Sapporo Medical Journal
Volume 57, Issue 5, May 2023

Alginate/lignin in the storage: The content of lignin and alginate, antioxidant activity and microorganisms

Nguyen Xuan Hoan[1], DinhHuu Dong [1], Pham Anh Dung [2], Dang Xuan Cuong[1,*]

Ho Chi Minh City University of Food Industry, Vietnam[1],
Ministry of Trade and Industry, Vietnam[2]

Abstract— Alginate/lignin is an antioxidant biomaterial synthesized from alginate of brown algae and lignin of maize by-product by spray drying method. Alginate/lignin was evaluated for lignin content, alginate content, and its antioxidant activity according to the storage time, for example, storage at 25°C for 18 months and accelerated aging at 55°C for 137 days. The results showed that alginate/lignin was stable in content and antioxidant activity for 24 storage months. The significant decrease (p<0.05) in lignin content, alginate content, and antioxidant activity changed according to the linear model for the two storage methods. Alginate/lignin content decreased by under 3% after 24 storage months. Microorganisms of alginate/lignin increased according to the storage time and faster in the storage method at 25°C, compared to the accelerated aging method.

Keywords: Alginate, antioxidant activity, lignin, microorganisms, storage.

1. Introduction

Alginate is a linear polysaccharide anion containing 1,4 bonds with basic units (-D-mannuronic acid (M) and 1,4 -L-guluronic acid (G)), occurring naturally in the cell wall of brown seaweed. Alginate is considered one of the most versatile biopolymers because of its wide range of applications and its role in drug-delivery polymer systems.[1] Alginates are commonly used in excipients in medicinal products because of their thickening, gelling, structural stabilizing, and time-controlled properties.[2]

Lignin has four types, water-soluble, ethanol-soluble, acid-soluble (klasson lignin), and alkaline medium (kraft lignin). All four types of lignin are insoluble in acid, so after obtaining lignin extracts from plants through the separation of lignin, cellulose, and hemicellulose, lignin precipitation was carried out by adjusting the pH medium.[3,4] Lignin obtained from a precipitation process will be purified for application in food, functional foods, and pharmaceuticals as an antioxidant, drug release control agent, and surfactant. [5]

Alginate/Lignin is a combination of biological polymers derived from plants with two basic polymers, lignin, and alginate, which has been researched and developed in recent years but has been applied in many different fields, such as pharmaceuticals, [6] functional foods [7] and agriculture.[8,9] Especially in functional foods and pharmaceuticals, studying and developing active alginate/lignin materials is a new direction that has attracted much attention from scientists and pharmaceutical enterprises.[10,11] This composite structure exhibits a biological

activity than lignin and alginate alone. This structure has shown high bioactivity with the ability to form films, create states for solutions, and create properties for materials, so they are applied in the production of coatings to support the treatment of open wounds and wounds and in food packaging to prevent UV-induced lipid oxidation.[4]Alginate/Lignin can also be used as a heavy metal absorbent material in the human body and in the aquatic environment.

Thus, the paper focused on the change of alginate, lignin, their antioxidant activity, and microorganisms according to the storage time with the other two storage methods.

2. Materials and Methods

2.1. Experiment design

For accelerated aging method

Alginate/Lignin is evaluated for quality stability according to the ASTM F1980-07 method. The lignin, alginate, antioxidant, and microbiological activities of alginate/lignin were analyzed every 15 days, and the time needed to evaluate was 137 days at 55^{o}C according to the accelerated aging method. The actual assessment time is as follows:

$$Accelerated\ aging\ time\ (day) = \frac{Actual\ storage\ time\ (month)}{Q_{10}^{[(T_{AA}-T_{RT})/10]}}$$

In there:

Q_{10}: aging coefficient. ranges from 1.8 to 2.5 and here is considered to be 2.

T_{AA}: accelerated aging temperature, here is 55^{o}C.

T_{RT}: product storage temperature, here is 25^{o}C.

Actual desired storage time (months): 24 months.

Accelerated aging factor: 9.19.

For the storage method at 25^{o}C

Alginate/Lignin was stored at 25^{o}C and analyzed once every three months for 18 months of storage. The lignin content, alginate content, antioxidant activity, and microorganism was evaluated on alginate/lignin for two storage method.

2.2. Analysis methods

Method of quantification

Quantification of lignin content: 01 g of lignin was dissolved in 100 mL of 0.1 M NaOH, and the measurement of absorbance at 280 nm [12].

Quantification of alginate content: Alginate content was quantified by colorimetric method with resorcinol.

Quantification of microorganisms

TCVN 11039-1:2015. Part 1: Determination of total aerobic microorganisms by plate counting technique.

