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The Effects of Germination and Roasting on Nutraceutical and Antioxidant Properties of *Jirani* Variety of Millet

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Abstract The objective of this study was to determine the effects of processing variables (germination time, roasting temperature and time) on the phytonutrients and antioxidants activity of millet. The germination time employed in this study ranged from 24 - 72 h, roasting temperature ranged from 112.5 -120.0°C and the roasting time ranged from 15-21 min based on an earlier preliminary study. All samples exhibited antioxidant properties and these properties were dose dependent. Positive correlations were obtained between the antioxidant activity of the samples and the content of the phytochemicals. Both germination and roasting time had an effect on the total antioxidant capacity of the germinated millet product. There was an interactive effect between the germination time and roasting time on the total antioxidant capacity and DPPH scavenging property. A negative interactive effect of germination time and roasting temperature as well as roasting temperature and roasting time on the total antioxidant capacity and DPPH values were observed. The germinated and roasted millet products showed the total antioxidant capacity of 39.30 - 66.01 mg/100g, DPPH value of 68.26 - 79.65 µg/ml and reducing power values of 0.353 - 0.441 µg/ml. The results demonstrated that germinated and roasted millet could be useful as an ingredient for functional food. The optimum conditions for processing millet into a functional food ingredient are germination time of 68.97 h, roasting temperature of 114.79°C and roasting time of 15.00 min, resulting at roasted millet product that possess 54.644 mg/100g of total antioxidant capacity, 72.152 µg/ml of DPPH value and 0.376µg/ml of reducing power values.

Keywords: *millet, phytonutrient, germinate, optimize, antioxidant capacity, reducing power, DPPH*

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1. Practical Application

The results of this study revealed that germinated and roasted *Jirani* millet grains possess high antioxidant properties and can be consumed to manage the onset and manage some age related non-communicable diseases. The processing technologies utilized involved cleaning, washing, germinating, kilning, drying, roasting, cooling, milling (optional) and packaging which have been successfully optimized to produce germinated and roasted *Jirani* millet of high antioxidant properties. Consequently most cereal based food formulations for adult and elderly population can be replaced with germinated and roasted *Jirani* millet or the germinated and roasted *Jirani* millet as spiced/flavored Snacks.

2. Introduction

Phytonutrients or phytochemicals are bioactive food components found in plants that contribute positively to human health such as in the prevention and/or treatment of disease and various physiological disorders [1]. Some of the important bioactive phytonutrients include polyphenols,

terpenoids, resveratrol, flavonoids, isoflavonoids, carotenoids, limonoids, glucosinolates, phytoestrogens, phytosterols, anthocyanins, ω-3 fatty acids, and probiotics. They possess anti-microbial, anti-oxidants, anti-inflammatory, anti-allergic, anti-spasmodic, anti-cancer and anti-aging properties [2,3,4].

Cereals are increasingly used in different food formulations and are good sources of phytochemicals and dietary fiber [5,6]. Millet, a cereal, is one of the most frequently utilized for food and feed formulations that has gained attention recently due to its resistance to pests and diseases, short growing season, and productivity under hardy and drought conditions when major cereals cannot be relied upon to provide sustainable yields. Additionally Millet is rich in phytates, tannins, phenolic compounds, resistant starch which are phytonutrients such that millet is believed to have high antioxidant properties [7,8,9]. However, millets are underutilized in many developed countries [10]. Several studies have been conducted on Finger Millets and have been reported to contain higher amount of phytonutrients such as tannins, phytates, saponin and others [11] than other variants of Millet grains. However, these phytonutrients remove can be reduced by processing methods such as germination, soaking in water, roasting, fermentation or a combination of those methods for optimum reduction of the phytonutrients to tolerable levels in the finished products [12,13,14,15,16].

Effect of processing techniques on antioxidant properties of finger millet have long been documented by many researchers [17,18,19] but *Jirani* variety of millet (LCICMW -4) which is important to Nigeria has not been adequately researched. Although, germination enhances the nutritive value of cereals and legumes [20]; few data are available on the effect of its antioxidant properties especially with *Jirani* variety of millet. Endogenous phenolic compounds in cereals have been reported to be modified during the germination of the grains and this may have a pronounced effect on the antioxidant potentials of the germinated product. This study therefore assessed the effects of processing parameters on the phytonutrients content and anti-oxidant activities, of differently processed *Jirani* variety of millet.

