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Abstract: Moisture adsorption isotherms of unroasted and roasted carob powder (Ceratonia siliqua L.) at 15, 25, and 40 °C were determinated by the static gravimetric method over the wide range of water activity from 0.06 to 0.90. GAB model fitted well the experimental adsorption data showing sigmoid shape curves for both samples. Claussius-Clapeyron equation showed that the adsorbed moisture is strongly bound to the surface of unroasted carob powder in low moisture content than that in roasted sample. The variation of the net integral enthalpy and entropy with the moisture content exhibited maximal and minimal values for unroasted carob powder at low moisture content, respectively. Gradual decrease in enthalpy and increase in entropy were observed for roasted carob powder. Combining the glass transition temperature lines with adsorption isotherms, it was found that roasted carob powder is stable up to 43 aw in comparison with unroasted carob powder (up to 38 aw).

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Dear editor,

We are sending you a manuscript entitled "***Thermodynamic properties and glass transition temperature of unroasted and roasted carob (Ceratonia silique L.) powder***". Carob powder is a valuable product with high content of dietary fibre and polyphenolic substances. Carob powder/flour is usually sold as such (after milling dried pods) or roasted in order to promote cocoa-like aroma and develop product with higher antioxidants content, as was mentioned in Introduction. We prepared manuscript with scientific data which were partly presented in The 10th International Conference on Water in Food. We have focused on the thermodynamic analysis of carob powder (unroasted and roasted) as a part of our research. To the best of our knowledge, there is no data available on that topic in scientific journal.

We hope that you will find this manuscript worth to the broader audience and suitable for publication in *Food Chemistry VSI: Water in Food)* as an **original research article**. The manuscript contains **4641** words, **2** tables, **4** figures and **37** references. In addition, supplementary materials were also prepared.

Yours sincerely

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***Highlights (for review)**

- GAB isotherms successfully described the experimental moisture content data
- Strongly bound water molecules in unroasted carob powder were observed
- Different integral thermodynamic properties were determined
- Roasted carob powder exhibited better stability

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Thermodynamic properties and glass transition temperature of roasted and unroasted carob (*Ceratonia siliqua* L.) powder

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1. Introduction

Carob powder is the product of the fruit of *Ceratonia siliqua L*. The powder is usually prepared from mature, dried carob pod (without seed) after milling to desired particle size. Carob powder is a good source of sucrose and other simple sugars (maltose, mannose), unsaturated fatty acids and minerals such as calcium, potassium and iron (Ayaz et al., 2009). High content of dietary fibre is the most important parameter, which makes the carob powder applicable in various food products such as pasta or bread (Biernacka, Dziki, Gawlik-Dziki, Rozylo, & Siastala, 2017; Turfani, Narducci, Durazzo, Galli, & Carcea, 2017). Carob powder is also used as a replacer of cocoa in cocoa- and chocolate-based products decreasing the content of caffeine and theobromine but keeps the cocoa-like aroma, particularly when roasting (Loullis & Pinakoulaki, 2018). In addition, carob powder is the rich source of polyphenolic substances exhibited promising effects on the human health acting such as antioxidant, antibacterial, anti-inflammatory, and anti-diabetic agents (Rtibi et al., 2017). The knowledge of moisture sorption behaviour of food matrix is crucial to select appropriate packaging material or storage condition. Several studies have reported about the moisture adsorption of various plant-based powders such as elecampe and burdock root powders (Cervenka, Kubinova, Juszczak, & Witczak, 2012), chironji karnels (Sahu et al., 2018), coffee (Baptestini, Correa, de Oliveira, Cecon, & Soares, 2017; de Oliveira et al., 2017), sesame seed (Kaya & Kahyaoglu, 2006) or wheat and chestnut flour (Moreira, Chenlo, Torres, & Prieto, 2010). Equilibrium relationship of the moisture content and water activity at the constant temperature has to be determine for calculation of various properties of sorbed water and thermodynamic characteristics of sorption process. There are many empirical equations proposed for describing the moisture adsorption of food material (Al-Muhtaseb, McMinn, & Magee, 2002) with Guggenheim-Anderson-de Boer's (GAB) equation as the most frequent

used mathematical description of experimental moisture sorption data, which can be applied in the wide range of water activities. GAB equation is also preferred as it provides monolayer moisture content, a key parameter corresponding to the stability of the food material.

The thermodynamic analysis of equilibrium moisture sorption data provides a closer insight into the microstructure of food material and describes the interaction in water-substrate interface. The isosteric heat of sorption, differential entropy, integral enthalpy and entropy describe the behaviour of water and the energy requirements of heat and mass transfer in biological material (Cervenka, Hlouskova, & Zabcikova 2015; McMinn, McKee, Ronald, & Magee, 2007; de Oliveira et al., 2017; Polatoglu, Bese, Kaya, & Aktas, 2011).

Despite the fact that water activity is an important parameter describing available water in biological material, it is not sufficient to describe the secondary processes of change-in-state in foodstuffs. Some deteriorative changes in food such as stickiness, caking, crystallization and structural collapse are related to its rubbery state. Hence, water activity has been often combine with glass transition temperature (T_g) to provide an integrated approach to study the role of water in foods (Tonon et al., 2009). The glass transition is a thermodynamic second-order phase transition, from glassy to rubbery state, which is related with the change in the heat capacity of the material that occurs over temperature range. The main difference between the two states is related to molecular mobility: very low in glassy and higher in rubbery state. In general, the glassy state can be considered as a more stable in the shelf life of foodstuff than glassy form (Mosquera, Moraga, & Martinez-Navarrete, 2012). The glass transition temperatures of biological materials depend mainly on the quantity of water, chemical composition and molecular weight of the solutes present in material (Rahman, 2006).

Although carob powder and flour are used in various formulas, there is no data about moisture sorption and thermodynamic properties in the literature. Therefore, the aim of this study is to determine the moisture adsorption behaviour of unroasted and roasted carob powder, to

elucidate their thermodynamic properties and glass transition phenomena in relation to their storage stability. The roasting process changes the thermodynamic of moisture adsorption is the hypothesis which is evaluated in this study.

