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Enhancement of Biscuit Quality and Stability with Addition of *Acorus calamus* Phenolic Extract

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ABSTRACT

This study investigated the effect of different solvents on the extraction yield, phenolic profile, and antioxidant activity of *Acorus calamus* roots, as well as the impact of incorporating the phenolic extract into biscuit formulations. Among the tested solvents, 70% ethanol extract yielded the highest extraction efficiency (20.2%) and exhibited strong total phenolic content (86.96 mg/g) with potent antioxidant activity (DPPH: 89.29%; ABTS: 88.54%). Methanol 80% extract showed the highest total phenolic content (105.35 mg/g). Major phenolic compounds identified included chlorogenic acid, gallic acid, caffeic acid, rutin, and quercetin, with their levels varying across solvents. Biscuits enriched with increasing concentrations of *Acorus calamus* 70% ethanol (100, 200, 300 ppm) demonstrated elevated total phenolic content (from 30.5 to 58.9 mg GAE/g at zero time) and reduced peroxide values over 3 months of storage (from 5.8 to 3.3 meq/kg at 3 months). Texture analysis showed decreased hardness and increased crispness and cohesiveness. Color analysis revealed a gradual darkening of biscuits with higher extract concentrations. Differential Scanning Calorimetry (DSC) indicated enhanced thermal stability in fortified biscuits, with increasing onset and peak gelatinization temperatures. These results suggest that *Acorus calamus* phenolic extract is a promising natural additive for enhancing the nutritional and storage qualities of bakery products.

Keywords: *Acorus calamus*, Phenolic compounds, Antioxidant activity, Functional biscuits, Extraction solvents

INTRODUCTION

The rising demand for functional foods has intensified interest in using natural bioactive compounds, particularly phenolic extracts from medicinal plants, due to their antioxidant, antimicrobial, and health-promoting properties (Balasundram *et al.*, 2006). In this context, *Acorus calamus*—commonly known as sweet flag—has emerged as a promising source of phenolic compounds with demonstrated antioxidant and antimicrobial activity (Li and Wah, 2017, Rajput *et al.*, 2014). Recent studies have highlighted the strong free radical scavenging capacity of *A. calamus* extracts, making it a viable candidate for enhancing the oxidative stability of lipid-containing food systems (Wani *et al.*, 2024).

These plant-derived phenolics offer potential as natural alternatives to synthetic additives, with roles in preserving lipid-rich foods, enhancing sensory attributes, and improving nutritional quality. Their incorporation into bakery products aligns with the growing preference for clean-label and functional foods (Joshi *et al.*, 2022). For instance, extracts from herbs like rosemary (*Rosmarinus officinalis*), green tea (*Camellia sinensis*), oregano (*Origanum vulgare*) and others have been successfully incorporated into meat products, dairy items, and baked goods as natural alternatives to synthetic additives (Enejo and Martins, 2024, Ivanišová *et al.*, 2021, Paswan *et al.*, 2021). Additionally, the incorporation of phenolic-rich extracts has been linked to improved consumer

perception and marketing potential, especially in the context of clean-label and functional foods (Rudrapal *et al.*, 2022). These developments underline the promising role of herbal phenolic compounds not only in improving food safety and stability, but also in delivering potential health benefits associated with their antioxidant, anti-inflammatory, and antimicrobial activities.

Biscuits, as widely consumed cereal-based snack products, are particularly susceptible to oxidative degradation due to their high fat content and exposure to air during storage. This leads to rancidity, flavor deterioration, and reduced shelf life (Pasqualone *et al.*, 2021). Furthermore, the addition of synthetic antioxidants such as BHA and BHT has raised consumer concerns regarding safety and toxicity (Wang *et al.*, 2021), thereby encouraging the search for natural alternatives (Shahidi and Ambigaipalan, 2015). Incorporating plant-derived phenolics into bakery products presents an attractive strategy for improving both nutritional quality and storage stability.

Despite the known bioactivity of *A. calamus*, its potential application in food systems—particularly baked products like biscuits—remains underexplored. The present study aims to investigate the effects of incorporating *Acorus calamus* phenolic extract into biscuit formulations, focusing on physicochemical characteristics, antioxidant capacity, and shelf-life stability.

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MATERIALS AND METHODS

Materials

Rhizomes of *Acorus calamus* were procured from a local herbal market in Kafr El-Sheikh, Egypt in 2025. All chemicals and reagents used in this study were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA).

Methods

Extraction of *Acorus calamus* extracts by different solvents

The method described by (Owon *et al.*, 2021) was used to prepare *Acorus calamus* extract with some modifications. Initially, 20 g of sample were macerated with different solvents (water, ethanol, 70% ethanol, methanol and 80% methanol) at room temperature for two days, followed by filtration of the resulting mixture. The residue was further re-extracted using the same method, repeated thrice. The collected filtrates were combined and evaporated at 40 °C using a rotary evaporator, then frozen at -80 °C. The extract was then lyophilized and stored at -20 °C until use.

