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# Fate of Phytochemicals during Malting and Fermentation of Type III Tannin Sorghum and Impact on Product Biofunctionality

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**ABSTRACT:** The aim of the present study was to assess the effects of sorghum bioprocessing into Gowé on iron bioavailability and antioxidant properties of the final products. Gowé is an African sour beverage, whose process combines malting and fermenting of sorghum grains. The effects of the durations of germination and fermentation on the phytochemicals were evaluated using a central composite design. The antioxidant capacity and iron bioavailability of the derived flour were also evaluated. During the germination process, the tannin content of the grain decreased from 429.5 to 174.1 mg/100 g DM, while the total phenolic content increased from 300.3 to 371.5 mg GAE/100 g DM. The phenolic acid contents of the flour were significantly modified as a result of the durations of germination and fermentation. Both germination and fermentation enhanced the antioxidant capacity of sorghum flour, and antioxidant characteristics were significantly correlated with the levels of total phenolics, tannins, and phenolic acids. Phytate content of sorghum grain decreased drastically from 1003 to 369.1 mg/100 g DM when the duration of germination or fermentation increased. This was associated with an increase in the bioavailability of iron.

**KEYWORDS:** *sorghum, gowé, antioxidant, germination, fermentation, polyphenols, phytate, Fe, health*

## ■ INTRODUCTION

Sorghum ranks fifth among cereals grown worldwide. It is an important nutrient source for millions of people in semiarid regions, where it is a main staple food.[1] Not only is sorghum able to adapt to harsh environmental conditions, i.e., it is tolerant to drought and low-input conditions, but it also contains a range of phytochemicals with different functional properties.[2] Potential health and pharmaceutical benefits of sorghum include cholesterol mitigation, reduction of cardiovascular diseases, and anticarcinogenic properties.[2,3] The fact that sorghum is gluten free is another advantage, and this explains bakers' and brewers' increasing interest in using sorghum, as it raises the possibility of producing bakery products and beers for celiacs.[4] The biofunctionality of sorghum products has been attributed to their phytochemicals such as phenolic compounds. Sorghum contains phytate and phenolics, which possess strong chelating effects of divalent minerals such as iron, thus inhibiting their absorption in the human gastrointestinal tract. The phytate concentration in sorghum grain averaged 1.2%,[1] and the grain total phenolics content, as measured by their reactive hydroxyl groups, is as high as 3.0%.[5] In sorghum, phenolics occur mainly as phenolic acids, flavonoids, 3-deoxyanthocyanidins, and occasionally condensed tannins in tannin-type sorghums.[3,6,7] Phenolic compounds are recognized to be health-promoting phytochemicals since they can act as antioxidants by radical scavenging.[8] The metal chelation ability of polyphenols is related to the presence of ortho-dihydroxy compounds. Among phenolic acids, molecules bearing catechol and galloyl groups (including caffeic, gallic, and chlorogenic acids) are reported to have a chelating effect on iron, while those without the catechol or galloyl group (including ferulic, vanillic, and syringic acids) did not form complexes with these minerals.[9] Phytate can act as an antinutritional factor by forming insoluble complexes with essential minerals such as Fe and Zn at physiological pH levels,[10] thus reducing their absorption in the gastrointestinal tract. Phytate may be partially responsible for the widespread mineral deficiencies observed in populations that subsist largely on sorghum and other cereals.[11] It has also been suggested that phytate may diminish the solubility, the digestibility, and the activity of proteins, such as digesting enzymes.[12] Consequently, processes that minimize the chelating ability of these phytochemical contents in foods may have nutritional benefits.

Gowé is an indigenous sour beverage produced from malted and fermented sorghum grain. The product has a natural sweet and sour taste and a soft texture appreciated by infants and adults alike. Basically, the processing of gowé involves soaking, germination, grinding, spontaneous lactic acid/alcoholic fermentation, and cooking. The processing method for gowé was accurately described.[13] The dominant microorganisms during the spontaneous fermentation of gowé are lactic acid bacteria and yeast species.[14] During spontaneous lactic acid fermentation of sorghum-based foods, several polyphenol oxidases are produced, leading to the oxidation of phenolics.[7] Phenolics are also hydrolyzed during sorghum germination, and this has been associated with variations in the antioxidant activity of the product.[5] Likewise, phytase activity increases

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during germination and lactic acid fermentation of sorghum, leading to phytate degradation.[15] The objective of the present study was to evaluate the effects of germination and fermentation durations of sorghum grain on its phenolics and phytate contents and on the biofunctionality of the resulting products using the antioxidant capacity and the bioavailability of iron as indexes. The example of the African sour beverage gowé was studied. During the successive germination and fermentation of sorghum, which is typical in the processing of gowé, interactions between factors may be important. These interactions cannot be detected using a one-factor-at-a-time approach. Thus, the response surface methodology with a central composite design was used. Such designs are used to estimate parameters of a full second-degree model.

