

The effect of stevia-fructose sweetener on the characteristics of biscuits made from lindur fruit (*Bruguiera gymnorrhiza*) and modified cassava flour

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Abstract

Biscuits, a popular snack enjoyed by individuals of all ages, are a product with a moisture content of less than 5%. There is potential to develop biscuits using local resources, such as lindur fruit (*Bruguiera gymnorrhiza*) from mangroves, combined with modified cassava flour (MOCAF). Stevia and fructose syrup are used in this formulation as sweeteners to replace sucrose. Therefore, this study aimed to investigate the impact of stevia and fructose syrup on the characteristics and shelf-life of biscuits made from lindur fruit flour (LFF) and MOCAF. The investigation was structured into two stages. First, biscuits were manufactured, and their characteristics were analyzed using a Completely Randomized Design (CRD). Three concentration levels of stevia (1.5%, 2%, and 2.5%) and fructose syrup (10%, 15%, and 20%) were used. The ratio of LFF to MOCAF was fixed at 20:80. The analyses, such as accelerated shelf-life testing (ASLT) and determination of critical moisture content, were conducted to assess the shelf-life of the best biscuit formulation, based on the optimal results obtained in the first phase for biscuit characteristics. The results showed that the optimal biscuits were achieved with the inclusion of 2% stevia and 20% fructose syrup, with 3.78% moisture, 2.3% ash, 0.91% fat, 4.06% protein, 89.27% carbohydrate content, and a breaking strength of 11.87 N. Sensory evaluations yielded scores of 4.20 for color, 4.03 for taste, 3.37 for texture, and 4.17 for aroma. The estimated shelf-life indicated that metalized plastic packaging was optimal, providing a maximum shelf-life of 21 days (0.70 months) at a relative humidity (RH) of 75%.

1. Introduction

Biscuits, a popular snack enjoyed by many individuals, are a product with a dry composition and a water content of less than 5%. (Leiva-Valenzuela *et al.*, 2018). Through the investigation of raw materials in specific regions and considering dietary needs, health benefits, and product shelf-life, biscuit formulations with various wheat and sugar alternatives have been developed (Chauhan *et al.*, 2016; Peter *et al.*, 2017; Di Cairano *et al.*, 2021). To adhere to strict dietary requirements, it becomes necessary to develop innovative substitutes for raw materials in foods made with flour, which currently have a high glycemic index (GI). With a GI of 55, wheat flour can be replaced by a combination of modified cassava flour (MOCAF) and Lindur Fruit Flour (LFF).

MOCAF is a type of flour derived from cassava

through a fermentation process (Ratnawati *et al.*, 2020; Faridah and Yuhelma, 2022). As one of Indonesia's key agricultural commodities, cassava plays a significant role in the country's food production sector. In 2017 alone, cassava production in Indonesia amounted to an impressive 19,053,748 tons (Ratnawati *et al.*, 2020). This high level of production underscores the importance of cassava as a versatile and widely cultivated crop, providing substantial raw material for the development of value-added products such as MOCAF, which has a range of applications in both the food and non-food industries. MOCAF is recognized as a promising alternative for gluten-free food products (Firdaus *et al.*, 2017; Patriani and Rosadi, 2023). Its availability offers a convenient option for those seeking gluten-free diets, particularly benefiting individuals with conditions such as celiac disease and autism spectrum disorder (Firdaus

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et al., 2017).

One potential approach to enhancing food security is by tapping into local food sources, such as the utilization of mangrove plants like the lindur fruit (Wilujeng et al., 2020; Rosulva et al., 2022). Lindur, a carbohydrate-rich fruit, has long been used by coastal communities as a vital food source during lean seasons, helping to mitigate the risk of food shortages (Afifah et al., 2022). Due to its high nutritional value, particularly its carbohydrate content, lindur holds significant potential as a new local food resource. It contains an impressive 85.10 g of carbohydrates per 100 g, along with a water content of 54.35%, ash content of 1.01%, fat content of 1.43%, and protein content of 1.83% (Jariyah et al., 2020). Given its high carbohydrate concentration, lindur fruit is especially suitable for processing into flour, offering a promising alternative to traditional carbohydrate sources and further contributing to local food resilience.

