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Optimization of fermentation conditions for *ting* production using response surface methodology

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Abstract

This study investigated the effect of fermentation conditions (time and temperature) of sorghum on the composition of *ting*, using the Doehlert design of response surface methodology (RSM). Fermentation temperature and time were optimized and pH, titratable acidity (TTA), total viable bacteria count (TBC), total lactic acid bacteria count (TLABC), total fungal and yeast count (TFYC), tannin content (TNC), total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (AA) were determined. Experimental and predicted values obtained were similar, with statistical indices indicating the validity of the models generated (R^2 between 93.45 and 99.71%, AAD values close to 0, B_f and A_f values close to 1). Numerical multi-response optimization of parameters suggested optimal fermentation conditions to be 34 °C for 24 hr. Physicochemical characterization of *ting* samples using scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR) showed slight changes in morphology, similarity in diffraction patterns and presence of different functional groups, respectively. Results of this study could provide information for the commercialization of quality *ting*.

Practical applications

Response surface methodology was used to study the influence of fermentation conditions on the quality of *ting* and optimal fermentation conditions were obtained at 34 °C for 24 hr. The findings in this study will be useful for *ting* processors to obtain a product with maximal beneficial composition and traits.

1 | INTRODUCTION

Sorghum is an important cereal crop and major source of food for millions of people. Sorghum like other cereals is transformed into edible forms using fermentation, known to enhance nutritional qualities, shelf life, bioavailability of nutrients, palatability, beneficial health promoting components, and consumer appeal (Adebiyi, Obadina, Adebo, & Kayitesi, in press; Taylor & Duodu, 2015).

Ting, is a fermented sorghum product commonly consumed in Botswana, South Africa, and other neighboring countries. It is known for its sour taste and unique flavor and it is used as a weaning food for infants as well as consumed during ceremonies (Sekwati-Monang & Gänzle, 2011). Different fermentation conditions for the preparation of *ting* have been reported in the literature (Madoroba et al., 2009; Sekwati-Monang & Gänzle, 2011), with variations in their fermentation process, which have no available standardized or optimized conditions.

A widely accepted optimization procedure is response surface methodology (RSM), which is a collection of statistical and mathematical methods for obtaining the optimum conditions of factors for desirable responses. The Doehlert design of RSM are easily applied to

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optimize variables more effectively as they have the ability to explore the whole of an experimental domain (Ferreira, dos Santos, Quintella, Neto, & Bosque-Sendra, 2004).

Scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) are techniques that can be used to study and understand the morphology, composition and possible structural changes during food processing. The use of such techniques can provide substantial information and have been used for fermented foods (Adebiyi, Obadina, Mulaba-Bafubiandi, Adebo, & Kayitesi, 2016; Amadou, Gounga, Shi, & Le, 2014). Few studies have been presented in the literature on characterizing the microbiota and selected chemical properties of *ting* (Madoroba et al., 2009, 2011; Sekwati-Monang & Gänzle, 2011). To our knowledge, none of these studies have optimized fermentation variables and subsequently investigated their effects on the composition and physicochemical properties of *ting*. Hence, this study focused on optimizing fermentation parameters (time and temperature) of *ting* and evaluating their physicochemical properties and microstructure.

2 | MATERIALS AND METHODS

2.1 | Raw material and sample preparation

Sorghum (*Sorghum bicolor* L.) grain cultivar (Titan) was purchased from Agricol (Pty) Ltd., Potchefstroom, South Africa. The sorghum grains were milled using a Perten Laboratory Mill 3600 (Perten Instruments, Sweden) and passed through a 2 mm aperture size sieve (Analysette 3 Spartan, Fritsch, Germany) to obtain the flour.

2.2 | Fermentation of *ting*

Sorghum flour was processed into *ting* by mixing sterile distilled water (40 °C) and the sorghum flour (1:1, w/v). The mixture was subsequently allowed to spontaneously ferment by endogenous microflora. For each experimental run, the fermentation process was done in triplicate.

2.3 | Optimization of *ting* production process

A response surface methodology (RSM) using the Doehlert design was used to model and optimize the effect of fermentation parameters on the parameters investigated. The independent variables were fermentation time (X_1) and fermentation temperature (X_2), with intervals of 24–72 hr and 20–34 °C respectively. The selection of the parameter levels was based on other studies in the literature on the production of *ting* (Madoroba et al., 2009; Sekwati-Monang & Gänzle, 2011). The two-factor Doehlert design gave a total of eight experimental runs (Supporting Information Table 1). Nine parameters including pH (Y_1), total titratable acidity (TTA, Y_2), total bacteria count (TBC, Y_3), total lactic acid bacteria count (TLABC, Y_4), total fungal and yeast count (TFYC, Y_5), tannin content (TNC, Y_6), total phenolic content (TPC, Y_7), total flavonoid content (TFC, Y_8), and antioxidant activity (AA, Y_9) were investigated.

Mathematical models describing the relationship between the process variables in terms of their linear, quadratic and interactive effects used were described by a second-order polynomial equation presented in Equation (1).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum \sum_{i<j}^{k} \beta_{ii} x_i x_j \quad (1)$$

where Y is the response, x_i and x_j are factors, β_0 the constant, β_i, β_{ii}, and β_{ij} the coefficients of linear, quadratic and interaction terms, respectively. The response surfaces were subsequently represented with model equations and respective coefficients obtained using Minitab 16 statistical software (Minitab Lt. Coventry, UK).

