Jurnal Kejuruteraan 38(5) 2026: 2411-2421
https://doi.org/10.17576/jkukm-2026-38(5)-25

Statistical Evaluation of Moisture and Time Effects on the pH of *Zingiber zerumbet* Extracts by Subcritical Water Extraction

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Received 4 December 2025, Received in revised form 12 April 2026
Accepted 12 May 2026, Available online 30 August 2026

ABSTRACT

Zingiber zerumbet is a medicinally important rhizome, with subcritical water extraction (SWE) offering a sustainable approach to recover its bioactive compounds. However, the influence of raw material moisture and process parameters on extract quality remains unclear. This study investigates two factors which affect the pH of Z. zerumbet extracts produced via SWE; (i) its initial rhizome moisture content (MC), (ii) the extraction time. Fresh rhizomes were extracted at subcritical condition for 5 - 25 min. MC and pH were measured, and statistical analyses were performed using General Linear Model (GLM) and Multiple Linear Regression (MLR). All extracts were slightly acidic (pH 5.85 ± 0.03 – 6.24 ± 0.02). GLM showed that extraction time significantly impacted pH ($F(4,9) = 55.92$, $p < 0.001$, partial $\eta^2 = 0.961$). The final multivariate model explained 98.3% of the pH variance (Adjusted R2=0.973), but the MC which shown explanatory bivariate association with pH was non-significant after controlling extraction time ($p=0.129$). Visual observations indicated stable, homogeneous extracts, with only darkening at longer extraction times. Overall, extraction time is the dominant factor influencing acidity of Z. zerumbet extracts under SWE, while initial moisture exerts only minor and inconsistent effects. These findings underscore the importance of controlling extraction duration for process optimization and product reproducibility, with moisture monitoring serving as a secondary safeguard. Future studies should expand on other SWE parameters and predictive validation to strengthen industrial applicability.

Keywords: Zingiber zerumbet; subcritical water extraction; moisture content; extraction time; pH value

INTRODUCTION

Zingiber zerumbet, commonly known in Malaysia as lempoyang, is a rhizomatous, perennial herb valued for its diverse medicinal properties attributed to numerous bioactive compounds. The plant is strongly aromatic and grows wild, typically reaching 1.8 to 2.1 m in height with an average spread of 1.2 to 1.8 m (Jantan et al. 2019). Traditionally, it has been used as a culinary spice, prepared as herbal tea, and employed as a folk remedy for ailments such as mild fever, diarrhea, and loss of appetite, among others (Ahmad et al. 2023; Chitkrachang et al. 2025; Premakumara et al. 2024). Owing to its medicinal potential, research on the extraction of its bioactive constituents has been conducted since the 1950s (Parihar and Dutt 1950) and continues to expand to the present day.

The utilization of the rhizomes for the extraction of bioactive compounds offers two different possibilities: fresh and dried rhizomes. In general, the dried rhizomes are favored for rhizome extraction due to several reasons such as longer shelf life, ease of storage, and lower susceptibility to spoilage and fungal attack (Kathiman et al. 2022; Wongpanit et al. 2018). In addition, the dried rhizomes are more readily available and can be stored longer, ensuring a constant supply for the extraction processes. However, multiple studies report that drying method and operating conditions modulate the degradation of bioactive compounds (Mieszczakowska-Frąc et al. 2021; Shahimoridi et al. 2025).

In contrast, the use of *Z. zerumbet* fresh rhizomes in natural product extraction has gained increasing attention as a sustainable and efficient alternative to traditional methods that rely on dehydrating them. Fresh rhizomes offer several advantages, including the preservation of heat-sensitive bioactive compounds, reduced processing steps, and lower energy consumption by eliminating the need for drying and grinding (Fahdi et al. 2025; Nordin et al. 2023; Rawat et al. 2023; Tian et al. 2020). Its high moisture content, typically ranging from 73.9 % to 86.9 % depending on maturity stage, also acts as a natural co-solvent that enhances solubility and transport of bioactive compounds (Ahmad et al. 1994; Chien et al. 2008).