In today's world, where sugar-rich foods are abundant, the preference for sweet, sugary treats has turned into a detrimental habit. This preference is contributing to the rise of diet-related non-communicable diseases, such as type II diabetes. In the 21st century, a significant number of people are affected by diet-related non-communicable diseases like type 2 diabetes. According to the World Health Organization's (WHO) latest report on diabetes, 422 million individuals were living with the condition in 2014 (Van Laar et al., 2021). New formulations can be created by substituting traditional sweeteners, such as sucrose and glucose syrup, with low-calorie alternatives like stevia and fructose syrup, which are safe for individuals with type II diabetes and lower calorie consumption (Jariyah et al., 2022; Raghavan et al., 2023). Stevia (*Stevia rebaudiana*) is a natural sweetener plant cultivated commercially across Asia, America and Europe. The sweetness of stevia comes from the glycosides found in its leaves. It is primarily used as a sweetener and flavor enhancer in the food and beverage industry (Putnik et al., 2020; Schiatti-Sisó et al., 2023). The compounds derived from stevia are widely regarded as an excellent sugar alternative, particularly for individuals with diabetes. Fructose has a relative sweetness ranging from 115 to 180, which is higher than glucose (50-70) and sucrose (100). To replace 100 g of sucrose in a beverage, approximately 33% less fructose is needed, given its relative sweetness of around 150 at higher concentrations. This substitution results in a beverage with fewer calories due to the reduced sugar content, but it also leads to an increased concentration of fructose (Brouns, 2020).

The low water concentration in biscuit formulations typically results in a distinctive crunchy texture. These characteristics render the product susceptible to moisture

absorption, leading to loss of crispness. Meanwhile, assessing shelf-life can help gauge product alterations over time. Using effective packaging, aimed at limiting the permeability of water vapor, light, and oxygen, and preventing chemical reactions between the packaging and the product, can be a strategy to maintain the quality of biscuits (Pasqualone et al., 2021). Determining the shelf-life of a food product can be achieved through the classic and accelerated methods. The classic method, known as Extended Storage Studies (ESS), is accurate but time-consuming and includes a comprehensive and costly study of quality criteria. In contrast, the Accelerated Shelf-Life Testing (ASLT) method, also known as Accelerated Storage Studies (ASS), offers the advantage of quick and accurate testing (Ni'mah et al., 2017). This method is particularly useful for testing foods susceptible to damage due to inadequate packaging that fails to prevent moisture evaporation from the air, potentially leading to increased water content and altered product texture. Therefore, using the ASLT method in this study can more effectively determine the shelf-life of the intended replacement biscuits.

The principal aim of the critical moisture content method is to determine the equilibrium moisture content (Me) of biscuits stored at various relative humidity (RH) levels. An isothermic absorption curve for the product is generated based on the relationship between the equilibrium moisture content and the RH at which they are stored. Isothermic sorption curves are instrumental in analyzing the pattern of moisture absorption in biscuits from the environment and estimating their shelf-life.

This study aimed to assess the impact of fructose syrup and stevia sweetener concentrations on biscuit properties, as well as their shelf-life using the ASLT method. Consumers prioritize shelf-life information, which is crucial and required on food packaging or labeling.

2. Materials and methods

2.1 Materials

Lindur fruit (*Bruguiera gymnorrhiza*) was sourced from the Wonorejo mangrove forest in Surabaya, while MOCAF was obtained from Ladang Lima Company, Surabaya, Indonesia. Other ingredients, including stevia, fructose syrup, margarine, baking powder, skim milk, sodium stearoyl lactylate, and eggs, were purchased from the local market. Various chemicals and reagents such as anthrone, glucose, Fehling's solutions A and B, hexane, distilled water, sulfuric acid (H₂SO₄), sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol 95%, lead acetate (Pb acetate), silica gel, magnesium chloride (MgCl₂), sodium nitrite (NaNO₂), sodium chloride

(NaCl), potassium chloride (KCl), barium chloride (BaCl₂), and potassium nitrate (KNO₃) were purchased from Sigma- Aldrich and were of analytical grade. Metalized plastic, polypropylene (PP), and aluminum foil were used for packaging biscuits.

2.2 Lindur flour preparation

Fresh lindur fruit was collected from mangrove farmers in Sidoarjo, East Java, Indonesia. The fruit was washed, peeled, sliced to a thickness of 0.1 cm, blanched at 90°C for 10 min, soaked in water three times with changes every 24 h, dried at 60°C for 10 h, milled, and sifted through an 80-mesh sieve. The resulting flour was referred to as Lindur fruit flour (LFF).

