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RESEARCH

Bee bread: sorption isotherms, thermodynamic characteristics of moisture adsorption and evaluation of adsorbed water

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Abstract

Bee bread is a value-added apiculture product produced from bee pollen by mixed lactic acid fermentation. Although many studies focused on the bioactive components and health effects of the bee bread, there is no study concerned with understanding its moisture adsorption properties. Herein, it was aimed to evaluate moisture adsorption properties and thermodynamics of bee bread using different sorption models at 25 and 35 °C. The water adsorption of bee bread had Type II characteristics, and the monolayer moisture content was calculated with *BET*, *GAB*, and *Caurie* models between 3.58 and 5.80 g/100 g. The *Peleg* and *Caurie* models ensured better prediction for adsorption. The stability of bee bread was high at 25 °C according to the smaller ratio of Type III to Type II-bound water. The entropy of adsorption was 16.01–25.78 kJ/mol.K and it decreased with the moisture adsorption. Besides, the moisture adsorption needs external energy from the environment because of $\Delta G > 0$.

1 Introduction

Bee bread is a natural apiculture product obtained from bee pollen in the honeycomb via fermentation of different microorganisms found in bee pollen such as *Pseudomonas* spp., *Lactobacillus* spp., *Saccharomyces* spp. [1]. Bee bread mainly contains water, carbohydrates, proteins, amino acids, fatty acids, vitamins, minerals, phenolic compounds, and co-enzyme Q_{10} [2]. It has been emphasized that the conversion of bee pollen to bee bread increases the nutritional value and ensures a more digestible product with high content of free amino acids and vitamins [1, 2]. Bee bread has many biological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer, anti-obesity, and anti-aging activities depending on its compositional properties [1]. For these reasons, it is an important food source for larvae and young bees producing royal jelly, and it has also a substantial potential for human nutrition and promoting health [2].

The bee bread production amount in apiaries is reported to be low and some recent studies have focused on developing new strategies to increase the amount of bee bread and also the income of beekeepers [3]. In this regard, appropriate preservation and storage of the limited amount of bee bread becomes an important issue. Water plays an important role in the preservation of food materials and its activity determines the occurrence of microbial growth, chemical, and biochemical reactions. Therefore, sorption isotherms and different sorption models are used to evaluate the state of water in foods and determine the conditions that will make the food more stable against microbial spoilage, and physical, chemical and sensorial deterioration. Furthermore, the determination of sorption isotherms of food navigates the design of drying equipment, optimization of operational parameters, selection of packaging materials and conditions, prediction of shelf-life, and storage stability [4].

To the best of our knowledge, there is no study related to determining the moisture adsorption properties of bee bread. Although there were some studies on moisture sorption properties of bee pollen [5] and honey powder [6], these products have different production ways, physical structures, and chemical compositions. These factors change the moisture adsorption characteristics and spoilage sensitivities of each product. Therefore, this study was constructed

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to evaluate moisture adsorption isotherms, thermodynamic characteristics of moisture adsorption, and the situation of adsorbed water in the bee bread by using different sorption models.

2 Materials and methods

2.1 Materials

Bee bread was purchased from the local market in Balıkesir, Türkiye. The sodium hydroxide, potassium acetate, magnesium chloride, potassium carbonate, sodium bromide, potassium iodide, sodium chloride, and barium chloride chemicals were supplied from Merck (Darmstadt, Germany), Sigma (Taufkirchen, Germany), and Isolab Laborgeraete GmbH (Wertheim, Germany).

2.2 Moisture adsorption procedure

The moisture adsorption of bee bread was detected with the static gravimetric method. According to this method, the oversaturated salt solutions were prepared at > 35 °C by dissolving sodium hydroxide (200 g/100 mL), potassium acetate (350 g/100 mL), magnesium chloride (60 g/100 mL), potassium carbonate (125 g/100 mL), sodium bromide (115 g/100 mL), potassium iodide (175 g/100 mL), sodium chloride (40 g/100 mL), and barium chloride (45 g/100 mL) salts in water, and prepared solutions placed into the desiccators. They were kept at 35 °C for 48 h to control the stabilization of salt crystals at the bottom of the solutions. After that, the water activities of these oversaturated salt solutions were measured with the water activity (a_w) analysis device (Novasina LabSwift-a_w, Switzerland) and compared with the values reported by Bell and Labuza (2000). The small glass beakers with 10 mL volume were placed onto the platform in the desiccators, and they were stabilized under the experimental conditions for 48 h [7]. The experiments to determine moisture adsorption characteristics of the bee bread at 25 and 35 °C were carried out at 6.90–87.20% relative humidity environments. Different relative humidity environments were formed with oversaturated sodium hydroxide (a_w at 25 and 35 °C: 0.082 – 0.069), potassium acetate (0.225 – 0.213), magnesium chloride (0.328 – 0.321), potassium carbonate (0.432 – 0.425), sodium bromide (0.576 – 0.546), potassium iodide (0.689 – 0.670), sodium chloride (0.753 – 0.749), and barium chloride (0.872 – 0.844) solutions [8].

