รายงานการวิจัยฉบับสมบูรณ์

การทำนายอายุการเก็บรักษาของน้ำมันกุ้งชนิดไมโครเอนแคปซูลในบรรจุภัณฑ์ที่เหมาะสม

Shelf life prediction of micro-encapsulated shrimp oil in suitable packaging

ประเภททุน ทุนพัฒนานักวิจัยใหม่ รหัสโครงการ KREF016103

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ได้รับการสนับสนุนเงินวิจัยจาก กองทุนวิจัย ประจำปีงบประมาณ พ.ศ. 2561

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แหล่งเงิน กองทุนวิจัย

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บทคัดย่อ

น้ำมันกุ้งชนิดไมโครเอนแคปซูลสามารถเติมลงในผลิตภัณฑ์อาหารต่าง ๆ เพื่อพัฒนาเป็นผลิตภัณฑ์อาหารสุขภาพแบบใหม่ ๆ ได้ การศึกษาซอร์ปชันไอโซเทอร์มความชื้น เพื่อประเมินอายุการเก็บรักษาผลิตภัณฑ์น้ำมันกุ้งชนิดไมโครเอนแคปซูลที่บรรจุในถุงพลาสติกที่เหมาะสม สามารถช่วยทำให้ง่ายต่อการนำมาใช้ประโยชน์ โดยงานวิจัยนี้ได้ทำการศึกษาซอร์ปชันไอโซเทอร์มความชื้น และจลนศาสตร์ของน้ำมันกุ้งชนิดไมโครเอนแคปซูลที่อุณหภูมิ 30 องศาเซลเซียส ระหว่างค่า a_w 0.113 และ 0.923 ด้วยการชั่งน้ำหนัก ซึ่งได้ใช้แบบจำลองทางคณิตศาสตร์ในการทำนายข้อมูลจากการทดลอง โดยช่วงเริ่มต้นของซอร์ปชันไอโซเทอร์มน้ำมันกุ้งชนิดไมโครเอนแคปซูลเพิ่มขึ้นอย่างรวดเร็ว และลดลงเมื่อเวลาเพิ่มขึ้น ซึ่งลักษณะของซอร์ปชันไอโซเทอร์มน้ำมันกุ้งชนิดไมโครเอนแคปซูลเป็นแบบที่ 3 มี GAB เป็นแบบจำลองทางคณิตศาสตร์ที่เหมาะสมที่สุดในการทำนายซอร์ปชันไอโซเทอร์ม เนื่องจากมีค่าเปอร์เซ็นต์ root mean square error (%RMSE) ต่ำที่สุด เมื่อทำการศึกษาอายุการเก็บรักษาน้ำมันกุ้งชนิดไมโครเอนแคปซูลที่เก็บในถุง polypropylene (PP) Nylon/linear low density polyethylene (Nylon/LLDPE) หรือ metalized polyethylene terephthalate (metalized PET) ที่อุณหภูมิ 30 องศาเซลเซียส/ความชื้นสัมพัทธ์ร้อยละ 75 และที่อุณหภูมิ 30 องศาเซลเซียส/ความชื้นสัมพัทธ์ร้อยละ 80 โดยการใช้แบบจำลอง GAB ในการทำนาย พบว่า น้ำมันกุ้งชนิดไมโครเอนแคปซูลที่เก็บในถุง metalized PET มีอายุการเก็บรักษานานที่สุด (507 วัน ที่ความชื้นสัมพัทธ์ร้อยละ 80 และ 725 วัน ที่ความชื้นสัมพัทธ์ร้อยละ 75) มีค่า %RMSE เท่ากับ 0.711 ซึ่งแบบจำลองทางคณิตศาสตร์สามารถทำนายอายุการเก็บรักษาของผลิตภัณฑ์ได้อย่างรวดเร็วและแม่นยำ

คำสำคัญ: น้ำมันกุ้งชนิดไมโครเอนแคปซูล; ซอร์ปชันไอโซเทอร์มความชื้นว แบบจำลองทางคณิตศาสตร์; อายุการเก็บรักษา; บรรจุภัณฑ์

