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Determination of Moisture Sorption Isotherm using Brunauer-Emmett-Teller (BET) and Guggenheim-Anderson-de Boer (GAB) Models, Germination Capacity and Index Vigor of Soybean during Storage

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ABSTRACT

Understanding the interaction between the equilibrium moisture content of a product and the surrounding air is crucial for guaranteeing product quality and preserving its characteristics throughout storage. The study of this tendency may be conducted utilizing sorption isotherms. This research aimed to study the moisture sorption characteristics of soybeans during storage, identify the most suitable mathematical model for the experimental data, and examine the relationship between the germination capacity and vigor index of soybeans with their moisture content and water activity. Moisture sorption characteristics of soybean, Anjasmara variety, were investigated at temperatures of 30°C, 40°C, and 50°C, and water activities of 0.08 to 0.80, and were modelled using two different mathematical models, Brunauer-Emmett-Teller (BET) and Guggenheim-Anderson-de Boer (GAB). Both two mathematical models, BET and GAB, were found to effectively describe the moisture sorption data based on regression analyses and goodness of fit. Each model was statistically evaluated by coefficient of determination and deviation standard. The moisture sorption behaviour of soybean seeds displayed a sigmoidal pattern. The GAB models have been suggested to estimate the moisture content at certain humidity levels at 30°C, 40°C, and 50°C temperatures. The BET models were suitable for temperatures between 30°C and 40°C. The vigor and germination indicated exhibited a progressive and optimal trend at a water activity level of 0.32, followed by a decline at all three temperatures.

Keywords:
Brunauer-Emmett-Teller model; Guggenheim-Anderson-de Boer model; Moisture sorption isotherm; Soybean; Vigor index

1. Introduction

Soybeans are a valuable agricultural commodity, accounting for roughly 85% of global soybean crop processing. Soybeans are produced worldwide to fulfil the nutritional requirements of humans

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and animals, oil extraction, and various industrial purposes [1]. Soybeans are valued for their high protein content (38 – 45%), which is used to produce a variety of food products such as tofu, tempeh, soy milk, and soy sauce, as well as a meat alternative in vegetarian diets. Soybeans have a protein level equivalent to animal proteins, making them a viable option for those wishing to limit their intake of animal-based proteins [2,3]. Soybeans, with about 18 – 19% oil content, are also high in oil and are often used in soybean oil manufacturing [4]. Furthermore, soybeans have been linked to possible health advantages such as cancer prevention, antidiabetic activity, and improved skin health [5].

Soybean moisture sorption isotherms reveal an equilibrium connection between moisture content and relative humidity. Therefore, it is critical to investigate soybean moisture sorption isotherms. This is beneficial for maintaining the product's quality and storage qualities because it explains the relationship between the soybeans' equilibrium moisture content and the air around them [6]. The food industry may optimize drying and processing methods by employing sorption isotherms to predict the stability of specific products [7]. Furthermore, these isotherms may provide information on the physiological processes that occur in healthy seeds, as well as how their sorption properties vary based on factors such as developmental stage, dryness tolerance, and dormancy status [8]. To maintain the long-term storage stability of dried soybean seeds, it is crucial to investigate sorption isotherms. This information may be used to describe and predict their storage behaviour [9]. Drying soybean seeds more effectively and with more excellent quality necessitates an understanding of sorption drying kinetics and the use of technologies [10,11].

Sorption isotherms of soybeans have been reported previously by Zeymer *et al.,* [6] by approaching the most appropriate mathematical model to describe the desorption and adsorption isotherm phenomena of soybean seeds. In the previous study, Zeymer *et al.,* [6] investigated the sorption isotherm of soybeans from a variety of DM 681691PRO from Brazil. Eight mathematical models were fitted to the experimental data, such as Chung Pfost, Copace, modified GAB, modified Halsey, Harkins-Jura, modified Henderson, and Smith. Zeymer *et al.,* [6] reported that the isotherms obtained for the desorption and adsorption process were classified as type III, and the modified Halsey model satisfactorily represented the desorption and adsorption phenomena of soybean grains. This study was focused on investigating the moisture sorption isotherm of soybean seed from the Anjasmara variety using Brunauer-Emmett-Teller (BET) and Guggenheim-Anderson-de Boer (GAB) models and to determine the germination capacity and vigor index of the soybean seeds.

2. Methodology

2.1 Materials

The study was conducted at the Indonesian Centre for Agricultural Postharvest Research and Development in Bogor, Indonesia. The main ingredient was soybean seeds, the Anjasmoro variety, purchased from the Indonesian Legume and Tuber Crops Research Institute in Malang, East Java.

Chemicals for adjusting the various water activity (a_w) levels, five saturating salt solutions, such as NaOH (Merck), $MgCl_2$ (Merck), NaBr (Merck), NaCl (Merck), and KBr (Merck) were used. These saturated salts could be used to adjust the water activity (a_w) condition at 30°C refer to the Table Greenspan values for saturated salts [12], respectively NaOH (0.08), $MgCl_2$ (0.32), NaBr (0.56), NaCl (0.75), and KBr (0.80).

The equipment used to investigate the sorption isotherm phenomenon included sorption containers, an analytical balance, incubators, oven dryers, tongs metal, a desiccator, and aw meters.

