JURNAL FARMASI
SAINS DAN KOMUNITAS
Journal of Pharmaceutical Sciences & Community

p-ISSN 1693-5683; e-ISSN 2527-7146

Vol. 20, No. 2, November 2023, pp. 130-138

Research Article

Stability Evaluation of Candlenut Oil Capsule: Disintegration, Iodine Value, Omega Content, and Drug Kinetics

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doi https://doi.org/10.24071/jpsc.004982

J. Pharm. Sci. Community, 2023, 20(2), 130-138

Article Info

Received: 09-08-2022
Revised: 18-05-2023
Accepted: 06-06-2023

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Keywords:
Candlenut oil; omega degradation; Omega fatty acid; Omega food; Oxidative stability; Supplement

ABSTRACT

Candlenut oil, known for its high omega-3 fatty acid content (up to 90%), shows great promise as a valuable source of omega. However, its rich unsaturated fatty acid composition makes it susceptible to oxidation and quality degradation. To address this, candlenut oil is encapsulated for use as a food supplement product. This research aims to investigate the stability of omega oil content in capsulated candlenut oil as well as the physical and chemical properties of gelatin and seaweed-based hard capsules used for encapsulation. Additionally, the study explores the optimal capsule material, opacity, and storage conditions for preserving the quality of candlenut oil products. Three key factors were considered: capsule material (gelatin and seaweed-based), capsule opacity (colored and transparent), and storage temperature (cool temperature with relative humidity of 33% and ±8°C, or room temperature with relative humidity of 65% and ±28°C). The evaluation of the best capsule material and optimum storing temperature of candlenut oil capsules was based on disintegration time, iodine value analysis, and omega content analysis. The results indicate that the disintegration times for gelatin and seaweed capsules were 5.80 and 5.78 minutes, respectively. Transparent gelatin capsules demonstrated the best performance in preventing oil quality degradation, as confirmed by the iodine value analysis. Both gelatin and seaweed capsules exhibited the ability to prevent the degradation of omega oil when stored at cool temperatures, with an estimated kinetic shelf life of 2.92 years.

INTRODUCTION

Omega-3 fatty acids, including DHA (docosahexaenoic acid), ALA (alpha-linolenic acid), and EPA (eicosapentaenoic acid), are essential components found in many food supplements. These fatty acids have been recognized for their potential to enhance brain function, including learning, memory, and brain development in children, as indicated by Stonehouse (2014). In addition, studies conducted by Chandola and Tanna (2014) shown the effectiveness of omega-3 fatty acids in reducing depression and mood disorders.

While these fatty acids offer significant benefits, the human body is incapable of producing omega-3 fatty acids on its own. However, according to Tur et al. (2012), they are also present in some fish and vegetables, such as salmon and plant oils. The daily intake recommendation for children is 100–200 mg per day (Fuentes-Albero et al., 2019). Several fish and seafood varieties can fulfill this requirement by providing approximately 240–1830 mg of omega-3 fatty acids (Gal, 2020). Despite the availability of abundant sources of essential fatty acids, studies indicate that four out of five children in Indonesia still lack adequate omega-3 fatty acid intake (Domasti, 2019). This can be attributed to the relatively high cost of salmon, which contains approximately 980 mg of omega-3 per 100g (Nichols et al., 2014), including its oil.

In addition to well-known sources like fish oils, various other sources, such as seaweed, algae, seeds, nuts, and soybean oil, are excellent sources of omega-3 fatty acids (Gal, 2020). Notably, Indonesia has a significant potential source of omega-3 fatty acids derived from a native plant called candlenut (*Aleurites moluccana*). Larasati's previous research (2019) revealed that candlenut oil contains 26.09% omega-3, 40.08% omega-6, and 24.31% omega-9 fatty acids. To facilitate the consumption of candlenut oil, several studies have explored its incorporation into various food products such as yogurt, bread, and tofu (Larasati, 2019; Saga, 2018; Isnaeni, 2017). However, it has been found that food processing methods involving heat and exposure to oxygen can lead to degradation of the omega content in candlenut oil (Tjhin, 2014).

