Research Article

Evaluation of Quality Parameters in Gluten-Free Bread Formulated with Breadfruit (*Artocarpus altilis*) Flour

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Flour from the fruit of breadfruit trees (*Artocarpus altilis*) holds the potential to serve as a wheat flour replacement in gluten-free product formulations. This study evaluated the impact of breadfruit flour and leavening agent on gluten-free bread quality. Breadfruit flour was first milled and characterized by the researchers prior to being used in this study. Experimental formulas were mixed with varying breadfruit flour inclusion (0%, 20%, 35%, and 50%) and leavening agent (yeast and baking powder). Quality parameters including density, specific volume, pH, water activity, color, and texture were assessed, and proximate analysis was performed to characterize the nutritional value of the bread. Significant differences ($p < 0.05$) were found in loaf density, specific volume, color (crust L^* and b^*; crumb L^*, a^*, and b^*), pH, water activity, and crumb firmness. Additionally, a consumer sensory study was performed on the most well-liked formulations. Consumer testing yielded significant differences ($p < 0.05$) between the yeast-leavened control (0% breadfruit flour) and yeast-leavened breadfruit bread (20% breadfruit flour). Nonceliac consumers rated the breadfruit treatment as significantly less acceptable than the control for all sensory characteristics assessed. These results indicate that breadfruit flour can be used at ≤20%, when leavened with yeast, to produce quality gluten-free bread. Future studies should be conducted to assess the impact of breadfruit variety and milling practices on breadfruit flour properties before further attempts are made to investigate how breadfruit flour impacts the gluten-free bread quality.

1. Introduction

Celiac disease is an autoimmune disorder that affects genetically susceptible individuals. It is caused by the ingestion of wheat gluten, as well as proteins in related cereals, such as barley, rye, and possibly oats. Portions of these proteins elicit an autoimmune response that causes inflammation of the upper small intestine, which can result in a variety of undesirable symptoms [1]. Studies in both the United States and Europe show that celiac disease affects about 1% of the population [2]. The only effective and available treatment is lifelong avoidance of gluten-containing foods by strict adherence to a gluten-free diet.

A GFD is a diet devoid of gluten-containing grains like barley, rye, and wheat. Alternative gluten-free grains one may consume on a GFD include but are not limited to rice, oats, quinoa, buckwheat, corn, and millet. Until recent years, very few value-added, processed, or packaged gluten-free foods existed at the retail level. Removing gluten from baked goods reduces a products' elasticity, extensibility, and water binding capacity [3]. When compared to gluten-containing products, gluten-free foods are perceived as having lower structural quality and lower palatability since the presence of gluten determines the overall appearance and textural properties of cereal-based products [3, 4].

Breadfruit (*Artocarpus altilis*) is widely available in tropical and subtropical regions across the globe, with the genus *Artocarpus* (Moraceae) being comprised of approximately 50 species [5]. As breadfruit does not contain the gluten proteins harmful to celiac patients, it may be a potential ingredient for use in gluten-free products. Additionally, many regions that grow breadfruit are dependent upon imports to support their food supply. Utilizing breadfruit flour to replace commonly imported flours such as wheat and rice could help creating increased food security in these regions.

It is widely accepted that gluten proteins are responsible for the gas-holding matrix that sets the structure in wheat bread [6]. Without these structure-forming proteins, it is a challenge to produce high-quality gluten-free bread. While there are a handful of commercially available gluten-free breads, these products have an undesirable firm texture, large crumb structure, bland taste, and poor shelf life [7]. Studies dating as far back as the 1920s document the effects of wheat flour composition and particle size on end-product quality [8]. However, such studies have not been carried out for the purposes of improving the quality of breadfruit products and specifically, gluten-free breadfruit bread. At present, it has been observed that breadfruit flour is not commercially available, except in local farmer's markets within the regions the breadfruit is grown, and there are no particular quality specifications regarding particle size, starch damage, or fiber content of the flour.

The aim of this study was to assess the functionality of breadfruit flour and investigate its impact on the quality and acceptability of gluten-free bread. This research will provide the breadfruit and gluten-free industries with a functionality profile of breadfruit flour, which can increase its presence in the market. Based on the well-documented effects of wheat flour and leavening properties on bread, it was hypothesized that the type and amount of the leavening agent and the inclusion level of breadfruit flour would affect the quality of gluten-free bread. Therefore, the overreaching goal of this research was to generate information on the quality parameters of breadfruit flour so that product developers can more effectively incorporate it into gluten-free baking formulations. Providing a value-added application for breadfruit flour can lead to increased demand, market price, and ultimately, greater dietary options for consumers who have celiac disease, gluten allergy, or gluten sensitivity.

2. Materials and Methods

2.1. Flour Preparation and Analysis. Since breadfruit flour is a relatively new ingredient, it was deemed necessary to first characterize the flour and perform preliminary testing to assess its functionality. Breadfruit of the Ma'afala variety (*Artocarpus altilis)* were grown and harvested by local farmers in Hawaii, shredded into thin (roughly 1 cm long) pieces, dried in a commercially available food dehydrator, and then packaged into 5 lb sealed plastic bags that were shipped to researchers at Kansas State University. The breadfruit pieces were milled into flour using a Buhler Laboratory Mill (Model: MLU-202, Uzwil, Switzerland) according to the Buhler Method for hard wheat (AACC method 26–22) with the appropriate roll gap settings (break rolls: left = 0.0047 in (0.10 mm), right = 0.0039 in (0.08 mm); reduction rolls: left = left = 0.0028 in (0.07 mm), right = 0.0012 in (0.03 mm)). The resulting flour was sieved through multiple screens, and the finest fraction was collected for use as experimental breadfruit flour.

The milled breadfruit flour was analyzed for particle size and starch damage (Table 1). Particle size was determined using a Beckman Coulter LS™ 13 320 Laser Diffraction Particle Size Analyzer (Beckman Coulter, Inc., Miami, FL), and starch damage was determined using the Megazyme Starch Damage Assay Procedure (AACC method 76.31; K-SDAM, Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Additionally, proximate analysis for moisture (AOAC 930.15), protein (AOAC 990.03), fat (AOAC 920.39), and fiber (AOAC 962.09) was performed on the flour to characterize its nutritional profile (Table 1).