2.3 Biscuits preparation

Biscuits were made from dough containing a ratio of 80% MOCFAF and 20% LFF (Jariyah, Winarti and Agrita, 2018a; Jariyah *et al.*, 2018b). The dough also included 1% salt, 0.25% baking soda, 8% egg yolk, 8% skim milk, 22% egg white, 0.5% sodium stearoyl lactylate, and 50% margarine, following the method described by Jariyah, Winarti and Agrita (2018a) with slight modifications (Jariyah *et al.*, 2018b). Biscuit dough was prepared with three concentrations of stevia sweetener (1.5%, 2%, and 2.5%) and fructose syrup (10%, 15% and 20%), as shown in Table 1. The dough was rolled to a thickness of 1.0 cm, cut into circles with a diameter of 3 cm, placed on a baking tray lined with parchment paper, and baked at 150°C for 15 min in an electric oven.

Table 1. Treatment of biscuit.

Code	Stevia sweetener (%)	Syrup fructose (%)
S1F1	1.5	10.0
S1F2	1.5	15.0
S1F3	1.5	20.0
S2F1	2.0	10.0
S2F2	2.0	15.0
S2F3	2.0	20.0
S3F1	2.5	10.0
S3F2	2.5	15.0
S3F3	2.5	20.0

S: sweetener stevia and F: Syrup fructose

2.4 De Garmo effectiveness test

The optimal treatment across all measured parameters is identified based on the De Garmo effectiveness value in Equation (1) (Garmo *et al.*, 1984).

$$\text{Product value (NP)} = N_E \times N_B \quad (1)$$

Where N_E = Effectiveness Value and N_B = Weight Value

2.5 Characteristics of the biscuit

The characteristics of biscuits, including proximate analysis, were determined to assess moisture, fat, protein, and ash content following the AOAC INTERNATIONAL Official Methods (2005). Carbohydrate content was calculated by difference. A total of 20 panelists evaluated the sensory analysis of the biscuits based on taste, color and aroma while 30 panelists were selected to evaluate the crispiness. Breaking strength was determined according to the method developed by Bala *et al.* (2015). Before estimating shelf-life, biscuit damage was determined by 50 panelists using a ranking test, with number one showing the least damage. The demographic panelist depicted in Figure 1. All panelists are between the ages of 21 and 25. Shelf-life estimation based on the critical moisture content method included the following.

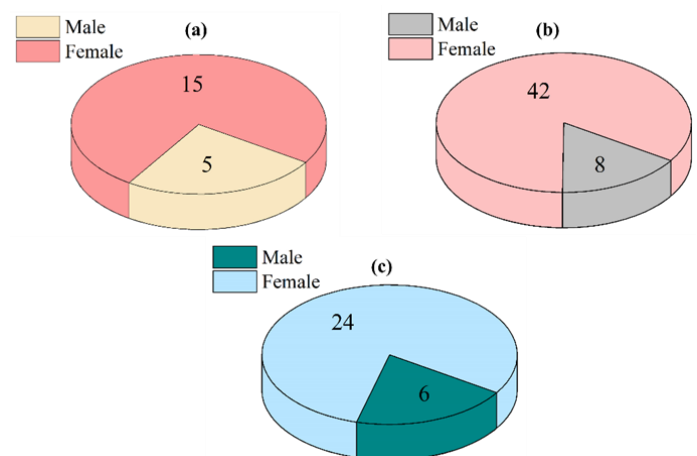


Figure 1. Demographic characteristics of panelists who participated in (a) sensory analysis of biscuits, (b) biscuit damage and (c) crispness test.

2.5.1 Accelerated shelf-life testing

The formula used to calculate shelf-life, based on Labuza's (1982) critical moisture content method, includes equation (2).

$$t = \frac{\ln\left(\frac{M_e - M_i}{M_e - M_c}\right)}{\left(\frac{k}{x}\right)\left(\frac{A}{W_s}\right)\left(\frac{P_0}{b}\right)} \quad (2)$$

Where t = The time needed in the packaging to move from the initial moisture content to the critical moisture content or the estimated shelf-life time (days), M_e = Moisture equilibrium product (g H₂O/g solids), M_i = Initial product moisture (g H₂O/g solids), M_c = Initial product moisture (g H₂O/g solids), k/x = Packaged water vapor permeability (g/m².day.mmHg), A = Package surface area (m²), W_s = Packaged product dry weight (g), P_0 = Saturated vapor pressure (mmHg) and b = The slope of the isothermic sorption curve (which is assumed to be linear between M_i and M_c).