Supporting this, Tian et al. (2020) demonstrated that fresh rhizomes yield higher levels of zerumbone and exhibit stronger antimicrobial and cytotoxic activities than dried rhizomes. Similarly, Fahdi et al. (2025) reported that a lotion formulated with essential oil derived from fresh rhizomes showed 82.45 % repellent efficacy, together with desirable organoleptic properties, homogeneity, suitable spread ability (5–7 cm), skin-compatible pH (6.18–7.46), and four-week stability. Collectively, these findings highlight the superiority of fresh rhizomes in extraction studies, as its endogenous moisture not only facilitates compound release but also preserves bioactivity, supporting greener and more effective applications in natural product development.

Subcritical water extraction (SWE) has emerged as an effective and environmentally friendly method for recovering bioactive compounds from plant-based materials (Majeed et al. 2024; Somat et al. 2025). SWE uses the water's adjustable properties; such as its lower dielectric constant and increased diffusivity, to extract both polar and non-polar compounds without the need of organic solvents. It operates at high temperatures (100 ^{o}C to 374 ^{o}C) and under 10 to 11 bar to keep water in the liquid phase (Ahmad et al. 2025; Cova et al. 2019; Khurun et al. 2024; Özel & Göğüş 2014). This technique is particularly suitable for herbal and nutraceutical industries, including this rhizomatous ginger species, *Z. zerumbet* (Ahmad et al. 2025; Albaayit 2021; Ibáñez et al. 2023a; Wahab et al. 2022).

The efficiency of SWE process is frequently linked to operational parameters such as temperature, pressure, and extraction time. However, raw material's properties, especially moisture content, are receiving more attention. According to several studies, utilizing a small amount of water as a co-solvent increases extraction efficiency because it can cause cells to swell, which increases solvent penetration (Cheng et al. 2021; Degot, Huber, Touraud, et al. 2021). It is likely that the water in the rhizomes interacts with subcritical water to enhance the solubilization of bioactive compounds (Kostrzewa et al. 2021). This impact of initial moisture content on the chemical profile of SWE extracts, particularly on measurable parameters like pH, is still not well explored, though.

Extract pH serves as a simple yet meaningful indicator of chemical nature and stability, reflecting the presence of organic acids, degradation products, and other solubilized compounds. pH shifts in SWE may signal hydrolysis or breakdown reactions (Gbashi et al. 2017; Plaza & Turner 2015), which could be influenced by both the initial moisture content of the raw material and the extraction time. Understanding how these variables interact helps to improve process optimization and possible applications by offering important insights into the stability and composition of the extract.

A combined statistical framework comprising the General Linear Model (GLM) and Multiple Linear Regression (MLR) was applied to examine the effects of initial moisture content and extraction time on the pH of *Z. zerumbet* extracts. The GLM was suitable as it incorporated both the fixed effect of extraction time and the covariate of moisture content, while also accommodating potential random effects such as batch-to-batch variability of rhizomes (Ruíz et al. 2023). This allowed a more realistic representation of the data structure and reduced the risk of confounding from uncontrolled biological variation.

Complementing this, MLR provided a predictive approach to quantify the relative contributions of moisture and time to pH variation. Despite limitations associated with a modest sample size, MLR highlighted the emerging patterns between raw material properties and extract quality, offering practical insights into the relationship between processing conditions and product characteristics (Ehteram & Banadkooki 2023). Together, these approaches established a foundation for future studies by employing larger datasets and factorial designs to validate and refine the observed trends.

The objective of this study was to examine the effects of initial moisture content and the extraction time on the pH of *Z. zerumbet* extracts produced via SWE. By assessing the relationship between these two factors and the resulting pH, the study aimed to generate preliminary insights into the influence of raw material properties and process duration on the chemical characteristics of the extract. To achieve this, both GLM and MLR were applied. These complementary approaches enabled the evaluation of whether moisture content and extraction time acted independently or in combination to alter extract acidity, thereby providing a foundational basis for future optimization of SWE processes involving any plant-based materials.