Additionally, mixing candlenut oil with other substances like milk and water during food production may contribute to a decrease in its omega content (Larasati, 2019). As a way to stop the omega content in candlenut oil from breaking down, researchers like Tiffany (2018), Meidiana (2019), and Wijaya (2019) have looked into microencapsulation techniques using HPMC, sodium caseinate, whey protein isolate, and wall materials made of carbohydrates. However, even with microencapsulation, some degradation of the omega content remains inevitable. A study by Listianto (2020) has shown the possibility of inserting candlenut oil into soft capsules to protect its omega content from heat and oxidation, eliminating the need for additional mixing with other substances. This encapsulation method offers the convenience of consuming an omega-3 supplement in a practical manner.

Candlenut oil encapsulation with HPMC shows degradation of omega-3 fatty acid content, which is possibly due to the spray drying method at which high temperatures are present (Tiffany, 2018). In addition, omega content degradation is also present in the encapsulation with sodium caseinate. This might happen due to the low efficiency of microencapsulation of freeze-dried products (Meidiana, 2019).

While some studies have focused on the production of candlenut oil soft capsules (Natalie, 2020) and pharmaceutical capsule shells (Halida, 2018), these efforts were not specifically intended for the development of candlenut oil capsules as food supplements. Furthermore, industrially manufactured candlenut oil capsules have been available as traditional medicines for slimming purposes and hair growth supplements, rather than as omega supplements. Therefore, this research investigates the potential of candlenut oil as an omega supplement by inserting it into different types of capsules and storing them at varying temperatures. The study aims to obtain the disintegration time, iodine value, and omega content of the capsules, as well as determine their shelf life and half-life.

METHODS

The materials used in this research included cold-pressed candlenut oil, size 0 seaweed-based hard capsule shells (Brataco Chemika), and size 0 gelatin-based hard capsule shells (Kapsulindo). For the analysis, the following chemicals were utilized: sodium thiosulfate for analysis, saturated potassium iodide for analysis, Wjis solution, chloroform for analysis, and starch (Merck).

The candlenut oil was filled into hard capsules with variations in material, opacity, and storage temperature. The capsule materials consisted of animal-based gelatin and plant-based seaweed. The capsule opacity was classified into transparent and colored capsules. The capsules were stored at two different temperatures: a cool temperature (±8°C) and a room temperature (±28°C). To facilitate identification, the samples were labeled using a three-letter code. The first letter represented the capsule material (G for gelatin, S for seaweed), the second letter indicated the capsule opacity (C for colored, T for transparent), and the last letter denoted the storage temperature (F for freeze/cool temperature, R for room temperature). In total, there were eight samples: GCF, GTF, GCR, GTR, SCR, STF, SCR, and STR.

Disintegration Test Analysis

The disintegration time of the capsule was analyzed at PT Sucofindo by using a disintegration tester.

Iodine Value Analysis

Iodine value analysis was performed following the method described by Dijkstra (2015), with some modifications. A total of 0.1g of candlenut oil was dissolved in 25 ml of Wijs solution and 10 mL of chloroform. The solution was then incubated for 30 minutes in the absence of light. Subsequently, 100 mL of distilled water and 20 ml of a 15% (w/v) potassium iodide solution were added to the solution. The resulting mixture was titrated against a 0.1 N sodium thiosulfate solution.

During the titration, the color of the solution changed from brown to yellow. To determine the endpoint, 1 mL of 1% (w/v) starch solution was added, causing the solution to turn dark blue in color. Titration with sodium thiosulfate was continued until the color transitioned from dark blue to transparent. A blank titration under the same conditions was also carried out. The iodine value was calculated using the following equation:

Iodine value = (Volume of sodium thiosulfate used for sample titration - Volume of sodium thiosulfate used for blank titration) × Normality of sodium thiosulfate solution × 0.127.

