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Use of Differential Scanning Calorimetry (DSC) as a New Technique for Detection of Adulteration in Honeys. 1. Study of Adulteration Effect on Honey Thermal Behavior

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Differential scanning calorimetry (DSC) was used to study the thermal behavior of authentic honeys (*Lavandula*, *Robinia*, and *Fir* honeys) and industrial sugar syrups. Thermal or thermochemical parameters such as the glass transition temperature (*T*g), enthalpies of fusion (ΔH_{fus}), and heat capacity variation (ΔC_p) were measured. The syrups and honeys showed significant differences in thermal phenomena, as well as in their amplitude and position on the temperature scale. Results showed good reproducibility of the method for all samples studied. The effect of adulteration of honey with different amounts of syrup (5, 10, 20, 40, and 60%) was investigated. A linear relationship was found between the percentage of added syrup and the glass transition temperature. A similar relationship was obtained from the enthalpy of fusion results in the temperature range of 40−90 °C. Under applied conditions, the effects of adulteration of honeys by industrial syrups appeared to be detectable from a level as low as 5%.

KEYWORDS: Honey; adulteration; DSC; heat of melting; glass transition; thermal behavior

INTRODUCTION

Honey adulteration has appeared on the world market since the 1970s, coincident with the industrial development of "high fructose corn syrups" (HFCS). HFCS constitute a subset of isoglucose syrups obtainable from various vegetable raw materials (such as wheat, beet, and cane). Historically (*1*), the substances most often incorporated into honey have been flour, paraffin wax, glucose, and sucrose. During the 1980s, the appearance of complex isoglucose syrups obtained from acid or enzymatic hydrolysis of farm-produced raw materials increased substantially. The ready availability of these syrups resulted in their being used extensively in the adulteration of honeys. The added syrups, which sometimes also contain products from partial fermentation, produce lower quality commercial products. Efforts to develop analytical methods to establish the authenticity of honey to ensure the consumer of product quality are ongoing. Different analytical methods have been developed to detect adulteration of honey with HFCS (*2*), but none was dedicated to the overall characterization of isoglucose syrups. Classical chromatographic methods suffer from severe limitations in the detection of these kinds of additions because of the similar profiles of the major sugars in these syrups.

Microscopic analysis appeared as an alternative methodology for the detection of visible exogenous elements in honey. This approach was adapted for the characterization of adulteration by cane sugar and cane sugar products (*3*, *4*). However, industrial microfiltration of syrups could remove the vegetable key markers such as sclerous rings or epidermic cells and others (*5*).

The application of stable carbon isotope ratio (IRMS) is currently considered to be the reference technique for the detection of adulterated honeys (*6*−*8*). However, IRMS remains linked to the detection of C4 plant sugar syrups.

Previous work carried out in our laboratory on the application of analytical methods defined both in the Codex Alimentarius and in European Directive 74/409 showed the limitations of the physicochemical determinations (i.e., pH, conductivity, humidity, acidity) for adulteration detection.

The limitations of classical analytical methods measuring chemical parameters led us to carry out experiments using new indicators derived from physical analysis such as thermal analysis. The most widely used technique in this area, differential scanning calorimetry (DSC), originated in the pharmaceutical (*9*−*11*) and polymer material industries (*12*−*18*).

Most of the physical or chemical transformations involve heat exchanges. Therefore, DSC analysis has a very broad field of

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Table 1. Thermal and Thermochemical Parameters Measured on Honeys

parameter	phenomena	description	unit
onset	glass transition, melting	start temperature	°C
midpoint	glass transition	temperature measured according to an international graphical norm	°C
ΔH	melting, crystallization, gelatinization	value of absorbed or emitted energy by phenomenon	$J \cdot g^{-1}$
ΔC_p	glass transition	measure of baseline deviation of curve due to physical and chemical property modifications	$J \cdot g^{-1} \cdot K^{-1}$

application (*19*−*21*). Even when no heat exchange occurs, as observed in glass transitions, the phenomenon gives rise to a baseline deviation of the thermoanalytical curve, related to a variation of heat capacity. Glass transition and heat capacity measurements provide detailed information about both the physical and calorimetric properties of a substance.

In the farm-products industry, many developments using DSC (*22*−*27*) have been recently investigated for the detection of alteration (*28*) or adulteration (*29*, *30*) and for quality control of food (*31*−*33*). In one particular study, Coni et al. (*29*) have shown that the presence of animal fats in butter can be detected at a level of 10% and above by subtracting the thermogram of pure butter from the thermogram of the sample.