MATERIALS AND METHOD

SAMPLE PROCUREMENT AND PRE-TREATMENT

Z. zerumbet rhizomes were harvested between April and May 2022 from a single farmer in Besut, Terengganu, Malaysia. The rhizomes were approximately 10 to 12 months old to ensure maturity and consistency of raw material. Plant material was supplied by Naturemedic Laboratories Sdn. Bhd., Chendering, Terengganu, and authenticated by the Herbarium, Universiti Sultan Zainal Abidin (voucher specimen UniSZA/A/000000006). Prior to extraction, the rhizomes were carefully inspected to take out spoilt or infested parts, then rinsed in detail under flowing tap water to eliminate soil and debris. The cleaned rhizomes were sliced into 2 to 3 mm thick pieces using a high-powered food processor (HR7320, Philips). The freshly prepared slices as shown in Figure 1 were immediately subjected to moisture content analysis and extraction to preserve its bioactive content.

(a) (b)

FIGURE 1. (a) *Z. zerumbet* fresh rhizomes (b) Slices of *Z. zerumbet* rhizomes

MOISTURE CONTENT ANALYSIS

Moisture content of *Z. zerumbet* fresh rhizomes was analyzed using a moisture analyzer (Precisa XM 66), as shown in Figure 2. To evaluate initial moisture levels, samples from different batches were arranged according to its assigned extraction times. This arrangement allowed the assessment of potential batch-to-batch variations that could influence subsequent extraction outcomes. For each measurement, approximately 10 g of cleaned rhizome slices, cut into 2 to 3 mm thickness, were evenly and finely spread on the weighing pan to ensure uniform drying and reproducible readings. The analyzer was operated under a standard drying program at 105 °C, with a weight change accuracy of < 0.01% (Mazian et al. 2018; Ray et al. 2022; William, 1999). Moisture content was automatically recorded once a stable value was reached, typically after ~92 min. All measurements were conducted in triplicate to ensure reliability. The measured moisture content for each batch/run was subsequently used as an independent variable in the statistical analysis to evaluate its effect, together with extraction time, on the pH of SWE extracts.

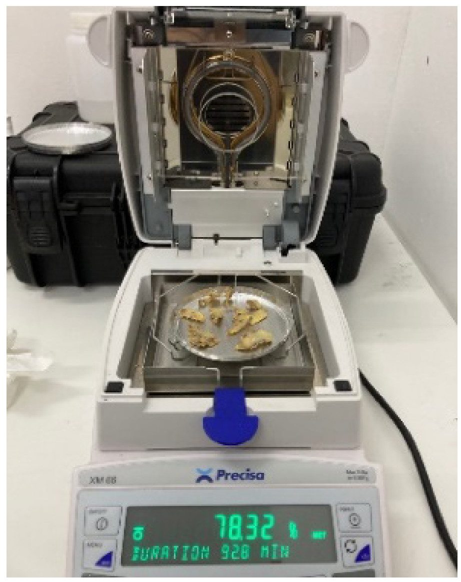


FIGURE 2. Moisture analyzer (Precisa XM 66)

SUBCRITICAL WATER EXTRACTION (SWE)

SWE was conducted on an industrial scale using a 70 L stainless steel batch system provided by Natxtract, AM Zaideen Ventures Sdn Bhd, Malaysia as shown in Figure 3. The extraction was performed following the single-factor experimental design (SFED) approach, where all other operating parameters remained constant except the extraction time, which was varied at 5, 10, 15, 20, and 25 min.

FIGURE. 3. Experimental setup of the 70 L SWE system and its main components

This SFED approach was chosen due to the constraints; (i) of the 70 L industrial-scale and raw material requirements, where each run required a minimum loading of approximately 8 kg of *Z. zerumbet* rhizomes to maintain the solids-to-solvent ratio of 1:4 (rhizomes : reverse osmosis water) and (ii) to keep the vessel approximately half-filled to ensure adequate headspace for safe pressure build-up. These constraints restricted the number of feasible runs and prevented factorial/response-surface experimentation at this stage.