ANOVA single-factor analysis was used in this study to find out what the differences were in the iodine value of capsulated candlenut oil that were measured once a week. This analysis aimed to assess whether there were significant variations in the decrease in iodine value over time. Additionally, slope tests and t-tests were conducted to compare the effects of capsule material, opacity, and storage temperature on the measured parameters.

Omega Content Analysis

The omega-3 fatty acid content was analyzed at PT Saraswanti Indo Genetech by using gas chromatography with a flame ionization detector (GC-FID).

RESULTS AND DISCUSSION

The consideration of choosing a hard capsule shell instead of a soft capsule shell was because of the production method, oxygen permeability, and moisture absorption. As this research was done on a laboratory scale, it is more convenient to utilize the hard capsule shell, as shown in Figures 1(a) and 1(b), while for the soft capsule to be produced, it should be formed and filled at once using a rotary die encapsulation machine (Gullapalli and Mazzitelli, 2017). Furthermore, soft capsules have higher oxygen permeability and moisture absorption compared to hard capsules due to the presence of plasticizers. This can potentially lead to the instability of the active pharmaceutical ingredient (API) within the capsule (Cole et al., 2008). Therefore, to ensure the stability and integrity of the candlenut oil and its omega-3 fatty acid content, hard capsule shells were deemed more suitable for this study.

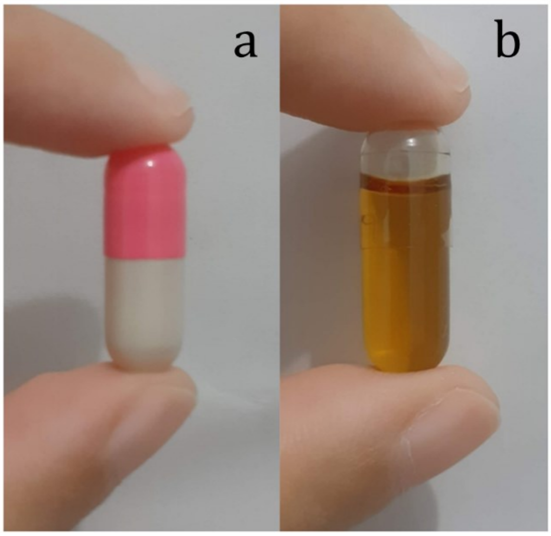

Figure 1. Colored capsule filled with candlenut oil (a) and transparent capsule filled with candlenut oil (b)

Table 1. Omega content of non-capsulated candlenut oil

Parameter	Unit	Result (value±SD)
Unsaturated Fatty Acids	%	90.16±0.03
Omega 3 Fatty Acids	mg/100g	25040.50±1.70
Omega 6 Fatty Acids	mg/100g	40018.40±39.30
Omega 9 Fatty Acids	mg/100g	25104.95±14.25

Table 2. Significant decrease in iodine value measured weekly

Capsule Type	P-Value (ANOVA single factor)
GCF	3E-04
GTF	3E-02
GCR	2E-04
GTR	2E-02
SCF	6E-04
STF	6E-05
SCR	4E-06
STR	2E-06

G=gelatin; S=seaweed; C=colored; T=transparent; F=freeze/cool temperature; R=room temperature

Disintegration Test Analysis

Disintegration time is an important physical property that evaluates the time required for a solid oral dosage form to break down into fine particles and dissolve in the body. In the case of capsule dosage forms, the disintegration time specifically refers to the duration it takes for the capsule shell to completely dissolve. In this study, both gelatin and seaweed-based capsules were analyzed to determine their disintegration times.

The disintegration time for gelatin-based capsules was found to be 5.80 minutes, while seaweed-based capsules exhibited a slightly shorter disintegration time of 5.78 minutes. It is important to note that both types of capsules fall within the United States Pharmacopeia (USP) range for dietary or food supplement capsules, which requires disintegration within 30 minutes. By meeting the disintegration time criteria set by USP, both gelatin and seaweed-based capsules demonstrate their suitability as oral dosage forms for delivering dietary or food supplements. The efficient disintegration of these capsules ensures that the encapsulated substances can be readily released and dissolved, facilitating their absorption and utilization in the body.