2.2. Formulation. Preliminary work was also performed to identify a gluten-free bread formula that would serve as the control and basis for all experimental formulas. A variety of gluten-free formulas from different sources were prepared, and the resulting bread was evaluated by a group of students at Kansas State University. The formula that gave the most desirable results, based on loaf volume, color, and sensory attributes, was selected and used for the purpose of this research. In this study, two variables were evaluated: breadfruit flour inclusion (0 (control), 20, 35, and 50%) and leavening agent (yeast and baking powder). All ingredient percentages were calculated as a baker's percent, meaning that their percentage is based off of the total weight of the flour blend. A total of eight ($n = 8$) formulas (Table 2) were prepared in duplicate in three replications.

All formulations were prepared using breadfruit flour (prepared as previously described); rice flour, tapioca starch, potato starch, and xanthan gum (Bob's Red Mill, Milwaukie, OR); cornstarch, sea salt, nonfat dry milk, butter, Grade A large eggs, cider vinegar, and honey (Great Value, Wal-Mart Stores Inc., Bentonville, AR); powdered whole egg (Primavera Foods, Cameron, WI); active dry yeast (Red Star Yeast, Milwaukee, WI) or double-acting baking powder (Clabber Girl Corporation, Terre Haute, IN); water; and a sweetness masking agent (Gold Coast Ingredients Inc., Commerce, California). The flour-weight basis of the baker's formula was derived from the flour blend, which consisted of the combined weight of the rice/breadfruit flour, tapioca starch, cornstarch, and potato starch. The addition of water to the formulation was modified for each flour treatment in order to standardize the consistency of each batter. For wheat bread, it is widely accepted that optimum water absorption may be determined with a Brabender farinograph or a mixograph. However, there are no such standard methods for water absorption optimization for gluten-free breads that do not form a dough. As a result, water optimization for this particular experiment was conducted by assessing how the bread performed during preliminary experimentation. It was discovered that a ratio of 1:2.5 percent breadfruit flour to water was adequate for adjusting the water content in experimental formulas.

2.3. Experimental Design. This experiment utilized a randomized complete block design. The blocks were the 6×2 slotted miniloaf pans. One pan containing the 8 treatments randomly assigned to slots was baked on the top rack of the

Table 1: Mean values for quality-impacting characteristics of milled experimental breadfruit flour.

	Particle size (μm)	Starch damage (%)	Fat (%)	Protein (%)	Fiber (%)	Moisture (%)
Breadfruit flour	58.211 ± 53.84	4.12 ± 0.10	0.45 ± 0.08	3.24 ± 0.01	31.93 ± 6.12	7.35 ± 0.07

Table 2: Formulations for experimental gluten-free bread made with breadfruit flour.

	Yeast leavened				Baking powder leavened			
	Breadfruit 0% (YC)	Breadfruit 20% (Y20)	Breadfruit 35% (Y35)	Breadfruit 50% (Y50)	Breadfruit 0% (BPC)	Breadfruit 20% (BP20)	Breadfruit 35% (BP35)	Breadfruit 50% (Y50)
Rice flour*	30.00	—	—	—	30.00	—	—	—
Breadfruit flour*	—	20.00	35.00	50.00	—	20.00	35.00	50.00
Tapioca starch*	32.68	37.68	30.18	22.68	32.68	37.68	30.18	22.68
Cornstarch*	35.74	40.74	33.24	25.74	35.74	40.74	33.24	25.74
Potato starch*	1.58	1.58	1.58	1.58	1.58	1.58	1.58	1.58
Xanthan gum	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Powdered whole egg	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Salt	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83
Masking agent	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Nonfat dry milk	4.87	4.87	4.87	4.87	4.87	4.87	4.87	4.87
Unsalted butter	8.79	8.79	8.79	8.79	8.79	8.79	8.79	8.79
Whole eggs	29.23	29.23	29.23	29.23	29.23	29.23	29.23	29.23
Cider vinegar	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33
Honey	17.23	17.23	17.23	17.23	17.23	17.23	17.23	17.23
Water	72.00	68.00	74.00	80.00	72.00	68.00	74.00	80.00
Yeast	0.70	0.70	0.70	0.70	—	—	—	—
Baking powder	—	—	—	—	20.00	20.00	20.00	20.00
Total (g)	240.02	236.02	242.02	248.02	259.32	255.32	261.32	267.32

*Components of flour blend.

oven, and a second pan containing the same 8 treatments randomly assigned (different from the pan on the top rack) was backed on the bottom rack; any slots not containing batter will be filled with water to promote even baking. This allowed for two subsamples of a treatment to be produced per replication with three replications performed in total.

2.4. Sample Preparation. Prior to preparing the experimental formulas, the dried yeast was reactivated and allowed to perhydrate for 5 minutes in the standardized amount of water (37.8°C) specific to each formula. Once the yeast had reactivated, the flour blend (rice or breadfruit flour, tapioca starch, cornstarch, and potato starch), xanthan gum, dried egg powder, salt, nonfat dry milk, and masking agent were placed into the bowl of a kitchen aid mixer, equipped with a flat beater attachment (Ultra Power, 300 W, Model KSM395; St Joseph, MI), and mixed together for 30 seconds at the lowest speed (i.e., "stir") to ensure any clumps were broken up. Next, the whole eggs, butter, cider vinegar, and honey as well as the leavening agent (baking powder or reactivated yeast-water mixture) were added to the mixing bowl; if the formula contained baking powder, then the standardized amount of water specific to that formula was also added. The batter was then mixed for 2 minutes at medium speed (i.e., speed 5″). After mixing, 120 g aliquots of each batter were weighed, placed (as previously described later in Experimental Design) into the individual slots of a greased 6 × 2 miniloaf baking pan, and proofed at 42°C with 85% relative humidity in a proofing cabinet (National Manufacturing Co., Lincoln, NE). Each batter was proofed to height, corresponding to 1 cm above the edge of the pan. Approximate proof time was about 60 minutes. After proofing, the batters were baked for 35 minutes in an electrically powered reel-type test baking oven (National Manufacturing Co., Lincoln, NE) preheated to 218.3°C (425°F) with convection. After baking, the loaves were removed from the pan and cooled for 1.15 hours on a wire rack at ambient temperature. All analyses were performed on the loaves immediately following the 1.15-hour cooling time.

