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Determination of the Shelf Life of Microencapsulated Extract of Microalgae *Nannochloropsis*Sp with Accelerated Shelf Life Testing Method

A.A. Made Dewi Anggreni[1], N. Semadi Antara[2*], L.P. Wrasiati[3], I.B.W. Gunam[4]

[1]Doctoral Student, Doctoral Program in Agricultural Science, Faculty of Agriculture, Udayana University Bali, Indonesia.

[2*]Lecturer, Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University, Bali, Indonesia.E-mail: semadi.antara@unud.ac.id

[3]Lecturer, Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University, Bali, Indonesia.

[4]Lecturer, Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University, Bali, Indonesia.

ABSTRACT

The shelf life of a product really needs to be considered before the product is marketed. The aim of this research was to determine the shelf life of microencapsulated – extractofmicroalgae *Nannochloropsis* sp. The shelf life prediction using accelerated shelf life testing (ASLT) method, calculated using Labuza model with critical moisture content approach.Analysis of the water sorpsionisothermic characteristics of the sample in the Henderson approach model. The shelf life of microencapsulated – extract of microalgae *Nannochloropsis*sp at 28 ^{o}C at 75% RH using low density polyethylene (LDPE) plastic packaging with 0.03 mm thickness is 0.93 months and 0.1 mm aluminum foil packaging is 8.14 months.

KEYWORDS

ASLT, Microencapsulated-extract of *Nannochloropsis* sp., Shelf Life.

Introduction

Determination of the shelf life of a product is needed before the product is marketed in addition to products that are safe for consumption by the public (Herawati, 2008).The shelf life is the length of time a product is safe for consumption.When a new product is produced, the quality of the product is considered to be 100%, and will decrease with the length of storage or distribution.During storage or distribution, food products will experience loss of weight, food value, quality, money value, growth power, and trust (Rahayu*et al.*, 2003; Suryani& Arya, 2017). The shelf life of food powder is not only specified by the package materials, but also by external environmental factors such as relative humidityand temperature (Yu *et al*., 2013). Determination of the shelf life needs to pay attention to the parameters of the shelf life, especially the critical point (Herawati, 2008).

Estimation of the shelf life can be done using several approaches, namely 1) literature value, 2) distribution abuse test, 3) distribution turn over, 4) accelerated shelf-life testing (ASLT), or 5) consumer complaints (Herawati, 2008).Estimation of product shelf life using the ASLT method is carried out by using parameters of environmental conditions that can accelerate the process of product quality degradation.The advantage of using the ASLT method is high accuracy and precision and requires a short testing time (3 to 4 months) (Floros and Gnanasekharan 1993; Lodang et al., 2020; Santoso et al., 2020).

There are two approaches to be able to estimate the shelf life of a product with the acceleration method, namely: 1) semi-empirical approach with the help of the Arrhenius equation, namely the kinetics theory which generally uses zero or one order for food products, and 2) the critical water content approach withdiffusion theory using changes in moisture content and water activity as expiry criteria. The mathematical equation model in the water content approach is derived from Fick's unidirectional diffusion law.The mathematical model that can be used is the Labuza (1982) model.Labuza (1982) model or critical water content model is a model based on physical changes, namely for food products that are sensitive to changes in water content.Analysis of the characteristics of the ISA can be done with a variety of model approaches.In this study, using the Henderson Model.

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Microencapsulated – extractofmicroalgae *Nannochloropsis*sp aremicrocapsules made from microalgae *Nannochloropsis*spextract which are encapsulated with maltodextrin and gelatin coating material. These microcapsules contain bioactive compounds that have properties for the human body (published separately).The ASLT method has been used to determine the shelf life of the microencapsulated extract and mangosteen rind powder for 120 days and 109 days at 4^oC storage temperature, respectively (Rahmawati*et al*., 2017; Miwada et al., 2019; Suriani, 2019).There is no information regarding the shelf life of the microencapsulated – extractofmicroalgae *Nannochloropsis*sp, so it is necessary to conduct research on this matter. The purpose of this study was to determine the shelf life of microencapsulated – extractofmicroalgae *Nannochloropsis* sp.

Materials and Methods

The materials used in this research include: microencapsulated – extractofmicroalgae *Nannochloropsis* sp., MgCl2 (Merck), K2CO3 (Merck), KI (Merck), NaCl (Merck), KCl (Merck), BaCl2 (Merck), distillate water.The equipment used is: magnetic stirrer, chamber, desiccator, autoclave (Tommy), glass tool (pyrex), clamp, gloves, thermometer and humidity meter, room hygrometer, aluminum plate, 0.15 mm thick aluminum foil, Low Density Polyethylene (LDPE) plastic 0.03 mm thickness.