Iodine Value Analysis

The primary objective of this study is to identify the optimal capsule type and storage conditions for a candlenut oil food supplement product. Candlenut oil is rich in omega-3 fatty acids, with a content of 90.16%, as indicated in Table 1. However, the high concentration of unsaturated fatty acids in candlenut oil makes it susceptible to degradation. The presence of double bonds in unsaturated fatty acids weakens the bond strength at the methylene position, thereby increasing its vulnerability to oxidation (Decker et al., 2012). By encapsulating the candlenut oil in a capsule, it is expected that the degradation of omega fatty acids can be minimized.

The iodine value is a measure of the degree of unsaturation of fatty acids. A higher iodine value indicates a higher level of unsaturation and implies a better quality of the omega-3 fatty acids present. The iodine value, on the other hand, measures the presence of double bonds in fatty acids (Dijkstra, 2015), while the peroxide value measures the amount of hydroperoxides formed during oxidation. By evaluating the iodine value and monitoring the degradation of omega fatty acids, this study aims to determine the most suitable capsule type and storage conditions that can effectively preserve the quality and integrity of candlenut oil as an omega-rich food supplement. These findings will contribute to the development of a stable and high-quality product that can deliver the desired nutritional benefits to consumers.

The results of the iodine value analysis are presented in Figure 2 (a) and Figure 2 (b) for the gelatin and seaweed capsules, respectively. Over the course of one month of observation, a consistent decrease in iodine value per week was observed for all types of capsules. Table 2 summarizes the significant decrease in iodine value for each capsule type.

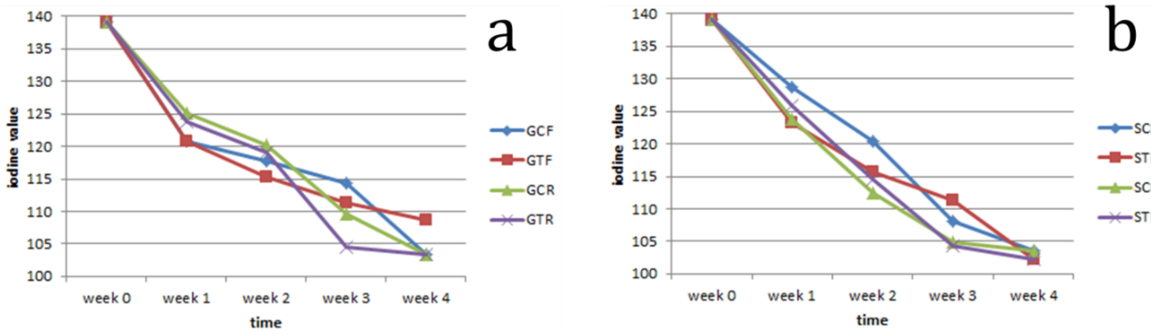

Figure 2. Iodine value of candlenut oil in gelatin capsule (a) and Iodine value of candlenut oil in seaweed capsule (b)

To compare the effects of capsule material, opacity, and storage temperature, a slope test was conducted. The comparison revealed a significant difference (P = 0.02) between the GTF and STF capsules, with GTF showing a lower slope. This indicates that the gelatin capsule is more effective in preventing the degradation of oil quality compared to the seaweed capsule. However, this difference was found to be significant only when the capsule was transparent and stored at a cool temperature.

Several factors may contribute to these findings. One possible reason is the incompatibility between the candlenut oil and the capsule material. Also, the capsules' ability to let oxygen pass through may have sped up the oxidation process. This is especially true for cellulose films, which are more oxygen-permeable than gelatin films because they are less rigid (Gullapalli and Mazzitelli, 2017). Furthermore, the humidity of the surrounding environment can also influence the quality of the oil inside the capsule. A study conducted by Liu et al. (2019) examined the effect of relative humidity on the oxidation of soybean oil and found that higher relative humidity levels correlated with increased oxidation rates.

