

Effects of Germination and Roasting on Polyphenolic Compounds of Finger Millet (*Eleusine coracana* [L.] Gaertn.)

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Abstract

Germination and roasting are routinely applied to finger millet (*Eleusine coracana* [L.] Gaertn.) before consumption in Kenya, yet their effects on polyphenols associated with the grain's reported antidiabetic potential have not been quantified for Kenyan-bred germplasm. This study evaluated the effect of germination duration (0, 24, 48 and 60 h) and roasting treatment (raw, 120°C for 5 min, 180°C for 1 min) on the total phenolic content (TPC), total flavonoid content (TFC) and the gallic, ferulic and vanillic acid contents of the representative KALRO variety Kakamega Wimbi-1. TPC was measured by Folin-Ciocalteu spectrophotometry, TFC by aluminium chloride colorimetry, and phenolic acids by RP-HPLC-PDA using methods validated in accordance with ICH Q2(R1). Data from three independent treatment batches were analysed by one-way ANOVA with Student-Newman-Keuls post hoc separation at $\alpha = 0.05$. Germination increased TPC approximately 6.3-fold (an estimated 18.16 to 115.23 mg GAE/100 g DW; the raw baseline lies below the TPC method LoQ) and TFC 7.9-fold (22.97 to 181.19 mg QE/100 g DW), both $p < 0.001$, with no significant difference between 48 h and 60 h for either parameter. Roasting raised TPC only 1.6-fold and non-significantly ($p = 0.076$) but raised apparent TFC 12.4-fold ($p < 0.001$). The dissociation between the two colorimetric signals, the magnitude of the TFC rise relative to the 2-4-fold enhancement attributable to release of bound flavonoids, and the visible browning of grain at 180°C together suggest that much of the roasting-associated TFC in-

crease may reflect Maillard reaction products chelating Al^{3+} rather than genuine flavonoid accumulation; because neither flavonoids nor Maillard products were directly quantified, this is offered as an interpretation. The acid-extractable ferulic acid reached a similar endpoint of 3.14 mg/100 g DW under both treatments, consistent with convergence of enzymatic and thermal hydrolysis on a shared arabinoxylan-bound pool, although free and bound fractions were not separately quantified. In Kak W-1 under the conditions tested, 48 h germination provided the clearest increase in measured polyphenolic indices, whereas the large roasting-associated TFC signal requires chromatographic confirmation before it can be interpreted as flavonoid enrichment.

Keywords

Finger Millet, Germination, Roasting, Maillard Reaction, Total Phenolic Content, Total Flavonoid Content

1. Introduction

Finger millet (*Eleusine coracana* [L.] Gaertn.) is a drought-tolerant cereal of considerable importance to smallholder farming and household nutrition in western Kenya, where it is consumed as stiff and fermented porridges and as traditional malted beverages [1] [2]. Its pigmented seed coat carries 0.2% - 3.0% polyphenols by dry weight, principally phenolic acids, flavan-3-ols and condensed tannins, and these compounds account for much of the grain's antioxidant, anti-inflammatory and antihyperglycaemic activity [3] [4]. Interest in the crop as a dietary intervention has grown alongside the rising burden of type 2 diabetes and cardiovascular disease in Kenya [5], because finger millet phenolics inhibit α -amylase and α -glucosidase and thereby moderate postprandial glycaemia [3] [6].

Grain is almost never eaten raw. Germination (malting) and roasting are the two operations most commonly applied at household and small-commercial scale [7], and both act directly on the polyphenol pool. Germination triggers a coordinated sequence of imbibition, reserve mobilisation and secondary-metabolite induction that can hydrolyse bound phenolics, synthesise new ones and degrade tannins that previously sequestered phenolic acids [8]-[10]. Roasting, by contrast, acts through competing physicochemical processes: thermal cleavage of ester bonds that liberates cell wall-bound phenolics, thermal degradation of heat-labile structures, and, above roughly 120°C, the Maillard reaction between reducing sugars and amino groups [6] [11] [12].

The direction and magnitude of these changes are not uniform. They depend on temperature, duration, moisture, genotype and the specific phenolic class measured, and reported outcomes for cereal roasting in particular are inconsistent [12] [13]. A recurring complication is that most processing studies rely on bulk colorimetric assays alone. The aluminium chloride assay used for total flavonoid content responds to any species capable of chelating Al^{3+} and producing absorbance

at 510 nm, and Maillard reaction products carry carbonyl, enol and reductone groups that can do exactly that [14]-[16]. Apparent flavonoid “increases” after high-temperature roasting may therefore be at least partly artefactual, but this possibility is seldom tested because the Folin-Ciocalteu and aluminium chloride assays are rarely reported alongside compound-specific chromatography for the same samples.

For Kenyan germplasm the evidence base is thinner still. Processing studies on finger millet have been conducted mainly on Indian, Nigerian, Nepalese and South African varieties using variable protocols and frequently without full method validation [9] [17]-[19], and nutritional work on the KALRO breeding collection has concentrated on proximate composition, minerals and antinutrients rather than polyphenols [20] [21]. There is consequently no validated, quantitative basis on which to advise Kenyan households, food processors or nutritionists on which processing operation actually preserves or enhances the health-promoting fraction of the grain.

This study therefore quantified the effects of germination duration (0, 24, 48 and 60 h) and roasting treatment (raw, 120°C for 5 min and 180°C for 1 min) on the total phenolic content, total flavonoid content and gallic, ferulic and vanillic acid contents of a representative KALRO variety, using validated spectrophotometric and RP-HPLC-PDA methods applied in parallel to the same extracts. Running both colorimetric assays alongside compound-specific chromatography also allowed the two bulk responses to be cross-checked against each other and against the chromatographic data, which proved important in interpreting the roasting results.

2. Materials and Methods

2.1. Plant Material

Grain of the finger millet variety Kakamega Wimbi-1 (Kak W-1) was obtained from the KALRO Food Crops Research Institute, Kisii Centre, western Kenya. Kak W-1 was selected as the experimental variety because it is a widely grown, well-characterised KALRO release whose baseline polyphenol content lies close to the mean of the twenty-one-variety KALRO panel, making it a representative rather than an extreme case. The use of a single representative variety for the processing experiment is supported by evidence that the qualitative direction of polyphenol change during germination and roasting is broadly conserved across finger millet varieties [6] [12] [20]. Grain was cleaned by sorting and winnowing to remove soil, stones, broken kernels and chaff.

Because both treatments must act on the intact kernel, roasting relying on the pericarp to moderate heat transfer and germination requiring a viable embryo, milling was carried out only after treatment. Each treatment level was prepared as three independent replicate lots ($n = 3$), germinated or roasted as separate batches rather than sub-sampled from a single treated batch. Treated grain was milled in a laboratory disc mill fitted with a 0.5 mm sieve and stored in airtight amber glass

containers in the dark until extraction.

