International Journal of Design & Nature and Ecodynamics
Vol. 21, No. 7, July, 2026, pp. 1971-1978
Journal homepage: http://iieta.org/journals/ijdne

Surface Morphology and Elemental Modification of Eggshell Flour Treated with Water and Fermentation

Raudhatu Shofiah[1*], Riska Dian Oktari[1], Amsar Maulana[2], Oksana Oksana[1]

[1] Department of Agrotechnology, Agriculture and Animal Science Faculty, Universitas Islam Negeri Sultan Syarif Kasim Riau, Pekanbaru City 28293, Indonesia
[2] Research Group of Food Safety, Research Center for Food Technology and Processing, National Research and Innovation Agency, Yogyakarta 55861, Indonesia

Corresponding Author Email: raudhatu@uin-suska.ac.id

https://doi.org/10.18280/ijdne.210711

Received: 12 April 2026
Revised: 14 June 2026
Accepted: 22 June 2026
Available online: 31 July 2026

Keywords:
ameliorant, calcium, eggshell, scanning electron microscopy-energy-dispersive X-ray, surface morphology, surface elemental

ABSTRACT

Eggshell waste consists of several elements potentially utilized as an ameliorant. Thus, environmentally friendly surface morphology and surface elements are required to increase its functional value, which was studied microscopically using scanning electron microscopy-energy-dispersive X-ray (SEM-EDX). This study has examined the chemical characteristics of surface morphology and elemental modification on eggshell flour (EF) with H_2O and fermentation using SEM-EDX. This study has used a quantitative descriptive approach in a completely randomized design (CRD) with three replications and two treatments (EF surface morphology and elemental modification process), namely H_2O and fermentation. The process of modifying EF with water and fermentation showed a significant effect on proximate properties (moisture, volatile matter (VM), and ash), pH, electrical conductivity (EC), and inorganic C (IOC). The modified eggshell flour (MEF)-fermentation process showed an increase in pH H_2O (8.40) and EC (0.86 dS m^{-1}), as well as higher cation exchange capacity (CEC) [31.60 cmol(+) kg^{-1}] and IOC (15.66%). The modification process through fermentation produced a denser surface structure with an irregular texture and a higher proportion of C. The MEF through fermentation increased the active surface elemental, nutrient availability, and adsorption capacity.

1. INTRODUCTION

Eggshell waste is a type of organic waste that is quite large, especially from household activities, food industries, and restaurants. More than 82 million tons of eggs are produced each year [1]. It is estimated that about 11-12% of the egg weight is shell, resulting in 9-10 million tons of eggshell waste per year worldwide [2, 3]. In Indonesia, broiler egg production in 2023 reached 6 million tons per year [4]. If it is assumed that 10% is shell, then about 600,000 tons of eggshell waste is generated each year [5]. Although eggshells have only been considered as ordinary kitchen waste, the high $CaCO_3$ in them provides great potential for reuse. Most eggshells are still dumped into the environment without treatment, which can lead to sanitation problems and environmental pollution.

The utilization of eggshells as an alternative material, specifically for ameliorants, has the potential to be developed. Eggshells also contain approximately 94% $CaCO_3$, 1% $CaPO_4$, 1% $MgCO_3$, and 4% organic matter [6]. The $CaCO_3$ can modify soil pH and reduce the amount of Al-exchange in the soil so that pH and availability of P increase in the soil (Reactions 1 and 2) [7].

$$CaCO_3(s) + H_2O(l) \rightleftharpoons Ca^{2+}(aq) + HCO_3^-(aq) + OH^- \quad (1)$$

$$Al^{3+} \text{ [soluble]} + 3OH - IAl(OH)_3 \text{ [insoluble]} \quad (2)$$

The 5,328 tons of $CaCO_3$ and 13.53 tons of $MgCO_3$ have not been optimally utilized. However, the main challenge of using eggshells in flour form is their low active surface area. The specific surface area (SSA) of untreated eggshells ranges from 1.0 to 3.5 m^2/g [8]. The small surface area limits the ability of this material to interact with other compounds, making its effectiveness as an ameliorant less than optimal. So, to increase the surface elemental and surface activity, various modification techniques have been developed, both physically, chemically, and biologically. The eggshell structure consists of a dense and compact layer of calcite crystals and an organic membrane that is only slightly porous [9, 10]. One approach that is environmentally friendly and economical is through treatment using H_2O and biological fermentation.

Water plays a role in softening the structure and removing contaminants, while microbial fermentation produces compounds such as lactic acid that can dissolve organic fractions and significantly alter the microstructure of the eggshell. Fermentation by microorganisms such as lactic acid bacteria has the advantage of being able to break down proteins and other organic compounds that are still attached to the eggshell surface [11-13]. This process helps in the formation of microscopic pores that increase the number of surface elements. The combination of fermentation and water washing is expected to produce materials with much better characteristics for functional applications, without the use of harmful chemicals or expensive thermal methods. Changes in structure and elemental composition that occur due to the modification process require instrument-based analysis such as scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX). The SEM allows observation of changes in surface morphology down to the micron scale, while EDX can provide quantitative data on the dominant elements on the surface. The combination of these two methods is an appropriate analytical approach to fully understand the effects of modification. This study has examined the chemical characteristics of surface morphology and elemental modification on eggshell flour (EF) with H_2O and fermentation by SEM-EDX.

2. MATERIALS AND METHODS

This research has been carried out at the Laboratory of Pathology, Entomology, Microbiology, and Soil Science (PEMTA), Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau, and at the Central Laboratory of Andalas University from April to May 2025.

2.1 Experimental design

This study has used a quantitative descriptive approach in a completely randomized design (CRD) with three replications and two treatments (EF structure surface morphology and elemental modification process), namely H_2O and fermentation.

