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Research Article

Baking Effect on Resistant Starch Digestion from Composite Bread Produced with Partial Wheat Flour Substitution

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The consumption of composite flour, such as green banana and corn flour, is related to maintain stable blood glucose levels, due to high resistant starch levels. However, most of these studies have conducted analyses of unprocessed food such as flour. Therefore, this study aimed to evaluate the effect of baking on resistant starch concentration and digestion from bread produced with partial wheat flour substitution. Response surface methodology was used to evaluate bread physical-chemical characteristics, and then, sensorial and nutritional qualities of the bread were evaluated. The feasibility of incorporating 40% of corn flour was demonstrated, while incorporation of 20% produced bread with similar characteristics to the control; for green banana flour, these levels were 20 and 10%, respectively. Resistant starch levels of composite breads were also enhanced by in vitro analyses. On the other hand, in vivo blood glucose levels evidenced that the ingestion of breads produced with partial wheat flour substitution by green banana or corn flour promoted a more important peak in blood glucose levels in comparison with control bread, which was never previously presented in the literature. Bread ingestion rapidly increased the blood glucose levels of rats; once during the baking process, starch granules become gelatinized and therefore easily digestible. Furthermore, this study also highlighted the lack and need for future investigation of wheat flour-substituted baked goods, in order to better understand mechanical properties formation and also product digestibility.

1. Introduction

Typically, bread comprises 60% wheat flour [1], but the wheat production in tropical countries does not fulfill this demand. According to the United States Department of Agriculture (USDA) [2], Egypt, Indonesia, Algeria, and Brazil imported approximately 11.5, 8.1, 7.7, and 6.3 million tons of wheat in 2015/16, respectively. Furthermore, especially in the developing countries, the population is growing at a higher rate [3]; therefore, alternatives to staple crops should be considered for future food production. Corn is among the most widely produced and consumed grains in the world, with corn production estimated to be 989.83 million tons in 2015/16 [2]. Corn (*Zea mays*) is a gluten-free cereal, used as a base to formulate numerous food products, such as "tortillas" in Mexico and "broa" in Portugal. In addition to being the main staple food in many countries, corn grain and its flour contain higher levels of total phenolic compounds, and it has a higher antioxidant capacity than other grains, for example, wheat, rice, and oats [4]. Corn grain and its flour contain phytochemicals in both free and soluble-conjugated forms as well as in insoluble complexes [4].

Tropical and subtropical countries are also major producers of fruits such as banana. Indeed, India, China, Philippines, Brazil, and Ecuador are the largest global producers of banana, with an average production of 19.39, 6.67, 6.43, 6.40, and 6.38 thousand tons in 1994–2014, respectively [5]. However, in Brazil, about 20% of the fruit is wasted and rejected [6, 7]. Furthermore, some studies have demonstrated that green banana flour has high levels of resistant starch, between 16 g/100 g of flour [8] and 40.9–58.5 g/100 g of flour [6]. Resistant starch is the product of starch degradation known to be resistant to α-amylase and glucoamylase digestion, with in vivo results showing that 75%–84% of the starch granules ingested reach the terminal ileum [9]. However, there are a limited number of studies available for the application of green banana flour in popular food products and their effects on blood glucose levels; most studies describe the effect of flour consumption and not the effect of processed foods.

Wheat, corn, and green banana flours mainly comprise starch. The susceptibility of starch to digestion is the functional property of particular interest for nutrition. Food process is closely related to starch digestibility, in terms of how the starch is baked or cooked [10], as well its intrinsic properties, such as variations in physiochemical, granular structure, and the relation between amylose and amylopectin. Amylopectin is the major constituent of starch, consisting of both crystalline (double helix) and amorphous parts [11, 12]. When food rich in starch is baked, for example, the crystalline amylopectin melts and goes to the amorphous form, which is rapidly digested and absorbed due to its accessibility to digestive enzymes. Since it is well-known that the susceptibility of crystalline amylopectin to digestion depends on its botanical origin [11] and amorphous amylopectin is more susceptible to enzymatic action than the crystalline form [8, 11], it is important to quantify the level of blood insulin after consumption of foods with a high starch content from different sources, moreover after food processes, which could change the starch structure and digestibility. However, most of proposed studies on the literature only analyses raw materials and not the processed food such as bread.

Therefore, this study aimed to incorporate corn and green banana flours into bread formulations, characterize their physical and sensorial properties, and then evaluate resistant starch levels and in vivo blood glucose levels of rats after consuming bread produced with composite flour in order to verify the functional health attributes attributed to unprocessed foods, such as the used flours.

2. Materials and Methods

2.1. Materials. Wheat flour (Bunge, Brazil), corn flour (Yoki, Brazil), green banana flour (Zona Cerealista, Brazil), salt (Cisne, Brazil), commercial baking improver (BI) S500 (composed by a mix of corn starch xylanase, emulsifier polysorbate 80, and estearoil-2-lactil lactate to sodium, ascorbic acid, azodicarbonamide, and α-amylase) from Puratos (Brazil), and fresh yeast (Fleischamann, Brazil) were used. All material was purchased from the same lot. The resistant starch content of flours and breads was measured using a colorimetric enzyme assay kit (KRSTAR kit, Megazyme International, Wicklow, Ireland), according to the AACC method no 32-40-01 [13] adapted [14].

2.2. Methods

2.2.1. Flour Characterization. Chemical composition: the moisture, crude fat, ash, crude protein, and crude fiber levels of corn, green banana, and wheat flour were determined using standardized methods [15].

(1) Total Phenolic Content. The phenolics were extracted from the flours following the method proposed by Bakan et al. [16]. Flour samples (approximately 1.5 g) were dissolved in 80% ethanol (30 mL), submersed in an ultrasonic bath for 60 min at room temperature, and centrifuged at 3500 rcf for 10 min. A second extraction was performed by adding 30 mL of 80% ethanol before centrifugation as before. The total phenolic content was determined using Folin–Ciocalteau reagent. Aliquots of the samples (0.1 mL) were added to 1 mL of Folin's reagent (1 : 10). After 5 min, 2.0 mL of calcium carbonate (20%) was added, the solution was rested for 1 h, and the absorbance was determined at 740 nm using a spectrophotometer (Biospectro, SP-22). The absorbance values were converted to mg of gallic acid/100 g of flour using a gallic acid standard curve.

2.2.2. Bread Production. The dough was prepared with flour (1200 g), salt (24 g), BI (12 g), fresh yeast (60 g), and water (696 g) [17]. First, all dry ingredients were mixed in a spiral vertical mixer (AM-12E, Famag Brasil, Brazil), then the yeast and the water were added, and the ingredients were mixed for 9 min. After mixing, the dough was covered with a plastic film for 10 min, then divided into 100 g portions, and re-covered with plastic film for 15 min, enabling dough relaxation. The breads were placed in a mold (MR500, Prática Technipan, Brazil) and transferred to a fermentation chamber (Klimaquip, Brazil) at 35°C and 85% relative humidity until the volume increased 2.5-fold its initial volume (approximately 30 min). Finally, breads were baked at 200°C for 14 min in an electric oven (E250, Prática Produtos, Brazil).

2.2.3. Experimental Design. The response surface methodology (RSM) was used to study the effect of wheat flour level substitution and baking improver concentration on bread properties (Tables 1 and 2). The effects of corn flour concentration (C_{CF}) (0–40 g/100 g of total flour) and baking improver concentration (C_{BI}) (12–33 g/1200 g total flour)

Table 1: The 2^2 factorial design and responses in the characterization of bread produced with different concentrations of wheat flour, corn flour (C_{CF} = g/100 g total flour), green banana flour (C_{GF} = g/100 g total flour), and baking improver (C_{BI} = g/1200 g total flour).