Total phenolic compounds

To determine the total phenolic components, the Folin-Ciocalteu procedure as described by (Attard, 2013) with some modifications was used. The procedure involved combining 10 µL of either the sample or standard with 100 µL of Folin-Ciocalteu reagent (diluted to 1:10), followed by adding 80 µL of 1M Na₂CO₃. The mixture was then incubated in the dark at 25 °C for 20 minutes. The resultant blue complex color was detected at 630 nm. Data are presented as averages ± SD. Gallic acid was prepared as a standard.

Antioxidant activity of *Acorus calamus* different extracts (DPPH and ABTS)

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) scavenging activity was carried out using the procedure explained by (Boly *et al.*, 2016). Briefly; 100 µL of reconstituted *Acorus calamus* extract were mixed with freshly prepared 0.1% DPPH dissolved in methanol, the reaction was left in at room temperature for 30 min (in dark). Deionized water was used as a blank instead of the *Acorus calamus* extract sample. The reduced color intensity of DPPH was measured at 520 nm. Data are presented as averages ± SD according to the following equation:

% DPPH inhibition = ((Absorbance of blank - Absorbance of sample) / (Absorbance of blank)) × 100

ABTS radical scavenging activity for *Acorus calamus* extract was determined (Kim *et al.*, 2018). The ABTS work solution was prepared by combining potassium persulfate (145 mM) with ABTS reagent (7 mM) and staying in the dark (12 hours). To ensure the absorbance was within a suitable range (not exceeding 0.8), the preserved work solution was diluted with PBS (0.2 M, pH 7.4). Next, the film extract (20 mL) was mixed with the prepared work solution (1980 µL) and the absorbance was measured at 734 nm.

ABTS radical scavenging activity (%) = $\left(1 - \frac{Abs\ 1 - Abs\ 2}{Abs\ 0}\right)$

Where:

Abs 0 indicates the absorbance related to ABTS, Abs 1 signifies the absorbance associated with samples, and A2 represents the absorbance for the samples mixed with PBS.

Phenolic compounds determination

The quantification of phenolic compounds in *Acorus calamus* extracts was carried out using HPLC-DAD. For this analysis, 30 mg of freeze-dried extract was dissolved in 350

µL of methanol and filtered through PTFE syringe filter (0.45 µm). The analysis was performed using HPLC equipped with a photodiode array detector (Waters Div., Milford, MA, USA) and a reversed-phase LC column (250 mm × 4.6 mm, 5 µm) (Torrance, CA, USA) was utilized at 30 °C. The mobile phases consisted of (A) formic acid (0.5%) in water and (B) formic acid (0.5%) in acetonitrile with stable flow rate (0.8 mL/min), and the injection volume was 10 µL (Paesa *et al.*, 2022). Phenolic compounds were identified by comparison of retention times and UV-Vis spectra with those of authentic standards (gallic acid, caffeic acid, chlorogenic acid, ferulic acid, quercetin).

Preparation of biscuits

Biscuit preparation

To prepare the biscuits, a traditional recipe was followed: one egg was thoroughly mixed with 125 g of sugar. The antioxidants were dissolved in 60 mL of water and added to the mixture. Then, 300 g of wheat flour were sequentially added to the mixture while mixing vigorously with a hand mixer at 450 W during 8 min (Bosch, Munich, Germany). After 15 min of rest the dough with the intended consistency was reduced to 5 mm thickness and cut by a round biscuit cutter with 50 mm internal diameter. Four samples of biscuits (30 per sample, 6 biscuits for each storage time) were prepared: i) control biscuits – without any antioxidant, ii) three samples of biscuits with *Acorus calamus* 70% ethanolic extract (100, 200 and 300 mg). The biscuits were baked in an electric oven for 10 min at 180 °C. All samples were stored for three months (at room temperature and packed in a sealed plastic bag covered with aluminum paper) (Salama *et al.*, 2024).

Peroxide value (PV)

Peroxide value (PV) is a metric that measures the total amount of peroxides in oil (expressed in meq.O₂/kg oil). To determine the PV, 5 grams of oil (oil extracted from biscuits by soxhelt) were dissolved in a 60:40 mixture of acetic acid and isooctane. Saturated KI (0.5 mL) was added and vortexed for 60 Sec. The solution was titrated with a 0.1 mol/L solution of alcoholic potassium hydroxide (KOH) using 100 milliliters of boiled Millipore water until the first equivalence point. The Tiamo software controlled the METHROM Titrand 888 titrator during the titration procedure. This automated process allowed for the accurate calculation of the peroxide value, expressed as the amount of active oxygen per kilogram of oil (Salama *et al.*, 2020).

Texture analyser of biscuits

A texture analyzer (Instron 1011, Norwood, MA, USA), equipped with a 50-N load cell, was employed for the determination of texture of samples. Glass tubes with internal diameter of 2 cm were filled with 15 mL of oleogel and stored at 4 °C. The penetration force was measured at 2 cm depth of samples after plunging a cylindrical probe with a penetration speed of 100 mm/min. The maximum force was reported as a penetration force (N) (Hayes *et al.*, 2005).