2. Materials and methods

2.1 Sample preparation

Dry carob pods (*Ceratonia silique* L.) without seed were purchased in local supplier. Pods were milled using knife mill Grindomix GM 200 (Retsch®, Haan, Germany) to obtain powder. The powder was passed through analytical sieve to get particles smaller than 600 µm, which were subsequently used for further experiment. The roasting condition was chosen with respect to the low content of toxic Maillard reaction products as was described by (Cepo et al., 2014), i.e. the carob powder was heated in drying oven at 130 °C for 30 min in 0.5 cm layer´s thickness. Both roasted and unroasted carob powder samples were stored in evacuated plastic bag at room temperature until used.

2.3 Chemical analysis

Crude fat (920.85), crude protein (920.87), reducing sugars (939.03), total dietary fibre (985.29) and ash content (923.03) were determined according to AOAC official methods (AOAC, 2007).

2.4 Moisture adsorption study

Prior the moisture adsorption study, carob powder was carefully dehydrated in closed desiccator with freshly dried silica gel until the weight constancy. Static gravimetric method was applied to examine equilibrium moisture content (EMC; g H_2O g^{-1} dry basis, db) at particular water activity level. Briefly, salt slurries (LiBr, LiCl, CH_3COOK, $MgCl_2$, K_2CO_3,

76 $Mg(NO_3)_2$, NaBr, $CoCl_2$, KI, NaCl, KCl, K2SO4) were prepared to induce water activity level
77 in the range from 0.058 to 0.980 as was described by (Greenspan, 1977). Approximately 1.0 g
78 of sample was placed above salt slurry in desiccator and equilibrated at 15, 25, and 40 °C
79 until the weight changed of less than 0.0005 g using analytical balance with an accuracy
80 ±0.0001 g. In order to prevent the mould growth, 0.5 g of thymol was placed in desiccators
81 with relative humidity > 60% (a_w > 0.60). The moisture content was determinate by AOAC
82 Official Method 930.04 (AOAC, 2007). Three replicates of equilibrium moisture contents at
83 each a_w level were used.
84
85 2.5 Isotherm modelling
86 Equilibrium moisture content was plotted as a function of water activity using the GAB
87 equation for both unroasted and roasted carob powder samples. The GAB equation was used
88 in the form:
89
90 $$M = \frac{M_0 C K a_w}{\left[\left(1 - K a_w\right)\left(1 - K a_w + C K a_w\right)\right]} \quad (1)$$
91
92 where M_0 (g g^{-1} db) is monolayer moisture content and C and K represent parameters.
93
94 2.6 Thermodynamic properties of moisture sorption
95 Net isosteric heat, q_{st} (kJ mol^{-1}) was obtained from Claussius-Clapeyron equation:
96 $$q_{st} = -R\frac{d\ln a_w}{d\left(1/T\right)} \quad (2)$$
97
98 where T is the temperature (K) and R is the universal gas constant (8.314 J $(mol\ K)^{-1}$). Net
99 isosteric heat is the important parameter describing the energy requirements for drying and the

state of water molecules in dried products. The relationship between q_{st} and differential entropy of sorption (S_d, kJ (mol K)$^{-1}$) is described by the equation:

$$(ln\, a_w)_M = -\frac{q_{st}}{RT} + \frac{S_d}{T} \quad (3)$$

Net isosteric heat and differential entropy were obtained from the slope and intercept of the fitted line ln(a_w) vs. 1/T at the constant moisture content (M), respectively.

2.7 Compensation theory

Linear relationship between q_{st} and S_d is the main assumption of applying the enthalpy-entropy compensation theory (McMinn, Al-Muhtaseb, & Magee, 2005), i.e.:

$$q_{st} = T_\beta S_d + \Delta G \quad (4)$$

where isokinetic temperature (T_β) and the change of the free energy (ΔG) were calculated using linear regression. At isokinetic temperature, all the reactions occurred at the same rate during sorption process. If $T_\beta \neq T_{hm}$ (harmonic temperature), the compensation theory is valid. Harmonic temperature was defined in the form (Krug, Hunter, & Grieger, 1976):

$$T_{hm} = \frac{n}{\sum_1^n (1/T)} \quad (5)$$

where n is the number of isotherms.

2.8 Integral enthalpy and entropy

123 Total energy required for binding the water molecules to the solid is described by the net
124 integral enthalpy (q_{int}). It is similarly calculated as differential enthalpy (i.e. isosteric heat) but
125 at constant spreading pressure ϕ

126 $$q_{eq} = -R\left[\frac{d\left(\ln a_w\right)}{d\left(1/T\right)}\right]_{\phi} \quad (6)$$

127

128 After plotting ln(a_w) against *1/T* at constant spreading pressure using linear regression
129 technique, the slope of the curve was used to estimate net integral enthalpy. The variation of
130 net integral enthalpy with moisture content shows whether the interactions of water/solid are
131 greater than those among water molecules (Vigano et al., 2012).
132 The difference between energy of liquid and solid phases can be described by spreading
133 pressure, which corresponds to the lowering of surface tension of the liquid during adsorption.
134 In this study, the spreading pressure was estimated using a procedure described in Kaya &
135 Kahyaoglu (2006) from the equation:
136

137 $$\phi = \frac{K_B T}{A_m}\int_0^{a_w}\frac{M}{M_0 a_w}d\left(a_w\right) \quad (7)$$

138

139 where K_B is the Boltzmann constant (1.38×10^{-23} J K^{-1}), T is temperature (K), A_m is the area of
140 the water molecule (1.06×10^{-19} m^2), M is the equilibrium moisture content, and M_0 is the
141 monolayer moisture content. When substituting GAB equation (Eq. 1) to the Eq. (7), the
142 following equation was obtained for the calculation of spreading pressure (Lago, Liendo-
143 Cardenas, & Norena, 2013):
144

145 $$\phi = \frac{K_B T}{A_m}\ln\left[\frac{1+CKa_w - Ka_w}{1-ka_w}\right]_{0.05}^{a_w} \quad (8)$$

The randomness of motion of water molecules is described by the net integral entropy (S_{int}) using the equation:

$$S_{int} = -\frac{q_{int}}{T} - R\ ln(a_w) * \quad (9)$$

where $(a_w)^*$ is the geometric mean water activity obtained at the constant spreading pressure at different temperatures (Torres, Moreira, Chenlo, & Vazquez, 2012).