Run	Variable X_1 (C_{CF})	Variable X_2 (C_{BI})	WCD	WCCb	WCCt	WL	SV	Hd	El	Co	Ym
1	−1 (0)	−1 (12)	46.6 ± 0.2	44.2 ± 1.4	16.9 ± 2.0	20.6 ± 1.1	6.02 ± 0.41	0.09 ± 0.02	0.98 ± 0.02	0.83 ± 0.02	0.11 ± 0.04
2	+1 (40)	−1 (12)	45.6 ± 0.2	44.8 ± 0.1	20.6 ± 0.7	19.4 ± 0.5	2.30 ± 0.01	0.84 ± 0.12	0.87 ± 0.04	0.53 ± 0.05	1.36 ± 0.21
3	−1 (0)	+1 (36)	45.6 ± 0.8	43.8 ± 0.2	11.6 ± 0.9	21.4 ± 0.8	5.97 ± 0.16	0.10 ± 0.01	0.94 ± 0.03	0.81 ± 0.03	0.12 ± 0.04
4	+1 (40)	+1 (36)	46.0 ± 0.1	43.2 ± 0.1	15.7 ± 1.5	20.7 ± 1.2	3.80 ± 0.07	0.29 ± 0.03	0.89 ± 0.10	0.53 ± 0.11	1.49 ± 0.52
5	0 (20)	0 (24)	45.1 ± 0.1	44.3 ± 0.1	18.9 ± 2.1	19.1 ± 0.4	4.26 ± 0.30	0.18 ± 0.02	0.94 ± 0.02	0.77 ± 0.02	0.23 ± 0.03
6	0 (20)	0 (24)	46.0 ± 0.1	43.6 ± 0.3	17.8 ± 1.1	20.0 ± 0.1	4.20 ± 0.38	0.19 ± 0.03	0.94 ± 0.03	0.75 ± 0.04	0.21 ± 0.05
7	0 (20)	0 (24)	51.8 ± 1.6	43.2 ± 0.3	15.3 ± 0.5	21.1 ± 0.4	4.53 ± 0.25	0.21 ± 0.02	0.92 ± 0.03	0.73 ± 0.03	0.25 ± 0.07
8	0 (20)	0 (24)	45.3 ± 0.1	43.4 ± 0.1	20.1 ± 1.2	19.6 ± 1.0	3.82 ± 0.13	0.22 ± 0.01	0.93 ± 0.03	0.76 ± 0.02	0.25 ± 0.06
Run	Variable X_1 (C_{GF})	Variable X_2 (C_{BI})	WCD	WCCb	WCCt	WL	SV	Hd	El	Co	Ym
1	−1 (0)	−1 (12)	45.8 ± 0.1	45.2 ± 0.2	16.8 ± 0.9	17.8 ± 0.5	3.63 ± 0.25	0.30 ± 0.04	0.95 ± 0.03	0.80 ± 0.03	0.16 ± 0.01
2	+1 (20)	−1 (12)	46.4 ± 0.1	43.8 ± 0.1	21.2 ± 1.09	16.3 ± 0.3	2.21 ± 0.03	0.94 ± 0.12	0.88 ± 0.02	0.67 ± 0.03	1.02 ± 0.06
3	−1 (0)	+1 (20)	46.5 ± 0.1	45.7 ± 0.2	13.5 ± 1.1	22.8 ± 0.3	6.22 ± 0.11	0.08 ± 0.01	0.91 ± 0.03	0.80 ± 0.03	0.08 ± 0.02
4	+1 (20)	+1 (20)	44.9 ± 0.2	44.5 ± 0.6	19.1 ± 2.6	16.6 ± 0.4	2.42 ± 0.06	0.71 ± 0.09	0.87 ± 0.01	0.69 ± 0.03	0.85 ± 0.14
5	0 (10)	0 (16)	46.3 ± 0.1	45.2 ± 0.2	13.3 ± 1.4	18.5 ± 0.7	4.09 ± 0.12	0.12 ± 0.01	0.91 ± 0.05	0.80 ± 0.01	0.14 ± 0.03
6	0 (10)	0 (16)	47.3 ± 0.1	44.3 ± 0.2	16.6 ± 1.0	18.6 ± 0.8	4.21 ± 0.11	0.23 ± 0.03	0.94 ± 0.01	0.80 ± 0.01	0.19 ± 0.02
7	0 (10)	0 (16)	46.3 ± 0.2	44.8 ± 0.2	19.4 ± 0.9	18.1 ± 0.6	4.07 ± 0.04	0.19 ± 0.01	0.88 ± 0.10	0.80 ± 0.02	0.20 ± 0.05
8	0 (10)	0 (16)	46.4 ± 1.6	43.4 ± 0.2	18.3 ± 0.8	18.7 ± 0.7	4.04 ± 0.20	0.22 ± 0.04	0.93 ± 0.03	0.80 ± 0.01	0.15 ± 0.03

X_1, X_2 independent variables; (−1), (0), and (+1) = coded values of experimental design; and (·) = decoded values of experimental design. WCD = dough water content (%), WCCb = crumb water content (%), WCCt = crust water content (%), WL = water weight loss (%), SV = specific volume (mL/g), Hd = hardness (kg), El = elasticity, Co = cohesiveness, and Ym = Young modulus (MPa).

Table 2: Continuation of the 2^2 factorial design and responses in the characterization of bread produced with different concentrations of wheat flour, corn flour (C_{CF}), green banana flour (C_{GF}), and baking improver (C_{BI}).

Run	Variable X_1 (C_{CF})	Variable X_2 (C_{BI})	CutF	L^*·Cb	a^*·Cb	b^*·Cb	L^*·Ct	a^*·Ct	b^*·Ct
1	−1 (0)	−1 (12)	45 ± 6	68.1 ± 3.3	−0.3 ± 0.1	10.7 ± 1.1	61.0 ± 3.4	14.0 ± 1.9	36.5 ± 1.4
2	+1 (40)	−1 (12)	25 ± 8	71.1 ± 0.2	5.6 ± 0.1	40.0 ± 0.4	71.7 ± 3.0	11.9 ± 1.6	42.3 ± 1.7
3	−1 (0)	+1 (36)	40 ± 6	82.6 ± 0.4	0.9 ± 0.2	18.8 ± 0.5	60.7 ± 2.6	17.5 ± 1.0	42.1 ± 0.5
4	+1 (40)	+1 (36)	17 ± 3	73.3 ± 3.0	3.6 ± 0.3	36.1 ± 2.1	73.8 ± 7.1	10.2 ± 1.7	31.7 ± 4.6
5	0 (20)	0 (24)	32 ± 6	75.8 ± 0.5	2.7 ± 0.3	30.1 ± 1.0	71.9 ± 4.7	11.9 ± 2.5	33.8 ± 3.9
6	0 (20)	0 (24)	33 ± 4	75.8 ± 0.9	2.5 ± 0.2	29.6 ± 0.8	66.2 ± 3.2	14.5 ± 1.3	36.3 ± 2.9
7	0 (20)	0 (24)	31 ± 5	75.3 ± 0.5	2.6 ± 0.2	29.8 ± 0.6	71.0 ± 1.9	12.2 ± 0.9	36.9 ± 3.5
8	0 (20)	0 (24)	30 ± 5	75.3 ± 0.7	3.0 ± 0.2	30.5 ± 0.8	70.2 ± 4.5	11.8 ± 2.5	33.6 ± 4.0
Run	Variable X_1 (C_{GF})	Variable X_2 (C_{BI})	CutF	L^*·Cb	a^*·Cb	b^*·Cb	L^*·Ct	a^*·Ct	b^*·Ct
1	−1 (0)	−1 (12)	57 ± 9	78.3 ± 1.8	2.1 ± 0.2	20.6 ± 0.5	66.1 ± 3.7	12.9 ± 2.6	35.9 ± 2.1
2	+1 (20)	−1 (12)	27 ± 5	43.9 ± 2.0	4.0 ± 0.2	16.0 ± 0.7	48.4 ± 2.4	8.9 ± 1.0	25.9 ± 1.6
3	−1 (0)	+1 (20)	32 ± 4	64.9 ± 2.3	−0.4 ± 0.1	9.7 ± 0.5	61.3 ± 2.6	13.2 ± 1.7	39.1 ± 1.5
4	+1 (20)	+1 (20)	28 ± 2	45.2 ± 1.4	3.9 ± 0.3	15.7 ± 0.5	43.3 ± 2.8	11.0 ± 2.1	26.9 ± 1.8
5	0 (10)	0 (16)	34 ± 7	51.5 ± 1.6	2.2 ± 0.2	14.1 ± 0.9	53.7 ± 3.5	10.7 ± 1.6	32.0 ± 1.7
6	0 (10)	0 (16)	41 ± 7	58.1 ± 3.4	3.7 ± 0.3	15.5 ± 1.3	57.3 ± 2.8	10.7 ± 0.6	28.4 ± 3.2
7	0 (10)	0 (16)	36 ± 7	58.6 ± 2.2	3.6 ± 0.2	15.2 ± 0.7	57.3 ± 3.9	12.0 ± 1.4	29.6 ± 3.5
8	0 (10)	0 (16)	37 ± 6	59.2 ± 2.2	3.6 ± 0.2	15.4 ± 1.0	53.2 ± 1.2	11.8 ± 1.9	32.0 ± 1.6

X_1, X_2 independent variables; (−1), (0), and (+1) = coded values of experimental design; (·) = decoded values of experimental design. CutF = cutting force; L^*·Cb = crumb L^*, a^*·Cb = crumb a^*, b^*·Cb = crumb b^*, L^*·Ct = crust L^*, a^*·Ct = crust a^*, and b^*·Ct = crust b^*.

on the dependent variables were evaluated first, and then, the effects of green banana flour concentration (C_{GF}) (0–20 g/100 g of total flour) and baking improver concentration (C_{BI}) (12–20 g/1200 g total flour) on the dependent variables were evaluated. Higher levels of C_{CF} and C_{GF} were previously determined, with the center points of runs 5–8 used to determine the experimental error and data reproducibility.

Statistical analysis (95% confidence level) was performed using Statistica 9.1 software. First, a Pareto diagram was used to evaluate the effects of each individual independent variable and its interactions on each dependent variable. In this diagram, the value in which $t_{calculated}$ is equal to $t_{tabulated}$ completes the diagram, providing the value from which the effects are significant at the 95% confidence level ($p = 0.05$). All determinations for the

Table 3: Regression coefficient and ANOVA for the different dependent variables evaluated as a function of the independent variables, corn flour concentration (C_{CF} = g/100 g total flour), green banana flour concentration (C_{GF} = g/100 g total flour), and baking improver concentration (C_{BI} = g/1200 g of total flour).