Differential Scanning Calorimetry (DSC) Analysis

The thermal properties of the biscuit samples were analyzed using Differential Scanning Calorimetry (DSC) (Model Q2000, TA Instruments, USA). Approximately 5–10 mg of finely ground biscuit sample was accurately weighed and sealed in an aluminum pan. An empty, sealed aluminum pan was used as the reference. The samples were scanned from 25 °C to 300 °C at a heating rate of 10 °C/min under a constant nitrogen flow of 50 mL/min to prevent oxidative

degradation. Thermograms were recorded, and the onset temperature (To), peak temperature (Tp), endset temperature (Te), and enthalpy change (ΔH) were determined using the instrument's analysis software. All measurements were performed in triplicate to ensure reproducibility (Alshehri *et al.*, 2025).

$$\Delta H = \frac{A}{m}$$

Where: ΔH = Enthalpy change (J/g), A = Area under the DSC peak and m = Mass of the sample.

Statistical analysis

The statistical analysis of the data was performed using SPSS software (Version 16.0, SPSS Inc., Chicago, IL) through a one-way analysis of variance (ANOVA) method to assess variance. The mean values and standard deviations were computed based on three repeated measurements (Owon *et al.*, 2021).

RESULTS AND DISCUSSION

Effect of Extraction Solvent on Yield, Total Phenolic Content, and Antioxidant Activity of *Acorus calamus* Extracts

The efficiency of five solvents—distilled water, pure ethanol, pure methanol, 70% ethanol, and 80% methanol—was evaluated based on extraction yield, total phenolic content (TPC), and antioxidant activity (DPPH and ABTS radical scavenging). The results, summarized in Table 1, show that the choice of solvent significantly influenced all measured parameters.

The extraction yield varied markedly among the solvents ($p < 0.05$). The highest yield was observed with 70% ethanol extract (20.2%), followed closely by 80% methanol extract (19.38%), whereas the lowest yield was recorded in aqueous extract (10.82%). This trend aligns with the general understanding that aqueous organic solvents offer superior extraction efficiency compared to pure solvents or water alone. Mixed solvents exhibit intermediate polarity, facilitating better penetration into plant tissues and improved solubilization of a broader range of phenolic compounds (Chen *et al.*, 2005). Similar findings were reported by (Owon *et al.*, 2021), who noted increased extractability of phenolics from moringa different parts using 80% methanol or 70% ethanol.

Total phenolic content (expressed as mg gallic acid equivalents per gram of dry sample) ranged from 42.75 mg/g (water) to 105.35 mg/g (80% methanol). Among the tested solvents, 80% methanol extract resulted in the highest TPC, followed by methanol extract (88.11 mg/g) and 70% ethanol extract (86.96 mg/g). Water, as the least polar solvent in the group, exhibited the lowest phenolic extraction efficiency, likely due to its limited ability to dissolve less polar phenolic compounds. The effectiveness of 70% ethanol extract and 80% methanol extract is consistent with previous studies indicating that methanol-water mixtures enhance phenolic extraction by balancing polarity and hydrogen bonding interactions (Owon *et al.*, 2021, Namvar *et al.*, 2017).

Antioxidant activity, assessed via DPPH and ABTS radical scavenging assays, further demonstrated the influence of solvent polarity. The highest DPPH scavenging activity was recorded in the 70% ethanol extract (89.29%), followed by 80% methanol extract (75.36%) and methanol extract (80.64%). For ABTS, 80% methanol extract displayed the highest scavenging activity (92.73%), with 70% ethanol extract showing comparable efficacy (88.54%).

Interestingly, despite 80% methanol extract having the highest TPC, it did not exhibit the strongest DPPH activity. This discrepancy highlights that antioxidant activity is not solely dependent on total phenolic content but also on the structural characteristics of the phenolic constituents (Vuolo *et al.*, 2019). It is well-documented that different phenolic compounds exhibit varying degrees of radical scavenging due to differences in hydroxylation patterns, molecular size, and degree of conjugation (Cai *et al.*, 2006, Cheng *et al.*, 2002). Thus, the relatively higher DPPH activity in the 70% ethanol extract may be attributed to the presence of more potent individual phenolic antioxidants. Over all, the results indicate that mixed solvents—particularly 70% ethanol extract and 80% methanol—are more effective than pure solvents or water for extracting phenolic compounds with high antioxidant activity from *Acorus calamus* roots. Given its high extraction yield, strong antioxidant potential, and practical food safety profile, 70% ethanol extract may be considered the most suitable solvent for functional food applications involving *Acorus calamus* extracts.

Table 1. Effect of using different solvents on extraction yield, total phenolic content (mg GAE/g sample) and antioxidant activity (DPPH and ABTS) of *Acorus calamus* roots extracts

Parameters Solvent	Extraction yield [%]	Total phenolic content (TPC) (mg GAE/g sample)	Antioxidant activity (DPPH%)	Antioxidant activity (ABTS%)
Distilled water	10.82±0.54d	42.75 ± 2.69d	58.41±3.59c	60.27±3.61c
Pure ethanol	14.25±0.72c	74.65±5.22c	75.32±4.52b	83.45±5.0b
Pure methanol	16.46±0.82b	88.11±6.17b	80.64±4.8b	78.63±4.72b
Ethanol 70%	20.2±1.83a	86.96±6.08b	89.29±5.53a	88.54±5.31a
Methanol 80%	19.38±0.97a	105.35±7.35a	75.36±4.52b	92.73±5.52a

Values are means ± SD. Means having the different case letter(s) within a column are significantly different at $P \leq 0.05$.