2.2 Equilibrium Relative Humidity (ERH)

Saturated salts for adjusting the various a_w levels were used, such as NaOH (0.08), $MgCl_2$ (0.32), NaBr (0.56), NaCl (0.75), and KBr (0.80). These saturated salts could adjust the a_w condition at 30^{o}C. Refer to the Table Greenspan values for saturated salts [12,13] , respectively.

Soybean seeds were subjected to an isotherm sorption experiment utilizing five saturated salt solutions. Until equilibrium relative humidity (ERH) was reached, the balancing procedure was carried out in sorption containers with saturated salt solutions using water activity (a_w) conditions ranging from 0.08 to 0.80. Ten grams of soybean were placed in a sorption container filled with saturated salt and incubated at 30°C, 40°C, and 50°C. Two-day intervals of weighing were performed until a steady state was reached. According to Al-Khalili [13], the ERH requirement was met if the change in moisture content on three consecutive weightings was less than 2 mg/g db and less than 10 mg/g db under high a_w circumstances (a_w>0.9). After ERH was reached, the soybeans were investigated its moisture content [14]. Samples of moisture content were calculated using % db, and three replications of the experiment were conducted to measure the equilibrium moisture content.

Fig. 1. Soybean seeds sorption isotherm experiment using several saturated salts

2.3 Sorption Isotherm Models

This research used the Brunauer, Emmet, and Teller (BET) and Guggenheim-Anderson-de Boer (GAB) equations as their models. The a_w and moisture content values acquired at the examined temperatures were modified using the BET and GAB equations to identify the best approximation of the soybean seeds isotherm sorption.

2.3.1 BET model

According to Eq. (1), the modified BET equation model was used to evaluate the suitability of the soybean seed's isotherm sorption data.

$$M = \frac{C_b\, a_w\, M_o}{(1-a_w)(1+(C_b-1)\, a_w)} \quad (1)$$

$$\frac{a_w}{(1-a_w)\, M} = \frac{1}{C_b M_o} + \frac{C_b-1}{C_b M_o}\, a_w \quad (2)$$

Where M is the moisture content of soybean seeds on a dry basis (% db), M_o is the monolayer moisture content (%), a_w is water activity, and C_b is the BET constant, an adsorption energy constant in the monolayer equation, the amount of sorbate sorbed by 1 g of sorbent at sorbate activity a_w. [15].

2.3.2 GAB model

The quadratic equation of the GAB model was used to evaluate the suitability of the soybean seeds isotherm sorption data, according to Eq. (3).

$$\frac{M}{Mo} = \frac{CKa_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \tag{3}$$

$$\frac{a_w}{M} = \frac{1}{CKMo} + \frac{(CK-2K)}{CKMo}a_w + \frac{(K^2-CK^2)}{CKMo}\,a_w^2 \tag{4}$$

Where M is moisture content, % dry basis, M_o is the monolayer moisture content, a_w is water activity, C is the monolayer water adsorption energy, and K is the multilayer water energy constant (above monolayer water).

2.4 Vigor Index of Soybean Seed

The vigor index was investigated following the previous methods [16], using Eq. (5):

$$\text{Vigor index (\%)} = \text{Seedling length } \times \text{Germination (\%)} \tag{5}$$

2.5 Germination Capacity of Soybean Seed

Germination capacity was determined following Mangena and Mokwala [17] method. Soybean seeds were placed on moistened filter paper in a single layer. The filter paper was placed in a petri dish or similar container. The dishes were covered to maintain humidity and prevent contamination. Soybean seeds were incubated at a consistent temperature, usually around 25^{o}C, and kept away from direct sunlight and extreme temperatures. After a set period of 7 days, the number of soybean seeds that have germinated was calculated, and the germination capacity can be calculated following Eq. (6):

$$\text{Germination capacity (\%)} = \frac{\text{Number of germinated seeds}}{\text{The total number of seeds sown}} \times 100\% \tag{6}$$

2.6 Microstructure of Epidermis Surface and Kernel of Soybean Seeds

The seed epidermis surface and kernel of soybean seeds microstructure were analysed using a Scanning Electron Microscope (SEM). The seeds were coated with gold-palladium alloy (85:15) in the JEOLJEE 48 vacuum evaporator and observed under JEOLJSMT-35 SEM.

2.7 Data Analysis of Soybean Seeds

The Brunaer-Emmet-Teller (BET) and Guggenheim-Anderson-de Boer (GAB) equations were used in this research to describe isotherm sorption data from soybeans. According to Eq. (7), the modified BET equation model was used to evaluate the suitability of soybean seed sorption isotherm data.

$$\frac{a_w}{(1-a_w)M} = \frac{1}{C_b M_o} + \frac{C_b - 1}{C_b M_o} a_w \quad (7)$$

Where: M is dry basis moisture content (% db), Mo is monolayer moisture content (%), a_w is water activity, and C_b is adsorption energy constant in the monolayer layer. Eq. (7) can be simply into a linear regression equation, where $y = \frac{a_w}{(1-a_w)M}$, $x = a_w$, with coordinate intersection point is $\frac{1}{C_b M_o}$, and $\frac{C_b - 1}{C_b M_o}$ is the gradient (slope) of the graph.

