



Alginate Extraction from *Turbinaria murayana* Seaweed as a Feed Additive for Poultry

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Abstract | This study was aimed to evaluate the effective methods of alginate extraction from *Turbinaria murayana* seaweed as a potential feed additive for poultry. The experimental design employed a Completely Randomized Design (CRD) consisting of three extraction methods as treatments, in which each treatment was replicated six times. The procedure of extraction tested included acid, alkaline, and calcium methods. Measured variables were alginate yield, moisture content, ash content, viscosity, molecular weight, and functional group characterization of alginate using Fourier Transform Infrared (FTIR) analysis. The data were statistically analyzed using Analysis of Variance (ANOVA) of CRD and differences among treatments were further assessed using Duncan's Multiple Range Test (DMRT). The results demonstrated that the extraction methods significantly influenced ($p < 0.01$) the alginate yield, moisture content, ash content, viscosity and molecular weight. The acid extraction method yielded the highest quality alginate, with a yield of 26.93%, moisture content of 13.44%, ash content of 23.74%, viscosity of 15.75 cPs, and a molecular weight of 76,786.97 g/mol. FTIR analysis confirmed that the functional groups of the extracted alginate were consistent with the standard alginate compound. Based on the evaluated parameters, the quality of alginate obtained through the acid extraction method met the criteria established by the Food Chemical Codex 2004.

Keywords | Alginate, Extraction, Feed additive, Poultry, *Turbinaria murayana*

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INTRODUCTION

The poultry industry plays strategic roles in meeting the global demands for efficient and sustainable animal protein. One of them is the use of antibiotics as feed additives for increasing the growth of poultry. However, excessive use of feed additives, particularly antibiotics, has raised global concerns due to the emergence of antibiot-

ic-resistant bacterial strains and antibiotic residues in food products. These impacts not only threaten human health but also disrupt ecosystem balance and the sustainability of the livestock enterprise. The use of antibiotics has been shown to have negative consequences for the environment, animal health, and consumer safety (Arsene *et al.*, 2022). In response to this situation, the Indonesian government banned the use of antibiotics as growth promoters starting

in January 1st, 2018 through the act number 14/2017 by the Ministry of Agriculture. This has driven the development of natural compound-based innovations to support livestock productivity without negative side effects.

One promising natural compound is alginate, a natural polymer derived from brown seaweed. Alginate exhibits various biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties (Liu *et al.*, 2019; Fu *et al.*, 2023). This compound has shown to inhibit the growth of pathogenic bacteria in poultry gastrointestinal tracts by forming a protective layer that prevents pathogen adhesion and proliferation (Zhu La *et al.*, 2023; Fu *et al.*, 2023). Additionally, alginate functions as a prebiotic, promotes lactic acid bacteria populations, lowers gastrointestinal pH, and suppresses pathogenic bacteria growth in the small intestine and cecum. Using alginate as a feed additive alternative to antibiotics not only enhances poultry performance but also supports gastrointestinal health (Liu *et al.*, 2019). Previous studies have shown that the inclusion of alginate oligosaccharides (AOS) at 200mg/kg in diets improves feed intake, production performance, and lactic acid bacteria populations while reducing *Escherichia coli* populations in the intestine and cecum (Zhu La *et al.*, 2023).

The brown seaweed *Turbinaria murayana* is a potential source of alginate. This seaweed grows abundantly along the shores of Sungai Nipah Beach, Pesisir Selatan Regency, yet remains underutilized. According to Mahata *et al.* (2023), the brown seaweed growing on Sungai Nipah Beach can be harvested at a rate of 1 ton per week. The alginate content in *T. murayana* has been reported to reach 34.93% (Reski *et al.*, 2024). In addition to its antimicrobial properties, alginate from this seaweed also has the potency to reduce fat and cholesterol levels in meats and eggs. The use of *T. murayana* seaweed flour at 15% in broiler diets has been reported to maintain production performance while reducing abdominal fat (Reski *et al.*, 2022). Fermented *T. murayana* seaweed included at up to 20% in diets for quails and laying hens has also been shown to maintain egg quality while lowering egg yolk cholesterol levels (Reski *et al.*, 2023).

