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APPLICATION OF DSC AS A TOOL FOR HONEY FLORAL SPECIES CHARACTERIZATION AND ADULTERATION DETECTION

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Abstract

The thermal behaviour of authentic honeys and sugar syrups (industrial and homemade) was investigated by DSC. To confirm the first previous results concerning the effect of adulteration on the thermal behaviour of authentic honeys, 30 honey samples (Robinia, Lavender, Chestnut and Fir) were analyzed by DSC and their T_g were measured following a suited experimental protocol. The results indicated that this parameter was useful to characterize and to distinguish significantly these varieties between them. Applied to honey samples artificially adulterated with different industrial syrups, DSC showed a detection level of 5–10% depending on the type of syrup. An endothermic phenomenon occurring between 40–90°C during the heating was studied by TMDSC and a new thermal transition similar to a glass-transition was highlighted.

Keywords: adulteration, DSC, glass transition, heat of melting, honey, TMDSC, thermal behaviour

Introduction

The dietary frauds in particular the adulteration are practices in constant progress. Adulteration consists of adding external chemical substance(s) into a food product that contains naturally similar substance(s). The authentication of food products (nature and origin) is of primary importance for both consumers and industries, at all levels of the production process. From the legislative point of view, quality standards have been established through the requirement of quality labels that specify the chemical composition of each product. From the economic point of view, products authentication is essential to avoid unfair competition that can create a destabilized market and disrupt the regional economy and even the national economy. The detec-

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tion of adulteration is a technical problem. Indeed, the basic question is the following: How to detect an adulterant with approximately the same chemical composition of the food product in which it is included?

Numerous techniques are usable [1], but three approaches can be distinguished for the utilization of these techniques. The first approach consists of determining the ratio between some chemical constituents, and assumes these ratios are a constant component of the particular food. From this point of view, it seems to make sense that any addition of any substance(s) into foods will modify the value of these ratios or will highlight an anomaly in its chemical composition. This approach is frequently associated with a large set of analyses and the use of chemometrics. In this area, many pattern classification procedures such as principal component analysis, linear discriminant analysis, or artificial neural networks, can be applied to the dataset in order to compare similarities or differences between sample data and original data. Theoretically, after a multivariate analysis taking into account many analytical factors, adulterated samples form singular groups that can be easily distinguishable from authentic samples.

An alternative approach to this problem could be to search for a specific marker in the product, which could be a chemical constituent (complexes, molecules, nucleic acids) or morphological component (plant cells) that proves either the adulteration or authenticity of the food.

The third possibility for detection of adulteration in foods is the global approach of the food products. The method consists of using analytical techniques derived from physical analysis by considering the whole of the sample in order to show the effects of the adulteration on the physicochemical properties of the sample. Rather than measuring one chemical parameter such as pH, conductivity, water content, ash content, etc… a physical analysis, such as thermal analysis observes the thermal behaviour of the food. The thermal behaviour is followed over changing temperature and/or time.

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

Considering that the detection of adulteration requires knowledge of the food products' physical and chemical properties, our approach followed two steps. We first used DSC for determining the thermal behaviour of pure honeys and pure syrups, and secondly, we used DSC to detect modification of the thermoanalytical curve due to adulteration of honey by syrup addition. In order to complete our understanding of some observed thermal phenomena, we used modulated temperature-DSC.

Material 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.* [2] and confirmed by organoleptic tests. Syrup samples were obtained from French industrial suppliers (Dorsman S.A.R.L.; Ickovich S.A.).

Preparation

Because honey is not a pure and homogeneous material, a suited 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, Germany) during 20 min. To ensure the preparation of the samples was repeatable and for a given sample, time and speed rotation were constant, the mechanical device was connected to a computer (RS232 port) and controlled with a specific software. In order to avoid air incorporation in sample, mixing was applied with a constant speed rotation between 80–120 rpm depending on the viscosity of samples. A mass amount between 5–10 mg was sealed in pierced aluminum crucibles (100 μL).

Differential scanning calorimetry

Apparatus

A Mettler-Toledo DSC model 822^e with ADSC option was used to follow the thermal behaviour of the samples. The differential scanning calorimeter was previously calibrated using indium and zinc standards for temperature and power calibration [3, 4]. The autosampler available on the Mettler-Toledo DSC 822^e was used to automate the experimental procedure. The heating and cooling were made under a constant nitrogen flow (200 mL min^{-1} outside the oven, 100 mL min^{-1} inside the oven).