## ■ MATERIALS AND METHODS

**Chemicals and Reagents.** 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-*s*-triazine (TPTZ), trifluoroacetic acid, potassium persulfate, sodium hydroxide, gallic acid, Folin-Ciocalteu reagent, bacterial alpha-amylase, pepsin, piperazine-*N*,*N*′-bis[2-ethansulfonic acid] sodium salt (PIPES), pancreatin, and bile extract were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Cyanidin 3-glucoside came from Extrasynthese (Genay, France). Formic acid, acetonitrile, acetone, and methanol were purchased from Carlo Erba (Val de Reuil, France). All chemicals and solvents used in the study were of HPLC grade.

**Plant Materials.** A sorghum variety [*Sorghum bicolor* (L.) Moench] used by farmers and locally known as Koussoubagou, with pigmented testa, was provided by a farmer in Banikoara Region, Benin. This sorghum variety corresponds to a type III tannin sorghum according to the classification proposed by Earp et al.[16] based on tannin content of the grain. The seeds were grown on a tropical ferruginous-type soil in 2009 during the natural season of the Guinea Savannah climate of West Africa. The annual rainfall in the regions varies from 985 to 1473 mm with an average of 1237 mm.[15]

**Experimental Design.** An orthogonal rotatable central composite design[17] for $K = 2$ factors and five levels was used to estimate the simultaneous effect of two process variables on sorghum phytochemicals, pH, and antioxidant capacity, in a quadratic function. The variables (factors) were the duration of germination (0−72 h) and the duration of fermentation (0−72 h). The responses were pH, phytate, tannin, total phenolic, and phenolic acid contents, and antioxidant capacity. The design generated 14 experiments, distributed as follows: 4 kernel points, 4 star points, and 6 replications of the center point. The design matrix and variable combinations are presented in Table 1.

**Experimental Processing.** Two hundred grams of cleaned sorghum grains was soaked in distilled water (1/5, w/v) for 10 h. The grains were then drained for 5 min, laid on a polyethylene sheet, and allowed to germinate during the predefined period (Table 1). The grains were sprayed with distilled water twice daily. At the end of germination, the sprouted grains were dried at 50 °C in an oven for 10 h. The whole dried sprouted grains were milled in a laboratory mill (IKA M20, Labortechnik, Staufen, Germany) to obtain a flour that passed through a 500 $\mu$m sieve. For fermentation, the flour was mixed with distilled water (45% w/w) in a plastic bucket covered with a lid, kneaded into dough, and allowed to ferment spontaneously at room temperature during the predefined period in the experimental design. The fermented dough was dried and ground to flour as described above. Samples were packed in polyethylene bags and stored at −20 °C until analysis.

**pH Measurement.** Around 2 g of flour sample was suspended in 20 mL of distilled water, and the pH was measured at ambient temperature using a Mettler Toledo pH meter with automatic compensation of temperature.

**Determination of Tannins and Total Phenolic Compounds (TPCs).** Phenolic compounds were extracted from 50 mg of flour in 1.5 mL of HCl/methanol (1% v/v) for 1 h under continuous stirring at room temperature. The mixture was centrifuged at 5000*g* for 10 min, and the supernatant was recovered. The pellet was re-extracted as described above, and both supernatants were pooled. Condensed tannins were measured according to the method of Porter et al.[18] To 0.5 mL of extract were added 3 mL of butanol/HCl (95:5 v/v) and 100 $\mu$L of ferric ammonium sulfate solution (2% in HCl, 2 N). Hydrolysis was performed in screw-capped tubes. The mixture was heated at 95 °C for 50 min in a water bath. After cooling, the optical density was measured at 550 nm against a reagent blank. Cyanidin 3-glucoside was used as standard. Total phenolics were measured following the method of Singleton and Rossi[19] modified as follows: to 300 $\mu$L of extract were added 4.2 mL of distilled water, 0.75 mL of Folin-Ciocalteu's reagent (Merck, Germany), and 0.75 mL of sodium carbonate solution (200 g $L^{-1}$). After incubation for 30 min, the optical density was measured at 760 nm against a reagent blank. Gallic acid was used as standard, and the results were expressed as mg gallic acid equivalent (GAE) per 100 g of sample DM.

