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Impact of ultrasonication and fermentation on oxalate reduction in Beneng taro (*Xanthosoma undipes* K. Koch.) flour

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Abstract. Beneng taro (*Xanthosoma undipes* K. Koch.), an indigenous tuber from Banten, Indonesia, has considerable potential as a gluten-free starch source; however, its utilization is limited by its high oxalate content, which reduces mineral bioavailability and may pose health risks. This study evaluated the effectiveness of combining ultrasonication and yeast fermentation to reduce oxalate levels in Beneng taro flour. A Box–Behnken Design (BBD) was employed to examine the effects of sonication time (0–30 min), yeast concentration (1–2%), and fermentation time (24–72 h). The untreated flour contained 39.41% oxalate. Under optimized conditions (30 min sonication, 1% yeast concentration, and 48 h fermentation), oxalate levels decreased to 7.32%, corresponding to an 81.44% reduction. The regression model was statistically significant (F= 28.49; $p<0.0001$; R=0.8860), indicating good predictive capability. The reduction mechanism is attributed to acoustic cavitation during ultrasonication, which disrupts cell structures and enhances mass transfer, followed by physicochemical changes during fermentation. This combined non-thermal approach provides an effective strategy to improve the safety of Beneng taro flour for gluten-free applications.

1 Introduction

The demand for gluten-free and sustainable food ingredients has increased due to the growing prevalence of gluten intolerance and interest in alternative carbohydrate sources [1]. This trend has encouraged the exploration of indigenous crops with high starch content for gluten-free flour development. Beneng taro (*Xanthosoma undipes* K. Koch.), an indigenous tuber from Banten Province, Indonesia, has attracted attention as an underutilized carbohydrate source with potential applications in gluten-free and supplementary food products. Beneng taro also contains higher protein and dietary fiber

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than several local taro varieties, increasing its potential as a functional food ingredient [2]. In addition, Beneng taro contributes to local food diversification and regional food security.

Despite its potential, the utilization of Beneng taro flour is limited by the presence of antinutritional compounds, particularly oxalates [3]. Oxalates can bind essential minerals such as calcium and reduce their bioavailability. The oxalate content of Beneng taro flour has been reported to range from 0.33% to 0.44%, which is relatively higher than that of commonly consumed tubers such as cassava and sweet potato (generally below 0.08% and 0.20% respectively) [4]. Excessive oxalate intake has also been associated with kidney stone formation, highlighting the need for effective oxalate reduction strategies. Conventional methods such as soaking, boiling, and chemical treatments have been applied to reduce oxalates [5]. However, these methods often require prolonged processing times (typically 2–24 h), which may lead to nutrient losses and undesirable changes in functional properties [3]. Therefore, alternative processing technologies that are more efficient and capable of maintaining product quality are needed.

Ultrasonication is a non-thermal processing technology that modifies plant tissues through acoustic cavitation [6, 7]. The collapse of cavitation bubbles generates localized pressure and temperature gradients that disrupt cell structures and enhance mass transfer [8], facilitating the release of soluble compounds, including oxalates [9]. The effectiveness of ultrasonication is strongly influenced by sonication time because longer treatment increases structural disruption and mass transfer efficiency. Fermentation is also widely used to modify the chemical composition of plant materials through microbial activity. Its effectiveness depends on microbial concentration and fermentation time, which determine metabolic activity and the extent of biochemical transformation. In the context of oxalate reduction, fermentation may promote physicochemical changes, including increased solubilization and leaching of oxalates [10]. The combination of ultrasonication and fermentation may therefore provide a complementary approach for oxalate reduction. However, studies on the combined application of these techniques in Beneng taro flour remain limited. Therefore, this study aimed to optimize the ultrasonication and fermentation conditions for oxalate reduction in Beneng taro flour using box–behnken design (BBD).