These findings suggest that gelatin capsules provide better protection against oil quality degradation compared to seaweed capsules. However, this difference is only significant when the capsules are transparent and stored at cool temperatures. The observations highlight the importance of considering various factors, such as capsule material, opacity, and storage conditions, in order to maintain the quality and stability of candlenut oil as an omega supplement.

Omega Content Analysis

Table 1 provides the omega content of non-capsulated candlenut oil, which is found to be similar to the results reported in a previous study by Meidiana (2019). In that study, self-extracted candlenut oil exhibited a yield of 90.89% omega-3 fatty acids. In contrast, the candlenut oil used in this current study was purchased from an e-commerce platform. Despite the different sources, the omega-3 content of the candlenut oil remains relatively high and comparable.

The analysis of omega content was performed specifically for seaweed capsules due to considerations related to lifestyle, beliefs, and market scope. Considering that the majority of the Indonesian population follows the Muslim faith, there is a preference for halal-certified products. Gelatin, derived from animal sources, often raises questions regarding its halal status. Therefore, opting for plant-based capsules, such as seaweed capsules, is deemed more appropriate and aligned with dietary preferences. Moreover, the choice to analyze the omega content in seaweed capsules aligns with the growing trend of individuals adopting vegetarian or vegan lifestyles. Increased awareness of issues relating to animal cruelty and the effects of animal agriculture on climate change is what has caused this shift. By offering plant-based capsules, the study caters to this expanding market and provides an option that resonates with the values and choices of individuals seeking vegetarian or vegan dietary supplements.

The omega-3 content of candlenut oil was measured every 2 weeks throughout a one-month observation period. The results are depicted in Figure 3 (a) for SCF, Figure 3 (b) for SCR, Figure 3 (c) for STF, and Figure 3 (d) for STR. Interestingly, all candlenut oil samples stored in seaweed capsules exhibited a relatively stable and consistent omega content during the observation period. Unlike the iodine value, which indicated a decrease in double bonds, the omega content did not show a significant decrease over time.

This disparity between the iodine value and omega content could be attributed to the different aspects these measurements evaluate. The iodine value specifically quantifies the presence of double bonds in the fatty acid structure, providing an overall indication of the degree of unsaturation. In contrast, the omega content focuses on the detection of double bonds specifically located at the methyl end of the unsaturated fatty acids. So, the iodine value shows the level of unsaturation for all double bonds, while the omega content only shows the presence of double bonds related to omega fatty acids (Przybylski and Eskin, 2011). Considering these differences, it is noteworthy that the omega content of candlenut oil stored in seaweed capsules remained relatively stable throughout the observation period. This finding suggests that the choice of seaweed capsules as the encapsulation material and the selected storage conditions were effective in maintaining the omega content of the candlenut oil.

Compared to previous studies conducted by Saga (2018) and Meidiana (2019), the process of capsulating candlenut oil in this study has proven effective in preventing degradation of the omega content. (Saga, 2018) focused on the microencapsulation of candlenut oil and incorporated the encapsulated oil into a bread formulation. Meanwhile, Meidiana (2019) employed freeze drying for the microencapsulation of candlenut oil. Both studies aimed to protect the oil from oxidation by the encapsulation method. In the current study, the encapsulation of candlenut oil in hard capsules was successful in preserving the omega content. The encapsulation process created a barrier between the oil and external substances, preventing direct interactions that could lead to oxidation.

To compare the omega content, Table 3 presents a comparison between the findings of this study and the aforementioned studies (Saga, 2018) and (Meidiana, 2019). The relatively stable levels seen in this study's results show that capsulation of candlenut oil effectively preserves the omega content. This further emphasizes the importance of encapsulation in preventing the degradation of valuable components such as omega-3 fatty acids in food products.