3. Materials and Methods

3.1. Materials

3.1.1. Raw Material Collection

The millet grains (*Jirani* LCICMW - 4) was supplied by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Kano, Nigeria.

3.1.2. Processing of Millet Grains

Millet grains were washed with potable water using the grain to water ratio of 1:3. The seeds were drained and allowed to germinate on a bed thickness of about 30 mm (open air at 28 ± 2°C) for period (24, 48 and 72 hr). The germinated millet were subsequently roasted using an electric oven (Multilayer Deck, Electron, Model - 0532321-1) at different temperatures (105,112 and 120°C) and time (15, 18 and 21 min) combination based on the process design matrix generated in using as stated in the design matrix (Table 1)

3.1.3. Experimental Design

The RSM technique reported by [21] was utilized in evaluating the simultaneous effect of processing variables (germination time, roasting temperature and roasting time) on the phytonutrient and antioxidant properties of millet (*Jirani* LCICMW - 4 variety) using the Design Expert Software package (version 6.0.6) Stat-Ease, Inc. MN USA. Table 1 presents the design matrix that was used to identify the effects of processing conditions on phytonutrients content and antioxidant properties of millet.

Table 1. Box-Behnken design matrix used to evaluate the effects of process variables on phytonutrient content and antioxidant properties of millet measured

Runs	A:Malting Time (Hr)		B:Roasting Temp (°C)		C:Roasting Time (Min)	
	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded
1	+1	72.00	0	112.50	-1	15.00
2	-1	24.00	+1	120.00	0	18.00
3	-1	24.00	-1	105.00	0	18.00
4*	0	48.00	0	112.50	0	18.00
5	-1	24.00	0	112.50	-1	15.00
6*	0	48.00	0	112.50	0	18.00
7	0	48.00	-1	105.00	-1	15.00
8*	0	48.00	0	112.50	0	18.00
9	+1	72.00	+1	120.00	0	18.00
10	0	48.00	+1	120.00	-1	15.00
11	+1	72.00	-1	105.00	0	18.00
12	-1	24.00	0	112.50	+1	21.00
13*	0	48.00	0	112.50	0	18.00
14	0	48.00	-1	105.00	+1	21.00
15	+1	72.00	0	112.50	+1	21.00
16*	0	48.00	0	112.50	0	18.00
17	0	48.00	+1	120.00	+1	21.00

*Midpoint repeated five times.

3.2. Biochemical Analysis

The biochemical analyses of the samples were carried out to determine the DPPH radical scavenging activity, ferric ion reducing antioxidant power assay and total antioxidant capacity. DPPH as commonly abbreviated for the organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl and the DPPH assay is routinely practiced for assessment of free radical scavenging potential of an antioxidant molecule and considered as one of the standard methods for the evaluation of antioxidant properties [22]. Total antioxidant capacity is frequently used to assess the antioxidant response against free radicals. The methods of the analysis are as described below.

DPPH radical scavenging activity assay: The methods of Cuendet *et al*. [23] and Burits and Bucar [24] were used for the determination of DPPH radical scavenging activity in the Jirani millet processed products. An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/ ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature for 30 minutes. The decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was determined at 517 nm. The scavenging effect was calculated using the expression:

$$\%Inhibition = \left[A0 - A1\right] X \frac{100}{A0}$$

Where: A0 = Absorption of the blank sample;
A1 = Absorption of the extract.

3.2.1. Ferric Ion Reducing Antioxidant Power Assay

This was determined according to the documented procedures of Cuendet *et al*. [23] and Burits and Bucar [3]. One ml of different concentrations of the extracts ranging from 20 to 100µg/ml which were in deionized water was mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml). After the incubation of the mixture at 50^{o}C for 20mins, aliquots of trichloroacetic acid (2.5ml) were added to the mixture, which was then centrifuged at 300rpm for 10min. the upper layer of solution (2.5ml) was removed and mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance of the sample and the blank (without adding extract) was measured at the 700nm.