The process was run at the low end of pressurized subcritical conditions (100 to 110 °C), with pressure maintained at 10 to 11 bar to keep water in the liquid phase, to balance extraction efficiency with the preservation of heat-sensitive constituents. 8 kg of cleaned rhizomes were put in a stainless-steel sieve basket for each run, submerged in thirty-two liters of reverse osmosis water, and heated to the desired temperature and pressure. A stopwatch was used to record the extraction time from the moment the desired temperature and pressure were achieved.

FREEZE-DRYING SAMPLE

Following extraction, the heated crude extracts were first cooled in a cooling vessel and then transferred into polyurethane (PU) bottles. The extracts were frozen until freeze-drying was carried out, which was done with a slightly modified version of a previously described method (Hanafi et al. 2021).

The main goals of freeze-drying were to; (i) stabilize the aqueous SWE extracts for storage and (ii) produce a dry extract that could be reconstituted at a fixed concentration for further analyses. This will improve comparability across extraction times.

The resulting freeze-dried extracts were labeled according to its respective extraction times and stored in airtight PU bottles for subsequent analysis. The process is illustrated in Figure 4, which shows (a) crude extract, (b) freeze-dried crude extract, and (c) bottled freeze-dried product.

(a) (b) (c)

FIGURE 4. Physical appearance of the extract at different stages : (a) crude extract, (b) freeze-dried crude extract, and (c) bottled freeze-dried product

PH DETERMINATION

Determining the pH of the samples is essential in comprehending its chemical characteristics, whether it is acidic or alkaline. The pH of the samples in this study was measured using a glass electrode pH meter (the Eutech™ PC 700 multiparameter meter from Thermo Scientific™) as shown in Figure 5.

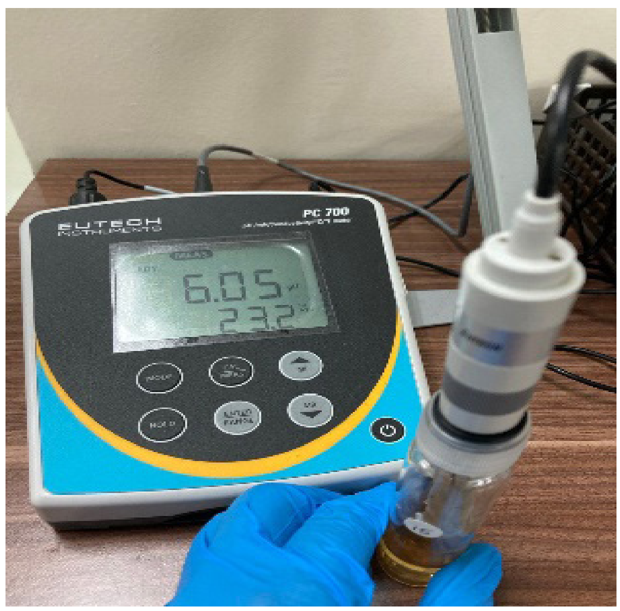


FIGURE. 5. pH analysis

Freeze-dried extracts were measured at room temperature after being reconstituted in distilled water at a fixed concentration of 1 mg/mL (Thomas 2018). Each sample was measured three times (triplicate), and the mean pH values were calculated and recorded. Although it is recognized that freeze-drying and reconstitution may influence absolute pH values (e.g., loss of volatile acidic/basic constituents or CO_2-related effects), all samples were processed and reconstituted under the same conditions, thus relative comparisons among extraction times remain internally consistent.

STATISTICAL ANALYSIS

The influences of extraction time and initial moisture content on the pH of extracts were analyzed using IBM SPSS Statistics version 8.5. The dependent variable was pH (as measured after SWE), the fixed factor was the extraction time (5, 10, 15, 20, and 25 min), and the covariate was the initial moisture content of fresh rhizomes (in order to account for baseline batch-to-batch variations). Two complementary approaches were employed; (i) General Linear Model (GLM; Univariate/ANCOVA) and (ii) Multiple Linear Regression (MLR). To evaluate both extraction time and moisture content correlation with extract pH, the GLM incorporated extraction time (5-25 min) as its fixed factor and moisture content (measured for each batch/run) as its covariate. It was to obtain covariate-adjusted mean pH values across extraction times (IBM SPSS Statistics v8.5) too. Estimated marginal means (EMMEANS) were compared using the Least Significant Difference (LSD) adjustment, which applies pooled error variance from the GLM to test pairwise differences among time levels (Mehrbod et al. 2021).

