

EVALUATING THE PROBIOTIC POTENTIAL OF FERMENTED INULIN EXTRACTION WASTE FROM LOCAL TUBERS FOR USE AS A FEED ADDITIVE

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Abstract

This study aims to evaluate the potential of fermenting inulin extraction waste as a feed additive, based on lactic acid, dry matter, pH, and *Fleish* points. The research was conducted from March to June 2022 at the Animal Husbandry and Fisheries Laboratory, Tidar University. The experimental design used was a Completely Randomized Design with four treatments: T1 (uwi, *Dioscorea alata*), T2 (ganyong, *Dioscorea alata discolor*), T3 (gadung, *Dioscorea hispida*), and T4 (umbi ungu, *Ipomoea batatas*), each replicated five times. The variables observed included lactic acid content, dry matter, crude protein, pH, and *Fleish* points of the fermentation of inulin extraction waste. Data were analyzed using Analysis of Variance (ANOVA), with Duncan's multiple range test applied if significant differences were found. The analysis was performed using IBM SPSS 21. Results showed that inulin extraction waste from local tubers significantly affected ($p < 0.05$) lactic acid content, pH, and *Fleish* points, but had no significant effect ($p > 0.05$) on dry matter content. T3 and T4 treatments produced the highest lactic acid levels and *Fleish* points, and the lowest pH values. T3 and T4 produced the highest lactic acid content, *Fleish* points, and the lowest pH value.

Keywords: feed additive, inulin, local tuber

INTRODUCTION

Indonesia has a variety of tubers that are still not fully exploited for their greatest potential. This is caused by the community's lack of knowledge and inskills to processing local tubers into functional food ingredients with greater use value, one of which is as a source of inulin. Inulin is a carbohydrate with a high fiber content that cannot be processed by digestive enzymes but can be fermented by microflora (probiotics) in the digestive tract (Zubaidah & Akhadiana, 2013).

The extraction of inulin from tubers such as *Dioscorea alata*, *Dioscorea hispida*, *Dioscorea alata discolor*, and *Ipomoea batatas* generates waste by-products. If the waste is going to be used fresh, it is likely to spoil quickly due to its high water content. It needs to be handled to preserve its qualities. Handling options include drying and fermentation.

Dried inulin extraction waste has a high nitrogen-free extract (NFE) content, indicating

its potential as a substrate for microbial fermentation. The microbial cultures are expected to become probiotics and can be used as feed additives for livestock. There is currently a lack of research on the utilization of inulin industry by-products as animal feed. This study is therefore of particular interest, as preliminary analyses suggest that inulin residues possess appreciable nutritional value and may serve as a promising substrate for probiotic growth.

Probiotics are feed additives that have beneficial microbes. It can improve intestinal microbial balance by influencing the host and manipulating the microflora, thereby enhancing livestock health and production (Utami et al., 2020). The purpose of this study was to utilize and increase the nutritive value of inulin extraction waste to be used as feed additives and to determine the content of lactic acid, dry matter, pH, and *Fleish* points from fermented inulin extraction waste from tubers.

MATERIALS AND METHODS

The tools used in this study included analytical balances, beaker glass, Erlenmeyer flasks, burettes, pipettes, pH meters, analytical drying ovens, porcelain crucibles, blenders, a Kjeldahl unit, and a distillation unit. The materials used in this study were inulin extract waste from tubers (*Dioscorea hispida*, *Dioscorea alata*, *Dioscorea alata discolor*, and *Ipomoea batatas*), commercial Heryaki probiotic liquid was known to contain *Lactobacillus* sp., *Bacillus* sp., *Monascus fumus*, and *Candida ethanolica* (Rahayu et al. 2023), molasses, distilled water, chemicals for lactic acid analysis (NaOH 1 N and phenolphthalein), and crude protein analysis (H₂SO₄, selenium, NaOH, boric acid, methyl red indicator, and 0.1 N HCl).

Inulin extraction is carried out based on Zubaidah & Akhadiana (2013). Fresh tubers were cleaned, cut, and blended with distilled water (1:4 w/v) to form a slurry. The slurry was heated at 80 °C for 30 min, cooled, and filtered. The filtrate was mixed with 80% ethanol (40% of filtrate volume) and stored at -10 °C for 18 h to precipitate inulin. After thawing for 2 h, the mixture was centrifuged at 5000 rpm for 15 min. The obtained inulin precipitate was oven-dried at 60 ± 6 °C to constant weight. The sludge that did not pass through the filter was used as the waste material and served as the research object in this study. In the next stage, the inulin waste was dried and then ground into a fine powder. The next step is to process the waste into a feed additive by adding 3% molasses and 0.5% liquid probiotic, by weight, to the inulin extraction waste from tubers. All ingredients are mixed until homogeneous, then placed in a silo and compacted until there is no oxygen space that could inhibit fermentation. For fermentation, it is then stored at room temperature and kept away from direct sunlight for 21 days.