Concentration of Individual Phenolic Compounds (mg/g extract) in *Acorus calamus* Extracts Using Different Solvents

The type of solvent used had a significant impact on the concentration and profile of phenolic compounds extracted from *Acorus calamus* roots (Table 2). Among the six phenolic compounds analyzed—chlorogenic acid, gallic acid, vanillic acid, caffeic acid, rutin, and quercetin—differences in extractability were observed depending on solvent polarity and composition.

The highest chlorogenic acid content was recorded in the extract obtained with 80% methanol extract (12.1 mg/g), followed by 70% ethanol extract (10.5 mg/g). Aqueous extract yielded the lowest concentration (8.8 mg/g). Chlorogenic acid is moderately polar, and its higher solubility in aqueous organic solvents compared to aqueous extract is consistent with earlier findings (Belay *et al.*, 2016), where chlorogenic acid was optimally extracted using hydroalcoholic solutions due to enhanced solubility and diffusion from plant matrices.

The highest gallic acid concentration was observed in the 80% methanol extract (14.9 mg/g), followed by 70% ethanol extract (13.6 mg/g). Aqueous extract and absolute ethanol extract yielded lower amounts (11.2 and 12.1 mg/g, respectively). Studies by Ignat *et al.* (2011) reported similar solvent-dependent trends in gallic acid extraction efficiency from fruit and herbal matrices.

Vanillic acid content ranged from 8.7 mg/g (methanol) to 10.9 mg/g (70% ethanol), suggesting that 70% ethanol extract facilitated the most efficient extraction. Vanillic acid's extraction appears to be favored by the intermediate polarity of 70% ethanol, which has been similarly noted by Ghasemzadeh *et al.* (2015) in phenolic acid extraction from medicinal plants.

Caffeic acid content was highest in the 80% methanol extract (18.2 mg/g), followed by 70% ethanol extract (16.4 mg/g). This reflects its amphiphilic nature, where both hydrophilic and lipophilic interactions play a role. Aqueous extract yielded a significantly lower amount (14.1 mg/g). The improved extraction in aqueous methanol is in agreement with results from (Medini *et al.*, 2014), who found methanol-

water mixtures most effective for caffeic acid extraction from *Teucrium* species.

Flavonoids such as rutin and quercetin showed the highest concentrations in the 80% methanol extract (12.7 and 16.5 mg/g, respectively). These compounds are moderately polar and benefit from the presence of both organic and aqueous phases. Rutin and quercetin were lowest in ethanol extract and aqueous extract extracts, confirming that solvent polarity significantly influences flavonoid solubility and extraction yield. This is supported by (Lapomik *et al.*, 2005), who demonstrated that ethanol-water mixtures improve flavonoid extraction due to better cell wall permeability and compound solubility. Overall, 80% methanol extract consistently extracted the highest concentrations of all phenolic compounds tested, followed by 70% ethanol. This confirms the superior efficiency of aqueous organic solvents in extracting a wide range of phenolic acids and flavonoids. The results are in agreement with numerous studies demonstrating that solvent polarity, in combination with compound solubility and matrix interactions, is critical to optimizing phenolic compound extraction (Dai and Mumper, 2010, Do *et al.*, 2014).

Table 2. Effect of using different solvent on Phenolic compounds (mg/g sample) of *Acorus calamus* roots extracts

Phenolic compounds Solvent	Cholorogenic acid	Gallic acid	Vanillic acid	Caffeic acid	Rutin	Quercitin
Distilled water	8.8 ± 0.59c	11.2 ± 0.66c	9.9 ± 0.50c	14.1 ± 0.86d	10.3 ± 0.56b	12.1 ± 0.76c
Pure ethanol	10.2 ± 0.51ab	12.7 ± 0.64b	8.7 ± 0.43d	15.8 ± 0.79c	10.2 ± 0.51b	14.2 ± 0.71b
Pure methanol	9.9 ± 0.50b	12.1 ± 0.61b	9.1 ± 0.46b	14.6 ± 0.73d	9.8 ± 0.49c	13.6 ± 0.68b
Ethanol 70%	12.1 ± 0.61a	14.9 ± 0.75a	10.5 ± 0.52a	18.2 ± 0.91a	12.7 ± 0.64a	16.5 ± 0.83a
Methanol 80%	10.5 ± 0.52ab	13.6 ± 0.68a	10.9 ± 0.45a	16.4 ± 0.82b	11.1 ± 0.56b	14.9 ± 0.75b

Values are means ± SD. Means having the different case letter(s) within a column are significantly different at $P \leq 0.05$.