2.9 Differential scanning calorimetry and modelling of the glass transition curves

The glass transition temperatures of roasted and unroasted carob samples at different moisture contents were measured by differential scanning calorimeter DSC 204F1 Phoenix (Netzsch, Germany). Prior to analyses, calorimeter was calibrated by using a multipoint method (Hg, In, Sn, Bi, Zn and CsCl). Samples (8–10 mg) were sealed in aluminium pans and an empty pan was used as reference, while liquid nitrogen was used for sample cooling before runs. Thermal program consisted in a two cycle-scan model with the temperature ranging from - 80 to 200 °C at a rate of 10 °C min^{-1}. All analyses were taken in two independent scans. Obtained thermograms were analysed using NETZSCH *Proteus*® thermal analysis software (Netzsch, Germany). The glass transition temperature (T_g) was taken as the midpoint of the baseline shift in the second scan. The plasticizing effect of water on the glass transition temperature was described by the following Gordon-Taylor model:

$$Tg = \frac{T_{gs}X_s + kT_{gw}X_w}{X_s + kX_w} \quad (10)$$

where T_g is experimental glass transition temperature (°C), T_{gs} is glass transition temperature for anhydrous solid (°C), T_{gw} is glass transition temperature of pure water (°C), X_s is a mass fraction of the solute, X_w is a mass fraction of water and k is a constant depending on the

system respectively. Glass transition temperature of pure water was taken as – 135 °C (Moraga, Talens, Moraga, & Martinez-Navarrete, 2011).

2.10 Statistical analysis

The parameters of GAB isotherms and Gordon-Taylor equation were evaluated using non-linear regression analysis (OriginPro v. 5.0, OriginLab Corp., Northampton, MA, USA) using Revenberg-Marquardt iteration algorithm until minimal values of the residual sum of squares (RSS) were obtained. The goodness of fit of the experimental data was evaluated by the coefficient of determination (r^2), and by the mean relative percentage deviation (E, %) between experimental (M_{exp}) and predicted values (M_{pred}) moisture content using the formula

$$E = \frac{100}{n}\sum_{i=1}^{N}\frac{\left|M_{exp} - M_{pred}\right|}{M_{exp}} \quad (11)$$

The model is acceptable if E value is below 10%. Statistical differences were evaluated using One-way analysis of variance (ANOVA). All the statistical treatments of the data were performed at the probability level $p = 0.05$.

3. Results and discussion

3. 1 Proximate composition of carob powder samples

The main composition of both unroasted and roasted carob powder is presented in Suppl. 1. As can be seen, roasting at 130 °C for 30 min did not influence the content of crude protein, crude fat and total dietary fibre. Reducing sugars decreased from 13.12. to 12.51 % after thermal treatment ($p>0.05$) and the small but significant increase in ash content from 3.29 to 3.70 % has been observed ($p<0.05$). The both decrease in sugar content and increase in ash

content were similar to roasting of carob pod powder in temperatures ranged from 110 °C to 150 °C, probably due to the involvement in Maillard reaction (Boublenza et al., 2017).

3.2 Moisture adsorption isotherms

Equilibrium moisture content of carob powder was plotted against water activity and isotherms were constructed by applying GAB equation (Eq. 1) via non-linear regression (Fig. 1). The estimated parameters of the GAB isotherm are presented in Table 1. together with high value of r^2 (0.990–0.997) and low mean relative percentage deviations E (5.76–8.91 %) for both samples at all the temperatures.

As can be seen from Fig. 1, equilibrium moisture content is increasing with the increase of water activity for both samples. The type II isotherms (sigmoid shape) were observed for carob powders according to the BET classification (Brunauer, Emmett, & Teller, 1938). Significantly higher moisture content at 15 °C was determined for unroasted carob powder in the whole water activity range (p<0.05), while isotherms for 25 °C and 40 °C were almost identical (p>0.05). Roasted carob powder exhibited similar EMC up to 0.43 a_w for all the temperatures followed by significantly higher EMC for isotherm at 15 °C in the range from 0.43 to 0.90 a_w (p<0.05). The latter is caused by the exothermic character of sorption process as was confirmed for other food products (Moreira, Chenlo, Torres, & Prieto, 2010; Baptestini, Correa, de Oliveira, Cecon, & Soares, 2017; Polatoglu, Bese, Kaya, & Aktas, 2011). Roasted carob powder adsorbed less moisture than unroasted counterparts at all the temperatures probably due to the structural and chemical changes initiated by the high temperature during roasting process. The interactions between the carbohydrates and proteins or the increase of protein hydrophobicity may occur during temperature treatment. It follows the limited number of sorption sites capable to adsorb molecules as found for Argentinean Algarobba pods (Prokopiuk, Martinez-Navarrete, Andres, Chiralt, & Cruz, 2010), Yerba mate

leaves (Cervenka, Hlouskova, & Zabcikova, 2015) and coffee (Rocculi et al., 2011). However, higher EMC values above ~ 0.70 a_w at 15 °C were observed for roasted carob powder in agreement with the study of Roculli et al. (2011). The upper part of isotherm corresponds to the adsorption of bulk water into the large pores of solid causing swelling and solute dissolution. It may suggest that roasting process increase the number of pores (or their volumes), hence the equal volume of water resulted in higher adsorption. However, several authors subjected moisture adsorption and particle size distribution to examination and found that there is no significant relationship (Baptestini, Correa, de Oliveira, Cecon, & Soares, 2017; de Oliveira, Correa, de Oliveira, Baptestini, & Vargas-Elias, 2017). They found the differences in moisture sorption characteristics among coffee samples with various degree of roasting but did not find correlation with particle size. Therefore, some chemical modification could be also responsible for higher moisture adsorption of roasted carob powder at high water activities.