2.4 | Model validation

The different statistical parameters utilized in validating the adequacy of the models generated, were average absolute deviation (AAD), bias factor (B_f), and accuracy factor (A_f) (Equations (2)–(4)), respectively, as well as the coefficient of determination (R^2).

$$\text{AAD} = \frac{\left[\sum_{i=1}^{N} \left(\frac{|Y_{i,\text{exp}} - Y_{i,\text{cal}}|}{Y_{i,\text{exp}}}\right)\right]}{N} \quad (2)$$

$$B_f = 10^{\frac{1}{N}\sum_{i=1}^{N} \log\left(\frac{Y_{i,\text{cal}}}{Y_{i,\text{exp}}}\right)} \quad (3)$$

$$A_f = 10^{\frac{1}{N}\sum_{i=1}^{N} \left|\log\left(\frac{Y_{i,\text{cal}}}{Y_{i,\text{exp}}}\right)\right|} \quad (4)$$

2.5 | pH and TTA

At the end of each fermentation process, pH of the *ting* was measured using a pH meter (pH 510, Eutech Pte Ltd, Singapore). Titratable acidity was determined by titrating a mixture of 2 g of *ting* sample and 20 ml distilled water against 0.1 N NaOH, using phenolphthalein as an indicator, the TTA was expressed in g of tartaric acid/kg sample.

2.6 | Estimation of viable microbial counts

For viable microbial counts, 1 g of the respective *ting* sample was added to 9 ml sterile distilled water and vortexed. Counts were determined by surface plating tenfold serial dilutions of *ting* sample on plate count agar (Oxoid, South Africa), potato dextrose agar (Merck, South Africa), and MRS agar (Sigma Aldrich, Germany) in petri dishes for bacterial (TBC), fungal and yeast (TFYC) and lactic acid bacteria (LAB) counts, respectively (Madoroba et al., 2011; Njobeh et al., 2009; Nyambane, Thari, Wangoh, & Njage, 2014). Plates were incubated (IncoShake, Labotec, South Africa) at 30 °C for 72 hr for TBC, 25 °C for 120 hr for TFYC, and anaerobically at 35 °C for 72 hr for TLABC.

2.7 | Tannin content, total phenolic content, total flavonoid content, and antioxidant activity assay

2.7.1 | Extraction

To a 0.3 g of freeze-dried milled *ting* sample, 10 ml of acidified methanol (1% HCl in methanol) was added in a centrifuge tube (Kayitesi, de Kock, Minnaar, & Duodu, 2012). The content was sealed with an

aluminum foil, stirred for 2 hr and centrifuged at 3,500 rpm for 10 min (Eppendorf 5702R, Merck South Africa). The supernatant was decanted and kept while the residue was re-extracted using 10 ml acidified methanol as earlier described. The extraction process was repeated until a total of 30 ml acidified methanol (1% HCl in methanol) was used. After extraction, the supernatants were pooled together and stored at −4 °C prior to analysis.

2.7.2 | Analytical procedure

Tannin content

Using the methods of Price, Van Scoyoc, and Butler (1978), 1 ml of extract was added to a test tube containing 5 ml of an equal volume of 8% HCl in methanol and 1% vanillin (Merck, South Africa). The resulting mixture was vortexed and incubated in a water bath (30 °C) for 20 min. A blank was also done repeating the earlier step but this time using 5 ml of 4% HCl. The absorbance of the mixture was read at 500 nm using a spectrophotometer (Biomate, Thermo Spectronic, Rochester). Catechin (Sigma Aldrich, Germany) was used as a standard and results obtained expressed in mg catechin equivalents (CE)/g.

Total phenolic content

The TPC of the *ting* sample was determined according to Folin–Ciocalteu method as described by Ainsworth and Gillespie (2007). To 500 μl of distilled water, 10 μl of the extract was added and reacted with 50 μl of the Folin–Ciocalteu phenol reagent (Sigma Aldrich, Germany). This was allowed to stand in the dark for 3 min followed by the addition of 200 μl of 20% Na_2CO_3 (g/v) and finally 245 μl of distilled water and mixed. The mixture (300 μl) was accurately pipetted into a 96-well microplate, wrapped in aluminum foil and further incubated in the dark for 30 min and absorbance read at 750 nm wavelength on a microplate reader (iMark, Biorad, South Africa). Gallic acid (Sigma Aldrich, Germany) was used as a standard and results obtained expressed in mg gallic acid equivalents (GAE)/g.

Total flavonoid content

Using the method of Ar-Farsi and Lee (2008), TFC was determined by mixing 30 μl of the extract with 20 μl of 36 mM $NaNO_2$ followed by incubation in the dark for 5 min. Thereafter, 20 μl of 94 mM $AlCl_3$ was added and after incubation for another 5 min (in the dark), 100 μl of NaOH was added. The absorbance of the mixture was read at 450 nm on a microplate reader (iMark, Biorad, South Africa). Catechin (Sigma Aldrich, Germany) was used as a standard and data obtained were expressed as mg CE/g.

Antioxidant activity

The free radical scavenging potential of *ting* sample was determined using the ABTS modified methods of Awika, Rooney, Wu, Prior, and Zevallos (2003) and Kayitesi et al. (2012). To 20 μl of the extract, 180 μl of ABTS free radical cation solution (equal volumes of 7 mM ABTS and 2.45 mM $K_2S_2O_8$ previously incubated for 12 hr) was added and incubated for 5 min in the dark. Absorbance of the solution was measured at 750 nm on a microplate reader (iMark, Biorad, South Africa). Trolox (Sigma Aldrich, Germany) was used as a standard solution and results obtained expressed as μM trolox equivalents (TE)/g sample).

2.8 | Physicochemical characterization

For both SEM and XRD, representative samples were obtained by vigorously homogenizing the freeze-dried *ting* samples obtained from each experimental run prior to analysis.

