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Effect of Spent Yeast Fortification on Physical Characteristics of Cassava-Wheat Composite Bread

Amaraegbu A, Adewale P and Ngadi M*

Department of Bioresource Engineering, McGill University, Canada

Abstract

In this study, the effect of substituting wheat flour with 10%, 20%, and 30% cassava flour (CF) and the addition of 5, 10, and 15 g SBY (protein concentrates) on cassava-wheat composite bread was studied. Density and specific volume of the bread were influenced ($p<0.05$) by the percentage composition of CF. The breadcrumb total color difference was significantly ($p<0.05$) influenced by the addition of SBY. In the crust, only the brightness remained significantly different between the control and SBY fortified composite bread. The parameters a*, b* and L* of the fortified bread crusts were not significantly different. The desirability score of the two major variables: % composition of CF was 20% and SBY concentration was 10 g as optimum values of the fortified cassava–wheat composite bread. The Microstructure of 5 and 10 g of fortified bread were similar but different from that of 15 g fortified bread. Findings from this study revealed suitability of SBY in composite bread making to improve physical properties of the bread.

Keywords: Protein fortifier; Cassava flour; Composite bread; Physical properties; Microstructure

Introduction

Nutritive facts of food products have been the focus of most consumers. In order to improve the quality of wheat flour based bread, composite bread is produced from wheat flour by the addition of low protein flours. Due to an apparent reduction in protein content of composite bread, the effects of different protein fortifying materials have interested many researchers. However, finding a readily available fortifying ingredient that is economical and sustainable is a challenge. Spent brewer's yeast is one of the major by-products from the brewing industry which is readily available. It is a valuable source of cheap fiber and very rich in β-glucans [1-3]. β-glucans are natural cell wall polysaccharides which constitute a major structural component of bakers and brewing yeast [4]. Thammakiti et al. [2] reported a successful procedure to extract high purity β-glucans from spent brewer's yeast and equally examined the suitability of its functional properties for food products. The authors found that the β-glucan obtained from their study had a higher apparent viscosity, water-holding capacity, and emulsion stabilizing capacity, but very similar oil binding capacity as compared with commercial β-glucan from baker's yeast. The authors suggested that the β-glucan obtained from brewer's yeast could be used in food products as a thickening, water-holding, or oil-binding agent and emulsifying stabilizer. For the modification of nutritive value, texture and stability, β-glucan has been reported as an interesting hydrocolloid because of its positive effects on human and animal health such as cholesterol-lowering, antifibrotic, anti-inflammatory, antimicrobial, and antidiabetic [4-6]. Therefore, investigation of the effects of incorporation of spent brewer's yeast in the cassava-wheat flour bread formulations is a worthwhile investment.

Most of the supplement flours (e.g. cassava flour (100 g of cassava = 1.2 g of protein), maize flour (100 g of maize flour = 6.9 g of protein), etc.) to wheat flour to form composite bread are of low protein content Begum et al. [7]. For instance, the effect of the addition of protein concentrates from natural and yeast fermented rice bran on the rheological and technological properties of wheat bread was investigated by Chinma et al. [8]. The authors reported that the springiness, cohesiveness and resilience values of wheat bread were not significantly different from composite bread. Their results further revealed that composite bread surface had embedded granules of protein-like deposits with small spores. Pasting characteristics of wheat-chia blends for bread formulation was examined by Švec et al. [9]. Similarly, Begum et al. [7] studied protein fortification and use of cassava flour for bread formulation. The authors observed a strong correlation between sensory scores and soy protein fortification techniques. Their results equally showed that wheat flour could be replaced partially by cassava flour in the composite bread preparation by applying fermentation and soy protein fortification techniques. However, low-cost protein fortifiers such as spent brewer's yeast (SBY) have not been well researched in the literature for the production of composite bread. Recently, Martins et al. [6] studied the effect of spent yeast fortification on physical parameters, volatiles and sensorial characteristics of home-made bread. The fortification of home-made bread using spent yeast was reported to have an influence on the crumb darkened and increased crumb and crust springiness and had an impact on the volatile profile of the home-made bread. In an attempt to improve gluten-free bread quality, Blanco et al. [10] enriched their composite bread from rice flour and hydroxypropyl methylcellulose with acidic food additives. The authors concluded that addition of monosodium phosphate to the dough increased the volume of the gluten-free bread, which subsequently improved the appearance, odor, taste and texture of the composite bread. The objectives of this study were to enhance the protein content of composite bread produced from cassava-wheat flour blends using SBY and to investigate the effects of SBY on the physical properties of the composite bread.

Materials and Methods

Materials

Commercial wheat flour with the nutritive fact of 4 g of protein in

***Corresponding author:** Ngadi M, Department of Bioresource Engineering, McGill University, 21111 Lakeshore Rd., Ste-Anne-de-Bellevue, QC, H9X 3V9, Canada, Tel: 1-514-398-7779; E-mail: michael.ngadi@mcgill.ca

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30 g of wheat flour was purchased from Walmart store in Montreal, Canada. Cassava flour with 7.75% moisture, 1.57% ash, 2% protein and 91% starch was obtained from a local tropical market. Cassava flour was mixed with wheat flour at three levels (10%, 20% and 30%) for the preparation of dough [11]. The spent brewer's yeast of 45g of protein per 100g of spent yeast was procured from Lallemand Bio- Ingredient, Montreal, Canada. The inactive spent brewer's yeast contained less than 300 colonies forming unit (CFU/g) which is far below the standard plate count (< 10000 CFU/g). The amount of spent brewer's yeast used was determined by calculating the amount of protein lost from the cassava-wheat blend. Other required ingredients (table salt, granulated sugar, shortening, Fleishmann's active dry yeast) were purchased from a Walmart store in Montreal. For the control sample, 20% cassava flour blend was used to make the bread without the addition of spent brewer's yeast.