2.5. Bread Analysis. After cooling, loaves were weighed and loaf volume was measured by rapeseed displacement (AACC method 10–05). Loaf specific volume (loaf volume (mL)/loaf weight (g)) was calculated. A HunterLab MiniScan (Model MiniScan EZ 4500 L, Hunter Associates Laboratory Inc., Reston, VA) was used to measure the color of the crust and crumb of each treatment sample after cooling. The device was calibrated with a light trap and white tile provided by Hunter Associates Laboratory Inc. Readings were taken from three spots on the loaf (end, middle, and opposite end). The type of illuminant used was C, average daylight, with a 10° standard observer. "L^*," "a^*," and "b^*" values were given as output. "L^*" is the measurement for lightness (0 = black and 100 = white). Red and green colors are indicated by the "a^*" value ($+a$ = red and −a = green). The "b^*" value indicates yellow ($+b$) and blue (−b) colors. Once the specific volume of each treatment loaf was determined, the loaf was sliced transversely using an in-house manufactured slice regulator and bread knife to obtain four slices of 25 mm thickness. The third bread slices from each experimental loaf were assessed for crumb grain characteristics using a C-Cell instrument (Calibre Control International Ltd., Appleton, Warrington, United Kingdom). Texture profile analysis (TPA) (Bourne 1978) of the crumb was performed on the second slice from each experimental loaf using a texture analyzer (TA-XT2, Stable Micro Systems, Godalming, United Kingdom) equipped with a 38 mm Perspex cylinder probe along with a 30 kg load cell. The TPA was carried out with a constant speed of 2 mm/s (applying to the pretest speed, test speed, and posttest speed) for a distance of 10 mm, corresponding to 40% compression of the 25 mm slices. There was a 5 second wait time between the first and second compression cycles; the trigger force was 20 g. After cooling, bread pH was analyzed using 15 g of crust-free crumb of applicable baked product separated into small pieces) in a dry Erlenmeyer flask and add 100 mL cooled, distilled water. The flask was agitated until the bread was suspended and free of lumps. The suspension was maintained for 30 min using a magnetic stirrer. The suspension was left to stand and settle for 10 min; then, the supernatant liquid was decanted into the electrode vessel, and pH was immediately determined, by placing a potentiometer and electrodes, that have been calibrated against known buffer solutions, into the supernatant liquid (AACC method 02-52.01). Water activity (a_w) was determined for each loaf by placing the loaf into a plastic sample dish then inserting it into a calibrated AquaLab Series 3 water activity meter (Decagon Devices Inc., Pullman, WA). Both a_w and sample temperature are displayed on the instrument's screen and were recorded [9]. Proximate analysis for moisture (AOAC 930.15), protein (AOAC 990.03), fat (AOAC 920.39), and fiber (AOAC 962.09) was performed on the finished loaves to characterize their nutritional profile.

2.6. Consumer Sensory Study. Since the researchers did not have the sufficient resources (i.e., breadfruit flour) to prepare samples of all 8 treatments for a large consumer panel, a small informal preliminary panel was used to identify two (n = 2) experimental gluten-free bread formulas that would undergo a larger consumer panel. Five (n = 5) panelists were randomly selected to evaluate all 8 treatments for overall acceptability, and the two most preferred treatments (YC and Y20) were selected for a final 100 consumer sensory study.

The two most preferred formulas (YC and Y20) were then assessed in a consumer study that was carried out in the Kansas Value-Added Foods Lab at Kansas State University. A total of 108 untrained panelists volunteered to participate in this study, including 5 suffering from celiac disease and/or a gluten allergy or sensitivity. Prior to participating in the study, each panelist signed an informed consent statement that informed them of the purpose and guidelines of the study (see Appendix A in Supplementary Materials). Panelists were also required to complete a numbered prescreening form containing information about their age, gender, highest education completed, if they suffer from any food allergies, the frequency they purchase bread products, frequency they purchase gluten-free products, and frequency they purchase gluten-free bread products (see Appendix B in Supplementary Materials). Any participant who indicated they had a food allergy, intolerance, or sensitivity to anything other than gluten was not allowed to participate in the study. Degree of liking of organoleptic sensory properties including appearance, color, flavor, texture in mouth, aftertaste, and overall acceptance was assessed. YC and Y20 loaves were prepared as previously described in "sample preparation". Each participant was served one interior slice from each of the four treatment loaves, on a 3-digit coded plate, one treatment at a time, in a random order. At the time the treatment samples were distributed, numbered ballots bearing identical 3-digit codes, matching those on the sample plates, were given to the panelists. Panelists were instructed to evaluate each sample in the order they were provided to them (to eliminate possible bias) and complete the ballots according to the instructions listed on them. Each ballot contained a 9-point hedonic scale for the previously listed organoleptic sensory properties (appearance, color, flavor, texture in mouth, aftertaste, and overall acceptance). These 9-point hedonic scales displayed degree of liking corresponding to the specific attributes (9 being "like extremely," 5 being "neither like nor dislike," and 1 being "dislike extremely"). When panelists finished tasting and rating the samples, they had the opportunity to write additional comments to suggest improvements and make any other comments concerning the samples (see Appendix C in Supplementary Materials).

2.7. Statistical Analysis. The moisture content, ash content, protein content, fat content, fiber content, starch damage, and particle size of breadfruit shreds were performed in duplicates. Measurements of batter pH, weight, and volume as well as loaf weight, volume, color (crust and crumb), texture, pH, and water activity were repeated in triplicates. Replications of each flour/leavening treatment were baked in duplicate loaves, and 2 slice views were evaluated for crumb characteristics with a C-Cell instrument. Proximate analysis was performed once on the duplicate loaves from the first replication.

All data were analyzed using SAS, Software Release 9.4 (SAS, Institute Inc., Cary, NC, 2013). When treatment effects were found significantly different, the least square means with Tukey–Kramer groupings were used to differentiate treatment means. A level of significance was observed at $\alpha \leq 0.05$. The level of significance is indicated in parentheses. Multiple linear regression was carried out to determine significance of interaction between variables. Pearson's correlation coefficients were used to determine if positive or negative correlations existed between the different terms analyzed. Paired t-tests were performed on data from the sensory testing ballots to see if the two treatments being analyzed were significantly different from each other. A level of significance was observed at $\alpha \leq 0.05$.