2.5.2 Moisture content

Moisture content was determined according to AOAC Official Method 925.10 using oven drying at 105°C until constant weight.

2.5.3 Ash content

Ash content was determined according to AOAC Official Method 923.03 by incineration at 550–650°C until constant weight.

2.5.4 Fat content

Fat content was determined according to AOAC Official Method 920.39.

2.5.5 Protein content

Protein content was determined according to AOAC Official Method 979.09.

2.6. Measurement of initial moisture content (M_i)

The initial moisture content (M_i) of biscuits was determined using the air-oven method at 105°C to constant weight according to AOAC Official Method 925.10.

2.7. Measurement of critical moisture (M_c)

Biscuit samples were stored for 0, 30, 45, 60, 75, 90, 105, and 120 min without packaging in a closed container at 100% RH ($a_w = 1$) (de Morais *et al.*, 2018). This process generated a series of samples with different storage times, and each was subjected to repackaging for a sensory preference test conducted by 30 panelists regarding crispness and moisture measurement using the oven method. The sensory crispness results were connected to the logarithmic moisture results, allowing for the derivation of a linear equation used to examine the critical moisture.

2.8 Measurement of moisture equilibrium (M_e)

Equilibrium moisture measurement was conducted to produce an isothermic sorption curve for biscuits, showing the pattern of water vapor absorption from the environment. This was achieved using salt of CH_3COOK , MgCl_2 , K_2CO_3 , NaNO_2 , NaCl , KCl , BaCl , KNO_3 at 30°C, RH of 75-90%, and apparent water content (a_w). The isothermic sorption curve was obtained by plotting the equilibrium moisture against the equilibrium RH value or a_w . Subsequently, the curve was tested using six equation models, including Hasley, Chen-Clayton, Henderson, Caurie, Oswin, and Guggenheim-Anderson-de Boer (GAB) (Patil *et al.*, 2021). The model accuracy was assessed by calculating the Mean Relative Determination (MRD).

$$\text{MRD} = \frac{100 \sum_i}{n} = 1 \frac{M_i - M_{pi}}{M_i} \quad (3)$$

Where M_i = Experimental of moisture, M_{pi} = Calculation of moisture and n = Sum of data

When MRD was less than 5, the isothermic sorption model accurately described the actual situation. For MRD values between 5 and 10, the model was reasonably accurate, while MRD exceeding 10 showed the model's inadequacy in describing the actual condition. The selected model was then used to determine the slope value of the isothermic sorption curve.

2.9 Determination of supporting parameters

The packaging area (A/m^2) was calculated by multiplying the length and width of the packaging to obtain the area in m^2 . The water vapor permeability of the packaging was assessed using a moisture cup. Subsequently, silica gel was added to the cup until it reached a height of approximately 1.25 in or 31.75 mm. The packaging was cut to fit the shape of the moisture cup, and its sides were sealed using parafilm to prevent air gaps. The cup was weighed over a 5-day period to monitor changes in weight, and the water vapor transmission rate was calculated.

The total solids value (W_s) was obtained by subtracting the initial moisture from the total weight of biscuits. The weight of the product in each package was measured and adjusted for moisture content, resulting in the weight of solids per package (W_s) expressed in grams. The shelf-life of biscuits was calculated by inputting the experimental data into Equation 1, using RH of 75% and 85%.

3. Results and discussion

Biscuits made from MOCAF and LFF with 2% stevia sweetener and 20% fructose syrup had the best results based on the de Garmo method's effectiveness test. The analysis of biscuits' characteristics showed moisture content of 3.78%, ash content of 2.31%, protein content of 4.07%, fat content of 0.92%, carbohydrate content of 89.27%, breaking strength of 11.87 N, and sensory scores of 4.03 for taste and 3.37 for texture (Table 2). The obtained biscuit gives lower breaking strength compared with other studies (Arun *et al.*, 2015; Onacik-Gür *et al.*, 2015). Based on these results, the ASLT method was used to assess shelf-life.

3.1 Damage parameters of biscuits

According to the questionnaire results, the primary cause of biscuit product degradation was the loss of

Table 2. Characteristics of biscuits.