2.2. Roasting

Grain was roasted in a laboratory muffle oven at 120°C for 5 min and at 180°C for 1 min, with raw grain as the control. Roasted grain was cooled rapidly in a desiccator at room temperature to prevent moisture re-absorption, then milled and stored. These temperature-time combinations were selected to represent commonly used roasting conditions in Kenyan finger millet food preparation: 120°C for 5 min approximates a moderate household pan-roasting regime, whereas 180°C for 1 min approximates high-temperature short-duration commercial flash-roasting [6] [11]. Because the two roasting conditions differ in both temperature and duration, the roasting factor is treated throughout as a combined temperature-time treatment, and observed differences between conditions cannot be attributed to temperature alone.

2.3. Germination

Grain was steeped by full immersion in distilled water for 12 h. The water was drained and the grain spread on a moistened hessian (gunny) bag and covered with a second moistened bag, held at room temperature (22°C - 26°C) with periodic wetting to maintain grain moisture in the 38% - 45% range optimal for sprouting [8]. Samples were withdrawn at 0, 24, 48 and 60 h measured from the start of steeping [22]. The 0 h sample was untreated grain taken before steeping and served as the raw baseline; a soaking-only (steeped but non-germinated) control was not included, so the earliest germination-associated changes cannot be separated from the effect of steeping alone. All concentrations are expressed per final dry weight, and because germination is accompanied by respiratory dry-matter loss, values expressed per unit initial dry grain may differ from those reported here; this is noted as a limitation. Germinated grain was rinsed with distilled water, sun-dried, then oven-dried at 45°C for 24 h, milled and stored. The 60 h upper limit was chosen because polyphenol enhancement in finger millet typically plateaus or declines beyond 60 - 72 h [8] [9].

2.4. Extraction and Analysis

Phenolics were extracted following Pradeep and Sreerama [23]. Flour (5.00 g) was mixed with 1% HCl in methanol at 1:50 (w/v), heated at 80°C for 20 min with constant stirring, and centrifuged at 4000 g and 25°C. A second extraction was performed under identical conditions, the combined supernatants filtered through Whatman No. 1 paper, concentrated on a rotary evaporator at 40°C and 40 mbar, made up to 10 mL with 80% aqueous methanol and stored at -20°C. All operations were performed under subdued light. This acidified-methanol extraction recovers the free and soluble conjugated (acid-extractable) phenolic forms, whereas insoluble cell wall-bound phenolics, which require alkaline or enzymatic hydrolysis for release, remain largely in the extraction residue and were not quantified.

TPC was determined by the Folin-Ciocalteu method at 760 nm against a gallic acid calibration (0 - 200 µg/mL) and expressed as mg gallic acid equivalents per 100 g dry weight [24] [25]. TFC was determined by the aluminium chloride colorimetric method at 510 nm against a quercetin calibration (0 - 200 µg/mL) and expressed as mg quercetin equivalents per 100 g dry weight [26]. Measurements used a double-beam UV-visible spectrophotometer (model 6305, Jenway, UK) with matched 1 cm quartz cuvettes.

Gallic, ferulic and vanillic acids were quantified by RP-HPLC-PDA on a Waters 2695 separations module with a Waters 2996 photodiode array detector and a Kinetex C18 column (150 mm × 4.6 mm, 2.6 µm; Phenomenex, USA) at 30°C, following Adelakun and Duodu [27]. Phenolic acids were separated isocratically over 30 min at 0.4 mL/min with water:acetonitrile:acetic acid (87.5:12.5:0.1, v/v/v) and detected at 280 nm, with external calibration over 5 - 100 mg/L (5, 10, 25, 50 and 100 mg/L). Compound identity was confirmed by retention-time matching and PDA peak-purity comparison against authentic standards, and reagent and mobile-phase blanks were injected at the start of each run and after every ten samples.

Table 1. Method validation parameters for the analytes quantified in this study.

Analyte	Regression equation	R ²	LoD	LoQ	Recovery (%)	RT %RSD	Intra-assay precision (%RSD)
TPC (spectrophotometric)	$y = 0.0145x - 0.1008$	0.980	7.62	23.10	—	—	—
TFC (spectrophotometric)	$y = 0.0005x - 0.0002$	0.9997	0.32	0.96	—	—	—
Gallic acid (HPLC)	$y = 7562.17x - 852.46$	0.998	0.61	1.85	99.6	1.37	0.75
Ferulic acid (HPLC)	$y = 7319x - 476.25$	0.9996	0.64	1.95	100.1	0.98	0.86
Vanillic acid (HPLC)	$y = 6888x - 2525.3$	0.9957	0.43	1.30	98.2	0.86	1.10

Spectrophotometric calibrations (TPC, TFC) were run over 0 - 200 µg/mL and HPLC calibrations over 5 - 100 mg/L (5, 10, 25, 50 and 100 mg/L). LoD and LoQ are expressed per 100 g dry weight (TPC as mg GAE/100 g DW, TFC as mg QE/100 g DW, phenolic acids as mg/100 g DW) and were calculated as $LoD = 3.3 \times (\sigma/S)$ and $LoQ = 10 \times (\sigma/S)$. Recovery is the mean analytical recovery from three-level spiking ($n = 15$); intra-assay precision is the concentration repeatability (%RSD, $n = 5$). RT %RSD is retention-time repeatability ($n = 5$); recovery, RT %RSD and intra-assay precision are not applicable to the spectrophotometric assays (—). In the regression equations, y is absorbance (TPC at 760 nm; TFC at 510 nm) or chromatographic peak area (HPLC analytes), and x is the concentration of the calibration standard, expressed in µg/mL for the spectrophotometric assays (gallic acid and quercetin standards, 0 - 200 µg/mL) and in mg/L for the HPLC analytes (5 - 100 mg/L); the two units are numerically equivalent (1 µg/mL = 1 mg/L).

All methods were validated for specificity, linearity, repeatability and the limits of detection and quantification in accordance with ICH Q2(R1) [28]. All calibrations were linear ($R^2 = 0.980$ for TPC, 0.9997 for TFC and 0.9957 - 0.9996 for the HPLC analytes). The lower slope of the TFC calibration ($S = 0.0005$ AU·mL/µg) relative to TPC ($S = 0.0145$ AU·mL/µg) reflects the lower molar absorptivity of the quercetin- Al^{3+} chelate at 510 nm compared with the molybdotungstate complex at 760 nm (AU, absorbance units). Accuracy, assessed as analytical recovery from

spiking authentic standards into the raw matrix at three levels ($n = 15$), was 98.2% - 100.1% across the phenolic acids (mean recoveries: gallic acid 99.6%, ferulic acid 100.1%, vanillic acid 98.2%), and intra-assay concentration precision was $\leq 1.10\%$ RSD ($n = 5$); retention-time repeatability was 0.86% - 1.37% RSD ($n = 5$). The limits of detection and quantification, recovery and precision for each analyte are summarised in **Table 1**, and the observed linearity, recovery and precision values met the predefined method-performance criteria used in this study ($R^2 \geq 0.98$; recovery 80% - 120%; %RSD $\leq 5\%$). Because free and bound phenolic fractions were not separately isolated, the phenolic-acid concentrations reported represent the acid-extractable pool recovered by acidified-methanol extraction.