Monolayer moisture is the important parameter defining moisture strongly adsorbed to the surface of the food matrix. The monolayer values were estimated to 0.065–0.082 g g^{-1} db and 0.047–0.063 g g^{-1} db for unroasted and roasted carob powder, respectively. These values were close to monolayer values of wheat and chestnut flour (Moreira, Chenlo, Torres, & Prieto, 2010), prosopis pod (Prokopiuk, Martinez-Navarrete, Andres, Chiralt, & Cruz, 2010) and chironji kernels (Sahu et al., 2018). As can be seen from Table 2, monolayer values for roasted carob samples were significantly lower in comparison with unroasted carob samples at the same temperature similarly to our previous study (Cervenka, Hlouskova, & Zabcikova, 2015).

3.3 Differential thermodynamic properties of moisture adsorption

The net isosteric heat of adsorption was calculated according the Eq. (2) and plotted against moisture content (Fig. 2). The curves showed that the net isosteric heat decreased with the increase of EMC as was described in foods (McMinn, McKee, Ronald, & Magee, 2007; Moreira, Chenlo, Torres, & Prieto, 2010). At low moisture content, surface of the solid exposed active sites strongly bound the water molecules and formed mono-molecular layer. Once these highly-energy active sites are occupied, the lower-energy active sites are exposed with the increase of moisture content (McMinn, McKee, Ronald, & Magee, 2007). At particular EMC point, the heat of sorption remained stable indicating the multilayer adsorption process. Unroasted carob powder exhibited higher net isosteric heat of adsorption then that of roasted carob powder in the range of moisture content from 0.08 to 0.12 g g^{-1} db (Fig. 2A). Net isosteric heat of adsorption for unroasted carob powder was 24.9 kJ mol^{-1} at 0.08 g g^{-1} db followed by sharp decline to 10.1 kJ mol^{-1} at 0.10 g g^{-1} db, while small decrease from 6.8 to 6.2 kJ mol^{-1} was obtained for roasted carob powder with the increase of moisture content from 0.08 to 0.10 g g^{-1} db. Above particular moisture content (i.e. 0.12 g g^{-1} db), roasted carob powder showed lower net isosteric heat of adsorption (2.0 kJ mol^{-1}) than that for unroasted counterparts (4.8 kJ mol^{-1}). It corresponds with our previous findings for green and roasted Yerba mate leaves (Cervenka, Hlouskova, & Zabcikova, 2015). Fig. 2B shows the effect of moisture content on differential entropy, S_d, calculated from Eq. (3). Differential entropy for both carob samples increased with the increase of moisture content similarly to medium-light roasted coffee in a study of de Oliveira et al. (2017). They explained the increase in S_d by the formation of water molecule layers during adsorption process. At high equilibrium moisture content, the active sites are saturated and S_d values approaches to zero (differential entropy of pure water).

3.4 Compensation theory

The values of net isosteric heat of sorption was linearly dependent on differential entropy values for both carob powder samples as shown in Suppl. 2. Therefore, the enthalpy-entropy compensation theory is valid and can be described by the equations q_{st} = 357.1 S_d + 0.41 (r^2 = 0.999) and q_{st} = 518.5 S_d – 1.77 (r^2 = 0.998) for unroasted and roasted carob powder, respectively. The harmonic mean temperature (T_{hm}) was calculated from the Eq. (5) and found as 299.6 K. The isokinetic temperatures T_β were (357.1 ± 1.1) K and (518.5 ± 7.9) K for unroasted and roasted carob powders, respectively. Since the condition $T_{hm} < T_\beta$ was confirmed, the adsorption process is enthalpy-driven. In addition, positive free Gibbs energy for unroasted carob powder (ΔG = 0.41 kJ mol^{-1}) indicates nonspontaneous adsorption process while spontaneity was evident for roasted powder (ΔG = -1.80 kJ mol^{-1}) in the whole range of EMC. Enthalpy-driven and nonspontaneous adsorption process was determined in oatmeal biscuit and flakes (McMinn, McKee, Ronald, & Magee, 2007) or Yerba mate leaves (Cervenka, Hlouskova, & Zabcikova, 2015), while the spontaneity of adsorption was observed for Turkish fermented sausage (Polatoglu, Bese, Kaya, & Aktas, 2011).

3.5 Spreading pressure and integral thermodynamic of adsorption

The spreading pressure for both carob powder samples was calculated according to Eq. (7) and its variation with water activity is presented in Suppl. 3. The values of ϕ increased with the increase of a_w and decreased with the increase of temperature as was found in other foods (Polatoglu, Bese, Kaya, & Aktas, 2011; Kaya & Kahyaoglu, 2006). Spreading pressure represents the free energy at the surface of sorption sites of the solid and its high values indicates higher affinity of water molecules to active sites. Unroasted carob powder has lower spreading pressure at a given a_w values in comparison with roasted carob powder in the whole water activity range. Similar conclusions were observed when examining unroasted and roasted dehulled sesame seed (Kaya & Kahyaoglu, 2006).

293 The net integral enthalpy (q_{int}) was calculated using Eq. (9), by plotting ln(a_w) against 1/T for
294 a specific spreading pressure. In Fig. 3, the net integral enthalpy of adsorption of carob
295 powders was plotted as a function of moisture content. For unroasted carob powder, the
296 enthalpy increases until maximum value of 24.37 kJ mol^{-1} at moisture content of 0.12 g g^{-1} db
297 followed by gradual decrease with further increase in moisture content (Fig. 3A). At low
298 moisture content, water is adsorbed on the most accessible sites on surface of carob powder
299 causing swelling of material. The swelling is responsible for opening of new high energy
300 locations where new water binding can be developed. At higher moisture content (above 0.12
301 g g^{-1} db), high-energy binding sites are occupied by water molecules remaining less
302 favourable locations to be covered with them. Therefore, net integral enthalpy decreased with
303 the increase of moisture content. This behaviour was also observed in brown seewead
304 (Moreira, Chenlo, Sineiro, Sanchez, & Arufe, 2016) or Swiss cheese microparticles (Silva,
305 Borges, da Costa, & Queiroz, 2015). On the other hand, enthalpy variation with moisture
306 content for roasted carob powder showed different pattern. A steep decline from maximum
307 value of 27.15 kJ mol^{-1} to 11.24 kJ mol^{-1} was observed at low moisture content in the range
308 from 0.03 to 0.06 g g^{-1} db (Fig. 3A). This trend was also observed in oatmeal biscuits
309 (McMinn, McKee, Ronald, & Magee, 2007). Increasing trend of net integral enthalpy at low
310 moisture content indicates the greater water-solid interactions compared to the interactions of
311 water molecules (Kaya & Kahyaoglu, 2006).
312 The net integral entropy was calculated using the same Eq. (9) but from the intercept value.
313 Initially, the entropy was observed to decrease with the increase of moisture content from
314 0.04 to 0.12 g g^{-1} db for unroasted carob powder (Fig. 3B). It ranges from -16.34 to -73.75 kJ
315 (mol K)$^{-1}$. In very low EMC, water molecules are initially bonded to highly-active sites in
316 solid surface keeping certain degree of movements. While those readily available sites are
317 occupied, additional water molecules attached to less favourable active sites followed by their