Methods

Preparation of bread with composite flour

The composite bread was produced modifying a method described by Taofik et al. [9]. Cassava flour was added to wheat flour at three levels (10%, 20%, and 30%) w/w flour basis (Table 1). The dough was baked in a pre-programmed bread maker (black and decker B3200, Middleton, USA). The machine was pre-programmed as follows: (1) all ingredients (cassava-wheat flour % composition, 4% sugar, 1% salt, 1.3% active dry yeast and 4% shortening) w/w flour basis (Table 2); (2) Kneaded/mixed for 30 min; (3) fermented for 120 min at 40 °C; (4) baked at an incremental temperature to a maximum temperature of 105 °C in the fermentation pan for 45 min as monitored by Hotmux thermocouple (DCC Corporation, Westfield Ave., Lower Level Pennsauken, NJ, USA). The baking process which included, mixing, kneading, leavening and baking took approximately 3 hours 15 min to make a complete cycle. After cooling, the bread samples were packed in polyethylene bags and. Samples were stored in Ziploc bags at room temperature (25 °C) in preparation for further analysis.

Composite bread physical properties

Evaluation of bread mass, volume, and specific volume: The mass of the bread was determined by weighing the whole baked bread (sample) with a sensitive electronic weighing balance (0.001 g accuracy). The bread dimensions were measured using a digital caliper and a ruler. The caliper was used to determine the height (thickness) of the bread. Due to the variability of the bread's height, length, and breadth, these three dimensions were measured at three different locations of the bread to estimate the mean value. Specific volume was calculated as cm^3/g by dividing the volume of the bread loaf by its weight. The bread density is the reciprocal of specific volume.

Level	Cassava four (%)	Spent yeast required to replace the protein lost (g)
1	10	5
2	20	10
3	30	15

Table 1: Ingredients used for preparing composite bread (Levels of experimental design for dough formulation).

Dough component	Amount (g)	Amount (%)
Water	232.00	61.8
Salt	3.75	1.0
Shortening	15.00	4.0
Sugar	15.00	4.0
Active dry yeast	5.00	1.3

Table 2: Ingredients used for preparing composite bread (Constant ingredients in dough formulation).

Determination of bread crumb and crust color: A Minolta spectrophotometer CM 3500d (Minolta, Ramsey, NJ, USA) was used to determine the color parameter lightness (L), redness (+a), greenness (-a), yellowness *(+b),* Blueness (-b) of the baked loaves crust and crumb. The colorimeter was calibrated using a white calibration plate and zero calibration black boxes before use. The machine was then standardized with 100% wheat flour bread before the measurement of bread crust and crumb. The crumb color was determined after cutting the bread to expose the crumb in triplicate at different locations. Similarly, the crust color was determined in triplicate by taking the average result of three measurements (the sides were excluded). The colorimetric results were used in equation 1 to determine the brown index (BI) and total color difference (ΔE) of the crust and crumb (equation 2) which was estimated according to Maskan [12]:

$$\mathbf{BI} = \frac{[100\mathbf{x} - 0.31]}{0.17} \quad (1)$$

$$\textbf{Where, } \mathbf{x} = \frac{\mathbf{a} + 1.75\mathbf{L}}{5.645\boldsymbol{L} + \boldsymbol{a} - 3.012\boldsymbol{b}}$$

$$\Delta\mathbf{E} = \sqrt{(\mathbf{L_0} - \mathbf{L})^2 + (\mathbf{a_0} - \mathbf{a})^2 + (\mathbf{b_0} - \mathbf{b})^2} \quad (2)$$

Textural analysis - crumb softness: The softness of the bread crumb was analyzed using a tensile testing machine (Instron-4502, Instron Corporation, USA) controlled by a computer software (Instron Series IX, version 8.25). A portion of each breadcrumb [2.5 (length) × 2.5 (breadth) × 2.5 (thickness)] cm was cut from the central portion of loaves with a bread knife on which the test was carried out. Care was taken during cutting in order to obtain a clean and undistorted surface. The breadcrumb softness was examined using a compression test by a cylinder plunger with a diameter of 5 mm to a depth of 12.5 mm to reach a load of 50 N. The slices were placed on a flat surface beneath the probe. The probe was brought down until it touched the surface of the slice. The slices were compressed at a deformation speed of 50 mm/min, the compression curves were recorded. The amount of force exerted on the bread slice was as a function of the distance traveled by the probe before it returned its initial position at the same speed. The results obtained were calculated using Instron Series IX software, version 8.25 (Instron Corporation, USA), which recorded the plunger force as a function of time, to determine softness of the breadcrumb. The compression data of each breadcrumb sample was collected in triplicate.

Scanning electron microscopy

The microstructure of the composite bread enriched with three levels of SBY (5, 10 and 15%) and a control (Bread without SBY) were examined through a scanning electron microscope (SEM) (JEOL JSM-6460LV, Tokyo, Japan) operated at 20.0 kV. Composite bread samples (cassava-wheat) of 20% cassava flour fortified with SBY were prepared for scanning electron microscopy. Bread samples were cut into approximately 5 × 5 × 2 mm^3 cubes with a sharp razor. Each sample was mounted on aluminum stubs, coated with a thin layer of gold before being scanned and digital images were acquired at 50 × magnification over regions of 1280 × 1040 pixels. Three images were captured on each sample over square regions of 5 × 5 mm^2.

Statistical analysis

A full factorial design was used to verify effects of % composition of cassava flour and SBY on the quality of composite bread production from cassava-wheat flour (Table 1). The bread produced was analyzed separately and was allowed to cool down in a desiccator for a maximum of 4 h at room temperature prior to analysis. Results were reported as averages of each of the three replications (all treatments were evaluated in three batches). Statistical analysis was carried out to investigate factor effect leverage and desirability using JMP® 11.2.0 (Statistical Analysis Systems, SAS Institute Inc., Cary, NC, USA). Leverage Plot reports the effect of each factor on the response variable and gives insight on possible multicollinearity observations. Desirability functions were used to find the optimal value for each factor.