To investigate whether the moisture-pH relation varied across time levels, a Time×Moisture interaction term was also incorporated. However, since the extraction followed an SFED/time-based design and moisture content was not experimentally changed, any interaction inference was considered exploratory rather than confirmatory.

A Time×Moisture interaction term was also included to explore whether the moisture–pH relationship differed across time levels; however, because moisture content was not experimentally manipulated and the extraction followed an SFED/time-based design, any interaction inference was interpreted as exploratory rather than confirmatory.

Subsequently, MLR was applied as a complementary, predictive analysis with pH as the outcome and the same predictors including the interaction term to mirror the GLM specification (Çalhan et al. 2023). The MLR yielded an explicit prediction equation and model-fit indices (R^2, adjusted R^2), quantifying variance in pH explained jointly by moisture and time. Although the samples' sizes were modest, the combined GLM and MLR approaches provided convergent inferential and predictive evidence thus establishing a methodological basis for future studies with larger, more tightly controlled designs.

RESULTS AND DISCUSSION

MOISTURE AND TIME EFFECTS ON PH OF EXTRACTS

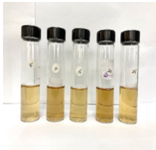
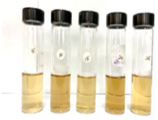
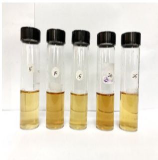
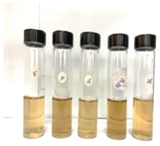
The average moisture content of fresh *Z. zerumbet* rhizomes before extraction is shown in Table 1, along with the corresponding pH values of the extracts made using SWE at different extraction times. The initial moisture content was at its lowest at 20 min and at its highest at 25 min, ranging from 72.53 ± 1.55% to 76.77 ± 0.49% respectively. All extracts fell into the slightly acidic range, with corresponding pH values ranging from 5.85 ± 0.03 to 6.24 ± 0.02.

A notable trend observed was the inverse relationship between pH and moisture content. The extract exhibited the lowest pH (5.85 ± 0.03) at 25 min, when the highest moisture content (76.77 ± 0.49 %) was recorded, indicating increased acidity. On the other hand, the highest pH value (6.26 ± 0.02) was recorded at 20 min, along with the lowest moisture content (72.53 ± 1.55%).

All extracts maintained an acidic character within a narrow range despite variations in pH and moisture content. This suggests a stable chemical environment that is typical of aqueous extracts based on *Z. zerumbet*. This slightly acidic character too, may contribute positively to the extracts' stability and preservation potential. Particularly, in food or pharmaceutical applications where pH plays an important role in product safety and efficacy.

As the extraction time increased, the extracts only showed minor visual changes. The color appeared generally consistent across all samples, with only a slight tendency toward a darker shade at the longer durations (20 and 25 min). This slight variation might be related to gradual solubilization of compounds from the phenolic or Maillard reaction as a result of prolonged thermal exposure (Kheirkhah et al. 2019). Nonetheless, there was no evidence of phase separation or instability, suggesting that the extracts were constantly uniform and physically stable.