Drug Kinetics

In this study, the determination of the shelf life and half-life of candlenut oil capsules was based on the analysis of the omega content. Shelf life represents the time taken for the active pharmaceutical ingredient (API), in this case, candlenut oil, to decrease to 90% of its initial amount. Half-life, on the other hand, indicates the time taken for the API to decrease to 50% of its initial amount. To determine the shelf life and half-life, the omega content data from the capsules stored under the worst-case scenario, i.e., transparent seaweed capsules stored at a cool temperature (STF), were utilized. This scenario exhibited the highest degradation of omega content among the different capsule types and storage conditions analyzed.

Linear plotting was performed using the data from STF to establish the kinetics of omega content degradation. The correlation coefficient (R2) was used as a measure of the goodness of fit for different kinetic models. The first-order reaction model demonstrated the highest R2 value of 0.9683, indicating a linear relationship between the concentration of omega fatty acids and their degradation over time. Based on the analysis, it can be concluded that the degradation of omega content in candlenut oil is linearly dependent on the concentration of omega fatty acids. This information allows for the determination of the shelf life and half-life of the candlenut oil capsules, providing valuable insights into the stability and quality of the product over time.

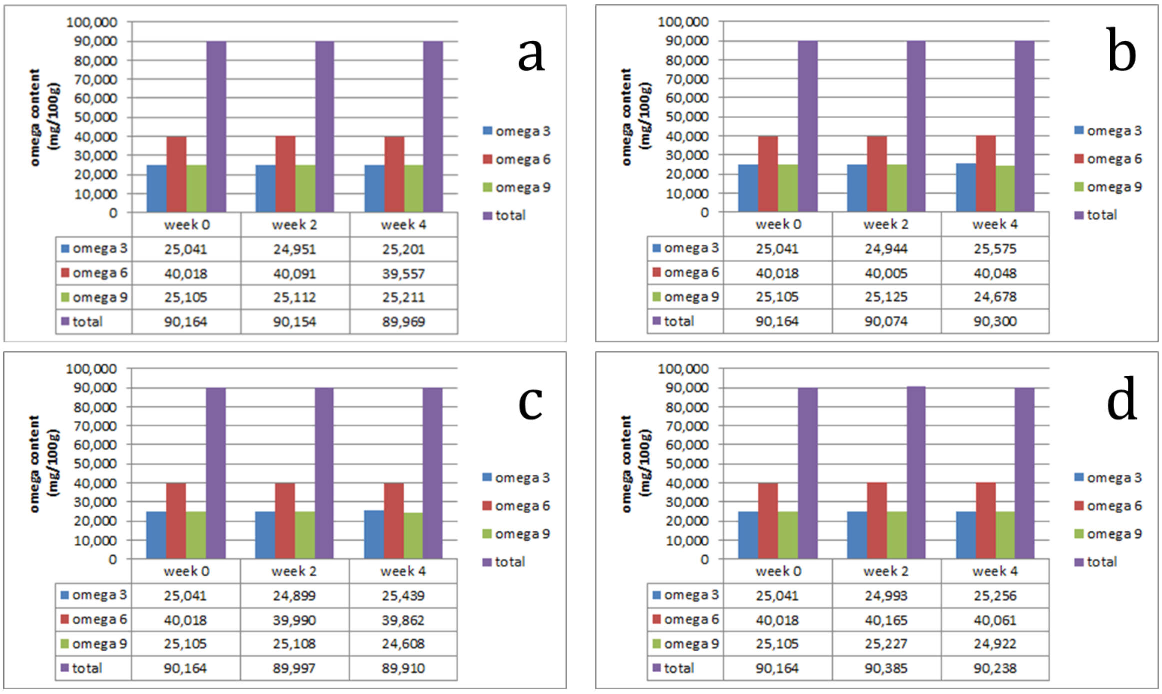

Figure 3. Omega content of candlenut oil in SCF (a), Iodine value of candlenut oil in seaweed capsule (b), Omega content of candlenut oil in STF (c), and Omega content of candlenut oil in STR (d)