Code	Proximate and breaking strength of biscuit						Sensory score			
	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Breaking strength (N)	Aroma	Taste	Color	Texture
S1F1	3.06	2.39	3.78	0.68	90.01	5.36	3.90	3.17	4.00	3.57
S1F2	3.34	2.59	3.76	0.69	89.18	5.86	3.70	3.73	3.90	3.27
S1F3	3.65	2.36	3.93	0.59	89.56	11.41	3.77	4.07	3.73	3.30
S2F1	3.09	2.40	4.39	0.91	89.05	5.56	3.77	3.87	3.80	3.47
S2F2	3.34	2.35	4.03	0.77	89.71	6.23	3.93	4.10	3.93	3.57
S2F3	3.78	2.31	4.07	0.92	89.27	11.87	4.17	4.03	4.20	3.37
S3F1	3.09	2.71	3.68	0.80	89.93	5.66	3.80	4.13	3.80	3.90
S3F2	3.40	2.24	3.19	0.70	90.74	6.94	3.97	3.90	3.90	3.87
S3F3	3.77	2.28	3.40	0.76	90.22	11.85	3.83	3.60	3.87	3.37

crispness (texture) (Figure 2). A total of 29 respondents identified non-crispy as the main factor affecting biscuits' quality, followed by 14 respondents who selected the taste factor, 5 respondents selected the altered color, and 2 respondents selected the changed aroma. Biscuit products, including snacks, crackers, and chips, were expected to be neither soggy nor crunchy (de Moraes *et al.*, 2018; Davidson, 2021; Patil *et al.*, 2021). Therefore, it could be deduced that the loss of crispness was primarily due to an increase in the biscuits' moisture content.

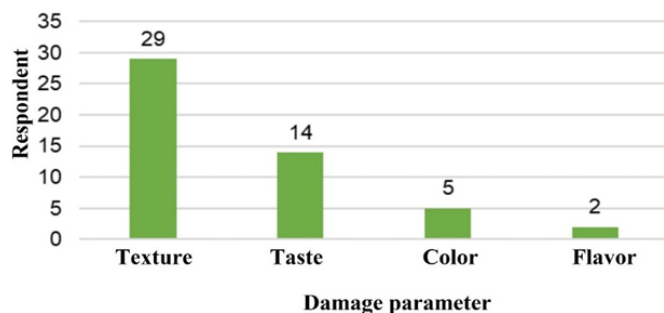


Figure 2. Biscuits damage parameter.

3.2 Initial moisture (M_i)

Moisture significantly impacted the quality of the dry product, as the RH of the storage environment could be easily altered (Szymańska *et al.*, 2015; Lufu *et al.*, 2020). The moisture content of biscuits played a crucial role in determining their shelf-life. Biscuits containing fructose syrup and stevia sweetener (2%; 20%) had a moisture content of 0.0364 g H₂O/g solids, compared to commercial biscuits, which had a moisture content of 0.0301 g H₂O/g solids (Kaur *et al.*, 2015; Labuza and Altunakar, 2020). Biscuits made from LFF and MOCAP generally had lower moisture content compared to commercial varieties. The composition and types of ingredients used, specifically the use of LFF and MOCAP, along with the addition of stevia sweetener and fructose syrup, could significantly influence the characteristics of this product.

3.3 Critical moisture (M_c)

Sensory evaluation and moisture content analysis were conducted to assess the level of crispness of biscuits and determine the critical moisture content (Table 3). The critical moisture was determined at an assessment score of 3, showing "somewhat disliked" by the panelists. The sensory score appeared to decrease over the observation period, from 3.43 to 2.73 at 120 min, while the moisture content increased from 0.0386 g H₂O/g solids to 0.0958 g H₂O/g solids.

The linear regression resulted in $y = -0.0807x + 0.3198$, $R^2 = 0.9731$, with $x = 3$, showing a critical moisture level of 0.0777 g H₂O/g solids. This regression showed the inverse relationship between product moisture and sensory score, suggesting consumer dissatisfaction with the product.

Table 3. Moisture and sensory scores of biscuit products.

Time (min)	Moisture (g H ₂ O/g solid)	Sensory score
0	0.0386	3.43
30	0.0576	3.27
45	0.0640	3.17
60	0.0682	3.13
75	0.0760	3.07
90	0.0807	3.00
105	0.0875	2.87
120	0.0958	2.73

3.4 Equilibrium moisture

In an ambient RH range of approximately 75–90% and a 30°C isothermic sorption curve, the equilibrium moisture content was calculated. To account for the storage conditions of biscuits made from MOCAP and LFF, this isothermic sorption curve was also considered to measure a_w values. Table 4 presents the obtained equilibrium water content and the corresponding time needed to reach it.