318 further localization. When water molecules covered all the active sites on the surface, the
319 minimum of integral entropy was achieved forming the first layer. Further increase of net
320 integral enthalpy with the increase of EMC is due to the formation of multilayer. Regarding
321 roasted carob powder, net integral entropy decreased from -67.34 kJ $(mol\ K)^{-1}$ to -27.19 kJ
322 $(mol\ K)^{-1}$ in EMC ranged from 0.03 to 0.06 g g^{-1} db. For both samples, net integral entropy
323 tends to reach the entropy of free water. Based on the results, we may assume that roasting
324 process changed the surface of the sample by exposing all the hidden high energy locations to
325 the direct interaction with water molecules (without swelling of material). This statement was
326 also supported by similar maximal and minimal values of net integral enthalpy and entropy,
327 respectively, for unroasted and roasted carob powder.
328
329 3.6 Glass transition temperature
330 Values of *T*g obtained for roasted and unroasted carob samples as a function of equilibrium
331 moisture content are shown in Suppl. 4. As expected for both materials T_g values decreased as
332 equilibrium moisture content (or water activity) increased. It can be related with the
333 plasticizing effect of the water, contributing to the storage and stability of the foodstuff. Water
334 acts as a plasticizer by reducing the T_g due to reduction of the inter- and intra-macromolecular
335 forces (Shi, Lin, Zhao, & Zhang, 2015). The similar trend was observed in our previous study
336 for burdock and elecampe roots (Cervenka, Kubinova, Juszczak, & Witczak, 2012).
337 Experimental data of T_g varied from – 45.0 to 62.6 °C for unroasted samples and from - 38.3
338 to 67.9 °C for roasted material. The roasted carob powder showed higher glass transition
339 temperatures as compared to unroasted material, which is related with higher hygroscopicity
340 of unroasted sample. For example, equilibrium moisture content of unroasted carob at 0.23 a_w
341 is 0.044 g g^{-1} db while 0.028 g g–^{1} db for roasted carob powder at 25 °C. The lower T_g values
342 obtained for unroasted material can be also related to the higher concentration of reducing

343 sugar content (see Suppl. 1), although not statistically significant (p> 0.05). It is known that
344 low molecular weight compounds such as simple sugars or organic acid reduced glass
345 transition temperature (Roos, Karel, & Kokini, 1996). Moreover, the higher value of T_g
346 obtained for roasted material may be results of changes like a Maillard reaction occurring
347 during roasting process. Differences in the thermal properties before and after roasting
348 process were observed for coffee beans (Rivera et al., 2011). The experimental data of T_g
349 fitted well to the Gordon-Taylor model showing high values of r^2 (above 0.980). The
350 parameters obtained from the model are presented in Table 2. Values of the constant T_{gs},
351 corresponding to glass transition of anhydrous material were 61.8 and 65.7 °C for unroasted
352 and roasted carob powder, respectively. The predicted values of T_g are similar to the
353 previously reported values obtained for other materials with high level of dietary fibre such as
354 date flesh (Rahman, 2004) or freeze-dried pineapple (Telis & Sobral, 2001). Higher T_g values
355 obtained for unroasted material confirm that it may contains less low molecular weight
356 components than unroasted sample. The k parameter controls degree of curvature of T_g
357 dependence on water content in a binary system and can be related with the strength of
358 interaction between water and foods solids. Higher value of k indicate a greater plasticizing
359 effect of water on solids (Shi, Lin, Zhao, & Zhang, 2015). The calculated values of k for
360 roasted and unroasted carob were 6.05 and 5.68, respectively. These values may indicate that
361 plasticizing effect of water was more significant in the roasted material then in unroasted one.
362
363 3.7 Critical conditions of storage
364 When combining the glass transition temperature concept with sorption isotherms, one can
365 obtain a useful tool for estimation of critical values for aw and moisture content. For
366 estimation of the critical moisture content in carob powder samples, the adsorption isotherms
367 using GAB equation at 25 °C and the plots of T_g vs. a_w were constructed (Fig. 4A-B).

Applying this concept, critical water activity and critical moisture content for the glass transition of unroasted and roasted carob powders were 0.38 a_w (corresponded to 0.077 g g^{-1} db) and 0.43 a_w (corresponded to 0.058 g g^{-1} db), respectively. It seems that roasted carob powder can be considered as most stable since it showed higher critical water activity. The glassy state of the product would be ensured up to 0.43 a_w upon storing.

4. Conclusion

To the best of our knowledge, this study represents the first report describing the moisture adsorption characteristics of carob powder (*Ceratonia siliqua* L.). Since thermally treated carob powder/flour is also available in the market, two types (unroasted and roasted) of carob powders were used to elucidate the differences in thermodynamic properties of moisture adsorption. The isotherms were evaluated at temperature range of 15–40 °C and were of type II commonly observed in the most foods. The GAB model was found to be suitable for adsorption of both carob powder samples. At 15 °C, unroasted carob powder was susceptible to adsorb more moisture in the whole a_w range, while roasted carob powder was more hygroscopic above 0.43 a_w at the same temperature. Moisture adsorption was similar at 25 °C and 40 °C for both carob samples. Both the net isosteric heat and differential entropy decreased with the increase in moisture content showing that the adsorbed water molecules are strongly bound to the surface at low moisture content for unroasted carob powder in comparison with that of roasted carob powder. Enthalpy-entropy compensation theory was successfully applied and suggests that the adsorption process is enthalpy driven. The study of integral thermodynamic properties of adsorption process for carob powder samples revealed particular differences, i.e. maximal and minimal values for net integral enthalpy and entropy, respectively, were observed for unroasted carob powder. It means that probably swelling of the material occurred during the adsorption process. The glass transition was determined in

0.077 and 0.058 g of moisture per gram of dry basis for unroasted and roasted carob powder, respectively. The combination of Gordon-Taylor and GAB models into one plot revealed that roasting of carob powder at 130 °C for 30 min made this product more stable for storage purposes. Therefore, we may conclude that the hypothesis was confirmed. In addition, further investigations of chemical stability (polyphenols, fat) at different storage conditions are needed.