DSC analysis

The experimental conditions (temperature range, type of crucible, temperature programming, heating rate (20°C min^{-1}), cooling rate (10°C min^{-1}), and weighed mass of sample) was described in [5]. Three DSC runs were carried out to determine several thermal and thermodynamic parameters (T_g, T_{mel}, $\Delta_{cal}H$, ΔC_p) as well as the melting behaviour of the samples at higher temperatures.

TMDSC analysis

We use modulated temperature scanning calorimetry (TMDSC) in order to improve the understanding of specific thermal phenomena, which occurred in our samples. Particularly, we studied the glass-transition and a thermal phenomenon appearing between 40–90°C-temperature range. The parameters for the temperature modulation used for this task were: heating rate of 7°C min^{-1}, a modulation amplitude of ±1°C, and a period of 60 s. Samples were cycled by heating from –65 to –30°C to study the glass-transition, and from 20 to 100°C to study the endothermic phenomenon.

Calculations and statistical tests

The statistical validation of our protocol was previously demonstrated in [5], but the main results are recalled here in the 'Characterization of the thermodynamic parameters' section.

Results and discussion

Thermal behaviour of honeys and syrups

Figures 1 and 2 show typical DSC-curves of honey (Lavender) and sugar syrup (beet and isoglucose) respectively. In most cases, three thermal phenomena were observed in thermoanalytical curves of honeys:

i) a baseline deviation of the thermoanalytical DSC curve, characterized by a change in heat capacity and interpreted as a glass transition (–44 to –36°C); the glass transition temperature is taken at the onset of the thermal effect and is called T_g,

ii) an endothermic phenomenon (relatively weak) in the temperature range of 40 to 90°C, called transition 2,

iii) a very wide and intense endothermic peak in the temperature range of 100–120°C to 180–220°C, called transition 3.

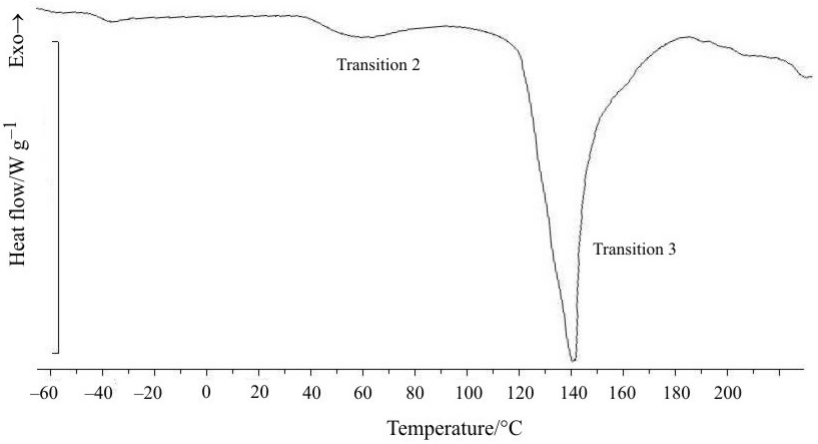

Fig. 1 DSC scan (linear scan temperature) for Lavender honey

Significant differences in the T_g position and intensity have been observed between honeys and between honeys and syrups (cane and beet). The differences were both qualitative and quantitative. For example, the mean T_g was respectively –40.7, –42.9, –36.7 and –32.0±0.5°C for Robinia, Lavender, Fir honeys and cane syrup. This observation was completed by noting the presence or the absence of characteristic phenomena (glass transition observed and measured for honeys but non-observed for isoglucose syrups). For beet and cane syrups (moisture: 50%), DSC analyses showed the presence of a typi-

cal endothermic peak in the range –20 to 0°C (Cf. Fig. 2, transition 1) linked to the melting of ice of the sample [6]. The associated enthalpy change (noted $\Delta_{cal}H_1$), corresponding to the melting of the frozen water, was not observed in honeys because the water is included in the sugar network.