Results and Discussion

Bread mass, volume, and specific volume

The loaf size parameters of the cassava-wheat composite bread (volume, mass, density, and specific volume) were successfully estimated in this study. Figures 1A-1D shows the results of some physical properties of the bread loaf. The results of bread mass, volume, density, and specific volume range from 506 to 587 g, 1540 to 2038 cm^3, 0.250 to 0.391 g/cm^3, and 2.55 to 3.90 cm^3/g, respectively as shown in Figure 1. All these parametric values of the bread varied significantly ($p<0.05$) based on percentage composition of cassava flour but not in the case of SBY. The bread mass is not significantly ($p>0.05$) affected by SBY concentrations (Figure 1A). The correlation analysis explained a high correlation coefficient ($r \leq 0.98$) correlation between the cassava flour and mass of loaves. Increase in cassava flour concentration caused an increase in the mass; this is as a result of insufficient carbon dioxide, and water binding capacity of cassava flour due to its high concentration of damaged starch [13-15]. A reverse case was observed for volume. There was a downward trend in the volume (Figures 1B and 1C), and specific volume as the concentration of cassava flour (%) composition increased (Figure 1D). Both CF and SBY concentrations significantly ($p<0.05$) influenced the volume and specific volume of the bread. This is as a result of the attenuation of gluten content of the wheat flour with the CF which contains no gluten. Decrease in gluten concentration leads to an early offset of gelatinization [5], plasticized the starch- protein network, causing the gas cell walls to become firmer and tougher, making it difficult for expansion to occur [13-15].

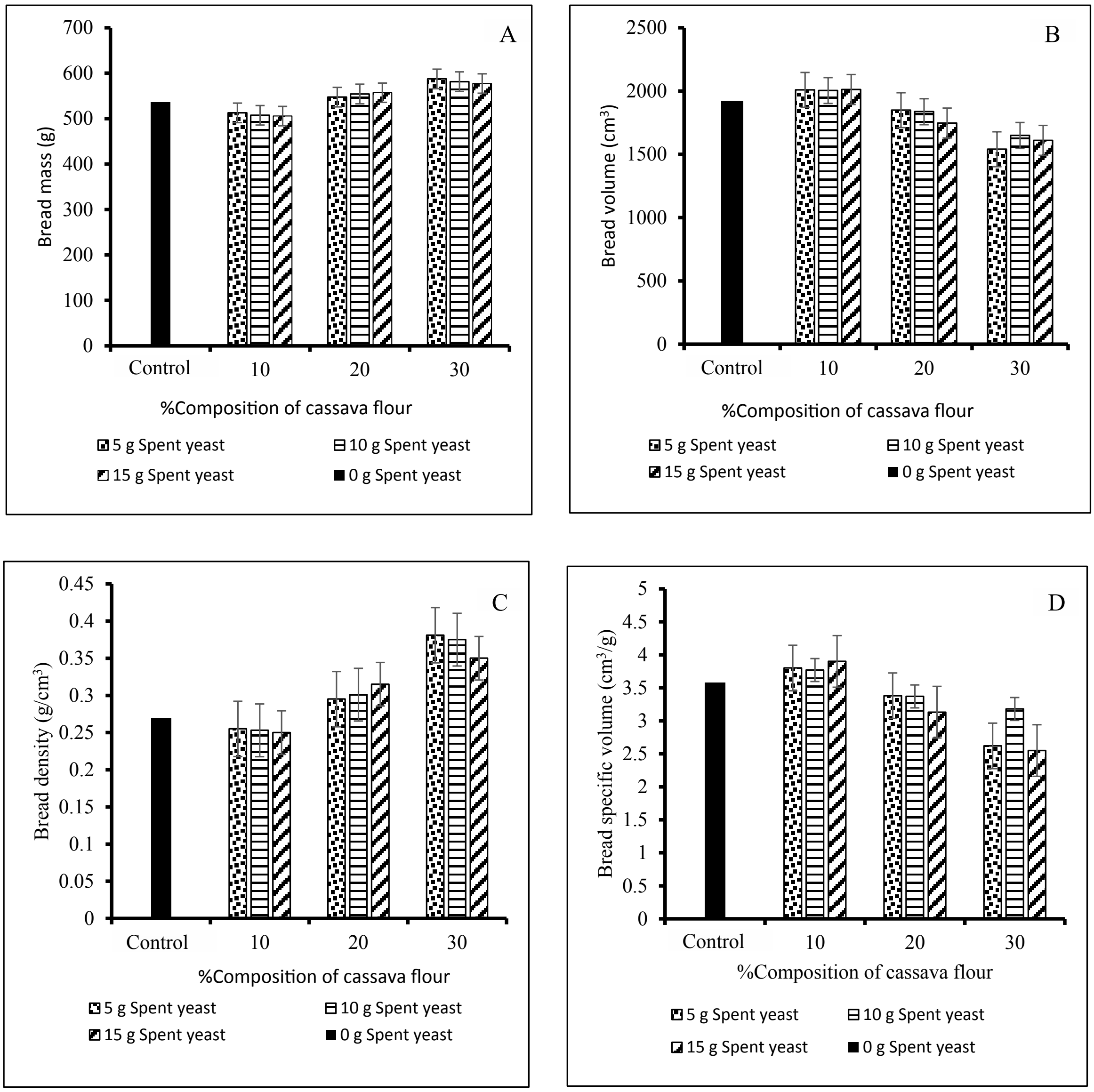

Figure 1: Bread size parameters (A) Bread mass (B) Bread volume (C) Bread density (D) Bread specific volume.