2.2 Materials

2.2.1 Eggshells

The eggshells that have been used are broiler eggshell waste from restaurants and bakeries. The waste was washed thoroughly with water and dried in direct sunlight until the inner layer was dry for 2 × 24 hours. The dried waste was crushed using a mortar, then sieved using a 100-mesh sieve (0.149 mm or 149 microns or 0.0059 inches). The EF that has passed the sieve is then subjected to a surface morphological and elemental modification process with H_2O and fermentation.

2.2.2 Surface morphology and elemental modification

Surface morphology and elemental modification on EF have been carried out with two processes, namely (1) modification using H_2O at 100 ℃; 50 g of EF is added with H_2O in a ratio of 1:2 and shaken for 1 hour at 300 rpm in a water bath. Then, the solids were separated from the suspension using Whatman filter paper number 40 and continued with the drying process in an oven at 105 ℃. Meanwhile, (2) for microbial modification (fermentation), 50 g of eggshell powder was mixed with 50 mL of effective microorganisms (EM4) solution and 50 g of dissolved brown sugar (1:1:1, w/v/w). The EM4 inoculum consisted of a consortium of beneficial microorganisms, primarily *Lactobacillus* spp., photosynthetic bacteria (*Rhodopseudomonas* spp.), yeasts (*Saccharomyces* spp.), actinomycetes, and fermentative fungi, as specified by the manufacturer. Distilled water was added to adjust the moisture content to approximately 60%. The initial pH of the fermentation mixture was 6.5 ± 0.2. The mixture was thoroughly homogenized, transferred to an airtight polypropylene fermentation vessel, and sealed to maintain predominantly anaerobic conditions and minimize gas exchange with the atmosphere. Fermentation was carried out at room temperature (28 ± 2 ℃) for 21 days. Fermentation progress was monitored periodically by measuring the pH, which gradually decreased to 3.8–4.2 due to microbial activity. Fermentation was considered complete when the pH remained stable for three consecutive days, no further gas production was observed, and the material exhibited a characteristic sweet-sour fermentation aroma, with no signs of contamination or mold growth. After fermentation, the solid fraction was separated from the suspension using Whatman No. 40 filter paper and washed with distilled water to remove any remaining dissolved components. The resulting solid was then dried in an oven at 60 ℃ to constant weight to maintain its physicochemical properties, then ground to pass through a fine sieve and stored in airtight polyethylene bags before characterization.

2.3 Modified eggshell flour and statistical analysis

Modified eggshell flour (MEF) analysis includes proximate, pH, electrical conductivity (EC), cation exchange capacity (CEC), liming potential, and inorganic C (IOC). Proximate analysis, including moisture, ash, volatile matter (VM), and fixed carbon content, was performed following AOAC standard procedures. pH and EC were measured in a 1:10 (w/v) suspension of EF and deionized water using a calibrated pH meter and conductivity meter after equilibrium was reached. The CEC was determined using the 1 M ammonium acetate saturation method (NH_4OAc, pH 7.0). Liming potential was evaluated as calcium carbonate equivalents (CCE) using a standard acid neutralization and back titration method. IOC was determined by acid digestion and quantification of carbon derived from carbonate using a total carbon analyzer or equivalent standard analytical procedure [14]. Surface morphology analysis was performed using a Carl Zeiss EVO 10 SEM equipped with an EDX system. Observations were made at accelerating voltages of 10-20 kV, with magnifications between 1000× and 10,000×. The elemental composition of the surface was analyzed qualitatively and semi-quantitatively using an Oxford Instruments AZtec system-based EDX detector.

All analyses were statistically analyzed using Excel 2023 and SPSS 23 software. The statistical analysis used was analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). After detecting significant effects with ANOVA, DMRT was used as a post hoc test to identify specific group means that differed from each other. Principal Component Analysis (PCA) was used to visualize the overall clustering pattern and to assess modification-dependent separation among samples. Model validation was performed using permutation testing (n = 3), which confirmed the statistical significance of the model ($p < 0.001$). The significance level was determined as follows: if F count > F table at the 5% level, the result is significant, and if F count > F table at the 1% level, the result is highly significant.

3. RESULTS AND DISCUSSION

The modification process showed a significant effect on the proximate (moisture, VM, and ash) composition of EF (Figure 1). Proximate analysis of MEF through modification with water (MEF-water) and fermentation (MEF-fermentation) showed significant differences in moisture, VM, and ash content. The moisture of MEF-water was higher (9.00%) than that of MEF-fermentation (3.50%), indicating that fermentation can reduce the moisture content in the material. This reduction in moisture content is important because materials with low moisture content tend to be more stable and have better shelf life [15].

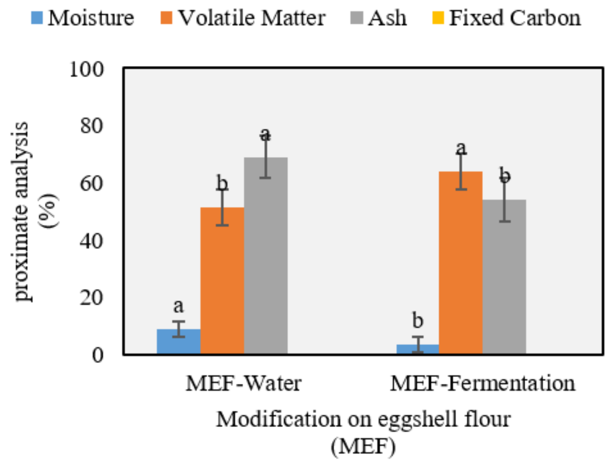

Figure 1. Proximate analysis of modified eggshell flour (MEF)

The VM in MEF-fermentation (63.93%) was much higher than that in MEF-water (51.52%). The fermentation process increases the content of volatile organic components, which are likely derived from the degradation of proteins and lipids in eggshells by microorganisms [16]. High VM content reflects the material's potential as an energy source or bioactive ingredient after fermentation. Meanwhile, ash content decreased in MEF-fermentation (54%) compared to MEF-water (69%). Ash represents inorganic mineral content, mainly calcium carbonate ($CaCO_3$) in eggshells [17]. This decrease may indicate that some minerals may dissolve or undergo chemical transformation during fermentation, which can affect the availability of nutrients in certain applications, such as feed additives or organic fertilizers. Fixed carbon was not found in either modification, indicating that eggshell material does not store carbon in a stable solid form as in lignocellulosic biomass. This is consistent with the main properties of eggshells, which are mostly composed of calcium carbonate (about 94%), with only a small organic fraction [18]. Thus, the solid carbon component is not significant in EF. Modification by fermentation is more effective in reducing moisture content and increasing VM content, although there is a slight decrease in mineral content. This may have implications for the use of fermented EF as a bioactive ingredient or organic fertilizer that is more easily degradable than water-modified products.