2.5. Statistical Analysis

Values are reported as mean $\pm$ standard error of three independent treatment batches ($n = 3$). Treatment effects were tested by one-way ANOVA at $p < 0.05$, with normality and homogeneity of variance checked by the Shapiro-Wilk and Levene tests. Where the overall effect was significant, means were separated by the Student-Newman-Keuls test at $\alpha = 0.05$; SNK was preferred over Tukey's HSD as a stepwise multiple-range procedure with greater power at small replicate numbers; SNK does not, however, provide strong control of the family-wise (experimentwise) error rate across all pairwise comparisons, and the mean separations are interpreted with this limitation in mind [29]. Means sharing a superscript letter within a column do not differ significantly. Fold-changes were computed as the value at maximum processing intensity divided by the raw baseline for the same parameter, and the relationship between the two processes was examined by Pearson correlation of these fold-changes. Descriptive statistics were computed in Microsoft Excel 2019 and plots prepared in GraphPad Prism 9.

3. Results and Discussion

3.1. Effect of Germination

The polyphenol-enhancing effect of germination is understood to operate through three interrelated mechanisms: hydrolytic release of bound and conjugated phenolics into the extractable pool; de novo biosynthesis through induction of the phenylpropanoid pathway; and breakdown of antinutritional factors such as tannins that previously sequestered phenolic acids [8] [10] [23]. The effect of germination duration on the five measured parameters is presented in **Table 2**.

Total phenolic content. TPC increased progressively and substantially with germination duration (**Figure 1**). Raw grain contained 18.16 ± 0.16 mg GAE/100 g DW, rising to 72.26 ± 9.64 by 24 h, 102.67 ± 3.81 at 48 h and 115.23 ± 7.35 mg GAE/100 g DW at 60 h, an approximately 6.3-fold increase over the 60 h window; because the raw baseline (18.16 mg GAE/100 g DW) lies below the validated TPC LoQ (23.10 mg GAE/100 g DW), this fold-change is an estimate and is interpreted with caution. ANOVA confirmed a highly significant effect of duration ($p < 0.001$), and SNK separated the 0 h control from all three germinated time points,

while 48 h and 60 h did not differ from each other. The most pronounced change occurred between 0 and 24 h, an approximately four-fold rise, followed by a more gradual increase to 60 h.

Table 2. Effect of germination duration on the polyphenolic compounds of Kak W-1 (mean $\pm$ SE).

Germination time (h)	TPC	TFC	Gallic acid	Ferulic acid	Vanillic acid
0	18.16 [†] $\pm$ 0.16 ^c	22.97 $\pm$ 1.86 ^b	1.86 $\pm$ 0.08 ^a	1.96 $\pm$ 0.24 ^a	1.31 $\pm$ 0.07 ^a
24	72.26 $\pm$ 9.64 ^b	127.95 $\pm$ 8.63 ^a	1.80 $\pm$ 0.12 ^a	1.53 $\pm$ 0.18 ^a	1.26 $\pm$ 0.13 ^a
48	102.67 $\pm$ 3.81 ^a	164.48 $\pm$ 8.67 ^a	7.11 $\pm$ 1.62 ^b	2.32 $\pm$ 0.27 ^{ab}	2.46 $\pm$ 0.52 ^a
60	115.23 $\pm$ 7.35 ^a	181.19 $\pm$ 21.24 ^a	7.07 $\pm$ 1.75 ^b	3.14 $\pm$ 0.32 ^b	2.51 $\pm$ 0.60 ^a
p-value	<0.001	<0.001	<0.05	<0.05	ns (>0.05)

All values are mean $\pm$ SE (n = 3). TPC in mg GAE/100 g DW; TFC in mg QE/100 g DW; gallic, ferulic and vanillic acids in mg/100 g DW. Within each column, means followed by different superscript letters differ significantly (SNK, α = 0.05); “ns” denotes not significant. ANOVA: TPC F(3,8) = 46.282, p < 0.001; TFC F(3,8) = 33.390, p < 0.001; gallic acid F(3,8) = 6.464, p = 0.016; ferulic acid F(3,8) = 7.030, p = 0.012; vanillic acid F(3,8) = 2.949, p = 0.098. [†]Estimated value below the validated TPC LoQ (23.10 mg GAE/100 g DW); fold-changes calculated from this baseline are approximate.

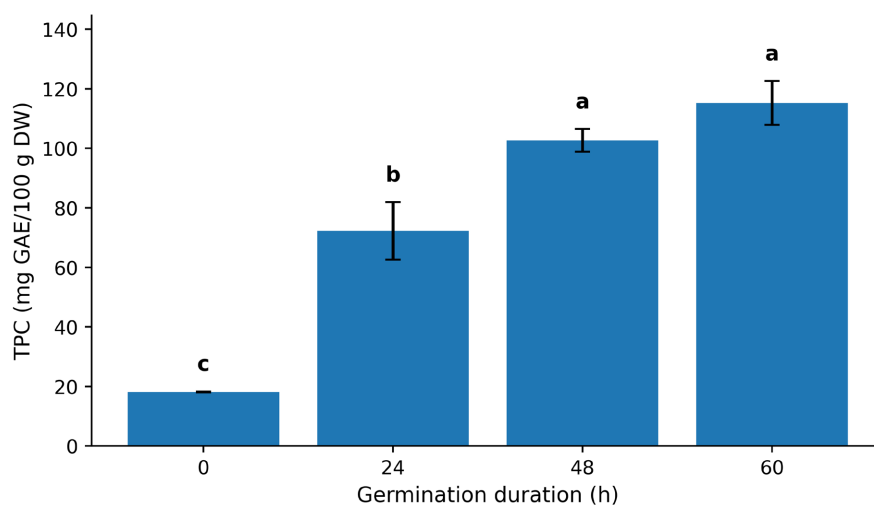


Figure 1. Effect of germination duration on total phenolic content. Bars are mean $\pm$ SE (n = 3); statistical separation is given in **Table 2**.

This biphasic pattern is consistent with an initial phase of rapid hydrolytic release of bound and conjugated phenolics during steeping and early sprouting, followed by a slower phase dominated by de novo phenylpropanoid biosynthesis as phenylalanine ammonia-lyase (PAL) expression peaks during coleoptile and radicle elongation [6] [8] [30]. PAL, the rate-limiting enzyme of the pathway, deaminates phenylalanine to trans-cinnamic acid and thereby supplies the carbon skeleton for all downstream phenolic acids and flavonoids [31] [32]; phenylalanine is itself liberated from storage-protein reserves by germination-activated proteases,

providing substrate for accelerated PAL flux.