TCVN 4882:2007 (ISO 4831:2006) Microbiology of food and animal feeding stuff – Methods for the detection and enumeration of *coliforms* – Counting technique with the highest probability.

TCVN 11039-6:2015. Part 6: Detection and enumeration of *Staphylococcus aureus* by colony counting technique.
TCVN 11039-5:2015. Part 5: Detection of *Salmonella*.
TCVN 7924-2:2008 (ISO 16649-2:2001) Microbiology of food and animal feeding stuff – Method for the enumeration of β-glucuronidase-positive *Escherichia coli* – Part 2: Colony counting technique at 44^{o}C using 5-bromo-4-clo-3-indolyl β-D-glucuronide.
TCVN 4991:2005 (7937:2004) Microbiology of food and animal feed - Quantitative method of *Clostridium perfringens* on agar plates - Colony counting technique.
TCVN 7903: 2008 (ISO 21871: 2006) Microbiology of food and animal feeding stuff - Determination method for the of presumptive small numbers of *Bacillus cereus* - most probable numerical technique and detection method.

Evaluation of antioxidant activity

The total antioxidant as described by Prieto et al. (1999), [13] took 100 µl of the sample, supplemented with 900 µl of distilled water and added 3 ml of solution A (0.6 M H_2SO_4, 28 mM sodium phosphate and 04 mM ammonium Molybdate). The mixture was kept for 90 min at 95°C. Then measure at 695 nm with ascorbic acid as standard.
Reducing power activity was determined by taking 500 µl of sample solution supplemented with 0.5 ml of phosphate buffer pH 7.2 and 0.2 ml of $K_3[Fe(CN)_6]$ 1%. Hold the mixture for 20 min at 50 °C, then, add 500 µl of 10% CCl_3COOH, 300 µl of distilled water and 80 µl of 0.1% $FeCl_3$. Finally, the mixture was measured at 655 nm with $FeSO_4$ as the standard. [14]

2.4. Data analysis

Each experiment was repeated three times and expressed as mean ± SD. Analysis of statistical, ANOVA and unusual removal was using MS.Excel 2013 software.

3. Results and discussion

3.1. Alginate content

For the accelerated aging method of storage, alginate content decreased from 742.9 ± 20.8 to 694.54 ± 25.00 mg uronic acid equivalent/g DW, corresponding to storage start to day 137th, respectively. Alginate content decreased by 6.5% at the storage day 137th, compared to the storage start. Alginate content at the storage day 60th, 90th, and 120th got the value of 97.8, 96.6, and 95.2%, compared to the storage start. The decrease in alginate content was according to the linear model, and a significant difference did not occur in alginate content during the storage days (p>0.05) (Tab. 1).
For the storage method at 25^{o}C, alginate content was in the range of 742.90 ± 20.8 and 726.76 ± 19.62 mg uronic acid equivalent/g DW, corresponded to storage start and month 18th, respectively (Tab. 2). After 18 storage months, alginate content only decreased by 2.17%, compared to the start material, and the difference was not statistical significance (p>0.05). The decrease of alginate content depended on numerous factors during the storage processing, for example, humidity, material nature, and packing material. [15,16]

Table 1. Alginate/Lignin content and antioxidant activity over storage time under accelerated aging

Storage days	Alginate content (mg uronic acid equivalent/g DW)	Lignin content (mg lignin equivalent/g DW)	Antioxidant activity (mg ascorbic acid equivalent/g DW)	Reducing power activity (mg $FeSO_4$equivalent/g DW)
0	742.9 ± 20.8	79.5 ± 2.56	248.72 ± 6.23	208.7 ± 5.96
15	739.2 ± 19.96	79.1 ± 3.01	242.86 ± 8.74	205.46 ± 8.63
30	735.9 ± 26.49	77.62 ± 3.26	238.27 ± 7.62	202.01 ± 5.05
45	732.33 ± 30.76	78.96 ± 3.55	234.69 ± 9.39	193.58 ± 6.39
60	726.42 ± 34.87	77.54 ± 2.87	228.7 ± 6.63	195.6 ± 7.04
75	724.89 ± 24.65	75.83 ± 2.20	226.75 ± 8.39	193.77 ± 5.43
90	717.65 ± 27.99	76.89 ± 3.23	223.17 ± 9.37	190.48 ± 7.62
105	719.22 ± 33.80	74.28 ± 3.57	219.68 ± 7.91	186.93 ± 7.85
120	707.18 ± 23.34	73.76 ± 2.51	213.75 ± 6.84	180.17 ± 4.50
137	694.54 ± 25.00	74.49 ± 2.23	217.59 ± 8.70	183.56 ± 6.06

Note: significant level ($p<0.05$).