The modification process showed a significant effect on the pH of EF (Figure 2). The pH of MEF showed differences in active pH (H_2O), potential pH (KCl 1M), and point of zero charge (PZC) between MEF-water and MEF-fermentation. The pH H_2O of MEF-water is 7.63, while MEF-fermentation is higher at 8.40. Fermentation can increase the alkalinity of the material, possibly due to microbial activity that produces alkaline compounds such as ammonia during the degradation process [19]. The pH KCl indicates the tendency for pH changes when the material interacts with the environment. The MEF-water has a higher potential pH (9.40) than MEF-fermentation (8.57). This difference indicates that MEF-water is more likely to act as an alkalizing agent when applied, while MEF-fermentation is more stable at near-neutral pH. This condition is particularly important in agricultural applications, where materials with near-neutral to alkaline pH can improve soil acidity [20].

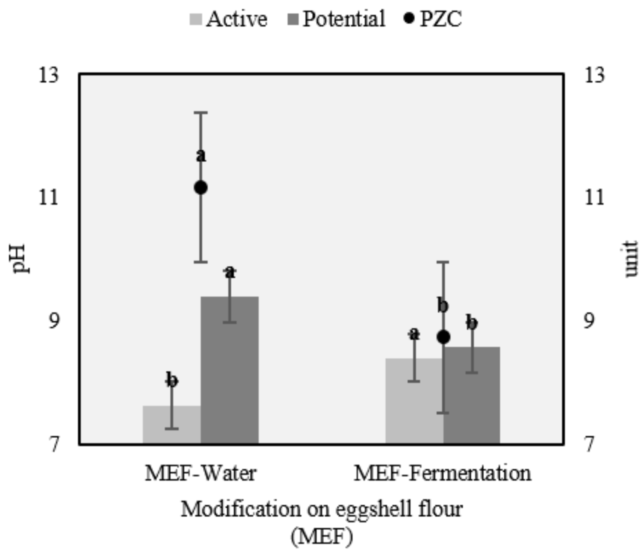

Figure 2. The pH of modified eggshell flour (MEF)

At the PZC, MEF-water has a higher value (11.17) than MEF-fermentation (8.73). The PZC describes the surface charge equilibrium point; the higher the value, the greater the tendency for the material to be positively charged at the natural pH of the environment (pH active). MEF-water tends to be more positively charged than MEF-fermentation, which has implications for its ability to adsorb anions [21, 22]. The differences in active pH, potential pH, and PZC between the two indicate that the fermentation process is capable of modifying the chemical properties of eggshells. Fermentation reduces PZC and potential pH, so that the surface of the material is more likely to be negatively charged under normal environmental conditions (pH < 8.7). This can increase the potential of MEF-fermentation as an adsorbent for heavy metal cations or positively charged ions. The MEF-water produces eggshell powder with more alkaline and positively charged characteristics, while fermentation produces material with higher active pH but lower PZC (pH active > pH PZC), making it more suitable for use as a neutralizing agent or adsorbent in environmental systems.

EC in MEF showed significant differences between MEF-water and MEF-fermentation (Figure 3). The MEF-water had a very low EC value of 0.17 dS m^{-1}, while MEF-fermentation was much higher at 0.86 dS m^{-1}. Fermentation increases the number of dissolved ions in the system, which has an impact on increasing the material's ability to conduct electricity [23]. The EC is closely related to the content of soluble minerals, such as calcium, magnesium, and other inorganic ions. In MEF-water, most minerals may still be in the form of calcium carbonate ($CaCO_3$), which is difficult to dissolve, resulting in low conductivity. Conversely, in MEF-fermentation, microbial activity and biochemical processes can dissolve some mineral compounds into ionic forms, such as Ca^{2+}, which contributes to an increase in EC [24]. The increase in EC value in MEF-fermentation has important implications for environmental and agricultural applications. Materials with higher EC can serve as a source of readily available calcium ions for plants. Modifying eggshell waste can improve soil fertility, especially in acidic soils, by increasing the availability of base elements. However, high EC also needs to be considered in the context of the application. Excessively high EC values can cause potential salinity in the soil, although in this case, a value of 0.86 dS m^{-1} is still considered low and safe for most plants. Therefore, MEF-fermentation can be considered as a soil amendment material with relatively low salinity risk, but with greater benefits in increasing nutrient ion availability compared to MEF-water. Fermentation is a more effective method for increasing the EC of eggshell powder through increased solute availability. This reinforces that the bioconversion process is capable of modifying the chemical properties of organic-inorganic waste such as eggshells into materials that are more reactive and useful in agriculture and environmental processing.