Table 4 presents that the value of the equilibrium water content increased along with the rise in water activity. The equilibrium moisture content was determined by placing the product in a desiccator with eight different salt varieties of varying degrees of saturation, ensuring a fair representation of each salt's RH. During storage, the weight of biscuits was subjected to changes as part of a process. The process, whether it was adsorption or desorption, relied on the moisture content of the product and the RH of the air.

Table 4. The equilibrium moisture (Me) of biscuits.

Saturated salt solution	RH (%)	a_w	Me Biscuit
CH ₃ COOH	22.5	0.225	0.0489
MgCl ₂	32.4	0.324	0.0669
K ₂ CO ₃	43.0	0.432	0.0718
NaNO ₂	64.0	0.643	0.0809
NaCl	75.1	0.751	0.1602
KCl	84.0	0.836	0.1752
BaCl ₂	90.3	0.903	0.2055
KNO ₃	93.0	0.923	0.2279

The water content value was plotted against the RH of each storage location to generate a curve. Figure 3 presents the isothermic sorption curve for biscuits made from LFF and MOCAF. The experimental yield curve shown in Figure 3 had a sigmoid appearance (shape of the letter S), influenced by factors such as the type of food, temperature, adsorption rate, and water displacement during adsorption or desorption processes. The interaction between water molecules and biscuits made from LFF and MOCAF was driven by the difference between the a_w of the product and the RH of the environment. Water vapor traveled from areas of high RH to low RH during storage, leading to a transfer

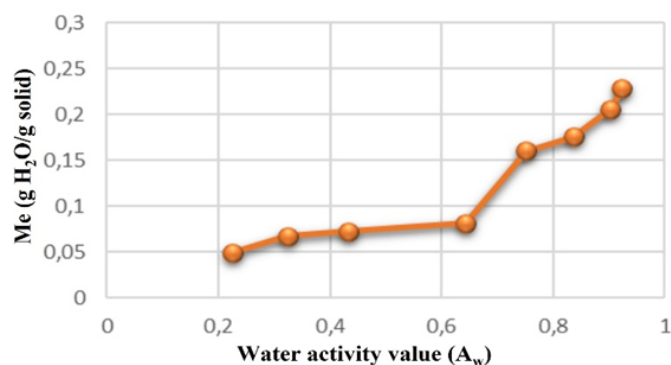


Figure 3. Isotherm absorption curve of biscuit.

Table 5. The equation for the isothermic absorption curve of biscuits.

Model	Equation	% MRD
Hasley	$\log(\ln(1/a_w)) = \log -2.0158 - 1.7206 \log Me$	9.74
Chen-Clayton	$\ln(\ln(1/a_w)) = 0.3545 - 5.5372 Me$	12.12
Henderson	$\log(\ln(1/(1-a_w))) = 1.2060 + 1.3314 \log Me$	8.77
Caurie	$\ln Me = -3.5549 + 2.3134 a_w$	8.43
Oswin	$\ln Me = -2.4302 + 0.4586 \ln(a_w/1-a_w)$	4.21
GAB	$Me = 0.7111 a_w / (1 - 0.8782 a_w) (1 + 15.6317 a_w)$	4.54

of the vapor into biscuits until equilibrium was achieved (Ergun *et al.*, 2010). Biscuits made with LFF and MOCAF had changes in weight during storage, showing a desorption and adsorption process of water vapor due to the disparity between the storage environment's RH and the a_w of the product. When the RH exceeded the a_w , there was the diffusion of water vapor into the product, resulting in increased weight, and vice versa. Therefore, the water content of biscuits made from LFF and MOCAF increased as a result of this weight gain.

3.5 Isothermic absorption model and model accuracy test

To achieve a smoother curve, an isothermic sorption equation model needed to be developed. This model covered plotting the calculated equilibrium water content and water activity value. To simplify calculations, the non-linear equations were transformed into linear ones ($y = a + bx$). Table 5 provides the equations for various models' isothermic sorption curves along with their corresponding MRD.

The use of the isothermic sorption curve equation model was necessary for accurately showing the relationship between water activity and equilibrium water content. Several mathematical equation models have been developed to describe the phenomenon of isothermic sorption. By calculating the MRD value for each model, it was possible to determine whether a model accurately, relatively accurately, or inaccurately represented the isothermic sorption curve based on the equilibrium moisture content data. The accuracy of the isothermic sorption equation was assessed to determine the correctness of the chosen model and to construct a sorption curve using the MRD computation. The closer the experimental isothermic sorption curve matched the mathematical models' curve, the more precisely the model captured the isothermic sorption phenomena.