3.2. Lignin content

Table 2. Alginate/Lignin content and antioxidant activity over storage time under storage conditions without accelerated aging

Storage days	Alginate content (mg uronic acid equivalent/g DW)	Lignin content (mg lignin equivalent/g DW)	Antioxidant activity (mg ascorbic acid equivalent/g DW)	Reducing power activity (mg $FeSO_4$ equivalent/g DW)
0	742.90 ± 20.8	79.50 ± 2.56	248.72 ± 6.23	208.70 ± 5.96
3	738.54 ± 22.16	79.14 ± 2.85	240.52 ± 10.10	200.93 ± 5.63

6	740.10 ± 21.46	78.20 ± 2.50	244.19 ± 6.10	203.26 ± 7.54
9	735.59 ± 22.03	77.31 ± 3.09	238.63 ± 7.87	201.73 ± 6.66
12	727.82 ± 24.75	78.82 ± 2.29	230.96 ± 8.31	196.54 ± 8.49
15	732.80 ± 26.38	79.64 ± 2.93	234.45 ± 6.56	193.88 ± 7.37
18	726.76 ± 19.62	77.48 ± 3.25	228.61 ± 9.14	195.47 ± 8.21

Note: significant level ($p<0.05$).

For the accelerated aging method of storage, lignin content was within 79.50 ± 2.56 (storage start) to 74.49 ± 2.23 mg lignin equivalent/g DW (day 137th). The decrease of lignin content was 6.3% at the storage day 137th, compared to the storage start. Lignin content decreased by 2.47, 3.28, and 7.22% in comparison to the storage start, corresponding to the storage day 60th, 90th, and 120th. The linear model appeared in the decrease in alginate content according to the storage time for two methods, and lignin content in consecutive timelines was not a significant difference (p>0.05) (Tab. 1).

For the storage method at 25^{o}C, lignin content decreased from 79.50 ± 2.56 (storage start) to 77.48 ± 3.25 mg lignin equivalent/g DW (month 18th) (Tab. 2). Lignin content only decreased by 2.17% after 18 storage months in comparison to the start material, and the decrease was not a difference in statistical significance (p>0.05). Application of lignin in the storage appeared in numerous previous publications, [17] but antioxidant lignin storage did not present in any notice.

Table 3. Microbiology of Alginate/Lignin over storage time under storage conditions without accelerated aging

Storage days	**Total aerobic microorganisms (CFUx 10^2/g)**	***Coliform* (CFU/g)**	***S. aureus* (CFU/g)**	***Salmonella* (CFU/25 g)**	***E. coli* (CFU/g)**	***C. perfringens* (CFU/g)**	***B. cereus* (CFU/g)**
0	0.30 ± 0.08	-	-	-	-	-	-
3	0.44± 0.10	-	-	-	-	-	-
6	0.62± 0.13	-	-	-	-	-	-
9	0.70 ± 0.10	-	-	-	-	-	-
12	0.93± 0.12	-	-	-	-	-	-

15	1.55±0.26	-	-	-	-	-	-
18	2.98± 0.17	-	-	-	-	-	-

Note: significant level (*p<0.05)*.

Table 4. Microbiology of Alginate/Lignin over storage time under storage conditions accelerated aging

Storage days	**Total aerobic microorganisms (CFUx 10^2/g)**	***Coliform*(CFU/g)**	***S. aureus* (CFU/g)**	***Salmonella* (CFU/25 g)**	***E. coli*(CFU/g)**	***C. perfringens* (CFU/g)**	***B. cereus*(CFU/g)**
0	0.30 ± 0.08	-	-	-	-	-	-
15	0.43± 0.10	-	-	-	-	-	-
30	0.46± 0.10	-	-	-	-	-	-
45	0.50± 0.09	-	-	-	-	-	-
60	0.52± 0.10	-	-	-	-	-	-
75	0.58± 0.11	-	-	-	-	-	-
90	0.63± 0.07	-	-	-	-	-	-
105	0.80 ± 0.12	-	-	-	-	-	-
120	0.90 ± 0.07	-	-	-	-	-	-
137	1.10 ± 0.14	-	-	-	-	-	-

Note: "-" non detected.

