Original Article

STATIC WATER VAPOR SORPTION PROPERTIES OF HONEY

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Abstract

Static sorption properties of pine honey (PH) and citrus honey (CH) were studied at 20, 30 and 40°C. Yeast formation was observed on the surface of honeys at water activity (a_w)>0.7 at all temperatures. Visible yeast formation (YF) took place earlier in PH than in CH under the same conditions due to its characteristic higher pH and lower monosacchride content. The temperature was insignificantly effective on YF ($p>0.05$). The honeys exhibited a sorption isotherm (SI) in the shape of a "J". Their SIs exhibited desorption and adsorption at $a_w<0.7$ and $a_w>0.7$, respectively and the desorption part was almost linear. $A_w=0.7$ emerged as a border between the absence and presence of YF and between the desorption and adsorption. The SIs of honeys were insignificantly affected by temperature ($p>0.05$). PH had a significantly lower SI than that of CH ($p\leq0.05$) due to its characteristic lower monosacchride content. GAB equation exhibited a good fit to the honeys' SIs. Sorption heat vs equilibrium moisture content (EMC) revealed monolayer, multilayer and loosely bound free water regions in the SIs of PH and CH.

Keywords: citrus honey, honey, isotherm, pine honey, sorption, water activity

INTRODUCTION

Water quality in foods has a critical role as in all materials of biological origin. Water activity (a_w) is an effective way for expressing the quality of water in foods. In practice, water in honey is expressed quantitatively by moisture content (MC) rather than qualitatively by water activity (a_w). MC checks honey for compliance with specs and pricing, and a_w helps to understand how available water is for microbiological and enyzmatic, among others, deterioration in honey.

a_w is the ratio between the vapor pressure of water in food (p) and the vapor pressure of pure water (p_w) at the same temperature (Eq. 1). It is related to the relative humidity of the air (RH) surrounding the food (Eq. 1). The value of a_w ranges between 0 and 1, and the deteorative activities in food are slower when it is lower.

$$a_w = \frac{p}{p_w} = \frac{RH}{100} \quad \text{Eq. (1)}$$

a_w and MC integrate each other in terms of the sorption properties of foods. Food sorption is studied during the kinetic sorption phase when water moves from the food to the surrounding air or vice versa and MC changes and then in static sorption phase when water in the food and surrounding air is at equlibrium and MC is constant.

The relation between a_w and equilibrium moisture content (EMC) provides the static sorption properties of honey. The maximum MC of 20% wb (wet basis) (25% db - dry basis) suggested by the Codex Alimentarius (1981) for most honeys is the EMC corresponding to $a_w\approx0.6$ when deteriotating activities, especially the metabolism of osmophilic yeasts may start. This one-point relation between a_w and EMC is good for estimating a_w using MC or vice versa, and how prone honey is to deterioration.

The a_w and EMC of many honeys were determined under practical conditions (20°C), and a linear correlation among them was established in most cases (Serin et al., 2018). They were within the

short range of a_w from 0.44 (Abramovic et al., 2008) to 0.70 (Cavia, 2004). This short-range relation is an extended version of the one-point relation and good for estimating a_w using MC or vice versa within the given range.

The one-point and short-range relations between a_w and EMC could be useful for limited cases. Sorption isotherm (SI) covers the one-point and the short range relation and is needed to understand static sorption properties of honey obtained from EMC vs a_w data for the full range of a_w (0–1). The knowledge of SI is critical for determining the optimum conditions for the production of honey in the hive, its harvesting, handling and storage in the apiary, its processing, packaging, handling and storage in the plant, and keeping in proper conditions in the market.

The SI of dew honeys has not been encountered and there have only been some cases of nectar honeys in literature. Martin (1958) obtained EMC vs RH for a nectar honey (clover) at 20-25°C for RH range of 32 and 81% to investigate the effect of a_w on the fermentation. Tabouret (1979) determined EMC vs a_w for a nectar honey (colza) at 19-25°C within a_w range of 0.2-1.0 to deduce the influence of a_w on the crystallization. Doull & Mew (1977) reported solid content vs RH for diluted samples of an unspecifed honeys at 34.5°C within RH range of 55 and 95% for optimizing egg hatching and survival of new larvaes in the hive. Rüegg and Blane (1981) determined MC vs a_w for diluted samples of some unspecified honeys at 25°C within a_w range of 0.55 and 0.78 for investigating the change in a_w with MC.

Work on the relation between EMC and a_w is too limited for honey and did not identify its static sorption properties. The aim of this work is to determine the SI of a honeydew and a nectar honey with commercial importance and study their static sorption properties under practical conditions.

MATERIALS AND METHODS

Honey samples

Liquid pine (dew) and citrus (nectar) honey were donated by Balparmak Bal (Altıparmak Food Industry, İstanbul, Turkey) in water vapor and air-tight jars. They were free from foreign materials and their detailed analysis were conducted by the donator (Tab. 1). Pine honey (PH) was a blend of honeys from various apieries around Muğla, Turkey in 2018. Citrus honey was a blend of honeys harvested from various apieries in the Çukurova (Cilician) region of Turkey in 2019. Samples were kept in original jars at room temperature (22-25°C) till use.