Research Implementation

Determination of the shelf life is determined through 2 stages, namely analysis of moisture sorption isothermic characteristics (MSI) and analysis of shelf lifeestimation.

1)Analysis of Moisture Sorption Isothermic Characteristics

MSI analysis was carried out by static gravimetric method by storing the sample at ERH 30 – 90%which was conditioned using a saturated salt solution in a chamber at a temperature of 28 ± 2 ^oC to obtain the product equilibrium moisture content at various a_w.Making the ISA curve through several stages, namely: preparation of a saturated salt solution, storage and weighing of the sample, making the water sorption isothermic curve, analyzing the the water sorption isothermiccharacteristics of the sample in the Henderson approach model (Fitriani, 2015).

Preparation of a saturated salt solution is done by weighing chemical salts (MgCl2, K2CO3, KI, NaCl, KCl, BaCl2) and gradually pouring it into a chamber that has been given distilled water and stirring until it is saturated.Furthermore, the chamber was closed immediately.The chamber was then stored in an incubator for 24 hours at a temperature of 28 ± 2^oC before use (Spiess and Wolf, 1987).After storing the chamber for 24 hours in the incubator, the ERH measurements were taken using a temperature and humidity meter 3 times to get the ERH conditions for each chamber.
The storage and weighing of the microcapsule samples were carried out as follows: 2 g of the sample with 3 replications for each chamber of the saturated salt solution.The sample was then put into the chamber and stored at a temperature of 28 ± 2 ^oC.Samples are weighed every day until they reach a balanced weight using digital scales.Before and during weighing, the sample is transferred from the chamber into the desiccator to balance the sample with a controlled atmosphere.The balanced weight is indicated by the difference between 3 consecutive weights of not more than 2 mg/g for samples stored at RH below 90% and no more than 10 mg/g for samples stored at RH above 90% (Adawiyah, 2006; Sukada et al., 2019; Miwada et al., 2018).The sample moisture content was calculated using the oven-drymethod (AOAC, 1995) and expressed on a dry basis.The moisture content in this condition is the equilibrium water content in the ERH used.The resulting data is used to construct a water sorption isothermic curve. Moisturesorption isothermic curve is made by plotting the experimental data between me as ordinate and aW as abscissa so that the MSI curve is formed.Water activity is obtained by dividing ERH by 100 (Labuza, 1980).

Analysis of the MSIcharacteristics of the sample in an approach model is carried out by entering the experimental value into the model approach (Henderson's Model).The linear form of the MSI equation model from Henderson's model is as follows: (Lamauro, 1984 *in*Rahayu*et al*., 2005).

$$\text{Log}[\ln(1/(1-aw))]=\log K+n \text{ Log } me$$

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The form y = a + bx is as follows:

$$y=\log(\ln(1/(1-aw)))$$
$$x=\log me$$
$$a=\log K$$
$$b=n$$

Plot between the Y axis and the X axis is carried out, so that the a and b values are obtained.The linear equation obtained is then returned to the original equation to obtain the MSIcharacteristics.

2)Shelf Life Estimation Analysis

Analysis of the estimation of shelf life is carried out according to Labuza (1982).The assumptions made in determining the shelf life are that RH and temperature are constant and the quality deviation of the microencapsulated – extract of the microalgae *Nannochloropsis*sp during storage is indicated by changes in the moisture content of the product.The calculation of the shelf life is calculated by equation (1).

$$t=\frac{\ln\left(\frac{Me-Mi}{Me-Mc}\right)}{\left(\frac{k}{x}\right)\left(\frac{A}{Ws}\right)\left(\frac{Po}{b}\right)} \quad (1)$$

Note:

t : product shelf life (day)

m_e : equilibrium moisture content of product (g H_2O/g solid)

m_i : initial moisture content of product (g H_2O/g solid)

m_c : critical moisture content of product (g H_2O/g solid)

k/x : WVTR/Po= Package water vapor permeability constant (g/m^2.mmHg/day)

WVTR adalah*water vapor transmission rate* (g/m^2/day) at a certain temperature and time (%)

A : Package cross-sectional area (m^2)

W_s : Microcapsule weight in the package (g)