The bee bread was dried at 45 °C and under 25 mbar pressure in a vacuum oven (Memmert VO200, Germany) before the experiments. The moisture content of bee bread was determined with the drying of 1.0 g ground sample at 60 °C (Memmert VO200, Schwabach, Germany) until reaching constant weight. The moisture content and water activity of bee bread were determined as 2.56% and 0.16, respectively. After that, about 0.5 g of dried bee bread was weighed into the beakers and moisture adsorption was measured from the weight changes until they reached constant values at 25 and 35 °C in 6.90–87.20% relative humidity environments. It was decided that the weight changes in the samples became stable for both temperatures at the end of 10 days [6, 8].

2.3 Moisture adsorption data evaluations with sorption models

The moisture adsorption properties of bee bread were evaluated with some sorption models used for food products. For this aim, the *BET*, *Caurie*, *GAB*, *Halsey*, *Henderson*, *Iglesias and Chirife*, *Kuhn*, *Smith*, and *Peleg* sorption models were selected, and their equations were presented in Table 1.

The X and X_0 are equilibrium and monolayer moisture content of bee bread, respectively. A, B, C, and D are constants used in sorption models. C_c and n symbols in the

Table 1 Equations of selected sorption models

Sorption models	Equations	References
BET	$X = \frac{X_0\, C\, a_w}{[(1-a_w+Ca_w)\,(1-a_w)]}$	[9]
Caurie	$\frac{1}{X} = \frac{1}{C_c\, X_0}\left[\frac{1-a_w}{a_w}\right]^{\frac{2}{n}}$	[10]
GAB	$X = \frac{X_0\, C\, k\, a_w}{[(1-k\, a_w)\,(1-k\, a_w+C\, k\, a_w)]}$	[11]
Halsey	$X = \left[-\frac{A}{ln(a_w)}\right]^{\frac{1}{B}}$	[11]
Henderson	$X = \left[\frac{ln(1-a_w)}{-A}\right]^{\frac{1}{B}}$	[12]
Iglesias and Chirife	$X = A + B\, \frac{a_w}{1-a_w}$	[12]
Kuhn	$X = \frac{B}{ln a_w} + A$	[11]
Smith	$X = A - [B \ln (1 - a_w)]$	[11]
Peleg	$X = A\, a_w^B + C\, a_w^D$	[13]

Caurie model are monolayer moisture density, and number of adsorbed monolayers, respectively. In addition, *C* and *k* symbols in *GAB* model are chemical potentials between mono-multilayers, and monolayer-free water, respectively.

X values were calculated by determining the weight difference between the initial weight of the sample and the weight at the end of the 10th day as a percentage based on the dry matter of the sample. The linear regression was applied to calculate the X_0 values and model constants in the *BET*, *Caurie*, *Halsey*, *Henderson*, *Iglesias and Chirife*, *Kuhn*, *Smith*, and *Peleg* models, whereas nonlinear regression was applied for the *GAB* model [5, 6, 14].

2.4 Goodness of fit of models to the sorption data

The goodness of fit evaluations for models were performed with the minimum mean absolute percentage error (*E%*), regression coefficient (R^2), and root mean square error (*RMSE*) parameters. They are calculated with the following equations. The goodness of fit is accepted for a model when the *E%* value is ≤10% [11]. Additionally, the acceptance of models was decided according to the $R^2 \geq 0.98$ and $0 < RMSE < 1$ values [14, 15].

$$E\% = \frac{100}{N} \sum {}_{i=1}^{N} \frac{|X_e - X_p|}{X_e}$$

$$R^2 = 1 - \frac{\sum (X_e - X_p)^2}{\sum (X_e - \bar{X}_e)^2}$$

$$RMSE = \sqrt{\frac{\sum (X_e - X_p)^2}{N}}$$

N, X_e, and X_p are the number of observations, experimental sorption data, and predicted data by related sorption models, respectively [6, 14, 15].