Experimental set-up

Experiments were conducted in glass jars (inner diameter: 10.5 cm, inner height: 18.0 cm) sealed against air and water vapor. Saturated solutions of various salts were prepared at 50°C. One saturated solution was filled up to quarter of a jar. The solutions provided RHs between 10 and 95% at 20, 30, and 40°C. The salts were lithium chloride (LiCl), potassium acetate (CH_3CO_2K), magnesium chloride ($MgCl_2$), potassium carbonate (K_2CO_3), sodium bromide (NaBr), magnesium nitrate ($Mg(NO_3)_2$), sodium chloride (NaCl), ammonium sulfate ($(NH_4)_2SO_4$), potassium chloride (KCl), potassium nitrate (KNO_3), and potassium sulfate (K_2SO_4). RH above 95% was obtained using distilled water. The a_w of solutions and distilled water were measured with the use of an a_w-meter (Novasina LabSwift, Switzerland) at experimental temperatures. A tripod with a polyethlene net on it was fixed within one jar to hold sample vessels. The height of the tripod was almost half of the jar.

Sample preparation

Polyethlene containers (inner diameter: 3 cm, inner height: 1.5 cm) were used as sample vessels. Containers without honey were kept in jars before experiments to condition them to experimental temperature and RH. Tares of the containers were taken with the use of a balance with a sensitivity of 0.0001 g (Ohaus, Adventurer Pro AV264C, Nanikon, Switzerland) just before honey samples were placed in them. The samples were placed in containers to cover the bottoms and their mass was between 1.5-2.0 g. Three honey samples were kept in a

Table 1.
Analyzes of pine honey (PH) and citrus honey (CH)*

	PH	CH
Color (mm)	63.0	8.0
Moisture (%)	16.6	18.1
HMF (mg/kg)	1.4	7.0
Acidity (meq. g/kg)	13.2	5.0
pH	4.7	3.9
Proline (mg/kg)	436	325
Conductivity (mS/cm)	1.08	0.15
Diastase Number (DN)	15.6	5.0
Fructose (%)	32.5	37.6
Glucose (%)	26.4	30.4
Fructose/Glucose	1.23	1.24
Fructose+Glucose (%)	58.9	68.0
Sucrose (%)	0.0	3.1
Maltose (%)	2.6	1.9
Turanose (%)	1.9	1.3
Erlose (%)	1.4	1.9
Isomaltose (%)	1.8	0.3
Maltotriose (%)	0	0
Melezitose	0	0
Total Disaccharide (%)	9.4	7.5
Higher Sugars (%)	8.7	1.5
C13 Honey (δ C13 ‰)	-24.5	-25.2
C13 Protein (δ C13 ‰)	-24.7	-24.9
C13 Difference(δ C13 ‰)	-0.2	0.3
C4 Sugar (%)	1.3	0
Naphthalene (ppb)	ODL	ODL
Sulfonamid group (ppb)	ODL	ODL
Tetracycline group (ppb)	ODL	ODL
Strepto group (ppb)	ODL	ODL
Fluoroquinolone group (ppb)	ODL	ODL
Macrolide group (ppb)	ODL	ODL
Chloramphenicol group (ppb)	ODL	ODL
Nitrofuran group (ppb)	ODL	ODL
Starch/Pollen (%)	1.8	1.3
Polen (%)	Dense	38

*Conducted by the donator, Balparmak Bal (Altıparmak Food Industry, İstanbul, Turkey)
ODL: Out of detection limit

jar. Experiments were conducted in temperature controlled cabinets (Velp Scientifica, Foc 225I, Usmate, Italy). The mass of samples was measured almost every 24 hr till equilibrium, for 90 days or till visible yeast formation (YF) on the surfaces. Mass measurements were conducted as quick as possible to minimize mass change in samples, and a tweezer was used to hold containers. The MC of honey samples were calculated as percentage on dry basis (% db) using the initial moisture content (Tab. 1) and the change in mass. The change in mass was assumed only due to the change of moisture.

Constructing sorption isotherms

Equilibrium between a honey sample and the surrounding air was decided when the difference between three consequitive mass measurements were smaller than 0.00%, and the average of the measurements was determined as EMC. That of samples not reaching the equilibrium by 90 days or having visible YF on the surface before 90 days was estimated with Curve Expert 1.4 software (Hyams Development) through the exploitation of the collected kinetic sorption data (MC vs time). The pattern of the kinetic sorption data at any condition was a concave downward curve approaching a MC. The best fitting equation to the curve was determined, and the approached MC was estimated to be EMC through the first derivative of the equation. SI was obtained from EMC vs a_w data. The significant difference ($p \leq 0.05$) between curves was tested by Correlation Analysis (Bivariate - Partial) with the use of SPSS Statistics 21 software (IBM). SI data from literature was digitized with the use of GetData Graph Digitizer 2.26 software (Informer Technologies).