Moreover, the ability of DSC to detect thermal phenomena with small transitions allows protein denaturation studies to be performed (*34*). Studies on meat (chicken, veal, and turkey), reported by Harwalkar et al. (*35*) showed protein denaturation from thermoanalytical curves recorded during conventional DSC scans. Thermal parameters extracted from these curves are useful for the identification and quality control of meats. More recently, Gringberg et al. (*36*) have reported a kinetic study of the denaturation of the Kunitz soybean trypsin Inhibitor (KTI) from tobacco leaves. The interpretation of the thermoanalytical curves allows one to calculate kinetic and thermodynamic parameters of the enzymatic reaction studied (ΔG, ΔH, ΔS, and k_d). As well as providing fundamental thermodynamic parameters, this work also confirms that DSC is a technique with a range of possibilities of uses in testing of biological products.

For degradation, decomposition, or stability determinations, DSC can be used as a routine screening tool in association with other analytical techniques. DSC has the advantages of the rapidity with which the measurements are made and the small amount of sample that is required.

Considering that the detection of adulteration requires knowledge of the food products' physical and chemical properties, our approach follows two steps. We first use DSC for determining the thermal behavior of pure honeys and pure syrups, and, second, we use DSC to detect modification of the thermoanalytical curve due to adulteration of honey by syrup addition. To our knowledge, no previous study about the detection of adulteration in honey by DSC has been reported.

MATERIALS AND METHODS

Samples. *Lavandula*, *Robiniae*, and *Fir* honeys were obtained from French beekeepers. The botanical origin of the samples was certified by quantitative pollen analysis according to the procedure of Louveaux et al. (*37*) and confirmed by sensory analysis. Syrup samples were obtained from French industrial suppliers (Dorsman S.A.R.L.; Ickovich S.A.).

Preparation. Because honey is not a pure homogeneous material, an experimental protocol was developed to reduce the sample heterogeneity. Honeys and syrups were stored at 4−6 °C and left to stand at room temperature for 12 h before analysis. Each sample was homogenized with a mechanical device (Eurostar, power control-visc, IKA Labortechnik) for 20 min. To avoid air incorporation into the sample, mixing was applied with a constant speed rotation between 80 and 120 rpm depending on the viscosity of the sample. The residual moisture of samples was determined by refractometry (Atago Abbe refractometer, Atago Co. Ltd., Tokyo, Japan) following the European method (*38*) and was found to be about 17.5 and 24.5% for honeys and syrups, respectively.

Differential Scanning Calorimeter. A Mettler-Toledo DSC model 822^e was used to follow the thermal behavior of the samples. The apparatus was equipped with a ceramic sensor FRS5 (heat-flux sensor with 56 thermocouples Au−Au/Pd). The differential scanning calorimeter was previously calibrated using indium and zinc standards for temperature and power calibration (*39*, *40*). The autosampler available on the Mettler-Toledo DSC 822^e was used to automate the experimental procedure. The measuring range was extended to −65 °C by a cooling Intra Cooler system (RP-100MT; Powerpoint 2000 LDT).

Calculations and Statistical Tests. For analysis of the melting enthalpy, the area between the heat flow curve and the extrapolated baseline and the ΔC_p were determined automatically by the STARe software (Mettler-Toledo) following the ASTM, IEC norm (*41*). The limits of integration were chosen to maximize the energy of melting under the thermoanalytical curve. Thermal and thermodynamic measurements are displayed in Table 1. A hypothesis test was used to decide whether the observed difference between two sample series was significant or not. The repeatability was evaluated on two series of measurements under multiple analytical conditions: temperature programming (10, 20, 30 °C/min) and number of replicates (5−10 replicates per series). We used an *F* test (*42*) (two-sided test) for comparison of variances and a *t* test (Student's test) for comparison of means.

Differential Scanning Calorimetry Analysis. We used calorimetric heat flow to obtain quantitative and qualitative data concerning the net heat changes produced by carbohydrates during their heating. As reference, an empty crucible was measured at the same time. Runs were conducted from −65 to 230 °C to obtain the complete thermal behavior of pure honeys and pure syrups from low temperature to high temperature. However, experiments on adulterated honeys were conducted, for practical reasons, in a temperature range from −65 to 130 °C to avoid burning of sugars and an eventual explosion of the crucible during the analysis. Some trials with medium-pressure crucibles were investigated but led to thermograms with low signal sensibility and were not considered further. A sample of ∼10 mg was sealed in a pierced aluminum crucible (100 μL) and heated under nitrogen flow (200 mL/min outside the oven, 100 mL/min inside the oven).