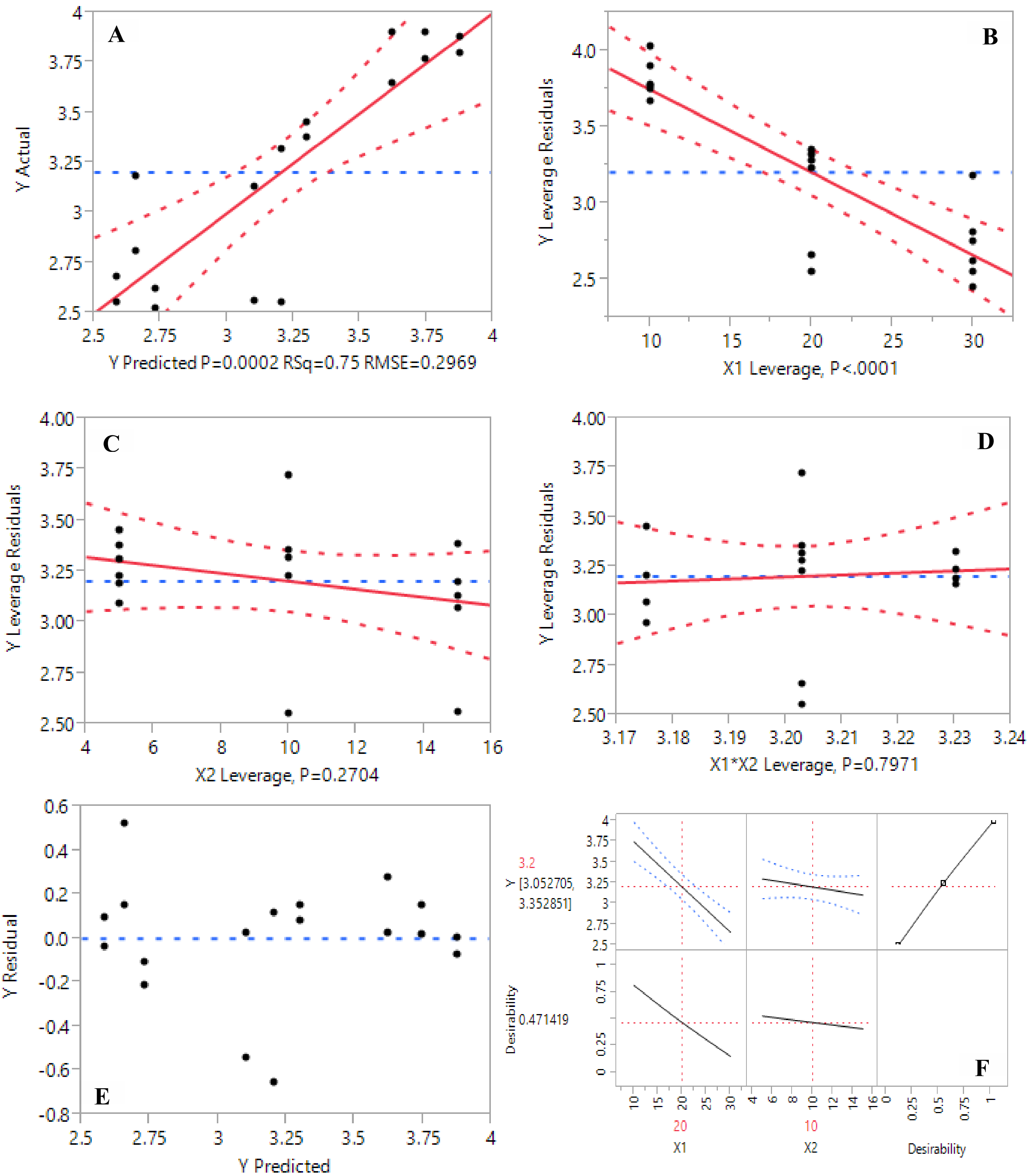

Figure 2: Effects leverage and desirability plots of bread specific volume based on (A) Yield Prediction (B) percentage composition of cassava flour (C) Spent yeast concentration (D) Factors interaction (E) Specific volume residual plot (F) Desirability of factors.