The correlation between EMC and a_w for honeys was investigated through the fitting performance of GAB equation to their SIs (Eq. 2).

$$EMC = \frac{m_0 \, C \, k \, a_w}{(1 - k \, a_w)(1 - k \, a_w + C \, k \, a_w)} \quad \text{Eq. (2)}$$

m_0 is monolayer moisture content (% db), C is a constant related to the difference between the sorption enthalpy of water in monolayer and

multilayer regions and k is a constant related to the difference between the sorption enthalpy of water in multilayer region and latent enthalpy of pure water.

The Heat of sorption for honeys was determined with Clasius–Clapeyron's equation (Eq. 3).

$$\frac{d(\ln a_w)}{d(1/T)} = -\frac{Q_s}{R} \quad \text{(Eq. 3)}$$

T is temperature (K), Q_s is net heat of sorption (kJ/kg water) and R is universal gas constant (0.4619 kJ/kgxK).

RESULTS

Visible yeast formation (YF)

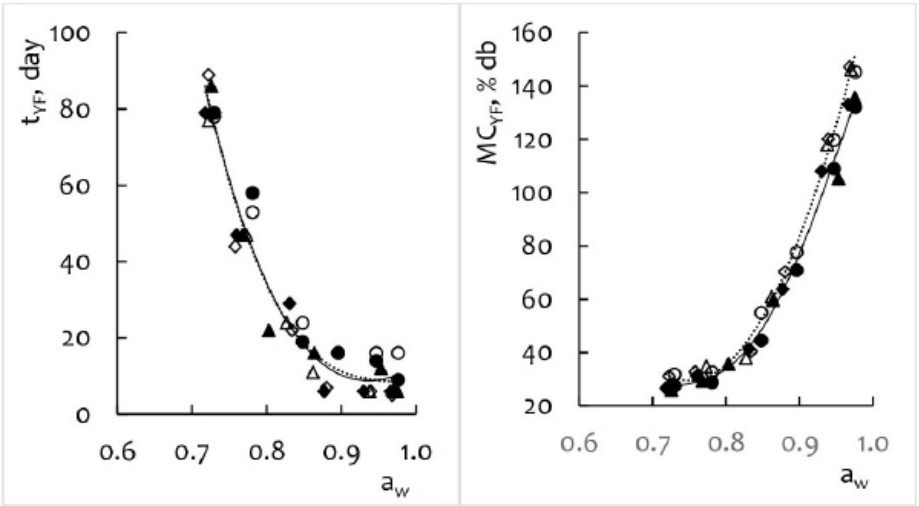
YF was observed on the surfaces of PH and CH for $a_w \geq 0.72$ at all temperatures. It was not observed for $a_w \leq 0.69$ (the highest a_w just below $a_w=0.72$ in the work) at any temperature. $a_w=0.7$ was practically denoted as the border between the absence and presence of YF.

Time for YF (t_{YF}) decreased as a_w increased and the effect of temperature was mixed at a given a_w for both PH and CH (Fig. 1a). Honey which exhibited YF earlier at a given temperature and a_w was also mixed according to data and polynomial fits in Fig. 1a. Then, PH and CH was assumed to have the same t_{YF} at the same temperature and a_w. For both PH and CH, the earliest YF was observed at $a_w=0.97$ and t_{YF} was around five days, and the last YF was observed at $a_w=0.72$ and t_{YF} was around 90 days.

MC values corresponding to t_{YF} (MC_{YF}) increased with increasing a_w and the effect of temperature on it was mixed at a given a_w for both PH and CH (Fig. 1b) as in the case of t_{YF}. CH had significantly ($p \leq 0.05$) higher MC_{YF} than PH at the same a_w according to data and polynomial fits in Fig. 1b. The smallest MC_{YF} for PH and CH was 25.9% db (20.6% wb) and 27.7% db (21.7% wb), respectively at $a_w = 0.72$. The greatest MC_{YF} for PH and CH was 135.7% db (51.1% wb) and 147.3% db (59.6% wb), respectively at $a_w=0.97$.

Sorption isotherm (SI)

The EMC of PH and CH at a given a_w was to be obtained at the end of the dynamic sorption phase providing MC vs time data. The MC of honeys decreased with time at $a_w<0.7$ and increased at $a_w>0.7$ during the dynamic phase. EMCs of samples at 30 and 40°C were experimentally obtained before 90 days at $a_w<0.7$ when desorption took place. Their EMCs were not obtained at a <0.7 within 90 days at 20°C. At $a_w>0.7$, when adsorption took place, the