Dependent variable for bread produced with corn flour	β_0	C_{CF}	C_{BI}	$C_{CF}\cdot C_{BI}$	R^2	$F_{calculated}$	$F_{tabulated}$ (p = 0.05)	$F_{Lack\ of\ adjustment}$	$F_{tabulated2}$ (p = 0.05)
Specific volume (SV)	4.36*	−1.47*	0.36	0.39	0.95	32.58	5.99	5.18	9.28
Hardness (Hd)	0.26*	0.24*	−0.14*	−0.14*	0.92	14.68	6.59	100.38	10.13
Elasticity (El)	0.93*	−0.05*	−0.01	0.02*	0.95	25.55	5.79	5.26	9.55
Cohesiveness (Co)	0.71*	−0.14*	−0.01	0.001	0.87	40.51	5.99	12.23	9.28
Cutting force (CutF)	31.58*	−10.60*	−3.23*	−0.66	0.99	161.57	5.79	0.61	9.55
Young modulus (Ym)	0.050*	0.067*	0.0025	0.0025	0.75	17.71	5.99	81.33	9.28
L^* (crumb) (L^*·Cb)	74.66*	−1.59	4.17*	−3.08*	0.95	16.28	5.79	115.55	9.55
a^* (crumb) (a^*·Cb)	2.57*	2.18*	−0.20	−0.81*	0.99	132.94	5.79	2.74	9.55
b^* (crumb) (b^*·Cb)	28.20*	11.64*	1.05	−3.02	0.96	48.05	5.99	135.30	9.28
L^* (crust) (L^*·Ct)	68.30*	5.95*	0.44	0.61	0.80	21.64	5.99	1.12	9.28
a^* (crust) (a^*·Ct)	12.99*	−2.35*	0.42	−1.30	0.83	9.77	5.99	1.86	9.28
b^* (crust) (b^*·Ct)	36.66*	−1.13	−1.24	−4.04*	0.74	10.42	5.99	3.42	9.28
Dependent variable for bread produced with green banana flour	β_0	C_{GF}	C_{BI}	$C_{GF}\cdot C_{BI}$	R^2	$F_{calculated}$	$F_{tabulated}$ (p = 0.05)	$F_{Lack\ of\ adjustment}$	$F_{tabulated2}$ (p = 0.05)
Specific volume (SV)	3.86*	−1.31*	0.70*	−0.60*	0.95	27.97	6.59	86.67	10.13
Water loss (WL)	18.43*	−1.94*	1.34*	−1.16*	0.99	172.90	6.59	0.13	10.13
Cutting force (CutF)	36.58*	−8.47*	−5.76*	6.54*	0.96	31.64	6.59	0.16	10.13
Young modulus (Ym)	0.36*	0.38*	−0.09	0.00	0.68	10.70	5.99	123.53	9.28
L^* (crumb) (L^*·Cb)	57.48*	−13.53*	−3.04	3.68	0.95	33.12	5.99	2.44	9.28
a^* (crumb) (a^*·Cb)	2.84–	1.58*	−0.67	0.60	0.81	9.53	5.99	3.27	9.28
b^* (crumb) (b^*·Cb)	15.28*	0.35	−2.79*	−2.65*	0.95	66.14	5.79	0.99	9.55
L^* (crust) (L^*·Ct)	55.07*	−8.95*	−2.45	−0.06	0.96	48.22	5.99	1.63	9.28
a^* (crust) (a^*·Ct)	11.41*	−1.54*	0.61	0.43	0.81	15.45	5.99	1.73	9.28
b^* (crust) (b^*·Ct)	31.22*	−5.56*	1.06	−0.56	0.90	37.70	5.99	1.01	9.28

$F_{calculated} > F_{tabulated1}$: significant model; $F_{Lack\ of\ adjustment} < F_{tabulated2}$: significant and predictive model, $p < 0.05$ (95% confidence); and *significance at 95% confidence interval.

effect or model calculations were performed by the residual error, since the pure error is used only for calculating the values of nonadjusted analysis of variance (ANOVA), that is, the pure error only indicates the variation of the center points. After this, only predictive responses, which presented significant effects at the 95% confidence level (p = 0.05), were evaluated (Table 3). Data were fitted to a first-order effects model as a function of the dependent variables (equation (1)).

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2, \quad (1)$$

where Y_i is the predicted response; β_0 is the constant coefficient; β_1 and β_2 are the linear coefficients; β_{12} is the interaction coefficient; and X_1 and X_2 are independent variables.

2.2.4. Bread Characterization

(1) Water Content. The water content of the dough, crumb, and crust were determined after drying the samples in an oven (FANEM, Fanem 515, Brazil) at 105°C for 24 h.

(2) Water Loss. The water loss was calculated using the weight ratio of the dough and the baked bread expressed as a percentage [17].

(3) Specific Volume. The specific volume of the bread was determined using a VolScan Profiler (Stable Micro Systems, Godalming, United Kingdom) at a set laser distance of 4 mm. The average SV was determined by calculating the ratio between the volume (mL) and the weight (g) of the bread.

(4) Texture Properties. Crumb texture properties were determined by texture profile analysis (TPA) and cutting force using a TA-XT Plus texturometer (Stable Microsystems, Surrey, United Kingdom). For TPA analyses, a 25 mm diameter cylindrical probe at a test speed of 1.70 mm/s was used. The bread slice was subjected to a double cycle of compression at a maximum deformation of 40%, and the responses obtained were hardness, elasticity, and cohesiveness. For cutting force analyses, bread samples (15 mm cross-sections) containing crumb and crust, positioned vertically, were sheared by a Warner–Bratzler blade [18]. The parameters defined for the test were kept constant at 4.00 m/s pretest velocity, 1.00 mm/s test velocity, and

5.00 mm/s posttest velocity, with a test activation force of 20.0 g. The Young modulus (Ym) was also determined by plotting force in function of the strain and selecting the linear region (N/% strain), followed by dividing this slope by the cross-section area of the sample, and then expressed in MPa.

(5) Color. The color parameters of the crust and crumb, lightness (L^*), a^*, and b^* were determined (in triplicate) using a colorimeter (HunterLab, MiniScan XE plus, Reston, USA) with illuminant D65 (daylight) and a 30 mm diameter cell opening.

(6) Sensorial Analyses. Similarly, in the second step of this study, selected bread samples were evaluated by 115 untrained panelists. This study was approved by the Ethics Committee of the Faculty of Animal Science and Food Engineering (FZEA/USP) (process 56390716.0.0000.5422). Prior to performing the test, panelists gave informed consent. Bread samples, including control, were evaluated for color, flavor, texture, and overall acceptability, using a nine-point structured hedonic scale where nine corresponded to "liked extremely" and one to "disliked extremely." Samples were randomly presented to the panelists on white plates with undisclosed codes. Consumer purchase intention was evaluated by assessing the possibility that consumers plan to or would be willing to purchase the product using a five-point scale (1 = "certainly would not buy" and 5 = "certainly would buy"). The panelists scaled the order of preference among the three samples evaluated.

2.2.5. Blood Glucose Levels in Rats. The in vivo study was conducted at the animal facility of the Department of Animal Pathology, Faculty of Veterinary Medicine and Animal Science of University of São Paulo (FMVZ-USP). The experiment was conducted in compliance with the guidelines of and approved by the Ethics Committee FZEA/USP (process 3307130916/2016). The experiments were conducted according to the methodology proposed by Kawai et al. [12]. Twenty-five male Wistar rats (6 weeks of age) had free access to water and food during acclimation (2 weeks). After a 12 h-fast, blood glucose readings were determined from the tail vein of mice using a glucose sensor (G-Tech Lite, Glucomed, Brazil). Rats were divided into five groups ($n = 5$ per group), and no significant differences in initial blood glucose were observed. The bread samples were homogenized with filtered water, and suspensions (100 mg/mL) were obtained. The suspensions were orally administered at 20 mL/kg by gavage, and blood glucose was assayed at 30, 60, 90, and 120 min after administration.

3. Results and Discussion

3.1. Flour Characterization. The proximate chemical compositions of the flours are presented in Table 4, and there were differences observed in protein, fiber and resistant starch, and total phenolic content between wheat, corn, and green banana flour. The proximate chemical composition of corn flour (Table 4) showed a profile very similar, considering the varietal differences, to those obtained by Sabanis and Tzia [19], whereas the total phenolic content of corn flour, approximately 7.8 mg of GAE/100 g of flour, was lower than the values reported by Méndez et al. [6]. This difference could be related to corn genotype, variety, and extraction method quantification. Resistant starch content in corn flour, 5.14% (Table 4), was higher than that observed for wheat flour (1.27%).

The green banana flour analyzed in this study had a protein content of 7.77% (dry matter, dm), higher than the values reported for different unripe bananas, estimated between 2.5% and 3% (dm) [8, 20, 21]. Similarly, green banana flour had a higher ash content of 6.34% (dm) compared to published results (2.6%–4.7%, dm) [8, 20, 21]. The total phenolic content of green banana flour, approximately 18.0 mg of GAE/100 g of flour, was also higher than that reported in the literature [21]. Finally, resistant starch in green banana flour (13.77%, dm), which is close to the highest level, is measured in eight different unripe green banana flours (15.54%, dm) [20], and it was higher than values observed for wheat and corn flour. Consequently, the corn and green banana flour evaluated in this study showed a higher total phenolic and resistant starch content in comparison to other flours and fruits, thus, potentially increasing those components in bread formulations, with green banana flour having the highest resistant starch and total phenolic content. Moreover, the high ash content of green banana flour could be attributed to the high mineral content. Therefore, it could also be expected that breads produced with corn and green banana flour, in relation to control bread, will have an increased nutritional content, and could potentially be developed for patients with specific nutritional needs.