Samples were introduced in the calorimeter at various start temperatures, and various heating rates were applied. Following literature reports (*43*, *44*), and after evaluation of its effect in the range of 2−50 °C/min, a heating rate of 20 °C/min was selected. This heating rate improves the calorimetric response (without a decrease of accuracy) and reduces the time of the analysis and, consequently, the risk of sample degradation. To apply the same experimental conditions to all samples, the cooling rate selected was 10 °C/min. Three DSC runs were carried out to determine various glass transitions (T_g) as well as the melting behavior of the samples at higher temperatures.

RESULTS AND DISCUSSION

Thermal Behavior of Honeys and Syrups. We used DSC to follow the effect of adulteration on the thermal behavior of pure honey. Sugars and water represent the main chemical constituents of honey (>95%). Proteins, flavors and aromas, pigments, and numerous volatile compounds constitute the minor components of honey. Pure substances can be characterized by

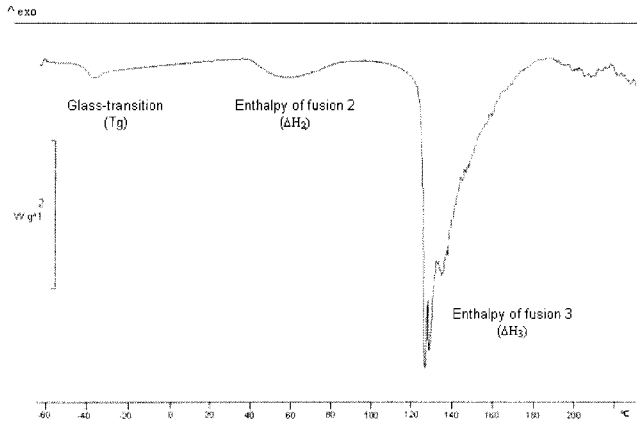

Figure 1. DSC scan (linear scan temperature) for *Lavandula* honey.

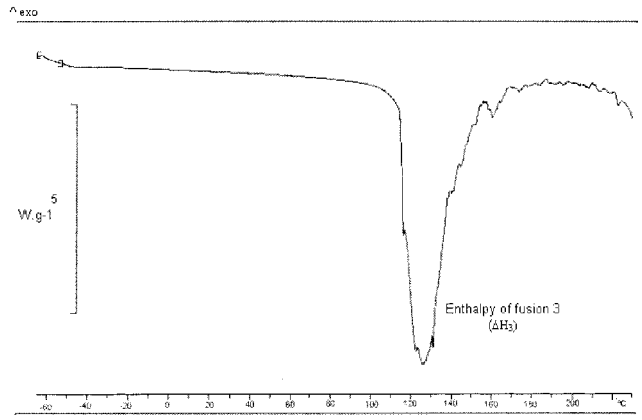

Figure 2. DSC scan for isoglucose sugar syrup.

a unique and sharp melting point. This is not true for honeys, as the chemical complexity as well as the thermal treatment used causes many differences in the thermoanalytical curves. Figures 1 and 2 show typical DSC curves of honey (*Lavandula)* and sugar syrup (isoglucose), respectively.

In most cases, three thermal phenomena were observed in thermoanalytical curves of honeys:

(a) A glass transition (−45 to −39 °C) is seen, often linked with a relaxation effect the amplitude of which depends on the thermal history of the samples. When a material is not sufficiently ordered to crystallize, a glass transition can generally be observed at low temperature. Thus, amorphous material can be characterized by their glass transition temperature (T_g). This phenomenon is well-known in polymers (*45*) as well as in sugars (*46*−*50*) and is observed in honey as well.

(b) An endothermic phenomenon (relatively weak) at 40−90 °C is observed, which could correspond to the melting of the water/sugars/starch complex or could be caused by a polymorph form of sugars. The temperature range of this phenomenon was constant in comparison to other thermal transitions. The phenomenon disappears systematically after a heating/cooling/heating sequence.

(c) A very wide and intense endothermic peak at 100−120 to 180−220 °C corresponding to the melting of sugars (mono-, di-, tri-, and oligosaccharides) is seen.

Table 2 shows the values obtained for these parameters. The glass transition temperature is characterized by a change in heat capacity, which induces a change in the baseline of the thermoanalytical DSC curve.