a b

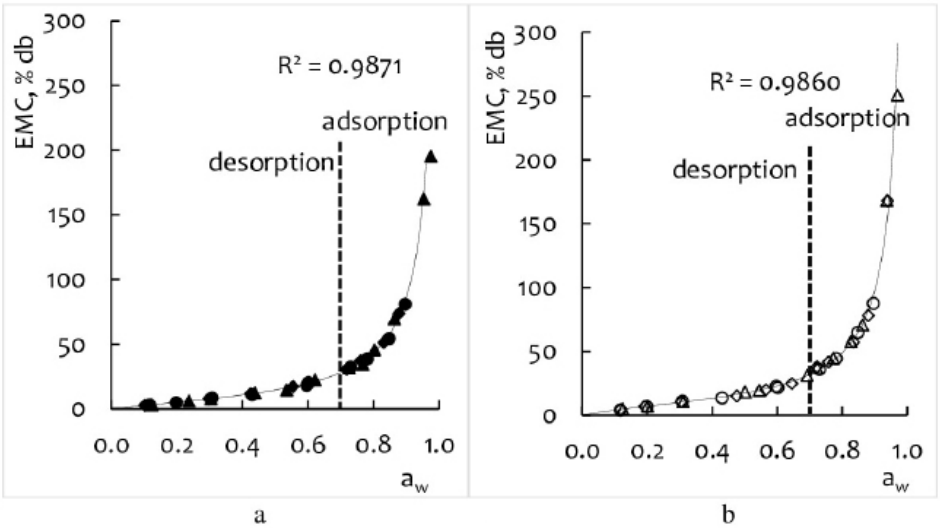
Filled: PH, Empty: CH, Bold line: PH*, Dotted line: CH*
Circle: 20 °C, Diamond: 30 °C, Triangle: 40 °C
*Fits all data.

Fig. 1. The effect of a_w on the time of visible yeast formation (t_{YF}) (a) and the corresponding moisture content (MC_{YF}) (b) in pine honey (PH) and citrus honey (CH).

EMCs of PH and CH were not experimentally obtained due to YF on their surfaces.

Some EMCs at a_w<0.7 and 20°C and also all EMCs at a_w>07 and all temperatures were estimated using the MC vs time data collected during the dynamic sorption phase. The estimation was performed as described in the "Materials and Methods." The employed method was checked using MC vs time data collected at a_w<0.7 and 30 and 40°C. The estimated EMCs did not deviate from the experimental EMCs more than ±1%. Then, SIs of PH and CH were constructed with the use of experimental and estimated EMC data together (Fig. 2).

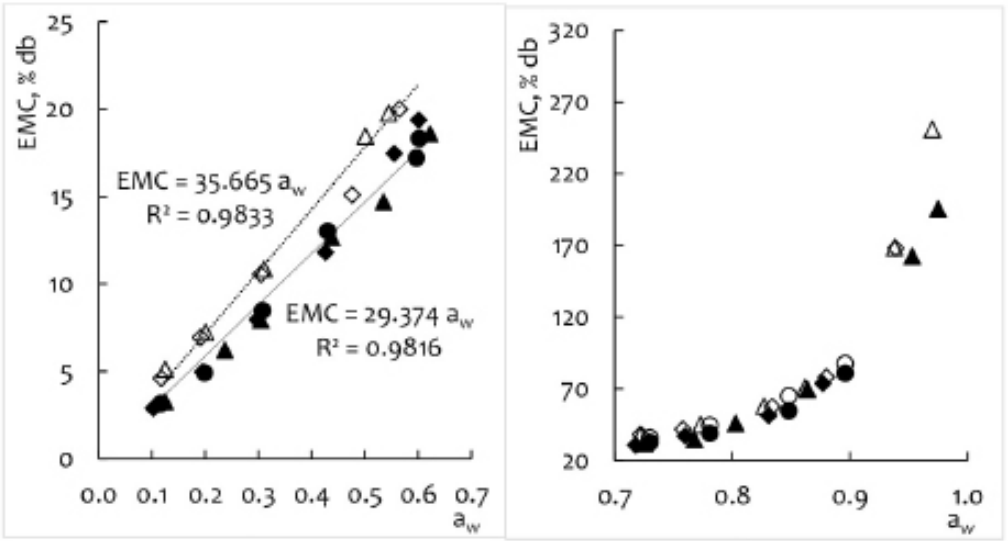
PH and CH exhibited SIs in the shape of "J" (Fig. 2). The lower part (a_w<0.7) and upper part



a b

Filled: PH, Empty: CH, Line: GAB equation *
Circle: 20 °C, Diamond: 30 °C, Triangle: 40 °C
*Fits all data.

Fig. 2. Sorption isotherm (SI) of pine honey (PH) (a) and citrus honey (CH) (b) and the fit of GAB Eq. (Eq. 2) to them.



a b

Filled: PH, Empty: CH, Bold line: PH*, Dotted line: CH*
Circle: 20 °C, Diamond: 30 °C, Triangle: 40 °C
*Fits all data.

Fig. 3. Desorption part (a) and adsorption part (b) of sorption isotherm (SI) of pine honey (PH) and citrus honey (CH).

(a_w>0.7) of the "J" practically corresponded to desorption and adsorption, respectively (Fig. 2). The smaller desorption data closely clustered due to the presence of the greater adsorption data on the same scale (Fig. 2). Fig. 2 was then divided into the desorption and adsorption parts (Fig. 3) for a clear presentation. PH always had a significantly lower ($p \leq 0.05$) EMC than CH at a given a_w, and consequently a lower SI than CH (Fig. 3).