2.8.1 | SEM analysis of the ting samples

The *ting* samples were mounted on an aluminum stub and sprayed-coated in a carbon coater (Quorum Q150TE, Quorum Technologies, UK). The samples were then transferred to SEM specimen chamber, subjected to electron beam and viewed under a scanning electron microscope under vacuum.

2.8.2 | XRD analysis of the ting samples

The *ting* samples were loaded into the XRD sample holder and pressed down using a stainless steel weight. The XRD pattern of the samples were examined using an X-ray diffractometer (Rigaku-UltimaIV, Japan) equipped with a divergence slit, operating at 40 kV and 40 mA at a scan speed of 1°/min.

2.9 | FTIR spectroscopy and chemometric analysis

For analysis on FTIR, spectra of each triplicate fermented *ting* sample were obtained in duplicates giving six spectra for each *ting* sample. This was done using a FTIR spectrophotometer [Thermo Scientific Smart iTR, (Attenuated Total Reflectance), Thermo Fisher Scientific Inc., United States]. Respective spectra with characteristic peaks in wave numbers from 400 to 4,000 cm^{-1} at 32 runs per scan were subsequently recorded. All the FTIR spectra were respectively pre-treated using the following transformation techniques (i) baseline correction, (ii) normalization, (iii) standard normal variate (SNV), (iv) smoothing using Gaussian filter, (v) smoothing using Savitzky–Golay, and (vi) derivation using Savitzky–Golay first derivative on Unscrambler X statistical software version 10.4.2 (Camo software, Oslo, Norway). Principal Component Analysis (PCA) and Cluster Analysis (CA) were subsequently done on the same chemometric software.

2.10 | Statistical analysis

Except for FTIR, all other analyses were done in triplicate. To determine the significance of the generated models, an analysis of variance (ANOVA) was conducted on Minitab 16 (Minitab Lt. Coventry, UK) and differences were considered statistically significant if $p \leq .05$. Response surface plots were obtained using Sigmaplot 12.5 (Systat Software Inc., California).

3 | RESULTS AND DISCUSSION

3.1 | Statistical models and validation

This study investigated the effects of independent process variables [fermentation time (X_1) and fermentation temperature (X_2)] of sorghum on the production of *ting*. Parameters determined were pH (Y_1), TTA (Y_2), TBC (Y_3), TLABC (Y_4), TFYC (Y_5), TNC (Y_6), TPC (Y_7), TFC (Y_8), and

TABLE 1 Coefficient of regression for the different mathematical models obtained

Coefficient	pH	TTA	TBC	TLABC	TFYC	TNC	TPC	TFC	AA
β_0	6.18	0.565	2,240,000	1,415,000	706,000	9.965	16.06	8.695	1.145
β_1	−0.45*	0.27833	1,150,000	1,017,000*	268,333	−1.9917	−4.44*	−1.1967*	−0.03500*
β_2	−0.677552*	0.43014	2,881,062*	2,083,141*	680,716*	−5.5745*	−7.1132*	−1.9977*	−0.05485*
β_{11}	−0.075	0.34	1,110,000	450,000	126,500	−0.5450	1.12	1.2500	−0.02
β_{22}	−0.14834	0.36335	3,190,187*	1,391,415*	−16,168	−2.7552	0.7467	0.9567	0.01
β_{12}	−0.24249	−0.04042	438,799	1,557,737*	138,568	−1.0335	0.4157	0.5658	−0.00577
R^2 (%)	98.77	93.45	97.95	99.11	98.28	98.58	99.71	96.80	99.63
AAD	0.01	0.12	0.01	0.11	0.19	0.12	0.02	0.12	0.01
B_f	1.00	1.00	1.01	0.98	0.96	1.04	1.00	1.07	1.00
A_f	1.01	1.13	1.10	1.12	1.23	1.11	1.02	1.10	1.00

β represents the coefficients of equations of the different models with β_0 representing the constant term, β_1 and β_2 the linear effects of fermentation time and temperature, respectively, β_{11} and β_{22} their quadratic effects and β_{12} their interactions. TTA = titratable acidity; TBC = total bacteria count; TLABC = total lactic acid bacteria; TFYC = total fungal and yeast count; TNC = tannin content; TPC = total phenolic content; TFC = total flavonoid content; AA = antioxidant activity. *Significant at $p \leq .05$.

AA (Y_9) and the different models representing each provided in Equations (5)–(13).

$$Y_1 = 6.18 - 0.45x_1 - 0.67552x_2 - 0.07500x_{1^2} - 0.14834x_{2^2} - 0.24249x_1x_2 \quad (5)$$

$$Y_2 = 0.565 + 0.27833x_1 + 0.43014x_2 + 0.34x_{1^2} + 0.36335x_{2^2} - 0.04042x_1x_2 \quad (6)$$

$$Y_3 = 2,240,000 + 1,150,000x_1 + 2,881,062x_2 + 1,110,000x_{1^2} + 3,190,187x_{2^2} + 438,799x_1x_2 \quad (7)$$

$$Y_4 = 1,415,000 + 1,017,000x_1 + 2,083,141x_2 + 450,000x_{1^2} + 1,391,415x_{2^2} + 1,557,737x_1x_2 \quad (8)$$

$$Y_5 = 706,000 - 268,333x_1 + 680,716x_2 + 126,500x_{1^2} - 16,168x_{2^2} + 138,568x_1x_2 \quad (9)$$

$$Y_6 = 9.9650 - 1.9917x_1 - 5.5745x_2 - 0.5450x_{1^2} - 2.7552x_{2^2} - 1.0335x_1x_2 \quad (10)$$

$$Y_7 = 16.06 - 4.44x_1 - 7.1132x_2 + 1.12x_{1^2} + 0.7467x_{2^2} + 0.4157x_1x_2 \quad (11)$$

$$Y_8 = 8.695 - 1.1967x_1 - 1.9977x_2 + 1.2500x_{1^2} + 0.9567x_{2^2} + 0.5658x_1x_2 \quad (12)$$

$$Y_9 = 1.145 - 0.035x_1 - 0.05485x_2 - 0.02x_{1^2} + 0.01x_{2^2} - 0.00577x_1x_2 \quad (13)$$