Alginate extraction methods from brown seaweed have been extensively studied, including acid, alkaline, and calcium methods (Peteiro, 2017; Bojorges *et al.*, 2023). The acid methods, such as the Vincent, Herter, and Bashford method (Prasetyaningrum and Purbasari, 2002), and Zailanie method (Zailanie *et al.*, 2001), have advantages in producing high-quality alginate with lower costs compared to other methods (Husni *et al.*, 2012). Acid, alkaline, and calcium extraction methods are widely used to obtain alginate from brown seaweed (Bojorges *et al.*, 2023). Studies on *Macrocystis pyrifera* have demonstrated that the acid methods is more effective than that of the other methods

(Peteiro, 2017). Alginate extraction from *Turbinaria murayana* seaweed has previously been conducted using the Zailanie method, yielding 13.51% (Reski *et al.*, 2021). This yield is relatively low, necessitating the identification of a more suitable extraction method to obtain a higher alginate yield than the previous approach.

However, no specific studies have evaluated alginate extraction methods from *T. murayana* to produce alginate as an antibiotic substitute feed additive for poultry. Therefore, this study aims to explore the effectiveness of different alginate extraction methods from *T. murayana* in producing high-quality alginate that supports poultry performance and health.

MATERIAL AND METHODS

RESEARCH IMPLEMENTATION

The study began with the preparation of materials and equipment. Seaweed flour was prepared from *T. murayana* seaweed obtained from Sungai Nipah Beach, Pesisir Selatan Regency. Fresh seaweed was soaked in fresh water for two days to remove dirt and salts. After soaking, the seaweed was sun-dried until it reached a moisture content of 12% (Reski *et al.*, 2024). The dried seaweed was then ground using a hammer mill machine to produce fine flour.

The resulting *T. murayana* flour was used as the base material for the alginate extraction process. Extraction was performed using three different methods: acid, alkaline, and calcium methods. Each method involved specific steps to optimize the quality of the alginate obtained. A detailed procedure for each extraction method is presented in Figure 1.

MATERIAL

This study utilized *T. murayana* seaweed flour as the primary material, obtained through drying and grinding processes. The chemicals used included:

- distilled water,
- 1% HCl solution.
- 5% HCl solution.
- 7.5% Na₂CO₃ solution.
- 0.1 M CaCl₂ solution.
- 96% ethanol.
- 96% isopropanol alcohol.
- filter paper.

The equipment employed in the study included a blender, digital balance, beakers, Erlenmeyer flasks, glass rods, funnels, porcelain dishes, a hotplate stirrer, and graduated pipettes. These tools were used throughout the various stages of the research to facilitate optimal alginate extraction.

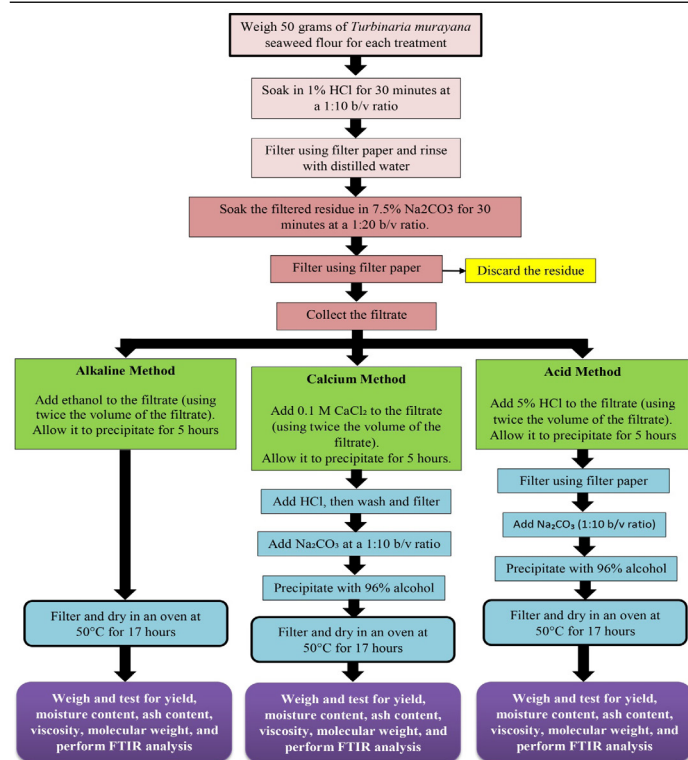


Figure 1: Procedure for alginate extraction using different methods (Modified from Peteiro, 2017).