Correlation between equilibrium moisture content (EMC) and water activity (a_w)

The SIs of PH and CH were significantly linear for the a_w-range of 0.1-0.7, i.e. the desorption part with high R^2 values (Fig. 3a). Their correlation between EMC and a_w in the full a_w-range of 0.1-0.9 was also tested with the use of the GAB equation (Eq. 2). EMC vs a_w data for both PH and CH at different temperatures were agregated because of the insignificant effect of temperature ($p<0.05$). The fitting performance of the GAB equation to the aggregated data was evaluated for R^2, relative error (RE) and distribution of error (DE) (Tab. 2).

DISCUSSION

Honeydew (pine) and a nectar honey (citrus) were used to observe how botanical origin affected the static sorption properties of honey. PH was chosen because Turkey produces 92% of the world's supply (USDA, 2015), and CH was preferred due to its specificity to the Mediterranean Climate Zone also covering Turkey.

The physicochemical, residue, microbiological, and microscopic analyses of honey samples (Tab. 1) showed that they complied with the Codex Alimentarius (1981) and were suitable for consumption and then investigating. Selected temperatures (20-40°C) covered a decent range which honey may subject to in the hive and during the handling, processing, storing, and consumption.

Table 2.

Constants of GAB equation (C, k, m_0), indicators for its fitting performance (R^2, RE, DE) and mean net sorption energy in the monolayer ($Q_{s,\,mono}$) and multilayer ($Q_{s,\,multi}$) water region for the static sorption of honey

	Pine[1]	Citrus[1]	Clover[2]	Colza[3]
a_w-range	0.1-0.9	0.1-0.9	0.3-0.8	0.2-0.9
C	2.96	4.73	4.64	5.29
k	0.9881	0.9936	0.9861	0.8356
m_0, % db	9.93	10.63	10.62	10.26
a_w <=> m_0	0.35-0.40	0.30-0.35	0.30-0.35	0.35-0.40
R^2	0.9871	0.9860	0.9924	0.9906
RE* (%)	4.73	4.33	0.51	8.30
DE*	Random	Random	Random	Random
$Q_{s,\,mono}$, kJ/kg	211	239	235	239
$Q_{s,\,multi}$, kJ/kg	84	113	111	116

*RE: Relative error = $\frac{1}{n}\sum_i^n \frac{|EMC - EMC_{GAB}|}{EMC} x100$

*DE: Distribution of error, (EMC - EMC_{GAB}) vs EMC

[1]This work, [2]Martin (1958), [3]Tabouret (1979)

EMC vs a_w data for honey was obtained with the use of either the static gravimetric or hygrometric method. Martin (1958) and Tabouret (1979) kept honey samples in various air atmospheres all having a constant RH. In such a static gravimetric practice, samples experience a dynamic sorption phase and then reach the static sorption phase. The MC and a_w of samples during the dynamic phase spontaneously change due to the exchange of water vapor with the surrounding air through either desorption or adsorption. MC and a_w decrease and increase in the case of the desorption and adsorption, respectively. Samples finally attain the static sorption phase of EMC and corresponding equilibrium a_w naturally. This way of experimentation may take a long time and samples may deteriorate before reaching equilibrium, but it mimics the natural sorption behavior and the journey of honey from hive to consumption. Doull and Mew (1977) and Rüegg and Blane (1981) inceased the MC of honey samples by diluting them with water and measured their MC and a_w. In this practice, the measurements of MC and a_w are taken as EMC and equilibrium a_w, respectively, which is quick and does not allow sample enough time for deterioration. It disturbs honey's composition, does not simulate its natural sorption behavior from hive to consumption and it is limited to the adsorption. The current work adopted the static hygroscopic method for the sake of mimicking the natural sorption behavior of honey, which is also the method for determining the SI of foods recommended by the European COST 90 project (Basu et al., 2006) and the American Society of Agricultural Engineers (ASAE, 2001).

The lowest a_w was 0.72 at which YF was observed. PH and CH stayed at a_w=0.72 for almost ninety days without YF and it was observable after ninety days (Fig. 1). YF was followed on the surfaces of samples at a_w=0.69 (the highest a_w just below a_w=0.72 in the work) for 180 days and was not observed. Based on the assumption that honey is processed or consumed within the span of 180 days in practice, conducting experiments for ninety days was assessed to be reasonable for about a_w<0.7 at 20°C where EMC was not reached.

The SI of PH and CH was obtained by combining the desorption (a_w<0.7) and adsorption parts (a_w>0.7) (Fig. 2). The SI of foods are generally obtained entirely through either desorption or adsorption through the adjustment of their MC to a higher or lower value beforehand, respectively according to the literature (Basu et al., 2006). Arranging the MC and a_w of foods moves them away from their accustomed natural state, and starting the sorption process from such an arranged state away from the natural state does not match the practice. Thus, the former approach was adopted in this work to get the SIs of PH and CH for the practicality.