TABLE 1. Moisture content of fresh rhizome prior to extraction and corresponding extract pH

	Extraction time				
	5 min	10 min	15 min	20 min	25 min
Moisture content prior to extraction (%)	75.10 ± 1.15	74.40 ± 0.53	74.33 ± 0.61	72.53 ± 1.55	76.77 ± 0.49
pH extract	6.04 ± 0.01	6.20 ± 0.01	6.04 ± 0.02	6.24 ± 0.02	5.85 ± 0.03
Acidity	Slightly acidic	Slightly acidic	Slightly acidic	Slightly acidic	Slightly acidic
Visual appearance and color					

All samples were consistently "slightly acidic", reflecting the mildly acidic environment of subcritical water, which can aid in the breakdown of plant cell walls and the release of bioactive compounds without significantly altering pH. Other than that, certain compounds in the sample may oxidize as the extraction time lengthen thus leading to the formation of products that could either raise or lower the pH (Díaz-Reinoso et al. 2023; Majeed et al. 2024).

Since the extraction was done using fresh rhizomes, it was assumed that the rhizome matrix's natural water content, which can also act as a co-solvent, would benefit the extraction mechanism (Kostrzewa et al. 2021). The water present in the rhizomes is likely to interacts with subcritical water, enhancing bioactive compound solubilization. (Degot, Huber, Hofmann, et al. 2021). Operating at high temperatures and pressures, subcritical water exhibits altered properties such as polarity and solvation ability, like that of organic solvents. It has been hypothesized that the additional moisture in the rhizomes contributes to the extraction medium and potentially facilitates the breakdown of cell walls and the release of intracellular compounds (Degot, Huber, Touraud, et al. 2021; Díaz-Reinoso et al. 2023).

GENERAL LINEAR MODEL

The effect of extraction time and initial moisture content on the pH of *Z. zerumbet* extracts was evaluated using a General Linear Model (Univariate, ANCOVA). The overall model was significant, $F(5, 9) = 101.63$, $p < 0.001$, accounting for 98.3 % of pH variance (adjusted $R^2 = 0.973$).

Extraction time exerted a highly significant influence on pH, $F(4, 9) = 55.92$, $p < 0.001$, with a very large effect size (partial $\eta^2 = 0.961$). This indicates that approximately 96.1 % of the variance in pH was attributable to extraction time. In contrast, initial moisture content did not have a significant effect, $F(1, 9) = 2.79$, $p = 0.129$, with a partial η^2 value of 0.237, accounting for only 23.7 % of pH variance. This suggests that batch-to-batch variability in baseline moisture did not reliably predict pH within this dataset.

Estimated marginal means (EMMEANS) (adjusted for moisture) demonstrated in Figure 6 shows a clear decreasing trend in pH with increasing extraction time. Covariates in the model were evaluated at a moisture content of 74.63 % with error bars representing 2 standard errors of the mean, approximating a 95 % confidence interval.

At 74.63 % mean moisture, the adjusted pH values were 6.034 (5 min), 6.203 (10 min), 6.044 (15 min), 6.264 (20 min), and 5.826 (25 min). Pairwise comparisons showed that the pH at 25 min was significantly lower than at any shorter extraction time ($p < 0.001$). Among the shorter time, 10 min and 20 min extracts yielded the highest adjusted pH values, while 5 min and 15 min extracts were slightly lower but still significantly higher than 25 min.

These findings indicate that prolonged extraction under subcritical water conditions resulted in a measurable reduction in pH, whereas variations in initial moisture content were not statistically influential under the tested conditions. The adjusted means highlight that the most pronounced decline occurred at 25 min, confirming that extraction time was the dominant factor driving acidity changes in the fresh rhizome extracts.