Research Title: Shelf life prediction of micro-encapsulated shrimp oil in suitable packaging
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Abstract

Micro-encapsulated shrimp oil (MSO) could be used to fortify food products, especifically for the developed a new functional foods. The evaluation of sorption isotherms for the determination of the shelf-life of MSO packaged in suitable plastic bags could facilitate their use. Moisture sorption isotherms and kinetics of MSO were evaluated at 30°C between the water activity (a_w) values of 0.113 and 0.923 by a static gravimetric method. The empirical models were determined to predict the experimental data. The initial stage of moisture sorption by the MSO was more rapid, which decreased with time. All the sorption curves were found to be type III. The most suitable model for predicting the moisture sorption isotherm of MSO was the GAB model because it had the lowest percentage root mean square error (RMSE). When the shelf-life of MSO packaged in polypropylene (PP), Nylon/linear low density polyethylene (Nylon/LLDPE) or metalized polyethylene terephthalate (metalized PET) pouches stored at 30°C and either 75% or 80% relative humidity (RH) were predicted by the GAB equation, the longer shelf-life of MSO (507 days in 80% RH and 725 days in 75% RH) was when they were packaged in metalized PET bag -having %RMSE of 0.711. The approximation of empirical models was achieved for accurate and rapid shelf-life prediction.

Keywords: Micro-encapsulated shrimp oil; Moisture sorption isotherm; Empirical model; Shelf-life; Packaging

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INTRODUCTION

Shrimp oil is one of the major sources of polyunsaturated fatty acids (PUFAs) and astaxanthins, which known for their health benefit, but are susceptible to oxidation. Encapsulation can be used to delay or inhibit the oxidation and mask unpleasant flavors and odors in the resulting products [1] and micro-encapsulation of oil can be produced using by spray drying [2]. Good quality microencapsules, with low a_w, ease of storage and handling are able can protect the active material against undesirable reactions [3]. However, many food ingredients that are added in the form of powders tend to have sticking and caking problems due to moisture absorption during storage. Water molecules permeate into the package, leading to an increase in moisture content of packed product with the result of decreasing its quality [4]. Moisture sorption isotherms are a useful mean for monitoring the drying, and shelf-life stability of packaged products, are associated to hydration of products [6] and represent the correlation between a_w and equilibrium moisture content (EMC) in food products at a constant temperature [5]. They are usually classified into types I, II, III, IV and V [7]. In general, dried food products are defined as Type II or III isotherms [8]. Previously sorption isotherms have been described by mathematical models that have been validated using statistical methods [8, 9]. Thus, it is a requirement to choose a suitable equation of moisture sorption isotherm for a specific product (MSO). This study was aimed at investigating the moisture sorption kinetics in order to select an empirical model for the moisture sorption isotherm and to evaluate the shelf-life of MSO packaged in bags made from different plastics.

MATERIALS AND METHODS

Materials

Sodium caseinate was procured from Vicchi enterprise Co., Ltd. (Bangkok, Thailand). Fish skin gelatin (bloom strength of 230-250 g) was procured from Lapi Gelatine S.p.A. (Milano, Italy). Glucose syrup was obtained from Charoenworrakit Co., Ltd. (Samut Prakan, Thailand).

All the plastic bags were purchased from a supermarket. Polypropylene (PP), Nylon/linear low density polyethylene (Nylon/LLDPE) and metalized polyethylene terephthalate (metalized PET) bags with the dimension of 3.0 cm x 2.0 cm with a thickness of 89, 82 and 85 μm, respectively, were used.

Preparation of micro-encapsulated shrimp oil (MSO)

The shrimp oil was extracted from the digestive gland (hepatopancreas) of Pacific white shrimp (*Litopenaeus vannamei*) using a mixture of isopropanol and hexane as per the method of Takeungwongtrakul *et al.*[10]. Fish gelatin, sodium caseinate and glucose syrup were mixed, at a ratio of 1:1:4 (w/w/w), to prepare the "wall materials" according to Takeungwongtrakul and Benjakul[2]. An SD-06A spray-dryer was used to produce micro-encapsulated shrimp oil (MSO) following the method of Takeungwongtrakul *et al.*[10]. MSO (4 g) was packaged in the different bags, described above, and heat-sealed.