2.5 Properties of adsorbed water

The number of adsorbed monolayers (n), monolayer moisture density (C_c), types (Type I, II, and III) of adsorbed water, and the total amount of adsorbed water were determined by constants of the *Caurie* model. These parameters were calculated with the following Eqs. [6, 16].

$$n = \frac{X_0}{C_c}$$

$$Type\ I = X_0$$

$$Type\ II = X_0 (n - 1)$$

$$Type\ III = X_0 (X_0 - n)$$

$$Total\ adsorbed\ water = {X_0}^2$$

2.6 The net isosteric heat, entropy, and Gibbs' free energy of sorption

The net isosteric sorption heat (q_{st}) indicates the required minimum energy to remove water from food material in the drying process [11]. It was determined by the *Clausius-Clapeyron* equation by plotting the $\ln(a_w)$ versus $(1/T)$ with assumptions that the system is at constant equilibrium moisture content, and the temperature has no effect on pure water vaporization heat and excess sorption heat [12, 17].

$$\frac{d(\ln a_w)}{d\left(\frac{1}{T}\right)} = -\frac{q_{st}}{R}$$

The differential sorption entropy (ΔS) shows interactions between water molecules and food components, and ensures to understand swelling, dissolution, and crystallization of materials during water adsorption. The ΔS is determined with following equation [11].

$$\ln a_w = -\frac{q_{st}}{R} + \frac{\Delta S}{R}$$

Additionally, Gibbs' free energy gives information about the way moisture adsorption occurs as a spontaneous process ($-\Delta G$) or requires energy ($+\Delta G$). The ΔG values are determined by the following Eqs. [13, 17].

$$\Delta G = RT \ln(a_w)$$

The R is the universal gas constant with 8.314×10^{-3} kJ/mol.K value and T is applied experimental sorption temperatures (K) [13].

3 Results and discussion

3.1 Sorption isotherms and equilibrium moisture content of bee bread

The moisture adsorption isotherms of bee bread obtained at 25 and 35 °C are shown in Fig. 1. There were two-bending points in the curve around 0.10–0.20 and 0.60–0.70 water activity levels. The adsorption isotherms at both temperatures had a sigmoid shape (S-shaped) and they were classified as Type II according to the *BET* classification. Besides, it has been evaluated that the constants *C* and *k* values determined by the *GAB* equation also support this evaluation. It has been highlighted that $C > 2$ and $0 < k < 1$ indicate