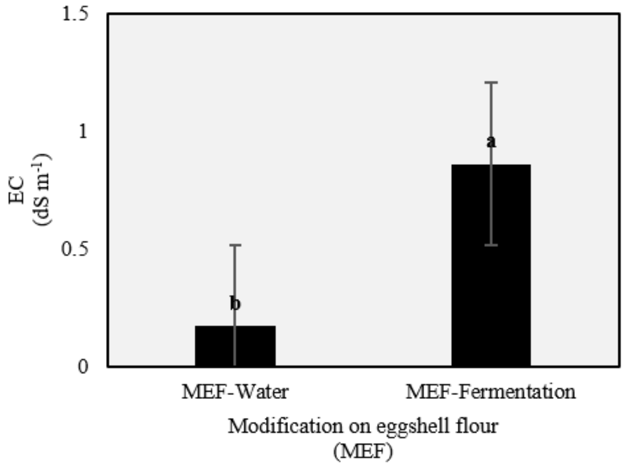

Figure 3. Electrical conductivity (EC) of modified eggshell flour (MEF)

The modification process showed a non-significant effect on the CEC of EF (Figure 4). The CEC value in MEF-water was 20.80 cmol(+) kg^{-1}, while MEF-fermentation reached 31.60 cmol(+) kg^{-1}. Fermentation can improve the ability of EF to retain and exchange cations, which is very important in the context of its application as a soil ameliorant or environmental adsorbent [25]. The higher CEC in MEF-fermentation may be due to changes in the chemical and physical structure of the material due to microbial activity during fermentation. This process can produce active organic compounds and open pores in the eggshell matrix, thereby increasing the surface area and multiplying the cation exchange sites [26]. This makes MEF-fermentation more effective in absorbing and releasing calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}) ions. High CEC is also closely related to increased nutrient retention capacity in soil. The application of MEF-fermentation to soil has the potential to improve fertility by increasing the availability of base nutrients and reducing the leaching of important ions. Eggshell waste can be used as a soil ameliorant to improve the chemical properties of acidic soils by increasing CEC and base saturation [27, 28]. High CEC is relevant to the adsorption of pollutants. Fermented MEF, which has a CEC that tends to be higher after fermentation but is not statistically different, is potentially more effective in adsorbing heavy metals such as Pb^{2+}, Cd^{2+}, and Cu^{2+} from wastewater, due to the presence of more ion exchange sites on the material surface. Eggshell-based materials have great potential in environmental remediation through ion exchange mechanisms [29]. Fermentation is a superior modification method compared to water treatment in increasing the CEC of EF. With a higher CEC, MEF-fermentation can be more beneficial both in agriculture as a soil conditioner and in waste as a biosorbent to reduce environmental pollution.

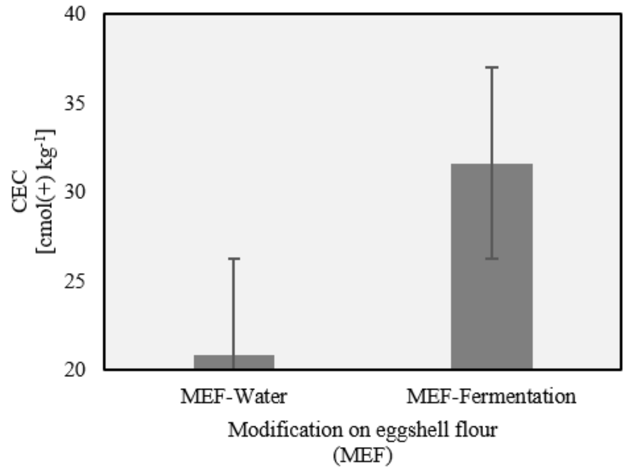

Figure 4. The cation exchange capacity (CEC) on the modification of eggshell flour

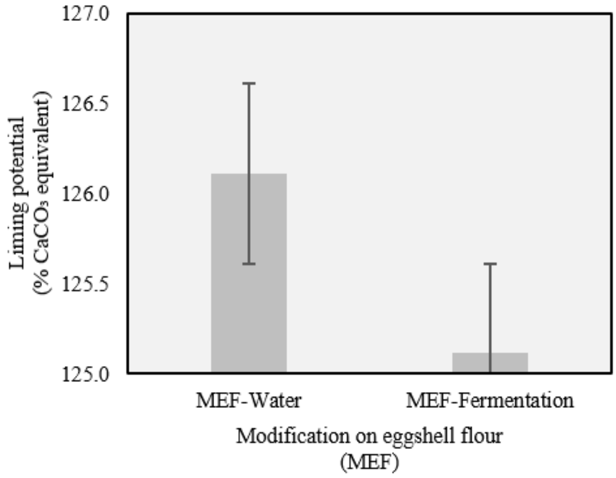

Figure 5. Liming potential for modified eggshell flour (MEF)

The modification process showed a non-significant effect on the liming potential of EF (Figure 5). The liming potential of MEF shows that the MEF-water value is 126.11% $CaCO_3$ equivalent, and the MEF-fermentation value is 125.11% $CaCO_3$ equivalent. This difference is relatively small, indicating that neither water treatment nor fermentation significantly affects the material's capacity to neutralize acidity. This high liming potential is consistent with the basic characteristics of eggshells, which are dominated by calcium carbonate ($CaCO_3$) content of approximately 94–97% [30]. The almost identical liming potential values between the two treatments indicate that the fermentation process does not significantly reduce the calcium carbonate content. Although fermentation can modify chemical and physical properties, the main component, $CaCO_3$, remains stable and retains its acid neutralization capacity. Eggshells have a high $CaCO_3$ equivalent lime value, making them a viable alternative to agricultural lime [31]. Liming potential is important for agricultural applications, especially on acidic soils. A liming potential value >100 indicates that eggshell powder can increase soil pH, reduce Al^{3+} saturation, and increase the availability of base nutrients (Ca^{2+}, Mg^{2+}, K^{+}). Thus, both MEF-water and MEF-fermentation have great benefits as acid soil ameliorants. Although the values are almost the same, a slight difference in liming potential (MEF-water is slightly higher) can be attributed to the possible dissolution of certain minerals during fermentation. The bioconversion process can dissolve a small portion of calcium into a more readily available ionic form, but slightly reduces the total $CaCO_3$ content in the material. However, a difference of 1.00% $CaCO_3$ is still very small and has no significant impact on the liming function. Both water modification and fermentation produce eggshell powder with high and relatively similar liming capabilities. This reinforces the use of eggshell waste as an environmentally friendly and economical source of natural lime, making it a viable alternative to conventional agricultural lime.