3. Results

A significant effect was noted ($p < 0.05$) for the specific volume of bread produced with the leavening agents studied (Table 3). Breads leavened with yeast (YC, Y20, Y35, and Y50) had significantly higher specific volumes when compared to breads leavened with baking powder (BPC, BP20, BP35, and BP50). A significant effect was noted ($p < 0.05$) for the specific volume of breads produced with all levels of breadfruit flour studied. Control loaves (YC and BPC) were found to have significantly higher specific volumes compared to all other treatments. Loaves containing 20% (Y20 and BP20), 35% (Y35 and BP35), and 50% (Y50 and BP50) breadfruit flour were all significantly different ($p < 0.05$) from one another, with specific volume decreasing as percent breadfruit flour increased.

Breadfruit flour treatment was found to significantly impact ($p < 0.05$) both cell diameter and cell volume. Leavening treatment had no significant effect on cell volume or diameter (Table 3). Loaves YC and BPC had significantly larger cell diameter and volume than all other treatments while loaves Y50 and BP50 had significantly smaller cell diameter and volume compared to all other treatments. Similarly, breads produced from yeast had a significantly larger cell diameter compared to bread leavened with baking powder. Images depicting the differences in cell diameter and volume are found in Figures 1 and 2 (in the Supplementary Material).

No significant effect was found for leavening or breadfruit flour treatment on cells per slice area (Table 3). A significant effect was found for leavening ($p < 0.05$) and breadfruit flour level ($p < 0.001$) on cell wall thickness (Table 3). Loaves leavened with yeast had significantly thicker cell walls than loaves leavened with baking powder ($p < 0.05$). Significant differences ($p < 0.05$) in cell wall thickness were observed at all levels of breadfruit flour inclusion. Among all breadfruit flour treatments, control loaves (YC and BPC) had the thickest cell walls. Loaves with 20% inclusion (Y20 and BP20) were found to have significantly thicker cell walls than those with 35% (Y35 and BP35; $p < 0.05$) and 50% (Y50 and BP50; $p < 0.0001$) inclusion.

Both leavening and breadfruit flour treatments were found to significantly impact bread slices hardness (Table 3). Yeast-leavened breads were significantly softer ($p < 0.0001$) in crumb texture compared to baking powder-leavened breads. Breadfruit flour treatment was found to have an overall significant effect ($p < 0.0001$) on crumb texture. Breads increased in crumb hardness as the breadfruit flour inclusion increased, and control loaves (YC and BPC) proved to have the softest crumb texture while loaves containing 50% breadfruit flour (Y50 and BP50) had the hardest crumb texture.

Yeast-leavened breads were found to have significantly lower ($p < 0.0001$) pH compared to baking powder-leavened breads. There was no significant effect for breadfruit flour treatment on bread pH. Leavening treatment was found to have a significant effect ($p < 0.05$) on the water activity of bread (Table 3). Breads leavened with baking powder were found to have significantly lower water activity compared to breads leavened with yeast. There was no significant impact of breadfruit flour treatment on water activity.

For crust L^*, a significant effect ($p < 0.05$) was observed for breadfruit flour treatment but not for leavening treatment (Table 4). Breads produced with greater inclusion of breadfruit flour (Y35, BP35, Y50, and BP50) were found to have significantly ($p < 0.05$) lighter crusts (i.e., higher L^*) and darker crumbs (i.e., lower L^*). Though leavening had no significant effect on the crust L^* values, yeast-leavened loaves (YC, Y20, Y35, and Y50) were found to have a whiter crumb (i.e., higher L^*) compared to baking powder-leavened loaves (BPC, BP20, BP35, and BP50). Neither leavening nor breadfruit treatment significantly impacted the crust a^* values (Table 4). Yeast-leavened loaves were found to have significantly lower ($p < 0.05$), positive a^* values (redness) than those leavened with baking powder. Crumb a^* values were significantly different between loaves with 50% breadfruit flour (Y50 and BP50) and all other treatments; however, it should be noted that significant impact observed for 50% breadfruit flour inclusion yielded increased redness (i.e., higher, positive a^*) in baking powder-leavened breads. Yeast-leavened breads were found to have significantly lower ($p < 0.05$), positive b^* (yellowness) values for crust and crumb compared to bread leavened with baking powder(Table 4). Control loaves (YC and BPC) were found to yield bread with significantly lower crust yellowness compared to formulas with higher breadfruit flour inclusion (Y35, BP35, Y50, and BP50). Though loaves YC and BPC were also significantly different in crumb b^* than all other treatments, it should be noted that YC has a significantly less yellow crumb than Y20, Y35, and Y50 while BPC has a significantly more yellow crumb than BP20, BP35, and BP50 (Table 4).

No significant effects were observed for breadfruit flour treatment on fat content, but BP50 was found to have significantly lower fat content ($p < 0.05$). Leavening treatment caused significant differences ($p < 0.05$) for protein content, specifically with yeast-leavened breads having significantly higher protein than baking powder-leavened breads. For breadfruit flour treatments, significant differences ($p < 0.05$) were observed at all inclusion levels with control loaves (YC and BPC) yielding significantly higher protein than loaves containing breadfruit flour (Table 5).

Table 3: Mean quality parameter values of experimental gluten-free bread formulas.