Table 3. Comparison of oil omega degradation with previous studies

Omega	(Saga, 2018)		(Meidiana, 2019)		Present study	
	Candlenut oil (mg/100g)	Omega in bread (mg/100g)	Before microencapsulation (mg/100g)	After microencapsulation (mg/100g)	Candlenut oil (mg/100g)	Omega in capsule (mg/100g)
Omega 3	25583.5	137.9	26315.9	14313.05	25040.5	25439.45
Omega 6	40864.6	399.1	40090.3	22012.25	40018.4	39862
Omega 9	23906.8	1821	24308.5	14209.25	25104.95	24608.45
Total omega	90354.9	2358	90714.7	50534.55	90163.85	89909.9
Omega decrease	97%		44%		0.28%*	

*lowest omega content decrease

Table 4. P-values of comparison between capsules (slope test)

Comparison	P-Value
GCF VS GCR[1]	0.25
GTF VS GTR[1]	0.10
GCF VS GTF[2]	0.32
GCR VS GTR[2]	0.99
SCF VS SCR[1]	0.36
STF VS STR[1]	0.45
SCF VS STF[2]	0.17
SCR VS STR[2]	0.58
GCR VS SCR[3]	0.68
GCF VS SCF[3]	0.13
GTR VS STR[3]	0.83
GTF VS STF[3]	0.02*

*significant difference
[1] comparison between storing temperature
[2] comparison between capsule opacity
[3] comparison between capsule material

To determine the shelf life and half-life of candlenut oil capsules based on first-order kinetics, the following formulas are used:

$$t_{90} = \frac{0.105}{k}$$

$$t_{50} = \frac{0.693}{k}$$

where,

t_{90} : shelf life
t_{50} : half life
k : rate constant; amount of omega degradation/unit time $\left(\frac{mg/100g}{day}\right)$

These formulas allow for the calculation of the shelf life and half-life of candlenut oil capsules based on the degradation kinetics determined from the omega content analysis. The rate constant (k) represents the rate at which the omega fatty acids degrade over time and is determined through linear plotting and analysis of the degradation data. The shelf life of a pharmaceutical product measures how long the drug can be stored prior to any significant decomposition of the drug occurring (Cairns, 2012).

The shelf life of candlenut oil capsules, based on the first-order kinetics analysis, is determined to be 1050 days, or approximately 2.92 years. This indicates that the capsules can be stored for up to 1050 days before experiencing significant decomposition or degradation of the active ingredients. Additionally, the half-life of the candlenut oil capsules is calculated to be 6930 days, or approximately 19.25 years. The half-life represents the time it takes for the concentration of the active ingredient to decrease to half of its initial amount. Based on these calculations, it can be concluded that with proper storage conditions, the candlenut oil capsules can be safely consumed within a period of 3 years without significant degradation of the active ingredients. It is important to adhere to recommended storage conditions, such as temperature and humidity, to ensure the longevity and quality of the product.

CONCLUSIONS

This study demonstrated that gelatin capsules, particularly when stored at cool temperatures, provided better protection against the degradation of candlenut oil compared to seaweed capsules. Capsulation effectively preserved the omega content of the oil, indicating its potential as a food supplement. The calculated shelf life of the candlenut oil capsules was 1050 days (2.92 years), indicating their stability over an extended period of time. The disintegration time for gelatin capsules and seaweed capsules was nearly identical, with a duration of approximately 5.80 minutes for gelatin capsules and 5.78 minutes for seaweed capsules. Proper storage conditions are crucial for maintaining the quality and longevity of the product. Overall, gelatin capsules stored at cool temperatures are recommended for preserving the omega content and ensuring the quality of candlenut oil as a food supplement.

ACKNOWLEDGEMENTS

The author would like to thank the Swiss German University for the Faculty Research Fund 2021.

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