Some variations of the T_g position and intensity occurred in honeys and cane and beet syrups. The differences were both qualitative and quantitative, with a significant difference in the transitions being observed in honeys and syrups. This observation was completed by noting the presence or absence of characteristic phenomena (glass transition observed and measured for honeys but not observed for isoglucose syrups). For beet and cane syrups (moisture = 50%), DSC analyses showed the presence of a typical endothermic peak in the range −20 to 0 °C linked to free water of the sample (*51*). This peak, with enthalpy noted as ΔH_1 in Table 2 and corresponding to the melting of the frozen water, was not observed in honeys because the water is included in the sugar network (cf. Figure 3).

For isoglucose syrups, (moisture ≈ 24%) the melting of frozen water was not observed. The glass transition of these syrups was also not present on the thermoanalytical curves, as it seemed to be situated below −65 °C, whereas it is observed at −40 °C (±3 °C) for honeys. Otherwise, preparations based on honey containing added syrups showed a noticeable decrease in T_g of the mixtures.

The observed difference between T_g of samples was ∼1−5 °C for nectar honeys and ∼3 °C for syrups. In contrast, differences between the T_g of nectar honeys and syrups are ≥10 °C. This observation showed the possibility of using the glass transition temperature to distinguish between honeys and syrups. However, there was no significant difference in T_g temperature between cane or beet syrups (−33.0 ± 2 °C) and honeydews (−37.5 ± 0.3 °C) represented by *Fir* honey in our study.

With the present protocol, enthalpies of fusion did not appear as the most appropriate parameters to discriminate among floral species but were essential for syrup recognition because they have the largest enthalpies of fusion of sugars (ΔH_3). The variation of the specific heat capacity (ΔC_p) did not appear to

Table 2. Transition Temperature (T_{onset}, $T_{midpoint}$), ΔC_p, and Enthalpy of Honeys and Syrups during Heating

			thermal phenomena					
			glass transition					
			T_g			fusion 1	fusion 2	fusion 3
sample		no. of replicates	T_{onset}	$T_{midpoint}$	ΔC_p	$\Delta H_{1,fus}$	$\Delta H_{2,fus}$	$\Delta H_{3,fus}$
syrups	beet syrup	4	–34.3	–33.4	0.9	–67.7	A[a]	–849.9
	cane syrup	2	–32.0	–31.6	0.3	–95.9	A	–997.5
	isoglucose 1	2	ND[b]	ND	ND	A	A	–560.8
	isoglucose 2	2	ND	ND	ND	A	A	–508.6
	isoglucose 3	2	ND	ND	ND	A	A	–466.3
	isoglucose 4	2	ND	ND	ND	A	A	–505.4
honeys	*Lavandula*	10	–41.1	–38.0	0.6	A	–23.7	–256.2
	Robinia	5	–42.5	–39.8	0.8	A	A	–213.1
honeydew	*Fir*	8	–37.5	–33.5	0.6	A	<2	–228.9

[a] A, not measured due to absence. [b] ND, not determined due to lack of accessibility by our cooling system.

Table 3. Statistical Values for Six Thermal Parameters (Carried out on *Lavandula* Honey)

	fusion 2		fusion 3		glass transition		
	T_{onset}	ΔH_2	T_{onset}	ΔH_3	T_{onset}	$T_{midpoint}$	ΔC_p
mean 1, $\bar{X}$ ($n = 6$)	35.4	–24.3	119.5	–251.6	–42.4	–38.8	0.73
mean 2, $\bar{X}$ ($n = 6$)	35.7	–24.2	119.2	–243.5	–42.3	–39.1	0.69
standard deviation 1 (SD1)	0.310	0.564	0.361	14.400	0.309	0.706	0.021
standard deviation 2 (SD2)	0.108	0.402	0.185	15.970	0.149	0.249	0.025
			merged series (n =12)				
mean	35.5	–24.2	119.5	–247.5	–42.4	–38.9	0.71
standard deviation (SD)	0.294	0.546	0.331	1.629	0.268	0.590	0.037
F test (F_{calcd})	3.92	1.96	0.26	1.23	4.32	8.04	1.20
t test (t_{calcd})	3.06	0.35	1.81	0.84	0.71	0.98	2.95
F_{theor} for ($\alpha = 0.01$) = 10.97,		for ($\alpha = 0.025$) = 7.15,				for ($\alpha = 0.05$) = 5.05	
t_{theor} for ($\alpha = 0.01$) = 3.17,		for ($\alpha = 0.025$) = 2.63,				for ($\alpha = 0.05$) = 2.23	
		H_0 is accepted if $F_{calcd} < F_{theor}$ and $t_{calcd} < t_{theor}$					