Conflict of interests

The authors have declared no conflict of interest

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Fig. 1 Equilibrium moisture content vs. water activity and moisture adsorption isotherms fitted by GAB equation (continuous lines) of A) unroasted and B) roasted carob powder at 15 °C (Δ), 25 °C (□) and 40 °C (○). Vertical bars represent standard deviation of replicates (*n*=3)

Fig. 2 Net isosteric heat A) and differential entropy B) of unroasted (□) and roasted (○) carob powder adsorption vs. the moisture content

Fig. 3 Integral enthalpy (A) and integral entropy (B) of unroasted (■) and roasted (○) carob powder adsorption vs. the moisture content.

Fig. 4 Variation of glass transition temperature (T_g) and moisture content with water activity for roasted (A) and unroasted (B) carob powders

Table 1

Table 1 Regression parameters of GAB equation applied to moisture adsorption isotherms

Carob powder		Temperature (°C)		
		15	25	40
unroasted	M_0	0.078 ± 0.007	0.082 ± 0.016	0.065 ± 0.007
	C	8.790 ± 0.771	2.489 ± 0.317	2.801 ± 0.364
	K	0.994 ± 0.025	0.970 ± 0.033	1.007 ± 0.010
	r^2	0.996	0.990	0.996
	E (%)	7.59	7.20	8.91
roasted	M_0	0.063 ± 0.009	0.047 ± 0.003	0.062 ± 0.007
	C	4.770 ± 0.539	2.574 ± 0.543	1.536 ± 0.518
	K	1.085 ± 0.011	1.044 ± 0.006	1.000 ± 0.010
	r^2	0.990	0.994	0.997
	E (%)	7.24	5.76	8.37

An average ± standard deviation; $M_{0,}$ monolayer moisture content (g g^{-1} db); C and K, constant of GAB equation; r^2, coefficient of determination; E, mean relative percentage deviation (%)

Table 2

560 Table 2 Nonlinear regression parameters of the Gordon-Taylor model for roasted and

561 unroasted carob

Parameter	Carob	
	unroasted	roasted
T_g (°C)	61.81	65.66
k	5.68	6.05
r^2	0.996	0.988

562

Figure captions

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Figure 1

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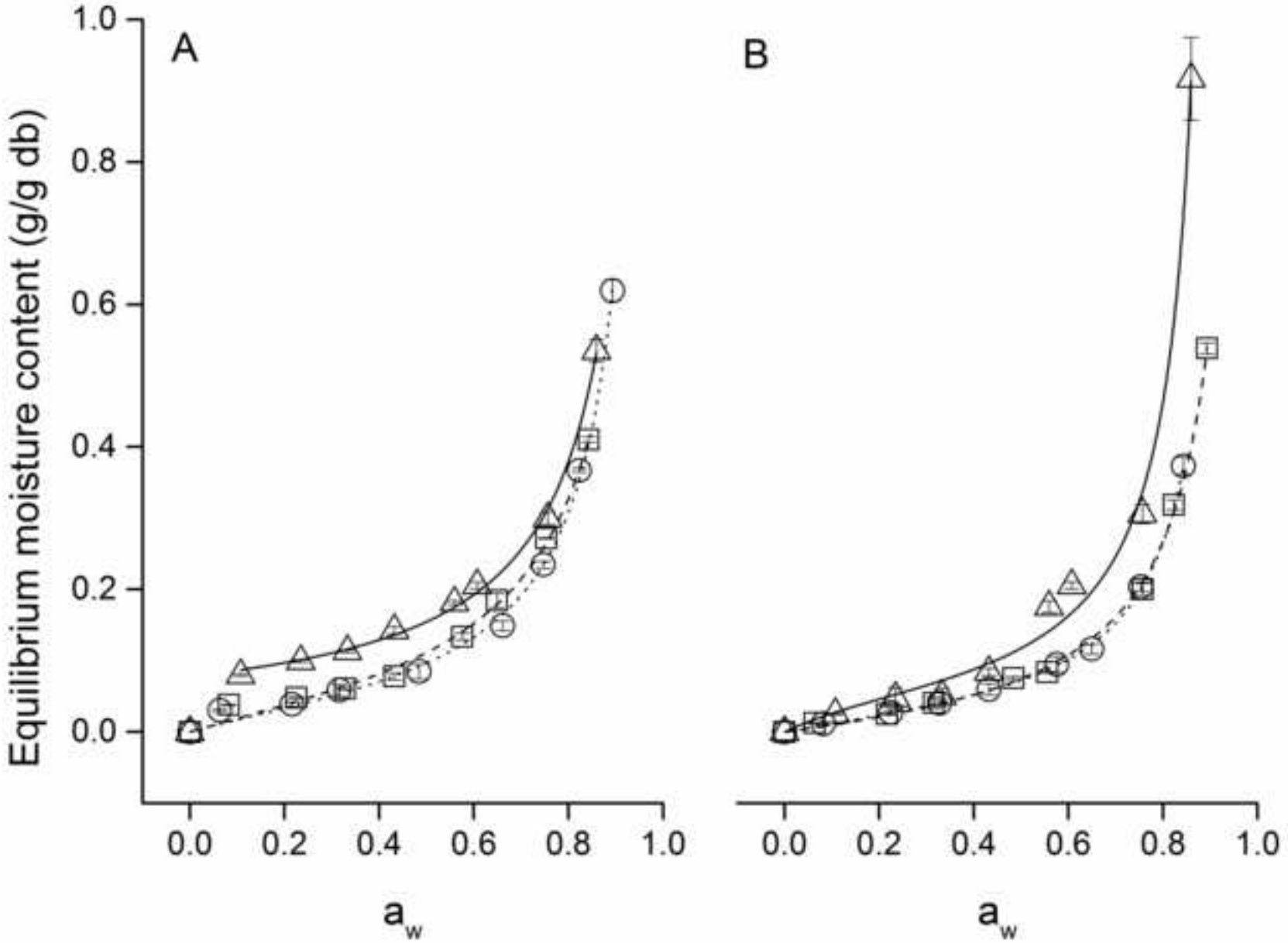