All calculated R^2 values in this study were above 90 (93.45–99.71%) (Table 1). R^2 values should be at least 80% to have a good fit of the model and the closer it is to 100%, the better the empirical model fits the actual data (Filli, Nkama, Jideani, & Abubakar, 2011; Sobowale, Adebiyi, & Adebo, in press). Other parameters of predictive models in biological systems that measure the relative deviation from the observed (experimental) and predicted (calculated) parameters were determined and results presented in Table 1. As observed, the closeness of the B_f and A_f to unity Equation (1)) and that of AAD to zero indicates reasonable agreements between the predicted and observed parameters (Desobgo, Stafford, & Metcalfe, 2015; Sobowale et al., in press).

3.2 | pH and TTA

The pH and TTA are important biochemical parameters peculiar to fermented foods. With increasing time and temperature, pH decreased (increased acidity) with a corresponding increase in TTA (Figure 1a,b). pH values obtained in this study were relatively higher compared with those reported by Sekwati-Monang and Gänzle (2011). This may thus be attributed to difference in sample sources, fermentation conditions, and the use of nondecorticated sorghum grains in this study. A decrease in TTA indicates an accumulation of organic acids with increase in microbial activity and metabolism of the fermenting organisms. The regression model describing the effect of fermentation time and temperature on pH and TTA is given in Equations (5) and (6), respectively, with their corresponding regression coefficient of determination values provided in Table 1. The values of AAD (0.01 and 0.12) depicted an agreement between experimental and predicted values further showing that the models adequately described the pH and TTA values (Table 2). Results also showed that the linear factors of fermentation time (X_1) and temperature (X_2) had a significant ($p \leq .05$) effect on pH of *ting* samples, while the quadratic effects were not significant, meanwhile these variables had no significant ($p \leq .05$) effect on TTA. A negative correlation of 0.936 (Table 3) between these parameters suggested that increased acidity resulted in increased amount of organic acids. While relatively higher TTA values were observed in other samples, lower TTA values in others suggests that the metabolic activities of the fermenting organisms at such conditions were relatively low.