**Table 1. Design Matrix and Combinations of Variables**

| treatment code | level code | | variable level | |
|---|---|---|---|---|
| | germination | fermentation | germination (h) | fermentation (h) |
| 1 | 0 | 0 | 36 | 36 |
| 2 | 0 | 0 | 36 | 36 |
| 3 | 0 | 0 | 36 | 36 |
| 4 | 0 | 0 | 36 | 36 |
| 5 | 0 | 0 | 36 | 36 |
| 6 | 0 | 0 | 36 | 36 |
| 7 | +1 | +1 | 57.63 | 57.63 |
| 8 | +1 | −1 | 57.63 | 14.37 |
| 9 | −1 | −1 | 14.37 | 14.37 |
| 10 | −1 | +1 | 14.37 | 57.63 |
| 11 | +$\alpha$ | 0 | 72 | 36 |
| 12 | -$\alpha$ | 0 | 0 | 36 |
| 13 | 0 | +$\alpha$ | 36 | 72 |
| 14 | 0 | -$\alpha$ | 36 | 0 |

**HPLC-MS$^n$ and DAD Analysis of Phenolic Acids.** Fifty milligrams of flour was extracted as described above using acetone/$H_2O$ (70:30, v/v) acidified with 0.1% formic acid. The solvent was removed from the extracts at 40 °C in a rotary evaporator, and the resulting residues were suspended in 2 mL of methanol. HPLC/MS$^n$ analysis was carried out on a Surveyor HPLC machine equipped with a UV6000LP DAD, model P4000 pumps, and an AS3000 autosampler, coupled to an LCQ ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, CA, USA). Separation was carried out at 30 °C in a 250 mm × 4.6 mm, 5 $\mu$m particle size, end-capped reverse-phase ACE C-18 column (AIT, Houilles, France). The LC/MS parameters used are described in a previous study.[20] The solvents were acetonitrile/water (8:992, v/v) slightly acidified with 20 $\mu$L of formic acid (solvent A) or with acetonitrile (solvent B). Gradient conditions were as follows: from 5% to 35% B in 50 min, from 35% to 50% B in 5 min, and from 50% to 80% B in 5 min, after which the column was washed and equilibrated for 15 min. The injection volume was 10 $\mu$L, and the flow rate was 0.7 mL/min. Detection was carried out at 280, 330, and 360 nm.

**Determination of Phytate Content.** After extraction from 0.2 g of flour with 10 mL of HCl (0.5 M) at 100 °C for 6 min, *myo*-inositol hexaphosphate (IP6) content was determined by high-performance anion-exchange chromatography, according to the method described by Lestienne et al.[21]

**Determination of Antioxidant Capacity.** The total antioxidant capacity of extracts obtained with HCl/methanol (1% v/v) was analyzed with the ABTS radical cation scavenging assay (Trolox equivalent antioxidant capacity (TEAC)) and the ferric reducing antioxidant power (FRAP) assay.

The TEAC assay was performed following a modified method by Pellegrini et al. [22] and Moore et al.[23] A stable stock solution of ABTS

**Table 2. Responses for Phenolics, Phytate, and Antioxidant Capacity[a]**

| treatment code | concentration (mg/100 g DM) | | | phenolic acids (% area relative to raw grain) | | | | | | | antioxidant capacity (mmol/100 g DM) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TPC | tannins | IP6 | caffeic acid | caffeoyl glycerol | dicaffeoyl glyceride | Ni | ferulic acid | *p*-coumaric acid | *p*-coumaroyl glycerol | TEAC | FRAP | pH |
| raw grains | 300.3 | 429.5 | 1003 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | nd | nd | 6.85 |
| 1 | 266.1 | 176.9 | 691.6 | 4 | 2 | 1 | 7 | 257 | 44 | 6 | 5.19 | 4.30 | 4.43 |
| 2 | 284.8 | 174.1 | 542.0 | nd | nd | nd | nd | nd | nd | nd | 5.05 | 4.26 | 4.06 |
| 3 | 270.3 | 202.1 | 734.6 | 6 | 3 | 2 | 13 | 239 | 55 | 4 | 5.27 | 4.52 | 4.41 |
| 4 | 233.4 | 178.2 | 622.5 | 6 | 2 | ud | 10 | 109 | 41 | 5 | 5.16 | 4.42 | 4.48 |
| 5 | 319.7 | 207.9 | 673.5 | 6 | ud | 2 | 12 | 279 | 62 | 4 | 5.09 | 4.35 | 4.31 |
| 6 | 266.3 | 186.5 | 695.3 | 8 | ud | 1 | 7 | 263 | 59 | 4 | 5.12 | 4.44 | 4.43 |
| 7 | 371.5 | 210.7 | 369.1 | 30 | 4 | 3 | 25 | 658 | 108 | 3 | 5.58 | 4.72 | 4.18 |
| 8 | 337.1 | 246.3 | 710.2 | 12 | 4 | 3 | 18 | 246 | 44 | 4 | 5.69 | 4.80 | 4.59 |
| 9 | 316.8 | 259.7 | 778.0 | 15 | 5 | 7 | 14 | 64 | 12 | 5 | 5.31 | 4.54 | 4.81 |
| 10 | 288.4 | 232.0 | 614.0 | 11 | 2 | 1 | 7 | 243 | 75 | 5 | 5.29 | 4.38 | 4.43 |
| 11 | 363.7 | 231.3 | 410.9 | 11 | 4 | 5 | 15 | 512 | 117 | 5 | 5.64 | 4.82 | 4.30 |
| 12 | 284.6 | 224.1 | 733.0 | 7 | 2 | 2 | 7 | 54 | 42 | 5 | 5.61 | 4.70 | 4.54 |
| 13 | 283.0 | 189.2 | 577.6 | 23 | 4 | 0 | 22 | 669 | 82 | 3 | 5.47 | 4.48 | 4.09 |
| 14 | 288.0 | 223.4 | 817.5 | 30 | 8 | 17 | 18 | 174 | 130 | 14 | 5.66 | 4.88 | 6.62 |