Flour inclusion level	Specific volume (mL/g)		Cell diameter (mm)		Cell volume (mm^3)		Cells per slice area ($cells/cm^2$)		Cell wall thickness (mm)		Slice area (mm^2)		Number of cells (mm)		Hardness (N)		pH		Water activity	
	Yeast	Baking powder	Yeast	Baking powder	Yeast	Baking powder	Yeast	Baking powder	Yeast	Baking powder	Yeast	Baking powder	Yeast	Baking powder	Yeast	Baking powder	Yeast	Baking powder	Yeast	Baking powder
0%	3.95 ± 0.24^{Aa}	3.04 ± 0.40^{Ab}	2.76 ± 0.26^{Aa}	2.46 ± 0.13^{Aa}	8.87 ± 1.38^{Aa}	9.12 ± 0.51^{Aa}	69.81 ± 13.82^{Aa}	64.18 ± 9.06^{Aa}	0.509 ± 0.015^{Aa}	0.501 ± 0.007^{Ab}	3524 ± 506^{Aa}	3191 ± 156^{Aa}	1790 ± 299^{Aa}	1696 ± 84.5^{Aa}	1.43 ± 2.14^{Aa}	2.32 ± 3.91^{Ab}	5.60 ± 0.24^{Ab}	7.25 ± 0.29^{Aa}	0.933 ± 0.02^{Aa}	0.876 ± 0.03^{Ab}
20%	3.14 ± 0.06^{Ba}	2.74 ± 0.43^{Bb}	1.95 ± 0.17^{Ba}	1.80 ± 0.12^{Ba}	5.91 ± 0.79^{Ba}	6.28 ± 0.41^{Ba}	65.06 ± 12.70^{Aa}	72.33 ± 15.15^{Aa}	0.469 ± 0.013^{Ba}	0.453 ± 0.008^{Bb}	2916 ± 276^{Ba}	2765 ± 112^{Ba}	1806 ± 98.5^{Aa}	1878 ± 116^{Aa}	2.27 ± 6.98^{Ba}	2.87 ± 7.13^{Bb}	5.59 ± 0.15^{Ab}	7.32 ± 0.23^{Aa}	0.910 ± 0.01^{Aa}	0.885 ± 0.02^{Ab}
35%	2.60 ± 0.48^{Ca}	2.37 ± 0.29^{Cb}	1.93 ± 0.10^{Ca}	1.52 ± 0.08^{Ca}	5.94 ± 0.50^{Ca}	4.62 ± 0.34^{Ca}	70.71 ± 13.05^{Aa}	79.38 ± 14.71^{Aa}	0.471 ± 0.005^{Ca}	0.423 ± 0.007^{Cb}	2577 ± 138^{Ca}	2160 ± 92.1^{Ca}	1681 ± 104^{Aa}	1745 ± 75.9^{Aa}	2.60 ± 2.79^{Ca}	3.51 ± 10.01^{Cb}	5.69 ± 0.14^{Ab}	7.36 ± 0.19^{Aa}	0.904 ± 0.02^{Aa}	0.895 ± 0.01^{Ab}
50%	2.31 ± 0.25^{Da}	2.08 ± 0.22^{Db}	1.54 ± 0.04^{Da}	1.40 ± 0.10^{Da}	4.35 ± 0.30^{Da}	3.90 ± 0.50^{Da}	76.91 ± 9.05^{Aa}	65.77 ± 10.48^{Aa}	0.443 ± 0.005^{Da}	0.404 ± 0.008^{Db}	2230 ± 165^{Da}	1946 ± 78.2^{Da}	1755 ± 128^{Aa}	1718 ± 84.2^{Aa}	3.01 ± 6.86^{Da}	3.85 ± 11.60^{Db}	5.61 ± 0.23^{Ab}	7.28 ± 0.14^{Aa}	0.920 ± 0.01^{Aa}	0.891 ± 0.01^{Ab}

For each column, mean values with the same upper case superscript are not significantly different ($p > 0.05$). For each row, mean values with the same lower case superscript are not significantly different ($p > 0.05$).

Table 4: L^*, a^*, and b^* color values of experimental gluten-free bread formulas.

Flour inclusion level	Crust L^* Yeast	Crust L^* Baking powder	Crust a^* Yeast	Crust a^* Baking powder	Crust b^* Yeast	Crust b^* Baking powder	Crumb L^* Yeast	Crumb L^* Baking powder	Crumb a^* Yeast	Crumb a^* Baking powder	Crumb b^* Yeast	Crumb b^* Baking powder
0%	39.11 ± 6.63^{Aa}	44.15 ± 10.59^{Aa}	15.08 ± 1.56^{Aa}	14.84 ± 1.53^{Aa}	24.58 ± 6.03^{Aa}	30.51 ± 6.23^{Ab}	77.40 ± 0.97^{Aa}	58.63 ± 2.17^{Ab}	0.96 ± 0.23^{Aa}	11.23 ± 0.68^{Ab}	23.26 ± 1.32^{Aa}	34.68 ± 0.36^{Ab}
20%	43.96 ± 5.14^{ABa}	45.69 ± 6.94^{ABa}	15.67 ± 0.44^{Aa}	15.04 ± 0.61^{Aa}	30.14 ± 2.63^{ABa}	33.95 ± 3.45^{ABb}	73.01 ± 0.76^{Ba}	58.63 ± 2.06^{Bb}	2.62 ± 1.11^{Aa}	10.40 ± 0.83^{Ab}	26.86 ± 1.11^{Ba}	34.58 ± 0.78^{Bb}
35%	46.56 ± 5.90^{Ba}	51.75 ± 7.23^{Ba}	15.62 ± 0.42^{Aa}	14.09 ± 1.45^{Aa}	31.75 ± 3.34^{BCa}	35.89 ± 2.00^{BCb}	70.47 ± 0.53^{BCa}	61.97 ± 0.85^{BCb}	3.94 ± 0.29^{Aa}	9.02 ± 0.60^{Ab}	29.46 ± 1.21^{Ba}	34.10 ± 0.61^{Bb}
50%	49.14 ± 5.02^{Ca}	52.81 ± 1.78^{Ca}	15.70 ± 0.45^{Aa}	14.76 ± 1.78^{Aa}	33.56 ± 2.35^{Ca}	37.04 ± 1.58^{Cb}	69.45 ± 0.77^{Ca}	63.87 ± 0.93^{Cb}	4.29 ± 0.24^{Ba}	7.95 ± 0.68^{Bb}	29.11 ± 0.67^{Ba}	33.65 ± 0.66^{Bb}

For each column, mean values with the same upper case superscript are not significantly different ($p > 0.05$). For each row, mean values with the same lower case superscript are not significantly different ($p > 0.05$).

Table 5: Mean proximate analysis values of experimental gluten-free bread formulas.