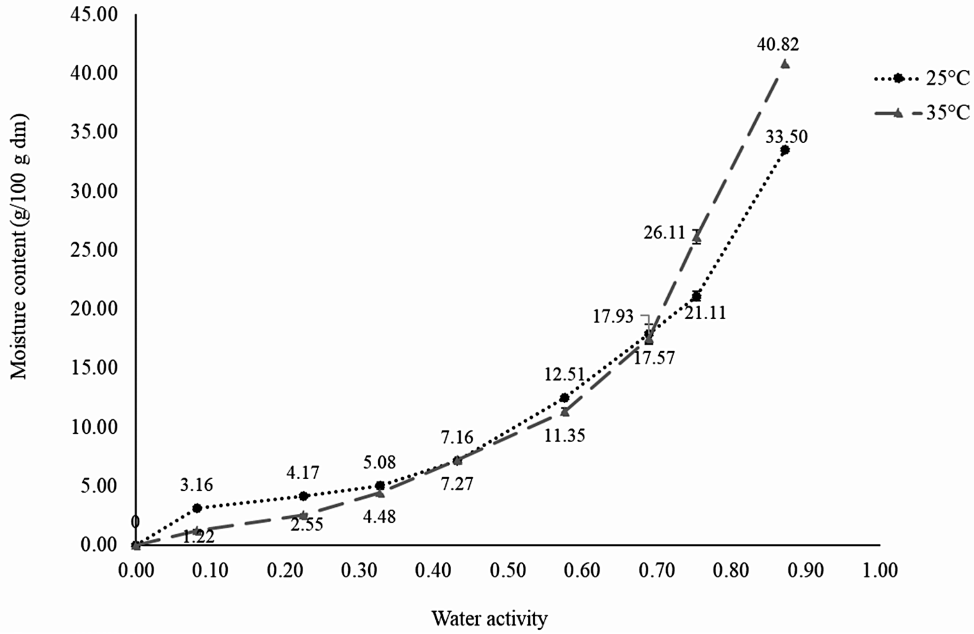

Fig. 1 Adsorption isotherms of bee bread at 25 °C and 35 °C temperatures

Brunauer class II [18]. It has been demonstrated that Type II isotherm is the characteristic moisture adsorption isotherm type of many foods especially rich in carbohydrates and/or proteins [19]. Bee bread contains high amounts of carbohydrates like fructose, glucose and saccharose, and proteins, peptides and amino acids [20]. This isotherm type is reported for different kinds of foods like cassava flour [21], bee pollen [5], chili pepper [22], honey powder [23], and propolis extract powder [24]. It consists as a consequence of sample capillarity status, colligative effects, and water molecules-sample surface interaction effects, and shows sample structure porosity and multilayer moisture adsorption in the sample [6].

The equilibrium moisture contents of bee bread were determined to be between 3.16–33.50 and 1.22–40.82 g/100 g at 25 and 35 °C, respectively (Fig. 1), and they increased with water activity increase for both temperatures. Furthermore, there was a sharp increase in water activities higher than 0.70 and the formation of a crossover point. As can be clearly see in Fig. 1 the moisture adsorption was higher at 35 °C above the 0.70 water activity. The temperature has decreasing effect on moisture adsorption depending on increasing kinetic energy among molecules. However, this phenomenon may change in sugar containing food products because of dissolution of sugars and/or the swelling of polymeric substances like polysaccharide and proteins at higher water content and temperatures [4, 25]. The swelling of polymeric compounds in foods creates free volumes within the structure causing an increase in the effectiveness of moisture sorption [25]. It was noted that bee bread is rich in low molecular weight sugars such as fructose and glucose [20], and these sugars may affect the change in moisture adsorption properties of bee bread. Similar crossover observations were reported at different water activity levels for amaranth flour [26], pregelatinized cassava snacks [27], date-pits [28], and Jerusalem artichoke powder [25]. The differentiation in crossover points has been associated with the variations of the swelling ability and the glass-rubber transition points at a critical water activity [28].

3.2 Sorption model parameters and fitting sorption data into the models

The obtained model parameters are presented in Table 2. The X_0 values calculated by the *BET*, *Caurie* and *GAB* models were 3.99–3.90, 5.80–4.70, and 4.53–3.58 g/100 g at 25 and 35 °C, respectively. The X_0 value is an indicator moisture content in terms of protection of food materials from physical changes, chemical deterioration, and microbiological spoilage. It has been stated that producing foods at moisture content equal to or lower than the X_0 value protects their quality and safety during storage. Based on this, it has been considered that the highest calculated X_0 value was 5.80 g/100 g among three sorption models and the bee bread can be stored as safe this moisture content limit at ≤ 35 °C. The importance of monolayer moisture content in the protection of physical, chemical, and microbiological situations like enzyme activity, non-enzymatic browning reactions, lipid oxidation, and textural characteristics has been emphasized in previous studies [29–31].

The *C* parameter in the GAB equation is energy constant and gives information about the hygroscopicity of the material. The low *C* values show little heat release during moisture adsorption by the material [32], whereas the high *C*

Table 2 Moisture adsorption evaluations of bee bread by different sorption models

Models	Parameters	25 °C	35 °C
BET	X_0	3.99	3.90
	C	20.20	9.02
	E%	6.94	37.25
	RMSE	0.39	0.85
	R^2	0.950	0.871
Caurie	X_0	5.80	4.70
	C_c	6.28	5.13
	n	3.35	2.34
	E%	14.32	7.76
	RMSE	1.29	0.79
	R^2	0.991	0.998
GAB	X_0	4.53	3.58
	C	11.14	9.47
	k	1.03	1.14
	E%	15.53	42.51
	RMSE	4.56	18.96
	R^2	0.931	0.830
Halsey	A	8.24	3.46
	B	1.13	0.77
	E%	10.54	11.85
	RMSE	1.90	3.57
	R^2	0.973	0.975
Henderson	A	0.03	0.08
	B	1.28	0.91
	E%	21.06	17.93
	RMSE	3.06	3.51
	R^2	0.963	0.979
Iglesias and Chirife	A	4.35	1.46
	B	4.61	7.56
	E%	22.33	17.86
	RMSE	2.03	1.11
	R^2	0.958	0.992
Kuhn	A	2.56	-1.36
	B	-4.53	-7.38
	E%	20.32	10.20
	RMSE	1.91	0.95
	R^2	0.962	0.994
Smith	A	-0.33	-3.66
	B	15.76	22.01
	E%	16.43	48.01
	RMSE	1.18	2.30
	R^2	0.986	0.968
Peleg	A	43.89	20.22
	B	3.50	1.29
	C	6.27	69.36
	D	0.29	6.11
	E%	4.69	10.64
	RMSE	0.60	1.81
	R^2	0.994	0.994