This study was conducted using a completely randomized design (CRD) with 4 treatments (using inulin extraction waste from tubers, namely *Dioscorea alata*, *Dioscorea alata discolor*, *Dioscorea hispida*, and *Ipomoea batatas* with added 3% molasses and 0.5% Heryaki probiotics (Septian et al., 2022), respectively) and replicated 5 times. The collected data were analyzed using analysis of variance (ANOVA) at the 5% significance level. When significant differences were found

among treatments, Duncan's Multiple Range Test (DMRT) was applied using SPSS version 27. The variables observed included lactic acid content, pH, dry matter, and Fleigh points.

Lactic acid observation used (Cappuccino & Sherman, 2014) method.

$$\text{Lactic acid (\%)} = \frac{V \times N \times 90}{vs}$$

Information:

V = NaOH volume for titration (ml)

N = NaOH normality

vs = sample volume (ml)

The pH of the probiotic powder sample was measured using a calibrated pH meter and standardized with buffer solutions at pH 4, 7, and 10 before use. The sample for each observation was 5-10 ml. The pH meter electrode was rinsed with distilled water and dried with a tissue. The electrode is immersed in the sample and left for a specified period. The pH value of the sample can be read when the pH meter is stable (Silaban et al., 2020).

Dried matter observed by the formula:

$$\%DM = \frac{\text{Samples after oven}}{\text{samples before oven}} \times 100$$

Fleigh points (FP) observed by Idikut et al., (2009) procedure, by the formula:

$$FP = 220 + (2 \times DM (\%) - 15) - (40 \times \text{pH}).$$

RESULTS AND DISCUSSION

Each inulin extraction waste source has a distinct nutritional profile. The detailed nutrient composition of each material is provided in Table 1.

Based on its nutritional composition, *Dioscorea hispida* demonstrates strong potential as a livestock feed, particularly as a feed additive, due to its high crude protein (CP), nitrogen-free extract (NFE), and total digestible nutrients (TDN) content, recorded at 8.70%, 79.34%, and 75.25%, respectively. The elevated levels of CP and NFE are likely to enhance the activity of lactic acid bacteria (LAB), as these nutrients serve as primary energy sources for bacterial growth. The presence of LAB plays a critical role in silage fermentation, contributing significantly to the production and preservation of high-quality fermented feed.

The different types of inulin extraction waste subjected to anaerobic fermentation

produced varying responses in terms of lactic acid content, dry matter, pH value, and Fleigh points. The results of the analysis of fermented inulin extraction waste are summarized in Table 2.

Lactic Acid

Lactic acid is widely recognized as an essential marker of effective fermentation. Its accumulation in fermented feed indicates active metabolism by lactic acid bacteria (LAB) and serves as a natural preservative, enhancing feed stability and safety. The lactic acid analysis of the fermented inulin-extraction residues revealed that tuber type significantly influenced lactic acid production ($p < 0.05$). As shown in Table 1, the highest lactic acid levels were observed in treatments T4 (1.09%) and T3 (1.07%), both of which were markedly higher than those in T2 (0.53%) and T1 (0.27%). The elevated lactic acid content in T4 is likely attributed to the high raffinose content in purple-fleshed tubers. Oligosaccharides in *Ipomoea batatas* are raffinose, which will later be degraded by lactic acid bacteria to produce lactic acid (Novelina et al., 2012). Microorganisms readily convert soluble carbohydrates into organic acids, such as lactic acid (Kuncoro et al., 2015).

The lack of a significant difference between treatments T4 and T3 can be attributed to the high starch content of *Dioscorea hispida* (63.56%). This abundant starch serves as a readily available substrate for *Lactobacillus* sp., which are known to produce amylolytic enzymes capable of hydrolyzing complex starch molecules. As lactic acid bacteria degrade starch, they generate short-chain oligosaccharides such as maltose, maltotriose, and α -D-glucose (Setiarto & Widhiyastuti, 2016), which can be rapidly fermented into lactic acid. The active role of *Lactobacillus* sp. in utilizing these hydrolysis products explains why T4 and T3 achieved similarly high lactic acid levels despite differences in tuber type.