IOC in MEF showed significant differences between MEF-water and MEF-fermentation (Figure 6). The IOC in MEF showed a striking difference between MEF-water and MEF-fermentation. The IOC in MEF-water was recorded at 5.10%, while in MEF-fermentation it was much higher at 15.66%. The fermentation process significantly increases the IOC content, which mainly comes from calcium carbonate ($CaCO_3$) compounds, the main component of eggshells [32, 33]. The increase in IOC in MEF fermentation can be explained by the bioconversion process involving microbial activity. During fermentation, microorganisms can release enzymes and metabolites that accelerate the decomposition of organic matter, thereby clarifying the dominance of the inorganic fraction in the material. The total $CaCO_3$ is relatively stable, and the contribution of measurable IOC is higher than that of plain water [34].

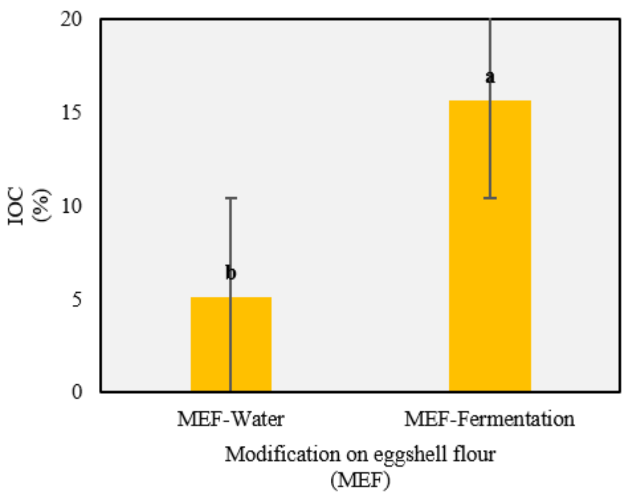

Figure 6. The inorganic C (IOC) in the modified eggshell flour (MEF)

The high IOC in MEF-Fermentation has important implications for its liming effect potential. $CaCO_3$, as the main source of IOC, plays a role in neutralizing soil acidity through the neutralization of H^{+} ions. Materials with high carbonate content are very effective in improving acidic soils, reducing Al^{3+} solubility, and increasing the availability of base nutrients [35]. In addition to its benefits in agriculture, the high IOC content also supports the use of MEF-fermentation in environmental remediation. The $CaCO_3$-based materials can be used to bind heavy metal ions through carbonate precipitation, thereby reducing metal toxicity in wastewater [36]. Thus, MEF-fermentation has greater added value than MEF-water in pollutant adsorption applications. Fermentation increases the IOC content of eggshell powder, which has a positive impact on its function as a soil ameliorant and biosorbent. Compared to MEF-water, MEF-fermentation is superior because it produces materials with more reactive chemical properties.

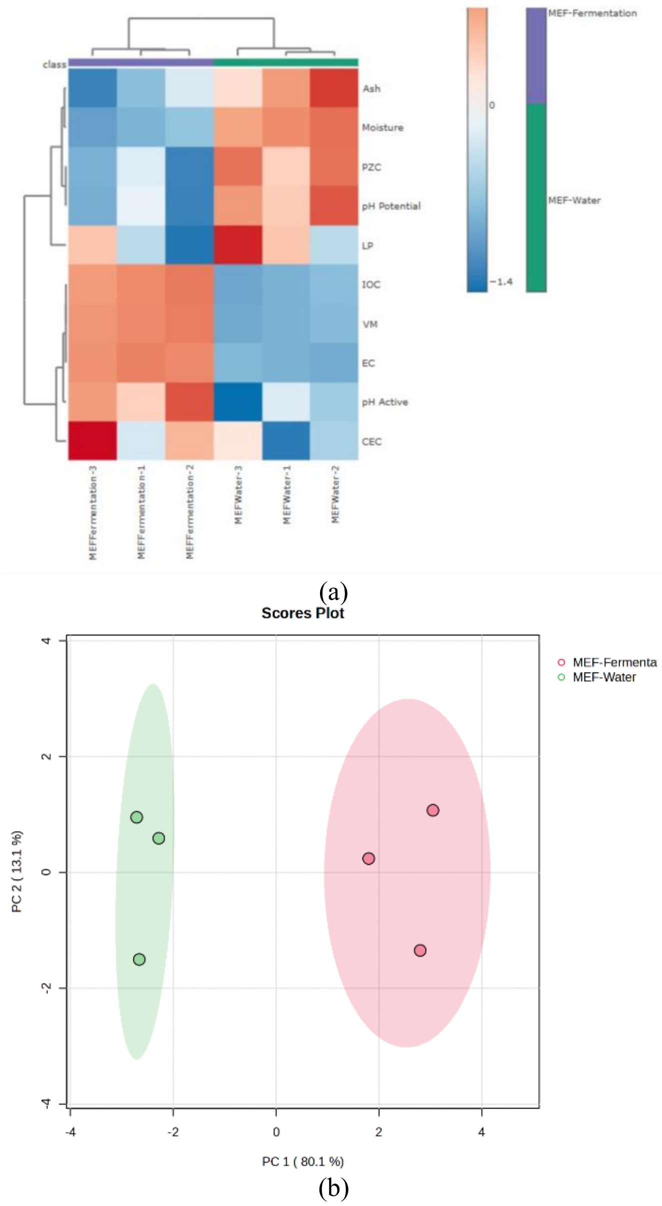

Figure 7. Principal Component Analysis (PCA) of modified eggshell flour (MEF) under MEF-water and MEF-fermentation conditions: (a) heatmap and (b) scores plot (PC1 vs PC2)

PCA and hierarchical clustering revealed clear physicochemical differences between EF modified by microbial fermentation (MEF-fermentation) and water treatment (MEF-water) (Figure 7). The first two principal components explained 93.2% of the total variance, with PC1 (80.1%) serving as the principal axis separating the two treatments. The absence of overlap between confidence ellipses and the clustering of adjacent replicates indicated that the modification method was the dominant source of variation, with high reproducibility and low within-treatment variability. Hierarchical clustering further confirmed this separation by grouping the samples into two distinct groups according to the modification method. The heatmap shows that the fermented EF exhibited relatively higher standard values of EC, CEC, IOC, VM, and active pH, while ash content, moisture content, pH, pH potential, and liming potential are relatively lower compared to water modification. These changes indicate that microbial fermentation modifies the mineral composition and surface chemistry of EF through partial carbonate solubilization and the formation of additional reactive surface functional groups. Consequently, fermentation improves ion exchange characteristics and alters surface reactivity, resulting in a physicochemical profile significantly different from that of water-treated EF. The multivariate analysis indicates that microbial fermentation is an effective strategy to improve the functional properties of EF for environmental remediation and agricultural applications.