Determination of initial and critical moisture content

Initial moisture content (IMC) was determined following the method of AOAC[11] and the critical moisture content (CMC) of MSO was determined by placing MSO into water desiccators at 30°C until MSO was assessed to have become unacceptable by 10 trained panelists. Panelists were trained to evaluate for appearance of MSO. Prior to the evaluation, the panelists were trained three times a week. Panelists were trained with standards for two sessions using a scale of 0 – 15, where 0 and 15 represent dry and wet MSO, respectively. The appearance of MSO exhibited the initial quality attribute that affect the acceptability of MSO.

MSO was evaluated for moisture content (% dry weight basis) every hour until MSO reached their CMC. The CMC was identified as the point at which the deterioration of MSO reached an unacceptable level. The CMC was reached when the MSO stick together to a level at which it would be rejected by the panelists.

Moisture sorption characteristics

Moisture curve and rates

Sorption isotherms were measured by a static gravimetric technique, using different saturated salt solutions [12]. MSO was pre-dried in a desiccator over phosphorous pentoxide (P_2O_7) and then were kept in separated desiccators with different saturated salt solutions. The desiccators were kept in an electric oven at 30±1°C, which gave equilibrated environments in the desiccators. All saturated salt solutions ($MgCl_2$, KI, KCl, NaCl and K_2NO_3) were selected to produce a broad range of a_w (0.324-0.923) [13]. A_w of storage conditions were confirmed by means of a humidity meter (Vaisala, Helsinki, Finland, Model HM70). Weights of MSO were determined as a function of time. Moisture content (g H_2O/100 g dry sample) of MSO were determined by placing samples in an oven at 105°C for 3 h. A_w was measured using an AquaLab Series 3 water activity meter (Decagon Devices, Inc., Pullman, WA, USA). Data of moisture adsorption were fitted into the following equation (Peleg [14]):

$$M_t = M_0 + t / (k_1 + k_2.t)$$

where M_t represents the moisture content after designate time of storage, M_0 represents the initial moisture content, k_1 represents the constant of Peleg rate (h/(g water/g solids)) and k_2 represents the constant of Peleg capacity (g solids/g water).

Sorption isotherm

The moisture sorption isotherm of MSO was determined over the a_w range of 0.113-0.923 using different saturated salt solutions in the desiccators as described above The salt solutions were K_2NO_3, KCl, NaCl, KI, $Mg(NO_3)_2$, $MgCl_2$ and LiCl giving the following a_w: 0.923, 0.836, 0.751, 0.679, 0.514, 0.324 and 0.113, respectively [13]. Sample weights, as a function of time, were determined until they reached equilibrium. The moisture contents (% dry weight basis) in the samples were measured using an oven at 105 °C for 3 h. A_w was evaluated using an Aqua-Lab Water Activity Meter. The percentage of the equilibrium moisture content (EMC) in the samples at each specific a_w was determined as using the method described by Rachtanapun [15]:

$$\%EMC = (W_e / W_i) \times (M_i + 1) - 1$$

where, W_e represents total weight (dry weight + water) at equilibrium (g), W_i represents the initial weight (g) and M_i represents the initial moisture content (g/g). Thus, a sorption isotherm was constructed by plotting between a_w and %EMC.

Moisture sorption isotherm modeling of MSO

The models of sorption isotherm of MSO were expressed as shown in Table 1. The model parameters were derived from the experimental results. The root mean square percentage error (%RMSE) is the predicting capability of a model in relation to the number of data points.