Table 4. Experimental Adulteration Trials: Evolution of Thermal Parameters of *Lavandula* Honey in Terms of Added Syrup Percentage (H in J·g^{-1}; T_{onset} in °C; ΔC_p in J·g^{-1}·K^{-1})

		fusion ($\Delta H_{2,fus}$)				glass transition (T_{onset})			
sample	% syrup	syrup 1	syrup 2	syrup 3	syrup 4	syrup 1	syrup 2	syrup 3	syrup 4
01	0	–23.7	–23.7	–23.7	–23.7	–41.12	–41.12	–41.12	–41.12
02	5	–19.1	–19.4	–17.9	–17.8	–42.89	–42.30	–42.14	–41.83
03	10	–14.3	–17.3	–14.1	–17.0	–43.95	–42.99	–43.45	–42.59
04	20	–14.7	–15.6	–15.9	–15.1	–44.93	–44.59	–45.24	–44.34
05	40	–8.9	–14.6	–11.0	–9.0	–48.43	–47.97	–47.50	–47.07
06	60	–0.6	–9.1	–3.3	–1.9	–50.16	–50.68	–50.41	–49.05
07	100	A[a]	A	A	A	ND[b]	ND	ND	ND

[a] A, not measured due to absence. [b] ND, not determined due to lack of accessibility by our cooling system.

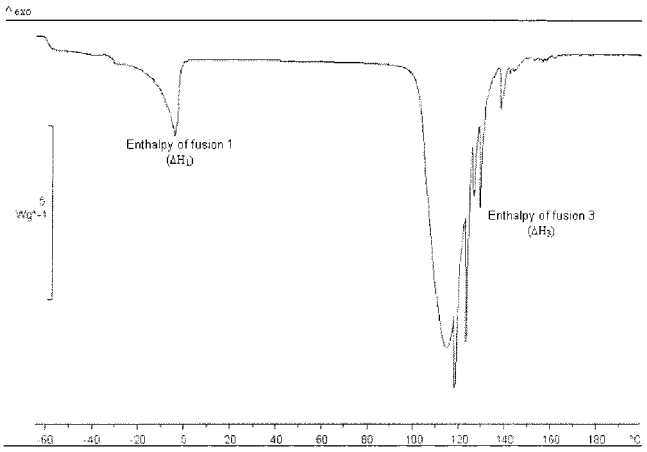

Figure 3. DSC scan for beet sugar syrup.

be a good parameter for distinguishing between floral species, but its absolute value, C_p, might be. Accurate measurements of C_p are in progress in our laboratory by the direct method and by the "sapphire" method. Results show a good reproducibility of measurements (cf. Table 3); therefore, the sets were merged to evaluate the standard deviation.

Effect of Experimental Adulterations on Thermal Behavior of Honey. Adulterations made with beet and cane sugar resulted in a significant displacement of the T_g of the mixture and a dramatic increase in the ΔH_3, but adulteration by isoglucose syrups was not so easily detected. Therefore, syrup additions at 5, 10, 20, 40, and 60% were carried out with four isoglucose syrups. Table 4 shows results obtained during thermal analysis of seven samples analyzed in triplicate. Although the fusion of sugars, noted "fusion 3", seems to be a good parameter for making a distinction between honeys and syrups (Table 2), this study did not use this parameter for practical reasons, in particular, because of the higher risk of crucible explosion when honeys or sugar-based products are used at the higher temperature. The higher temperature causes practical problems related to the heat-flux sensor being soiled by caramel from honey sugars. Therefore, we preferred to limit the temperature to a maximum of 130 °C and study parameters such as enthalpy of fusion 2 (ΔH_2) and T_g.

Enthalpy of Fusion 2. The effect of the adulteration with syrups on the enthalpy of fusion 2 follows a linear relationship. As an example, for isoglucose 2, $\Delta H_2 = 0.21S_{\%} - 21.3$ with $r^2 = 0.956$, where $S_{\%}$ is the percentage of added syrup and r^2 the coefficient of determination of the tendency curve. This observation was confirmed by a second set of experiments, and the sensitivity level of the method was within the range of 5−10% depending of the type of syrup used. For this percentage of added syrup, there was a decrease of the enthalpy of fusion (ΔH_2) of 3−5 J·g^{-1}, whereas the determination error associated with the measurement is ∼1 J·g^{-1}.