YF on the surface of PH and CH

Water vapor sorption starts at the surface of honey. Water is then simultaneously transferred between the surface and the inside due to a_w-difference. The rate of transfer slows down as the a_w-difference decreases. It ceases when the same a_w, therefore the same MC, is attained throughout the sample, which reaches equilibrium with the surrounding air, provided that it has a homogeneous structure and its volume is negligible compared to that of the surrounding air. If a_w on the surface is high enough, yeast formation occurs.

The absence of YF at a_w<0.7 and its presence at a_w>0.7 at all temperatures showed the insignificant effect of temperature on YF compared to a_w. It also revealed that a_w=0.7 is a critical value (a_{wc}) which forms a practical border between the absence and presence of YF. The insignificantly different t_{YF} and MC_{YF} values for a given honey at all temperatures at a given a_w (Fig. 1) were another indicator for the insignificant effect of temperature. The effect of a_w supressed that of temperature and made its effect on YF and t_{YF} insignificant. The insignificant effect of temperature left a_w as the dominant factor governing YF and t_{YF} and then the quality of PH and CH.

The minimum MC_{YF} (25.9% db or 20.6% wb for PH and 27.7% db or 21.7% wb for CH) (Fig. 1b) was little above the maximum MC suggested by Codex Alimentarius (1981) (25% db, 20% wb) which corresponds an a_w where yeast formation

probably starts in most honeys. Martin (1958), Tabouret (1979) and Yao et al. (2003) conducted the same experimantation, and the former two and the latter were concerned with the static and dynamic sorption of honey, respectively. Martin (1958) provided EMC vs a_w up to a_w=0.81 for clover honey at room temperature (20-25°C) and observed yeast formation on the surface at a_w=0.66 corresponding to MC of 27.4% db (21.5% wb). Tabouret (1979) listed EMC vs a_w for colza honey at room temperature (20-25°C) up to a_w=1.00 where EMC was 44.6% wb (80.5% db). The author did not mention yeast formation contrary to high a_w values. Yao et al. (2003) provided data of MC vs time for yapunyah and yellow box honeys at 30°C up to a_w=0.83 where the minimum MC was greater than 60% db (37.5% wb). The authors did not report yeast formation for both honeys contrary to high a_w values. Given the context of the yeast formation, this work's findings comply with the maximum MC signified by the Codex Alimentarius (1981), and MC and a_w remarked by Martin (1958).

The significantly higher MC_{FY} values for CH than PH at a given a_w (Fig. 1b) show its higher enduring ability against MC and thus its greater water holding capacity. This enduring ability could be attributed to its higher monosaccharide (glucose and fructose) content (Gleiter et al., 2006; Abromovic et al., 2008) and lower pH (Tab. 1) which delays YF on the surface.

SI of PH and CH

EMC data estimated for a_w<0.7 with the use of MC vs time data were in line with EMC data experimentally obtained. The estimated and experimental data constructed a concordent SI for PH and CH (Fig. 2). Estimation methods in literature were similarly exploited for obtaining the EMC and SI of foods and gave good results even in case of limited data (Tejada-Ortigoza et al., 2021). The estimation method used in the work was assessed to be acceptable for the given working conditions.

EMC vs a_w showed a linear curve up to a_w=0.6-0.7 (Fig. 2), which deviated from the linearity at a_w≈0.7 and steeply increased with increasing a_w. The region for a_w<0.7 is supposed to cover the monolayer and multilayer water, and the region for a_w>0.7 is supposed to cover the loosely bound free water (Spiess & Wolf, 1987).

a_w=0.7 emerged to be a_{wc} again in terms of the direction of the sorption and appeared to be a practical border between the desorption and adsorption. Yao et al (2003) implied that the transition between the desorption and adsorption processes occurred somewhere between a_w of 0.51 and 0.68 for yapunyah and yellow box honeys. The value of a_w for sorption transition in PH and CH was in line with that for yapunyah and yellow box honeys.

Most foods' SI appears in the shape of "S" or sigmoid. The SI of PH and CH with J-appearance (Fig. 2) is common in high-sugar containing foods (Al-Muhtaseb et al., 2002). EMC vs a_w data of Martin (1958) and Tabauret (1979) similarly exhibited a J-appearance for clover and colza honey.

The EMC of samples insignificantly differed ($p>0.05$) from one another at a given a_w at all temperatures. They were then aggregated and one SI was obtained for both PH and CH for all temperatures (Fig. 2 and 3). The insignificant effect of temperature on SI is an inherent result of the dominant role of a_w over temperature as aforementioned. In general, an increase in temperature causes a decrease in EMC at a constant a_w, e.g. a shift of SI to the right (Fig. 2). Deviation from this trend has been reviewed for such high-sugar containing foods (Al-Muhtaseb et al., 2002) as honey due to the increasing solubility of sugars with increasing temperature.