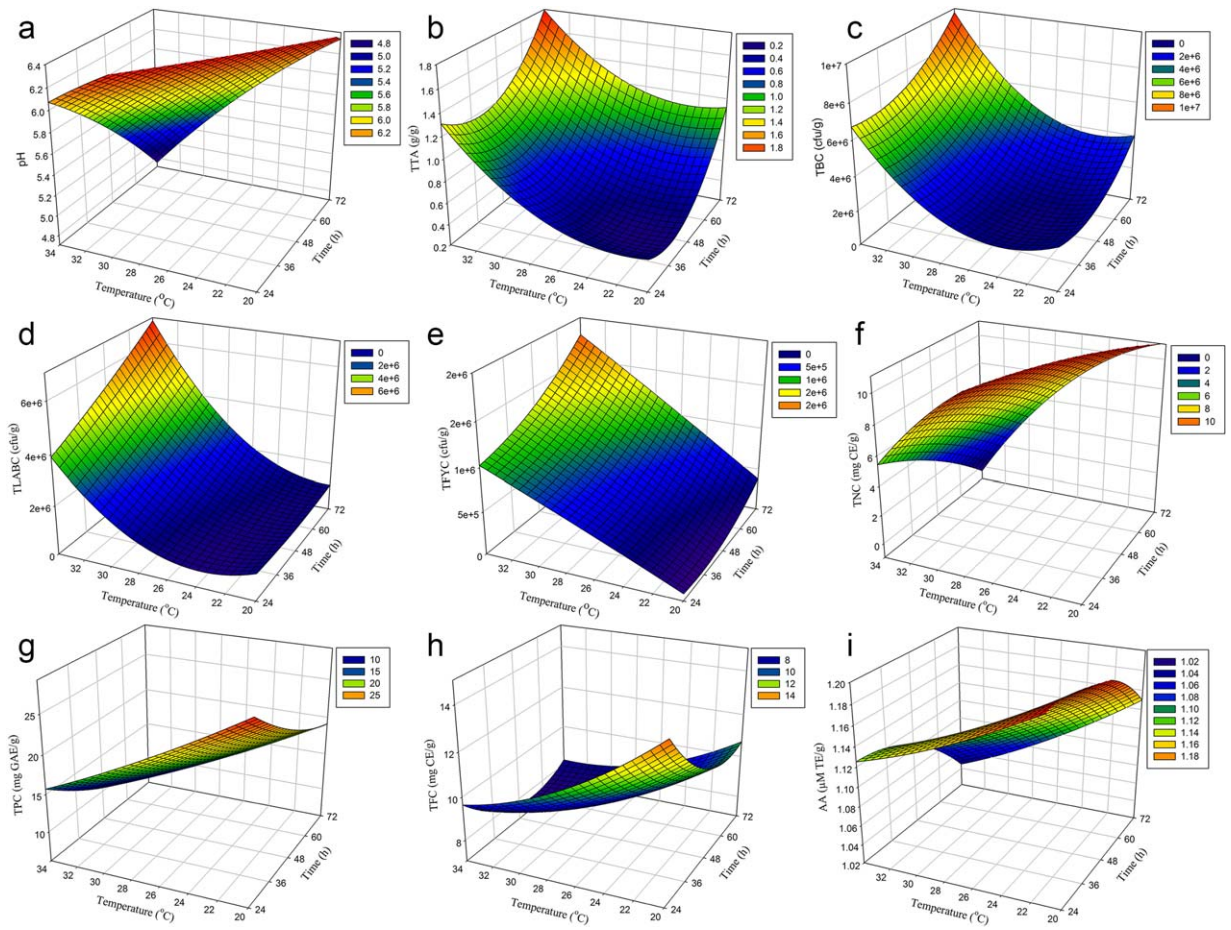

FIGURE 1 Response surface plots showing the effects of fermentation time and temperature on: (a) pH, (b) TTA, (c) TBC, (d) TLABC, (e) TFYC, (f) TNC, (g) TPC, (h) TFC, (i) AA of the ting samples

3.3 | Microbial load

Counts obtained on the different selective media used in this study (Table 2), suggested a diverse microbial flora on *ting* samples. LABs dominated the fermentation microbiota (Madoroba et al., 2011; Sekwati-Monang & Gänzle, 2011), while the relatively low counts of yeasts and fungi suggests that cereal fermentation favors the growth of LABs as compared with other group of microorganisms (Meroth, Hammes, & Hertel, 2003). As indicated by Adebiyi et al. (in press) spontaneous fermentation of cereals involves the competitive action of

TABLE 2 Experimental and predicted values obtained for the parameters investigated

Variables		pH		TTA (g/kg)		TBC ($\times 10^6$ cfu/g)		TLABC ($\times 10^5$ cfu/g)		TFYC ($\times 10^5$ cfu/g)		TNC (mg CE/g)		TPC (mg GAE/g)		TFC (mg CE/g)		AA (μM TE/g)	
X_1	X_2	Exp	Pre	Exp	Pre	Exp	Pre	Exp	Pre	Exp	Pre	Exp	Pre	Exp	Pre	Exp	Pre	Exp	Pre
48	27	6.18	6.18	0.56	0.57	2.25	2.24	14.3	14.2	7.07	7.06	9.96	9.97	16.07	16.06	8.71	8.70	1.15	1.15
48	27	6.18	6.18	0.57	0.57	2.23	2.24	14.0	14.2	7.09	7.06	9.97	9.97	16.05	16.06	8.68	8.70	1.14	1.15
72	27	5.59	5.66	1.29	1.18	4.87	4.50	27.1	28.8	11.7	11.0	7.95	7.43	12.42	12.74	8.43	8.74	1.09	1.09
60	34	5.20	5.14	1.31	1.42	7.80	8.17	57.3	55.6	14.4	15.1	0.97	1.49	9.02	8.70	7.96	7.64	1.08	1.08
24	27	6.62	6.56	0.52	0.63	1.83	2.20	10.2	8.48	4.95	5.64	10.89	11.41	21.94	21.62	11.46	11.14	1.16	1.16
36	20	6.69	6.76	0.50	0.39	2.40	2.03	7.61	9.33	1.31	0.62	13.66	13.14	25.14	25.46	11.98	12.30	1.21	1.21
60	20	6.58	6.52	0.60	0.71	2.43	2.80	7.73	6.01	1.41	2.10	11.52	12.04	20.98	20.66	10.93	10.61	1.18	1.17
36	34	5.73	5.79	1.28	1.17	7.01	6.64	30.2	31.9	11.9	11.2	4.90	4.38	12.46	12.78	8.03	13.18	1.12	1.11

X_1 = fermentation time (h); X_2 = fermentation temperature (°C). TTA = titratable acidity; TBC = total bacteria count; TLABC = total lactic acid bacteria; TFYC = total fungal and yeast count; TNC = tannin content; TPC = total phenolic content; TFC = total flavonoid content; AA = antioxidant activity; Exp = Experimental value; Pre = Predicted value.

TABLE 3 Pearson correlation between the investigated parameters

	pH	TTA	TBC	TLABC	TFYC	TNC	TPC	TFC	AA
pH		−0.914**	−0.889**	−0.934**	−0.966**	0.930**	0.968**	0.880**	0.950**
TTA	−0.914**		0.940**	0.849**	0.886**	−0.857*	−0.846**	−0.723*	−0.860**
TBC	−0.889**	0.940**		0.921**	0.852**	−0.928**	−0.812*	−0.682	−0.770*
TLABC	−0.934**	0.849**	0.921**		0.888**	−0.967**	−0.859**	−0.708*	−0.850**
TFYC	−0.966**	0.886**	0.852**	0.888**		−0.921**	−0.958**	−0.887**	−0.962**
TNC	0.930*	−0.857*	−0.928**	−0.967**	−0.921**		0.909**	0.788	0.877**
TPC	0.968**	−0.846**	−0.812*	−0.859**	−0.958**	0.909**		0.958**	0.955**
TFC	0.880**	−0.723*	−0.628	−0.708*	−0.887**	0.788*	0.958**		0.863**
AA	0.950**	−0.860**	−0.770*	−0.850**	−0.962**	0.877**	0.955**	0.863**	

**Correlation is significant at 1%.
*Correlation is significant at 5%.

endogenous microorganisms with LABs dominating. The presence of nutrients, organic acids and the acidic environment of the *ting* microbiota could have also supported the growth of other microorganisms. R^2 values (close to 100%) as observed on Table 1 indicate that the proposed mathematical models of the microbial colony count (Equations (7)–(9)) can explain more than 90% experimental observations as a function of the fermentation time and temperature. Using p values to establish the significance of each coefficient and interaction strength of each parameter, the linear factors of fermentation temperature (X_2) had a significant ($p \leq .05$) effect on TBC, TLABC, and TFYC of the *ting* samples (Table 1), while X_1 had a significant effect ($p \leq .05$) on only TLABC. The quadratic effect of both variables (X_1^2 and X_2^2) were significant ($p \leq .05$) on the TLABC of *ting* samples, whereas only X_2^2 had a significant effect ($p \leq .05$) on TBC. It can be observed from Figure 1c,d that the microbial counts increased with increasing fermentation temperature, but do not strongly depend on fermentation time (X_1). Nevertheless, the linear, quadratic, and interactive effect of fermentation temperature on microbial count was positive.

