



PROFILE OF PHYSICOCHEMICAL PARAMETERS IN THE DETERMINATION OF AUTHENTICITY AND DIAGNOSIS OF COLOMBIAN HONEYS: DSC AS A COMPLEMENTARY DIGNOSTIC TOOL

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REMARKS

Honey is a complex biological product, its composition is a function of botanical origin. The glucose, fructose and sucrose fractions account for 90% of the total solids. The product can be marketed as a viscous liquid, in combs, or in crystallized form, depending on the nature and type of honey.

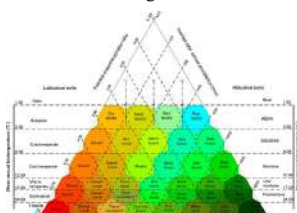


Fraud in commercial honeys is a criminal and intentional act committed to obtain unfair economic benefit and there are different types of honey adulteration. The quality criteria of honeys in general, have to be evaluated according to national or international legislation, to verify three concepts: 1. Authenticity, 2. Freshness and 3. Safety.

METHODS

Differential scanning calorimetry (DSC) techniques are establishing themselves as a rapid and simple diagnostic tool that does not require complex sample preparation operations for diagnosis. For this reason, their use has been evaluated in the characterization of Colombian honeys.

A total of 33 Colombian honeys were collected from different biogeographical zones of Holdridge's wild life: (*ml-WF*), (*pm-WF*), (*t-RF*), (*vh-LMF*), (*pm-VHF*), (*lm-DF*) and (*t-DF*) in Caribbean, Andean and Inter-Andean Region.



Holdridge's life zones system



Colombia life zones system

The phase transition properties of the honeys were studied using a differential scanning calorimeter (DSC, TA Instruments, USA, model TA 2010, with controlling module Thermal Advantage Version 1.1A) and cooling accessory, all samples were weighed (~ 10mg) in analytical scale (± 0.01 mg) inside hermetically sealed aluminum pans. Then were heated from -100 to 100°C, at 10°C.min. An empty aluminum pan was used as reference. The results were analyzed using the Universal Analysis 2000 software version 4.2E (TA Instruments). Reported data are the average of triplicate determination. Samples were analyzed for color (Pfund scale), water activity and sugar composition (fructose-to-glucose ratio by HPLC).

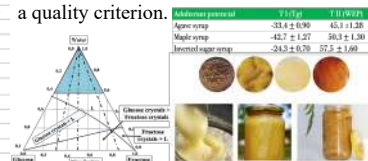
For HPLC analysis, 5 g of honey were dissolved in water, diluted to 100 mL with methanol and water, and filtered through a 0.45 μ m PTFE membrane. Samples were analyzed using a Shimadzu system with a RID-10A refractive index detector and a Phenomenex Luna® Omega 3 μ m Sugar 100 Å LC column (150 $\times$ 4.6 mm) at 30 °C. Isocratic elution (acetonitrile:water, 80:20) was performed at 1.3 mL/min, and sugars were quantified using calibration curves with standard solutions. The glass transition (Tg) and weak endothermic phenomenon (WEP) of Colombian honeys were compared with those of agave, maple, and invert sugar syrups. Specific rotation was also measured after dissolution and clarification using a ZUZI™ polarimeter (data not shown).

RESULTS

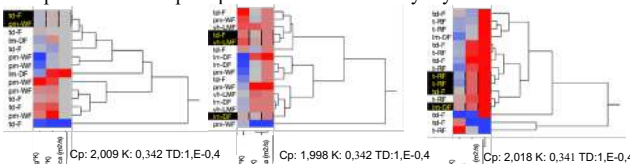
According to their physicochemical properties, honey can be considered as a complex high concentrated sugar solution. The significant effects of water on the physical state of sugars can be explained by state diagrams that relate the phase and state transition temperatures to water content. Water-sensitive amorphous systems may exhibit several changes in their physicochemical properties.