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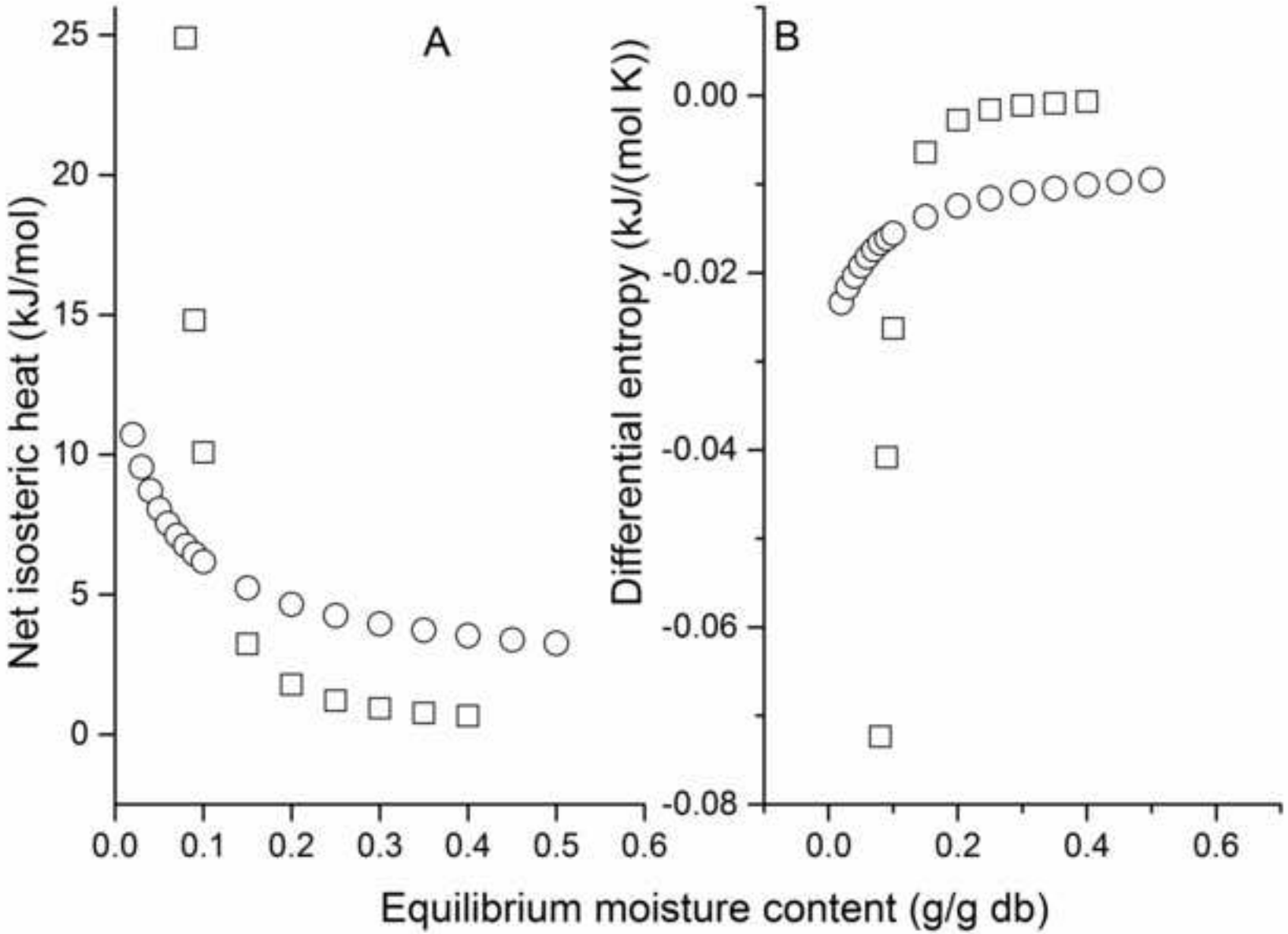