3.4 | TNC, TPC, TFC, and AA

Sorghum grains and subsequent products from them are known to be rich in tannins, flavonoids, phenolic compounds, and antioxidants and thus considered health promoting foods (Awika & Rooney, 2004; Taylor & Duodu, 2015). Experimental and predicted values of TNC, TPC, TFC and AA of *ting* samples are presented in Table 2 and their respective mathematical models presented in Equation (10)–(13). Figure 1f–i depicts the effect of fermentation time and temperature on these parameters. As observed, the effect of fermentation time (X_1) was significantly ($p \leq .05$) negative on all health promoting properties analyzed with the exception of TNC (Table 1). Similarly, the linear effect of fermentation temperature (X_2) had a significant ($p \leq .05$) effect on all the health beneficial properties. This thus, indicates that at increased temperature and sufficiently longer time, the concentrations and amounts of these parameters will decrease. Since the negative linear effect of X_1 and X_2 were significant ($p \leq .05$) on the health promoting parameters, while their corresponding quadratic factors were not, it can be suggested that both variables had a cumulative negative effect on the TNC, TPC, TFC and AA of *ting*. A general decrease of the investigated properties with increase in fermentation time and temperature could be attributed to reduced extractability of the phenolic compounds due to self-polymerization and/or interaction of these compounds with other macromolecules (Beta, Rooney, Marovatsanga, & Taylor, 2000; Taylor & Duodu, 2015). Such reduction and degradation of phenolic compounds in *ting* have also been reported by Svensson, Sekwati-Monang, Lutz, Schieber, and Gänzle (2010) and attributed to the actions decarboxylases, reductases, esterases, and the ability of LABs in fermented sorghum to metabolize phenolic compounds. A strong positive correlation between the AA and TPC (Table 3) strongly suggests that the phenolic contents of the *ting* samples, largely contributed to the antioxidant activities.

3.5 | Multiresponse numerical optimization

The surface plots (Figure 1a–i) shows the effect of different process variables (fermentation time and temperature) on the investigated parameters. A numerical optimization approach was adopted to determine the best experimental conditions for maximum release of phytochemicals accompanied by a good microbial growth, reduced pH, and high organic acid production. The numerical optimization of the process variables was done on Minitab 16 (Minitab Lt. Coventry, UK) and all parameters were investigated. The optimum derived conditions were fermentation time and temperature of 24 hr and 34 °C, respectively. The corresponding predicted parameters at this condition were pH (6.09), TTA (1.31 g/kg), TBC, (6.71×10^6 cfu/g), TLABC (2.35×10^6 cfu/g), TFYC (1.02×10^6 cfu/g), TNC (5.41 mg CE/g), TPC (15.66 mg GAE/g), TFC (9.64 mg CE/g), and AA (1.13 mg TE/g). To confirm the predicted values, the theoretical value was tested in triplicate using the optimal fermentation conditions obtained. Subsequent analysis gave the following results; pH (6.00), TTA (1.34), TBC ($6.19 \times$

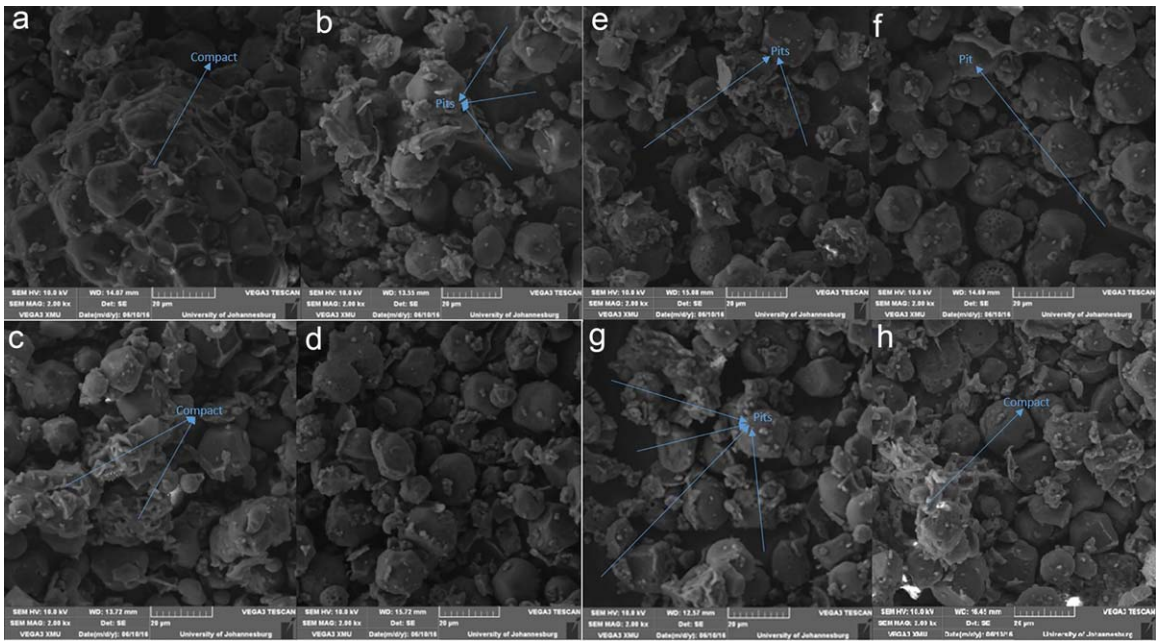

FIGURE 2 Scanning electron images of ting samples: (a) 20 °C 36 hr, (b) 20 °C 60 hr, (c) 27 °C 24 hr, (d) 27 °C 48 hr, (e) 27 °C 72 hr, (f) 34 °C 36 hr, (g) 34 °C 60 hr, (h) 34 °C 24 hr