Flour inclusion level	Fat (%) Yeast	Fat (%) Baking powder	Protein (%) Yeast	Protein (%) Baking powder	Fiber (%) Yeast	Fiber (%) Baking powder	Moisture (%) Yeast	Moisture (%) Baking powder
0%	4.30 ± 0.14^{Aa}	4.62 ± 0.10^{Aa}	4.70 ± 0.02^{Aa}	4.01 ± 0.01^{Ab}	5.62 ± 1.10^{Ab}	12.14 ± 1.29^{Aa}	36.11 ± 0.04^{Aa}	30.72 ± 0.01^{Bb}
20%	4.99 ± 0.09^{Aa}	4.35 ± 0.23^{ABa}	3.57 ± 0.01^{Da}	3.20 ± 0.04^{Cb}	3.72 ± 1.67^{Aa}	6.28 ± 0.41^{Ba}	33.95 ± 0.03^{Ca}	30.54 ± 0.11^{Bb}
35%	4.98 ± 0.06^{Aa}	4.67 ± 0.05^{Aa}	3.81 ± 0.01^{Ca}	2.52 ± 0.01^{Db}	2.96 ± 1.25^{Ab}	14.84 ± 0.38^{Aa}	33.99 ± 0.08^{Ca}	32.80 ± 0.87^{Aa}
50%	4.58 ± 0.37^{Aa}	3.99 ± 0.06^{Ba}	3.93 ± 0.00^{Ba}	3.50 ± 0.02^{Bb}	6.52 ± 3.41^{Aa}	2.90 ± 1.08^{Ba}	34.72 ± 0.01^{Ba}	34.20 ± 0.27^{Aa}

For each column, mean values with the same upper case superscript are not significantly different ($p > 0.05$). For each row, mean values with the same lower case superscript are not significantly different ($p > 0.05$).

Fiber content was significantly impacted ($p < 0.05$) by both leavening and breadfruit flour treatments (Table 5). Leavening treatment caused significant differences for fiber content with yeast-leavened breads generally having a lower fiber content than baking powder-leavened breads. Control loaves and those with 35% breadfruit flour (Y35 and BP35) were found to have significantly higher fiber than loaves with 20% (Y20 and BP20) and 50% (Y50 and BP50) inclusion. However, it should be noted that fiber content values were found to have greater and a larger range of variability compared to other proximate analysis values. In general, leavening treatment caused no significant differences in moisture content except in loaves BPC and BP20, which were observed to have significantly lower ($p < 0.05$) moisture content (Table 5). YC had significantly higher ($p < 0.05$) moisture content than Y20, Y35, and Y50. However, BP35 and BP50 were found to have significantly greater moisture content ($p < 0.05$) than BPC and BP20.

3.1. Consumer Sensory Study. Out of 108 volunteers in the consumer sensory testing, 74 were females, 32 were males, and 2 did not identify a gender. The age of the panelists ranged from 18 to 80 years, with 71.3% of the panelists belonging to the 18–25 age group. The general population ($n = 108$) can be divided into two distinct subgroups: persons suffering from celiac disease, gluten allergy, or gluten sensitivity ($n = 5$) and persons not suffering from celiac disease, gluten allergy, or gluten sensitivity ($n = 103$).

For the general population, significant differences ($p < 0.05$) were found for most sensory parameters tested (Table 6). A significant difference ($p < 0.0001$) was found in overall acceptability of YC (yeast-leavened, 0% breadfruit flour), compared to Y20 (yeast-leavened, 20% breadfruit flour), with mean YC scores being 1 point higher than Y20. Likewise, the appearance, color, flavor, and aftertaste of YC were also found to be significantly more liked ($p < 0.05$) compared to Y20. Similarly, panelists indicated they were significantly more likely to purchase YC versus Y20. Despite YC scoring significantly better than Y20 in most categories, there was no significant difference in texture likability between the two breads.

Statistically significant comparisons between the two distinct subgroups (i.e., consumers not suffering from versus suffering from Celiac disease, a gluten allergy, or gluten sensitivity) were attempted despite the small size of the

Table 6: Mean response of general panelist population to YC and Y20 formulas.

	Overall acceptability	Appearance	Color	Flavor	Texture	Aftertaste	Likelihood to purchase
Control (YC)	6.46 ± 1.67^{A}	6.72 ± 1.70^{A}	6.75 ± 1.60^{A}	6.51 ± 1.81^{A}	6.12 ± 2.06^{A}	6.01 ± 2.01^{A}	6.52 ± 3.22^{A}
Breadfruit treatment (Y20)	5.42 ± 2.16^{B}	6.27 ± 1.68^{B}	6.30 ± 1.58^{B}	5.34 ± 2.31^{B}	5.92 ± 2.11^{A}	5.16 ± 2.25^{B}	4.56 ± 3.60^{B}

Values with a common upper case letter are not significantly different ($p > 0.05$).

group who were identified as those suffering from celiac disease, gluten allergy, or gluten sensitivity ($n = 5$), keeping in mind that the findings may not be reproducible (Table 7). Overall acceptability and appearance of the control (YC) and experimental (Y20) were significantly different for nonsuffering consumers ($p < 0.05$), but no significant difference between the two breads was found for consumers suffering from celiac disease, gluten allergy, or gluten sensitivity. Mean scores for overall acceptability and appearance were higher for YC in nonsufferers, while scores were higher for Y20 in suffering consumers. Flavor was significantly different ($p < 0.0001$) between YC and Y20 for nonsufferers but not significantly different in suffering consumers; mean flavor scores for YC were higher among nonsufferers while among sufferers Y20 had a higher mean score. Texture was not found to be significantly different between YC and Y20 for neither sufferers nor nonsufferers. Mean texture scores were higher for YC among sufferers, while Y20 had higher a mean score among nonsufferers. Color was found to be significantly different ($p < 0.05$) between YC and Y20 for nonsufferers; sufferers found no significant difference in color between the two breads. Nonsufferers had a higher mean color score for YC, and sufferers had a higher mean score for Y20. Aftertaste was significantly different ($p < 0.05$) for nonsufferers but not significantly different in suffering consumers; mean aftertaste scores for YC were higher among nonsufferers while among sufferers Y20 had a higher mean score. Likelihood to purchase was found to be significantly different between YC and Y20 among nonsufferers but not significantly different among sufferers. For nonsuffering panelists, the mean likelihood to purchase score was higher for the control bread, while for sufferers, the mean score was higher for Y20.