values indicate high heat release because of the strong bond formation between polar sites having water binding capacity on the food surface and the monolayer water [33]. Besides, the k parameter relates to chemical potential between monolayer and free water in material [6]. It ensures describing the difference between the mono ($k \leq 0.5$) and multilayer ($k > 0.5$) adsorption properties [32]. It was noted that while the k constant has more entropic properties, the C constant is enthalpic in sorption, and it is adequate the association of temperature effect on moisture adsorption with the C value [34]. According to obtained results, increasing temperature had decreasing effect on the C values from 11.14 to 9.47. This decrease can be explained by the reduction of the water binding efficiency of polar sites due to the increased kinetic energy of water molecules induced by temperature. Accordingly, this energy causes the breaking of water-polar site bonds, and the C value gets decreased. It was stated that while increasing temperature increases molecular mobility and kinetic energy, it decreases the attraction between molecules and the thermodynamic stability of water molecules. All these effects result in the breaking of the bonds between water molecules and polar sites found in food products [35].

The results of linear and nonlinear regression analysis of goodness of fit values for each model are given in Table 2. The sorption models were evaluated according to their *E%*, *RMSE*, and R^2 results. These criteria were ensured by the *Peleg* sorption model results at 25 °C which means that the moisture adsorption characteristics of bee bread could be explained by this model. Besides, the *Caurie* and *Kuhn* sorption models could be used for moisture adsorption evaluation of bee bread at 35 °C. The *Peleg* is another model that can be used at 35 °C based on *E%* and R^2 values as 10.64% and 0.994, respectively.

The *Peleg* model is an empirical sorption model which is used for estimation of moisture adsorption and desorption properties of materials [32] up to 0.90 water activity level [6]. The A and C constants in the *Peleg* model are associated with the initial moisture adsorption rate and the moisture content after reaching the equilibrium of the material, respectively [36]. The A value changed from 43.89 to 20.22 whereas C value increased from 6.27 to 69.36 with temperature increase. It has been demonstrated that this model ensues better moisture adsorption prediction for foods having different physical and chemical properties such as whey protein edible films [36], freeze-dried powders including avocado, inulin and maltodextrin [37], Turkish dry-fermented sausage [38], and black elderberry and chokeberry [39].

The *Caurie* model is derived from the *BET* model and based on monomolecular physical moisture adsorption [40]. It is used in defining sorption properties of various materials up to 0.90 water activity [6]. The best fitting results were reported for the *Caurie* model in moisture adsorption by some apiculture products like multifloral bee pollen [5], and honey powder with maltodextrin, gum arabic, and whey protein [6]. Besides, the *Kuhn* sorption model was used for the explaining moisture adsorption characteristics of different food materials such as barley malt [41], amaranth–sorghum grains [4], *Shiitake* mushroom [42], and rice-based instant soup mix [43].

3.3 Properties of adsorbed water

Some properties of adsorbed water by bee bread at 25 and 35 °C are given in Table 3. According to the results, increasing environmental temperature decreased the number of adsorbed monolayers, density of water, and total adsorbed water. It has been reported that number of adsorbed monolayers and amount of bound water of solar, tray, and freeze-dried mint leaves decreased with increasing of temperature from 25 to 35 °C [44]. Besides, similar observations were declared for adsorbed water in bamboo shoot [45], Indian milk product called as Sandesh [46], wheat germ [47], and honey powders produced with maltodextrin, gum arabic and whey protein hydrocolloids [6]. This decreasing effect is associated with hydrogen-bond formation because water adsorption is an exothermic reaction and increasing temperature makes hydrogen-bound formation difficult and/or water can easily break up from polar sites of samples with increasing kinetic energy triggered by temperature [48].