In contrast, T1 exhibited the lowest lactic acid production, which may be associated with the extremely high starch content (79.34%) and higher initial pH (7.41). Excessive concentrations of easily fermentable carbohydrates can lead to osmotic stress, disrupting microbial homeostasis. For *Lactobacillus* sp., which are sensitive to

drastic increases in osmotic pressure, these conditions can inhibit metabolic activity and arrest fermentation prematurely. Septian et al. (2020) emphasized that excessive nitrogen-free extract (NFE) may halt microbial activity once fermentation reaches a stable phase. Similarly, Putri et al. (2016) reported that excessively high carbohydrate levels can damage bacterial cell walls, further reducing the viability of lactic acid bacteria and limiting their ability to convert substrates into lactic acid.

Although fermentation involves mixed microbial populations—including *Bacillus* sp., *Monascus fumeus*, and *Candida ethanolica**—the production of lactic acid is predominantly governed by *Lactobacillus* sp.. *Bacillus* sp. contribute through enzymatic hydrolysis of complex polysaccharides, thereby supporting LAB activity, whereas *Monascus fumeus* and *Candida ethanolica* assist in carbohydrate breakdown and secondary fermentation pathways. However, their metabolic outputs do not accumulate lactic acid as a primary product, making LAB the key determinant of lactic acid concentration across treatments.

The results of the lactic acid analysis confirmed that tuber type significantly influenced lactic acid production ($p < 0.05$). As shown in Table 2, T4 (1.09%) and T3 (1.07%) exhibited significantly higher lactic acid levels than T2 (0.53%) and T1 (0.27%). The superior performance of T4 may be associated with the high raffinose content in purple-fleshed tubers. Raffinose, a soluble oligosaccharide widely present in *Ipomoea batatas*, can be efficiently metabolized by *Lactobacillus* sp. into lactic acid (Novelina et al., 2012). The rapid fermentability of raffinose provides a favorable substrate not only for LAB but also for supporting microorganisms such as *Bacillus* sp. and *Candida ethanolica*, which enhance early carbohydrate degradation. As suggested by Cahyo Kuncoro & Farida Fathul (2015), soluble carbohydrates are readily utilized by microorganisms and converted into organic acids, such as lactic acid, thereby improving fermentation efficiency.

pH

The acidity level (pH) is a crucial indicator of fermentation quality, as it reflects the effectiveness of anaerobic microbial activity. A decrease in pH typically results from

the metabolic processes of *Lactobacillus* sp., which are the primary producers of lactic acid during fermentation. These bacteria convert fermentable carbohydrates into lactic acid, thereby lowering the pH and stabilizing the silage environment. Other microbes present in the fermentation system—such as *Bacillus* sp., *Monascus fumeus*, and *Candida ethanolica* may contribute to carbohydrate hydrolysis and secondary metabolite production, but they do not acidify the substrate as efficiently as lactic acid bacteria.

The results showed that tuber type significantly affected the pH values of the fermented materials ($p < 0.05$). Treatments T3 (4.24) and T4 (4.33) exhibited the lowest pH values, which were significantly lower than those of T2 (4.88) and T1 (6.44). Based on silage quality standards, the pH levels of T3 and T4 fall within the “good” category; T2 is classified as “moderate”; and T1 exhibits “poor” fermentation quality. Sandi *et al.* (2010) categorize fermentation pH values of 3.5–4.2 as very good, 4.2–4.5 as good, 4.5–4.8 as moderate, and >4.8 as poor.

The differences in pH values among treatments are likely influenced by the variability in carbohydrate content within the inulin extraction wastes. Substrates with balanced levels of fermentable carbohydrates tend to support optimal growth of *Lactobacillus* sp., resulting in greater lactic acid accumulation and lower pH, as observed in T3 and T4. Conversely, substrates with excessively high carbohydrate concentrations—such as those in T1—may impose osmotic stress on LAB, reducing their viability and limiting acid production. Additionally, as noted by Triwiyono *et al.* (2020), fermentation processes generate organic acids besides lactic acid, including acetic, propionic, butyric, succinic, and formic acids, all of which contribute to the final pH profile.

Overall, the interaction between substrate characteristics and microbial activity—particularly the efficiency of *Lactobacillus* sp. in acid production—explains the observed variation in pH among the treatments.