3.2. Bread Characterization

3.2.1. Water Content and Water Loss. According to ANOVA, the linear models fitted for dough, crumb, and crust water content (Table 3) were not statistically significant and predictive for both flours used. Similarly, no mathematical model was identified to describe the water loss for breads partially substituted by corn flour (Table 1).

Although the linear models could not be used to represent the effects of corn flour and baking improver concentration on the water content of the dough, crumb, and crust, as well as water loss, it was observed that the mean values presented for corn-wheat breads (calculated as the mean of the corresponding values presented in Tables 1 and 2) remained at approximately 46.5 ± 2.3 g of water/100 g of dough, 43.8 ± 0.6 g of water/100 g of crumb, 17.1 ± 2.9 g of water/100 g of crust, and 20.2 ± 0.8 g of water/100 g of bread, respectively. Similarly, mean values presented for green banana-wheat breads remained at approximately 46.2 ± 0.7 g of water/100 g of dough, 44.6 ± 0.8 g of water/100 g of crumb, and

Table 4: Proximate chemical composition and total phenolic content of the different flours used to produce breads with partial wheat flour replacement.

Compound	Flour		
	Wheat	Corn	Green banana
Moisture (g/100 g of flour)	11.19 ± 0.23	6.69 ± 0.15	5.12 ± 0.19
Dry matter (DM) (g/100 g of flour)	88.81 ± 0.23	93.31 ± 0.15	94.72 ± 0.19
Crude protein (g/100 g DM)	14.36 ± 0.22	8.31 ± 1.02	7.77 ± 0.32
Crude fiber (g/100 g DM)	0.16 ± 0.03	1.04 ± 0.19	3.75 ± 0.60
Crude fat (g/100 g DM)	1.22 ± 0.04	2.03 ± 0.08	1.15 ± 0.11
Ash (g/100 g DM)	0.62 ± 0.01	0.57 ± 0.04	6.34 ± 0.37
Resistant starch (g/100 g of DM)	1.27 ± 1.21	5.14 ± 2.47	13.77 ± 2.47
Dietary fiber* (g/100 g of fDM)	3.60	5.20	12.53
Total phenolic (mg GAE/100 g)	3.3 ± 0.4	7.8 ± 0.6	18.0 ± 1.13

*Not quantified: information provided by the suppliers.

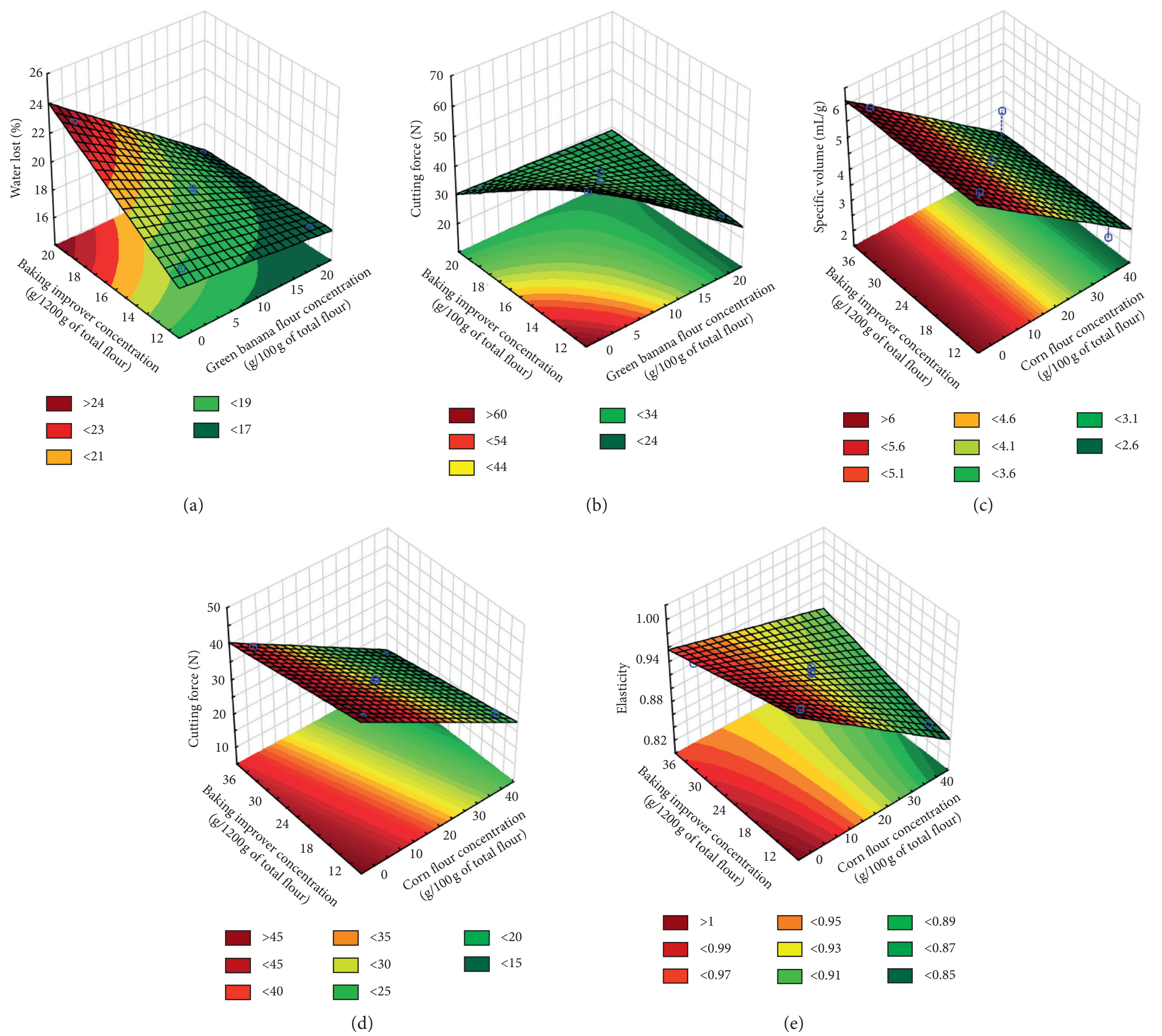

Figure 1: Response-surfaces for water loss (WL) (a) and cutting force (CutF) (b) effect of green banana flour concentration (C_{GF}) and baking improver concentration (C_{BI}) and response-surfaces for specific volume (SV) (c), CutF (d), and elasticity (El) (e) effect of corn flour concentration (C_{CF}) and baking improver concentration (C_{BI}).

17.3 ± 2.8 g of water/100 g of crust. Those values were in agreement with previous studies on bread formulated with only WF [22, 23], suggesting that corn and green banana flour can be used at the different levels evaluated without provoking a significant change in water content of the dough crumb and crust.

However, the linear model fitted for water loss (WL) for breads partially substituted by green banana flour was statistically significant ($F_{calculated} > F_{tabulated1}$) and predictive ($F_{Lack\ of\ adjustment} < F_{tabulated2}$) (Table 3), as represented by equation (1), enabling the plotting of response surface plots (Figure 1(a)). According to Figure 1(a), green banana flour and baking improver concentration directly affected the WL of the bread during baking. In the concentration range investigated, WL varied between 16.28% and 22.84% (Tables 1 and 2). The incorporation and the increase of green banana flour concentration provoked a decrease in the WL of breads during baking, which was also observed in previous study [23], and could be attributed to the high fiber content of green banana flour, as detailed in Table 3.

3.2.2. Specific Volume. The specific volume values ranged from 2.21 mL/g to 6.22 mL/g (Tables 1 and 2), agreeing with previous studies of bread formulated with only wheat flour [22, 23]. According to ANOVA, only the linear model fitted for specific volume of breads produced with corn flour (Table 3) was statistically significant ($F_{calculated} > F_{tabulated1}$) and predictive ($F_{Lack\ of\ adjustment} < F_{tabulated2}$). As shown in Figure 1(c), an increase in corn flour concentration (C_{CF}) decreased the SV of the bread, with no significant effect for the baking improver concentration (C_{BI}). This trend was consistent with the results observed by Sabanis et al. [19] for bread produced with partial wheat flour replacement by corn flour. The authors reported that as the corn flour concentration increased (0–50%), a decrease in specific volume was observed. Siddiq et al. [24] also observed a similar behavior in breads produced with partial substitution of wheat flour by defatted maize germ flour (0–20 g/100 g); a decrease in bread volumes from 318.8 mL to 216.3 mL occurred, comprising a 35% decrease in the specific volume (1.77 to 1.15 mL/g).

Although the corn flour concentration had a negative effect on the specific volume of bread formulated with corn-wheat flour, this effect was less pronounced when corn flour concentration levels remained lower than 20 g/100 g of flour (Figure 1(c)), producing bread with a specific volume similar to that of the control bread (produced with only wheat flour) (Figure 1(c)).

At this level of analyses, the specific volume was not related with the increased gas retention, as the crumb structure measurements were not significantly correlated between the different formulations. Volume enhancement seemed to be a consequence of enhanced elasticity of the gluten network, which could result in a higher gas retention capacity. The negative effect of increasing corn flour concentration on the specific volume was expected because it implies in a higher resistant starch and dietary fiber concentration, which leads to settle around gas phase cells and form physical barriers and increase in water content of the matrix. All of these decrease the retention of carbon dioxide, thereby decreasing the SV. Fleming and Sosulski [25] utilized scanning electron microscopy to observe little pores in the gluten fibrils and the cell walls of breads supplemented with different concentrated protein plants. Those authors proposed that these pores may permit the loss of gas during fermentation and baking. Moreover, the use of baking improver suggests that its increase in dough formulation could have favored the aggregation of gluten proteins and some hydrogen bonds with glutamine and complex with starch granules in corn-wheat dough, thereby increasing protein-starch interactions [26], thus enhancing the gas retention limit and expanding the loaf volume. Therefore, it could be proposed that at intermediary corn flour incorporation in the bread formulation (20%), these associations could create a more solid protein arrangement and improve the gluten-starch-lipid complex that subsequently will produce bread with similar texture and expanded volume.