The second model used is the GAB model, as seen in Eq. (8).

$$\frac{M}{Mo} = \frac{CKa_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \quad (8)$$

Where M is dry moisture content (% db), Mo is monolayer moisture content (%), a_w is water activity, C is monolayer water adsorption energy constant, and K is multilayer water energy constant (above monolayer water).

Eq. (8) can be simplified into a 2nd-order polynomial or quadratic equation with $\frac{a_w}{M}$ as the ordinate (y) and a_w as the abscissa (x), as seen in Eq. (9).

$$\frac{a_w}{M} = \frac{1}{CKMo} + \frac{(CK-2K)}{CKMo} a_w + \frac{(K^2 - CK^2)}{CKMo} a_w^2 \quad (9)$$

The accuracy of the mathematical model in comparison to the experimental data was evaluated by calculating the mean relative percentage deviation modules, denoted as E% in Eq. (10).

$$E\% = \frac{100}{N} \sum_{i=1}^{N} \frac{m_i - m_{pi}}{m_i} \quad (10)$$

In this context, N represents the quantity of experimental data, m_i denotes the experimental data obtained from isothermic sorption measurements, and m_{pi} signifies the predicted data generated by the model. Adequate accuracy in modelling is indicated by an E value that is below 10% [13].

3. Results and Discussion

Data collection for determining sorption isotherm was carried out on soybean seeds which could bind water. The moisture sorption isotherm of soybean seeds was generated at three different temperatures, 30°C, 40°C, and 50°C in the a_w range of 0.08 to 0.80. The equilibrium moisture contents obtained are presented in Figure 2. Equilibrium Moisture Content (EMC) tended to decrease with an increase in temperature at a particular a_w. The EMCs were plotted against a_w to obtain a moisture sorption isotherm. The moisture sorption isotherm of soybeans is depicted in Figure 2.

As per the Brunauer-Deming-Deming-Teller classification, most agricultural products exhibit type II sigmoid-shaped sorption isotherms. The EMC increased gradually in lower a_w activities, followed by a steep rise above 0.6 a_w, as shown in Figure 2. Soybean seeds show a sigmoid pattern (Figure 2), which is a pattern commonly found in amorphous systems. The sorption pattern of soybean seeds is type II, which has a moderate hygroscopic level. This finding was in line with many conventional seed and food products which showed a type II isotherm or sigmoidal pattern [7].

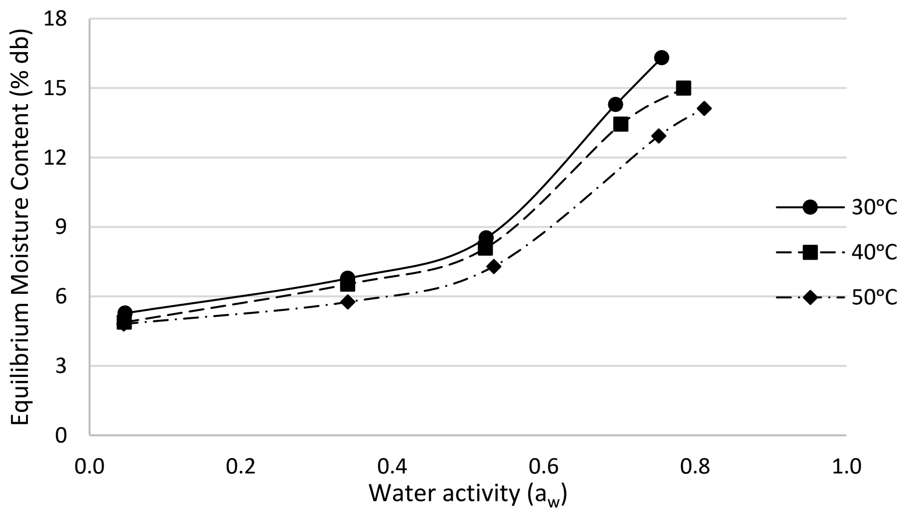


Fig. 2. Isothermic sorption curve of soybean seeds at temperatures of 30°C, 40°C and 50°C

3.1 Analysis of the Accuracy of the BET Equation Model

The BET model, a theory of multilayer adsorption, is a traditional isothermic model. When the a_w value is less than 0.5, the BET model may be used. The state of a monolayer, or a single layer of food elements, may, nevertheless, be adequately described in this way. Soybean seed adsorption energy constant (C_b), monolayer moisture content (Mo), and coefficient of determination (R^2) are all obtained by plotting a_w data against $a_w/(1-a_w)M$.