4. Discussion

Since preliminary experiments revealed that a blend of rice flour, tapioca starch, cornstarch, and potato starch produced bread most similar to conventional gluten-containing bread, this blend was used as a control formula, and rice flour was replaced at various inclusion levels with breadfruit flour. Commercially available gluten-free goods are commonly made from starch blends where rice or corn flour is the main ingredient. Rice flour is prominently utilized due to its availability, affordability, hypoallergenicity, neutral taste and color, digestibility, and low sodium content [10–13]. Among traditional starches, such as rice, breadfruit has been a staple food and traditional crop in the Pacific for more than 3000 years and is widely cultivated in the Caribbean and other tropical regions of the world [14]. Since many of these regions are food insecure and rely upon imports to provide the majority of their food supply [14], an important aspect of this research was to see if breadfruit flour can substitute in part for commonly used starch ingredients in gluten-free bread formulations. Leavening was selected as an experimental treatment because preliminary trials showed that yeast and baking powder successfully leavened bread but could produce different sensory characteristics. This difference prompted further investigation on how each would affect the quality of gluten-free bread made from breadfruit flour.

Specific volume is affected by many factors, including dough composition, processing conditions, and dough rheology—all properties that impact gas retention capabilities. There is a nutritional benefit to the incorporation of dietary fiber into gluten-free products (as well as other baked goods), which is met with the limitation of decreased volume [15–18]. It was therefore hypothesized by Moore and others [19] that this deleterious effect on volume could be expected to be even worse in gluten-free baked products. Though no significant correlation was found between loaf fiber content and specific volume in this study, it is worth noting that the fiber content of the milled breadfruit flour (~32%) is much larger compared to that of rice flour (2.4%) [20]. Additionally, breadfruit flour inclusion was found to be negatively correlated (Pearson's = −0.74) with specific volume ($p < 0.0001$). Particle size has also been shown to impact overall product quality and loaf volume in particular. Flour particle size was kept constant in this study because previous studies by others have noted a negative relationship between increased particle size and specific volume [21, 22]. Kim and others [23] supported this relationship with the finding that the small particle size of rice flour (<95μm) yielded cupcakes with the highest specific volume. Such results may explain why loaves containing rice flour had significantly higher ($p < 0.001$) specific volume than those containing breadfruit flour. Thus, it can also be concluded that increasing breadfruit flour inclusion decreases specific volume. However, if incorporated at a lower inclusion level, product developers may still be able to leverage breadfruit flour as an ingredient without drastically decreasing loaf volume.

The L^*, a^*, and b^* values together describe the flour color, the dominant factor in determining the crumb color. In fact, Pomeranz [24] observed that the flour color was correlated with the crumb color with a coefficient of 0.987. Within each attribute of color, flour is influenced by composition and most notably freedom from bran particles [25]. Color, either in crumb or crust, is a highly influential

Table 7: Mean response of panelist subpopulations to sensory quality of YC and Y20 formulas.

	Consumers not suffering from celiac disease, gluten allergy, or gluten sensitivity		Consumers suffering from celiac disease, gluten allergy, or gluten sensitivity	
	Control (YC)	Breadfruit treatment (Y20)	Control (YC)	Breadfruit treatment (Y20)
Overall acceptability	6.50 ± 1.64^{A}	5.34 ± 2.15^{B}	5.60 ± 1.95^{b}	7.00 ± 1.73^{a}
Appearance	6.75 ± 1.72^{A}	6.25 ± 1.67^{B}	6.00 ± 1.22^{b}	6.60 ± 2.07^{a}
Color	6.77 ± 1.60^{A}	6.26 ± 1.59^{B}	6.40 ± 1.82^{b}	7.00 ± 1.41^{a}
Flavor	6.56 ± 1.79^{A}	5.27 ± 2.29^{B}	5.60 ± 2.19^{b}	6.80 ± 2.39^{a}
Texture	6.17 ± 2.07^{A}	5.86 ± 2.09^{B}	5.20 ± 1.92^{b}	7.00 ± 2.55^{a}
Aftertaste	6.06 ± 2.02^{A}	5.11 ± 2.21^{B}	5.00 ± 1.41^{b}	6.20 ± 3.03^{a}
Likelihood to purchase	6.63 ± 3.19^{A}	4.46 ± 3.59^{B}	4.20 ± 3.35^{b}	6.60 ± 3.58^{a}

For the columns under not suffering, mean values with the same upper case superscript are not significantly different ($p > 0.05$). For the columns under suffering, mean values with the same lower case superscript are not significantly different ($p > 0.05$).

characteristic that can impact consumer acceptance of baked products [26]. It should be noted that pH appeared to affect ($p < 0.001$) the crumb color of the experimental loaves as it was negatively correlated with crumb L^* values (Pearson's = −0.88) and positively correlated with a^* and b^* values (Pearson's = 0.89 and 0.87, respectively). Loaves leavened with yeast were significantly darker in color than those leavened with baking powder. Coincidentally, loaves leavened with yeast had a significantly lower pH ($p < 0.0001$) than those leavened with either level of baking powder treatment. The production of acids during the yeast's fermentation process and naturally higher pH of sodium bicarbonate (a component of baking powder) are the causes of this observed experimental pH difference. Additionally, the Maillard reaction (responsible for nonenzymatic browning) is influenced by pH. The Maillard reaction is limited by its first step: the formation of the Schiff base between the carbonyl group of a reducing sugar and the free amino group of an amino acid, peptide, or protein. At higher pH values, nitrogen is more nucleophilic which allows it to react and form Schiff bases at a quicker rate, which ultimately increases the rate of nonenzymatic browning [27]. The color formation in the loaves leavened with baking powder can likely be attributed to this phenomenon.

Quality white pan breads are characterized by small, elongated gas cells with thin cell walls. Smaller cells, whether defined by volume or diameter, are desirable in gluten-free bread products, as greater numbers of small gas cells have been found to produce loaves of higher specific volumes [28]. Larger cell diameters are typically indicative of gas cell coalescence. Ahlborn and others [29] found that gas cell coalescence diminishes the presence of a web-like structure, which, if achievable in gluten-free bread, improves both visual and eating properties of the product. As corroborated by results for specific volume and crumb firmness, the small cell diameter and volume noted for breads produced from breadfruit flour—especially when used at a higher percentage—are indications of the extreme density of the loaves. It is unlikely that products of this density would be found acceptable by consumers.