[a]Ni = not identified; nd = not determined; ud = not detected.

radical cation was produced by reacting a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12−16 h before use. On the day of analysis, an ABTS radical cation working solution was obtained by diluting the stock solution in ethanol to reach an absorbance of 0.70 ± 0.02 AU at 734 nm. One hundred microliters of extract was mixed with 1.25 mL of the ABTS working solution, and absorbance was read at 734 nm after a 1 min reaction time. Results were expressed as TEAC in mmol of Trolox per g based on DM.

The FRAP assay is based on the reduction of the $Fe^{3+}$−TPTZ complex to the ferrous form at low pH. The reduction is monitored by measuring the change in absorption at 595 nm.[24] Briefly, 0.2 mL of sample extract was mixed with 1.3 mL of the FRAP reagent. Absorption was measured at 595 nm in a spectrophotometer (Hitachi, Japan, Type: U-1100) after 30 min of incubation at 37 °C. The FRAP reagent was prepared fresh daily and consisted of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at a ratio of 10:1:1 (v/v/v). FRAP values were obtained by comparing the change in absorption in the test mixture with doses obtained from increasing concentrations of $Fe^{3+}$ and expressed as mmol of $Fe^{2+}$ equivalent per g sorghum flour based on DM.

**Determination of Fe Content.** Total Fe content was determined by atomic absorption spectrophotometry (AA800, Perkin-Elmer, Les Ulis, France) after wet mineralization using an Ethos 1 microwave digestor (Milestone, Sorisole, Italy) for 15 min at 200 °C at a maximum power of 1000 W.

**Bioavailability of Iron.** Enzymatic *in vitro* digestions were carried out in two stages following the procedure described by Miller et al.,[25] modified by Wolfgor et al.,[26] and further adapted by Greffeuille et al.[27] Bioaccessible Fe corresponds to the percentage of dialyzable Fe, calculated on the basis of the total iron recovered at the end of the digestion. At the end of intestinal digestion, the digested samples were centrifuged at 10000*g* for 15 min at 4 °C to separate the insoluble and soluble fractions, in the pellet and supernatant, respectively. Soluble bound Fe was defined as soluble nondialyzable Fe. Dialysable and soluble bound Fe were calculated as follows:

$$\text{Dialysable Fe\%} = C_D(W_D + W_S)/(C_D W_D + C_S W_S + C_I W_I)\times 100$$

And

$$\text{Soluble bound Fe\%} = W_S(C_S - C_D)/(C_D W_D + C_S W_S + C_I W_I)\times 100$$

where $C_D$, $C_S$, and $C_I$ are iron concentrations in the dialysate, supernatant, and pellet fractions in μg/100 g and $W_D$, $W_S$, and $W_I$ are the weights (g) of dialysate, supernatant, and pellet fractions.

All samples were analyzed at least in quadruplicate, and values were averaged.

**Statistical Analysis.** Data were analyzed using the Minitab 14 (Minitab Inc., PA, USA) statistical program. A second-order polynomial model was used to describe the relationship between the responses ($Y$) and the variables ($X$) as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^{\,2} + b_4X_2^{\,2} + b_5X_1X_2$$

in which $b_0$ is a constant, $b_1$ and $b_2$ are the linear effect coefficients, $b_3$ and $b_4$ are the quadratic effect coefficients, and $b_5$ is the interaction effect coefficient. The fitted polynomial equations were expressed in a 3D response surface in which the response is presented on the vertical axis and the two factors are presented on the two horizontal perpendicular axes.

## RESULTS AND DISCUSSION

**Effect of Germination and Fermentation on Total Phenolics, Tannins, and Phytate.** The responses of the model for phenolic compounds, phytate, and antioxidant capacity are presented in Table 2. All treatments resulted in a marked decrease in phytate content and moderate decreases in tannin. The TPCs measured by their reactive hydroxyl groups increase significantly as the result of germination. The pH decreased in all samples to values between 4.0 and 5.0 except in sample 14, with no fermentation.