10^6 cfu/g), TLABC (2.01 × 10^6 cfu/g), TFYC (9.91 × 10^5 cfu/g), TNC (5.36 mg CE/g), TPC (15.48 mg GAE/g), TFC (9.67 mg CE/g), and AA (1.18 mg TE/g). These obtained results are closely related to the numerical optimized data obtained, thus showing that the regression models obtained could adequately predict the parameters.

3.6 | SEM and XRD of the *ting* samples

The SEM images of samples of *ting* were compared with investigate possible morphological changes after fermentation. It can be observed from the micrographs (Figure 2), that the granules were predominantly spherical, with fermentation causing a degradation in the granular structure. There was a gradual change from a more compact, fused and consistent structure to a more loosened, disoriented one, forming pits (Figure 2). This was more pronounced in *ting* samples fermented for longer time and higher temperatures, suggesting increased degradation and hydrolysis of components such as starch and amino acids.

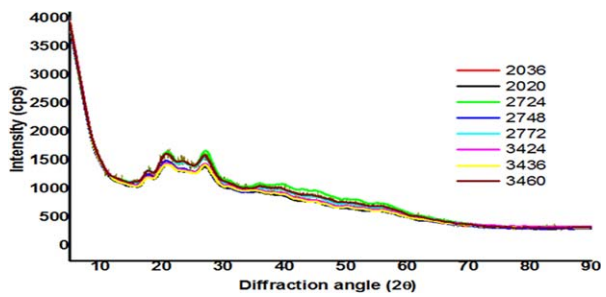

FIGURE 3 XRD patterns of the ting samples: 2036 (20 °C 36 hr), 2060 (20 °C 60 hr), 2724 (27 °C 24 hr), 2748 (27 °C 48 hr), 2772 (27 °C 72 hr), 3424 (34 °C 24 hr) 3436 (34 °C 36 hr), 3460 (34 °C 60 hr)

Differences in the diffraction pattern of *ting* samples as affected by the fermentation variables is presented in Figure 3. The diffractogram of all *ting* samples showed similarities and exhibited the same "A" pattern of diffraction, with significant peaks at angles of 2θ around 17.78, 21, and 27.07°. Differences in relative intensities could be attributed to the difference in the cellular and granular size (as observed with SEM) and the proportion and arrangements of the amylose and amylopectin components, which are known to have an impact on retrogradation and gelatinization.

3.7 | FTIR of the *ting* samples

FTIR spectroscopy was used to evaluate the composition of the obtained *ting* samples in terms of their functional groups, while band intensities could suggest their relative abundance. A comparative evaluation of the average spectra (Supporting Information Figure 1) revealed that the *ting* samples all had strong absorption peaks around 3,300, 2,900, 2,850, 1,740, 1,640, 1,520, and 1,007 cm^{-1}. Broad spectral peaks around 3,300 cm^{-1} is associated with O—H and polyhydroxyl bearing phenolic compounds suggesting the presence of quercetin, alcohols, and phenolic compounds present in fermented foods (Adebiyi et al., 2016; Taylor & Duodu, 2015). Bands occurring at 2,900 or and diffuse ones at 2,850 cm^{-1} could be attributed to alkanes (C—H stretching), aldehydes (H—C=O) and bound water in form of moisture, while peaks at 1,640 cm^{-1} represent conjugated carbonyl bonds which could be from flavonoids and esterified phytosterols (Adiana & Mazura, 2011). Peaks around 1,520 cm^{-1} represents the C=C—C signals of