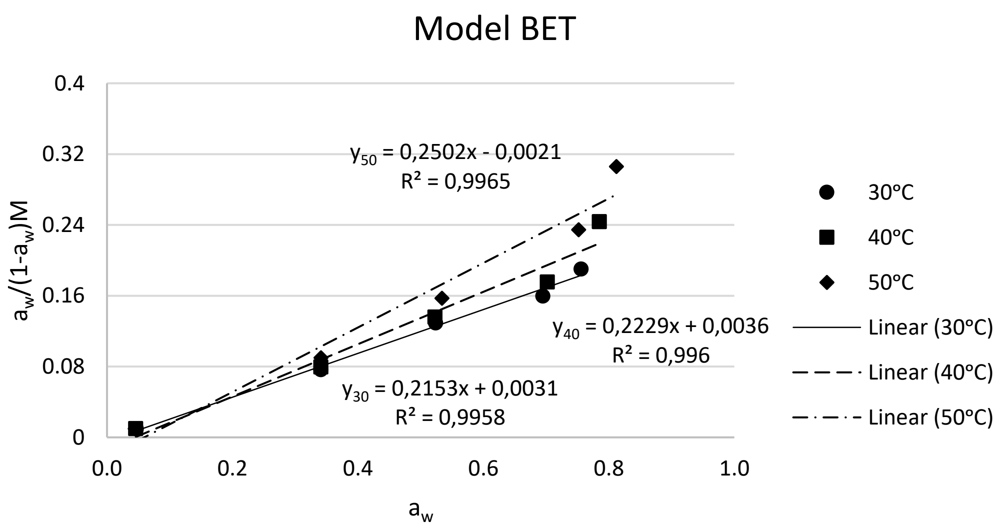


Fig. 3. Plot of a_w data vs $a_w/(1-a_w)M$ in the BET model

The plot with a_w as axis and $a_w/(1-a_w)M$ as ordinate revealed three straight lines for the three temperatures, 30°C, 40°C, and 50°C. The coefficient of determination (R^2) values for the three equations are similarly high, exceeding 0.995. This finding shows that plotting using the BET model provided a high level of accuracy so that dependent data approaches independent data.

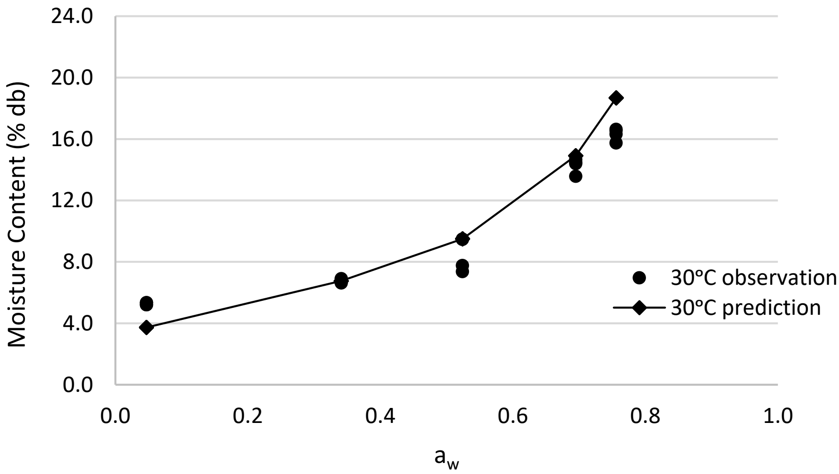
Table 1
The BET equations and constants for soybean seeds

Temperature (°C)	Equation of a_w to $a_w/(1-a_w)M$	R^2	BET constant		E%[a]
			C_b	Mo (% db)	
30	y = 0,2153x + 0,0031	0,9958	70,45	4,58	0,18
40	y = 0,2229x + 0,0036	0,9960	62,92	4,42	5,94
50	y = 0,2502x – 0,0021	0,9965	-118,14	4,03	22,53

[a] Deviation of experimental data with model prediction values (good accuracy if less than 10%)

C_b, the BET constant, from the three equations obtained, was 70.45 for a temperature of 30°C and 62.92 for a temperature of 40°C. Only a different C_b value was shown from the BET model at a temperature of 50°C, namely -118.14. C_b represents the adsorption energy of the first layer of adsorbate molecules on the adsorbent surface. It is a measure of the adsorption affinity of the adsorbent for the adsorbate. Meanwhile, the Mo results for the three temperatures are relatively almost the same, namely 4.58, 4.42, and 4.03 for 30°C, 40°C, and 50°C, respectively. Accuracy results from experimental data showed increasingly higher values with increasing storage temperature. Deviations of 0.18% and 5.94% were demonstrated by temperatures of 30°C and 40°C, indicating good accuracy for both temperatures. However, at a temperature of 50°C, the BET model could not depict the moisture content during storage, with a deviation rate of 22.53%, more than 10%.

Based on mathematical equations obtained from sorption isotherm experiments using the BET model, soybean moisture content at various water activities can be predicted. Figure 4a, Figure 4b and Figure 4c show plots of a_w values against moisture content (% db) from observations and predictions.