Because TPC at 48 h and 60 h did not differ significantly, most of the observed increase had occurred by 48 h; under the conditions tested, 48 h may therefore offer a practical time-saving germination duration for household- or small-scale malting. This accords with the accumulation kinetics reported under comparable steeping and sprouting conditions [9], with the plateau behaviour beyond 48 h observed in small millets [23], and with the 48 - 72 h window identified as optimal across several millet species [6]. The magnitude of the effect is notable: 48 h germination raised the TPC of Kak W-1 from a below-panel-mean raw value to approximately double the highest TPC recorded in an unprocessed twenty-one-variety KALRO screen. These findings show that germination can substantially increase measured TPC in Kak W-1 and support further evaluation of malted finger millet in food applications.

Total flavonoid content. TFC rose sharply from 22.97 mg QE/100 g DW at 0 h to 181.19 mg QE/100 g DW at 60 h (Figure 2), a 7.9-fold increase that was proportionately greater than the ≈ 6.3 -fold estimated TPC increase. ANOVA confirmed a highly significant effect ($p < 0.001$), with SNK separating 0 h from all germinated time points and no significant differences among 24, 48 and 60 h.

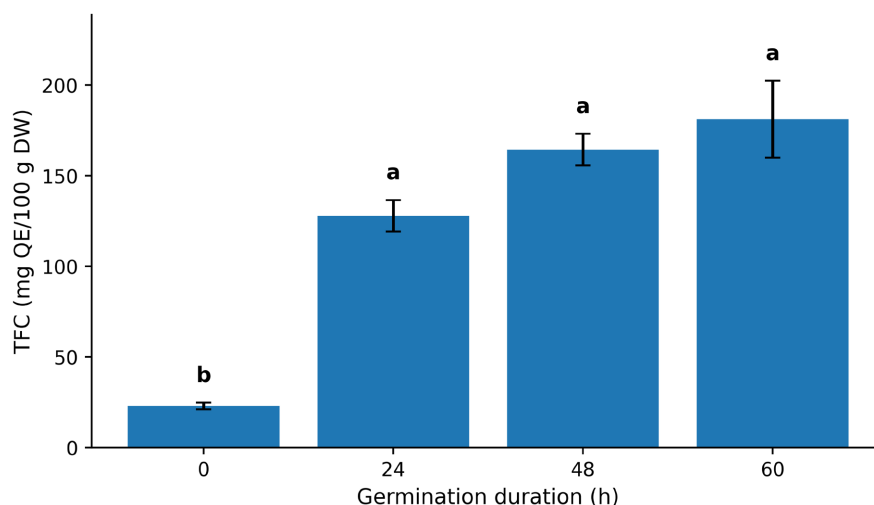


Figure 2. Effect of germination duration on total flavonoid content. Bars are mean $\pm$ SE ($n = 3$); statistical separation is given in Table 2.

Comparable germination-induced gains in phenolic and flavonoid content, accompanied by increased antioxidant activity, have been reported in germinated legumes [33] and in proso millet [34], indicating a conserved sprouting response across diverse seed types. The differential enhancement observed here is consistent with strong stimulation of the chalcone synthase (CHS)-mediated flavonoid branch of the phenylpropanoid pathway during seedling establishment [8] [31]. CHS catalyses condensation of three malonyl-CoA units with p-coumaroyl-CoA to form naringenin chalcone, the first committed flavonoid intermediate, and its induction during germination is well documented [30]. The very rapid early

rise, a 5.6-fold change within 24 h, supports the interpretation that early-phase flavonoid accumulation is dominated by β -glucosidase-mediated release of flavonoid glycosides into the free aglycone pool, followed by sustained de novo biosynthesis as CHS and flavonol synthase reach peak activity by 24 - 48 h [6] [8] [35]. The proportionately larger TFC gain also reflects the preferential response of the aluminium chloride assay to flavone and flavonol classes, which are direct CHS-pathway products, whereas the Folin-Ciocalteu assay additionally captures phenolic acids arising from earlier branch points that may be less strongly upregulated during germination.

Gallic acid. Gallic acid rose numerically with germination time, from 1.86 ± 0.08 mg/100 g DW at 0 h to 7.11 ± 1.62 at 48 h and 7.07 ± 1.75 mg/100 g DW at 60 h, an approximately 3.8-fold increase (Figure 3). The increase is biologically consistent with enzymatic hydrolysis of gallotannins by tannase (tannin acyl hydrolase), which cleaves the galloyl ester bonds of hydrolysable tannins and liberates gallic acid as the seed mobilises seed-coat tannin reserves [3] [10] [36].

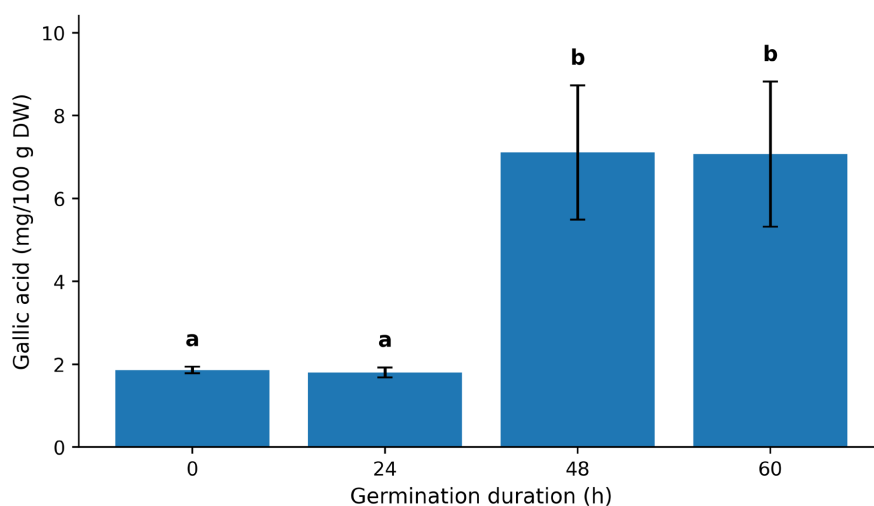


Figure 3. Effect of germination duration on gallic acid content. Bars are mean $\pm$ SE ($n = 3$).

A notable lag was observed: gallic acid was essentially unchanged at 24 h (1.80 versus 1.86 mg/100 g DW) before rising sharply at 48 h, suggesting that tannase induction in Kak W-1 occurs mainly after the first day of germination, consistent with the delayed activation kinetics reported for this enzyme in other cereals [8]. The overall ANOVA was significant, and SNK separated the 48 h and 60 h time points (superscript b) from the 0 h and 24 h baseline (superscript a), corresponding to an approximately 3.8-fold increase in acid-extractable gallic acid by 48 - 60 h. Within-group variability, expressed as the standard error of the mean ($n = 3$), was larger at 48 h (± 1.62) and 60 h (± 1.75) than at 0 h (± 0.08) and 24 h (± 0.12), consistent with variation in germination synchrony among replicate batches. Larger replicate numbers and tighter synchronisation would provide more precise estimates of this response.