3.2.2. Total Antioxidant Capacity Determination

The method of Prieto *et al*. [25] was used to determine the total antioxidant capacity of the Jirani millet samples. A solution of the sample extract (1ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 minutes. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm. The total antioxidant capacity was expressed as equivalent of ascorbic acid.

3.2.3. Data Analysis and Modeling

Five replicates at the center point for a 3-factor-3-level Box-Behnken experimental design was used to develop predictive models for the selected responses (phytonutrient content and antioxidant properties) determined in this study as reported by [21]. The 3 factors (i.e processing variables), levels and experimental design in terms of coded and uncoded are presented in Table 1. The following second-order polynomial equation of function Xi was fitted for each factor assessed:

$$Y = \beta 0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X^2_{ii} + \sum_{\substack{i=1 \\ i<j}}^{3} \sum_{j=1}^{3} \beta_i j X_i Xj$$

Where Y is the estimated response; $\beta 0$, βi, βii, βij are constant coefficients. Xi, Xj, which are defined as the coded independent variables include the germination time, roasting temperature and roasting time. The analysis was performed using uncoded units. Using Box-Behnken design, the number of tests for the germination time - roasting temperature - roasting time - combinations (germination time: 24 - 72 hrs, roasting temperature: 105 -120°C, roasting time: 15 - 21 mins) were 17 runs. In the center point of the model where the medium levels of three processing variables were represented, the experimental combination was repeated for five times (4th, 6th, 8th, 13th and 16th runs as indicated in Table 1 to estimate the experimental variance as suggested by the design.

4. Results and Discussion

4.1. Model Fitting from RSM

The effects of the process variables (germination time, roasting temperature and roasting time) on total anti-oxidant capacity (TA), 2, 2-diphenyl-1-picrylhydrazy (DPPH) and reducing power (RP) of the processed millet grains are as shown in Table 2 The independent and dependent variables were fitted to the second-order model equation and examined for the goodness of fit.

Analyses of variance (ANOVA) was used to determine lack of fit. The significance of the linear, quadratic and interaction effects of the independent variables on the dependent variables are as indicated in Table 3.

The results showed that the models for all the response variables were highly adequate because they had satisfactory levels of R^2 of more than 85% which indicated high variability in the generated data in this study. There is no significant lack of fit in all the response variables observed in this study.

4.2. Effect of Germination Time, Roasting Temperature and Roasting Time

The effect of different germination time, roasting temperature and roasting time on the phytonutrient content and antioxidant properties of processed millet grains are reported (Table 3) by the coefficient of the second-order polynomials. To aid visualization, the response surfaces for these response variables are as shown in Figure 1 - Figure 3.

Table 2. Box-Behnken design arrangement and experimental result for the response variables of the millet grains