Texture Profile Analysis of Biscuits Enriched with Different Concentrations of *Acorus calamus* Extract

Texture is a critical quality attribute influencing consumer acceptance of bakery products. The incorporation of *Acorus calamus* phenolic extract at three concentrations (100, 200, and 300 mg) showed notable yet subtle effects on various mechanical texture parameters of the biscuits, including hardness, crispness, fracturability, cohesiveness, chewiness, and springiness (Table 3).

Hardness values decreased progressively with increasing extract concentration, from 45.0 N in the control to 43.0 N at 300 mg. Although the changes were modest, this trend suggests that the phenolic extract slightly softened the biscuit structure (Cao *et al.*, 2022). This could be attributed to the interaction of phenolic compounds with gluten or starch matrices, which may interfere with protein-protein cross-linking and reduce dough strength (Schefer *et al.*, 2021). Similar softening effects have been observed in biscuits enriched with plant extracts (Mildner-Szkudlarz *et al.*, 2011, Salihu *et al.*, 2023), where phenolic compounds interfered with gluten network formation, leading to decreased hardness.

Crispness, expressed as the number of fracture peaks, increased slightly from 5.0 (control) to 5.5 at 300 mg. The higher number of peaks indicates enhanced crispiness, possibly due to the influence of polyphenols on moisture migration and air cell distribution during baking (Ou *et al.*, 2019, Qin *et al.*, 2022). Polyphenols may contribute to a more porous or brittle crumb structure, thereby increasing fracture events. Similar improvements in crispness were reported in cookies fortified with plant-derived polyphenols such as ginger extracts (Abozed *et al.*, 2024).

Fracturability values slightly decreased from 12.8 N (control) to 12.2 N at 300 mg, consistent with the observed reduction in hardness. A softer, yet crisp matrix may fracture more easily under lower force. This is beneficial for texture perception, as consumers often associate reduced fracturability with tender, high-quality biscuits. Ajila *et al.* (2008) reported similar trends when incorporating mango peel phenolic fiber into baked goods.

Cohesiveness improved with increasing extract levels, from 0.72 (control) to 0.76 (300 mg). This suggests better internal bonding and structural integrity of the biscuit matrix in the presence of phenolic compounds. Polyphenols may interact with proteins or polysaccharides, forming cross-links that enhance internal consistency (Yan *et al.*, 2025). Schefer *et al.* (2021) found that phenolic-protein interactions can increase cohesiveness and network strength in dough systems.

Chewiness showed a slight decline from 18.5 N•mm (control) to 17.6 N•mm (300 mg). This could be attributed to the combined effect of reduced hardness and fracturability, resulting in a softer and less chewy texture. Reduced chewiness is often desirable in biscuits and cookies, contributing to a more pleasant mouthfeel (Zoulias *et al.*, 2002, Pereira *et al.*, 2013, Pasqualone *et al.*, 2021).

Springiness increased slightly with extract addition, from 2.3 mm to 2.6 mm. This may be due to enhanced elasticity of the biscuit matrix, potentially promoted by polyphenol-protein interactions. Although not a dominant texture feature in biscuits, improved springiness can indicate a resilient and balanced structure, especially important in storage stability.

The addition of *Acorus calamus* phenolic extract resulted in mild yet positive modifications in biscuit texture.

At concentrations up to 300 mg, the extract improved cohesiveness, crispness, and springiness while slightly reducing hardness and chewiness. These changes suggest that phenolic extracts can enhance both the

sensory and mechanical quality of biscuits without compromising their structure.

This supports the potential of *Acorus calamus* extract as a functional ingredient in bakery applications.

Table 3. Texture analysis of biscuits prepared with different amounts of *Acorus calamus* roots extract

Sample Attribute	Control	100 mg <i>Acorus calamus</i> extract	200 mg <i>Acorus calamus</i> extract	300 mg <i>Acorus calamus</i> extract
Hardness (N)	45.0 ± 0.12a	44.5 ± 0.15b	43.7 ± 0.18c	43.0 ± 0.14c
Crispness	5.0 ± 0.04d	5.1 ± 0.04c	5.3 ± 0.03b	5.5 ± 0.01a
Fracturability (N)	12.8 ± 0.06a	12.7 ± 0.05a	12.4 ± 0.05b	12.2 ± 0.04c
Cohesiveness	0.72 ± 0.03a	0.73 ± 0.03a	0.75 ± 0.03a	0.76 ± 0.03a
Chewiness (N·mm)	18.5 ± 0.08a	18.3 ± 0.07b	18.0 ± 0.07c	17.6 ± 0.06d
Springiness (mm)	2.3 ± 0.01d	2.4 ± 0.01c	2.5 ± 0.01b	2.6 ± 0.01a

Values are means ± SD. Means having the different case letter(s) within a row are significantly different at $P \leq 0.05$.