EXPERIMENTAL DESIGN

This study employed an experimental method using a Completely Randomized Design (CRD) consisting of three treatments, in which each treatment was replicated six times. The treatments applied were three alginate extraction methods from *T. murayana* seaweed: the acid, alkaline, and calcium methods. The data obtained were analyzed using analysis of variance (ANOVA) with SPSS version 25 software. If there is the effect of treatments, the differences among treatments were detected using the Duncan's Multiple Range Test (DMRT) according to the procedure described by Steel and Torrie (1991).

MEASURED VARIABLES

The measured variables in this study included alginate yield, moisture content, ash content, viscosity, molecular weight, and functional group analysis of alginate. Alginate yield was calculated by comparing the obtained alginate's weight with the raw material's initial weight, then multiplying by 100% (Subaryono *et al.*, 2017). Moisture and ash content were analyzed using the proximate analysis method following the standards established by AOAC (1990). Alginate viscosity was measured by preparing a 1% (w/v) alginate solution in distilled water. The measurement was conducted using a Rapid Visco Analyzer (RVA) with a controlled temperature. The temperature was set at 20°C and a rotation speed of 130 rpm, with results expressed in centipoise (Husni *et al.*, 2012). The average molecular weight of alginate was calculated using the Mark-Houwink equation

($[\eta] = KMv^a$), with constants $K = 0,023 \text{ mL/g}$ and $a = 0.984$ for sodium alginate dissolved in distilled water. In this equation, $[\eta]$ represents intrinsic viscosity (mL/g), and Mv is the viscosity-average molecular weight (g/mol) (Lee and Mooney, 2012; Dodero *et al.*, 2019). Functional group analysis of alginate was performed using Fourier Transform Infrared (FTIR) spectroscopy, following the method described by Herdianto and Husni (2019).

STATISTICAL ANALYSIS

All data were analyzed using analysis of variance (ANOVA) in SPSS version 25. If the treatment effect were detected, the differences among treatment means were further analyzed using the Duncan Multiple Range Test method (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

The results of alginate extraction from *T. murayana* seaweed using different extraction methods are presented in Table 1.

Table 1: Mean values of alginate yield, moisture content, ash content, viscosity, and molecular weight obtained using different extraction methods (acid, alkaline, and calcium methods).

Alginate Extraction Methods	Alginate Yield (%)	Moisture Content (%)	Ash Content (%)	Viscosity (cPs)	Molecular Weight (g/mol)
Acid Method	26.93 ^c	13.44 ^a	23.74 ^c	15.75 ^a	76,786.97 ^a
Alkaline Method	37.86 ^b	14.02 ^a	37.63 ^b	7.32 ^b	32,446.55 ^b
Calcium Method	48.18 ^a	5.72 ^b	48.65 ^a	7.34 ^b	32,559.62 ^b
SEM	0.44	0,32	0.83	0.20	0.41

SEM: Standard Error of the Mean; ^{a,b,c} Means with different superscripts at the same column indicate highly significant differences ($p < 0.01$).

The functional group analysis of alginate extracted from *T. murayana* using Fourier Transform Infrared (FTIR) spectroscopy is presented in Figures 2, 3, and 4.

The results of the study showed that alginate extraction from *T. murayana* seaweed using different extraction methods had a highly significant effect ($P < 0.01$) on alginate yield, moisture content, ash content, viscosity, and molecular weight. Alginate yield as well as ash content of *T. murayana* seaweed from the calcium method is the highest compared to acid and alkaline methods. Meanwhile, the moisture content is the lowest at this calcium method. The viscosity and molecular weight from calcium method are not different from alkaline method, but they are different

from acid method. These viscosity and molecular weight of *T. murayana* from the acid method are the highest.