3.3. Total antioxidant activity

For the storage by the accelerated aging method, the total antioxidant activity got the value of 248.72 ± 6.23 and 217.59 ± 8.70 (mg ascorbic acid equivalent/g DW), corresponding to the start material and the storage day 137^{th}, respectively. The thing showed that the total antioxidant activity of alginate/lignin decreased to 87.48%, compared to the start material. The total antioxidant activity of alginate/lignin decreased by 8.05, 10.27, and 14.06% after storage days 60^{th}, 90^{th}, and 120^{th}, respectively, compared to the start material. The difference in the decrease

was statistical significance (p<005) (Tab. 1). The linear model of total antioxidant activity decrease appeared according to the storage time.

For the storage method at 25°C, total antioxidant activity was within 248.72 ± 6.23 (storage start) to 228.61 ± 9.14 mg lignin equivalent/g DW (month 18th) (Tab. 2). Total antioxidant activity decreased by 1.82, 7.14, and 8.09% after 6, 12, and 18 storage months, respectively, compared to the start material. The decrease in total antioxidant activity was a statistically significant difference (p<0.05) according to the storage time. The linear model was also found in total antioxidant activity decrease in the storage method at 25°C. Storage time usually changes the active substance and reduces biological activity. [18,19]

3.4. Reducing power activity

For the storage by the accelerated aging method, at the storage day 137th, the reducing power activity got the value of 183.56 ± 6.06 (mg $FeSO_4$ equivalent/g DW), corresponding to 87.95%, compared to the start material. The reducing power activity of alginate/lignin at the storage day 137th got 93.84%, compared to the storage day 60th. The decrease of reducing power activity was 6.28, 8.73, and 13.67%, corresponding to 60th, 90th, and 120th, respectively, and the thing occurred when compared to the start material. The statistical difference in the decrease in reducing power activity presented (p<005) (Tab. 1), and the linear model exhibited the decrease in reducing power activity was found according to the storage time.

For the storage method at 25°C, the change of reducing power activity during the storage time of 18 months was from 208.70 ± 5.96 (storage start) to 195.47 ± 8.21 mg $FeSO_4$ equivalent/g DW (month 18th) (Tab. 2). After 6th, 12th, and 18th storage month, reducing power activity got 97.39, 94.17, and 93.66% when compared to the start material, respectively. A statistically significant difference (p<0.05) was found in the decrease in total antioxidant activity according to the storage time. The total antioxidant activity decrease was simulated bylinear model in the storage method at 25°C. The change of reducing power activity was also described in previous studies on medicine plants [20,21], but reducing power activity of alginate/lignin did not saw.

3.4. Microorganisms

Tables 3 and 4 showed that *Coliform, S. aureus, Salmonella, E. coli, C. perfringens*, and *B. cereus* did not appear in alginate/lignin according to the storage time and occurred in both storage methods (Tab.1 and Tab. 2). Total aerobic microorganisms exist in alginate/lignin. Total aerobic microorganisms in consecutive timelines were in statistically significant difference according to the storage time (p<0.05) for the two storage methods. Microorganisms of alginate/lignin in other storage methods were statistical significance (p<0.05). After 137 days of storage by accelerated aging method and 18 months of storage at 25°C, total aerobic microorganisms of alginate/lignin were still within the permitted limit of healthy. The growth of total aerobic microorganisms in alginate/lignin when stored by the accelerated aging method was slower than that of storage at 25°C because the temperature of 55°C was not suitable for microbial growth.

Constraint

The study did not present metal according to the storage time.

4. Conclusion

Alginate/lignin is a stable antioxidant biomaterial according to the storage time at 25^{o}C for 18 months and at 55^{o}C for 137 days for accelerated aging. The content of lignin and alginate decreased and changed according to the linear model for the two storage methods. The difference in lignin content according to the storage time at 25^{o}C was significant (p<0.05), and occurred to alginate content. Lignin and alginate content in the accelerated aging method was also no exception. The antioxidant activity of alginate/lignin decreased more than the content of alginate and lignin during the storage time. Microorganisms in the storage condition at 25^{o}C increased faster than at 55^{o}C (accelerated aging). Antioxidant alginate/lignin could be usefull as pharmaceutical raw materials, functional foods or bio-absorbable materials.

5. Conflict of interest

No conflict of interest to declare.

6. Financial disclosure

The study did not receive any specific grant from funding agencies in the public, commercial, or not-forprofit sectors.

7. References

[1] Hanne H.T., Jan K. Alginate in drug delivery systems. Drug Dev Ind Pharm 2002;28(6): 621-630.

[2] Farhad A., Sevil V.M., Effat A., Elahe A., Elmira K., Seyyed M.B.F., Mohammadali T., Abolfazl A. Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. J BiolEng2020;14: 8.