3.2.3. Texture Properties. Texture properties were evaluated by the parameters of hardness, elasticity, cohesiveness, and cutting force (Tables 1 and 2). However, according to ANOVA, only the linear model fitted for cutting force of breads produced with both flours (Table 3) and the model fitted for elasticity of breads produced with corn flour were statistically significant ($F_{calculated} > F_{tabulated1}$) and predictive ($F_{Lack\ of\ adjustment} < F_{tabulated2}$) (Table 3).

Green banana flour and baking improver, in the concentration range studied, significantly affected the response of the cutting force of breads (Figure 1(b)). Similarly, cutting force of breads produced with corn flour was also significantly affected by corn flour and baking improver concentration (Figure 1(d)). The values obtained for the cutting force were affected negatively and significantly by the concentration of substitute flour and improver (Figures 1(b) and 1(d)). The composite green banana-wheat flour bread and corn-wheat flour bread were less resistant to cutting compared to the control wheat bread. Furthermore, it could be observed that the effect of adding corn and green banana flour had a greater impact on cutting force than the addition of BI.

The decrease in the cutting force values of breads produced with corn-wheat and green banana-wheat flour could be attributed to the greater fiber content of corn and green banana flour. The higher the fiber content, the higher the water absorption dough capacity, and consequently, the lower the force required to cut the bread crust. Furthermore, the higher levels of dietary fiber of corn and green banana flour probably decreased the gas retention capacity due to a weaker gluten network, with consequences on SV and texture properties. At this level of analyses, the decrease in cutting force with corn and green banana flour addition could be related to the specific volume and fiber content. Earlier studies have shown a similar relationship between bread specific volume and hardness.

The elasticity of the corn-wheat flour breads was also affected by increasing the corn flour concentration (C_{CF}) (Figure 1(e)) and the interaction of corn flour and baking improver concentrations (Table 3). This could be associated with the low level of gluten, lower gas retention capacity, or low quality in terms of gluten development, the protein responsible for the mass viscoelasticity. As the corn flour concentration increased from 0% to 40%, the elasticity decreased from 0.98 to 0.87 (Tables 1 and 2), in line with data reported by Păucean and Man [27] who observed that the partial replacement of wheat flour by 0%–40% corn flour resulted in loss of bread elasticity (from 0.88 to 0.63).

The use of baking improver had a negative effect on cutting force and elasticity, and as the baking improver concentration increased, the cutting force of the bread increased (Figures 1(b) and 1(d)) and the elasticity decreased (Figure 1(e)). Therefore, the incorporation of high levels of baking improver was not sufficient to achieve properties similar to those of the control bread. Despite baking improver favoring interactions with gluten-starch, those interactions were not sufficient to enhance dough development. Indeed, the texture properties analyses showed lower elasticity of the bread on addition of corn flour, indicating a less cohesive and unstable structure, which could lead to inferior elasticity, probably due to physical barriers around gas phase cells (dietary fibers, for example) and weakening of the gluten-starch network developed in corn-wheat breads, and therefore insufficient gas retention (Figure 2).

Unfortunately, as the model fitted for hardness of breads, as well as the other answers obtained by TPA, was not statistically significant and predictive (Table 3), they could not be related to specific volume of breads, as proposed in some others studies [28]. Thus, it seems that it is the most important factor responsible for superficial force, and therefore, cutting force changes in corn-wheat and green banana-wheat breads are the high level of fiber and/or resistant starch of corn and green banana flour, respectively, which could in turn influence water absorption, thermal properties, starch gelatinization, and therefore gluten network macromolecular structure, and consequently, the volume increment and texture properties (Figure 2).

Besides TPA parameters, texture analyses were also applied by a more straightforward mechanical test, allowing to determine Young's modulus (Ym) for the crumb (Tables 1 and 2). The linear models fitted for Ym of breads produced with both flours (Table 3) were statistically significant ($F_{\text{calculated}} > F_{\text{tabulated1}}$), however, not predictive ($F_{\text{Lack of adjustment}} < F_{\text{tabulated2}}$) (Table 3). Although the models could not be used to represent the effects of substituted flour concentration and BI concentration on the Young modulus of the crumb, it was observed that the values presented for breads (0.01–1.49) were in agreement with previous studies on bread formulated with only wheat flour [29–32]. Control crumb bread (bread with inly wheat flour) required the least amount of force to compress. The values reported for corn-wheat bread were 2.5 times (bread with 20% of corn flour) or yet 15 (bread with 40% of corn flour) times higher than the control wheat breads. While, values for green banana-wheat bread were between 2 (10% green banana flour) and 10 (20%) times higher than the standard wheat. Resistance to mechanical deformation of breads indicates, therefore, a higher rigidity of green banana-wheat bread, followed by corn-wheat bread, and control breads, independent of the concentration of substituted flour. Furthermore, the increase of Young modulus of composite bread seems to be linked with the resistant starch level in bread, once Pearson correlation between them present a good correlation, 0.98 for corn-wheat bread and 0.95 for green banana-wheat breads, and much better for them with specific volume (0.70 and 0.82, respectively), as normally presented in the literature [33]. No studies were found in the literature that evaluated the mechanical properties of bread made with partial replacement of wheat flour by any type of flour.

3.2.4. Color. The color of the crumb and crust was evaluated by L^*, a^*, and b^* (Tables 1–3). The values of L^*, a^*, and b^* of crumb observed for the control bread (Tables 1 and 2) agreed with those previously reported for similar breads [19, 24, 34]. The response surface for a^* of the bread crumb produced with corn and green banana flour (Table 4) revealed that increasing the level of flour substitution increased the a^* values of the bread crumbs. In relation to lightness (L^*) of crumb, for breads produced with green banana flour, L^* decreased significantly with increasing green banana flour concentration (Table 3). The baking improver concentration had a less-pronounced effect on crumb color parameters (Table 3). Siddiq et al. [24] also reported high values of a^* when higher defatted corn flour concentration was used to produce breads, whereas Fleming and Sosulski [25] and Alpaslan and Hayta [34] reported a lower a^* of crumb bagel/pretzel-type bakery compared to the control (bread made with wheat flour only). The more pronounced color of corn-wheat and green banana breads produced in this study could be attributed to a more pronounced Maillard reaction due to the high amount of glucose released by the amylases present in the flour and baking improver.

The crust color parameters, L^*, a^*, and b^*, for corn-wheat breads varied between 60.7 and 73.8, 10.2 and 17.5, and 31.7 and 42.3, respectively (Tables 1 and 2), while for green banana-wheat breads the values varied between 43.3 and 66.1, 8.9 and 12.9, and 25.9 and 35.9, respectively (Tables 1 and 2). The color values observed for bread crust produced with corn flour are in accordance with the study by De Farias et al. [35] who obtained L, a^*, and b^* values of bread crust between 65.7 and 71.6, 3.0 and 8.0, and 32.0 and 43.4. Moreover, it was observed that the increase of corn flour concentration increased L^* and decreased a^* and b^* of bread crust produced with corn flour (Tables 1 and 2). This yellow color increment could be due to color pigments presents in corn, mainly carotenes and xanthophylls [4]. The results were consistent with the literature [19, 24], indicating