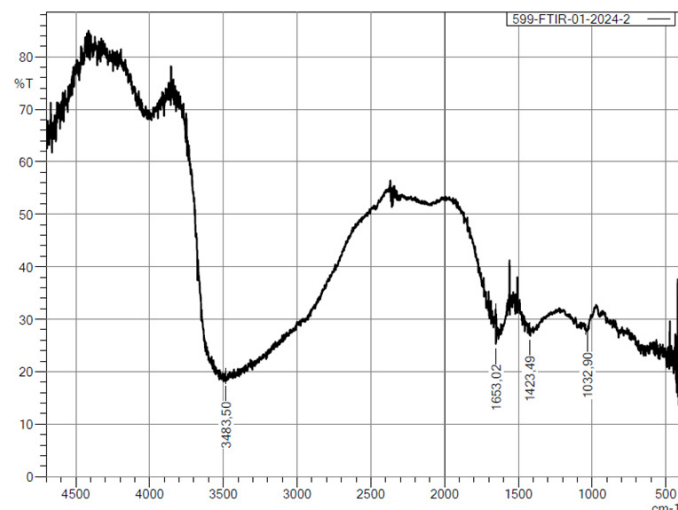


Figure 2: FTIR spectrum of alginate extracted using the acid method.

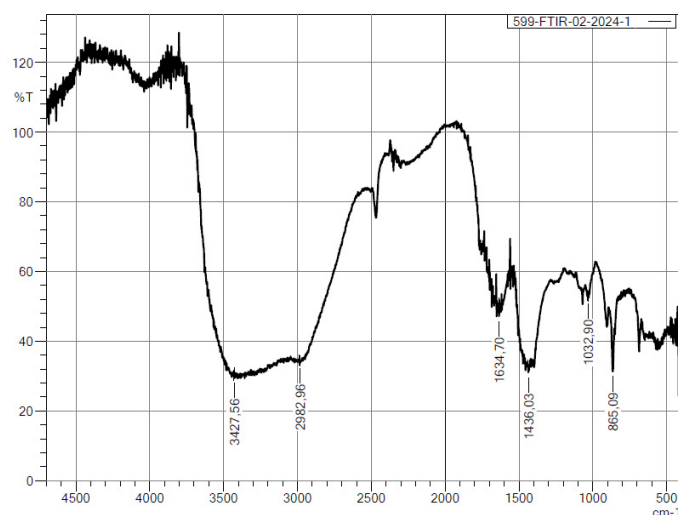


Figure 3: FTIR spectrum of alginate extracted using the alkaline method.

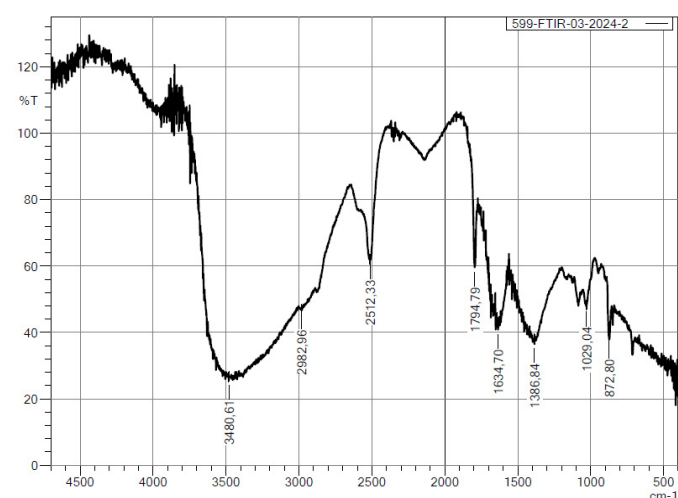


Figure 4: FTIR spectrum of alginate extracted using the calcium method.