Ferulic acid. Ferulic acid showed a significant progressive increase, from 1.96

± 0.24 mg/100 g DW at 0 h to 3.14 ± 0.32 mg/100 g DW at 60 h ($p < 0.05$; **Figure 4**), with SNK confirming 60 h as significantly higher than both 0 h and 24 h and 48 h as intermediate. The transient numerical dip at 24 h is biologically informative: during the earliest phase of germination, ferulic acid may be transiently consumed through re-esterification or oxidative incorporation into newly synthesised cell wall material, as the elongating radicle and coleoptile lay down fresh arabinoxylan-rich primary walls into which ferulic acid is covalently esterified at the C5 hydroxyl of arabinose residues [37] [38]. This early sequestration is subsequently outpaced by feruloyl-esterase-mediated release of cell wall-bound ferulic acid during the extensive arabinoxylan remodelling that accompanies seedling growth [8] [39].

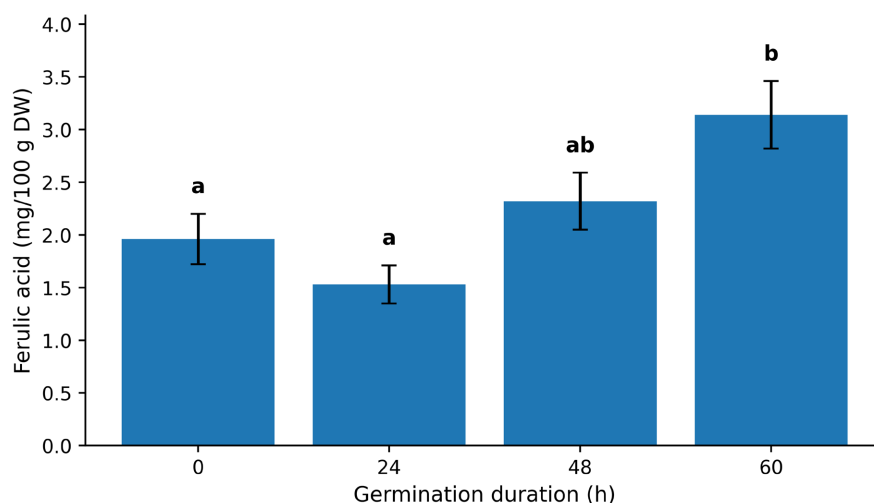


Figure 4. Effect of germination duration on ferulic acid content. Bars are mean $\pm$ SE ($n = 3$); statistical separation is given in **Table 2**.

Feruloyl esterase (EC 3.1.1.73) hydrolyses the ester bond linking ferulic acid to arabinoxylan, releasing ferulic acid into the acid-extractable pool [37]. This mechanism may explain why ferulic acid, uniquely among the three individual acids measured, showed a statistically significant germination response: it exists predominantly in cell wall-bound form in raw grain [40], and germination supplies precisely the enzymatic machinery required to liberate it. Given the reported inhibition of α -amylase and α -glucosidase by ferulic acid as a purified compound [6], and by plant polyphenolic compounds more broadly [41], this compositional change is of interest for the antidiabetic potential of germinated finger millet flour, although that potential was not measured in the present study.

Vanillic acid. Vanillic acid increased numerically at 48 and 60 h, from 1.31 to approximately 2.51 mg/100 g DW, but the effect was not statistically significant ($p > 0.05$; **Figure 5**). The pattern mirrors that of gallic acid, with large within-group variability at later time points limiting statistical power despite a clear numerical trend. Vanillic acid (4-hydroxy-3-methoxybenzoic acid) can be generated both as a direct product of phenylpropanoid metabolism and as a secondary me-

tabolite of ferulic acid catabolism through oxidative side-chain cleavage [39]. The near-identical time course of the two hydroxybenzoic acids, flat through 24 h and rising at 48 - 60 h, contrasts with the early dip-then-rise pattern of ferulic acid and is consistent with both being released from bound reserves by the same wave of late-germination glycosidase and esterase activation.

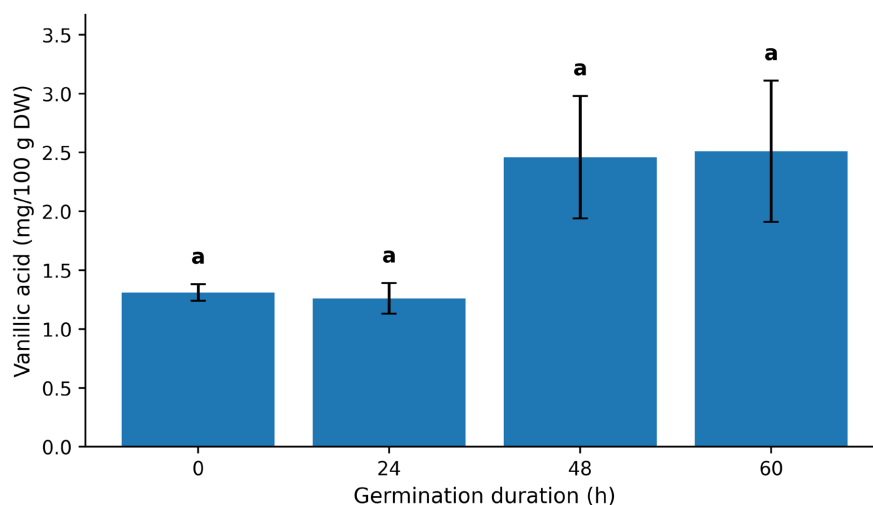


Figure 5. Effect of germination duration on vanillic acid content. Bars are mean $\pm$ SE ($n = 3$).

3.2. Effect of Roasting

Roasting acts on polyphenols through several competing processes operating simultaneously: thermal release of cell wall-bound phenolics, thermal degradation of heat-sensitive structures, and, at higher temperatures, the Maillard reaction between reducing sugars and amino acids that generates chromogenic melanoidins [6] [11] [16] [42]. The effect of roasting treatment (temperature and duration) on the five measured parameters is presented in **Table 3**.

Table 3. Effect of roasting treatment (temperature-time) on the polyphenolic compounds of Kak W-1 (mean $\pm$ SE).