2 Methods

2.1 Raw materials and sample preparation

Fresh Beneng taro tubers were obtained from local farmers in Lahat, South Sumatra, Indonesia. The tubers were peeled, washed, and chopped. Each experimental unit consisted of 250 g of chopped taro packed in polyethylene bags.

Ultrasonication treatment was conducted using an ultrasonic bath (Cole-Palmer, China) operating at a frequency of 40 kHz. The bags containing the taro samples were immersed in distilled water in the ultrasonic bath to ensure complete contact between the samples and the ultrasonic medium and treated for 0, 15, and 30 minutes. Following ultrasonication, the samples were directly fermented using commercial yeast (*Saccharomyces cerevisiae*, Fermipan®, Indonesia) at concentrations of 1–2% (w/w). The bags were then sealed while maintaining a small air space inside to facilitate fermentation. The samples were incubated at room temperature (±28°C) for 24–72 hours. After fermentation, the samples were dried at 60°C for 10 hours using a food dehydrator (Papalolo, China), milled (Panasonic, China) into flour, and sieved through an 80-mesh sieve to obtain a uniform particle size. The flour samples were stored in polypropylene containers prior to analysis.

2.2 Experimental design and statistical analysis

A box–behnken design (BBD) with three independent variables was applied: sonication time (X_1), yeast concentration (X_2), and fermentation time (X_3). The design consisted of 15 experimental runs with center point replication. Each treatment was performed in triplicate.

The response variable was oxalate content. Data were analyzed using Design-Expert software (version 13). A regression model was fitted, and statistical significance was evaluated using analysis of variance (ANOVA) at a confidence level of 95% ($p<0.05$). Model adequacy was assessed based on F-value, p-value, and coefficient of determination.

2.3 Oxalate analysis by titration method

Oxalate content was determined based on the method of Mishra et al. (2017) with slight modifications [11]. A 0.5 g sample was mixed with 30 mL of 0.5 N H_2SO_4 and heated at 100°C for 15 minutes. The mixture was filtered and diluted with distilled water. An aliquot of 10 mL filtrate was mixed with 40 mL of 0.5 N H_2SO_4 and reheated at 100°C for 5 minutes, followed by titration with 0.05 N $KMnO_4$ until a light red color endpoint was reached.

Prior to sample analysis, the $KMnO_4$ solution (0.05 N) was standardized using oxalic acid dihydrate ($C_2H_2O_4.2H_2O$). Approximately 0.0405 g of the standard compound was dissolved in 30 mL of 0.5 N H_2SO_4, heated at 100°C for 5 minutes, and subsequently titrated with $KMnO_4$ solution until a light red color persisted as the endpoint indicator. Oxalate content was calculated using the standard titration equation, and oxalate reduction (%) was determined as the percentage decrease relative to the initial oxalate content.

3 Results and discussion

3.1 Oxalate reduction

The experimental results obtained from the box–behnken design (BBD) are presented in Table 1. The table shows the complete combination of processing variables and their corresponding oxalate contents.

Table 1. Experimental design matrix and observed oxalate content.

Run	Sonication Time (min)	Yeast Concentration (%)	Fermentation Time (h)	Oxalate (%)
1	0	1.5	72	29.96
2	30	1.0	48	7.32
3	15	1.0	24	26.40
4	30	2.0	48	8.79
5	15	2.0	72	13.80
6	15	2.0	24	24.44
7	30	1.5	72	13.20
8	15	1.5	48	24.59
9	15	1.5	48	24.58
10	0	1.5	24	32.73
11	15	1.5	48	24.00
12	0	1.0	48	30.77
13	0	2.0	48	32.35
14	15	1.0	72	15.04
15	30	1.5	24	10.38

Each experimental run was performed in triplicate, and values represent mean measurements.

The results clearly demonstrate a substantial variation in oxalate content across treatment combinations, ranging from 7.32% to 32.73%. This wide range indicates that process parameters significantly influence oxalate reduction efficiency.