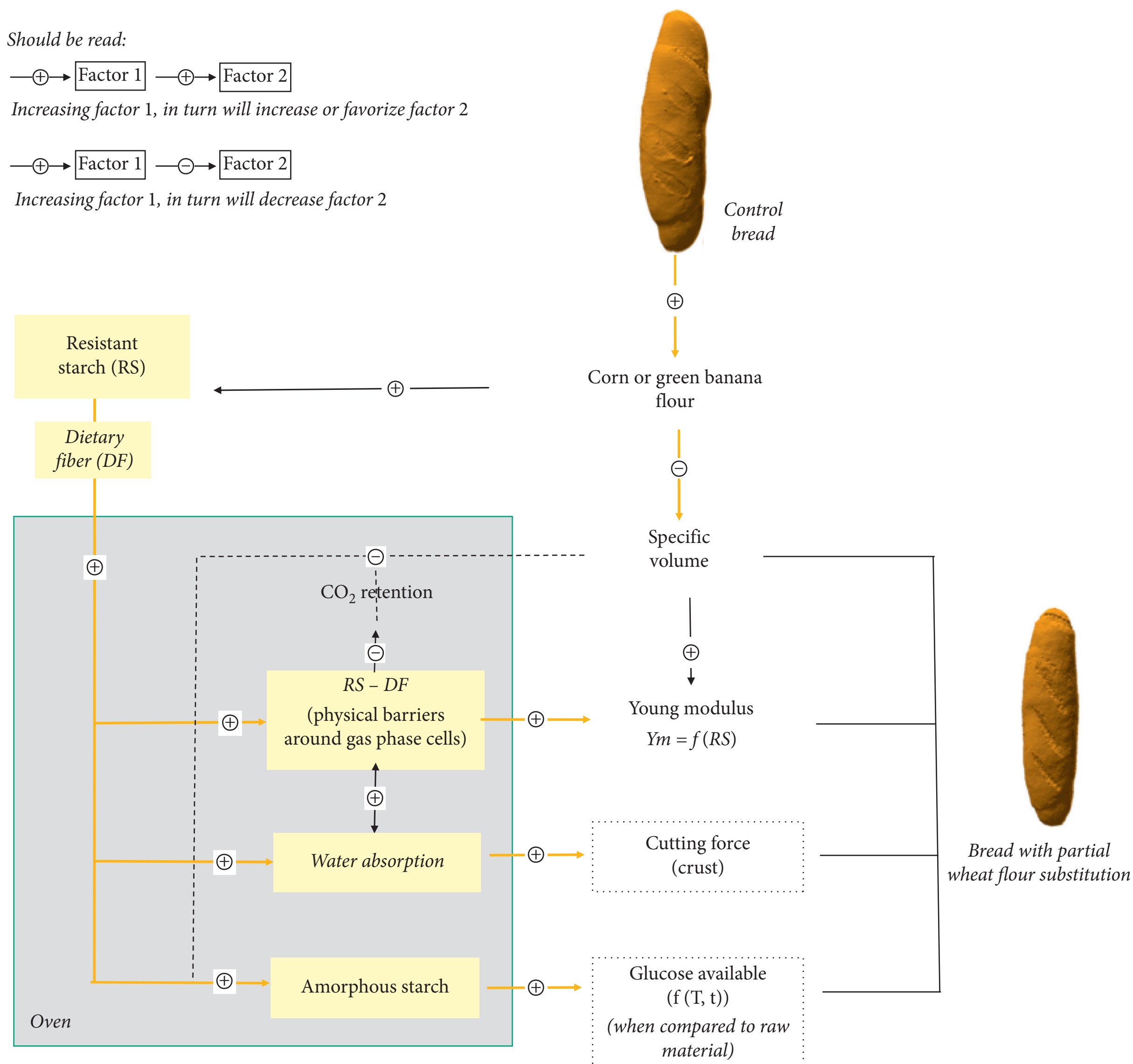

Figure 2: Flow chart to identify the macroscopic and macromolecular changes in structural, physical, and texture properties of enriched resistant starch composite bread produced with partial wheat flour substitution and its interrelationships.

that corn flour concentration and baking improver concentration significantly increased the lightness and the yellowness of the bread, which could be related to the phenol compounds and fiber present in corn flour. The color aspects of corn products in combination with carotenoid concentration (precursors of vitamin A) represent an important attribute to be evaluated by consumer acceptance [19].

Considering the color crust parameters of breads produced with green banana flour, a^*, b^*, and L^* of crust decreased significantly with increasing green banana flour concentration (Tables 1 and 2), which may be due to the higher amount of oxidized phenol compounds formed when high levels of green banana flour were used (Table 4). The darkness of the crust and crumb for breads produced with green banana flour could be a product of the Maillard reaction between reducing sugars and proteins. Similar results were reported by Mohamed et al. [23] for breads produced with different levels of banana powder incorporation.

3.3. Sensorial Analyses, Blood Glucose Levels in Rats, and Resistant Starch In Vitro Analyses. Statistical analyses showed that flour substitution level had a more important and significant effect on the physical responses evaluated, while baking improver concentration, generally, had a lower or insignificant effect. Therefore, to evaluate the effect of the bread intake in terms of sensorial aspects and blood glucose levels, as well as resistant starch in vitro, the baking improver concentration was kept constant, with only the flour concentration varied to better understand the effect of flour concentration on the glucose levels after bread intake.

3.3.1. Sensorial Analyses. Bread acceptance by consumers is closely related to its texture, color, taste, and flavor, which are critical quality attributes linked to baking process. In the present study, the acceptability of bread formulated with

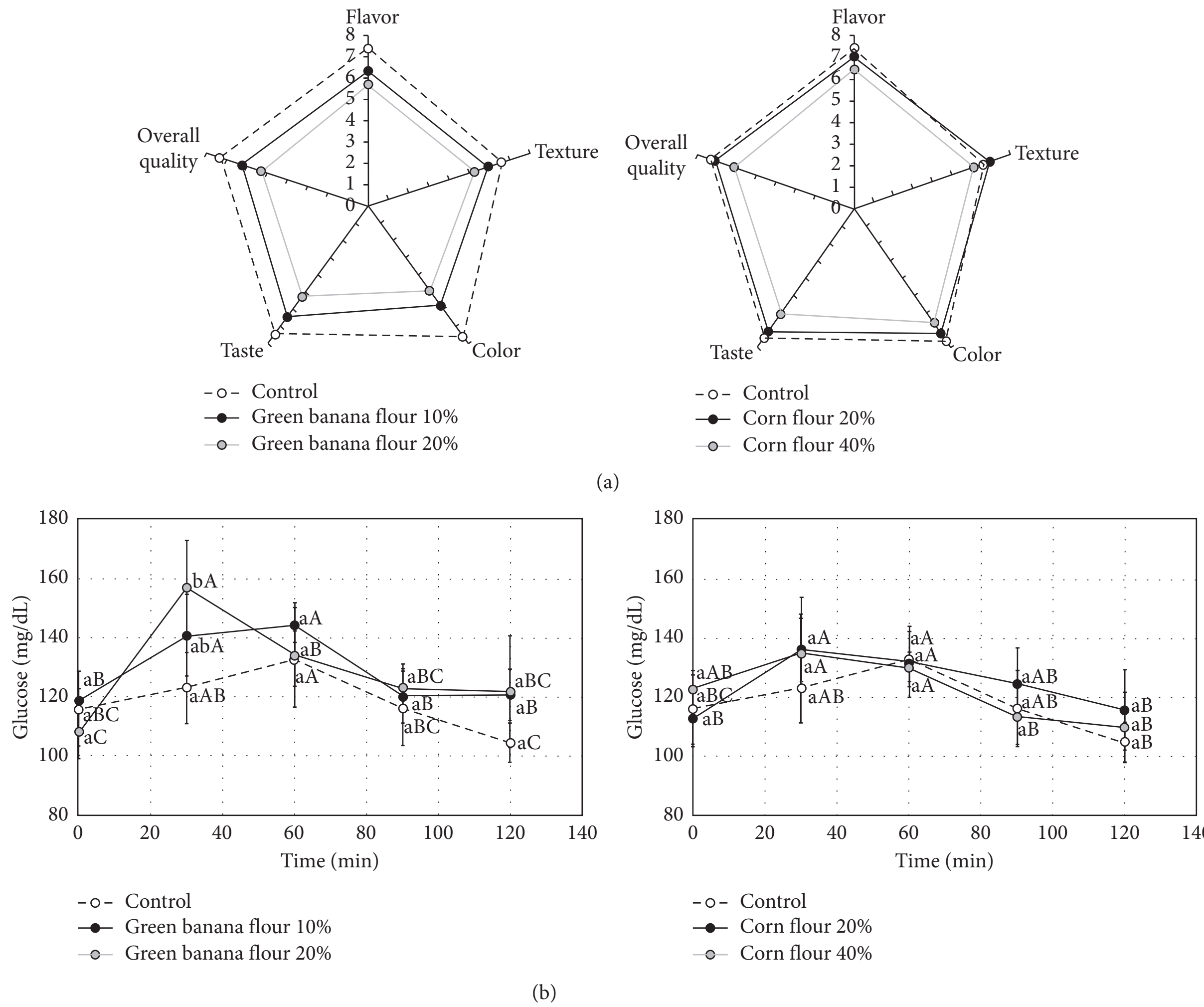

Figure 3: (a) In average sensorial analyses, the data are expressed as the mean of testers ($n = 115$) of the different bread samples. (b) Blood glucose levels after oral administration of bread samples in rats expressed as mean ± standard deviation ($n = 5$): (1) green banana (GF)-wheat flour breads and (2) corn (CF)-wheat flour breads. *Different lowercase letters indicate statistical difference between the different groups for the same digestion time. **Different capital letters indicate statistical difference between the different digestion times for the same group. ***Control group is the same for all in vivo experiments. Control = bread produced with only wheat flour + baking improver concentration of 1 g/100 g of flour; corn flour 20% = bread produced with corn flour concentration of 20 g/100 g + baking improver concentration of 1 g/100 g of flour; corn flour 40% = bread produced with corn flour concentration of 40 g/100 g + baking improver concentration of 1 g/100 g of flour; green banana flour 10% = bread produced with green banana flour concentration of 10 g/100 g + baking improver concentration of 1 g/100 g of flour; and green banana flour 20% = bread produced with green banana flour concentration of 20 g/100 g + baking improver concentration of 1 g/100 g of flour.

corn and green banana flour was compared with the control bread by sensory evaluation (Figure 3).

Samples formulated with corn flour at 20 g/100 g of flour were accepted as similar to the control bread by the consumers for flavor, color, taste, overall quality, and purchase intention (Figure 3). Păucean and Man [27] reported a similar trend, finding no significant difference between the control bread and bread formulated with CF up to 15 g/100 g of flour. For bread formulated with corn flour at 40 g/100 g of flour, the acceptance attributes generally received a lower score compared to the control bread (Figure 3). However, they were still considered acceptable, scoring 6 on a 9-point hedonic scale (the first score in the "liking" category) as the commercial or quality limit [36]. Considering this criterion, the samples of bread formulated with corn flour would be acceptable because all aspects contributing to overall satisfaction scored higher than 6 on the hedonic scale [24, 34].