Table 1. The nutrient content of the inulin extraction waste

Variables	Treatments			
	<i>Dioscorea alata</i>	<i>Dioscorea alata discolor</i>	<i>Dioscorea hispida</i>	<i>Ipomoea batatas</i>
Moisture (%)	8.36	7.67	10.50	8.77
Ash (%)	4.36	3.25	2.30	4.11
Crude Protein (%)	7.35	6.89	8.70	8.30
Extract Ether (%)	2.98	2.34	4.76	5.49
Crude Fibre(%)	9.00	10.42	4.90	12.88
Nitrogen Free Extract (%)	76.31	77.10	79.34	69.21
Total Digestible Nutrien (%)	69.40	68.60	75.25	67.80

Table 2. The nutritional content of the inulin extraction waste fermentation

Variables	Treatments			
	T1	T2	T3	T4
Lactic acid (%)	^a 0.27±0.00	^b 0.53± 0.01	^c 1.07± 0.01	^c 1.09± 0.01
pH	^c 6.44± 0.08	^b 4.88± 0.02	^a 4.24± 0.02	^a 4.33± 0.01
DM (%)	60.63±0.46	61.27±0.21	61.69±0.11	61.52±0.13
Fleigh	^c 68.66±2.85	^b 132.27±0.76	^a 158.69±0.98	^a 154.84±0.46

T1: *Dioscorea alata*, T2: *Dioscorea alata discolor*, T3: *Dioscorea hispida*, and T4: *Ipomoea batatas*

^{a,b,c} : Different superscripts within the same row indicate significant differences at the 0.05 significance level.

Table 3. pH value of inulin-waste before and after fermentation

Treatments	pH	
	Before	After
P1	7,41	6,44
P2	5,18	4,88
P3	6,26	4,24
P4	4,70	4,33

During fermentation, a decrease in pH was observed in the inulin extraction waste from tubers, as shown in Table 3. This pH reduction is primarily due to the growth and metabolic activity of lactic acid bacteria, which produce lactic acid as a major fermentation product. The accumulation of lactic acid lowers the pH and helps preserve the fermented material. As reported by Fadilah *et al.* (2018), the pH decline during fermentation can also result from the production of various organic acids by fermentative microbes, including malic acid, tartaric acid, citric acid, acetic acid, butyric acid, and propionic acid. These acids create an increasingly acidic environment, thereby contributing to the overall reduction in pH.

Dry Matter

Dry matter plays a crucial role in the success of feed fermentation. Substrates with high moisture content are more susceptible to rapid spoilage microorganism growth, while substrates that are too dry may result in a prolonged or incomplete fermentation. Furthermore, dry matter can affect to the Fleigh points. The higher the dry matter levels, the higher the Fleigh scores, which indicate better fermentation quality.

The analysis of dry matter content among the various types of inulin extraction waste showed no significant differences ($p > 0.05$), with values ranging from 60.63% to 68.88%. This consistency is likely due to the similar initial dry matter levels used in preparing the fermented inulin extraction wastes. In this study, variations in lactic acid concentration and pH did not appear to influence the dry matter content. Septian *et al.* (2022) reported that dry matter levels in fermented feed are often affected by differences in the amount of probiotics or liquid starters applied; however, in the present study, all treatments used the same

proportions of molasses and probiotics, namely 3% and 0.5%, respectively.

Fleigh Point

Fleigh points are used as an indicator of silage quality and are calculated based on pH and dry matter content (Septian *et al.*, 2022). The analysis of variance showed that tuber type significantly affected Fleigh scores in the fermentation of inulin extraction wastes ($p < 0.05$). This effect is likely related to the differences in pH among treatments, as Fleigh values are strongly influenced by both dry matter levels and acidity. Generally, higher dry matter content combined with lower pH results in greater Fleigh scores, reflecting improved silage quality.

According to Kiliç (1984) and Idikut *et al.* (2009), silage is classified as very good when Fleigh scores range from 85 to 100, good at 60–85, moderate at 55–60, sufficient at 25–55, and poor when scores fall below 25. Likewise, Ozturk *et al.* (2006) indicated that Fleigh scores above 85 represent very high-quality silage. In this study, treatments T3 and T4 recorded the highest Fleigh scores, reaching 158.69 and 154.84, respectively, followed by T2 with a score of 132.27. Treatment T1 produced the lowest score at 68.66.

Based on these classifications, treatments T2, T3, and T4 can be categorized as yielding very good fermentation quality, as all had Fleigh scores above 85, whereas T1 produced a good-quality fermented product. The lower Fleigh score in T1 is primarily due to its higher pH, reflecting reduced lactic acid production compared to the other treatments.

CONCLUSIONS

The study demonstrates that inulin extraction waste from various local tubers can be effectively utilized as a substrate for microbial fermentation. Significant differences

in lactic acid production, pH reduction, and Fleigh points were observed among treatments, with *Dioscorea hispida* and *Ipomoea batatas* showing the best fermentation performance. The overall decrease in pH across treatments confirms successful anaerobic fermentation. These findings indicate that tuber-derived inulin waste has strong potential to be developed as a probiotic-rich feed additive, supporting efforts to add value to underutilized local tubers in Indonesia.

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CONFLICT OF INTEREST

The authors whose names are listed have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in the manuscript.

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