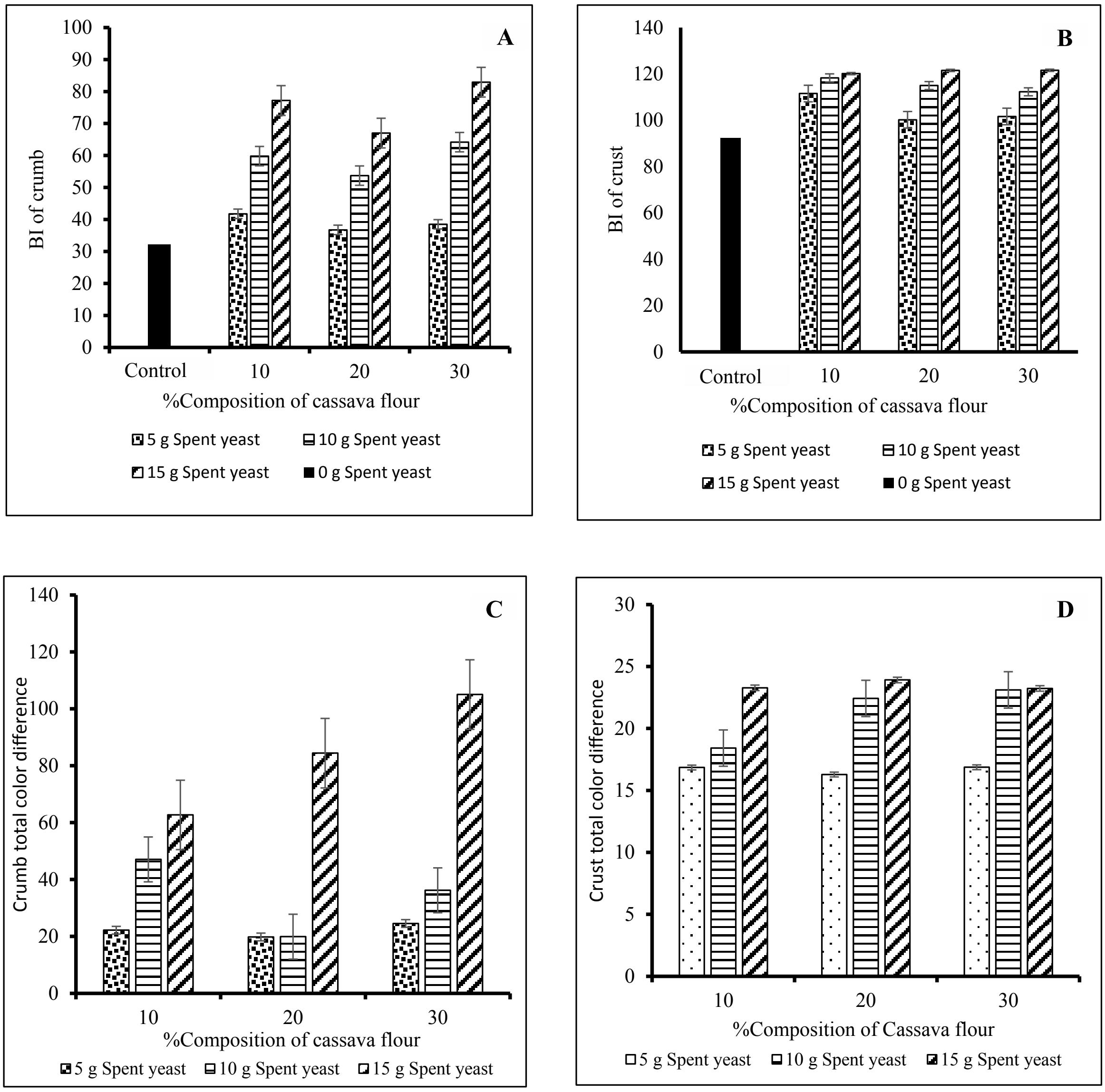

Figure 3: Crust and crumb color (A) Brown index of crumb (B) Brown index of crust (C) Crumb total color difference (D) Crust total color difference.

The higher the bread density the lower the specific volume and the bread density was a reciprocal of bread the specific volume. The expression to determine the specific volume of bread combines both mass and volume of the bread. Thus, further statistical analysis was carried out to understand the effects of CF and SBY concentration on the bread specific volume and their desirability towards the bread production. Figures 2A-2F shows the effect leverage and desirability plots of the percentage composition of CF and SBY on the bread properties based on the specific volume. The plot of actual versus predicted values, which shows the observed data against the predicted data of the bread specific volume, is shown in Figure 2A. The figure shows the leverage plot for the whole model with regression coefficient R^2 = 0.75, and RMSE = 0.2969 ($p<0.05$). The residual plot shows a random pattern around a horizontal axis, indicating a good fit of the model (Figure 2E). The effect leverage plot, (Figure 2B) shows the percentage composition of CF (X1) to significantly ($p<0.05$) influence the specific volume of the bread. The effect leverage plot, (Figure 2C) shows the concentration of SBY (X2) and interaction between percentage composition of CF and SBY (Figure 2D) to be insignificant ($p>0.05$) on the specific volume of the bread. This can be ascribed to the inactive nature of the SBY used. SBY is added to enhance the protein content of the bread. So, it has no effect on the risen of the bread dough. Similar results were reported by Martins et al. [6], that two-way ANOVA indicated no significant differences due to β-glucan fortification by dry spent yeast addition ($P>0.05$) on the bread volume. Figure 2F shows the statistical test carried out to investigate the actual effect of these factors using desirability function. The desirable values for percentage composition of CF and SBY concentration are 20% and 10 g, respectively with a corresponding desirable value for specific volume to be 3.2 cm^3/g.

Color properties of the bread crust and crumb

The effect of SBY on the color properties of composite bread crust and crumb were studied using two proximate parameters (brown index and total color difference) as shown in Figures 3A-3D. The addition of SBY significantly ($p<0.05$) influenced the color of the breadcrumb