For samples formulated with green banana flour at 10 g/100 g of flour, sensorial analyses also showed some similarity to the control bread for flavor and texture (Figure 3), whereas bread composed of green banana flour at 20 g/100 g of flour was less appreciated by consumers, and all attributes differed significantly from the control bread (Figure 3).

The purchase intent scores of the bread control and the bread formulated with corn flour at 20 g and 40 g/100 g of flour were 4.0, 3.9, and 3.1, respectively, indicating a high purchase intent for control bread and corn-wheat bread formulated with 20 g of corn flour/100 g of flour. Similar results were obtained for bread formulated with green banana flour.

These results highlight the commercial potential of bread formulations incorporating 20% of corn flour and 10% of green banana flour. Despite the fact that the bread produced with 40% of corn flour and 20% of green banana flour received lower scores, this product also represents a potential product for consumers.

3.3.2. Blood Glucose Levels in Rats. Several studies have reported in vitro procedures to simulate conditions in vivo to measure starch hydrolysis. However, as explained by Asp and Björck [37], the demarcation between available and resistant starch could be influenced by different factors, including incubation conditions. Therefore, the extent of disintegration of the substrate presented to the small intestine, the enzyme levels, and the effects of gastrointestinal transit are not totally imitated during in vitro measurements. There is a lack of studies concerning the blood glucose levels of processed foods made with corn or green banana flour, an important parameter to be assessed before introduction of such products into the diet.

The blood glucose levels after oral administration of bread samples in rats are shown in Figure 3. Statistical analyses showed a significant effect of time for all treatments, as well as for the different treatments and groups. For the control group, the blood glucose level increased after the administration of bread, peaking at 60 min after administration, and gradually decreasing thereafter. For the bread with 10% of green banana flour, the blood glucose level increased significantly at 30 min, peaked at 60 min, and decreasing thereafter. However, the administration of bread with 20% of green banana flour increased the blood glucose levels at 30 min, maintaining this increased level over the period of 60 min. Clearly, green banana-wheat bread quickly increased the blood glucose levels, maintaining the high levels for a longer time compared to control breads made with only wheat flour, in a dose-dependent manner.

For the corn flour, bread with 20% of corn flour increased the blood glucose levels at 30 min and at 60 min, decreasing afterwards. However, no significant differences were noted for bread with 40% of corn flour. Taken together, these results indicate that the breads partially produced with corn and green banana flour had a low capacity to reduce blood glucose levels, rather they seem to accelerate the rate of degradation compared to the control product (bread made only with wheat flour).

Banana starch, raw, i.e. uncooked, has been related to resistant to enzyme-catalyzed hydrolysis. According to Brown et al. [10], the cooking process could attenuate the ability of high-amylose meals to reduce insulin concentrations in rats. The increase of blood glucose levels of rats could be expected, since resistant starch from these matrices, bread or meals as proposed by Brown et al., due to the high temperature of the oven (100°C) (or cooking process) and the high water content of breads (44.9–51.8%, Tables 1 and 2), even after the baking (water content of crumb 43.2–45.7%, Tables 1 and 2), lose their crystallinity and become therefore amorphous. The amorphous structure of gelatinized starch enables greater availability for the α-amylase enzyme activity, which in turn makes this region more susceptible to enzymatic hydrolysis [38]. Moreover, according to the hydrothermal conditions of the baking process, bread and its different regions (crumb and crust, for example) may contain fully gelatinized, partially gelatinized, or ungelatinized starches [39]. Since gelatinization occurs during bread baking, it may be suggested that strategies that decrease gelatinization of the granules during baking may result in breads with a lower degree of gelatinization, and thus lower digestibility.

Table 5: Resistant starch content of breads with partial wheat flour replacement.

Bread formulation	Resistant starch (g/100 g of DM)
Control	1.94 ± 0.46
Corn flour 20%	1.34 ± 0.50
Corn flour 40%	2.57 ± 1.29
Green banana flour 10%	3.10 ± 0.92
Green banana flour 20%	5.66 ± 1.60

Control = bread produced with only wheat flour + baking improver concentration of 1 g/100 g of flour; corn flour 20% = bread produced with corn flour concentration of 20 g/100 g + baking improver concentration of 1 g/100 g of flour; corn flour 40% = bread produced with corn flour concentration of 40 g/100 g + baking improver concentration of 1 g/100 g of flour; green banana flour 10% = bread produced with green banana flour concentration of 10 g/100 g + baking improver concentration of 1 g/100 g of flour; and green banana flour 20% = bread produced with green banana flour concentration of 20 g/100 g + baking improver concentration of 1 g/100 g of flour.

However, the present study was the first to propose this behavior for resistant starch from green banana and corn flour after their use in bakery products [8, 40]. This difference could be attributed to the methodology of analyze, in vivo and in vitro analyses were used in the present study and in the cited literature, respectively; factors that affect starch digestion in humans and animals are very difficult to be reproduced in vitro [41]. Furthermore, the interactions may exist between starch and other matrices nutrients.

Therefore, for food industry and health consumers, the digestion performance of baked banana starch would be an important strategy to be verified; once, human starch consumption was much more important in these products than that of raw starch. To the best of our knowledge, there are no recent studies focusing on digestion properties of baked banana flour, although older citations imply that it may have a comparatively long digestion characteristic. Therefore, it could be proposed that the baking process may have led to increased amylose and amylopectin digestibility of starch in breads. This is in accordance to Englyst and Cummings [9] who proposed that the effect of slowly digestible starch could be also influenced by baking conditions.

3.3.3. Resistant Starch. The substitution of wheat flour for corn or green banana flour has changed the nondigestible starch fraction in breads, depending on the level incorporated. Resistant starch content in bread, measured by in vitro analyses after cooling, drying, and mincing the samples, was in a range of 1.9–5.7 g/100 g (Table 5). Breads formulated with corn flour at 40% had slight improvement on resistant starch compared to control sample, whereas at 20% no increase was observed. Otherwise, breads formulated with green banana flour exhibited marked and progressive improvement in resistant starch from 20% to 40%, which is in agreement with the findings reported by Aziah et al. [42]. The high content of resistant starch of green banana flour (Table 4), around 11 and 2.5 times higher than in wheat and corn flour, respectively, was the main responsible for the increment of resistant starch in green banana-wheat breads. Theoretical content calculated in breads, taken into account only flours as a source of resistant starch, were 1.3 (control), 2.0 (corn flour at 20%), 2.8 (corn flour at 40%), 2.5 (green banana flour at 10%), and 3.8% (green banana flour at 20%).

When starch granules are gelatinized, they become easily digestible, and it is widely known. However, when the food matrix temperature cools down and starts aging, the starch once more return in part to a crystalline configuration [43]. Therefore, it could be proposed that the level of resistant starch determined in this study by in vitro analyses, probably measured and sometimes “overestimated” the resistant starch due the aging of the bread samples, which allows amorphous structure of gelatinized starch to become again crystalline. Furthermore, foods matrices with a specific structure have the degree and amount of starch hydrolysis right dependent on the mode of sample division, i.e., a critical difference between in vivo and in vitro analyses [43]. Englyst et al. [43] evaluated the effect of mince and chewing on the extent of starch to resist to hydrolysis for different foods and observed that mincing gave higher results of resistance to starch hydrolysis for all evaluated foods. For example, using the in vivo method, 11%-12% of starch in polished rice was measured as resistant starch, whereas only 1%–3% starch from polished rice fed to ileostomy subjects was recovered in the effluent.

4. Conclusion

In recent years, the food industry has been engaged in developing new technologies for new products or processes, which diversify raw material choices, especially to enhance nutritional characteristics, at same time retaining the quality and competitiveness of the product. In this way, this study showed that it is technologically possible to produce bread with wheat flour replaced with corn (20%) or green banana (10%) flour acceptable to consumers. Incorporation of high levels of corn or green banana flours had a negative impact on gluten network formation, gas retention, and final bread quality. However, consumption of these breads gave rise to different blood glucose levels in rats than those previously reported, suggesting that the baking process may have led to increased amylose and amylopectin digestibility of starch in breads. Furthermore, the literature previously underlined that the development of the porous structure of bread is strictly connected to process. In this way, this study also highlighted this lack and need for future investigation for wheat flour substitution baked goods, in order to better understand mechanical properties formation and also product digestibility.

Data Availability

The data used to support this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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References

[1] W. Gisslen, *Professional Baking*, John Wiley & Sons, Hoboken, NJ, USA, 2012.

[2] USDA, *Grain : World Markets and Trade Foreing Agricultural Service 2017*, USDA, Washington, DC, USA, https://apps.fas.usda.gov/psdonline/circulars/grain.pdf.

[3] H. C. J. Godfray, J. R. Beddington, I. R. Crute et al., “The challenge of food security,” *Science*, vol. 327, 2012.

[4] K. K. Adom and R. H. Liu, “Antioxidant activity of grains,” *Journal of Agricultural and Food Chemistry*, vol. 50, no. 21, pp. 6182–6187, 2002.

[5] FAOSTAT, *FAOSTAT (Food and Agriculture Organization of United Nations-Statistics Division)*, 2017, http://www.fao.org/faostat/en/#data/QC/visualize.

