found in *miel campus*; the reference honey in this study.

Microbiological properties of WC honey

More than 73.47% of WC honey samples were contaminated with microbes. *Bacillus* sp. and 8 fungi species were identified in this study (Table 2); *Candida*, *Aspergillus*, *Geotrichum* and *Rhizopus* spp. being the most frequent fungi in decreasing order. This confirms other findings that fungi and spore forming bacteria may be present in honey (Anonn, 2003a; 2001-2004) for a limited period of time. Honey at concentrations of 17 – 100% by volume will completely kill or inhibit growth of microorganisms because of its intrinsic enzymatic glucose oxidation reaction and other physico-chemical properties (Jimenez, 1994; Anonn, 2003a; 2003b). The physico-chemical indices obtained in this study suggest that WC honey has high antimicrobial activity. However, microbial contamination during and or post processing rather than the indigenous microflora of honey itself can also result in spoilage or persistence of some bacteria in honey (Anonn, 2001-2004). Similar contaminations of other foodstuffs obtained from the local markets have been reported (Angrey, 2001). The microbes present in WC honey samples indicate contamination from secondary sources, during handling or adulterations. This is confirmed by their absence in honey harvested from the Bee Research Farm of the University of Dschang (*miel campus*), where processing and handling are always carried out in good hygienic conditions.

Crystallisation of WC honey

Only partial granulation was observed in all containers of WC honey on the 120th day of storage under constant light and at temperature of 21 – 22°C or 24°C. Complete crystallisation did not occur in these samples throughout the study period. However, granulation was complete by the 60th day of storage in all containers (except in dark plastics) when stored under constant darkness at 21 – 22°C. WC honey therefore has medium crystallisation tendency as complete crystallisation is most apparent before 12 months of storage in the dark (Crane, 1984). The 31.60 or 33.3% glucose content and 1.99 or 1.97 glucose/water ratio obtained in this study confirmed this tendency and also agree with Manikis and Thrasivoulou (2001) who reported that glucose/water ratio was the better index for prediction of crystallisation tendency of honey over glucose content.

Fast crystallisation of honey occurs when it has a high glucose/water ratio (2.1 or higher), good temperature (11 – 15°C), presence of solid nuclei or initial monohydrate glucose crystals for crystal growth and or slow stirring (Crane, 1975; Anonn, 2003a; 2001-2004). Other constituents and sugars of honey and storage conditions such as temperature, relative humidity, type of container as well as storage time also affect the tendency of honey to crystallise. Moderate temperatures (10 – 21°C) will generally encourage crystallisation while warm temperatures (21– 27°C) discourage crystallisation and degrade honey.

In this study, darkness seems to encourage granulation. Heat and light can destroy the glucose oxidase enzyme whose action on glucose leads to the formation of gluconic acid and hydrogen peroxide (Anonn, 2003b; 2001-2004) but the presence of phytochemicals from nectar will destroy the hydrogen peroxide, freeing water and oxygen in the process. Although low density polyethylene containers can allow moisture to escape to the surrounding atmosphere thereby contributing to the crystallisation process (Assil, 1991). The effect of exposure to light on honey crystallisation is not clear. Honey is not uniform all through and portions may differ in composition and levels of enzymatic or non-enzymatic factors. Nonetheless, honey is compatible to a wide variety of products since its composition is characteristic of its botanical orig-

Table 3. Effect of light and temperature on the crystallisation tendency of West Cameroon honey.

Storage duration (days)	Storage condition											
	Light at 21 – 22°C				Light at 24°C				Darkness at 21 – 22°C			
	CB	TB	DP	WP	CB	TB	DP	WP	CB	TB	DP	WP
30	-	-	-	-	-	-	-	-	±	±	±	±
60	-	-	-	-	-	-	-	-	+	+	±	+
90	-	-	-	-	-	-	-	-	+	+	+	+
120	±	±	±	±	±	±	±	±	+	+	+	+
150	±	±	±	±	±	±	±	±	+	+	+	+

CB: Coloured bottles; TB: Clear transparent bottle; DP: Dark plastic; WP: White translucent plastic.
+: Complete crystallisation; ±: Partial crystallisation; - : No crystallisation.

in, age and storage conditions (Anonn, 2003a; 2001-2004) and transparency or clarity of honey depends on the amount of suspended particles such as pollen. Result from this study suggest that constant light condition during storage inversely affect physical and chemical factors that stimulate granulation or enzymatic formation of initial monohydrate glucose crystal seeds for crystal growth. It was also apparent that the type of container used had no effect on WC honey crystallisation tendency.

CONCLUSION

From the above study on the survey of physico-chemical and microbiological characteristics of honey produced in the sudano-guinean zone of West Cameroon, the following conclusions are made:

1. The physico-chemical indices of West Cameroon honey are within the limits of the reference ranges recorded in temperate areas.
2. Honey obtained from the local markets of West Cameroon is contaminated with fungi and bacterial organisms indicating inadequate hygiene condition during harvesting, handling, processing and/or storage. The physico-chemical properties and possible enzymatic factors, of honeys from the region are similar to those produced elsewhere and likely also enough to kill or inhibit microbial establishment.
3. Honey produced in the sudano-guinean zone of West Cameroon has medium crystallisation tendency and granulates faster when stored in the dark.

ACKNOWLEDGEMENTS

The authors are grateful to the technical assistance of Mrs. Katte Bridget and staff of Soil Science, Animal Physiology and Nutrition laboratories of the University of Dschang, Cameroon.

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