ALGINATE YIELD

The mean alginate yields obtained were 26.93% (acid method), 37.86% (alkaline method), and 48.18% (calcium method). The highest alginate yield was produced using the calcium method, followed by the alkaline and acid methods. The high yield obtained with the calcium method is attributed to the ionic bonding of Ca^+ ions with the alginate filtrate during the precipitation process using CaCl_2 , as alginate readily binds with Ca ions. According to Maharani *et al.* (2017), alginate extraction using the calcium method produces a higher yield than the acid method due to the ionic bonding of Ca^{2+} ions from the CaCl_2 solution during the precipitation process. This is also supported by Husni *et al.* (2012), who stated that alginate yield using the calcium method is higher than that of the acid pathway. Latifi *et al.* (2015) further reported that the calcium pathway method yields higher alginate, 25–30%, compared to the acid method, which ranges from 12–16%. The alginate yield using the alkaline method (37.86%) in this study was higher than the 33% reported by Peteiro (2017). The alginate yield obtained using the acid extraction method in this study (26.93%) is comparable to the alginate yield from *Macrocystis pyrifera* as reported by Peteiro (2017), which was 27%, and higher than the alginate yield extracted from *Sargassum sp.* as reported by Latifi *et al.* (2015), which ranged from 12% to 16%. The alginate yield obtained from all three methods meets the standards for alginate as specified by the Food Chemical Codex (FCC, 2004), which requires yields more significant than 20%. Therefore, all three methods effectively extract alginate from *T. murayana* seaweed.

MOISTURE CONTENT

The mean moisture content of alginate obtained using different extraction methods in this study was 13.44% (acid method), 14.02% (alkaline method), and 5.72% (calcium method). The moisture content of alginate in this study meets the standard set by the Food Chemical Codex (FCC, 2004), which requires a moisture content of <15%. The moisture content of alginate extracted using the acid and alkaline methods showed no significant difference. However, the moisture content of alginate extracted using the calcium method was lower than that obtained using the acid and alkaline methods. This difference is due to the varying responses of alginate to precipitation processes using different solutions (acid, alkaline, and calcium). According to Pasaribu *et al.* (2020), the alginate moisture content is influenced by the soaking and precipitation processes as well as the type of solution used. Additionally, the moisture content of alginate is affected by the method and duration of the drying or sun-drying process (Latifi *et al.*, 2015). The moisture content of alginate in this study is comparable to that reported by Maharani *et al.* (2017), which ranged from 9.35 to 11.35% and is lower than the values reported by Addina *et al.* (2020).

The mean ash content of alginate obtained using different extraction methods in this study was 23.74% (acid method), 37.63% (alkaline method), and 48.65% (calcium method). The highest ash content was produced using the calcium method, followed by the alkaline and acid methods. The ash content of alginate extracted using the calcium and alkaline methods was higher than the quality standard for alginate set by the Food Chemical Codex (FCC, 2004), which is 18–27%. The high ash content in alginate produced using the calcium and alkaline methods is attributed to Ca and Na minerals bound to the alginate during precipitation. The calcium method uses a CaCl_2 solution, while the alkaline method uses Na_2CO_3 and ethanol for precipitation. Alginate readily binds with Ca minerals, which facilitates precipitation, whereas Na minerals tend to remain soluble, leading to higher ash content in alginate obtained using the calcium and alkaline methods. According to Herdianto and Husni (2019), the ash content of alginate is influenced by the mineral composition of the seaweed and the extraction solution used. Addina *et al.* (2020) further noted that the ash content of alginate is affected by the type of raw material, extraction process, and impurities remaining in the alginate. The ash content of alginate extracted using the acid method (23.74%) met the quality standard for alginate set by the Food Chemical Codex (FCC, 2004), which is 18–27%. This is due to the use of 1% HCl for initial soaking and 5% HCl for alginate precipitation in the acid method, which reduces the minerals in the seaweed during extraction and precipitation, as HCl can dissolve minerals present in alginate. Maharani *et al.* (2017) reported that alginate extraction using 1% and 5% HCl can reduce ash content by up to 6%. Factors contributing to reducing ash content include the washing process and adding 1% HCl during alginate precipitation (Pasaribu *et al.*, 2020). Alginate produced using the acid extraction method was superior to the calcium and alkaline methods and met alginate quality standards. The ash content of alginate obtained using the acid method in this study was consistent with the findings of Kamisyah *et al.* (2020), which reported an ash content of 20–30%, and Pasaribu *et al.* (2020), which reported 20.83–26.83%.