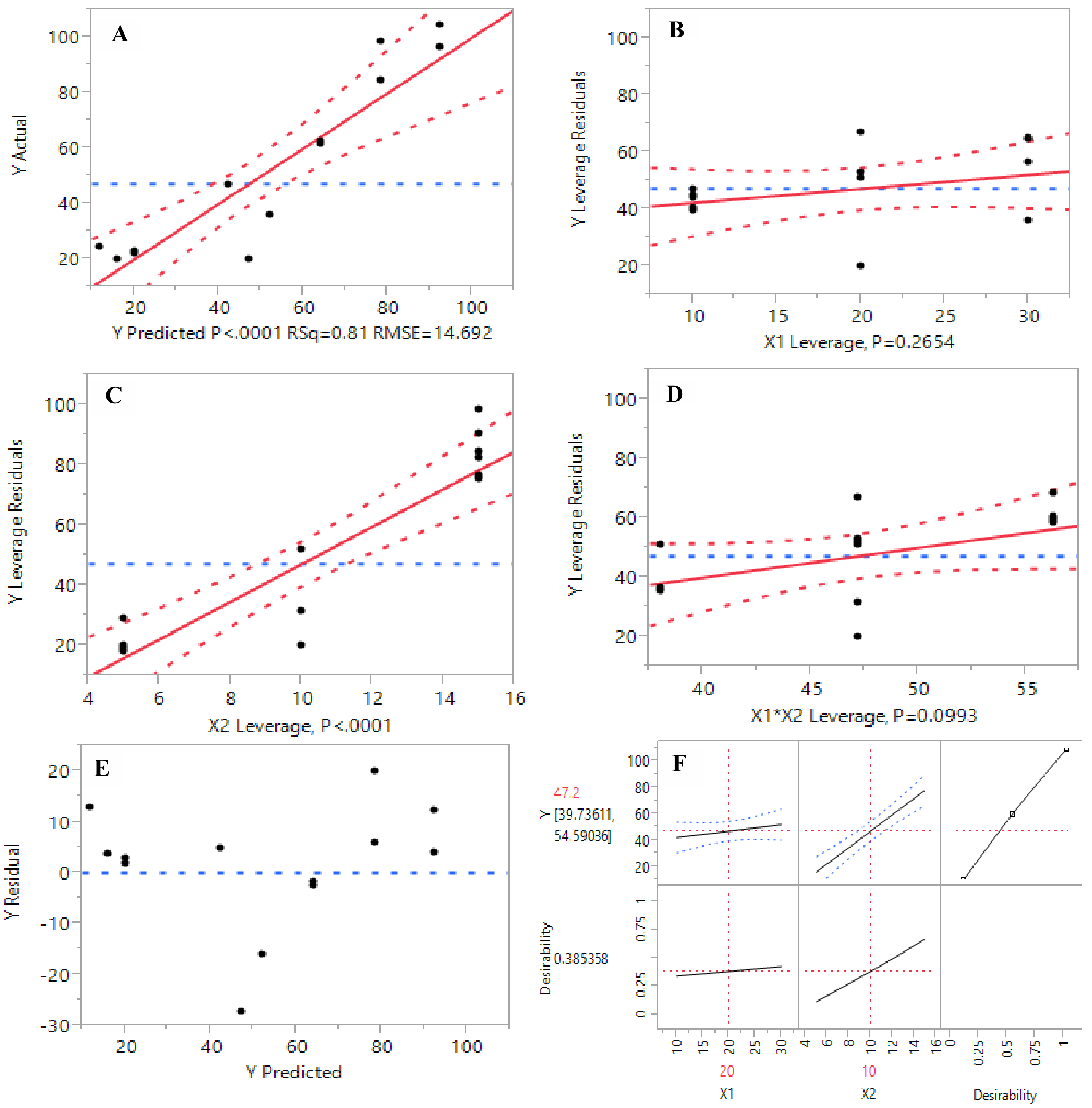

Figure 4: Effects leverage and desirability plots of total color difference based on (A) Crumb total color difference prediction (B) Percentage composition of cassava flour (C) Spent yeast concentration (D) Factors interaction (E) Total color difference residual plot (F) Desirability of factors.

but no significant influence was observed for the crust (Figures 3A and 3B). The L values decreased with the addition of SBY in bread while a, b, BI, and ΔE values increased. Total color difference parameter was used as a key analytic response to discuss the effect of SBY on the bread color. In ΔE, each of the bread samples was compared with the control bread (20% cassava flour-80% wheat flour without SBY). The control bread was lighter (L) in the crumb and crust with lower red color (a) than composite bread samples, while bread containing 15 g SBY had the lowest (L) and highest (a) values (Figure 3C). In addition, control bread was less yellow (b) than composite bread samples. The lower L and higher (a) values of composite bread may be due to their higher protein contents due to the addition of SBY which perhaps caused increased Maillard reaction during baking. The Maillard reaction is a chemical reaction between amino acids and reducing sugars that induce browning of food products and their desirable flavor. Bread color is a very good indicator of bread quality, how the individual ingredients affect the bread formulation, the storage conditions as well as the process variable [15-17]. The brown index shows less significance of the crust color than the crumb color, this is because the crust color was also affected by the heat (direct contact of the oven pan) than the crumb (Figures 3C and 3D). Figures 4A-4F) shows the effect leverage and desirability plots of the percentage composition of CF and SBY on the bread properties based on the total color difference of the crumb. The plot of actual versus predicted values, which shows the observed data against the predicted data of the bread specific volume is shown in Figure 4A. The figure shows the leverage plot for the whole model with regression coefficient $R^2 = 0.81$, and RMSE = 14.692 ($p<0.05$). The residual plot shows a random pattern around a horizontal axis, indicating a good fit of the model (Figure 4E). The effect leverage plot, (Figure 4B) shows the percentage composition of CF (X1) to not significantly ($p>0.05$) influence the total color difference of the bread. The effect leverage plot, (Figure 4C) shows the concentration of SBY (X2) as a significant ($p<0.05$) factor that influenced the total color difference of the breadcrumb. The interaction between percentage composition of CF and SBY (Figure 4D) had no influence ($p>0.05$) on the total color difference of the breadcrumb. This can be ascribed to the inactive nature of the SBY used. SBY was added to enhance the protein content of the bread. So, it has no effect on the risen of the bread dough. Desirability function was used to investigate the actual effect of independent variables on the composite bread quality. The desirable values for percentage composition of CF and SBY concentration are 20% and 10 g, respectively with a corresponding desirable value for the total color difference was 47.2 (Figure 4F).

Texture characteristics: crumb hardness

Figure 5 shows the breadcrumb hardness per percentage composition of CF and concentration of SBY. The crumb hardness is directly proportional to the percentage composition of CF and the concentration of SBY added. The 30% CF-wheat bread had the highest hardness and lowest springiness values while the lowest hardness and highest springiness was observed in 5 g SBY fortified bread. Figures 6A-6F shows the effect leverage and desirability plots of the percentage composition of CF and SBY on the bread properties based on the crumb hardness. The plot of actual versus predicted values, which shows the observed data against the predicted data of the bread specific volume, is shown in Figure 6A. The figure shows the leverage plot for the whole model with regression coefficient $R^2 = 0.99$, and RMSE = 0.197 ($p<0.05$). The residual plot shows a random pattern around a horizontal axis, indicating a good fit of the model (Figure 6E). The effect leverage plot, (Figure 6B) shows the percentage composition of CF (X1) to significantly ($p<0.05$) influenced the hardness of the breadcrumb. The addition of SBY (X2) to cassava-wheat flour in bread making significantly ($P<0.05$) increased bread hardness, which led to a slight reduction in its cohesiveness value (Figure 6C). A similar finding was reported by Totosaus et al. [18] in their investigation on the effect of lupinus (*Lupinus albus*) and jatropha (*Jatropha curcas*) protein concentrates on wheat dough texture and bread quality. The authors reported that added proteins in wheat dough compete with gluten for available water which results in increased hardness but decreased cohesiveness. In contrary, Martins et al. [6] reported that the effect of β-glucan fortification by dry spent yeast addition revealed no significant effects on texture and color parameters of the bread. The interaction between percentage composition of CF and SBY (Figure 6D) had no influence ($p>0.05$) on the hardness of the breadcrumb. However, the confidence level was almost at the borderline. It might be because of poor extensibility of the wheat-cassava network and the dilution of the gluten contributed to this effect. This could also be as a result of SBY activation during baking and kneading period. In the study of Gómez