The bound water was characterized by the three types as Type-I, Type-II, and Type-III. Type-I water is strongly bound and non-freezing water, and has no solvent property. Type-II water is non-freezing and weakly bound water, and it has solvent ability. Type-III is loosely bound with limited freezing property, and it has a solvent effect [16]. The Type-I, II and III bound waters were 5.80, 13.61, 14.20 g/100 g at 25 °C, whereas they were 4.70, 6.28 and 11.13 g/100 g at 35 °C, respectively. Besides, it has been stated that the stability of the product is high when the ratio of Type III-bound water to Type II-bound water molecules gets smaller [16]. These ratios were 1.04 and 1.77 at 25 and 35 °C, respectively. Based on these ratios, it can be concluded that the sample is more stable at 25 °C. In addition, the moisture content of bee bread should be lower than 5.80 g/100 g (Type-I) for the protection against microbial spoilage, and physical, sensorial, and chemical deterioration.

3.4 The net isosteric heat, entropy, and Gibbs' free energy of sorption

The net isosteric heat, entropy, and Gibbs' free energy of sorption are crucial parameters for the dehydration and hydration processes' optimization, and they explain the energy requirements and interactions between food and water molecules [49]. The net isosteric heat, entropy, and Gibbs' free energy of sorption results as a function of equilibrium moisture content are presented in Fig. 2. It was determined that the net isosteric heat and entropy of adsorption ranged between 6.32–11.22 kJ/mol and 16.01–25.78 kJ/mol.K, respectively, and these parameters decreased with increase in moisture content. The heat of sorption is an indicator because it shows intermolecular forces between the active sites in food and water molecules. It is high in low moisture content levels due to the strong binding energy between active sites on the surface of the materials and water. Then, it decreases with increasing moisture content levels in the later stages of adsorption because of weak binding energy between water molecules [50].

Entropy shows the status of order or disorder during water adsorption or desorption in the material [12], and thermal energy situations during moisture adsorption can be evaluated by the differential entropy determination [51]. The similar decreasing trend with net isosteric heat by moisture increase was also observed in the differential entropy values, and it reached to nearly constant minimum value after a 40% moisture content. The differential entropy strongly depends on moisture content, and it is high at low moisture content levels because the availability of active sites for binding water molecules [49]. In the other case, water molecules lose their mobility depending on the saturation of active sorption sites and therefore increasing moisture content causes a decline in the sorption entropy [51]. Similar results have been declared for tiger nuts [52], cowpea [33], and sweet cherry [53].

The Gibbs' free energy is an indicator of the affinity of active sites to water molecules, and explains that the water adsorption by the sample is carried out spontaneous (negative), or non-spontaneous (positive) way [50]. According to the obtained results, the Gibbs' free energy values were ≥ 0 at both temperatures, and it decreased from 3.54 kJ/mol to 1.54 kJ/mol at 25 °C and 3.28 kJ/mol to 1.38 kJ/mol at 35 °C with the increase in moisture content. It can be evaluated that the moisture adsorption by bee bread is a non-spontaneous process, and it needs external energy from the

Table 3 Some properties of adsorbed water by bee bread at 25 °C and 35 °C

Temperature (°C)	Number of monolayers	Density (g/cm^3)	Type I (g/ 100 g)	Type II (g/ 100 g)	Type III (g/ 100 g)	Total adsorbed water (g/100 g)
25	3.35	6.28	5.80	13.61	14.20	33.60
35	2.34	5.13	4.70	6.28	11.13	22.11