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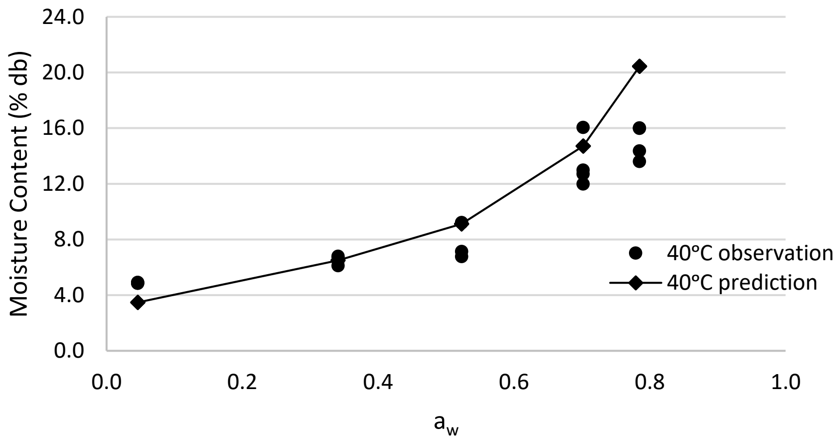

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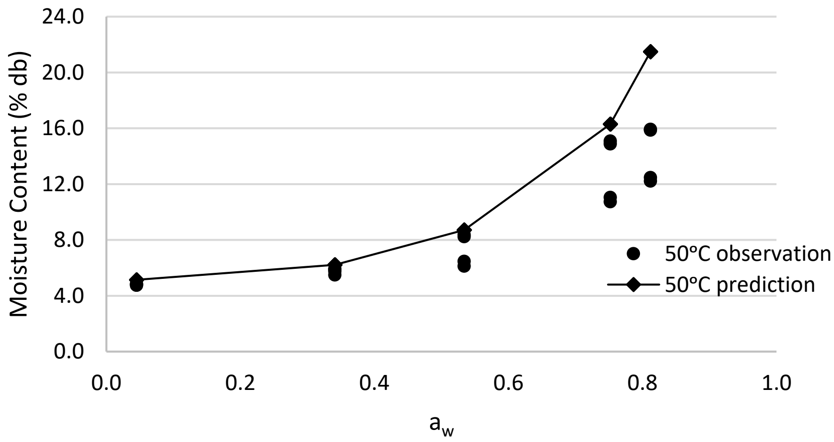


(c)

Fig. 4. Plot of a_w value against moisture content (% db) from observations and predictions using the BET model for temperature a). 30 °C, b). 40 °C, and c). 50°C

3.2 Analysis of the Accuracy of the GAB Equation Model

The Guggenheim-Anderson-de Boer (GAB) model is a three-parameter sorption equation used to interpret the adsorption of water by materials, such as soybean seeds. It is capable of describing the full shape of the isotherm and yields meaningful physical parameters, including the monolayer capacity, from which the water-accessible specific surface area can be obtained. The equation is particularly useful for analysing the moisture sorption properties of material. The GAB model provides a framework for understanding the interaction between the soybean seeds and the adsorbed water molecules, which can be influenced by factors such as temperature and water activity.

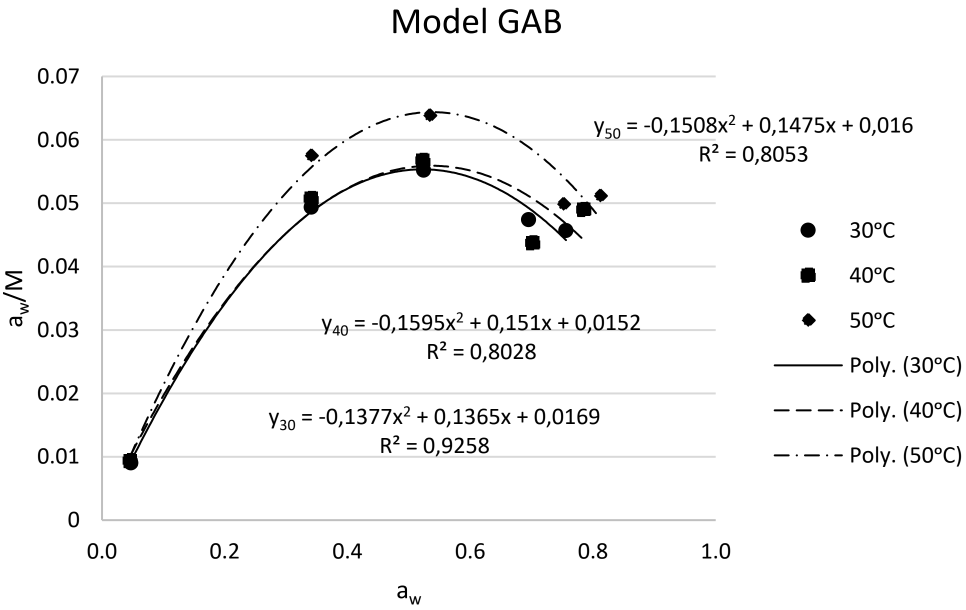


Fig. 5. Plot of a_w data vs a_w/M in the GAB model

Plotting between a_w versus a_w/M for the collected data, both for temperatures of 30°C, 40°C, and 50°C, provides a parabolic curve with a quadratic equation. The coefficient of determination (R^2) ranged between 0.80 for curves with temperatures of 40°C and 50°C. Meanwhile, a temperature of 30°C produced higher accuracy, with R^2 of 0.9258.