Sample 1 = biscuit with 100 mg *Acorus calamus* extract, Sample 2 = biscuit with 200 mg *Acorus calamus* extract and sample 3 = biscuit with 300 mg *Acorus calamus* extract

Color Profile of Biscuits Fortified with Varying Levels of *Acorus calamus* Extract

The incorporation of *Acorus calamus* phenolic extract into biscuit formulations resulted in noticeable changes in color parameters (L^* , a^* , b^*) compared to the control (Table 4). The lightness value (L^*) significantly decreased from 74.20 in the control to 68.70 in the sample containing 300 mg extract. This decline in L^* value indicates a progressive darkening of the biscuits with increasing extract concentration. The darker coloration can be attributed to the presence of polyphenolic compounds in the extract which are known to undergo browning reactions during thermal processing (Žilić *et al.*, 2016). Moreover, the Maillard reaction between amino acids and reducing sugars may be enhanced by phenolic compounds, further contributing to color development (Zhu *et al.*, 2010, Han *et al.*, 2024).

The a^* values, representing the red-green axis, showed a gradual increase from 3.50 in the control to 4.60 in the 300 mg treatment, indicating a shift toward redder tones. This increase can be explained by the oxidation of phenolic compounds and the formation of red-brown pigments during baking.

Conversely, the b^* values (yellow-blue axis) decreased with higher levels of extract, from 22.60 in the control to 19.40 at 300 mg, suggesting a reduction in the yellow coloration of the biscuits. This could be due to the masking effect of the darker phenolic compounds and the degradation of naturally occurring yellow pigments during baking.

Table 4. Color Measurement of Biscuits Enriched with *Acorus calamus* Phenolic Extract

Measurement Sample	L^* (Lightness)	a^* (Red-Green)	b^* (Yellow-Blue)
Control	74.20 ± 0.80a	3.50 ± 0.22d	22.60 ± 0.54a
Sample 1	72.80 ± 0.70b	3.80 ± 0.18c	21.80 ± 0.43b
Sample 2	70.90 ± 0.60c	4.20 ± 0.26b	20.70 ± 0.46c
Sample 3	68.70 ± 0.60d	4.60 ± 0.11a	19.40 ± 0.42d

Values are means ± SD. Means having the different case letter(s) within a column are significantly different at $P \leq 0.05$. Sample 1 = biscuit with 100 mg *Acorus calamus* extract, Sample 2 = biscuit with 200 mg *Acorus calamus* extract and sample 3 = biscuit with 300 mg *Acorus calamus* extract

These results are consistent with earlier studies where the addition of plant-based polyphenol sources to baked products caused a reduction in lightness and yellowness, along with an increase in red tones (Leicht *et al.*, 2025, Difonzo *et al.*, 2022).

Differential Scanning Calorimetry (DSC) Parameters of Biscuits Enriched with Varying Concentrations of *Acorus calamus* Phenolic Extract

The Differential Scanning Calorimetry (DSC) thermograms of biscuits incorporated with different concentrations of *Acorus calamus* phenolic extract are summarized in Table 5.

All thermograms exhibited endothermic transitions characteristic of starch gelatinization, and shifts were observed in thermal parameters (T_o , T_p , T_c) and enthalpy (ΔH) with increasing concentrations of phenolic extract.

The onset temperature (T_o) increased progressively from 55.20 °C in the control sample to 57.60 °C in the biscuit containing 300 mg extract. Similarly, the peak temperature (T_p) and conclusion temperature (T_c) also showed upward trends, reaching 65.00 °C and 73.80 °C, respectively, in the 300 mg sample. This suggests that the phenolic compounds present in *Acorus calamus* extract may enhance the thermal stability of the starch-protein matrix in the biscuit dough.

The increase in thermal transition temperatures is likely due to the interaction of phenolic compounds with macromolecules such as starch and gluten proteins. These interactions may lead to reduced water availability and hindered swelling and gelatinization of starch granules (Girard and Awika, 2020). Furthermore, phenolics can form hydrogen bonds with hydroxyl groups on starch chains, which delays the thermal disruption of crystalline regions (Zhu, 2015).

The enthalpy change (ΔH), which represents the energy required for starch gelatinization, decreased from 6.82 J/g in the control to 5.76 J/g in the 300 mg extract sample. The decline in ΔH with increasing extract concentration indicates that less energy was needed for gelatinization, potentially due to the partial disruption of the starch's crystalline structure by phenolic compounds prior to the heating process. This effect may also result from competitive water binding by phenolics, thereby reducing water availability for starch gelatinization.

These findings are consistent with those of Ribas-Agustí *et al.* (2018), Manzoor *et al.* (2024), who reported that the incorporation of plant-derived polyphenols into cereal matrices altered thermal behavior by modulating molecular interactions and water dynamics. The thermal analysis confirms that *Acorus calamus* extract influences the baking behavior of biscuits by increasing thermal stability and modifying gelatinization properties, which may contribute to improved structural integrity and bioactive retention during processing.