Table 1 Moisture sorption models for fitting experimental data

Model names	Model equations	References
BET	$M = m_o C a_w / [(1 - a_w) + (C - 1)(1 - a_w) a_w]$	Brunauer*et al.*[16]
GAB	$M = m_o C k a_w / [(1 - k a_w)(1 - k a_w + C k a_w)]$	Van Den Berg[17]
Peleg	$M = a.a_w^b + c.a_w^d$	Peleg[18]
Lewicki	$M = [F/(1 - a_w)^G] - [F/(1 + a_w^H)]$	Lewicki[19]
Oswin	$M = k[a_w/(1 - a_w)]^c$	Oswin[20]
Smith	$M = C_1 + C_2.\ln(1 - a_w)$	Smith[21]

Shelf life prediction of the packaged MSO

MSO (4 g) was packed in the different plastic bags (PP, Nylon/LLDPE and metalized PET) that were then heat sealed. MSO is very sensitive to changes in %RH. A little change in RH has affected the amount of water in MSO. So, the packaged MSO were stored at 30°C and either 75% RH or 80% RH for 13 weeks.

Water vapour permeability (WVP) determination

WVP was determined by a modified ASTM method[22] as modified by[23].

Calculation and verification of shelf-life of the packaged MSO

The simplest shelf-life calculation is when the isotherm is treated as a linear function in the linear model[24]. The model can be calculated as follows:

$$t = [(l.w_d.\ B) / (P.A.p_s)].\ln [(a_{wo} - a_{w\,t=0}) / (a_{wo} - a_{w\,t=t})]$$

where w_d represents the dry weight in the packaged food, l represents the film thickness, P represents the water vapour permeability coefficient, A represents the package surface area, p_s represents the saturated vapour pressure of water at the storage temperature, a_{wo} represents the water activity of storage conditions, $a_{w\,t=0}$ represents the initial water activity of product, $a_{w\,t=t}$ represents the critical water activity of product at time = t, B represents a slope of the moisture sorption isotherm.

The samples were examined for the EMC every week. The value of %RMSE was used to evaluate the fitting ability of the experimental with the predicted moisture content. The lower the %RMSE values the better the fit.

Statistical analysis

Experiments were performed in triplicate and data were subjected to analysis of variance (ANOVA) and mean comparison by using a Duncan's Multiple Range Test using the Statistical Package for Social Science (SPSS for windows, SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Moisture content and a_w of MSO

MSO initially had moisture content of 1.13 ± 0.01% (dry basis), which corresponded to the low a_w (0.35 ± 0.01). The CMC and critical a_w were 7.78 ± 0.07% (dry basis) and 0.71 ± 0.02, respectively. MSO is susceptible to absorb moisture from the surrounding atmosphere and is considered a moisture sensitive food. High a_w in food products prone to shorter storage life because of high free water for biochemical reaction associated with deterioration [25].

Moisture sorption kinetics

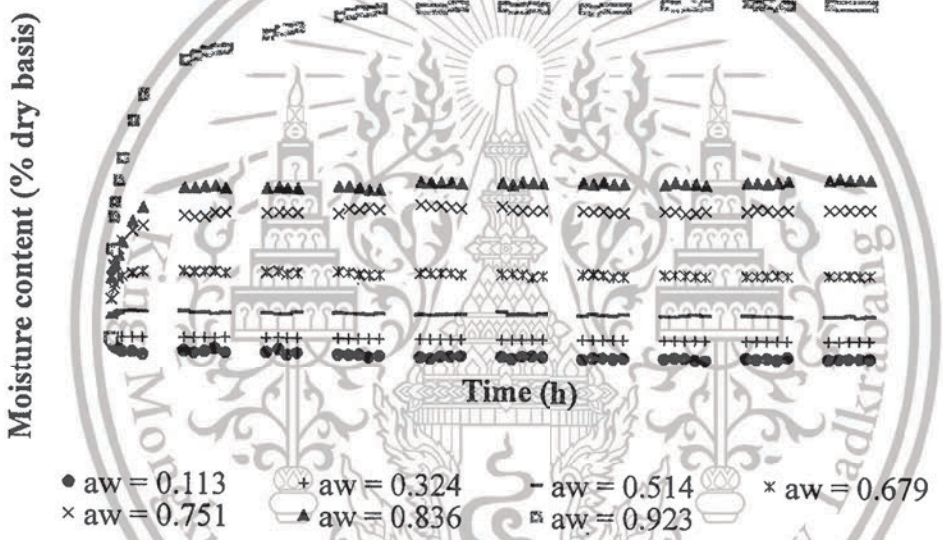

Figure 1 Moisture sorption curves of MSO at various water acitivity as a function of time