Table 2
The GAB equations and constants for soybean seeds

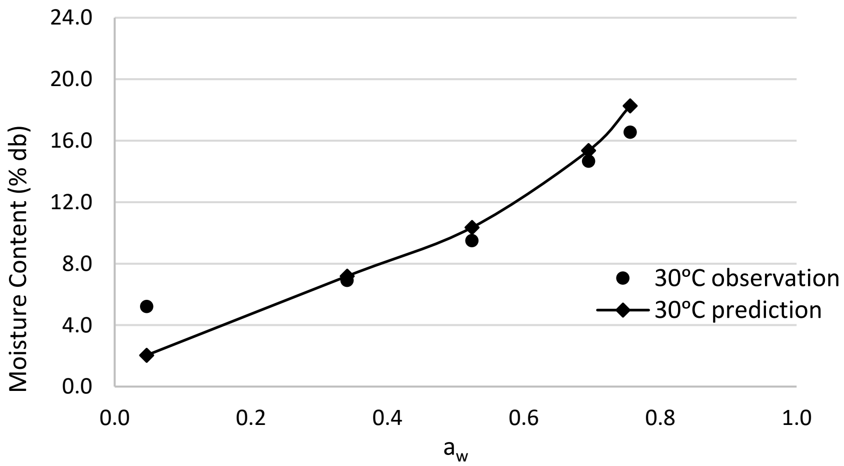
Temperature (°C)	Equation of a_w to a_w/M	R^2	GAB constant			E%[a]
			C	Mo (% db)	K	
30	$y = -0,1377x^2 + 0,1365x + 0,0169$	0,9258	10,91	5,98	0,91	6,51
40	$y = -0,1595x^2 + 0,151x + 0,0152$	0,8028	12,31	5,55	0,96	0,29
50	$y = -0,1508x^2 + 0,1475x + 0,016$	0,8053	11,93	5,64	0,93	8,63

R^2 for the quadratic regression equation $aw/M = \alpha + \beta x + \gamma x^2$
[a] Deviation of experimental data with model prediction values (good accuracy if less than 10%)

The three equations were quadratic equations, with C ranging from 10.91 to 12.31. Monolayer moisture content (Mo) ranged from 5.55 to 5.98% db. The multilayer water energy constant (K) for all three curves was 0.91, 0.96, and 0.93 for temperatures of 30°C, 40°C, and 50°C, respectively. The use of the GAB model provided small deviations with an accuracy level of below 10% for the three storage temperatures. This finding shows that the GAB model can depict the moisture sorption isotherm of soybeans during storage. Previous reports stated that the modified Halsey model was found to satisfactorily represent both the desorption and adsorption phenomena, indicating its effectiveness in capturing the equilibrium moisture content changes at different temperatures and relative humidity levels [6].

Based on mathematical equations obtained from sorption isotherm experiments using the GAB model, soybean moisture content at various water activities can be predicted. Plots of a_w values against moisture content (% db) from observations and predictions are shown in Figure 6a, Figure 6b and Figure 6c.

The moisture sorption isotherm of other legumes has been studied. Moisture sorption isotherm of legumes, especially soybean grains, has been reported. The desorption and adsorption isotherm were classified as type III due to the high oil content [6]. The modified Halsey model was found to represent the sorption phenomena of soybean grains accurately. The equilibrium moisture content of soybean grains increased with an increase in water activity and decreased with an increase in temperature. The sorption isotherm of other legumes has also been reported. The sorption isotherms of cowpeas were found to exhibit a type III behaviour, with a decrease in equilibrium moisture content (EMC) as temperature increased [18]. The Peleg equation was identified as the best model for predicting the sorption behaviour of cowpeas [6]. The adsorption isotherm of cowpea flour was classified as type II according to the BET and GAB models. The sorption isotherm of legume seeds has been used to predict product stability, optimize drying processes, and understand physiological processes. Jian & Jayas [19] conducted a study on the desorption and adsorption isotherms of red kidney beans using static and dynamic methods, and the best-fitted equations to describe the isotherms as the modified Chung-Pfost and modified GAB [19]. Jian and Jayas [19] also developed empirical, semi-theoretical, and finite element method models to simulate the water sorption of red kidney beans and calculated the effective water diffusivity. While in chickpeas, the moisture sorption isotherm has been studied using various models. A multilayer model with two energy levels based on statistical physics and theoretical considerations was proposed as the best model for describing the data in the whole range of relative humidity. The equilibrium moisture content of chickpeas was found to be an essential parameter in predicting changes in moisture content during storage. The sorption isotherms of chickpeas were classified as type II according to the BET and GAB models [20]. The GAB model was found to provide the best fit for the entire range of water activities. The monolayer moisture content of chickpeas ranged from 1.82 to 6.25 g/100 g solids [21].