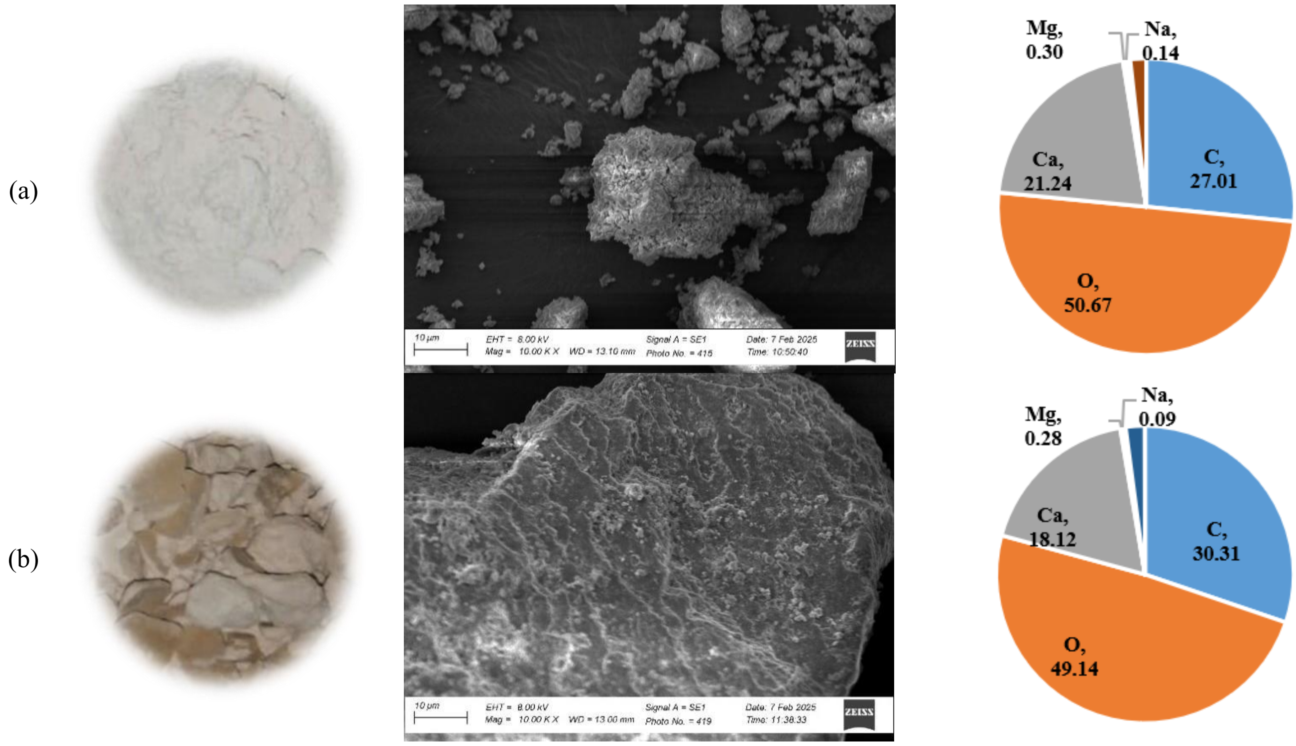

Figure 8. Scanning electron microscopy-energy-dispersive X-ray (SEM-EDX) analysis of modified eggshell flour (MEF): (a) MEF-water and (b) MEF-fermentation

The SEM shows morphological differences between EF modified with H_2O and with fermentation (Figure 8). MEF-water shows a more granular and porous surface, indicating a relatively fragmented particle structure. In contrast, MEF-fermentation showed a denser surface with an irregular texture, indicating structural changes due to microbial activity during fermentation [37]. This indicates that fermentation can enhance the demineralization and deproteinization processes in EF. The EDX for MEF-water showed Ca of 21.24%, C 27.01%, and O 50.67%. Meanwhile, in MEF-fermentation, the Ca content decreased to 18.12%, C increased to 30.31%, and O decreased slightly to 49.14%. This decrease in Ca indicates the release of calcium carbonate during the fermentation process, which is supported by the increase in carbon content. This phenomenon is common because fermentation involves the production of organic acids that can dissolve $CaCO_3$ [38].

The higher oxygen (O) content in the modified with H_2O compared to fermentation indicates that the washing process with water still maintains the oxide composition, while fermentation causes chemical bond changes through protein degradation and mineralization. This is in line with previous findings that biological fermentation can modify chemical bonds in biomaterials, thereby changing the proportions of their constituent elements [39]. The increase in carbon content in MEF fermentation is an indicator of the presence of organic compound residues from microbial metabolism absorbed in the eggshell matrix. Fermented EF has the potential to have different functional properties, for example, as a source of organic calcium or an adsorbent material with higher surface activity. This modification can also expand the application of EF in the environmental and food sectors [40]. SEM-EDX showed that the fermentation method was more effective in modifying the structure and chemical composition of EF than water alone. Fermentation not only reduced the Ca content but also increased the proportion of carbon, indicating a significant change in the inorganic-organic composition of the material. Therefore, the fermentation method is superior for producing EF with specific functional characteristics.