Roasting treatment	TPC	TFC	Gallic acid	Ferulic acid	Vanillic acid
Raw	18.16 [†] $\pm$ 0.16 ^a	22.97 $\pm$ 1.86 ^c	1.86 $\pm$ 0.08 ^a	1.96 $\pm$ 0.24 ^a	1.31 $\pm$ 0.07 ^a
120°C/5 min	25.36 $\pm$ 4.82 ^a	207.42 $\pm$ 14.23 ^b	3.04 $\pm$ 0.49 ^a	1.69 $\pm$ 0.38 ^a	2.28 $\pm$ 0.47 ^a
180°C/1 min	29.30 $\pm$ 0.56 ^a	283.90 $\pm$ 10.42 ^a	1.90 $\pm$ 0.13 ^a	3.14 $\pm$ 0.24 ^b	1.44 $\pm$ 0.20 ^a
p-value	0.076	<0.001	0.062	0.024	0.171

All values are mean $\pm$ SE ($n = 3$). Units as in **Table 2**. Within each column, means followed by different superscript letters differ significantly (SNK, $\alpha = 0.05$). ANOVA: TPC $F(2,6) = 4.082$, $p = 0.076$; TFC $F(2,6) = 171.695$, $p < 0.001$; gallic acid $F(2,6) = 4.588$, $p = 0.062$; ferulic acid $F(2,6) = 7.459$, $p = 0.024$; vanillic acid $F(2,6) = 2.403$, $p = 0.171$. [†]Estimated value below the validated TPC LoQ (23.10 mg GAE/100 g DW); fold-changes calculated from this baseline are approximate.

Total phenolic content. TPC increased numerically with roasting treatment (temperature-time combination), from an estimated 18.16 mg GAE/100 g DW in

raw grain (below the validated TPC LoQ of 23.10 mg GAE/100 g DW) to 25.36 at 120°C and 29.30 mg GAE/100 g DW at 180°C, but the overall ANOVA effect did not reach significance ($p = 0.076$; **Figure 6**). The observed pattern is consistent with competing processes during thermal treatment: thermally mediated release of bound phenolics, which can increase measurable TPC, and concurrent degradation of heat-sensitive phenolic structures, which can decrease it. With three independent batches per treatment, precision for small effects was limited; accordingly, the numerical increase is interpreted as non-significant under the present design. The finding is in general agreement with reports that the net TPC change during finger millet roasting depends strongly on the specific temperature-duration combination and the initial phenolic matrix [6] [11].

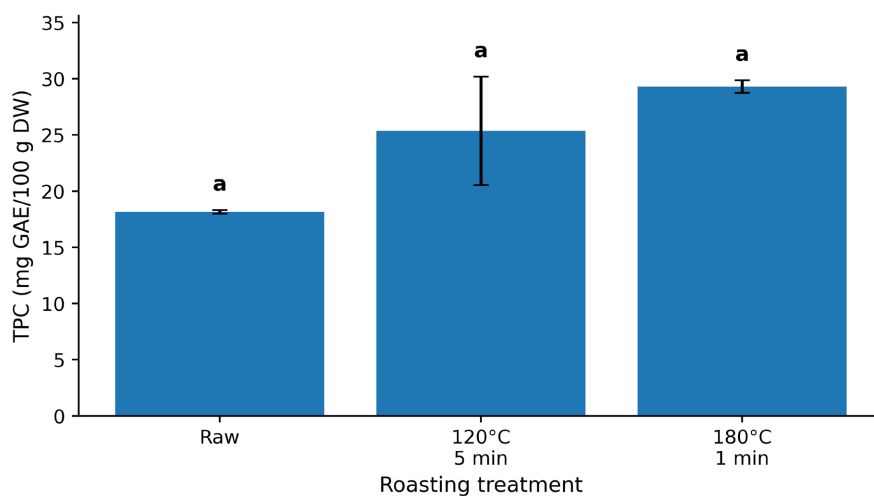


Figure 6. Effect of roasting treatment on total phenolic content. Bars are mean $\pm$ SE ($n = 3$).

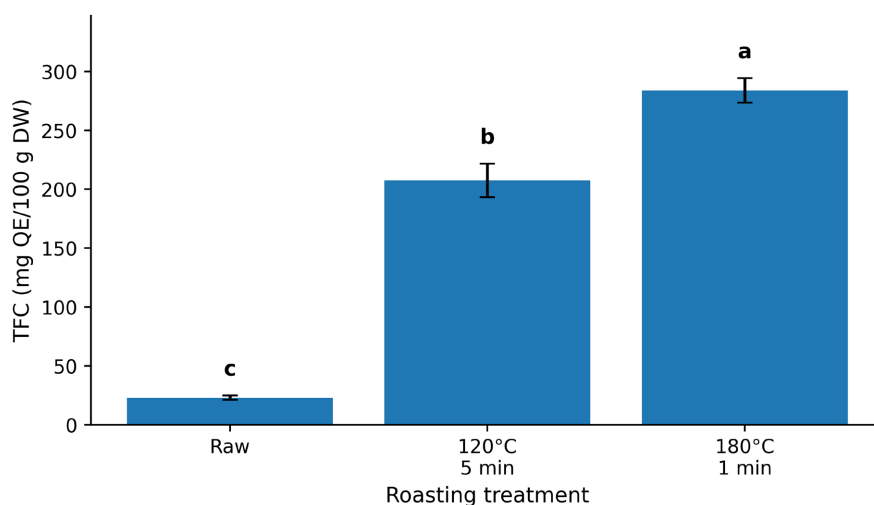


Figure 7. Effect of roasting treatment on total flavonoid content. Bars are mean $\pm$ SE ($n = 3$); statistical separation is given in **Table 3**.

Total flavonoid content and Maillard interference. Measured TFC rose 12.4-fold across the three treatments, from 22.97 ± 1.86 mg QE/100 g DW in raw grain

to 283.90 ± 10.42 mg QE/100 g DW at 180°C ($p < 0.001$; **Figure 7**). The result is statistically unambiguous but requires careful biological interpretation. The magnitude of the rise relative to the non-significant TPC change strongly suggests that a large fraction of the increase does not reflect genuine flavonoid accumulation but rather the formation of interfering chromogenic compounds during high-temperature roasting.

The most plausible source is the Maillard reaction, the non-enzymatic condensation of reducing sugars with the amino groups of free amino acids and proteins, which becomes prominent above approximately 120°C and accelerates sharply at 180°C [15] [16] [43]. The reaction generates brown, high-molecular-weight melanoidins bearing carbonyl, enol and reductone groups capable of binding the Al^{3+} ions used in the aluminium chloride assay, producing absorbance at 510 nm independent of genuine flavonoid content [14] [42].

Three lines of evidence support this interpretation. First, the magnitude of the TFC rise far exceeds the 2-4-fold flavonoid enhancement typically achievable through release of bound flavonoids alone in roasted cereals [6]. Second, the Folin-Ciocalteu assay, which responds to general reducing capacity rather than specifically to Al^{3+} -chelating groups, did not rise proportionately, so the TFC-specific signal increase is not mirrored by a comparable TPC-specific increase. Third, the visible browning of the grain at 180°C is itself a direct indicator of melanoidin formation. Maillard reaction products do possess intrinsic antioxidant capacity [16] [42], so the elevated reading at 180°C may correspond to a genuine increase in the total antioxidant capacity of the roasted flour; but that capacity arises from melanoidins rather than from flavonoids as such, and the two should not be conflated in nutritional labelling or product claims. Targeted HPLC measurement of individual flavonoid aglycones in raw versus roasted samples is required to separate the two contributions definitively. Because neither individual flavonoids nor Maillard products were directly quantified and both colorimetric assays are non-specific, the Maillard-interference mechanism is presented here as an interpretation consistent with the data rather than as a directly demonstrated pathway.