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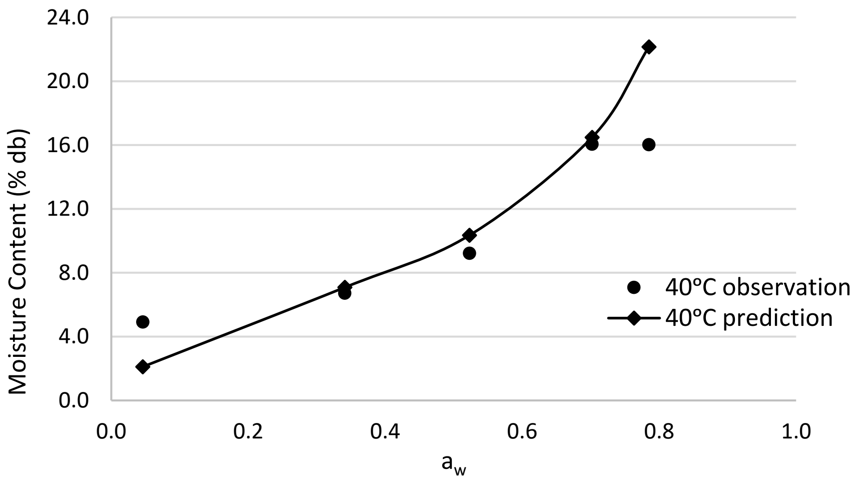

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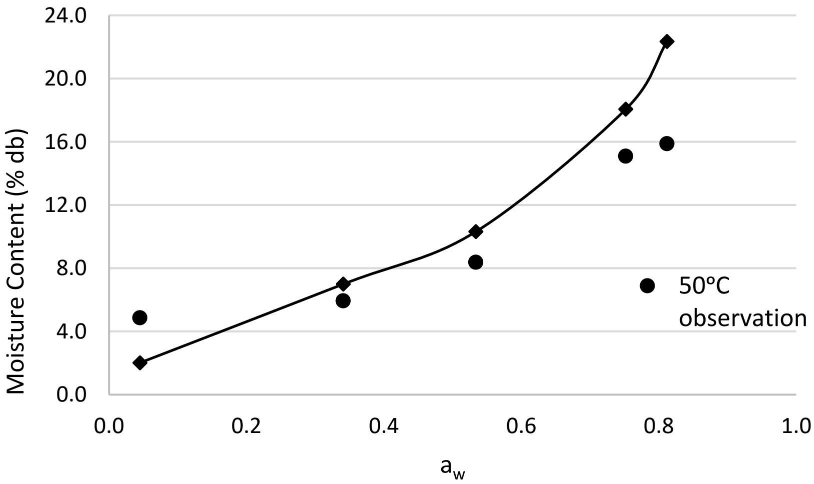

(c)

Fig. 6. Plot of a_w value against moisture content (% db) from observations and predictions using the GAB model for temperature a). 30°C, b). 40°C, and c). 50°C

3.3 The Relationship between Soybean Seed Condition and Vigor Index and Germination Capacity

Soybean germination capacity refers to the number of soybeans that could completely germinate. It measured the viability and vigor of the soybean, indicating the percentage of soybeans that were capable of sprouting and growing into healthy plants [22]. While, the vigor index is an important measure of seed quality, as it determines the potential for rapid and uniform emergence of plants [23]. It was collectively representative of the quality of a seed lot based on various factors that influence seed viability and vigor [24]. Germination capacity and seed vigor index are interdependent, as both factors influence the overall performance of the seeds. High germination capacity and a favourable seed vigor index indicate that the seeds are of high quality and have the potential to perform well under specific growing conditions.

Table 3 presents the relationship between water activity (a_w) and moisture content of soybean seeds in various environmental conditions (30°C, 40°C, and 50°C) and soybean seed germination capacity and vigor index. Figure 7 shows the relationship between germination capacity and moisture content of soybean seeds.

Table 3
Water activity, moisture content, vigor index, and germination capacity of soybean seeds at various temperatures

Water activity (a_w)	Moisture Content (%)			Vigor index (%)			Germination capacity (%)		
	30°C	40°C	50°C	30°C	40°C	50°C	30°C	40°C	50°C
0.07	2.06	1.90	0.14	65	70	60	65	70	65
0.32	7.54	8.05	8.60	85	75	85	85	75	85
0.56	12.81	13.95	16.72	65	55	35	65	60	40
0.75	16.98	18.62	23.15	50	20	0	55	25	0
0.80	18.07	19.85	24.84	25	0	0	28	0	0

In general, the vigor index and germination capacity of soybean seeds increased with 0.07 to 0.32 of water activity and 0.4-2.06% to 7.54-8.6% of moisture content. The vigor index and germination capacity reached their maximum at 0.32 of water activity, and the moisture content ranged from 7.54 to 8.60%. Furthermore, the vigor index and germination capacity continued to decrease with increasing water activity and moisture content. The decrease in vigor index and germination power became sharper with increasing temperature (Figure 7).