The isothermic sorption equation model showed that various isotherms could accurately, moderately accurately, and imprecisely characterize the full isothermic sorption curve. This assertion was supported by the MRD calculation, which measured the agreement between experimentally determined equilibrium water content and model-based equilibrium water content. Based on the MRD analysis, it was evident that the

Oswin model had an MRD value of 5, while the GAB model had the lowest MRD values, standing at 4.2100 and 4.5413, respectively. The results showed the accuracy of both the Oswin and GAB models in predicting the isothermic sorption curve of biscuits made from LFF and MOCAF.

3.6 Slope value and isothermic curve

The slope value (b) of the isothermic sorption curve was calculated within the linear section, chosen between the initial water content region and the essential water content area (Labuza, 1982). Described by a linear equation of $y = a + bx$, the slope showed the relationship between water activity and equilibrium water content. Figure 4 presents how the slope value of the isothermic sorption curve was determined using the Oswin model, with a slope of 0.2934 and an R^2 of 0.876. This slope value was then used to calculate shelf-life, supporting the effectiveness of the linear regression method in food isotherms, particularly within the a_w range of 0.2 and 0.6.

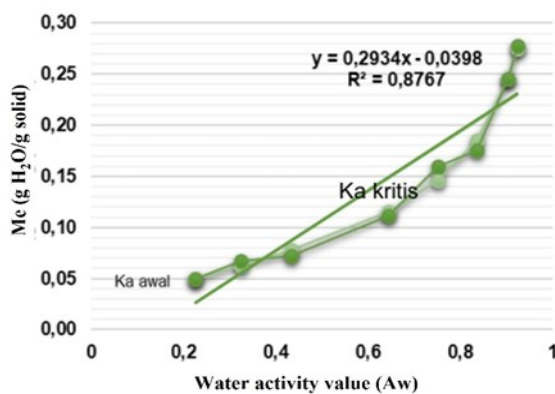


Figure 4. The slope of the Isothermic Absorption Curve of biscuits based on the Oswin model.

3.7 Water vapor permeability and package area

In this study, the change in silica weight over five days was used to calculate the permeability value of the packaging (k/x). The k/x values for aluminum foil, metalized plastic, and PP packaging were 0.2087 $\text{gH}_2\text{O}/\text{day}/\text{m}^2.\text{mmHg}$, respectively, with acceptable values ranging between 0.1559 and 0.2352 $\text{gH}_2\text{O}/\text{day}/\text{m}^2.\text{mmHg}$.

The package area measured $13 \times 9 \text{ cm}^2$, equating to 0.0117 m^2 for all three packaging types. A larger packaging area allowed more moisture to enter and spread out, delaying the attainment of critical water content, thereby extending the product's shelf-life (Malhotra et al., 2015; Sharma et al., 2017). Taking into consideration the initial moisture content of biscuits made from MOCAF and LFF, the solid weight per package was calculated, resulting in 48.24 g per pack. According to the saturated water vapor table, the pure

vapor pressure at 30°C was 31.82 mmHg (Malhotra et al., 2015)

3.8 Shelf-life

The period between the production of food ingredients and their rejection by consumers due to quality flaws is known as shelf-life. Several reviews examined the relationship between shelf-life and critical moisture content to determine the duration it took for an item to reach critical moisture levels. Understanding the pattern of water absorption and establishing the critical moisture content value was essential. An increase in RH led to a rise in water content, affecting the product's quality.

By inputting the RH value from storage conditions into the selected model equation, particularly the Oswin model, the equilibrium moisture content value was determined. In this study, shelf-life of biscuits was evaluated at 75% RH and 30°C . Initially, the equilibrium moisture content ($\text{g H}_2\text{O}/\text{g solid}$) and the RH were used to construct the Oswin equation model curve, which was then adopted to calculate shelf-life. To determine the slope value, an isothermic sorption curve was generated. The Oswin curve was described by the equation $\text{Ln } M_e = -2.4302 + 0.4586 \ln(a_w/1-a_w)$.