Glass Transition Temperature. Linear relationships are also obtained for the evolution of the glass transition in terms of percentage of added syrup. For example, for isoglucose 2 the equation is $T_g = -0.16S_{\%} - 41.4$ with $r^2 = 0.997$, where $S_{\%}$ is the percentage of added syrup and r^2 the coefficient of determination of the tendency curve. The addition of industrial sugar syrup was detected by a statistically significant temperature gap. For experimental adulteration, we selected the heating rate that presented the biggest temperature gap within as short as possible an analysis time. The origin of this temperature gap was mainly due to the water introduced into honey from the added syrup. This fraction of water incorporated in quench-cooled samples acts as a plasticizer on the T_g. Te Booy et al. (*52*) showed the strong effect of residual moisture on T_g in freeze-dried sucrose-containing formulations. Kantor et al. (*51*) observed both the plasticizing effect of water on T_g (also

depending of the kind of solute) and the possibility of using T_g determination coupled with the C'_g determination (maximally freeze-concentrated solution) for the characterization of honeys using state diagrams of the water−honey system. Our results concerning the effect of sugar syrup additions to honey on the glass transition temperature are in agreement with those obtained by Te Booy et al. (*52*) on sucrose solutions and by Kantor et al. (*51*) on water/honey mixtures.

Conclusion. DSC is a powerful technique for characterizing the thermal behavior of honeys and for detecting the effect of adulteration on physicochemical and structural properties of samples.

Used concomitantly with the second enthalpy of fusion (occurring between 40 and 90 °C), the glass transition temperature, T_g, is one of the most potentially useful parameters for characterizing honeys and syrups and for distinguishing between them. The T_g value, being strongly dependent on the amorphous phases of the sample, will respond to modification of the chemical composition and the implicit structural modification caused by the addition of exogenous material. Thus, adulteration of the honey will cause inevitable changes in both T_g and ΔH_2 values. Under laboratory conditions, adulterations by industrial sugar syrups can be detected from 5−10% additions depending on the measured parameter. The observed effects could be used to develop a new method for adulteration detection in honey, provided the natural variability among honey varieties is established.

Future work dealing with the contribution of DSC to the detection of adulteration in commercial honeys will be presented in the next paper. Results obtained by DSC and those obtained by other techniques such as microscopic analysis or IRMS will be compared for validation.

The ultimate aim of this work is to develop a means for screening commercial samples to establish the authenticity of honeys.

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LITERATURE CITED

(1) Sanford, M. T. Honey Adulteration, Fact Sheet ENY-103; Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida; http://edis.ifas.ufl.edu.

(2) Abdel-Aal, E.-S. M.; Ziena, H. M.; Youssef, M. M. Adulteration of Honey with High-Fructose Corn Syrup: Detection by Different Methods. *Food Chem.* **1993**, 209−212.

(3) Kerkvliet, J. D.; Shrestha, M.; Tuladhar, K.; Manandhar, H. Microscopic Detection of Adulteration of Honey with Cane Sugar and Cane Sugar Products. *Apidologie* **1995**, *26*, 131−139.

(4) Kerkvielt, J. D.; Meijer, H. A. J. Adulteration of honey: relation between microscopic analysis and δ^{13}C measurements. *Apidologie* **2000**, *31*, 717−726.

(5) Antinelli, J.-F.; Clément, M.-C.; Moussa, I.; Cordella, C.; Faucon, J.-P. Détection de Canne à Sucre dans les Miels par Analyses Isotopique et Microscopique: Etude et Comparaison. *Ann. Fals. Exp. Chim.* **2000**, 951.

(6) White, J. W.; Winters, K.; Martin, P.; Rossmann, A. Stable Carbon Isotope Ratio Analysis of Honey: Validation of Internal Standard Procedure for Worldwide Application. *J. AOAC Int.* **1998**, *81*, 610−619.

(7) White, J. W. Internal Standard Stable Carbon Isotope Ratio Method for Determination of C-4 plants Sugars in Honey: Collaborative Study, and Evaluation of Improved Protein Preparation Procedure. *J. Assoc. Off. Anal. Chem.* **1992**, *75*, 543−548.

(8) White, J. W.; Winters, K. Honey Protein as Internal Standard for Stable Carbon Isotope Ratio Detection of Adulteration of Honey. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 907−911.

(9) Benzler, B.; Nitschke, T. Application of thermal analysis in the pharmaceutical industry *LaborPraxis* **1996**, *20* (9), 70−72, 75.

(10) Clas, S.-D.; Dalton, C. R.; Hancock, B. C. Differential scanning calorimetry: applications in drug development. *PSTT* **1999**, *2* (8), 311−320.