Notably, the lowest oxalate content was observed at 30 min sonication, 1% yeast, and 48 h fermentation (Run 2), while the highest value occurred at 0 min sonication with shorter fermentation duration (Run 10). These findings suggest that both physical pretreatment (sonication) and processing duration (fermentation time) play critical roles in reducing oxalate levels.

To clearly illustrate the effectiveness of the treatment, a comparison between initial and optimized oxalate content is presented in Table 2.

Table 2. Comparison of oxalate content before and after treatment.

Condition	Oxalate (%)
Untreated flour	39.41
Optimized treatment	7.32
Reduction (%)	81.44

The untreated sample contained 39.41% oxalate, which decreased to 7.32% under optimized conditions, corresponding to an 81.44% reduction. This confirms the high effectiveness of the combined ultrasonication–fermentation treatment compared to conventional methods, which typically report lower reduction efficiencies. Previous studies reported oxalate reductions of approximately 12–51% using conventional soaking or boiling treatments in tuber crops [3, 9]. In comparison, the present study achieved more than 80% reduction, indicating that the combined treatment substantially improved oxalate removal efficiency.

The statistical parameters and regression coefficients of the fitted regression model are summarized in Table 3. The fitted model was highly significant, with an F-value of 28.49 ($p<0.0001$).

Table 3. Statistical parameters and model coefficients of the regression model for oxalate reduction.

Model statistics	Value
F-value	28.49
p-value	<0.0001
R^2	0.8860
Adjusted R^2	0.8549
Predicted R^2	0.7828
Standard deviation	3.40
Mean	21.22
Coefficient of variation (%)	16.02
Adeq Precision	15.3929
Intercept	21.22
Sonication time (A)	−10.77
Yeast concentration (B)	−0.0168
Fermentation time (C)	−2.74

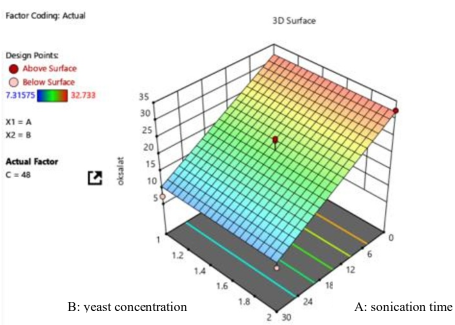
The coefficient of determination ($R^2=0.8860$) indicated that approximately 88.6% of the variation in oxalate content could be explained by the model, demonstrating good predictive capability of the regression model. Among the tested variables, sonication time (A) had the strongest influence ($p<0.0001$), followed by fermentation time (C) ($p=0.0433$), while yeast concentration (B) showed no significant effect ($p=0.9891$) within the tested range. This suggests that increasing yeast concentration beyond a certain level does not further enhance oxalate reduction, likely due to substrate limitation.

The regression analysis further confirmed the dominant influence of sonication time on oxalate reduction. Sonication time showed a strong negative coefficient (−10.77), indicating that longer sonication substantially reduced oxalate levels. Fermentation time also contributed negatively (−2.74), although to a lesser extent, whereas yeast concentration showed minimal influence under the tested conditions.

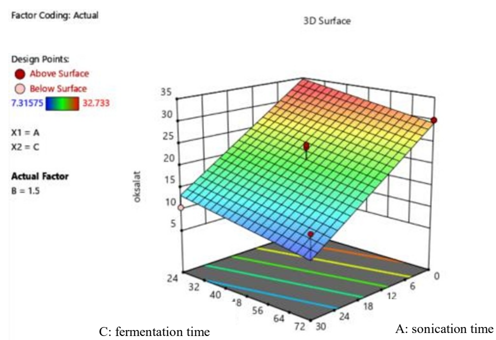
Similar trends have been reported in ultrasound-assisted oxalate reduction studies, where sonication time was identified as the most influential processing parameter due to its effect on cavitation intensity and mass transfer efficiency [9].