Po : Saturated vapor pressure (mmHg)

b : slope of the isothermic sorption curve

Determination of the shelf life is carried out in the following manner: the microcapsules are weighed 2 g each, then put into aluminum foil packaging with a thickness of 0.1 mm.The dimensions of the packaging are 5 cm long and 4 cm wide.The product is stored in a storage room with a temperature of 28 ^oC and RH 75%.The tests that need to be done to determine the shelf life of the microencapsulated – extractof microalgae *Nannochloropsis* sp.using the Labuza equation is:

a. Product Equilibrium Moisture Content (m_e)

The product equilibrium moisture content was determined at room temperature conditions of 28^{o}C and room RH of 75%.

b. Initial Moisture Content (m_i)

The initial moisture content was determined as follows: 2 g of the sample was put into a plate with a known weight, then put in an oven at 105^{o}C.After 3 hours, the plate containing the sample was removed from the oven, cooled in a

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desiccator then weighed and repeated every half hour until it reached a constant weight (AOAC, 1995). The initial moisture content is determined by the following equation and is expressed on a dry basis.

$$\text{Moisture content (dry base)} = \frac{W3}{W2} \times 100\%$$

$$\text{Moisture content (wet base)} = \frac{W3}{W1} \times 100\%$$

$$\text{Total Solids} = \frac{W2}{W1} \times 100\%$$

Note:

W1: The initial weight of the sample
W2: Constant weight of sample
W3: Lose weight

c. Critical Moisture Content (m_c)

Testing of critical moisture content is carried out by looking at the changes that occur in the sample during the study.A total of 2 g samples with 3 replications were placed on a petri dish and stored in a room with 75% RH and a temperature of 28^{o}C.Observation of samples of critical water content is carried out every day until it reaches a critical condition.The critical condition is a parameter of quality damage, that is, a food product can no longer be accepted and is considered out of date.The parameter of microcapsule quality deterioration was measured when the microcapsules were clumped.

d. PackageWater Vapor Permeability (k / x)

The package water vapor permeability constant was obtained from literature studies.Water vapor permeability of 0.15 mm thickness aluminum foil is 0.02 g/m^2.mmHg/day (Histifarina, 2004) and of 0,03 mm thickness LDPE plastic is 0,675 g/m^2.mmHg/day (Anandito, *et al*., 2010).

e. Package Cross-sectional Area (A)

The cross-sectional value of the package is calculated by multiplying the dimensions of the package using the equation below:

$$A = 2 \text{ (Length xWidth)}$$

f. Saturated Vapor Pressure (Po)

The value of saturated water vapor pressure at 28^{o}C is 28.349 mmHg (Labuza, 1982).

g. Packaged weight of microencapsulated – extract of microalgae *Nannochloropsis*sp (Ws).

The weight of microcapsule in packs is 2 g per package.

h. Slope of Curve (b)

The value of the slope (b) is obtained from linear regression of the water sorption isothermic curve of Henderson's model.

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Result

Water Sorption Isothermic Curve

The correlation between equilibrium moisture content of microencapsulated – extract of Microalgae *Nannochloropsis* sp.with $a_w$is presented in Figure 1.

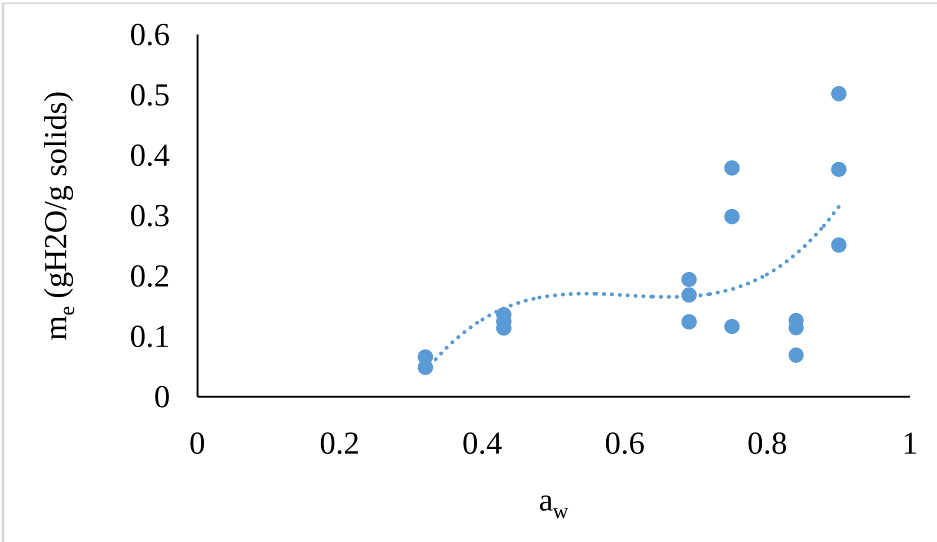

Figure 1. Isothermic Water Sorption Curve of microencapsulated – extract of Microalgae *Nannochloropsis* sp.