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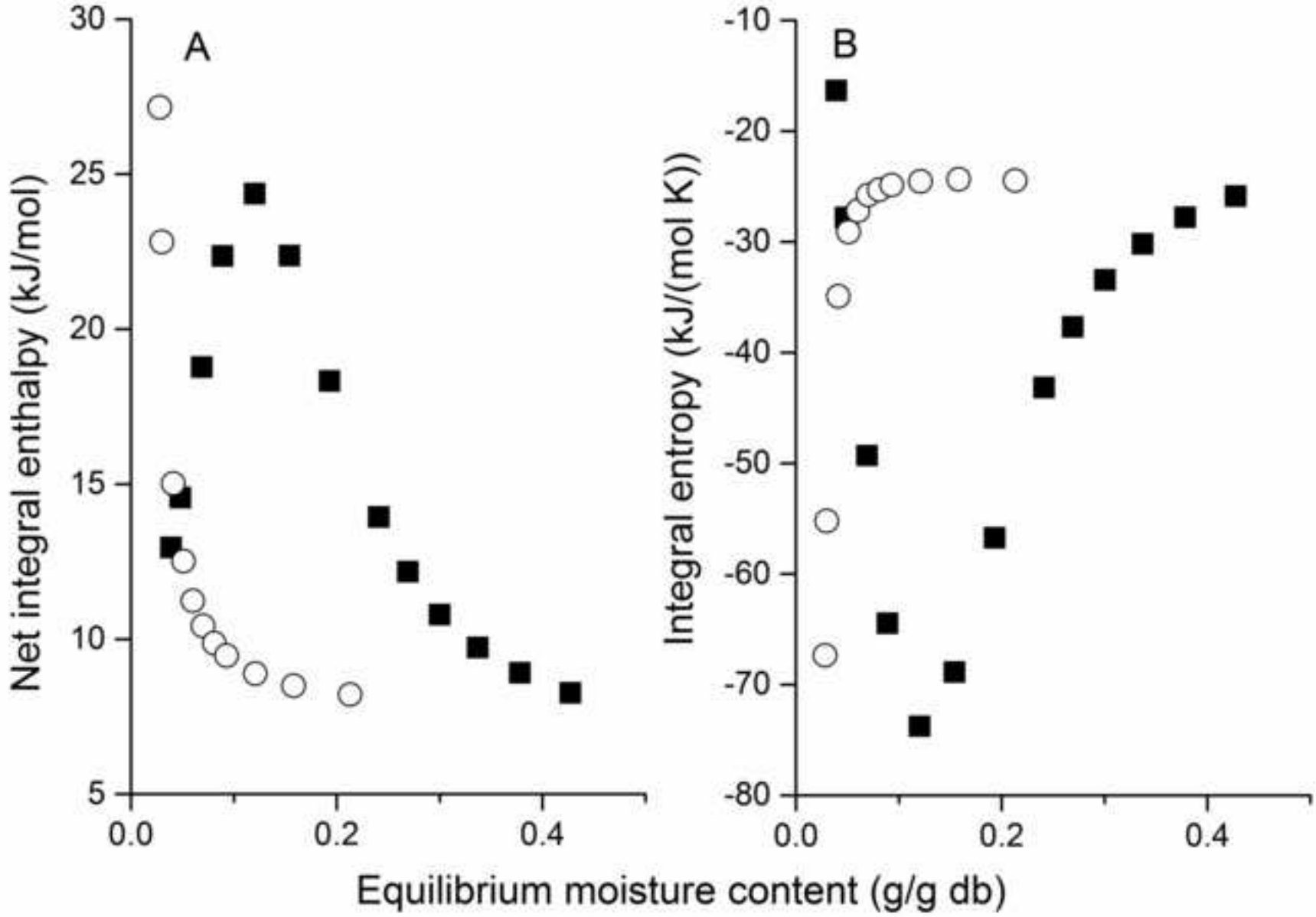

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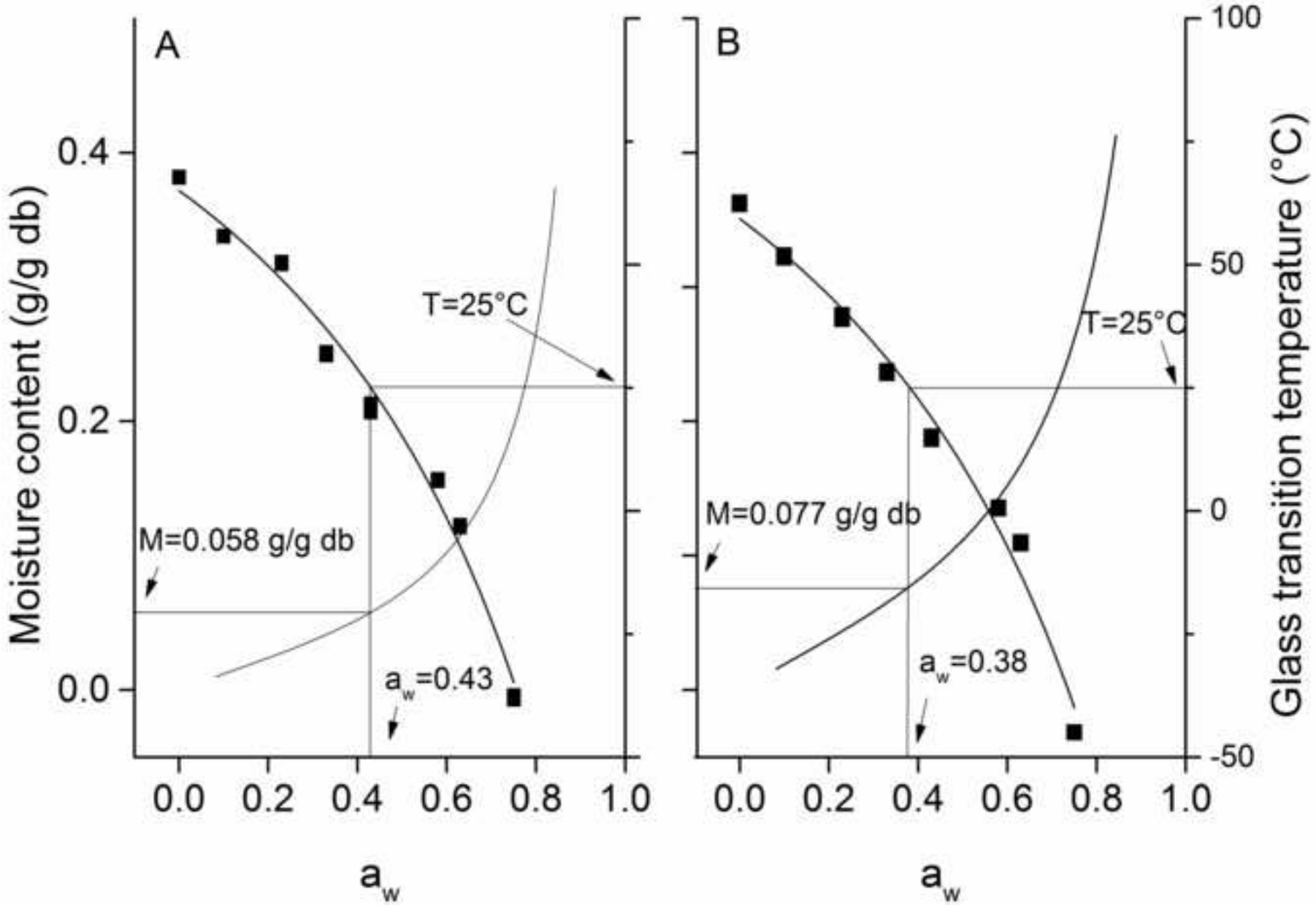

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***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Libor Červenka, in behalf of all authors