The sorption kinetic curves of MSO are given in Figure 1. Moisture sorption was rapidly increased during the initial stage, while less moisture was adsorbed as storage time progressed and their moisture content plateaued, which was likely to be the equilibration a_w in each condition. At a_w of 0.113-0.923, more time was required to reach each equilibrium. Baucour and Daudin [26] reported that mass transfer is very slow at high a_w, indicating that it difficult to reach equilibrium in a_w of 0.9–1.0. The sorption kinetic of MSO under the experimental situation was predicted with Peleg's model, which associated the hydration behavior of MSO. The coefficient of determination were found to be high in all cases (r^2> 0.99), which are a desire fit to the the experimental data. The constants of Peleg's rate (k_1)and capacity (k_2) are shown in Table 2.

Food products generally kept at a higher RH have lower values of k_1 and k_2 [27]. Turhan *et al.* [28] reported that k_1 is associated with mass transfer. The results indicate that a decrease in k_1 of

MSO was also found when a_w was increased, which was accordance with an increase in initial rate of water absorption. k_2 is associated with the maximum water absorption capacity [28] and decreased as a_w increased from 0.324 to 0.923, exhibiting the water absorption capacity of MSO increased with increasing a_w. However, k_1 showed a higher value at a_w 0.836, suggesting a lower degree of initial water adsorption rate [27]. This is due to a slow mass transfer at high RH. Thus, the effect of a_w on water absorption of MSO most likely depends on type and composition of wall material.

Table 2 Sorption kinetic model constants and coefficient of determination for MSO

Water activity	MSO		
	k_1	k_2	R^2
0.324	27.684	18.169	0.9917
0.679	0.0491	0.3719	0.9997
0.751	0.2546	0.1875	0.9998
0.836	0.3255	0.1537	0.9991
0.923	0.2217	0.0728	0.9996

k_1 = the constant of Peleg rate, k_2 = the constant of Peleg capacity, R^2 = the coefficient of determination

Moisture sorption isotherm of MSO

Change in the experimental and predicted EMC of MSO in a_w range of 0.113–0.923 is given in Figure 2. The EMC was increased at higher a_w, which is classified as a Type III isotherm. Foods containing high-sugar levels give Type III isotherm and they absorb relatively small amount of water at low a_w, especially beyond 0.60 [29]. The EMC of MSO increased above the a_w of 0.514. A slight increase in EMC with increasing a_w was found at a_w lower than 0.514. At high a_w ($a_w > 0.514$), the components of the wall material in MSO showed the highest effect on the sorption behavior, making a high equilibrium water uptake. At low a_w, water could be absorbed only at the surface. As a_w increased, the dissolution of soluble constituents caused the increase in moisture content [30]. Water molecules penetrated through the pores of the swollen structure and then are mechanically trapped in the voids especially at higher a_w, which could be affected by the stability of the microporous structure [31]. The results reconfirmed that the constituents were able to interact directly with the water molecules, which affected the moisture sorption ability of MSO. The behavior was similar to that reported for beta glucan-rich biscuits [32] and freeze-dried and spray-dried passion fruit pulp powders [33]. Barreiro *et al.* [34] reported that the EMC of barley malt sharply increased at higher a_w of 0.5, mainly at higher amounts of enzyme activity and sugars. At lower a_w, milk proteins showed preference to the sorption sites and at higher a_w, lactose

affected the protein because its EMC increased at higher a_w [32]. The incorporation of lipids can reduce water sorption because lipids have low water uptake capacity [35].