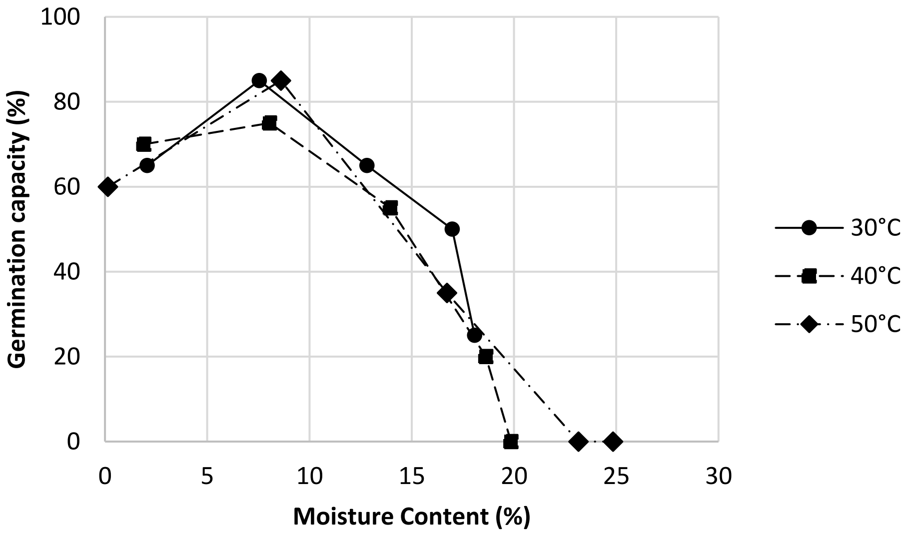

Fig. 7. Germination capacity of soybean seeds at various moisture contents and temperatures

The storage conditions of soybean seeds can significantly affect their germination capacity and vigor index [25]. Soybean seeds should be stored in a cool, dry environment with a water activity of 0.32 and a moisture content of 8% to maintain their maximum germination potential. Storing seeds at higher relative humidities can lead to increased moisture content and potential mold growth, while storing seeds at lower relative humidities can lead to reduced moisture content and reduced germination capacity [25].

3.4 Microstructure of Epidermis Surface and Kernel of Soybean Seed

Microscopic structure is an important aspect that needs to be studied to explain the phenomenon of seed quality degradation during storage. Soybean seeds consist of two main parts, namely the epidermis (seed coat) and seed flesh (endosperm). The epidermis plays a major role in determining seed quality because it is the outermost part that is in direct contact with environmental conditions.

The soybean epidermis is composed of a complex mixture of chemical compounds such as flavonoids, proteins, peptides, amino acids, alkaloids, terpenoids, steroids, and other components which have a role in protecting the seeds from microbial and insect attacks [26].

The outermost layer of the epidermis is a wax cuticle that acts as the first barrier to imbibition. According to Shepherd and Griffiths [27], the wax cuticle layer consists of two parts, namely a very stable layer, and a layer that is unstable environmental influences. This layer is uneven and has several open structures with a porous interior. The inside of the epidermis has a hollow structure consisting of a network of circles/polygons whose inside is empty. This structure causes soybean seeds to absorb moisture from the surrounding environment very easily, which can reduce the quality of the seeds.

The epidermis layer consists of the palisade, parenchyma, and aleurone layers. The palisade layer contributes to determining the mechanical strength of the epidermis [28]. The inside of the seed (endosperm) has a very porous structure with very small pore sizes (hundreds of nanometres). This structure can cause seed deterioration quickly. Moist air that penetrates through the epidermis will then enter the endosperm layer and cause a decrease in seed germination.

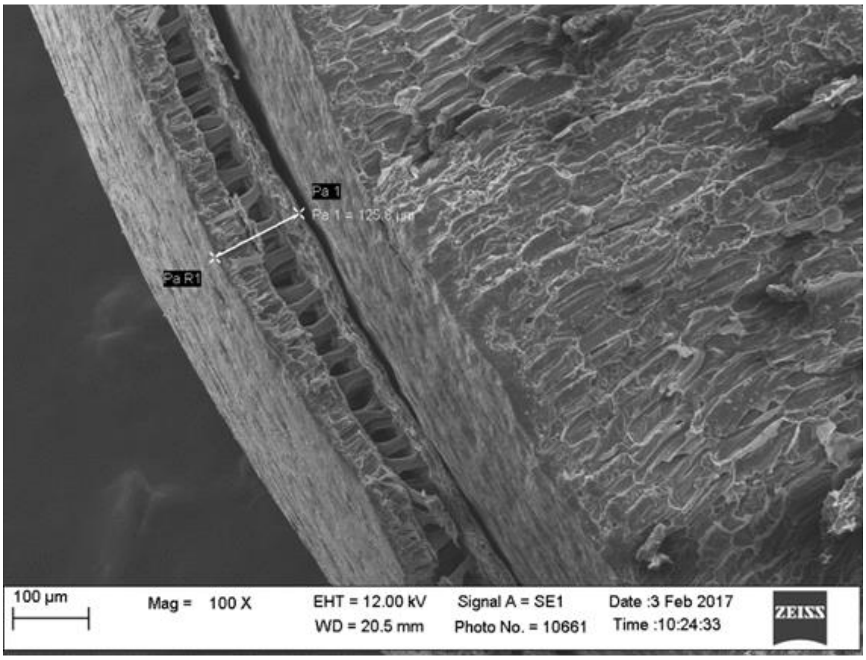

Fig. 8. Microscopic structure of soybean seeds: epidermis (seed coat), and endosperm (seed flesh)

4. Conclusion

The present study was conducted to understand the sorption characteristics of soybean at 30°C, 40°C, and 50°C and describe the various properties of sorbed water, such as monolayer moisture content. The moisture sorption isotherm exhibited a type II sigmoid shape, which is typical for most agricultural products. The vigor index and germination capacity reached their maximum at 0.32 of water activity, and the moisture content ranged from 7.54 to 8.60%, with 75 – 85% for both vigor index and germination capacity.

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