4. CONCLUSION

The process of MEF with water and fermentation showed a significant effect on proximate properties (moisture, VM, and ash), pH, EC, and IOC. The MEF-fermentation process showed an increase in H_2O pH (8.40) and EC (0.86 dS m^{-1}), as well as higher CEC [31.60 cmol(+) kg^{-1}] and IOC (15.66%). The modification process through fermentation produced a denser surface structure with an irregular texture and a higher proportion of C. The MEF through fermentation increased the surface elemental, nutrient availability, and adsorption capacity.

ACKNOWLEDGMENT

Acknowledgment to the Institute of Research and Community Service of Universitas Islam Negeri Sultan Syarif Kasim Riau for the financial support grant number 1031 in 2024.

REFERENCES

[1] Abbaspour, N. (2024). Fermentation's pivotal role in shaping the future of plant-based foods: An integrative review of fermentation processes and their impact on sensory and health benefits. Applied Food Research, 4(2): 100468. https://doi.org/10.1016/j.afres.2024.100468

[2] Abdelgalil, S.A., Kaddah, M.M.Y., Abo-Zaid, G.A. (2024). Eggshell waste bioprocessing for sustainable acid phosphatase production and minimizing environmental hazards. Journal of Biological Engineering, 18: 26. https://doi.org/10.1186/s13036-024-00421-8

[3] Alrosan, M., Al-Massad, M., Obeidat, H.J., et al. (2025). Fermentation-induced modifications to the structural, surface, and functional properties of quinoa proteins. Food Science and Biotechnology, 34: 3317-3329. https://doi.org/10.1007/s10068-025-01930-y

[4] Babalola, B.M., Wilson, L.D. (2024). Valorization of eggshell as renewable materials for sustainable biocomposite adsorbents—An overview. Journal of Composites Science, 8(10): 414. https://doi.org/10.3390/jcs8100414

[5] Darkun, K., Febrina, L., Lutfansa, A. (2022). Utilization of a mixture of eggshells and husk ash to reduce environmental impact. Environmental Research, Engineering and Management, 78(3): 110-118. https://doi.org/10.5755/j01.erem.78.3.31084

[6] Dong, H.L., Huang, L.Q., Zhao, L.D., et al. (2022). A critical review of mineral–microbe interaction and co-evolution: Mechanisms and applications. National Science Review, 9(10): 1-21. https://doi.org/10.1093/nsr/nwac128

[7] Esmaeili, A., Barzegar, M., Yazdi, A.P.G., Karimi, A. (2025). Eggshell powder as a mineral source for calcium fortification of brownie. Applied Food Research, 5(2): 101232. https://doi.org/10.1016/j.afres.2025.101232

[8] Sumiati, Fadilah, R., Darmawan, A., Nadia, R. (2025). Challenges and constraints to the sustainability of poultry farming in Indonesia. Animal Bioscience, 38(4): 802-817. https://doi.org/10.5713/ab.24.0678

[9] Fathi, M.B., Rahmatabadi, Z.T. (2024). Eggshell microstructure, shell quality indices, mineralogy, and UV–Vis absorbance of domestic eggs of Iran. Journal of Photochemistry and Photobiology, 21: 100235. https://doi.org/10.1016/j.jpap.2024.100235

[10] Guan, X.Y., Guo, J.T., Zhang, H., et al. (2022). Effect of co-existing cations and anions on the adsorption of antibiotics on iron-containing minerals. Molecules, 27(22): 8037. https://doi.org/10.3390/molecules27228037

[11] Guru, P.S., Dash, S. (2014). Sorption on eggshell waste—A review on ultrastructure, biomineralization and other applications. Advances in Colloid and Interface Science, 209: 49-67. https://doi.org/10.1016/j.cis.2013.12.013

[12] Hashem, M.A., Sahen, M.S., Bhowmik, P., Zahin, M.E.H., Ahammad, F. (2024). Calcination of eggshell could be a lime source for leather processing in a tannery. Waste Management Bulletin, 2(1): 83-88. https://doi.org/10.1016/j.wmb.2023.12.005

[13] Huang, Y., Long, P., Wang, G.S., et al. (2023). Ion-Exchange model for the leaching process of ion-adsorption-type rare-earth ores considering the influence of anions. Minerals, 13(12): 1475. https://doi.org/10.3390/min13121475

[14] Jannah, M., Wakiah, N., Sandi, O.M., Islamawan, P.A., Wulan, N.L.N. (2025). Microorganisms in probiotic beverage fermentation: Health benefits and mechanisms in dairy and non-dairy products. Jurnal Inovasi Pangan Dan Gizi, 2(1): 1-18. https://doi.org/10.61511/jipagi.v2i1.1773

[15] Leogrande, R., Vitti, C., Castellini, M., Mastrangelo, M., Pedrero, F., Vivaldi, G.A., Stellacci, A.M. (2021). Comparison of two methods for total inorganic carbon estimation in three soil types in Mediterranean area. Land, 10(4): 409. https://doi.org/10.3390/land10040409

[16] Liu, Y.Z., Hu, K.J., Pan, M.L., Dong, W., Wang, X.J., Zhu, X.Y. (2025). Research and application of green technology based on microbially induced carbonate precipitation (MICP) in mining: A review. Sustainability, 17(17): 7587. https://doi.org/10.3390/su17177587

[17] Lunstrum, A., Berelson, W. (2022). CaCO3 dissolution in carbonate-poor shelf sands increases with ocean acidification and porewater residence time. Geochimica et Cosmochimica Acta, 329: 168-184. https://doi.org/10.1016/j.gca.2022.04.031

[18] Manyatshe, A., Sibali, L.L. (2025). A review on egg waste-based adsorbents for the removal of organic and inorganic contaminants from aqueous solution. Heliyon, 11(3): e42278. https://doi.org/10.1016/j.heliyon.2025.e42278

[19] Masime, J.O., Ogur, E.O., Mbatia, B.N., Aluoch, A.O., Otieno, G. (2022). Characterization of eggshells nanocatalyst: Synthesized by bottom-up technology. Walisongo Journal of Chemistry, 5(2): 202-211. https://doi.org/10.21580/wjc.v5i2.13434