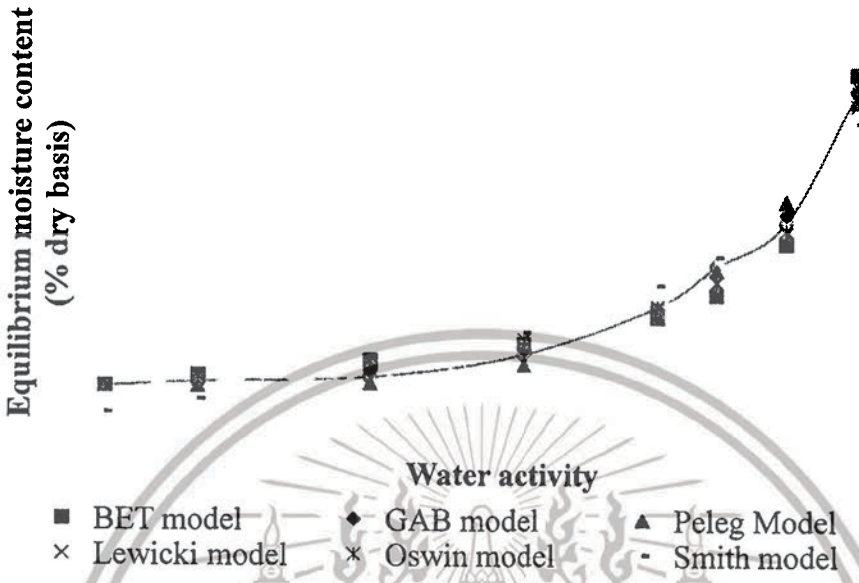

Figure 2 Moisture sorption isotherm of MSO

Modeling of sorption isotherm

The sorption isotherm was predicted using six sorption isotherm equations (BET[39], GAB[17 39], Peleg[18], Lewicki19, Oswin[20] and Smith[21]). The isotherm models, equation constants and root mean square percentage error (%RMSE) for each model of MSO are summarized in Table 3. The regression analysis was fitted to the constant values. The GAB model had the lowest values of 0.64 %RMSE, which was used to fit the moisture sorption isotherm of spray-dried MSO at 30°C, followed by the Smith, BET, Lewicki, Peleg and Oswin models, in that order. The results indicate that the GAB model was the most appropriate to predict the behavior of moisture sorption for MSO. In general, the GAB model shows the most accepted model for foods and M_o shows the amount of adsorbed water in a monolayer on the adsorbent surface. It is also used to measure the availability of active sorption sites in MSO. It is a crucial quality factor for the planning optimal storage conditions. The parameter C_G represents the stronger bonds between water molecules at monolayer and the binding sites on the absorbent surface. In addition, the k is a correction parameter for multilayer molecules relative to the bulk liquid. When k is 1, the molecules beyond the monolayer act as pure water [36]. Heat evaporation of the multilayer molecules is the same as pure water (k = 1) [37]. Sormoli and Langrish [38] used the GAB model to fit the moisture sorption data and found it to be more effectively than other models they tried. The ability of the GAB model to fit the moisture sorption isotherms of foods with a wide range of a_w values was documented by Timmermann [39]. The monolayer moisture content shows the maximum shelf-life of dried products with a_w values of between 0.2 and 0.3 and the lipid oxidation can

be initiated above this moisture content [40]. Changes in such factors as stickiness and caking of food powders occurred at a range of a_w from 0.35 to 0.5 [38]. However, the CMC of food products in regards to microbial growth terms was recommend to be 0.6 [40]. Thus, the appropriate a_w for MSO should be at $a_w < 0.3$ and moisture content of lower than 12.14% (on a dry weight basis) was recommended (Table 3). The Smith model provides the sorption isotherm of biological substances. Al-Muhtaseb *et al.*[41] reported that the Smith model was used to fit the experimental data between a_w values of 0.35 and 0.9. The BET model could be used to estimate the monolayer water content in the food products, which was applicable at a_w range of 0-0.5 [42]. Nevertheless, the Lewicki model was applicable to a high range of a_w levels. The water content leads to infinity as a_w reached 1.0 [19]. The Oswin model expressed the moisture isotherms throughout the all the range of a_w [20]. Nonetheless, this present study found that the maximum %RMSE value was best fitted with the Oswin model. The non-sigmoid and sigmoid isotherms could be predicted using the Peleg model [18], since this model could predict the moisture sorption isotherm more effective than the GAB model. The %RMSE data from the Peleg model were higher than those of GAB model.