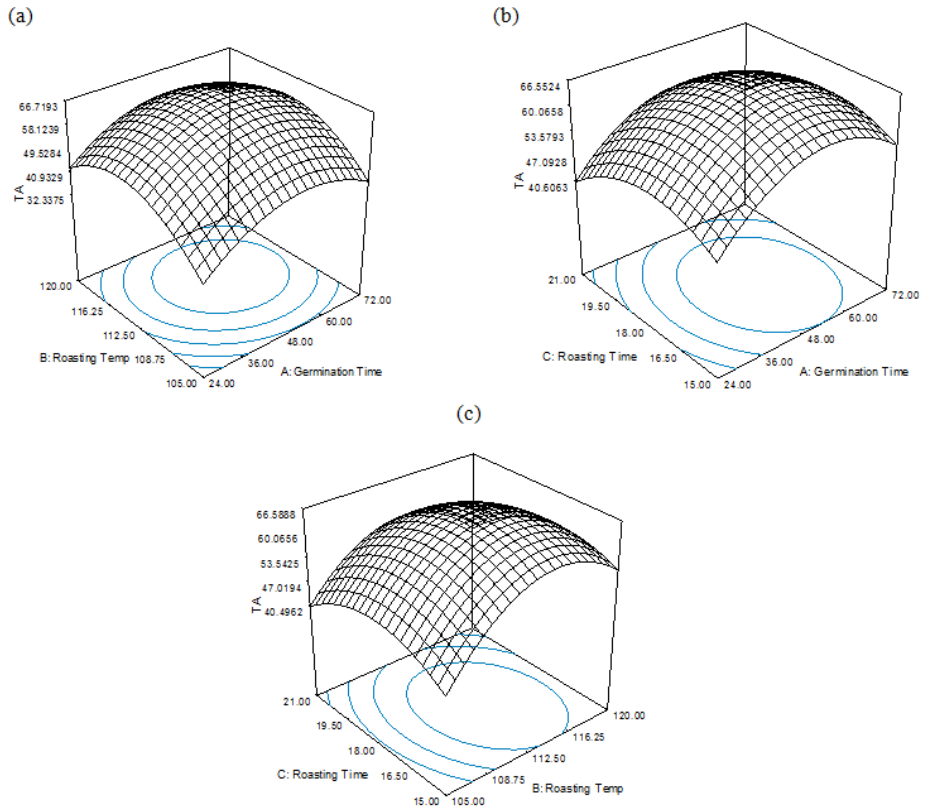
Runs	A:Germination Time (Hrs)	B :Roasting Temp (Deg C)	C:Roasting Time (Mins)	Response		
				TA (mg/100g)	DPPH (µg/ml)	RP
1	72.00	112.50	15.00	57.68	79.75	0.485
2	24.00	120.00	18.00	45.00	70.35	0.381
3	24.00	105.00	18.00	39.30	69.73	0.353
4	48.00	112.50	18.00	66.01	73.87	0.377
5	24.00	112.50	15.00	39.13	72.65	0.383
6	48.00	112.50	18.00	66.01	73.87	0.377
7	48.00	105.00	15.00	39.62	73.39	0.390
8	48.00	112.50	18.00	66.01	73.87	0.377
9	72.00	120.00	18.00	42.51	74.13	0.428
10	48.00	120.00	15.00	56.70	75.72	0.441
11	72.00	105.00	18.00	39.62	73.39	0.390
12	24.00	112.50	21.00	36.52	70.84	0.383
13	48.00	112.50	18.00	66.01	73.87	0.377
14	48.00	105.00	21.00	37.62	63.39	0.353
15	72.00	112.50	21.00	53.73	77.28	0.415
16	48.00	112.50	18.00	66.01	73.87	0.377
17	48.00	120.00	21.00	46.90	68.26	0.390

Table 3. Analysis of variance (ANOVA) showing the linear, quadratic interaction and the lack of fit of the response variables

Source of Variation	DF	Sum of squares		
		TA (mg/100g)	DPPH (µg/ml)	RP
Model	9	2038.30	182.86	0.017
A	1	141.04	55.02*	0.000594*
B	1	152.69	9.16	0.000297*
C	1	42.14	59.08*	0.000312*
A^2	1	549.48*	9.29	0.0000122*
B^2	1	709.25*	50.26*	0.0000152
C^2	1	257.57*	0.21	0.000213*
AB	1	1.97	0.00036	0.0000025
AC	1	0.45	0.11	0.000123*
BC	1	15.21	1.61	0.0000049
Residual	7	222.74	30.63	0.0000414
Lack of Fit	3	222.74	30.63	0.000041
Pure error	4	0.000	0.00	0.000
Cor total	16	2261.04	213.52	0.017
Model F Value		7.12	4.64	31.81
Model p V		0.0085*	0.0276	<0.0001
R^2		0.9015	0.8566	0.9761
Adjusted R^2		0.7748	0.6721	0.9455
PRESS		3563.83	490.06	0.000616