Table 5. Thermal Analysis (DSC) of Biscuits Enriched with *Acorus calamus* Phenolic Extract

Thermal °C Sample	Onset Temp (To, °C)	Peak Temp (Tp, °C)	Conclusion Temp (Tc, °C)	Enthalpy Change (ΔH, J/g)
Control	55.20 ± 0.33d	63.10 ± 0.45c	71.50 ± 0.36c	6.82 ± 0.15a
Sample 1	56.10 ± 0.15c	63.80 ± 0.40c	72.20 ± 0.53b	6.45 ± 0.14b
Sample 2	56.80 ± 0.22b	64.30 ± 0.38b	72.90 ± 0.45b	6.08 ± 0.13c
Sample 3	57.60 ± 0.41a	65.00 ± 0.35a	73.80 ± 0.23a	5.76 ± 0.12d

Values are means ± SD. Means having the different case letter(s) within a column are significantly different at $P \leq 0.05$. Sample 1 = biscuit with 100 mg *Acorus calamus* extract, Sample 2 = biscuit with 200 mg *Acorus calamus* extract and sample 3 = biscuit with 300 mg *Acorus calamus* extract

Changes in Peroxide Value (meq O₂/kg) of Biscuits Enriched with *Acorus calamus* Phenolic Extract During Storage

Peroxide value (PV) is a widely accepted indicator of primary lipid oxidation, which is directly associated with the development of rancidity and deterioration in fat-containing bakery products. The changes in peroxide value of biscuits enriched with different concentrations (100, 200, and 300 mg) of *Acorus calamus* phenolic extract during three months of storage at room temperature are shown in Table (6).

Table 6. Peroxide value of the prepared biscuits storage for three months

Biscuits Samples Storage Time	Control	Sample 1	Sample 2	Sample 3
0 Month	1.5 ± 0.05a	1.0 ± 0.05b	0.7 ± 0.03c	0.6 ± 0.03d
1 Month	3.5 ± 0.10a	2.8 ± 0.09b	2.2 ± 0.08c	1.7 ± 0.07d
2 Months	5.8 ± 0.20a	4.2 ± 0.18b	3.2 ± 0.13c	2.5 ± 0.10d
3 Months	7.9 ± 0.25a	5.8 ± 0.22b	4.4 ± 0.18c	3.3 ± 0.12d

Values are means ± SD. Means having the different case letter(s) within a row are significantly different at $P \leq 0.05$. Sample 1 = biscuit with 100 mg *Acorus calamus* extract, Sample 2 = biscuit with 200 mg *Acorus calamus* extract and sample 3 = biscuit with 300 mg *Acorus calamus* extract

A progressive increase in PV was observed in all biscuit samples during storage, reflecting the natural oxidative degradation of lipids. However, the rate of increase was significantly lower in samples containing the phenolic extract compared to the control. After three months, the control sample showed the highest PV (7.9 meq O₂/kg), while the 300 mg extract sample exhibited the lowest PV (3.3 meq O₂/kg).

The results demonstrate a dose-dependent antioxidant effect of *Acorus calamus* extract, with higher concentrations more effectively suppressing lipid oxidation. The phenolic compounds present in the extract, such as chlorogenic acid, gallic acid, rutin, and quercetin, are known to scavenge free radicals and chelate metal ions that catalyze oxidative reactions (Kaurinovic and Vastag, 2019). These compounds donate hydrogen atoms to lipid radicals, terminating propagation steps of oxidation (Shahidi and Ambigaipalan, 2015). The presence of these active molecules likely contributed to the improved oxidative stability observed in the enriched biscuit samples. Similar protective effects against oxidation have been reported in other bakery systems fortified with plant polyphenols. For instance, Aksoylu *et al.* (2015) demonstrated that biscuits supplemented with blueberry, grape seed powder and poppy seed extracts showed significantly lower peroxide values during storage due to the high phenolic content. Likewise, Shoukat *et al.* (2025) reported that pomegranate peel extract effectively reduced PV in functional cookies stored under ambient conditions. So, the incorporation of *Acorus calamus* phenolic extract significantly reduced the peroxide value of biscuits during three months of storage, in a concentration-dependent manner. This confirms the strong antioxidant potential of the

extract and highlights its effectiveness in retarding lipid peroxidation, thereby improving the shelf stability of baked products.

Total Phenolic Content (mg GAE/g) of Biscuits Fortified with Ipecac and *Acorus calamus* Extracts During Three Months of Storage

The incorporation of *Acorus calamus* extract significantly enhanced the total phenolic content (TPC) of biscuit samples compared to the control. At zero time, the TPC values increased with increasing extract concentration, ranging from 30.5 mg GAE/g at 100 mg to 58.9 mg GAE/g at 300 mg, whereas the control exhibited only 5.8 mg GAE/g (Table 7). This substantial enhancement is attributed to the high phenolic composition of *Acorus calamus*, which includes bioactive compounds such as rutin, gallic acid, chlorogenic acid, and quercetin. These compounds are known for their antioxidant activity and relative thermal stability, which likely contributed to their retention even after baking.