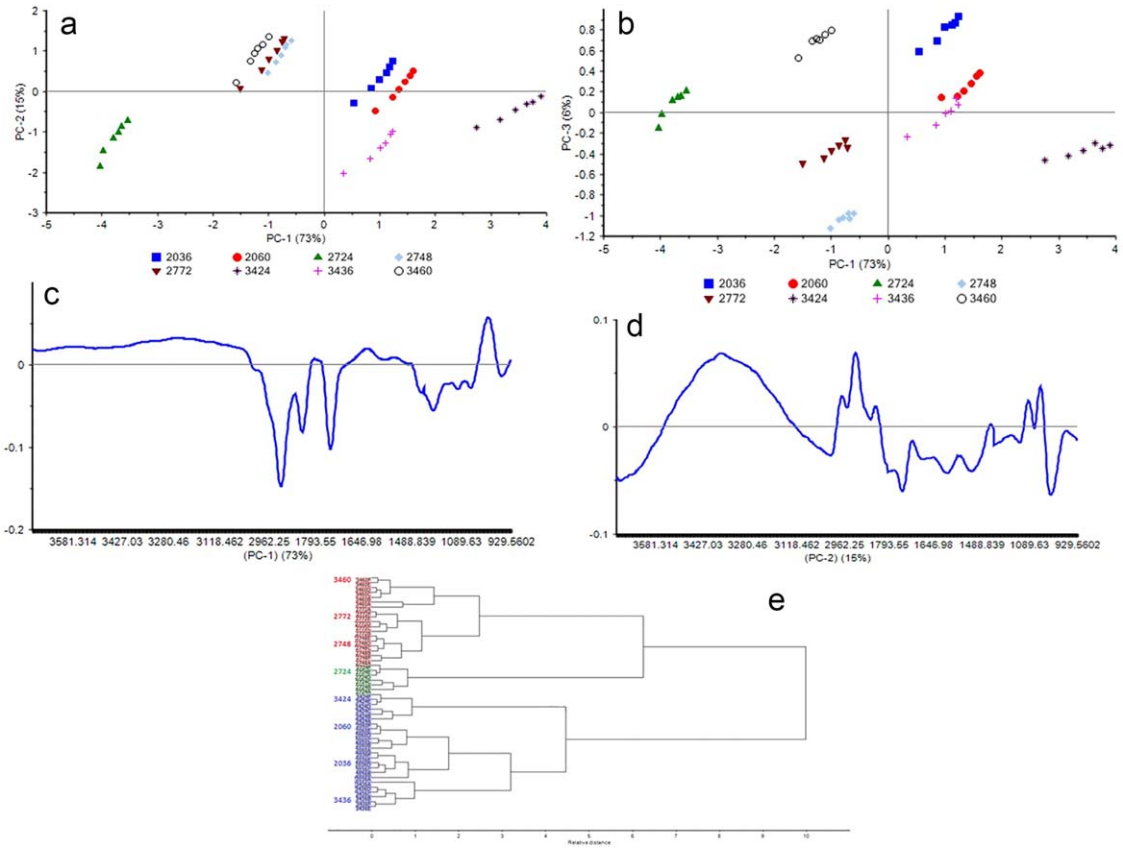

FIGURE 4 Plots of multivariate data analysis of the ting samples: (a) PCA scores plot (PC-1 and PC-2); (b) PCA scores plot (PC-1 and PC-3); (c) PC-1 loadings plot; (d) PC-2 loadings plot; (e) CA dendrogram

condensed tannins, sharp peaks at 1,143 cm^{-1} can be assigned to the —OH aromatic bond present in catechins, while the intense peaks at 1,007 and 930 cm^{-1} suggest the presence of phenolic compounds (flavonoids, tannins, glucosyl moieties), carbohydrates and amines containing the stretching vibrations of =C—O—C, C—C, or vibrational C—O—H bonds (Duodu, Tang, Grant, Wellner, & Belton, 2001; Sinelli, Spinardi, Di Egidio, Mignani, & Casiraghi, 2008). The high intensity bands in this region can be explained by the high amounts of flavonol glycosides and a possible accumulation of proteins (in form of amino acids) in the *ting* samples.

Regions associated with these described bands were selected, pretreated and subjected to PCA using different transformation techniques (Supporting Information Figure 2). The best pretreatment technique in terms of sample grouping coupled with relatively high variation of principal components (PCs) was the SNV pretreated data set (Figure 4a). As observed from Figure 4a, the first two principal components (PCs) accounted for 88% of the total variation. While PC-1 with 73% of the total variation differentiated *ting* samples fermented at 20 °C (for 36, 60 hr) and 34 °C (for 24, 36 hr) on the right, PC-2 with 15% separated the samples fermented at 27 °C (for 48, 72 hr), 34 °C for 60 hr, and majority of samples at 20 °C (for 36, 60 hr) above, from other *ting* samples below. This is also reflected on the discrete grouping formed along the PCs. The clustering as depicted in Figure 4a further confirm the observations on other parameters investigated in this study. Samples fermented for shorter fermentation times generally clustered together and the same was observed for those fermented for relative longer times, with temperature also playing a significant role. An additional PC-3 contributed 6% to the variation, bringing the total variation of the PCs to 94% and effectively differentiated samples fermented at 27 °C (for 48, 72 hr) from *ting* obtained at 34 °C for 60 hr (Figure 4b).

The loadings plot (Figure 4b) revealed that peaks at 2,900, 2,850, and 1,740 cm^{-1} largely contributed to the grouping of *ting* fermented at 27 °C (for 48, 72 hr) and 34 °C for 60 hr (Figure 4c). As observed from Figure 4d, the peak around 3,300 cm^{-1} was the major contributor to the discrimination of samples fermented at 20 °C, while peaks at 1,007 and 930 cm^{-1} influenced samples fermented at 34 °C for 36 hr. The cluster analysis (CA) presented as a dendrogram in Figure 4e, shows three-defined clusters, grouped in terms of sample similarity, and followed patterns of the PCA.

4 | CONCLUSIONS

Using a Doehlert RSM approach, this study investigated the effects of fermentation variables on some selected parameters, that is, pH, titratable acidity, microbial count, tannin content, total phenolic content,

total flavonoid content, and antioxidant activity. Numerical optimization of factors for the fermentation process established that optimal processing temperature and time condition for *ting* production was 34 °C for 24 hr. At these conditions, maximal phenolic, tannin, flavonoid contents, and antioxidant activity were derived, complemented with good microbial growth, reduced pH, and high production of organic acids. The reduced pH and high TTA levels at this optimal condition, would extend shelf life and preserve the *ting* better. Physicochemical characterization of *ting* first reported in this study, showed slight changes in the microstructure and similarity in the diffraction pattern of the differently obtained *ting* samples. FTIR analysis further confirmed the presence of different functional groups, while chemometric analysis effectively showed differentiation and variations in *ting* samples analyzed. Results from this study will be beneficial for the production of high quality *ting* for subsequent consumption.

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