Table 3 Sorption isotherm model constants and percentage of root mean square error for MSO

Sorption isotherm model	Constant	%RMSE
BET	m_o = 2.69	1.14
	C = 5.53	
GAB	M_0 = 12.14	0.64
	k = 0.87	
	C_G = 0.29	
Peleg	a = 22.24	1.90
	b = 4.34	
	c = 22.24	
	d = 4.34	
Lewicki	F = 52.29	1.21
	G = 0.38	
	H = 0.28	
Oswin	k = 5.01	2.12
	c = 0.76	
Smith	C_1 = -2.93	0.90
	C_2 = -12.47	

The experimental and the predicted models of moisture sorption isotherm for MSO are shown in Figure 3. Validation of the established model was evaluated by comparing the predicted moisture contents with the experimental counterpart in MSO. The predicted data usually banded around the straight line, suggesting the mathematical model was suitable to describe the sorption behavior of MSO.

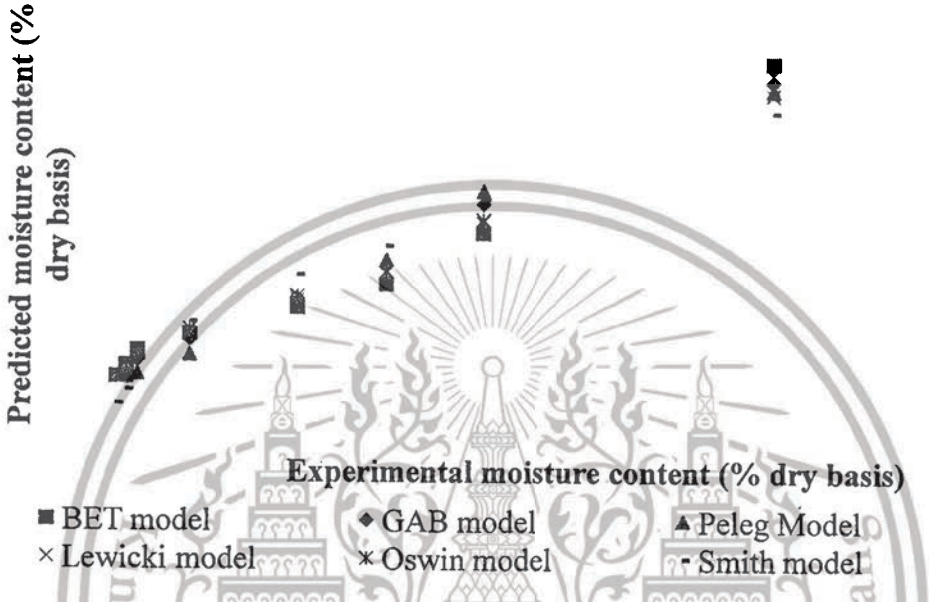

Figure 3 Comparison between experimental moisture content and predicted moisture content of MSO by various sorption models