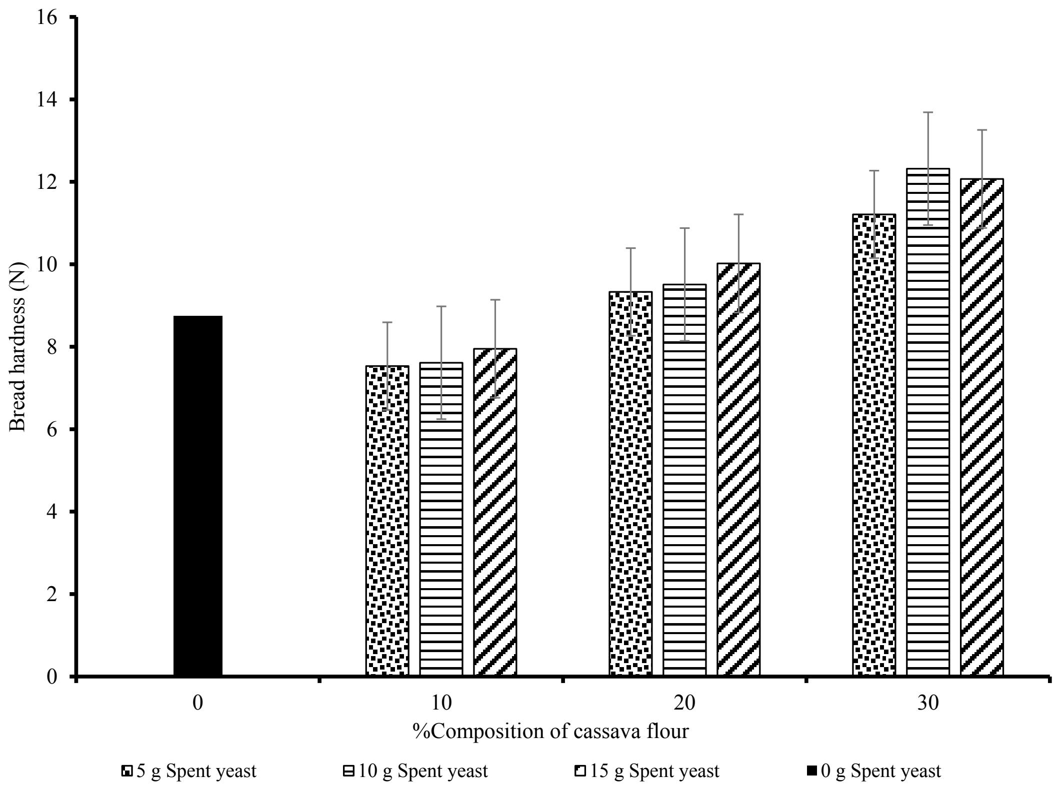

Figure 5: Bread crumb hardness.

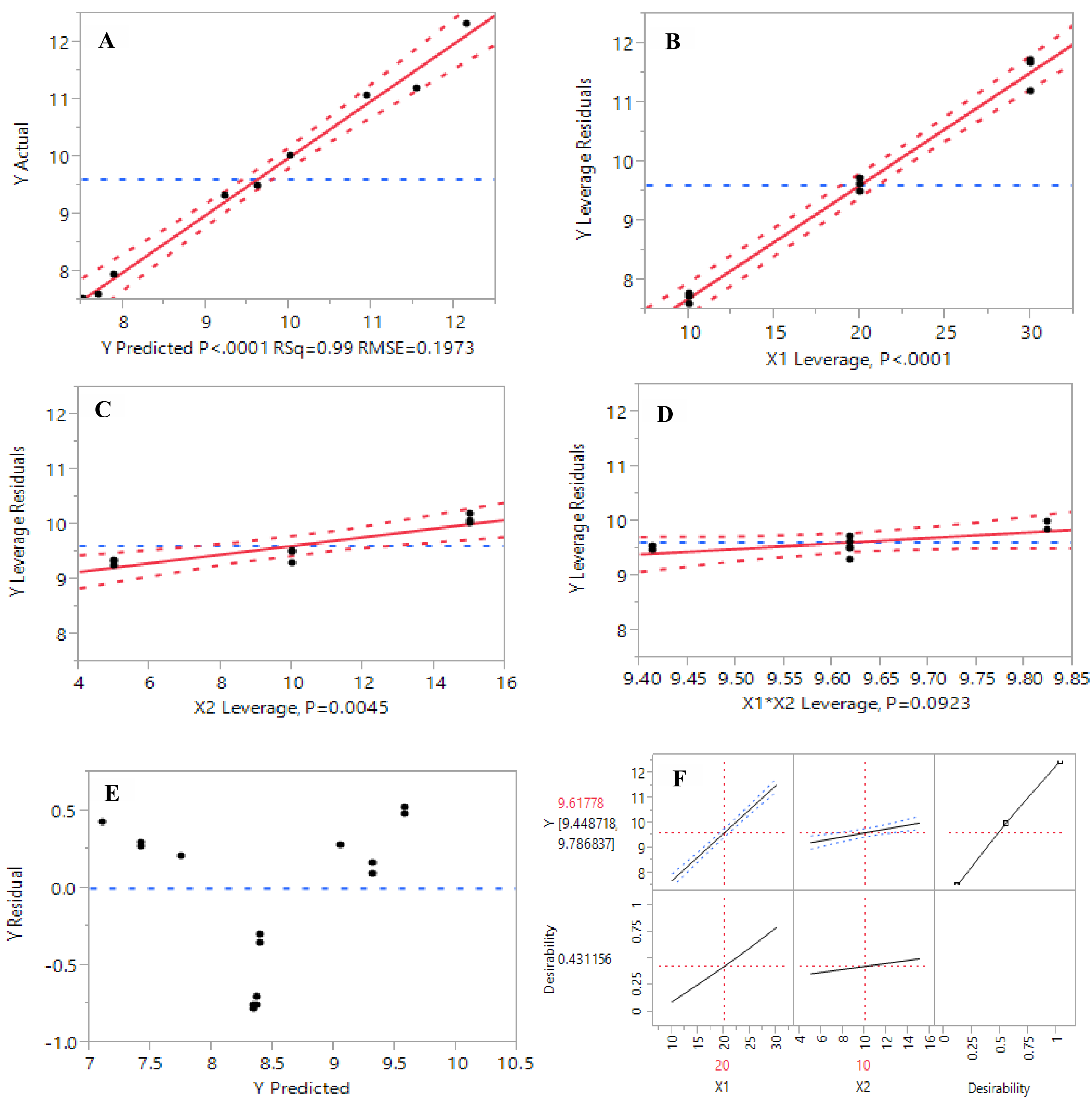

Figure 6: Effects leverage and desirability plots of crumb hardness based on (A) Hardness prediction (B) Percentage composition of cassava flour (C) Spent yeast concentration (D) Factors interaction (E) Hardness residual plot (F) Desirability of factors.