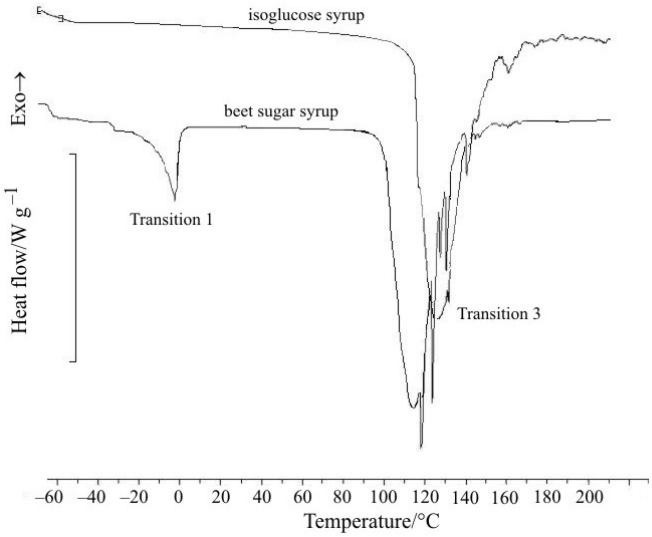

Fig. 2 DSC scan for isoglucose and beet sugar syrups

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 between –36.7 and –42.9°C for honeys.

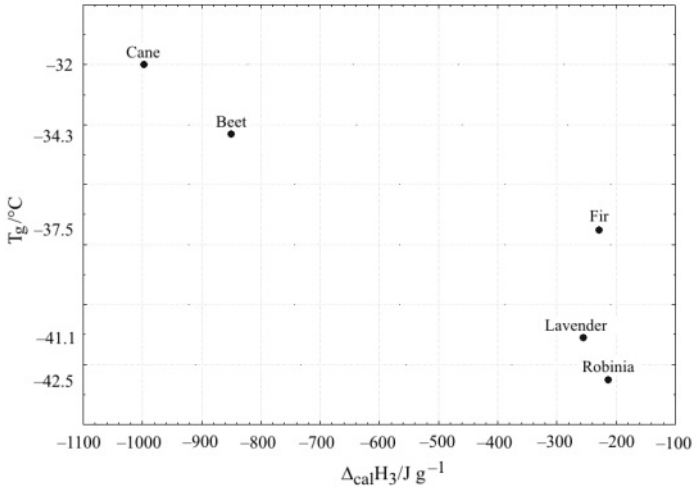

Fig. 3a Plane-projection of two factors. T_g/°C *vs.* $\Delta_{cal}H_3$/J g^{-1}. Distinction between syrups and honeys from thermal and thermodynamic parameters

The observed difference between T_g of samples was about 1–6°C for nectar-honeys and about 3°C for syrups. In contrast, differences between the T_g of nectar-honeys and syrups are 10°C or more (Cf. Fig. 3a). This observation showed the possibility of using the glass transition temperature to distinguish between honeys and syrups. Besides, there was no significant difference in T_g temperature between cane or beet syrups (–33.0±2.0°C) and honeydews (–36.7±0.3°C) represented by Fir honey in our study (Fig. 3a).

From the general point of view, higher is the thermal and thermodynamic behaviour differences between pure honeys and syrups (industrial or homemade), easier is the detection of the adulteration. Therefore, it is of prime importance to find the best parameters able to distinguish pure honeys from syrups and a fortiori, from adulterated honeys. If these parameters are efficient for this task, it is legitimate to wonder whether they will be efficient in the discrimination between honey floral species. With the present protocol, transition 3 did not appear as the most appropriate parameters to discriminate between floral species but was useful for syrup characterization because the thermal effect associated to this transition have the largest enthalpy change $\Delta_{cal}H_3$. Most part of this thermal effect can be attributed to the melting of sugars. Figure 3a illustrates this aspect. If the glass-transition of the isoglucose syrups had been measured in our experiments, the corresponding points should have appeared in the middle bottom of the graph, because $\Delta_{cal}H_3$ of these syrups is about 500 J g^{-1}.

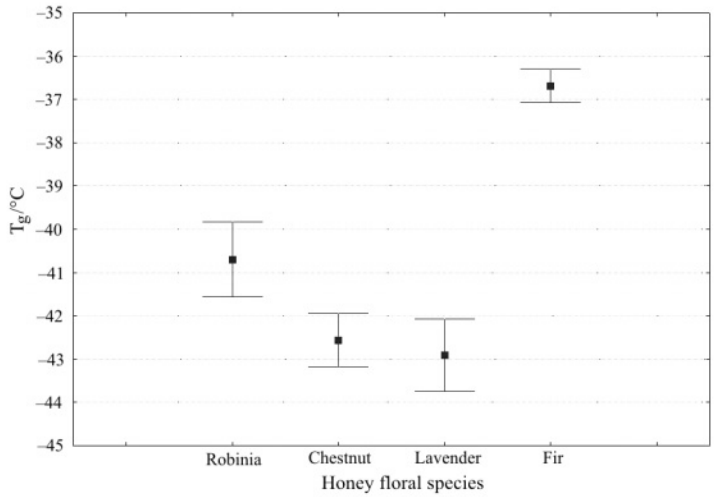

Fig. 3b Mean glass-transition temperature (T_g) of several unifloral honey species. ■ – Mean of group. Error vertical bars correspond to 0.95*SE where *SE* is the standard error of the measurements