Crumb firmness (N) is a key attribute in baked goods, as it is strongly associated with consumers' perception of bread freshness [29]. In white pan bread, most consumers prefer a soft, resilient, and short crumb [25]. Sabanis and others [26] observed that the fiber addition level significantly impacted crumb firmness of gluten-free bread at the $p < 0.0001$ level. Researchers have also cited an explanation for increased firmness based upon the possible thickening of the cell wall due to fiber content [30]. In the present study, no significant relationship was found between fiber content and crumb firmness or fiber content and cell wall thickness. However, a cell wall thickness was found to have a significant ($p < 0.0001$) negative correlation with crumb firmness (Pearson's = −0.83). As breads produced from yeast and lower inclusion levels of breadfruit flour had the softest crumb, these treatments are recommended for production of gluten-free breadfruit bread. While it seems that a use of rice flour would be most beneficial in producing high quality gluten-free bread, the use of breadfruit flour at lower inclusion levels of 20% does yield high-quality bread. Additionally, substitution of 20% breadfruit flour can serve as an extender in regions where shipments of wheat or gluten-free flours are infrequent.

The pH is a key attribute in baked goods, as it is strongly associated with consumers' perception of bread flavor. In the present sensory study, consumers mentioned disliking toward a "strong" flavor they detected. Based on their comments, it is likely that the "strong" flavor they detected was due to the by-product flavor compounds produced during the fermentation process. Yeast, as well as chemical leavening agents, can influence the structure, color, flavor, and pH of baked goods. The most influential fermentation products of yeast are volatile compounds like alcohols, aldehydes, and ketones and nonvolatile compounds like acids, esters, sugars, phenolic compounds free fatty acids, and lipids [31]. However, yeast also produce a range of other secondary metabolites such as glycerol, organic acids, flavor compounds, and precursors that impact aroma and flavor [32]. Birch and others [33] studied the influence of seven commercial compressed baker's yeasts on the formation of bread aroma and found significant differences in the aroma profile of the bread crumb with fermentation time, between the breads. Furthermore, they stated that the choice of baker's yeast is a very important decision for the bakers with respect to fermentation activity and aroma formation potential.

Water activity and glass transition are used to define the role of water in quality baked goods, as it is strongly associated with consumers' perception of bread freshness. Water is crucial to the plasticizing and solvent transformations that occur in baked bread. The organoleptic properties of baked goods change during storage, mostly due to moisture migration, and result in the quality loss known as "staling". Staling, or retrogradation of starch, causes the crumb and crust of bread to increase in hardness [34]. In the present study, significantly lower water activity was observed in breads leavened with baking powder compared to yeast. However, no significant relationship was found between water activity and crumb firmness (*N*). As previously mentioned, crumb hardness is a key attribute in baked goods, as it is strongly associated with consumers' perception of bread freshness [29]. In white pan bread, most consumers prefer a soft, resilient, and short crumb [25].

Based on the entire population ($n = 108$) surveyed, sensory testing revealed that the control (YC; yeast-leavened, 0% breadfruit flour) was preferred over the experimental treatment (Y20; yeast-leavened, 20% breadfruit flour) in overall acceptability, appearance, color, flavor, aftertaste, and likelihood to purchase. Neither YC nor Y20 bread was found to be significantly more acceptable over the other in the category of texture despite the fact that bread made with rice flour was found to be significantly softer than bread made with any treatment level of breadfruit flour. It is important to note that the significant difference in crumb hardness does not affect likability of these two breads by consumers. For a product to be launched on the market, it is generally desired for it to score an average of 7 or greater on a 9-point hedonic scale for overall acceptability [35]. However, this may not necessarily apply to launching of gluten-free foods. Based on the general population ($n = 108$) in this study, none of the samples achieved a mean score of 7 or higher for any of the quality parameters assessed, and high variation was observed for most of the parameters. According to Lawless and Heymann [35], the difference in perception of sensory parameters is often an issue with untrained panelist testing. In their comments, consumers often stated that they preferred the control (YC) because it most resembled conventional gluten-containing bread. Thus, it can be inferred that breadfruit containing bread would not sell better than current gluten-free breads available on the market, especially considering rice flour is the main component of these commercially available gluten-free breads. Despite the small size of the subgroup who were identified as those suffering from celiac disease, gluten allergy, or gluten sensitivity ($n = 5$), it is interesting to note that they seem to perceive sensory parameters in a different manner than the nonsuffering subgroup ($n = 103$). However, the suffering panelists did not have a large enough sample size and, and thus, no statistically sound conclusion can be made as to which bread would be preferred by people suffering from celiac disease, gluten allergy, or gluten sensitivity.

5. Conclusions

Overall, this research demonstrates that breadfruit flour inclusion impacts the quality of gluten-free bread. Breadfruit flour has a high fiber content and no gluten, making it an appealing substitute for rice flour in gluten-free formulations. Increased breadfruit flour inclusion causes decreased volume yielding a denser loaf and a firmer crumb, which is not preferred by consumers. However, when incorporated at lower levels into a gluten-free flour blend, breadfruit flour can increase the color and nutritional value of gluten-free bread compared to bread made from rice flour-based starch blend. The agent used to leaven gluten-free breadfruit bread was also found to greatly impact how quality characteristics develop. These findings point to the importance of understanding the impact of starch damage, particle size, and fiber content of gluten-free flour as well as the leavening agent on bread performance. Because breadfruit flour has an inherently high fiber content, which is detrimental to loaf quality, it may be ideal for use as a fiber boosting ingredient rather than the major flour component in gluten-free foods. Similarly, if the drying and milling processes are further investigated and optimized to ensure high quality, breadfruit flour may serve as a promising flour extender in the food insecure areas where breadfruit is grown. Additionally, sensory attributes the result from breadfruit flour inclusion, and those from fermentation-based leavening can impact the flavor and aroma of gluten-free bread. Since neither of the two most preferred breads achieved a 7 for overall acceptability or sensory characteristics, the experimental gluten-free formulas presented in this study will need improvement if they are to produce bread that can be considered competitive with commercially available gluten-free breads.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

The supplementary materials of this manuscript include Figures 1 and 2 as well as Appendices A, B, and C. Figures 1 and 2 depict images of the crumb structure and volume of the experimental loaves. Appendix A contains the informed consent statement form used in the consumer sensory analysis portion of this study. Appendix B outlines the consumer demographic survey used to prescreen and characterize the panelists in the sensory analysis portion of this study. Appendix C contains the ballot used by consumers to evaluate the experimental loaves for liking and sensory properties during the sensory analysis portion of this study. (*Supplementary Materials*)

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