et al. [19] on the effect of extruded wheat bran on dough rheology and bread quality, the authors reported that hardness of bread crumb was related to the gumminess, chewiness parameters and hydration effect which partly accounted for the hard texture of composite bread samples. Jensen et al. [13] results showed that, depending on the type of cassava flour, up to 30% of the wheat flour could be replaced without any significant differences from control bread and the addition of fibers resulted in a lower volume and harder and more cohesive crumb structure. In this study, a statistical test carried out to investigate the actual effect of independent variables using desirability function showed the desirable values for percentage composition of CF and SBY concentration to be 20% and 10 g, respectively with a corresponding desirable value for bread hardness as 9.62 N (Figure 6F). Thus, the best cassava-wheat composite bread can be made by the addition of 20% CF and 10 g of SBY.

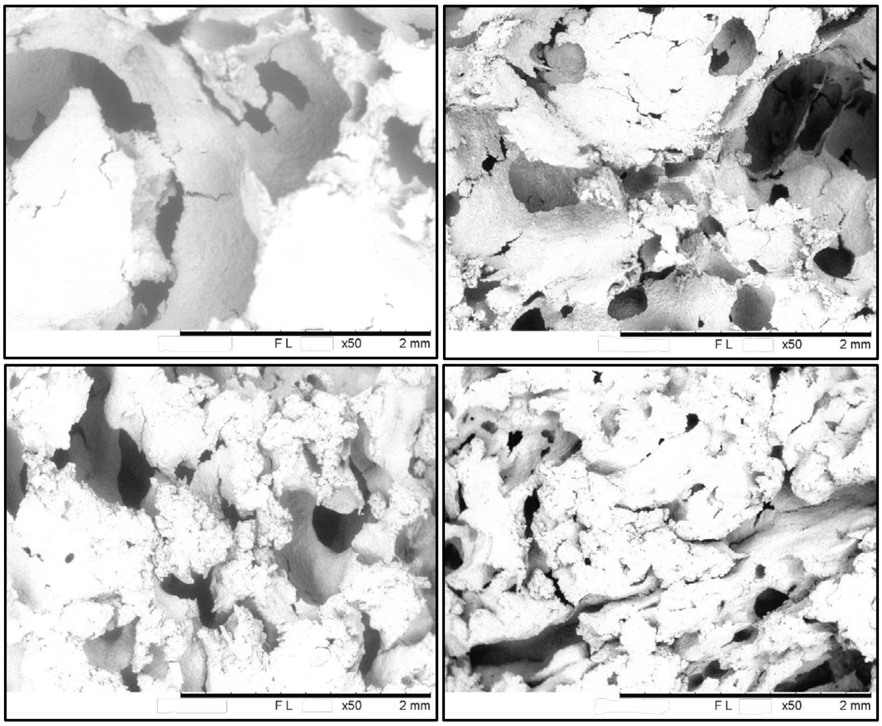

Figure 7: Scanning electron micrographs of crumb of three SBY-fortified composite bread (cassava-wheat) and control (without SBY) at the magnification × 50 (a) Control (without SBY) (b) 5% SBY, (c) 10% SBY (d) 15% SBY.

Microstructure of cassava-wheat bread

The microstructure of composite bread samples was examined after 24 h of storage in a desiccator to keep the samples dry before digital imaging on SEM. The effect of different concentrations of SBY in bread formulation on the morphological features of crumb structure was visualized. Figures 7A-7D shows the SEM micrographs of breadcrumb section. A qualitative information on pore structure from the SEM showed that all the bread samples were characterized by heterogeneously distributed pores of different sizes. In addition, SBY, the pores appeared to be slightly smaller as compared with control samples. The pores of bread fortified by 5 g SBY appeared to be the largest out of the levels of treatment for SBY concentrations (Figure 7B). The pore size decreased as the SBY concentration increased while the smallest pores distribution was predominant at 15 g SBY (Figure 7D). The microstructure of 5 g SBY fortified bread was similar to that of 10 g SBY while 15 g SBY was distinct from either concentration of SBY. The higher the concentration of SBY in the formulation the more compact the breadcrumb. This result affirms our findings in section 4.3, desirable value for SBY concentration to be 10 g. Control bread apparently differ from the SBY fortified bread (Figure 7A) with more opened structure, rigid and fragile features. Crumb firmness was observed in SBY fortified samples. The crumb firmness increased with increase in the concentration of SBY. This is one of the positive impacts of SBY on the breadcrumb apart from its nutritive values. Similar findings were reported by Gomez et al. [19] from their study of dough properties and bread quality of wheat–barley composite flour as affected by β-glucanase. The authors reported that the macroscopic characteristics (firmness and springiness) and microstructure of the dough displayed a remarkable improvement when β-glucanase was added; however, a higher level of β-glucanase (0.06% and 0.08%) could result in sticky dough and consequently lower final product quality.

Conclusion

This study has been able to show that varying spent yeast and cassava flour concentration to produce a cassava-wheat composite bread leads to a significant difference in the physical properties of composite bread produced. The study clearly showed that spent yeast up to 10 g of protein replacement will cause no negative changes in the bread quality or marketability of the bread but rather adds to the richness in brownness. The bread quality such as volume, mass, softness gradually diminished as the cassava flour increased. This study showed SBY (10 g of protein) and CF (20%) stood a good chance to compete with whole wheat in the market in terms of nutrition, quality, and health. The protein content was calculated based on the percentage composition of the flours and spent yeast used for bread making. There was no protein analysis done to determine the protein content of the composite bread. Therefore, further studies are required to accurately determine the response of sensory and protein content of composite bread as they for optimizing consumer acceptability.

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