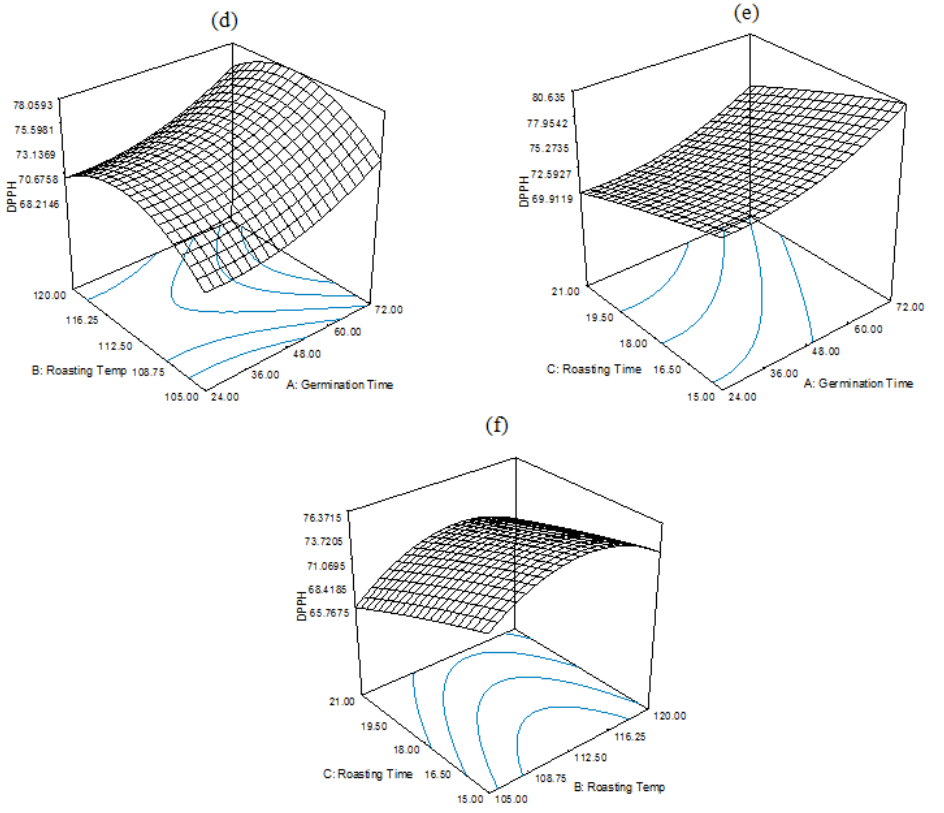
Table 4. Estimated Coefficients of the Fitted Regression Equation for Responses

Parameter	Responses		
	TA (mg/100g)	DPPH (µg/ml)	RP
B0	66.01	73.87	0.38
A - Germination Time	4.20	2.62	0.027
B - Roasting Temperature	4.37	1.07	0.019
C - Roasting Time	-2.30	-2.72	-0.020
A^2	-11.42	1.48	0.0017
B^2	-12.98	-3.46	-0.00060
C^2	-7.82	-0.22	0.022
AB	-0.70	0.030	0.00025
AC	-0.34	-0.16	-0.018
BC	-2.95	0.64	-0.00035



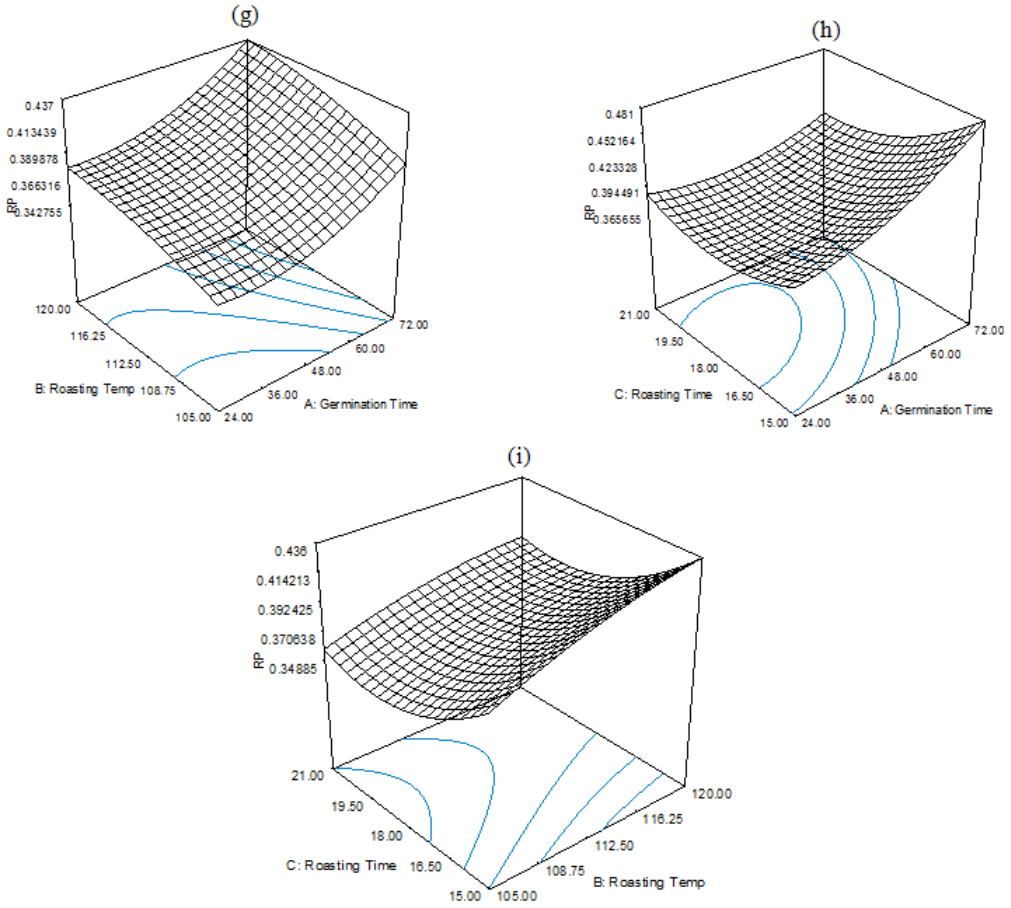
$$TA = +66.01 + 4.20*A + 4.37*B - 2.30*C - 11.42*A^2 - 12.98*B^2 - 7.82*C^2 - 0.70*A*B - 0.34*A*C - 1.95*B*C$$