Table 7. Total phenolic content of the prepared biscuits storage for three months

Sample Total Phenolic Content (mg GAE/g)	Control	Sample 1	Sample 2	Sample 3
0 Month	5.8 ± 0.20d	30.5 ± 1.1c	44.8 ± 1.4b	58.9 ± 1.9a
1 Month	5.4 ± 0.18d	29.9 ± 1.0c	44.2 ± 1.3b	58.2 ± 1.8a
2 Months	4.9 ± 0.15d	29.2 ± 0.9c	43.7 ± 1.3b	57.4 ± 1.7a
3 Months	3.2 ± 0.10d	27.8 ± 0.9c	42.5 ± 1.3b	56.4 ± 1.8a

Values are means ± SD. Means having the different case letter(s) within a row are significantly different at $P \leq 0.05$. Sample 1 = biscuit with 100 mg *Acorus calamus* extract, Sample 2 = biscuit with 200 mg *Acorus calamus* extract and sample 3 = biscuit with 300 mg *Acorus calamus* extract

Over the storage period of three months, a gradual decline in TPC was observed in all samples, although the fortified samples exhibited significantly better retention compared to the control. The control sample showed a marked reduction in TPC, from 5.8 to 3.2 mg GAE/g (~44.8% decrease), indicating susceptibility to oxidative degradation. In contrast, the 300 mg *Acorus calamus* sample retained 56.4 mg GAE/g, reflecting only a modest loss (~4.2%). This suggests that the phenolic compounds in *Acorus calamus* are relatively stable and effectively preserved within the biscuit matrix.

The observed stability can be explained by the presence of polymeric or conjugated phenolics that are less prone to degradation, and by the potential interactions between polyphenols and macromolecules such as starch and proteins, which may form protective complexes. Furthermore, antioxidant synergism among the phenolic constituents may enhance their collective stability during storage (Zhang *et al.*, 2020, Chen *et al.*, 2022).

Similar trends have been reported in previous studies. Shoukat *et al.* (2025) demonstrated that cookies enriched with pomegranate peel extract exhibited high initial TPC and strong retention. Davidov-Pardo *et al.* (2012) also observed that the addition of grape seed extract significantly improved the antioxidant potential and phenolic stability in cookies. These

findings support the present results, confirming that *Acorus calamus* extract effectively enhances and stabilizes phenolic content in biscuits. The results indicate that *Acorus calamus* extract is an effective natural antioxidant source for functional bakery products, contributing to both enhanced initial phenolic levels and their sustained retention during storage.

CONCLUSION

The findings of this study demonstrate that the choice of extraction solvent significantly influences the yield, total phenolic content, and antioxidant activity of *Acorus calamus* root extracts, with hydroalcoholic solvents showing superior efficiency. The phenolic-rich extracts were successfully incorporated into biscuit formulations, resulting in enhanced antioxidant capacity, thermal stability, and oxidative resistance during storage. These results underscore the potential of *Acorus calamus* as a functional ingredient for developing nutritionally enriched and shelf-stable bakery products.

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تحسين جودة وثبات البسكويت بإضافة مستخلص الفينولات من نبات عود ايكور (*Acorus calamus*)

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المخلص

قامت هذه الدراسة بدراسة تأثير المذيبات المختلفة على كمية المستخلص الكلي، والمحتوى الفينولي الكلي، والنشاط المضاد للأكسدة لجذور نبات عود ايكور (*Acorus calamus*)، بالإضافة إلى تأثير إضافة المستخلص الفينولي في خلطات البسكويت. أظهرت النتائج أنه من بين المذيبات المختبرة، حقق الإيثانول بتركيز ٧٠٪ أعلى كفاءة استخلاص (٢٠،٢٪)، وأظهر محتوى عاليًا من الفينولات الكلية (٨٦،٩٦ ملجم/جم) مع نشاط قوي مضاد للأكسدة، بينما أظهر مستخلص الميثانول ٨٠٪ أعلى محتوى فينولي كلي (١٠٥،٣٥ ملجم/جم). تضمنت المركبات الفينولية الرئيسية التي تم تحديدها حمض الكلوروجينيك، حمض الجالليك، حمض الكافيك، الروتين، والكيرسيتين، مع اختلاف مستوياتها حسب نوع المذيب. أظهرت عينات البسكويت المدعمة بتركيزات متزايدة من مستخلص عود الأيكور (١٠٠، ٢٠٠، ٣٠٠ ملجم) ارتفاعًا في محتوى الفينولات الكلية (من ٣٠،٥ إلى ٥٨،٩ ملجم حمض جالليك/جم في وقت الصفر)، وانخفاضًا في قيم البيروكسيد خلال ٣ أشهر من التخزين (من ٥،٨ إلى ٣،٣ ميلي مكافئ/كجم بعد ٣ أشهر). وكشف تحليل اللون عن تناقص تدريجي في شفافية البسكويت مع زيادة تركيز المستخلص. وأشارت تحاليل المسح الحراري التفاضلي (DSC) إلى تحسن في الاستقرار الحراري للبسكويت المدعم، مع زيادة درجات حرارة بدء وذروة التبلور. تشير هذه النتائج إلى أن مستخلص الفينولات من نبات عود ايكور يُعد مضافًا طبيعيًا واعدًا لتعزيز القيمة الغذائية وثبات التخزين في المنتجات المخبوزة.

الكلمات الدالة: عود الأيكور، المركبات الفينولية، النشاط المضاد للأكسدة، بسكويت وظيفي، مذيبات الاستخلاص