Figure 1 shows that the higher the a_w value, the higher the moisture content of the product.Figure 1 also shows that the lowest m_e is 0.04878 gH2O/g solid at the lowest a_w (0.32) and the highest m_e is 0.251462 gH2O / g solid at the highest a_w (90.3).The results showed that the resulting water sorption isotherm curve was in the form of type II sigmoid, following the general water sorption isotherm curve pattern of dry food.

The Characteristics of the Water Absorption Isotherm in the Henderson Model

The moisture content obtained from the experiment produces an imperfect curve, therefore the experimental moisture content data is entered into a model (Henderson's model) in order to obtain a perfect curve.The equation from the mathematical model is converted into a linear equation to determine the coefficients needed to facilitate calculations.In the Henderson model, first a plot between the x-axis is log m_e and the y-axis is log (ln (1 / (1-aw))) resulting in the Henderson equation for the microencapsulated – extract of *Nannochloropsis*sp in linear form:

$$\text{Log}\ (\ln (1/1-a_w)) = 1.18 + 1.1781 \log m_e$$

The linear equation is then returned to the original equation form to be used to predict the water content of equilibrium of microencapsulated – extract of *Nannochloropsis*sp in the entire a_w range to be:

$$1 - a_w = \exp\ (1.18 \text{ x } m_e \text{ x } 1.1781)$$

The experimental equilibrium moisture content obtained using the Henderson equation is presented in Figure 2. Figure 2 shows thaty = 0,2572x - 0,0483
R^2 = 0,9506. The slope is 0.2572.

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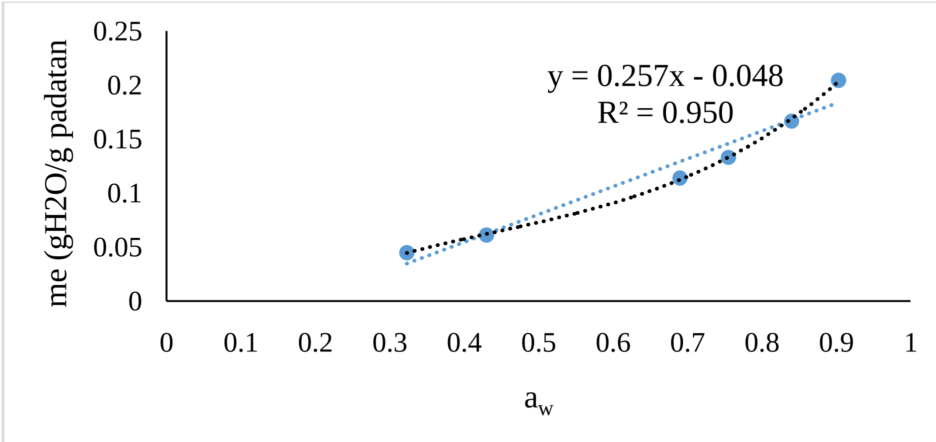

Figure 2. Water Sorption Isotherm Curve using Henderson's Model

Critical Moisture Content

Microencapsulated – extract of microalgae *Nannochloropsis*sp reached critical moisture content on the 21st day of storage. As a dry product, the microcapsules suffered from product damage with a lot of water absorption, the product became lumpy.The critical moisture content parameter is the condition of microencapsulated – extract of microalgae *Nannochloropsis*sp, which is visible first, namely the clumping product which indicates that the microcapsules have absorbed a considerable amount of moisture from the environment.The critical moisture content of Microencapsulated – extract of microalgae *Nannochloropsis*spwas 0.1209 gH2O/g of solid on the 21st day of storage.

Shelf LifeEstimation

The values required in calculating the product shelf life are presented in Table 1.

Table 1. Parameters and Parameter Value of Shelf Life Estimation of the Labuza Method