Shelf-life prediction of MSO

The predicted shelf-life of MSO in different bags and storage conditions is shown in Table 4. The commercial metalized PET and PP bags extended the shelf-life of MSO more efficiently than the commercial Nylon/LLDPE bag. However, the shelf-life of MSO packaged in PP bag was slightly lower than that of metalized PET bag. The atmosphere inside the Nylon/LLDPE film bags had a higher WVP than the other two ($p< 0.05$), which was more likely related with its lower thickness (Table 4). However, there was no significant difference ($p> 0.05$) in WVP between metalized PET and PP films. WVP is exceedingly important property of packaging used for moisture sensitive food [15]. The results indicated that the different bags showed the influence of WVP on the shelf-life of MSO. The verification of shelf-life for MSO packaged in metalized PET bag was performed at 30°C, 75% RH for 13 weeks. The EMC between the predicted and experimental values were plotted against storage time as indicated by product stability curves (Figure 4). The %RMSE estimated the quality of model fitting. The low %RMSE values indicated the small difference between the predicted and experimental data. The mathematical model successfully predicted the EMC

evolution profile with the %RMSE of 0.711. The linear model could thus be considered a reliable tool for predicting the EMC for MSO in the bags.

Table 4 Thickness and permeability coefficients of films and predicted shelf-life of MSO in different bags under various storage conditions

Package	Thickness (μm)	WVP ($\times 10^{-3}$ g.mm/day.m^2.mmHg)	Relative humidity (%)	Shelf life (days)
PP	89 ± 0.00a	6.09 ± 0.37b	75	722
			80	505
Nylon/LLDPE	82 ± 1.59c	9.31 ± 1.60a	75	436
			80	305
Metalized PET	85 ± 0.00b	5.76 ± 1.06b	75	725
			80	507

Data are expressed as mean ± SD (n=3)

Lowercase letters in the same column indicate significant difference ($p < 0.05$).

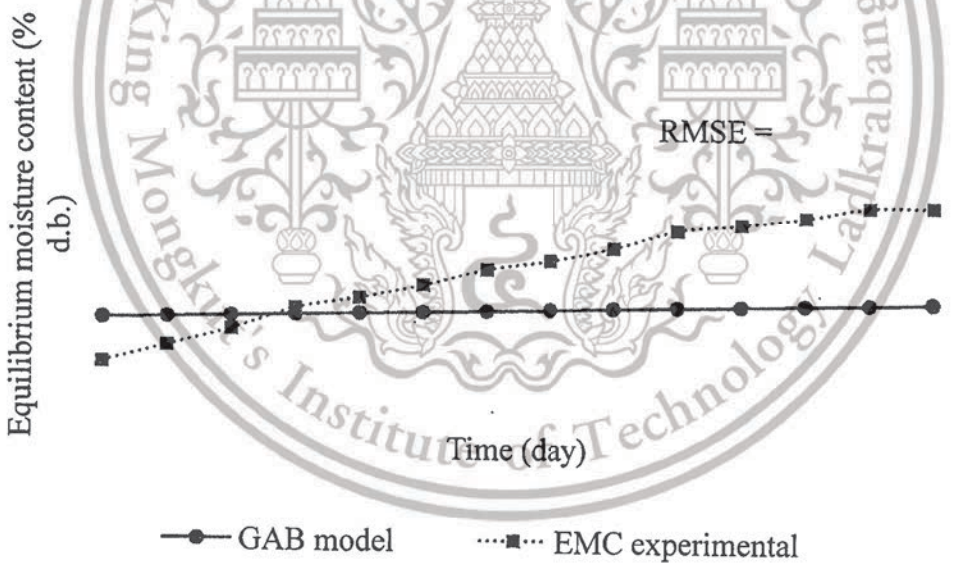

Figure 4 Predicted EMC and experimental data for MSO packed in metalized PET bags and stored at 30°C/75%RH

CONCLUSIONS

The isotherms and moisture sorption kinetics of MSO were made using various types of saturated salt solutions. The initial stage of the moisture sorption kinetics of MSO occurred more rapidly. However, the amount of water absorbed decreased with time. MSO exhibited Type III isotherm. The GAB model was the most appropriate model to estimate the moisture sorption isotherms of MSO. The predicted shelf-life of packaged MSO in metalized PET bag at 30°C and 75% RH was fitted to the actual shelf-life. Thus, the approximation of empirical models was achieved for estimating accurate and rapid shelf-life predictions.

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RESEARCH EXPERIENCES

List of International Publications

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