VISCOSITY

The mean viscosity of alginate obtained using different extraction methods in this study was 15.75 cPs (acid method), 7.32 cPs (alkaline method), and 7.34 cPs (calcium method). The acid method observed the highest viscosity, followed by the calcium and alkaline methods. The viscosity of alginate extracted using the acid method was consistent with the values reported by Kamisyah *et al.* (2020), which ranged from 8.74–8.91 cPs, and Pasaribu *et al.* (2020), which reported values of 8.23–11.42 cPs. The higher viscosity observed with the acid method compared to the calcium and alkaline methods is due to the absence of calcium alginate

formation using CaCl_2 , preventing excessive alginate chain fragmentation (Maharani *et al.*, 2017). In contrast, precipitation using the alkaline method with ethanol is less efficient, as ethanol has a very high polarity, making it less effective at binding hydroxyl groups. This results in the alginate solution exhibiting properties closer to highly polar water (Pasaribu *et al.*, 2020). The viscosity of alginate produced using the acid extraction method (15.75 cPs) meets the alginate quality standards for low viscosity alginate as specified by Doderio *et al.* (2019), which is 4–12 cPs and medium viscosity 2000 cPs.

MOLECULAR WEIGHT

The mean molecular weight of alginate obtained using different extraction methods in this study was 76,786.97 g/mol (acid method), 32,446.55 g/mol (alkaline method), and 32,559.62 g/mol (calcium method). These results indicate a highly significant difference in molecular weight between alginate extracted using the acid method and those extracted using the alkaline and calcium methods. The higher molecular weight of alginate in the acid method is also influenced by its higher viscosity compared to the alkaline and calcium methods. Viscosity is closely related to molecular weight; higher viscosity corresponds to a higher molecular weight of alginate (Subaryono, 2010). The molecular weight of alginate produced in this study meets the molecular weight standards established by Lee and Mooney (2012), which range from 32,000–400,000 g/mol, and Subaryono (2010), which range from 32,000–200,000 g/mol.

Table 2: Wavenumber values of functional groups in alginate extracted using different methods.

Alginate Extracted Using the Acid Method cm^{-1}	Alginate Extracted Using the Alkaline Method cm^{-1}	Alginate Extracted Using the Calcium Method cm^{-1}	Reference Wavenumber cm^{-1}	Interpretation of Functional Groups cm^{-1}
3483.50	3427.56	3480.61	3500 ^a	Hydroxyl Group O-H
1653.02	1634.70	1634.70	1620 ^a	Asymmetric COO^-
1032.90	1032.90	1029.04	1023.4 ^b	Carboxyl Group IC-O
1423.49	1436.03	1386.84	1410 ^a	Symmetric COO^-

Note: ^aJu *et al.* (2002); ^bSergios *et al.* (2010).

FUNCTIONAL GROUP ANALYSIS OF ALGINATE (FTIR)

The functional group analysis of alginate extracted using different methods—acid, alkaline, and calcium—was conducted using FTIR (as shown in Figures 2, 3, and 4), and the results are presented in Table 2. The compounds found in the alginate extracted in this study include hydroxyl groups (O-H), asymmetric COO^- groups, symmetric COO^- groups, and carboxyl groups (C-O). This is consistent with Ju *et al.* (2002), who stated that alginate compounds have three specific peaks: hydroxyl bonds at absorption regions around 3500 cm^{-1} , asymmetric COO^- at around 1620