Figure 1. Effect of (a) Germination time and roasting temperature, (b) germination time and roasting time, (c) Roasting temperature and roasting time on total anti-oxidant properties along with their second-order polynomial model equations predicting effects of the process variables



$$DPPH = +73.87 + 2.62*A + 1.07*B - 2.72*C + 1.48*A^2 - 3.46*B - 0.22*C^2 + 0.030*A*B - 0.16*A*C + 0.64*B*C$$

Figure 2. Effect of (d) Germination time and roasting temperature, (e) germination time and roasting time, (f) Roasting temperature and roasting time on DPPH properties along with their second-order polynomial model equations predicting effects of the process variables



$$RP = +0.38 + 0.027*A + 0.019*B - 0.020*C + 0.017*A^2 - 6.000E\text{-}003*B^2 + 0.022*C^2$$

$$+2.500E\text{-}003*A*B - 0.018*A*C - 3.500E\text{-}003*B*C$$

Figure 3. Effect of (g) Germination time and roasting temperature, (h) germination time and roasting time, (i) Roasting temperature and roasting time on RP properties along with their second-order polynomial model equations predicting effects of the process variables

4.2.1. Total Anti-oxidant (TA) Capacity of Processed Millet

An antioxidant can be defined as any substance that significantly delays or prevents oxidation of that substrate [26,27]. Figure 1 showed the 3D response surface for TA. It can be observed that the TA of the processed millet grains was dependent ($p<0.05$) on the quadratic effect of germination time, roasting temperature and roasting time (Table 3). It was found out that increase in the germination time (runs 5 and 1) and roasting temperature (runs 7 and 16) resulted into an increase in the total antioxidant capacity (39.13 to 57.68) and (39.62 to 66.01) respectively. However, increase in the roasting time (runs 1 and 15) resulted into a decrease in the total antioxidant capacity values (57.68 to 63.73). A similar result on germination of pigmented rice has been observed [28].

Phenolic compounds in grains usually play a significant role in determining their antioxidant properties as most phenolic acids have been reported as strong antioxidants due to their ability to donate hydrogen and electrons, and to form stable radical intermediates for preventing oxidation of other compounds [29,30]. Sprouting and seedlings have been reported [31] to be responsible for increase in nutritional value of seeds in terms of phytochemicals that are beneficial to health. Therefore Jirani millet investigated in this study can be consumed as germinated grains [32,33], vegetable [34] or as a food additive.