(11) Poirier, L. J. Characterization of pharmaceuticals by thermal analysis. *Int. Lab.* **1991**, *21* (1), 26, 28−30, 32, 34.

(12) Yamamoto, K.; Momota, M.; Katayama, S.; Narita, K. Determination of the Purity of Multicomponent Organic Substances by DSC. *Anal. Sci.* **1998**, *14*, 599−602.

(13) Gehenot, A.; Rao, R. C.; Maire, G. *Int. J. Pharm.* **1988**, *45*, 13−17.

(14) Clas, S.-D.; Cotton, M.; Moran, E.; Spagnoli, S.; Zogafri, G.; Vadas, E. B. Assessment of the physical stability of lyophilized MK-0591 by differential scanning calorimetry. *Thermochim. Acta* **1996**, *288*, 83−96.

(15) Pires, J.; Brotas De Carvalho, M.; Carvalho, A. P.; Guil, J. M.; Perdigon-Melon Heat of Adsorption of *n*-Hexane by Thermal Gravimetry with Differential Scanning Calorimetry (TG-DSC): A Tool for Textural Characterization of Pillared Clays. *Clays Clay Miner.* **2000**, *48* (3), 385−391.

(16) Morgan, D. R.; Kalachandra, S.; Shobha, H. K.; Gunduz, N.; Stejskal, E. O. Analysis of a Dimethacrylate Copolymer (Bis-GMA and TEGMA) Network by DSC and ^{13}C Solution and Solid-State NMR Spectroscopy. *Biomaterials* **2000**, *21*, 1897−1903.

(17) Sbirrazzuoli, N.; Girault, Y.; Elégant, L. Kinetic investigation of the polymerization of an epoxy resin by DSC and temperature profile determination during cure. *Angew. Makromol. Chem.* **1993**, *211*, 195−204.

(18) Vyazovkin, S.; Sbirrazzuoli, N. Effect of viscosity on the kinetics of initial cure stages. *Macromol. Chem. Phys.* **2000**, *201*, 199−203.

(19) Lachenal, G. Pittcon '98: thermal analysis and the birth of thermal microanalysis. *Analusis* **1998**, *26* (4), M86−M88.

(20) Dollimore, D. Thermal Analysis. *Anal. Chem.* **1992**, *64*, 147R−153R.

(21) Bergner, K.; Albano, C. Thermal Analysis of Peat. *Anal. Chem.* **1993**, *65*, 204−208.

(22) Aguilera, J. M.; Gloria, H. Determination of Oil in Fried Potato Products by Differential Scanning Calorimetry. *J. Agric. Food Chem.* **1997**, *45*, 781−785.

(23) Cornillon, P. Characterization of Osmotic Dehydrated Apple by NMR and DSC. *Lebensm.-Wiss -Technol.* **2000**, *333*, 261−267.

(24) Goff, H. D. The use of thermal analysis in the development of a better understanding of frozen food stability. *Pure Appl. Chem.* **1995**, *67*, 1801−1808.

(25) Sbarato, L. Fast analytical methods. *Ind. Aliment.* **1996**, 35 (344), 43−45.

(26) Kerr, W. L.; Reid, D. S. The Use of Stepwise Differential Scanning Calorimetry for Thermal Analysis of Foods. *Thermochim. Acta* **1994**, *246*, 299−308.

(27) Curini, R.; D'Ascenzo, G.; Materazzi, S.; Chiacchierini, E.; De Angelis Curtis, S.; Lucchetti, M. C. Characterization of Coffees by Thermoanalytical Methods. *Ann. Chim.* **1991**, *81* (11−12), 625−614.

(28) Fiala, M.; Honikel, K. O. Application of differential calorimetry. 2. Detection of additives in meat. *Fleischforschung* **1995**, *75*, 1013−1018.

(29) Coni, E.; Pasquale, P.; Coppolelli, P.; Bocca, A. Detection of Animal Fats in Butter by Differential Scanning Calorimetry: A Pilot Study. *J. Am. Oil Chem. Soc.* **1994**, *71*, 807−810.

(30) Tunick, M. H. DSC Analysis of Dairy Products. *Proceedings of the 26th Conference of the North American Thermal Analysis Society*, Sept 13−15, 1998; Wilkins: Cleveland, OH, 1998.

(31) Gloria, H.; Aguilera, J. M. Assessment of the Quality Oils by Differential Scanning Calorimetry. *J. Agric. Food Chem.* **1998**, *46*, 1363−1368.