Additional results obtained with four categories of honeys Robinia, Chestnut, Lavender and Fir, using respectively 6, 8 and 10 samples, are presented in Fig. 3b. The usefulness of the T_g as an efficient parameter to distinguish honey floral species is also confirmed by these experiments, where the non-overlapping between the dif-

Table 1 Statistical values for six thermal parameters

	Transition 2		Transition 3	Glass transition		
	T_{onset}	$\Delta_{cal}H_2$	T_{onset}	T_{onset}	$T_{midpoint}$	ΔC_p
Mean 1, $\overline{X}$ (n=6)	35.4	–24.3	119.5	–42.4	–38.8	0.73
Mean 2, $\overline{X}$ (n=6)	35.7	–24.2	119.2	–42.3	–39.1	0.69
Standard Deviation 1 (SD1)	0.310	0.564	0.361	0.309	0.706	0.021
Standard Deviation 2 (SD2)	0.108	0.402	0.185	0.149	0.249	0.025
		Merged series: n=12				
Mean	35.5	–24.2	119.5	–42.4	–38.9	0.71
Sandard Deviation (SD)	0.294	0.546	0.331	0.268	0.590	0.037
F-test (F calculated)	3.92	1.96	0.26	4.32	8.04	1.20
.t-test (t calculated)	3.06	0.35	1.81	0.71	0.98	2.95
F theoretical for (α=0.01)=10.97		For (α=0.025):7.15			For (α=0.05):5.05	
.t theoretical for (α=0.01)=3.17		For (α=0.025):2.63			For (α=0.05):2.23	
H_0 is accepted if $F_{calculated}<F_{theoretical}$ and $t_{calculated}<t_{theoretical}$						

ferent class of honeys can be noted, except for Chestnut and Lavender. Therefore, this observation does not cause any limitation of the analytical approach because the real confusion between these two honey categories is not possible in practical cases.

Characterization of thermodynamic parameters

Because honeys may differ from one sample to another according to multiple natural factors, the repeatability of the thermal effects obtained for honeys was established. 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 with multiple analytical conditions: temperature programming (10, 20, 30°C min^{-1}), number of replicates (5–10 replicates per series). We used an *F*-test [7] (two-sided test) for comparison of variances and a *t*-test (Student-test) for comparison of means. Results show a good repeatability of measurements (Cf. Table 1). Therefore, the sets were merged to evaluate the standard deviation.

Effect of experimental adulterations on thermal behaviour of honey

The results of adulterations made with beet and cane sugar (not presented here) resulted in a significant displacement of the T_g of the mixture and a dramatic increase in the $\Delta_{cal}H_3$, so the detection of adulteration by this type of syrups is easy. That is why, we oriented our study more particularly towards the adulteration by isoglucose syrups which is a more difficult problem. Therefore, syrup additions at 5, 10, 20, 40 and 60% were carried out with four isoglucose syrups.

Transition 2

The effect of the adulteration with syrups on the transition 2 follows a linear relationship. Figure 4 shows an example (for isoglucose 2) of the effect of different additions of syrup on the transition 2. This observation was confirmed by a second set of experiments and the sensitivity level of the method was within the range 5–10% depending of the type of syrup used. For this percentage of added syrup, there was a decrease of the absolute value of the enthalpy change $\Delta_{cal}H_2$ of 3–5 J g^{-1}, while the determination error associated with the measurement is about 1 J g^{-1}.