[20] Ngayakamo, B., Onwualu, A.P. (2022). Recent advances in green processing technologies for valorisation of eggshell waste for sustainable construction materials. Heliyon, 8(6): e09649. https://doi.org/10.1016/j.heliyon.2022.e09649

[21] Owuamanam, S., Cree, D. (2020). Progress of bio-calcium carbonate waste eggshell and seashell fillers in polymer composites: A review. Journal of Composites Science, 4(2): 70. https://doi.org/10.3390/jcs4020070

[22] Pagonis, N., Flegkas, D., Itziou, A., et al. (2024). Upcycling of eggshell waste into calcium phosphates for use in sustainable biomedical engineering applications. Eng, 5(4): 3540-3550. https://doi.org/10.3390/eng5040184

[23] Radandima, A., Arofah, S.K., Amalia, H.A.R., Nurbaiti, U. (2021). Review: Nanocomposite of bioactive glass/forsterite from raw material sand and egg shell for bone and dental implants. Journal of Physics: Conference Series, 1918(2): 022018. https://doi.org/10.1088/1742-6596/1918/2/022018

[24] Regasa, A., Haile, W., Abera, G. (2025). Effects of lime and vermicompost application on soil physicochemical properties and phosphorus availability in acidic soils. Scientific Reports, 15: 25544. https://doi.org/10.1038/s41598-025-02053-4

[25] Salami, S.A., Ross, S.A., Patsiogiannis, A., Moran, C.A., Taylor-Pickard, J. (2022). Performance and environmental impact of egg production in response to dietary supplementation of mannan oligosaccharide in laying hens: A meta-analysis. Poultry Science, 101(4): 101745. https://doi.org/10.1016/j.psj.2022.101745

[26] Sari, D.P., Warnita, Muhsanati. (2023). Impact of eggshells on growth and production of soybean (Glycine max(L) Merril) in ultisol. International Journal of Environment, Agriculture and Biotechnology, 8(2): 164-171. https://doi.org/10.22161/ijeab.82.18

[27] Saud, S., Tang, X.J., Fahad, S. (2024). The consequences of fermentation metabolism on the qualitative qualities and biological activity of fermented fruit and vegetable juices. Food Chemistry: X, 21: 101209. https://doi.org/10.1016/j.fochx.2024.101209

[28] Sebonela, L.K., Elephant, D.E., Sithole, N.J. (2024). Eggshells improve soil pH and P availability in sandy loam and sandy clay loamy soil. Agronomy, 14(11): 2539. https://doi.org/10.3390/agronomy14112539

[29] Sharma, R., Garg, P., Kumar, P., Bhatia, S.K., Kulshrestha, S. (2020). Microbial fermentation and its role in quality improvement of fermented foods. Fermentation, 6(4): 106. https://doi.org/10.3390/fermentation6040106

[30] Siddiqui, S.A., Erol, Z., Rugji, J., et al. (2023). An overview of fermentation in the food industry - looking back from a new perspective. Bioresources and Bioprocessing, 10: 85. https://doi.org/10.1186/s40643-023-00702-y

[31] Singh, B., Camps-Arbestain, M., Lehmann, J. (2017). Biochar: A Guide to Analytical Methods. CSIRO Publishing.

[32] Sionek, B., Szydłowska, A., Küçükgöz, K., Kołożyn-Krajewska, D. (2023). Traditional and new microorganisms in lactic acid fermentation of food. Fermentation, 9(12): 1019. https://doi.org/10.3390/fermentation9121019

[33] Sun, W.L., Shahrajabian, M.H., Lin, M. (2022). Research progress of fermented functional foods and protein factory-microbial fermentation technology. Fermentation, 8(12): 688. https://doi.org/10.3390/fermentation8120688

[34] Sunardi, S., Ariawan, D., Surojo, E., Prabowo, A.R., Akbar, H.I., Carvalho, H. (2023). Assessment of eggshell-based material as a green-composite filler: Project milestones and future potential as an engineering material. Journal of the Mechanical Behavior of Materials, 32(1): 20220269. https://doi.org/10.1515/jmbm-2022-0269

[35] Getahun, G.T., Etana, A., Munkholm, L.J., Kirchmann, H. (2021). Liming with $CaCO_3$ or CaO affects aggregate stability and dissolved reactive phosphorus in a heavy clay subsoil. Soil & Tillage Research, 214: 105162. https://doi.org/10.1016/j.still.2021.105162

[36] Therdthai, N., Soontrunnarudrungsri, A., Khotchai, W. (2023). Modified eggshell powder using thermal treatment and its application in Ca-fortified dog biscuits. Heliyon, 9(2): e13093. https://doi.org/10.1016/j.heliyon.2023.e13093

[37] Torres-Mansilla, A., Hincke, M., Voltes, A., et al.(2023). Eggshell membrane as a biomaterial for bone regeneration. Polymers, 15(6): 1342. https://doi.org/10.3390/polym15061342

[38] Turyasingura, M., Wakatuntu, J., Lubwama, M., Jjagwe, J., Hensel, O., Olupot, P.W. (2023). Optimisation of eggshell-zeolite composite as a potential surfactant adsorbent for hand-washing wastewater. Case Studies in Chemical and Environmental Engineering, 7: 100284. https://doi.org/10.1016/j.cscee.2022.100284

[39] Zambrano, M.V., Dutta, B., Mercer, D.G., MacLean, H.L., Touchie, M.F. (2019). Assessment of moisture content measurement methods of dried food products in small-scale operations in developing countries: A review. Trends in Food Science & Technology, 88: 484-496. https://doi.org/10.1016/j.tifs.2019.04.006

[40] Xia, Y., Feng, J.N., Zhang, H.B., et al. (2024). Effects of soil pH on the growth, soil nutrient composition, and rhizosphere microbiome of Ageratina adenophora. Peer J, 12: e17231. https://doi.org/10.7717/peerj.17231