The lower SI of PH than that of CH is related to the botanical origin. The lower EMC of honeydew honeys than those of nectar honeys at the same a_w was reported by Schroeder et al. (2005), Gleiter et al. (2006) and Abramovic et al. (2008). Serin et al (2018) also observed the same for pine and nectar honeys. Honeydew honeys characteristically have a lower EMC than nectar honeys at the same a_w due to a lower monosaccharide (glucose and fructose) content (Gleiter et al., 2006; Abromovic et al., 2008) as seen for PH and CH in Tab. 1.

Correlation of EMC vs a_w for PH and CH

Serin et al. (2018) found a linear correlation between EMC and a_w for 257 pine and 706 nectar honeys including 129 citrus honey samples for the a_w-range of 0.4-0.6. Serin et al. (2018) also reported linear correlations for numerous honeys in literature for almost the same a_w-range (0.4-0.7). All these works had been based on the simultaneous measurement of MC, and a_w, the linear correletions were given for a_w vs EMC, and the unit of EMC was in % wb. The linearity between EMC vs a_w was observed for the desorption part of SIs of PH and CH (Fig. 3) for a_w<0.7 as in the literature (Serin et al., 2018). The linear range included the reported short a_w-range of 0.4-0.7. MC and a_w of a honey sample could practically be estimated through the linear equation by measuring a_w or MC for a_w<0.7.

Few studies on model fitting to honey SI were found in literature besides that by Martin (1958) and Tabouret (1979) on EMC vs a_w data. The SI of PH and CH was modelled with the use of the GAB equation (Eq. 2), which is the most used equation for modelling the SI of foods. It features the largest fitting range (up to a_w=0.93) compared to other known models, theoretical background and other attributes discussed elsewhere (Basu et al., 2006). It was suggested by the European Project COST 90 on Physical Properties of Foods (Wolf et al., 1984) and the American Society of Agricultural Engineers (ASAE , 2001).

The fit of the GAB equation to food SIs is physically and mathematically sound provided that C is greater than 2 and k is between 0 and 1 according to a detailed analysis by Blahovec (2004). GAB constants for both PH and CH and also for other honeys from the literature met these conditions (Tab. 2).

The C constant in the GAB equation is related to the difference between the sorption enthalpy in the monolayer and multilayer water regions (Eq. 2). The C of PH was smaller than that of CH, and the C of other honeys was in agreement with that of CH (Tab. 2). The k constant is related to the difference between the sorption enthalpy in the multilayer water region and pure water, and the k of PH was comparable with that of CH and other honeys. The m_o is the EMC at which the rate of deteriorative reactions are minimum in foods which have the longest shelf-life at EMC≤m_o except for fatty foods. The values of m_o and a_w<=>m_o were reasonable for PH and CH, and other honeys (Tab. 2). m_o was around 10% db (9.1% wb) and a_w<=>m_o was within the range of 0.30-0.40 for PH and CH, and other honeys (Tab. 2). However, the m_o of PH was smaller than that of CH, and the m_o of other honeys was closer to that of CH. The discrepancy in some GAB constants between PH and CH, and other honeys could be attributed to botanically lower monosaccharide content (glucose and fructose content) of PH (honeydew honey) than CH, and clover and colza honeys (nectar honeys) (Gleiter et al., 2006; Abromovic et al., 2008).

In addition to meeting the conditions (Blahovec, 2004), the visual fit of PH and CH to SIs (Fig. 2), and consistency among PH, CH and other honeys in terms of constants (Tab. 2), the fitting performance of the GAB equation of PH and CH to SIs was tested. It fit SIs of PH and CH with high R^2 values greater than 0.98, RE was smaller than 5% and DE was random (Tab. 2). The fit of the GAB equation to other honeys exhibited almost the same performance as PH and CH (Tab. 2). The indicators used pointed that GAB equation fit to the SI of PH and CH, and other honeys.

Based on the fit of the GAB equation, the sorption heat was determined for PH and CH with the Clasius–Clapeyron equation (Eq. 3). It gave the net sorption energy (Q_s) above the latent heat of pure water at a given MC. Q_s was calculated using EMC obtained from the GAB equation (Eq. 2) for a given a_w. The calculation was performed for the average temperature used in the work (30°C, 303 K), and a_w was incremented by 0.1 starting from 0.1. Fig. 4 shows the change of Q_s vs EMC which has three regions. Q_s exhibited a linear steep decrease up to EMC≈10% db (a_w=0.3-0.4) in the region I, kept the linear decrease with a smoother slope up to EMC≈20% db (a_w=0.6-0.7; Fig. 2) in the region II and finally asymptotically approached to zero after EMC≈20% db in the region III (Fig. 4). The figure revealed three regions in the static sorption

behavior of PH and CH that matched their SIs in Fig. 2.