The fit of the polynomial equation and the estimated linear regression coefficients are presented in Table 3. Analysis of variance revealed significant effects ($p < 0.05$) of germination duration ($X_1$) on the concentration of total phenolics. In particular, the linear and the quadratic effects of this factor were significant with respect to TPC concentration of the flour (Table 3). The tannin content was affected only by the quadratic term of germination. Both germination and fermentation durations affected the phytate concentration of

Table 3. Model Coefficients and Their Significance[a]

| coefficient[b] | TPC | tannins | IP6 | caffeic acid | caffeoyl glycerol | dicaffeoyl glyceride | Ni | ferulic acid | *p*-coumaric acid | *p*-coumaroyl glycerol | TEAC | FRAP | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $b_0$ | 227.2432 | 190.9437 | 657.0855 | 6.3829 | 2.0848 | 1.0394 | 10.1101 | 227.3876 | 49.0424 | 4.2528 | 5.9310 | 5.1195 | 4.2355 |
| $b_1$ | 1.1390[c] | −0.1105 | −4.1132[d] | 0.1117 | 0.0149 | 0.0195 | 0.1662 | 6.5933[d] | 0.9171 | −0.0160 | −0.0213 | −0.0184[c] | −0.0042 |
| $b_2$ | −0.0113 | −0.5825 | −4.3828[d] | 0.0127 | −0.0409[d] | −0.1608[d] | 0.0316 | 6.8567[d] | 0.2287 | −0.0945[c] | −0.0236 | −0.0222[c] | −0.0242[d] |
| $b_3$ | 0.0477[c] | 0.0384[c] | −0.0742 | 0.0029 | 0.0007 | 0.0013 | 0.0015 | 0.0376 | 0.0158 | 0.0001 | 0.0004[d] | 0.0003[d] | −0.0000 |
| $b_4$ | 0.0178 | 0.0219 | 0.0228 | 0.0165[d] | 0.0031[d] | 0.0054[c] | 0.0081[d] | 0.1444[d] | 0.0362 | 0.0025 | 0.0003[d] | 0.0002[d] | 0.0007[c] |
| $b_5$ | 0.0335 | −0.0042 | −0.0946 | 0.0118[c] | 0.0016[c] | 0.0033 | 0.0067 | 0.1247 | 0.0003 | −0.0010 | −0.0000 | −0.0000 | −0.0000 |
| $R^2$ (%) | 66.3 | 64.5 | 85.3 | 87.8 | 97.0 | 85.8 | 82.8 | 96.0 | 53.2 | 65.2 | 87.3 | 84.3 | 80.5 |

[a]Data reported in this table are the measured (fitted) values of the coefficients $b_0$, $b_1$, $b_2$, $b_3$, $b_4$, and $b_5$, which are explained in detail in the Statistical Analysis section. Ni = not identified. [b]$b_0$, constant; $b_1$ and $b_3$ coefficients for germination; $b_2$ and $b_4$ coefficients for fermentation; and $b_5$, coefficient for interaction (germination × fermentation). [c]Significant at $p < 0.05$. [d]Significant at $p < 0.01$.

the flour with respect to their linear term. As could be expected, the pH of the flour decreased significantly as a result of fermentation duration, whereas the duration of germination had no significant effect (Table 3). Figure 1 shows the trend in sorghum phytochemical concentrations of the flour as a function of the durations of germination and fermentation. The TPC content of the flour increased significantly under the effect of germination (Figure 1a) probably due to hydrolysis of polymeric phenolics to lower polymerization degrees,[28,29] thus exhibiting higher levels of assayable phenolic hydroxyl groups.[15] Tannin content significantly decreased as the result of germination. Most of the change in the tannins content of the grain took place during the first 60 h of germination (Figure 1b). Phytate concentration significantly decreased as a result of longer germination and fermentation (Figure 1c). Hydrolysis of IP6 into its lower inositol phosphates occurred during germination and fermentation. During the development of the sprout, the degradation of phytate occurs naturally, as it provides phosphorus for the growth of the young plant. This is achieved through the activation of an endogenous phytase during the germination process.[15] In addition, during fermentation of cereal doughs, lactic acid bacteria and yeasts, which are the main functional microflora found in gowé,[14] are able to produce phytase and thereby reduce the phytate content of food products. Moreover, by decreasing the pH of the dough, fermentation could provide the optimum pH for phytase activity which ranges between pH 4.5 and 5.5 depending on the origin of the phytase.[30]