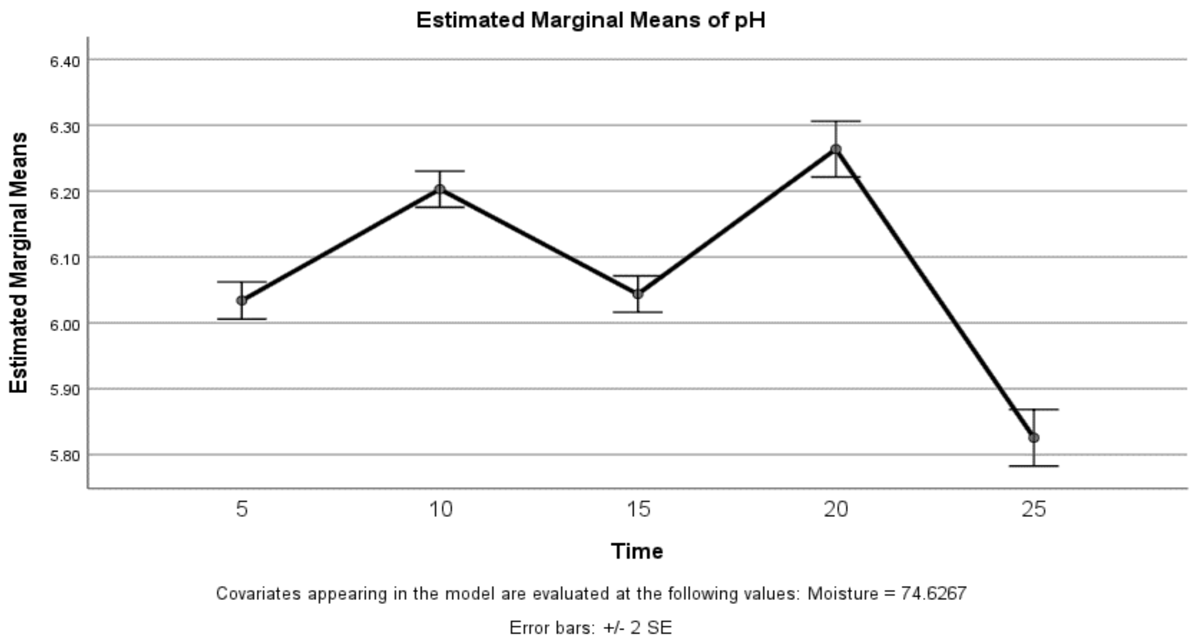


FIGURE. 6. Estimated marginal means of pH across extraction time

MULTIPLE LINEAR REGRESSION ANALYSIS

MLR was applied to evaluate the simultaneous influence of initial moisture content and extraction time on the pH of *Z. zerumbet* extracts. Moisture content (% wet basis) and extraction time (min, dummy-coded with 25 min as the reference group) were entered as predictor variables, while pH served as the quantitative response. This approach was selected for its ability to model both individual and combined contributions of input parameters to extract acidity.

In the first step of the analysis, when moisture content was considered in isolation, it significantly predicted pH (B = – 0.065, p = 0.002), accounting for 55.0 % of pH variance. This indicated that higher initial moisture content was associated with lower pH values. However, after extraction time was added to the model, the regression coefficient for moisture content became positive (B = 0.013) but not significant (p = 0.129), showing that its effect on pH was no longer reliable once extraction time was controlled.

However, in the second step, when extraction time was introduced into the model, the explained variance increased markedly to 98.3 % (Adjusted R^2 =0.973). In this final model, extraction time emerged as the dominant predictor of pH, while the effect of moisture was no longer significant (B = 0.013, p = 0.129). These results indicate that prolonged extraction under subcritical water conditions consistently lowered the pH of the extracts.

Taken together, the findings demonstrate that although initial moisture content appeared influential when assessed alone, its effect was overshadowed once extraction time was accounted. Thus, extraction time represents the primary driver of acidity changes in the extracts, whereas batch-to-batch variation in baseline moisture content exerted only a minor and inconsistent influence under the conditions tested. These outcomes highlight the need to prioritise extraction parameters, particularly extraction duration, while still recognising raw material variability as an important factor to be addressed in future optimisation studies.

The small sample size of the MLR reduces statistical power and may limit the stability and generalizability of coefficients, particularly for moisture content (and any interaction terms, where applicable). The model is proof that extraction time is the dominant predictor of pH under the conditions investigated and guides the design of future factorial studies with larger datasets; however, the regression estimates should be interpreted as preliminary.

STATISTICAL AND PREDICTIVE EVIDENCE FOR EXTRACTION TIME AS THE DOMINANT DRIVER OF PH REDUCTION

Both GLM and MLR consistently identified extraction time as the primary determinant of pH in *Z. zerumbet* extracts. The GLM (ANCOVA) highlighted that extraction time explained over 96 % of pH variance, while moisture content was not statistically significant once time was controlled. This reinforced that process duration, rather than baseline raw material variability, was the dominant factor influencing extract acidity.