Region I in Fig. 4 represents a monolayer water region where water is strongly bound with an enthalpy of vaporization considerably higher than that of pure water, and it is not available for deteroative reactions. Monolayer water has the strongest binding, and the highest energy is required in this region for desorption in honey. The m_o was ≈10% db and a_w<=>m_o was between 0.3 and 0.4 for PH and CH and (Tab. 2, Fig. 4). The a_w<=>m_o was reported between 0.2 and 0.4 in foods (Spiess & Wolf, 1987). Region II in Fig. 4 represents a multilayer water region where water is binded less firmly than in region I. Its vaporization enthalpy is slightly higher than that of pure water, and its upper end was observed at EMC≈20% db and a_w≈0.7 for PH and CH (Fig. 2, Fig. 4). The multilayer water region is reported between a_w<=>m_o and 0.7 in foods (Spiess & Wolf, 1987). Region III in Fig. 4 represents loosely bound free water. The sorption energy in this region is related to the adsorption (Fig. 2) and has no practical importance since PH and CH are not supposed to be kept at a_w > 0.7 due to YF.

The limits of the monolayer and multilayer water regions in Fig. 2, Tab. 2, Fig. 4 and literature exhibited a good match. The sorption energy in these regions is related to the desorption which is exotermic and important during honey storage for determining the cooling load. The behavior of Q_s versus EMC for other honeys qualitatively and quantitatively complies with the scheme given for PH and CH (Tab. 2).

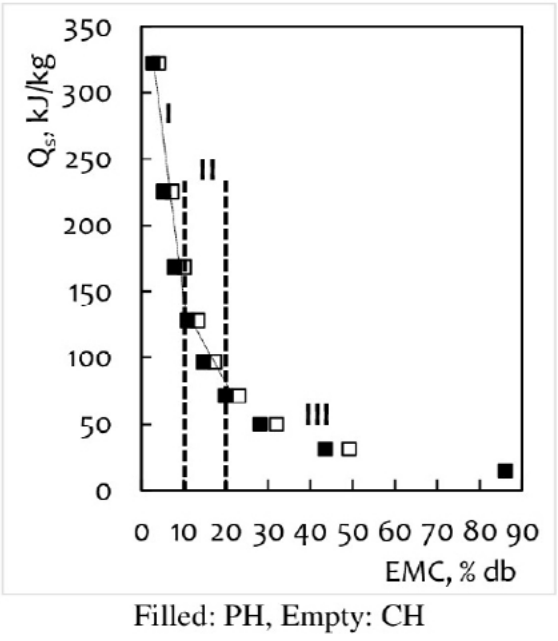


Fig. 4. Effect of moisture content (EMC) on net heat of sorption (Q_s) for pine honey (PH) and citrus honey (CH).

Tab. 2 shows average Q_s values in the monolayer ($Q_{s,\ mono}$) and multilayer ($Q_{s,\ multi}$) water regions for PH, CH, and other honeys. The $Q_{s,\ mono}$ and $Q_{s,\ multi}$ of PH was lower than that of CH, and those of other honeys were comparable with that of CH (Tab. 2). The lower Q_s values for PH than CH and other honeys could be attributed to its botanically lower monosaccharide content (glucose and fructose content) than CH, and clover and colza honeys (nectar honeys) (Gleiter et al., 2006; Abromovic et al., 2008;). Because the monosacchide content was lower both less strongly binded water and energy were required for the desorption.

In conclusion, YF was observed on the surface of PH and CH at a_w>0.7 at 20, 30 and 40°C within five to ninety days. The a_w was the dominating parameter on YF and was observed earlier in PH than CH at the same a_w due to its botanically higher pH and lower monosaccharide content. PH and CH exhibited SIs in the shape of "J" and were insignificantly affected by temperature. The SIs of PH and CH exhibited desorption a_w at <0.7 and adsorption at a_w>0.7. The a_w=0.7 appeared to be a border between the absence and presence of YF and between desorption and adsorption. PH exhibited a lower SI than that of CH due to its botanically lower monosaccharide content. The desorption part of SI of PH and CH was almost linear. The GAB model fit the SI of PH and CH with physically and mathematically consistent and meaningful constants. The change in net sorption energy (Q_s) vs EMC revealed the monolayer, multilayer and loosely bound free water regions in the SI of PH and CH. The static sorption properties of PH and CH complied with those of nectar honeys and high-sugar foods in the literature. PH as a honeydew honey exhibited the effect of its botanical origin on the behavior of SI by

being different than those of CH and other nectar honeys.

Nomenclature

a_w	Water activity
a_{wc}	Critical water activity
C	Sorption constant in GAB eq.
CH	Citrus honey
DE	Distribution of error
EMC	Equilibrium moisture content, % db
k	Sorption constant in GAB eq.
m_o	Monolayer moisture content in GAB Eq., % db
MC	Moisture content, % db or wb
MC_{YF}	Moisture content corresponding to $t_{YF,}$(% db)
p	Vapor pressure of pure water
p_w	Vapor pressure of water in food
PH	Pine honey
RE	Relative error
RH	Relative humidity, %
R^2	Coefficient of determination
Q_s	Net heat of sorption, kJ/kg
$Q_{s, mono}$	Net heat of sorption in the monolayer water region, kJ/kg
$Q_{s, multi}$	Net heat of sorption in the multilayer water region, kJ/kg
SI	Sorption isotherm
t_{YF}	Time of YF, day
YF	Visible yeast formation on the surface

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