cm⁻¹, and symmetric COO⁻ at around 1410 cm⁻¹. Yulianto (2007) also reported that the wavenumber range for hydroxyl groups (O-H) is between 3200–3500 cm⁻¹, and for carbonyl groups (C=O) is between 1600–1680 cm⁻¹. The hydroxyl group in alginate extracted using the acid method appeared at an absorption region of 3483.50 cm⁻¹, using the alkaline method at 3427.56 cm⁻¹, and using the calcium method at 3480.61 cm⁻¹. These results align with Yulianto (2007), who stated that the absorption region for hydroxyl groups is between 3200–3500 cm⁻¹, and with Maharani *et al.* (2017), who reported a range of 3425.58–3456.44 cm⁻¹. The asymmetric COO⁻ group in alginate extracted using the acid method appeared at 1653.02 cm⁻¹, using the alkaline method at 1634.70 cm⁻¹, and using the calcium method at 1634.70 cm⁻¹. These results are consistent with Ju *et al.* (2002), who reported 1620 cm⁻¹; Yulianto (2007), who reported 1600–1680 cm⁻¹; and Herdianto and Husni (2019), who reported 1623.74–1629.79 cm⁻¹. The symmetric COO⁻ group in alginate extracted using the acid method appeared at 1423.49 cm⁻¹, the alkaline method at 1436.03 cm⁻¹, and the calcium method at 1386.84 cm⁻¹. These results closely align with Maharani *et al.* (2017), who reported 1411.89–1419.61 cm⁻¹, and Herdianto and Husni (2019), who reported 1420.33–1488.11 cm⁻¹. The carboxyl group (C-O) in alginate extracted using the acid method appeared at 1032.90 cm⁻¹, the alkaline method at 1032.90 cm⁻¹, and the calcium method at 1029.94 cm⁻¹. These results are consistent with Maharani *et al.* (2017), who reported 1023.40–1033.85 cm⁻¹, and Herdianto and Husni (2019), who reported 1031.88–1093.70 cm⁻¹. Alginate extracted using different methods (acid, alkaline, and calcium) met the quality standards for alginate-based on functional group analysis. The best results were obtained from alginate extracted using the acid method, as its wavenumbers closely matched the reference standards for alginate compounds.

Based on the results of this experiment, the acid extraction method produced alginate with yield, moisture content, ash content, viscosity, and molecular weight that met the quality standards set by the FCC (2004), which include an alginate yield of >20%, moisture content <15%, ash content 18–27%, viscosity 4–2000 cPs, and molecular weight 32,000–400,000 g/mol. Although the alkaline and calcium methods resulted in higher yields than the acid method, the ash content of the alginate obtained from these methods did not meet the quality standards established by the FCC (2004).

CONCLUSIONS AND RECOMMENDATIONS

The alginate extraction method from *Turbinaria murayana* seaweed using the acid pathway was the most effective method for producing alginate that meets quality stand-

ards. The alginate produced using the acid method had a yield of 26.93%, moisture content of 13.44%, ash content of 23.74%, viscosity of 15.75 cPs, molecular weight of 76,786.97 g/mol, and functional group analysis using FTIR that aligned with the standard alginate compound. These values comply with the quality standards for alginate as specified by the Food Chemical Codex in which alginate >20%, moisture content <15%, ash content 18–27%, viscosity 4–12 cPs and molecular weight 32,000–400,000 g/mol (FCC, 2004).

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NOVELTY STATEMENT

This study highlights the novelty of utilizing *Turbinaria murayana*, an underutilized brown seaweed species, as a sustainable source of high-quality alginate for poultry feed additives. Among the evaluated extraction methods acid, alkaline, and calcium, the acid pathway proved the most effective, producing alginate with superior yield, viscosity, molecular weight, and functional group characteristics as confirmed by FTIR analysis, all meeting the standards set by the Food Chemical Codex (FCC, 2004). This research not only introduces *Turbinaria murayana* as a new raw material for alginate production but also supports its potential as a natural alternative to antibiotics in poultry feed, addressing global concerns over antibiotic resistance and environmental sustainability.

AUTHOR'S CONTRIBUTIONS

All authors listed in this manuscript have contributed to this article's research and preparation. Sepri Reski, Maria Endo Mahata, Ahadiyah Yuniza, and Yose Rizal collectively participated in the preparation and conceptualization of the research, as well as data collection and processing. All authors have agreed to submit this manuscript to the Advances in Animal and Veterinary Sciences journal.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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