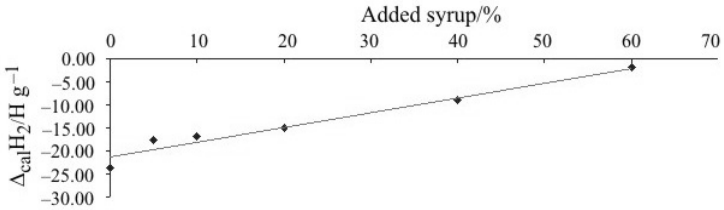

Fig. 4 Effect of added syrup on the enthalpy change of transition 2 of a pure honey

Glass transition

Linear relationships are also obtained for the evolution of the glass-transition in terms of percentage of added syrup (Cf. Fig. 5). The addition of industrial sugar syrup was detected by a statistically significant temperature-gap. 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 sample acts as a plasticizer on the T_g values.

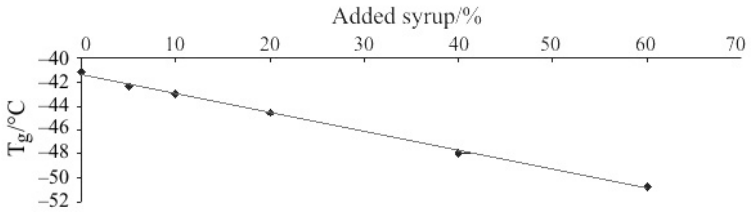

Fig. 5 Effect of added syrup on the glass-transition temperature of a pure honey

Numerous authors reported both the plasticizing effect of water and the water activity (a_w) effect on T_g [8–12]. 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.* [8] on sucrose solutions and by Kantor *et al.* [6] on water/honey mixtures.

Because the glass transition is a non-equilibrium thermodynamic transition, the glass transition temperature is expected to vary with the heating rate. Experiments have been made using four heating rates (β), for the authentic sample and for the adulterated sample (10% of syrup). Then, T_g was measured for each scan. As expected, higher is the heating rate, higher is the glass transition temperature, this increase follows a linear relationship. The comparison of authentic sample and adulterated sample shows a significant deviation between two straight-lines. Higher is the percentage of adulteration, higher is the distance between the two straight-lines. The next step of this work is the determination of the limits of conformity based on these experiments applied on each honey variety.

TMDSC

Contrary to conventional DSC where the heating rate is constant and the temperature follows the linear relationship: $T=T_0+\beta_0 t$, the temperature-modulated DSC (TMDSC) provides a superposition of a periodic modulation to a constant heating and the temperature is expressed as $T=T_0+\beta_0 t+f(t)$ whereas T_0 is the initial temperature, β_0 is the heating rate, t is the time and $f(t)$ is either a saw tooth function, a sine function or a stepwise form. Therefore, in the sine case, the temperature function can be written as: $T(t)=\beta_0 t+T_a\sin(\omega t)$. The TMDSC approach allows the simultaneous use of a low underlying heating rate that increases the temperature resolution, and a high instant heating rate that improves the sensitivity (amplification of weak transitions). After mathematical treatment with Fourier transform, the result is the separation of the total

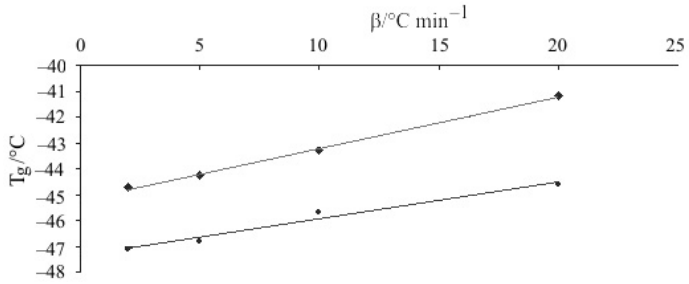

Fig. 6 Plot of the glass-transition temperature *vs.* heating rate. Line at top – pure honey (r^2=0.9975); Line at bottom – honey+10% of syrup (r^2=0.9739)

heat flow signal into components that are often called the reversing and non-reversing signals. These two signals are related to heat capacity and kinetic components of the response according to: $dQ/dt=C_p dT/dt+f(t,T)$ where dQ/dt is the total heat flow, dT/dt is the temperature scanning rate and $C_p dT/dt$ represents the reversing signal. $f(t,T)$ is a time-temperature dependant function representing the kinetically controlled component in which all thermal events such as the enthalpic relaxation appears. The curve where this phenomenon appears is also called non-reversing component or non-reversing signal [13–15].

In our experiments, we use TMDSC both to separate the glass-transition from the enthalpic relaxation (in order to measure more precisely the glass-transition temperature) and to extend our understanding of the endothermic phenomenon, called transition 2, which occurs between 40–90°C.