The MLR analysis complemented these findings by providing a predictive perspective. Moisture content alone initially showed a significant inverse relationship with pH, but its effect disappeared when extraction time was added, increasing the explained variance to 98.3 %. Thus, while GLM emphasised hypothesis testing and effect sizes, MLR strengthened the interpretation through predictive modelling, with both approaches agreeing that extraction time is the critical driver of pH reduction.

Beyond the statistical confirmation of extraction time as the key determinant, these findings align with the fundamental chemistry of SWE. Prolonged exposure to high temperature and pressure promotes thermal hydrolysis and oxidative degradation of labile compounds (Antony & Farid 2022; Mieszczakowska-Frąc et al. 2021).

This could explain the generation of organic acids that directly lower the pH. In contrast, the initial moisture of the rhizome, while potentially facilitating solvent penetration and cell wall rupture (Belwal et al. 2020; Degot, Huber, Touraud, et al. 2021; Wahab et al. 2022), exerts only a modest influence compared to the overwhelming effect of extraction duration. This explains why its contribution is masked when both factors are analyzed simultaneously.

From an operational perspective, this has important implications for process control and optimization. Extraction time is a fully controllable parameter that can be precisely adjusted by operators, making it a reliable target for standardization across batches. On the other hand, moisture content is frequently influenced by several factors that are more difficult to control on a larger scale.

These include varietal genetic diversity, harvested rhizome maturity, cultivation methods, post-harvest handling, harvest timing, local environmental conditions, and storage conditions. Specific soil conditions in various geographical areas may also contribute to the observed differences (Baby et al. 2009; Ibáñez et al. 2023b; Tian et al. 2020). Even though it has secondary effect, uncontrolled moisture variability may still cause variations in extract pH, suggesting that some degree of raw material quality control is still required.

These insights emphasize the importance of prioritizing extraction time as a critical process variable in the context of industrial application to guaranteed stability, acidity, and retention of bioactive compound in *Z. zerumbet* extracts. Simultaneously, regularly checking the moisture content of raw materials could also act as an extra precaution against inconsistency.

To capture possible saturation or threshold effects, future research should extend this work by testing non-linear regression models and adding additional parameters such as extraction temperature and pressure. Furthermore, the application of predictive validation techniques, such as independent dataset testing or cross-validation, would improve these models' generalizability and robustness.

CONCLUSION

This study demonstrated that the primary factor affecting the pH of *Z. zerumbet* extracts made under SWE conditions is extraction time. When examined separately, initial rhizome moisture content showed an inverse relationship with pH; yet, once extraction time was taken into consideration in both GLM and MLR models, this relationship disappeared. These extraction time explained more than 96.1 % of pH variance, confirming its role as the primary driver of acidity changes.

While moisture acts more indirectly by promoting solvent penetration and compound release, the chemical decrease in pH with extended extraction is consistent with hydrolytic and oxidative processes that produce organic acids under high temperature and pressure. From the perspective of processing, rhizome moisture content variations can still cause small fluctuations and should be tracked as part of raw material quality control, but extraction time is a controllable parameter that can be standardized for repeatable results.

Overall, these findings highlight that to optimized SWE for *Z. zerumbet,* extraction duration must be given priority, and moisture variability must be acknowledged as a variable that must be controlled. Future research should include more extraction parameters, predictive validation approaches, and a wider range of raw material sources to further refine process control and enhance the consistency, stability, and potential applications of *Z. zerumbet* extracts in food, nutraceutical, and pharmaceutical products.

ACKNOWLEDGEMENT

The authors express their sincere gratitude for the collaborative efforts, facility support, valuable expertise, and generous funding from JICA-MJIIT UTMKL and Natxtract, AM Zaideen Ventures Sdn Bhd. These contributions have been instrumental in the successful completion of this research project.

DECLARATION OF COMPETING INTEREST

None.

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