Parameter	**Score**	**Note**
equilibrium moisture content of Microencapsulated – extract of microalgae *Nannochloropsis* sp (m_e)	0,133 g H_2O/g solid	Obtained from the equilibrium water content of Henderson's model at 75% RH
Initial moisture content of Microencapsulated – extract of microalgae *Nannochloropsis* sp (m_i)	0,038 g H_2O/g solid	Obtained using the AOAC method
Critical moisture content of Microencapsulated – extract of microalgae *Nannochloropsis* sp (m_c)	0,121 g H_2O/g solid	The critical moisture content is the water content when the microcapsule is damaged, indicated by the clumping of the product on the 21st storage day
Package permeability constant (k/x)	LDPE 0,03 mm = 0,675 gH_2O/m^2.mmHg/day Aluminium Foil 0,1 mm = 0,0768 gH_2O/m^2.mmHg/day	Histifarina, 2004
Package cross-sectional area (A)	(4 cm x 5 cm) = 20 cm^2 = 0,002 m^2	
Microcapsule weight in the package	2 g	
Saturated vapor pressure (Po)	28,349 mmHg	Table of saturated vapor pressure by Labuza (1982) at 28 ^oC
Slope (b)	0,257	The slope is obtained from the Handerson's linear regression model

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Based on the above parameters, after being put into equation (1)

Annotation:

Product equilibrium moisture content (m_e)	= 0.133 g H2O/g solid
Initial moisture content of the product (m_i)	= 0.038 g H2O/g solid
Product critical moisture content (mc)	= 0.121 g H2O/g solid
Packaging permeability (k / x)	= LDPE plastic 0.03 mm: 0.675 gH2O/m2.mmHg/day, Aluminum Foil 0.1 mm: 0.0768 gH2O/m2.mmHg/day
Package cross-sectional area (A)	= 20 cm2 = 0.002 m2
Product dry weight	= 2 g
Saturated vapor pressure (P0)	= 28.349 mmHg
Slope (b)	= 0.257

Then:

a. Low density polyethylene (LDPE)

$$t = \frac{\ln \dfrac{(0{,}133 - 0{,}038)}{0{,}133 - 0{,}121}}{0{,}675 \times (0{,}002/2) \times (28{,}349/0{,}257)}$$

$$t = 27{,}79 \text{ days} = 0{,}93 \text{ month}$$

b. Aluminium foil

$$t = \frac{\ln \dfrac{(0{,}133 - 0{,}038)}{0{,}133 - 0{,}121}}{0{,}077 \times (0{,}002/2) \times (28{,}349/0{,}257)}$$

$$t = 244{,}22 \text{ days} = 8{,}14 \text{ months}$$

Discussion

Microencapsulated – extract of microalgae *Nannochloropsis*spare hygroscopic products, which are able to absorb water vapor from the environment into the product.During the research, there was a process of absorption of water vapor from the chemical salt solution into the microcapsules until the product state was constant.The resulting water sorption isotherm (ISA) curve is in the form of type II sigmoid, following the general ISA curve pattern of dry food.Wijaya *et al* (2014) reported that the results of the instant *Ledok* water sorption isothermic test have a type II sigmoid shape.The sigmoid shape is due to the capillary effect and the interaction between the surface of the material and water molecules (Adawiyah, 2006). The S pattern is caused by the accumulation effect of hydrogen bonds, Raoult's law, and the interaction between the surface of the material and water molecules (Labuza, 1984).

The shelf life of a product is influenced by the nature of the product, the type of packaging and the storage conditions for the product.Things that need to be known to be able to predict the shelf life of the product are the product equilibrium moisture content, the initial moisture content of the product, the critical water content, the package water vapor permeability constant, the cross-sectional area of the package, the dry west of the product, the saturated vapor pressure and the slope of the ISA curve so that it can bepresumed through diffusion theory (absorption of gas by products).

Based on Table 1 and equation (1), the results of calculating the shelf life of the microencapsulated – extract of microalgae *Nannochloropsis* sp.at a temperature of 28 ^{o}C at RH 75% using low density polyethylene (LDPE) packaging with a thickness of 0.03 mm is for 24.79 days or 0.93 months and 0.1 mm aluminum foil packaging is 244.22 days or 8.14month.Aluminum foil packaging has lower permeability than LDPE packaging so that the possibility of water vapor absorption from the surrounding environment is also very small.The results of this study are in line with the research of Wijaya *et al.*(2014) which states that the shelf life of instant ledok packed with aluminum foil is longer than that of products with HDPE plastic.The expiration period of lycopene extract from tomatoes mixed with 5:10 maltodextrin was 12.93 weeks (Daniel *et al.*, 2017).

The shelf life of a food product will be longer with the use of a larger packaging area.This is because the entry of water vapor into the packaging will be spread more widely in the packaging so that it can slow down the condition of the product to reach a critical condition (Kusnandar, 2006).

Conclusion

The shelf life of microencapsulated – extract of microalgae *Nannochloropsis*spat 28 ^{o}C at 75% RH using low density polyethylene (LDPE) plastic packaging with 0.03 mm thickness is 0.93 months and 0.1 mm aluminum foil packaging is 8.14 months.

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