(32) Niediek, E. A. Amorphous Sugar, Its Formation and Effect on Chocolate Quality. *Manuf. Confectioner* **1991**, *No. 6*, 91−95.
(33) Roudaut, G.; Dacremont C.; Le Meste, M. Is Tg Responsible for the Crispness Loss of Cereal Based Foods During Storage? *Biopolym. Sci. Food Nonfood Appl.* **1999**, 283−289.
(34) Relkin, P. Differential Scanning Calorimetry: a useful tool for studying protein denaturation. *Thermochim. Acta* **1994**, *246*, 371−386.
(35) Harwalkar, V. R.; Ma, C.-Y. *Thermal Analysis of Foods*; Elsevier Applied Science: London, U.K., 1990; pp 92−98.
(36) Gringberg, V. Y.; Burova, T. V.; Haertlé, T.; Tolstoguzov, V. B. Interpretation of DSC on Protein Denaturation Complicated by Kinetic and Irreversible Effects. *J. Biotechnol.* **2000**, *79*, 269−280.
(37) Louveaux, J.; Maurizio, A.; Vorwohl, G. Methods of Melissopalynology. *Bee World* **1978**, *59* (4), 139−157.
(38) Bogdanov, S. Determination of Moisture, Refractometric Method. A*pidologie* **1997**, extra issue, 11−13.
(39) Gmelin, E.; Sarge, St. M. Calibration of Differential Scanning Calorimeters. *Pure Appl. Chem.* **1995**, *67*, 1789−1800.
(40) Sabbah, R.; El Watik, L. New reference materials for the calibration (temperature and energy) of differential thermal analysers and scanning calorimeters. *J. Therm. Anal.* **1992**, *38*, 855−863.
(41) ASTM (American Society for Testing and Materials). IEC (International Electronic Commission).
(42) Kellner, R.; Mermet, J.-M.; Otto, M.; Widmer, H. M. *Analytical Chemistry, Part IV.12.1: Computer-Based Analytical Chemistry (COBAC), Analytical Quality and Performance*; Wiley-VCH Verlag: Weinheim, Germany, 1998.
(43) Acquistucci, R.; Bucci, R.; Magri, A. D.; Magri, A. L. Thermal Analysis of Food Carbohydrates by Determination of Starch Gelatinization Phenomena. *Fresenius' J. Anal. Chem.* **1997**, *357*, 97−100.
(44) Breitschuh, B.; Windhab, E. J. Direct Measurement of Thermal Fat Crystal Properties for Milk-Fat Fractionation. *J. Assoc. Off. Anal. Chem.* **1996**, *73*, 1603−1610.
(45) Redfern, J. P. Polymer studies by simultaneous thermal analysis techniques. *Polym. Int.* **1991**, *26* (1), 51−58.
(46) Carstensen, J. T.; Van Scoik, K. Amorphous to Crystalline Transformation of Sucrose. *Pharm. Res.* **1990**, *7*, 1278−1281.
(47) Arvanitoyannis, I.; Blanshard, J. M. V.; Ablett, S.; Izzard, M. J.; Lillford, P. J. Calorimetric Study of the Glass Transition Occuring in Aqueous Glucose: Fructose Solutions. *J. Sci. Food Agric.* **1993**, *63*, 177−188.
(48) Raemy, A.; Kaabi, C.; Ernst, E.; Vuataz, G. Precise Determination of Low Level Sucrose Amorphism by Microcalorimetry. *J. Therm. Anal.* **1993**, *40*, 437−444.
(49) Raemy, A.; Schweizer, T. F. Thermal Behaviour of Carbohydrates Studied by Heat Flow Calorimetry. *J. Therm. Anal.* **1983**, *28*, 95−108.
(50) Wang, G. M.; Haymet, A. D. J. Trehalose and Other Sugar Solutions at Low Temperature: Modulated Differential Scanning Calorimetry (MDSC). *J. Phys. Chem. B* **1998**, *102*, 5341−5347.
(51) Kantor, Z.; Pitsi, G.; Thoen, J. Glass Transition Temperature of Honey as a Function of Water Content as Determined by Differential Scanning Calorimetry. *J. Agric. Food Chem.* **1999**, *47*, 2327−2330.
(52) Te Booy, M. P. W.; de Ruiter, R. A.; Meere, A. L. J. Evaluation of the Physical Stability of Freeze-Dried Sucrose-Containing Formulation by Differential Scanning Calorimetry. *Pharm. Res.* **1992**, *9* (1), 109−114.

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