**Effects of Germination and Fermentation on Phenolic Acids.** The phenolic acids were separated and quantified using HPLC-MS$^n$ and DAD. The retention times, the mass spectra, the UV spectra, and the assignment to different phenolic compounds of the different peaks recorded are presented in Table 4. Three hydroxycinnamic acids including caffeic acid, *p*-coumaric, and ferulic acids and six derived glycerol esters were tentatively assigned. Svensson et al.[7] identified similar glycerol esters in red sorghum. Peak 3 could not be assigned, but showed the characteristic spectrum of a phenolic acid. Caffeic acid, caffeoyl-glycerol, dicaffeoyl-glycerol, and *p*-coumaroyl-glycerol were significantly affected by the duration of fermentation (Table 3). The highest value for caffeic acid (30% area compared to raw grain) was obtained for products germinated and fermented for 57.6 h (treatment 7), and the lowest values (between 4% and 6% area compared to raw grain) were obtained for products corresponding to the center points, i.e., germinated and fermented for 36 h (Table 2). Figure 1d shows changes in the concentration of caffeic acid of the flour as a function of the germination and fermentation durations. At fermentation duration between 0 and 30 h, caffeic acid content decreased. Longer fermentation increased caffeic acid content. This effect was greater for fermentation durations from 40 to 72 h than for germination. A similar trend was observed for *p*-coumaric acid (Figure 1e). In contrast, longer fermentation durations resulted in a decrease in caffeoyl-glycerol, dicaffeoyl-glycerol, and *p*-coumaroyl-glycerol contents (Figure 1f). It has already been shown that esterases of microbial origin are capable of releasing esterified hydroxycinnamic acids from cereal grains.[31,32] Recently Svensson et al.[7] showed that, during lactic acid fermentation, i.e., the most common type of fermentation used during gowé processing, phenolic acids and phenolic acid esters as well as flavonoid glucosides can be metabolized as a result of the increasing activities of phenolic acid reductase, phenolic acid decarboxylase, and glucosidase.

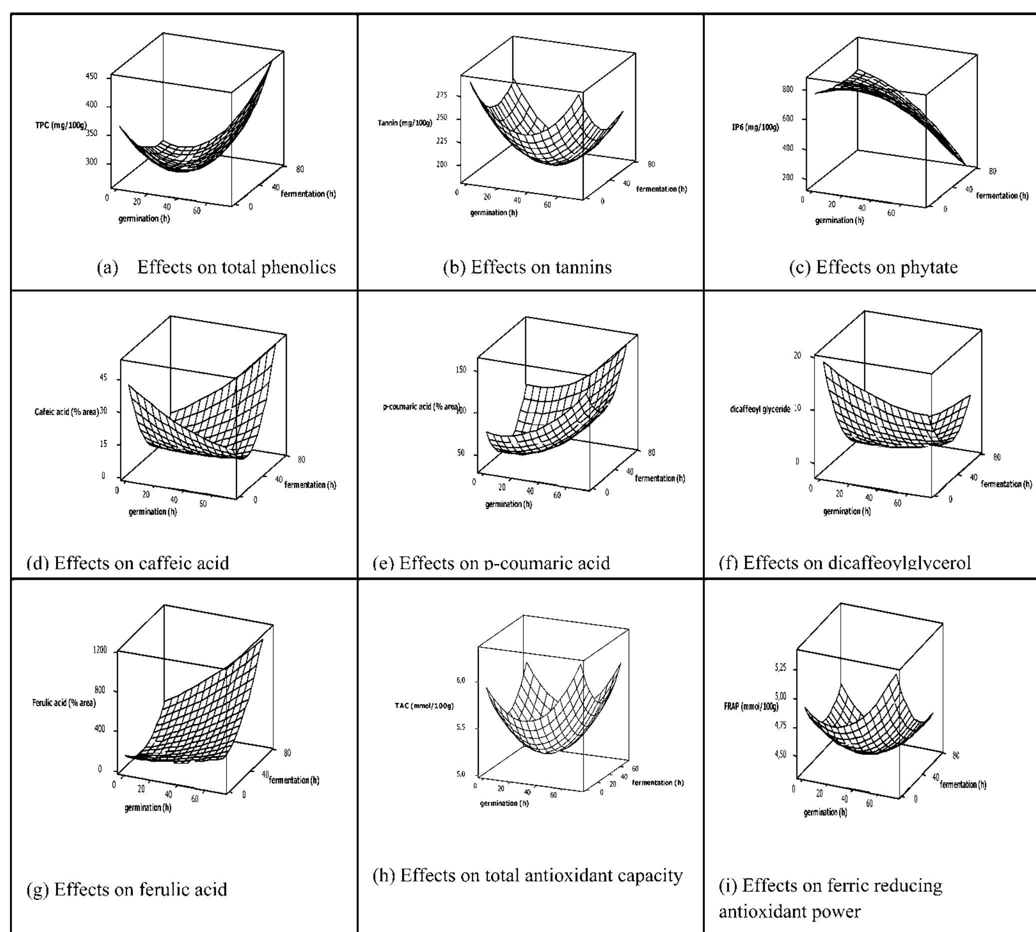


**Figure 1.** Response surface curves showing the effects of germination and fermentation periods on (a) total phenolics, (b) tannins, (c) phytate, (d) caffeic acid, (e) *p*-coumaric acid, (f) dicaffeoylglycerol, (g) ferulic acid, (h) total antioxidant capacity (TAC), and (i) ferric reducing antioxidant power (FRAP) in sorghum flour.