[3] Mengyao Y., Muhammad S.UrR., Tingxuan Y., Asad U.K., PiotrOleskowicz-Popiel, Xia X., Ping C., Jian X. Treatment of different parts of corn stover for high yield and lower polydispersity lignin extraction with high-boiling alkaline solvent. BioresourTechnol 2018; 249: 737-743.

[4] Neeraj M., Aurélie C., François R., Stéphane G., Fabine S., Giulio M., Jin-Ping G. An overview on the use of lignin and its derivatives in fire retardant polymer systems, 1st ed., Matheus P., Eds.: IntechOpenPublishers, Inc: United Kingdom, 2017;

[5] Avinash P.I., Anuj K.C., Silvio S.d.S. Lignocellulosicbiorefining technologies. John Wiley & Sons Publishers, Inc: USA, 2020;376 pages.

[6] Juan D.-R., Sarah A.S., Andreas R., Zoilo G., Ryan F.D., Eneko L. Lignin and cellulose blends as pharmaceutical excipient for tablet manufacturing via direct compression. Biomolecules 2019; 9(9): 423.

[7] Xiaojia H., Huey-Min H. Nanotechnology in food science: Functionality, applicability, and safety assessment. JFDA 2016; 24(4): 671-681.

[8] Francisco F.-C., Gerardo P.M.-D., Matilde V.-S., Manuel F.-P. Preparation and characterization of azadirachtin alginate-biosorbent based formulations: Water release kinetics and photodegradation study. J Agric Food Chem 2015; 63(38): 8391-8398.

[9] Domitille L.-L., Karina S., Renate W., Georg M.G., Gibson S.N. Enzymatic synthesis of 100% lignin biobased granules as fertilizer storage and controlled slow release systems. ACS Sustainable ChemEng 2019; 7(14): 12621–12628.

[10] [Keshaw R.A., Harit J. Physico-chemical properties of lignin–alginate based films in the presence of different plasticizers. Iran Polym J 2016; 25: 661–670.

[11] Karna R., Mi-Jung C., Geun-Pyo H. Micro- and nano-scaled materials for strategy-based applications in innovative livestock products: A review. Trends Food SciTechnol2018; 71: 25-35.

[12] Olumoye A., Jawad J., Marzouk B., Andrea M.R., Naima E.M.,Yacine B. Quantification and variability analysis of lignin optical properties for colour-dependent industrial applications. Molecules 2018; 23: 1-21.

[13] Prieto P., Pineda M., Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem1999;269: 337–341.

[14] Zhu Q.Y., Hackman R.M., Ensunsa J.L., Holt R.R., Keen C.L. Antioxidative activities of oolong tea. J Agric Food Chem 2002;50: 6929–6934.

[15] Ahmad R., Ghasem O.S., Somayeh Z., Mohammad J.S., Ahmad R.S. Effect of storage time of extended-pour and conventional alginate impressions on dimensional accuracy of casts. J Dent (Tehran). 2014; 11(6): 655–664.

[16] Steven O.H.DDS., Richard F.Jr. Effects of long-term storage on properties of an alginate impression material. J Prosthet Dent 1997; 77(6): 601-606.

[17] José L.E.-A., Patricia I.T.-C., Jorge L.O.-M., Alejandro V.-R., Sergio F.-G., Armando Z.-C.E. Lignin in storage and renewable energy applications: A review. J Energy Chem 2018; 27(5): 1422-1438.

[18] Dang X.C., Vu N.B., Tran T.T.V., Le N.H. Effect of storage time on phlorotannin content and antioxidant activity of six *Sargassum*species from Nhatrang Bay, Vietnam. J ApplPhycol 2016; 28(1): 567-572.

[19] Nydia E.B.-C., Marilena A.-R, Javier V.-C., Janet A.G.-U. Changes in cellular antioxidant and anti-inflammatory activity after 12 months storage of roasted maize-based beverages supplemented with nejayote solids. J Cereal Sci2019; 89: 102807.

[20] Sheila O.-A., Sílvia L., Ofélia A., Tiago A.F., Ilda C., Sofia C., Sara C. Influence of the storage in bottle on the antioxidant activities and related chemical characteristics of wine spirits aged with chestnut staves and micro-oxygenation. Molecules. 2022; 27(1): 106.

[21] Yung-Sheng L., Wen-Ying H., Pang-Yen H., Shiou-Yih H., Ying-Yi L., Cheng-You C., Min-Yun C., Shu-Ling H. Effects of storage time and temperature on antioxidants in juice from *Momordicacharantia* L. and *Momordicacharantia* L. var. abbreviata Ser. Molecules 2020; 25(16): 3614.

This work is licensed under a Creative Commons Attribution Non-Commercial 4.0 International License.