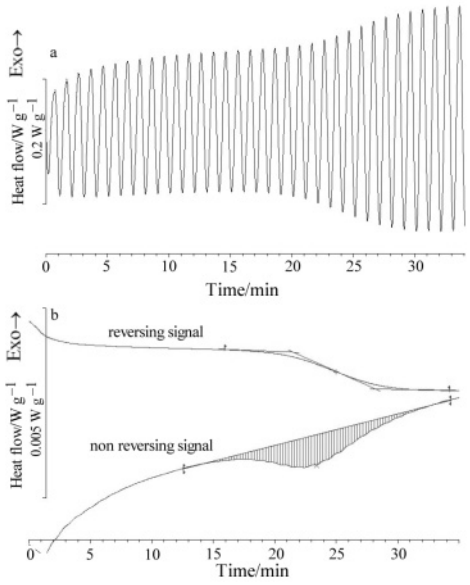

Fig. 7 a – Original modulated signal and b – transformed signal for the glass-transition of a honey sample (Lavender). Temperature range: –65 to –30°C

Figure 7a shows the TMDSC signal obtained in the temperature range of the glass-transition. Deconvoluted signals presented in Fig. 7b, show the characteristic base line deviation associated with the glass-transition in the reversing component of the signal, and an endothermic effect recorded in the non-reversing component which can be attributed to the enthalpic relaxation of the glass. Separation of these two thermal effects makes it possible to obtain higher reproducibility in the determination of the glass transition temperature (T_g).

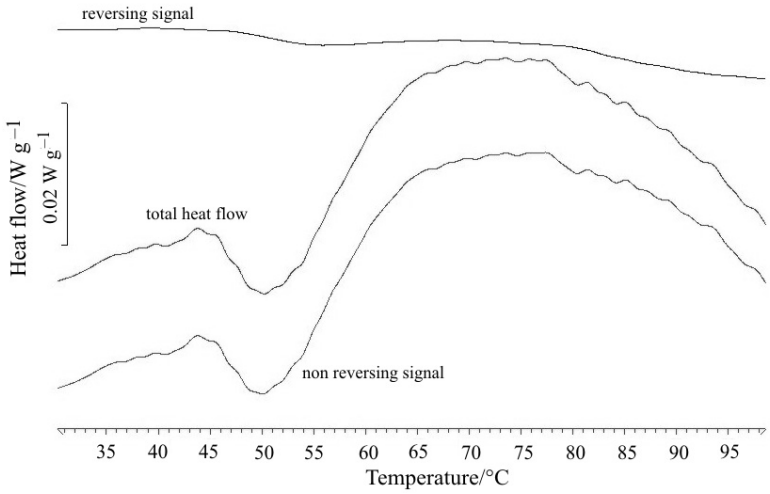

Fig. 8 Total heat flow, reversing and non-reversing heat flow obtained from original TMDSC scan of a sample (Lavender). Temperature range: 35–100°C

In a very similar way, deconvolution of the signal measured in the temperature range of transition 2 (40–90°C, Fig. 8) allows the separation of the thermal effect associated to the glass transition recorded in the reversing component of the signal, from the endothermic effect recorded in the non-reversing signal. According to the temperature range [16–21], this endothermic peak can be attributed to the gelatinization of a part of honey compounds, i.e. starch-sugars-water, while another components undergo simultaneously a thermal transition (recorded in the reversing signal) similar to a glass transition of the amorphous part of the material. Measurement and quantification of this two phenomena was not possible using conventional DSC.

This endothermic effect is followed by an exothermic effect before transition 3. Other kind of analyses is now processed to confirm whether this phenomenon can be attributed to a partial crystallization of the material.

Conclusions

Differential scanning calorimetry is a powerful technique for characterizing the thermal behaviour of honeys and for detecting the effect of adulteration on physicochemical and structural properties of samples.

This work shows the interest of conventional DSC for quantifying the effect of the adulteration by industrial sugar syrups on authentic honeys. Various parameters such as glass-transition temperature and enthalpies changes can be used to reach the aim and it has been shown that our method can be used at various heating rates without losing the ability to detect the plasticizing effect of the adulterant.

Finally, the use of the modulated temperature DSC for a better understanding of certain phenomena is well suited. The approach can give some explanations concerning the chemical reactions and structural transformations occurring to the samples during the heating.

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