**Table 4. Tentative Identification of Phenolic Acids and Some Esterified Derivatives in Malted Fermented Sorghum Flour**

| peak | $t_R$ (min) | UV[a] | $[M - H]^-/M$ | $MS^2$ | tentative identification |
|---|---|---|---|---|---|
| 1 | 22.4 | 300sh, 323 | 179/180 | | caffeic acid |
| 2 | 23.9 | 300sh, 325 | 253/254 | 179, 161, 135 | caffeoyl-glycerol |
| 3 | 24.5 | 290sh, 320 | 371/372 | 249 | not identified |
| 4 | 28.4 | 300sh, 313 | 237/238 | 163, 119 | *p*-coumaroyl-glycerol |
| 5 | 28.9 | 300sh, 310 | 163/164 | | *p*-coumaric acid |
| 6 | 31.5 | 297sh, 322 | 193/194 | | ferulic acid |
| 7 | 45.9 | 300sh, 326 | 415/416 | 253, 179, 161 | dicaffeoyl-glyceride |
| 8 | 51.2 | 300sh, 318 | 399/400 | 253, 163 | *p*-coumaroyl-caffeoyl-glycerol |
| 9 | 52.2 | 300sh, 327 | 429/430 | 235, 193, 161 | feruloyl-caffeoyl-glycerol |
| 10 | 57.6 | 300sh, 320 | 413/414 | 235, 193, 163 | *p*-coumaroyl-feruloyl-glycerol |

[a]sh: shoulder.

The concentration of ferulic acid was affected by both germination and fermentation. In particular, the linear terms of these parameters ($p < 0.01$) and the quadratic effect of fermentation ($p < 0.05$) significantly contributed to the increase in the ferulic concentration in the flour (Figure 1g). The coefficient of determination ($R^2$) was 0.96 for ferulic acid (Table 3).

**Effects of Germination and Fermentation on Antioxidant Capacity.** Plant pigments and other phytochemicals have been associated with beneficial health effects, including prevention of cardiovascular diseases and cancer.[33] In this context, the antioxidant properties of extracts of sorghum flour were assessed. Total antioxidant capacity is the ability of different antioxidants to scavenge free radicals. The ABTS radical cation scavenging assay and the FRAP assay were used to evaluate the antioxidant capacity of sorghum flours. The TEAC and the FRAP values varied with the treatment, and a large proportion of variation was explained by the model. The coefficients of determination ($R^2$) were 87.3 and 84.3 for TEAC and FRAP, respectively. The highest value for TEAC (5.69 mmol/100 g of flour) was obtained with treatment 8 (i.e., 57.63 h germination time and 14.37 h fermentation time), and the lowest (5.14 mmol/100 g) was obtained from the central point (36 h germination time and 36 h fermentation time). The FRAP values varied from 4.26 to 4.88 mmol $Fe^{2+}$ equiv/100 g DM. The antioxidant capacity of sorghum flour was thus significantly influenced by both germination and fermentation durations. The linear and the quadratic effects of these factors affected the antioxidant capacity of the processed sorghum



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Table 5. Pearson Correlation Matrix between Phenolic Compounds and Antioxidant Properties

| | tannins | TPC | *p*-coumaric | caffeic | ferulic | TEAC |
|---|---|---|---|---|---|---|
| TPC | 0.588[a] | | | | | |
| *p*-coumaric acid | −0.019 | 0.402 | | | | |
| caffeic acid | 0.214 | 0.415 | 0.658[a] | | | |
| ferulic acid | −0.218 | 0.505 | 0.601[a] | 0.498 | | |
| TEAC | 0.599[a] | 0.555[a] | 0.493 | 0.590[a] | 0.271 | |
| FRAP | 0.620[a] | 0.552[a] | 0.524[a] | 0.557[a] | 0.127 | 0.930[b] |

[a]Significant at the 0.05 level (2-tailed). [b]Significant at the 0.01 level (2-tailed).

flour. The antioxidant capacities of germinated and fermented sorghum flour are comparable to those of several antioxidant sources such as red and black rice[34] and red onion.[35] The TEAC and FRAP values significantly decreased during the first 40 h of germination and fermentation and increased thereafter (Figure 1h, i). Trends in antioxidant characteristics resembled those of tannin content and were significantly correlated (Table 5). This suggests that tannins may contribute to the antioxidant properties of sorghum flour.[36] During fermentation, lactic acid bacterial strains (such as *Lactobacillus plantarum*, *Lactococcus brevis*, and *Pediococcus*) are capable of decarboxylating hydroxycinnamic acids such as *p*-coumaric and caffeic acids.[37] It has been reported that the carboxyl group exerted a negative effect on the antioxidant capacity of phenolic acids.[38] Thus, the decarboxylation of phenolics that may occur during the spontaneous fermentation of gowé could be another way to enhance the antioxidant properties of processed sorghum flour. However, further investigations should evaluate the effects of this decarboxylation on the organoleptic quality of the final product.