Individual phenolic acids. ANOVA detected a statistically significant roasting effect for ferulic acid ($F(2,6) = 7.459$, $p = 0.024$), whereas the effects for gallic acid ($F(2,6) = 4.588$, $p = 0.062$) and vanillic acid ($F(2,6) = 2.403$, $p = 0.171$) were not statistically significant. The gallic acid response was numerically biphasic, highest at 120°C (3.04 ± 0.49 mg/100 g DW) and returning close to the raw value at 180°C (1.90 ± 0.13 mg/100 g DW; **Figure 8**). This pattern is consistent with moderate-temperature ester hydrolysis of gallotannins releasing gallic acid at 120°C , followed by thermal decarboxylation of gallic acid at 180°C , which generates pyrogallol with loss of CO_2 [6]. Gallic acid is heat-sensitive above approximately 150°C , so decarboxylation could reduce the measurable acid-extractable concentration even while tannin hydrolysis continues to release it, giving the observed numerical peak at the intermediate temperature. Because the overall roasting effect on gallic acid did not reach statistical significance ($F(2,6) = 4.588$, $p = 0.062$),

all three treatments share the same superscript (a) in **Table 3**; the apparent peak at 120°C should therefore be interpreted as a numerical pattern rather than a statistically confirmed treatment effect.

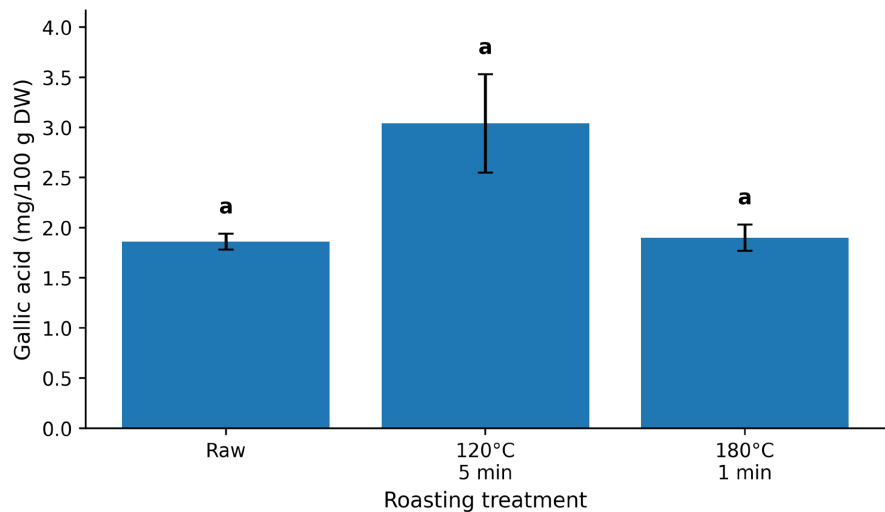


Figure 8. Effect of roasting treatment on gallic acid content. Bars are mean $\pm$ SE ($n = 3$); statistical separation is given in **Table 3**.

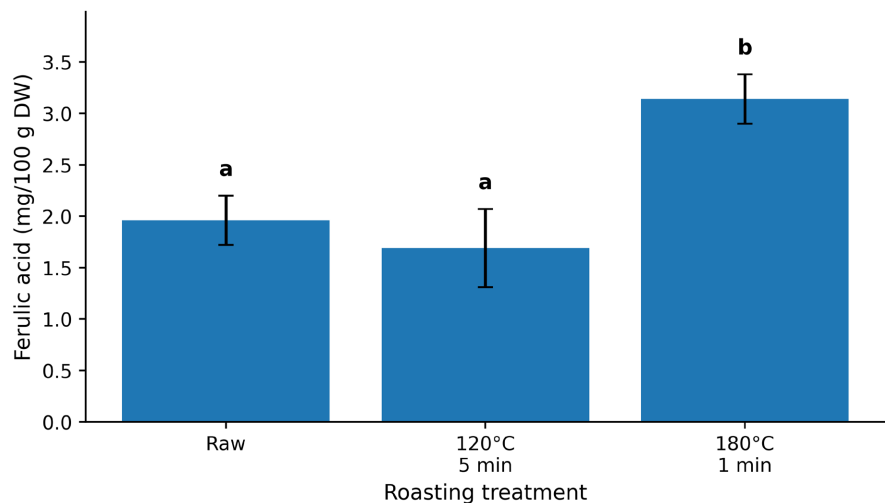


Figure 9. Effect of roasting treatment on ferulic acid content. Bars are mean $\pm$ SE ($n = 3$); statistical separation is given in **Table 3**.

Acid-extractable ferulic acid was significantly higher at 180°C (3.14 ± 0.24 mg/100 g DW) than in raw grain (1.96 ± 0.24) or at 120°C (1.69 ± 0.38 ; **Figure 9**). This is attributable to thermally driven hydrolysis of the ester bonds linking ferulic acid to arabinose residues of arabinoxylan cell-wall polysaccharides, a well-established phenomenon in high-temperature dry roasting of cereal grains [11] [37] [39]. At 180°C the thermal energy is sufficient to cleave the relatively labile feruloyl-arabinose ester linkage, releasing bound ferulic acid into the measurable acid-extractable pool. The slight non-significant decrease at 120°C relative to raw grain

is consistent with partial esterification or oxidation of ferulic acid at moderate temperatures before thermal hydrolysis becomes dominant, a biphasic pattern that parallels the early-germination dip described above.

Vanillic acid, which is more thermally stable than gallic acid, showed only a modest and non-significant rise at 120°C followed by a return towards baseline at 180°C (**Figure 10**), consistent with its intermediate thermal lability and with its dual origin as both a direct phenylpropanoid product and a ferulic acid catabolite [39] [40].

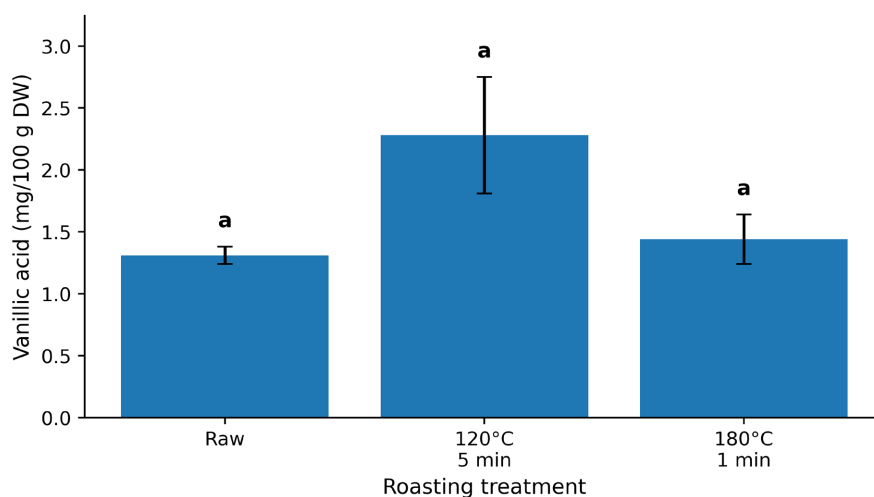


Figure 10. Effect of roasting treatment on vanillic acid content. Bars are mean $\pm$ SE ($n = 3$).