Color	W/L	α_m	F	G	F/G	T1	T2
bo-DF	0.604	37.2	25.8	1.44	-45.6	45.8	
pm-WF	0.606	42.7	27.9	1.53	-45.8	48.4	
t-RF	-	-	-	-	-	-	
td-F	0.602	41.8	27.9	1.50	-45.5	45.6	
vh-LMF	-	-	-	-	-	-	
W/L	α_m	F	G	F/G	T1	T2	
bo-DF	0.581	35.5	28.1	1.27	-40.9	49.6	
pm-WF	0.599	37.1	26.7	1.39	-45.3	47.1	
t-RF	-	-	-	-	-	-	
td-F	0.615	37.0	29.6	1.25	-40.1	52.5	
vh-LMF	0.645	32.5	28.4	1.15	-46.1	44.9	
W/L	α_m	F	G	F/G	T1	T2	
bo-DF	0.563	40.1	29.0	1.38	-41.5	43.7	
pm-WF	-	-	-	-	-	-	
t-RF	0.594	45.5	25.2	1.82	-44.1	53.7	
td-F	0.615	38.2	30.2	1.27	-46.3	50.0	
vh-LMF	-	-	-	-	-	-	
W/L	α_m	F	G	F/G	T1	T2	
bo-DF	0.608	35.4	35.3	1.00	-40.0	48.2	
pm-WF	0.582	41.2	28.5	1.45	-43.3	49.9	
t-RF	-	-	-	-	-	-	
td-F	0.588	42.1	27.5	1.54	-45.3	50.8	
vh-LMF	0.563	41.3	31.4	1.32	-41.3	55.6	

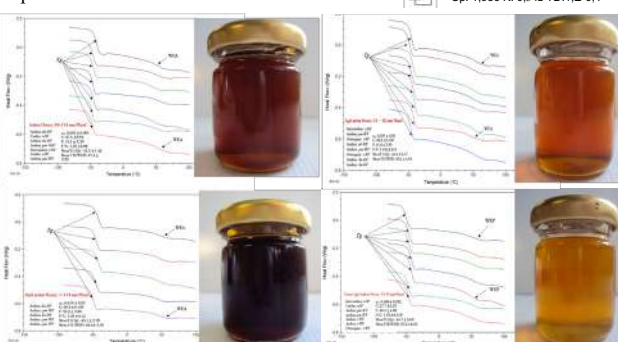
The use of DSC techniques in the detection of honeys adulterated with various types of syrups and hydrolysates in different percentages is well known. The determination of parameters such as pH, electrical conductivity, water and ash in the characterization of honey takes a back seat when thermal analysis is used as a quality criterion.



The heat flow curves obtained by DSC showed typical honey behavior with two main thermal events. First, a glass transition (-45 to -39°C) associated with a relaxation event, the amplitude of which depends on the sample's thermal history. This transition reflects the amorphous nature of honey, as materials lacking sufficient order to crystallize generally exhibit Tg at low temperatures. Second, a weak endothermic phenomenon (WEP) between 40.6 and 59.0°C was observed, likely related to melting of water-sugar complexes or polymorphic sugar forms. This transition is reproducible within a narrow temperature range but disappears after a heating/cooling/heating cycle. The WEP region is strongly influenced by temperature and helps explain variations in honey crystallization kinetics.



Syrup content directly influences the observed values of Tg and WEP, showing a linear relationship: higher syrup levels result in higher values (data not shown). Thermophysical properties therefore provide a useful classification method.



This study contributes to the typification of *A. mellifera* honeys from Colombia. Variations in DSC thermal properties, optical rotation, and reducing sugars proved to be effective markers for honey characterization, while thermal parameters enabled the classification of honey groups. Moreover, principal component analysis (PCA) of DSC data demonstrated strong potential for detecting fraudulent honeys.

Profile of physicochemical parameters in the determination of authenticity and diagnosis of Colombian honeys: DSC as a complementary diagnostic tool

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Honey is produced from flower nectar and plant secretions, as well as from the excretions of sucking insects, which the bees collect, transform and combine with specific substances and ripen inside the comb. Amorphous water-sensitive systems can exhibit various changes in their physicochemical properties. DSC is a tool to evaluate this type of interactions, with interesting correlations associated with the quality of materials such as honey. Fructose/glucose and physicochemical parameters considered by the International Honey Commission were evaluated. Rotary power, thermal conductivity, heat capacity, viscosity, differential scanning calorimeter (DSC) and phase transition properties of the honeys were studied using instrumental methods. A glass transition, which must be associated with the amorphous dry solids in honey, was observed to occur at very low temperature, between -67.2 and -42.61 °C, with T_g varying linearly with the water content of the samples. This study demonstrated that the variation of thermal properties DSC, sugar and rotary power are powerful techniques to characterize the behavior of honeys, reducing sugars, proportion and thermal parameters of DSC analysis allow the identification of groups of honeys, unifloral and honeydew. Principal component analysis contributes to the identification of fraudulent honeys.