This equation allowed the determination of the equilibrium water content at a 75% RH distribution, which was 0.1227 $\text{g H}_2\text{O}/\text{g solids}$, 0.1668 $\text{g H}_2\text{O}/\text{g solids}$, and 0.2025 $\text{g H}_2\text{O}/\text{g solids}$. Initially, biscuits made from MOCAF and LFF had a moisture level of 0.0364 ($\text{g H}_2\text{O}/\text{g solid}$). Through the study of physical changes observed during storage, it was possible to ascertain the critical moisture content of the product made with both flours. Biscuits absorbed the moisture from their environments until this crucial limit was reached. Once the critical moisture content was reached, consumers no longer found the crispness of biscuits made from MOCAF and LFF acceptable. The product was placed in an area with a 75% RH and checked every 2 h until a physical change, such as sluggishness, occurred, at which point the water content was determined. Biscuits made from MOCAF and LFF required a moisture content of 0.0777 ($\text{g H}_2\text{O}/\text{g solid}$). When the moisture content of the product was equal to its critical moisture content, the shelf-life of the item was generally determined. Water vapor was absorbed until it reached equilibrium with the environment, affecting both LFF and MOCAF biscuits.

The Labuza equation (1982) was used to compute the critical moisture content method for estimating shelf-life, as outlined in Equation 2, assuming storage conditions maintained a 75% RH. This assumption was

in line with typical storage conditions for biscuits, showing the room's RH. The results of calculations using the chosen Oswin model showed the shelf-life of biscuits made from LFF and MOCAF (Table 6).

Table 6 provided that biscuits made from MOCAF and LFF, packaged in metalized plastic, aluminum foil, and PP plastic, had shelf lives of 21, 15, and 13 days, respectively, at 75% RH. The choice of packaging significantly affected shelf-life due to the increasing water vapor in the environment, which elevated the vapor entering the product to reach an equilibrium (Marangoni *et al.*, 2010). Different packaging types influenced shelf-life alongside environmental RH, as each type had a distinct permeability value. Factors, including food ingredient properties, chosen models, temperature, RH, and packaging permeability, collectively impact a product's shelf-life (Hisham *et al.*, 2016; Robertson *et al.*, 2016; Bhogayata and Arora, 2018; Forsido *et al.*, 2021)

The predictive accuracy of the isothermic sorption method for biscuits made from LFF and MOCAF was significantly influenced by the packaging's low water vapor permeability. However, shelf-life and the packaging permeability value were inversely related. Biscuits packaged with metalized plastic had longer shelf-life than those in aluminum foil and PP due to their lower permeability. PP packaging tended to compromise crispness more easily than metalized plastic and aluminum foil due to its less brittle molecular composition, resulting in higher air and oxygen transmission efficiency.

4. Conclusion

This study demonstrated that biscuits made from a combination of MOCAF LFF offer promising potential as a gluten-free alternative with improved shelf-life, especially when packaged in metalized plastic. The

optimal formulation included 2% stevia and 20% fructose syrup, resulting in biscuits with desirable sensory and physical properties, including a moisture content of 3.78%, protein content of 4.07%, and breaking strength of 11.87 N. The application of accelerated shelf-life testing (ASLT) provided accurate estimations, indicating that metalized plastic packaging was most effective, extending the shelf-life of the biscuits to 21 days at 75% relative humidity, compared to 15.68 days for aluminum foil and 13.91 days for PP plastic packaging. These results provide valuable insights into the formulation and packaging of gluten-free biscuit alternatives, highlighting the importance of packaging materials in extending shelf-life and maintaining product quality.

Conflict of interest

The authors declare no conflict of interest.

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Table 6. Shelf-life used three types of packaging at various storage RH based on the Oswin model

Calculation Variables Shelf-life:	PP plastic	Metalized plastic	Aluminium Foil
(Mi) Initial moisture (g H ₂ O/g solids)	0.0364	0.0364	0.0364
(Mc) Critical moisture (gH ₂ O/g solids)	0.0777	0.0777	0.0777
Oswin Equation Model	Ln Me = -2.4302 + 0.4586 ln(aw/1-aw)		
(Me) equilibrium moisture (gH ₂ O/g solids)	0.3305	0.5204	0.6702
(Po) Saturated vapor pressure at RH distribution (mmHg)	31.824	31.824	31.824
(A) Packing area (m ²)	0.0117	0.0117	0.0117
(b) Isothermic slope	0.2934	0.2934	0.2934
(Ws) Dry weight per pack (g)	48.24	48.24	48.24
(k/x) Packaging permeability gH ₂ O/m ² /day/mmHg)	0.4141	0.2745	0.3674
STORAGE LIFE (day)	13.91	21	15.68
STORAGE LIFE (month)	0.46	0.70	0.52

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