3.2 Effect of processing variables

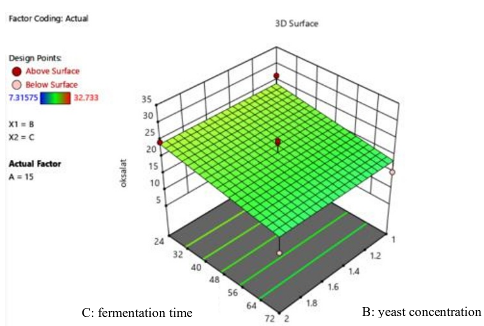
To visualize the interaction effects between processing variables, three-dimensional response surface plots are presented in Fig. 1a–c.



a) Sonication time and yeast concentration



b) Sonication time and fermentation time



c) Yeast concentration and fermentation time

Fig. 1. Response surface plots illustrating the interaction effects of processing variables on oxalate content: (a) sonication time and yeast concentration, (b) sonication time and fermentation time, and (c) yeast concentration and fermentation time.

Fig. 1a shows a pronounced decrease in oxalate content with increasing sonication time, regardless of yeast concentration. Similar observations were reported by Srivastava et al. [9], who found that ultrasonication enhanced oxalate reduction in elephant foot yam through improved mass transfer and structural disruption caused by cavitation effects. The relatively flat gradient along the yeast axis confirms its minimal effect, supporting the ANOVA findings.

Fig. 1b indicates a strong combined effect between sonication and fermentation time on oxalate reduction. Longer sonication enhances structural disruption, while extended fermentation promotes further oxalate removal through biochemical processes. Similar interaction patterns between ultrasonication intensity and processing duration have been reported in ultrasound-assisted oxalate reduction studies on tuber crops [9].

Fig. 1c shows that the interaction between yeast concentration and fermentation time was relatively weak, further confirming that yeast concentration was not a limiting factor within the studied range.

The reduction mechanism could be explained through the combined effects of physical and biological processes. Ultrasonication induces acoustic cavitation, generating microbubbles that collapse violently and disrupt plant cell structures. This enhances the release of soluble oxalates and improves mass transfer. Fermentation further reduced oxalate content through acidification (lowering pH), increased solubility of oxalate salts, and leaching into the surrounding medium [12]. Fermentation-assisted reduction of antinutritional compounds has also been reported in fermented vegetable and tuber products, where microbial activity promoted acidification and increased solubilization of oxalates [8, 12]. Overall, the combination of these processes led to substantially higher reduction efficiency compared to conventional single-treatment approaches.

Despite the promising results, this study was conducted at laboratory scale and did not evaluate the economic feasibility or industrial scalability of the process. Box–Behnken Design was selected because it is efficient for optimization involving three variables while requiring fewer experimental runs than some other response surface methodologies, making it suitable for laboratory-scale optimization studies. In addition, physicochemical properties such as starch functionality, mineral bioavailability, and sensory quality were not investigated. Further studies are therefore required to assess the applicability of this combined treatment under industrial processing conditions and its effects on overall flour quality.

4 Conclusion

The integration of ultrasonication and yeast fermentation effectively reduced the oxalate content of Beneng taro flour. The optimized processing condition, consisting of 30 minutes of sonication, 1% yeast concentration, and 48 hours of fermentation, achieved an oxalate reduction of 81.44%. The statistical model developed using a Box–Behnken Design (BBD) showed good predictive capability for describing the relationship between process variables and oxalate reduction. The reduction mechanism is associated with structural disruption caused by ultrasonic cavitation followed by biochemical and physicochemical modifications during fermentation. These findings demonstrate that the combined non-thermal processing approach can significantly improve the safety of Beneng taro flour and support its utilization as a gluten-free ingredient derived from indigenous food resources. Further studies are recommended to evaluate the scalability of this process and its effects on other functional properties and sensory characteristics in food applications.

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