4.2.2. 2, 2-diphenyl-1-picrylhydrazy (DPPH) of the Processed Millet

It can be observed (Figure 2) that the DPPH of the processed millet grains was dependent ($p<0.05$) on the interactive effect of germination time and roasting time (Table 3). The DPPH values of the samples increased with increase in germination time (runs 5 and 1) from 72.65 to 79.75 and roasting temperature (runs 11 and 15) from 73.39 to 77.28 while increase in roasting time (runs 10 and 15) resulted in a decrease in DPPH from 75.72 to 68.26. The germinated and roasted millet products showed antioxidant properties in view of the DPPH values. It was found that roasting temperature and germinating time individually had significant effects on the total antioxidant capacity and DPPH scavenging property of the germinated millet. Both a decrease and an increase in the total antioxidant capacity during germination were observed and these may be attributable to the activity of some unspecified enzymes or a decrease in the phenolic content of the germinated grains. The enzymatic hydrolysis of tannin-protein and tannin-enzyme complex contributes to the removal of polyphenols during germination of pearl

millet. However, Xiang *et al*. [35] also noted that the total phenolic and flavonoid content increased during germination of sweet corn (Zea mays L.) and this was closely related and significantly contributed to an increase in the antioxidant activity in the germinated sweet corn.

4.2.3. Ferric Ion Reducing Antioxidant Power of the Processed Millet

This study aimed to determine the extent of scavenging for free radical mediated chain reactions of a given food product. The chain reaction process have been documented to involve initiation, propagation, branching and termination steps [21] which can be initiated by some external agents such as heat, light, ionizing radiation and chemical reaction. The ferric ion reducing power measures the potential contributions of a given food product to preventing metal ion catalyses for free radical mediated chain reaction [22]. Additionally determination of specific antioxidants activity usually gives different values as the procedures used for determination are often dependent on different reaction mechanism [23]. Figure 3 showed the 3D response surface for RP of the processed millet grains investigated in this study. The RP of the processed millet grains did not depend ($p<0.05$) on any of the processed variables and the reducing power of the sample increased with increases in the germination time (runs 2 and 9) from 0.381 to 0.428 and roasting temperature (runs 7 and 10) from 0.39 to 0.441. However, it decreased with the increase in the roasting time (run 5 and 12) from 0.415 to 0.383.

4.3. Optimization of Process Variables

Total anti-oxidant, DPPH and reducing power were increased up to 66.01, 79.65 and 0.353 respectively. As a result, germination time of 68.95 hr, roasting temperature of 114.77℃ and roasting time of 15.00 mins with desirability of 0.860 was selected as the most optimized sample.

Thus, the resulting optimized product would probably have a total antioxidant capacity of 56.28 mg/100g, DPPH of 79.75 µg/ml and reducing power of 0.478. However, Sharma *et al*. [24] reported germination temperature of 25 ℃ and soaking time of 15.84 mins as the best combination for germination of foxtail millet to improve phytochemical content.

5. Conclusions

Germinated millet product with high antioxidant properties can be produced by optimizing germination period, roasting temperature and roasting time. A germination period of 68.95hr, 114.77℃ roasting temperature requiring 15 minutes roasting time was established in this study for optimum germinating and roasting conditions for *Jirani* millet grains. The study had indicated that the total antioxidant capacity of the processed millet was dependent ($p< 0.05$) on the quadratic effect of germination time, roasting temperature and roasting-time. Also an increase in the germination time and roasting temperature resulted into an increase in the antioxidant capacity values in the processed *Jirani* millet while an increase in the roasting time of the *Jirani* millet resulted into a decrease in the total antioxidant capacity of the processed millet grains. The ferric reducing power of the germinated and roasted *Jirani* millet grains did not depend significantly ($p< 0.05$) on the germination time, roasting time and roasting temperatures explored in this. However, there was an increase in the ferric reducing power of the processed *Jirani* millet grains with germination time. Additionally the DPPH value of the processed millet grains was dependent ($p< 0.05$) on the interactive effect of germinating time and roasting time of the grains. Roasting temperature and germinating time also individually had significant effects on the total antioxidant capacity and DPPH scavenging property of the germinated millet grains.

The germinated and roasted *Jirani* millet product obtained in this study can therefore be used as a dietary antioxidant supplement, which can help to scavenge free radicals. This helps to inhibit formation of reactive oxidants thereby preventing the onset of free radicals induced disorders and diseases. The developed product in this study is suitable as an ingredient for functional food product development or as a nutraceutical product.

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Conflict of Interests

There are no conflicts of interests to be declared.

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