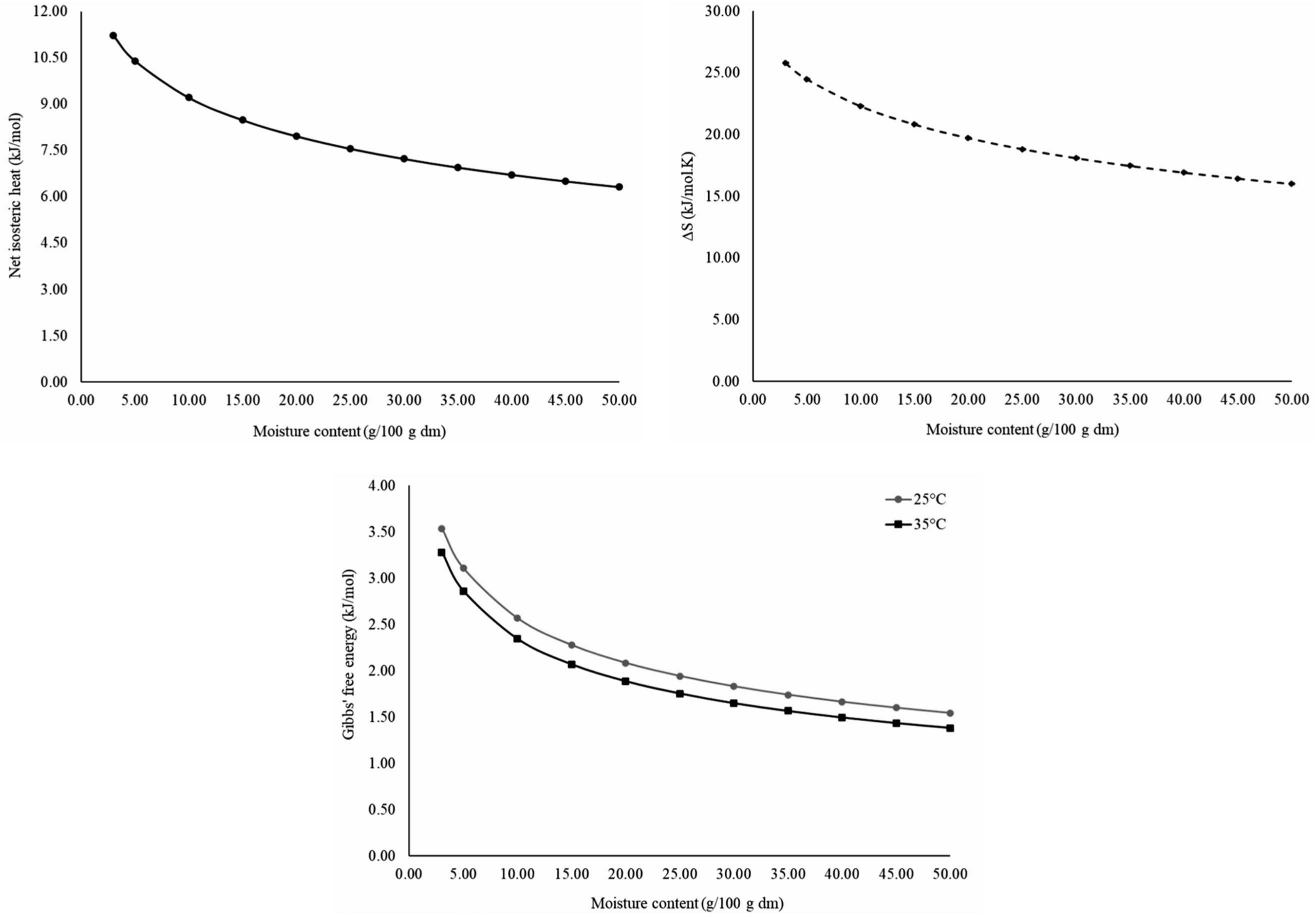

Fig. 2 The net isosteric adsorption heat, sorption entropy, The Gibbs' free energy values of bee bread at different moisture contents

environment. Furthermore, it has been noted that required water binding energy decreases at high temperatures [49], and the obtained results were in line with this statement. The positive Gibbs' free energy results have been reported for different kinds of foods such as cowpea [33], cherry powder [51], fig [50], and quinoa grains [49].

4 Conclusions

The adsorption isotherm of bee bread showed a sigmoidal shape as reported for most food materials and the *Peleg* and *Caurie* sorption models were in good agreement for explaining moisture adsorption behaviors of bee bread at 25 and 35 °C for 0.069–0.872 water activity levels according to *E%*, *RMSE*, and R^2 values. As for the thermodynamic evaluations, net isosteric heat and sorption entropy decreased with the increase in moisture because of low binding energy between water molecules and the unavailability of active sites for binding water molecules. Besides, the positive Gibbs' free energy values of bee bread indicated that moisture adsorption by bee bread is a non-spontaneous process, and it needs external energy from the environment. It can be concluded that the sample is more stable at 25 °C, and the moisture content of bee bread should be lower than 5.80 g/100 g for protection it against microbial spoilage, and physical, sensorial, and chemical deterioration.

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Data availability The data are available on request, but they are not publicly shared.

Declarations

Ethics statement This article does not contain any studies with human or animal objects.

Consent to participate Not applicable.

Consent for publication Author has consented to the publication of this manuscript.

Competing interests The author declares no competing interests.

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