3.3. Comparative Assessment of the Two Processes

Table 4 summarises the raw baseline, the value at maximum processing intensity and the corresponding fold-change for each parameter under both processes, together with the dominant mechanism, so that the two operations can be compared directly.

Correlating the germination fold-changes with the roasting fold-changes across the five shared parameters gave a positive, moderate-to-strong Pearson coefficient ($r \approx 0.74$). This association was, however, driven almost entirely by TFC, the parameter with the largest response under both processes; excluding the Maillard-confounded TFC value collapsed the correlation to $r \approx 0.27$. Interpreted cautiously given the small number of parameters ($n = 5$, and $n = 4$ without TFC), this descriptive analysis reinforces the central finding of the study: apart from a similar acid-extractable ferulic acid endpoint, germination and roasting are largely mechanistically independent, and the apparent similarity in their bulk effects is an artefact of Maillard interference in the roasting TFC signal rather than evidence of shared underlying chemistry.

Under the conditions tested, germination produced a substantially larger and statistically significant increase in TPC than roasting. The 6.3-fold TPC increase and 7.9-fold TFC increase during germination are consistent with enzymatic phenolic release and de novo phenylpropanoid biosynthesis. Roasting at 180°C pro-

duced a non-significant TPC rise and a 12.4-fold apparent TFC increase that is interpreted cautiously because of potential Maillard-product interference. Both treatments raised the acid-extractable ferulic acid to a similar endpoint of 3.14 mg/100 g DW at their respective maximum intensities, consistent with convergence of thermal ester hydrolysis and feruloyl-esterase catalysis on an arabinoxylan-associated pool; because free and bound fractions were not separately quantified, equal extracted concentrations do not establish that both treatments released the same bound pool. A comparable divergence between sprouting and thermal processing has been reported in oilseeds, where soaking, germination and roasting exerted markedly different effects on phenolic composition and antioxidant activity [44], and the strong dependence of processing-induced change on the specific technology applied is consistent with the wider literature on polyphenol stability during food processing [13].

Table 4. Comparison of germination and roasting effects on the polyphenolic compounds of Kak W-1.

Parameter	Raw	Germination 60 h (fold-change)	Roasting 180°C (fold-change)	Dominant mechanism
TPC	18.16 [†]	115.23 (6.3×; p < 0.001)	29.30 (1.6×; p = 0.076)	Hydrolytic phenolic release plus de novo biosynthesis (germination); competing release and degradation (roasting)
TFC	22.97	181.19 (7.9×; p < 0.001)	283.90 (12.4×; p < 0.001)	CHS-pathway biosynthesis, interpreted (germination); Maillard-product interference, interpreted (roasting)
Gallic acid	1.86	7.07 (3.8×; p < 0.05)	1.90 (1.0×; 1.6× at 120°C; p = 0.062)	Tannase-mediated hydrolysis; thermal decarboxylation above ≈150°C
Ferulic acid	1.96	3.14 (1.6×; p < 0.05)	3.14 (1.6×; p = 0.024)	Feruloyl-esterase release and thermal ester hydrolysis, inferred to act on a shared arabinoxylan-associated pool
Vanillic acid	1.31	2.51 (1.9×; ns)	1.44 (1.1×; p = 0.171)	Late-phase hydrolytic release; thermally stable

Values are means (mg GAE/100 g DW for TPC; mg QE/100 g DW for TFC; mg/100 g DW for the phenolic acids). Fold-change = maximum-intensity value ÷ raw baseline. The p-value for each treatment effect (one-way ANOVA, $\alpha = 0.05$) is given in parentheses; “ns” denotes not significant. For gallic acid the roasting peak occurred at 120°C, shown in parentheses. [†]Estimated value below the validated TPC LoQ (23.10 mg GAE/100 g DW); fold-changes calculated from this baseline are approximate.

Three generalisations follow from the present data. First, reported increases in total flavonoid content following high-temperature cereal roasting [11] [12] should be confirmed by compound-specific chromatography before being interpreted as genuine flavonoid enrichment. Second, bulk colorimetric assays are best interpreted together rather than in isolation; divergence between Folin-Ciocalteu and aluminium chloride responses may provide a useful indication of assay interference that warrants chromatographic follow-up. Third, for Kak W-1 under the

conditions tested, germination produced a larger increase in TPC than the roasting treatments, while the large roasting-associated TFC response requires chromatographic confirmation. Extension to other varieties and grain sources will be required before these findings are generalised. Related work on the same KALRO germplasm has also reported changes in mineral bioaccessibility after processing [20] [45], and improved functional or sensory characteristics have been reported for malted finger millet products [18] [46]-[48].

4. Conclusion

Overall, for Kak W-1 under the conditions tested, 48 h provided a practical shorter germination duration, as extending germination to 60 h did not significantly increase TPC or TFC. Roasting produced a non-significant 1.6-fold TPC rise but a 12.4-fold apparent TFC increase that is interpreted as potentially reflecting Maillard reaction products chelating Al^{3+} in the aluminium chloride assay rather than flavonoid accumulation alone. The acid-extractable ferulic acid reached a similar endpoint under both treatments, consistent with enzymatic and thermal hydrolysis acting on arabinoxylan-associated ferulate, although free and bound fractions were not separately quantified. Thus, germination produced the clearest increase in measured polyphenolic indices in Kak W-1, whereas roasting-associated TFC increases should be verified chromatographically before being generalised. Future work should extend both treatments to a wider panel of varieties, quantify individual flavonoids in raw and roasted samples, and couple compositional measurements with *in vitro* digestion and enzyme-inhibition assays to evaluate biological relevance.

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Author Contributions

Fraser Onyango Juma conceived and designed the study, conducted experimental and analytical work, analyzed the data, and drafted the manuscript. Hudson Nyambaka and Peter Chege supervised the research, guided data interpretation, and critically revised the manuscript. Chrispus Oduori provided germplasm and agronomic guidance. Everlyne Wanzala and Judith Munga contributed to analytical methodology and nutritional interpretation. Hudson Nyambaka and Everlyne Wanzala contributed to selecting the study area. John Kinyuru and Judith Munga advised on processing conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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