**Relationship between Phenolics and Antioxidant Capacity.** Tannins and TPC were significantly correlated with antioxidant capacity (Table 5). Correlation experiments to predict the antioxidant properties have been performed by many authors, resulting in different observations. Sorghum flour with higher levels of tannins and TPC had a greater antioxidant capacity.[3,5,36] A high correlation was found between TEAC and TPC in strawberries ($r = 0.95$),[39] in red cabbage ($r = 0.98$),[40] in red raspberries ($r = 0.74$),[41] and in milling fractions of pigmented wheat and barley ($r = 0.96$)[42] as well as red and black rice varieties ($r = 0.93$).[34] TEAC strongly correlated with FRAP ($r = 0.93$; $p < 0.01$). Pairwise correlations between the different assays to measure the antioxidant capacity were found between TEAC and FRAP at $r = 0.81$ in red raspberries,[41] at $r = 0.95$, $p \leq 0.001$ in strawberries,[39] and at $r = 0.90$, $p < 0.0001$ in milling fractions of pigmented wheat and barley[42] as well as at $r = 0.93$ in red and black rice varieties.[34] *p*-Coumaric acid significantly correlated with FRAP ($r = 0.52$; $p < 0.05$), caffeic acid ($r = 0.66$), and ferulic acid ($r = 0.66$). Caffeic acid significantly correlated with levels of antioxidant, whereas no significant relationship was found between ferulic acid and antioxidant capacity. Indeed, the antioxidant activity of caffeic acid has been reported by several authors.[43,44]

**Effects of Germination and Fermentation on Fe Bioavailability.** Measurements were made on three strongly contrasting flours made from raw grains, one center point (no. 6), and the kernel point no. 7, which corresponded to one of the most drastic treatments. Figure 2 shows the effects of germination and fermentation on total, bioaccessible, and bound Fe contents. The total Fe content of the sorghum sample remained constant throughout the process and averaged 3.53 mg/100 g DM. The bioaccessible Fe content increased

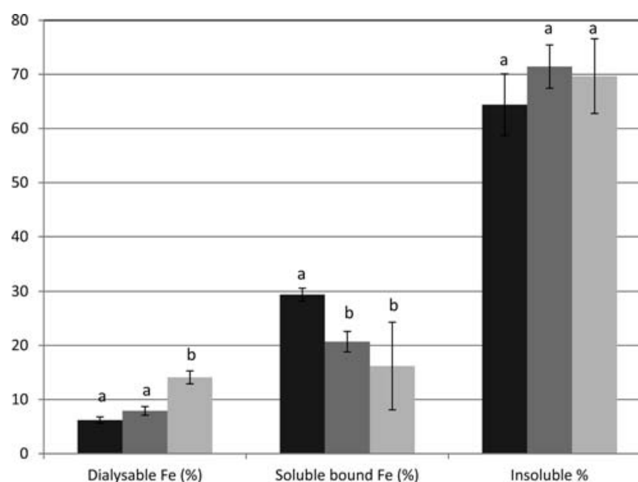


**Figure 2.** Effects of germination and fermentation periods on dialyzable and soluble bound Fe fractions after *in vitro* digestion of sorghum flour: (black) untreated grain; (dark gray) grain germinated 36 h and fermented 36 h; (light gray) grain germinated for 57 h and fermented for 57 h. Columns with the same letter in the same group did not significantly differ according to the LSD.

significantly ($p < 0.05$) from 6.2% in raw grains to 14.1% in grain germinated and fermented for 57 h. Clearly, germination and fermentation improved the Fe bioavailability of the sorghum flours as previously reported.[15] Simultaneously, the bound Fe content decreased, showing that the extra dialyzable Fe was removed from soluble bound Fe, but not from the insoluble Fe fraction, which remained unchanged. This result can be at least partially attributed to the change in phytate content, which decreased as a result of germination and fermentation (Table 2). Indeed, the raw sample with the high phytate content also exhibited high bound Fe content. Several authors reported that *myo*-inositol hexakisphosphate is the major inhibitor of divalent minerals' such as Fe and Zn absorption from plant foods, and reducing the levels of phytic acid in meals of plant origin could greatly improve the absorption of these minerals.[15,45] The decrease in tannins content may also contribute in an increase in dialyzable iron. The effect of changes in concentrations of total phenolic compounds on Fe bioavailability was apparently less clear.

In conclusion, germination and fermentation of sorghum grain enhanced the antioxidant capacity and mineral bioavailability in sorghum through degradation of phytate and changes in phenolic compound contents. Sorghum products such as gowé have potential health benefits.

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## ABBREVATIONS USED

IP6, *myo*-inositol hexakisphosphate; PIPES, pepsin, piperazine-*N*,*N*′-bis[2-ethansulfonic acid] sodium salt; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); DM, dry matter; FRAP, ferric reducing antioxidant power assay; GAE, gallic acid equivalent; TAC, total antioxidant capacity; TEAC, Trolox antioxidant capacity assay; TPC, total phenolic content; TPTZ, 2,4,6-tripyridyl-*s*-triazine

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