[6] T. B. Tribess, J. P. Hernández-Uribe, M. G. C. Méndez-Montealvo, E. W. Menezes, L. A. Bello-Perez, and C. C. Tadini, “Thermal properties and resistant starch content of green banana flour (Musa cavendishii) produced at different drying conditions,” *LWT-Food Science and Technology*, vol. 42, no. 5, pp. 1022–1025, 2009.

[7] P. Zhang, R. L. Whistler, J. N. Bemiller, and B. R. Hamaker, “Banana starch: production, physicochemical properties, and digestibility—a review,” *Carbohydrate Polymers*, vol. 59, no. 4, pp. 443–458, 2005.

[8] E. Juarez-Garcia, E. Agama-Acevedo, S. G. Sáyago-ayerdi, S. L. Rodríguez-ambriz, and L. A. Bello-pérez, “Composition, digestibility and application in breadmaking of banana flour,” *Plant Foods for Human Nutrition*, vol. 61, no. 3, pp. 131–137, 2006.

[9] H. N. Englyst and J. H. Cummings, “Digestion of the carbohydrates of banana (Musa paradisiaca sapientum) in the human small intestine,” *The American Journal of Clinical Nutrition*, vol. 44, no. 1, pp. 42–50, 1986.

[10] M. A. Brown, L. H. Storlien, I. L. Brown, and J. A. Higgins, “Cooking attenuates the ability of high-amylose meals to reduce plasma insulin concentrations in rats,” *British Journal of Nutrition*, vol. 90, no. 4, pp. 823–827, 2003.

[11] A. C. Dona, G. Pages, R. G. Gilbert, and P. W. Kuchel, “Digestion of starch: in vivo and in vitro kinetic models used to characterise oligosaccharide or glucose release,” *Carbohydrate Polymers*, vol. 80, no. 3, pp. 599–617, 2010.

[12] K. Kawai, K. Matsusaki, K. Hando, and Y. Hagura, "Temperature-dependent quality characteristics of pre-dehydrated cookies: structure, browning, texture, in vitro starch digestibility, and the effect on blood glucose levels in mice," *Food Chemistry*, vol. 141, no. 1, pp. 223–228, 2013.

[13] AACC International, "Approved methods of analysis," in *Method 32-4001 Resistant Starch in Starch Samples and Plant Materials*, AACC International, Saint Paul, MN, USA, 11th edition, 2002.

[14] M. A. Gularte and C. M. Rosell, "Physicochemical properties and enzymatic hydrolysis of different starches in the presence of hydrocolloids," *Carbohydrate Polymers*, vol. 85, no. 1, pp. 237–244, 2011.

[15] AACC Intternational, *Official Methods of Analysis of AOAC International, Gaithersburg: Cereal Foods*, 2020, http://143.107.154.66:3410/sfxlcl41/?sid=tandf&iuid=75464&aulast=&date=1995&title=Official methods of analysis methods.

[16] B. Bakan, A. C. Bily, D. Melcion et al., "Possible role of plant phenolics in the production of trichothecenes by Fusarium graminearum strains on different fractions of maize kernels," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 9, pp. 2826–2831, 2003.

[17] F. M. Vanin, C. Michon, and T. Lucas, "Effect of the drying rate on the complex viscosity of wheat flour dough transforming into crust and crumb during baking," *Journal of Cereal Science*, vol. 58, no. 2, pp. 290–297, 2013.

[18] A. Gåmbaro, P. Varela, A. Giménez, A. Aldrovandi, S. M. Fiszman, and G. Hough, "Textural quality of white pan bread by sensory and instrumental measurements," *Journal of Texture Studies*, vol. 33, no. 5, pp. 401–413, 2002.

[19] D. Sabanis and C. Tzia, "Effect of rice, corn and soy flour addition on characteristics of bread produced from different wheat cultivars," *Food and Bioprocess Technology*, vol. 2, no. 1, pp. 68–79, 2009.

[20] R. V. da Mota, F. M. Lajolo, B. R. Cordenunsi, and C. Ciacco, "Composition and functional properties of banana flour from different varieties," *Starch-Stärke*, vol. 52, no. 2-3, pp. 63–68, 2000.

[21] C. Sarawong, R. Schoenlechner, K. Sekiguchi, E. Berghofer, and P. K. W. Ng, "Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour," *Food Chemistry*, vol. 143, pp. 33–39, 2014.

[22] L. G. Carr and C. C. Tadini, "Influence of yeast and vegetable shortening on physical and textural parameters of frozen part baked French bread," *LWT-Food Science and Technology*, vol. 36, no. 6, pp. 609–614, 2003.

[23] A. Mohamed, J. Xu, and M. Singh, "Yeast leavened banana-bread: formulation, processing, colour and texture analysis," *Food Chemistry*, vol. 118, no. 3, pp. 620–626, 2010.

[24] M. Siddiq, M. Nasir, R. Ravi, M. S. Butt, K. D. Dolan, and J. B. Harte, "Effect of defatted maize germ flour addition on the physical and sensory quality of wheat bread," *LWT-Food Science and Technology*, vol. 42, no. 2, pp. 464–470, 2009.

[25] S. E. Fleming and F. W. Sosulski, "Microscopic evaluation of bread fortified with concentrated plant proteins," *Cereal Chemistry*, vol. 55, pp. 373–382, 1978.

[26] F. R. Jacobsberg, S. L. Worman, and N. W. R. Daniels, "Lipid binding in wheat-flour doughs: the effect of datem emulsifier," *Journal of the Science of Food and Agriculture*, vol. 27, no. 11, pp. 1064–1070, 1976.

[27] A. Păucean and S. Man, "Influence of defatted maize germ flour addition in wheat: maize bread formulations," *Journal of Agroalimentary Processes and Technologies*, vol. 19, no. 3, pp. 298–304, 2013.

[28] Y. Chen, L. Zhao, T. He, Z. Ou, Z. Hu, and K. Wang, "Effects of mango peel powder on starch digestion and quality characteristics of bread," *International Journal of Biological Macromolecules*, vol. 140, pp. 647–652, 2019.

[29] A. Le-Bail, K. Boumali, V. Jury, F. Ben-Aissa, and R. Zuniga, "Impact of the baking kinetics on staling rate and mechanical properties of bread crumb and degassed bread crumb," *Journal of Cereal Science*, vol. 50, no. 2, pp. 235–240, 2009.

[30] J. Gao, S. L. Tay, A. H. S. Koh, and W. Zhou, "Dough and bread made from high- and low-protein flours by vacuum mixing: part 2. Yeast activity, dough proofing and bread quality," *Journal of Cereal Science*, vol. 77, pp. 275–283, 2017.

[31] M. G. Scanlon, H. D. Sapirstein, and D. Fahloul, "Mechanical properties of bread crumb prepared from flours of different dough strength," *Journal of Cereal Science*, vol. 32, no. 3, pp. 235–243, 2000.

[32] C. J. A. M. Keetels, T. van Vliet, and P. Walstra, "Relationship between the sponge structure of starch bread and its mechanical properties," *Journal of Cereal Science*, vol. 24, no. 1, pp. 27–31, 1996.

[33] M. G. Scanlon and M. C. Zghal, "Bread properties and crumb structure," *Food Research International*, vol. 34, no. 10, pp. 841–864, 2001.

[34] M. Alpaslan and M. Hayta, "The effects of flaxseed, soy and corn flours on the textural and sensory properties of a bakery product," *Journal of Food Quality*, vol. 29, no. 6, pp. 617–627, 2006.

[35] L. R. G. d. F. Feitosa, J. F. Maciel, T. A. Barreto, and R. T. Moreira, "Avaliação de qualidade do pão tipo francês por métodos instrumentais e sensoriais," *Semina: Ciências Agrárias*, vol. 34, no. 2, pp. 693–704, 2013.

[36] A. M. Muñoz, "Sensory evaluation in quality control: an overview, new developments and future opportunities," *Food Quality and Preference*, vol. 13, no. 6, pp. 329–339, 2002.

[37] N.-G. Asp and I. Björck, "Resistant starch," *Trends in Food Science & Technology*, vol. 3, pp. 111–114, 1992.

[38] S. Dhital, F. J. Warren, P. J. Butterworth et al., "Mechanisms of starch digestion by α-amylase-structural basis for kinetic properties," *Critical Reviews in Food Science and Nutrition*, vol. 57, no. 5, pp. 875–892, 2017.

[39] P. Bredariol, M. Spatti, and F. M. Vanin, "Different baking conditions may produce breads with similar physical qualities but unique starch gelatinization behaviour," *LWT*, vol. 111, pp. 737–743, 2019.

[40] E. Agama-Acevedo, J. J. Islas-Hernández, G. Pacheco-Vargas, P. Osorio-Díaz, and L. A. Bello-Pérez, "Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour," *LWT-Food Science and Technology*, vol. 46, no. 1, pp. 177–182, 2012.

[41] N. Faisant, A. Buléon, P. Colonna et al., "Digestion of raw banana starch in the small intestine of healthy humans: structural features of resistant starch," *British Journal of Nutrition*, vol. 73, no. 1, pp. 111–123, 1995.

[42] A. A. N. Aziah, L. H. Ho, A. A. Noor Shazliana, and R. Bhat, "Quality evaluation of steamed wheat bread substituted with green banana flour," *International Food Research Journal*, vol. 19, no. 3, pp. 869–876, 2012.

[43] H. N. Englyst, S. M. Kingman, and J. H. Cummings, "Classification and measurement of nutritionally important starch fractions," *European Journal of Clinical Nutrition*, vol. 43, no. 2, pp. 33–50, 1992.