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Descriptive and instrumental analysis of gluten free bread from green banana, pumpkin seed and cassava composite flours

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Abstract
Gluten-free breads have been developed but little is known about consumer perception and the bread sensory characteristics. Locally available food products green banana, pumpkin seed and cassava flours which are gluten-free and have ideal baking qualities are underutilized in commercial bread production. The main objective of this study was to formulate, develop and determine sensory profile both consumer and instrumental characteristics of seven gluten-free breads made from green banana, pumpkin seed and cassava composite flours. A mixture design was used to formulate seven variations of bread that included 100% each of banana, pumpkin seed and cassava flours, composites with 50:50 Banana: Pumpkin seed, Banana: Cassava and Pumpkin seed: cassava, one sample with ⅓ of banana: pumpkin seed: cassava, while the eighth 100% wheat was a control. The sensory characteristics of bread were evaluated using a descriptive panel and instrumental texture analysis. Acceptability was evaluated by 55 consumers using a 9 point hedonic scale. Green banana bread was the hardest with 11.07 N compared to wheat bread with 4.31 N. Cassava bread was only 6% and 8% less springy and cohesive, respectively than wheat bread. All the gluten-free breads, except green banana were the same as wheat bread in chewiness with a range of 2.53 to 5.52 N. Principal Component Analysis (PCA) explained 86% of the total variation in bread samples, of which 57% separated wheat from gluten free breads, while 29% separated bread types with pumpkin seed and those without. The best sensorial characteristics are imparted by pumpkin seed flour and identification of various textural parameters contributes significantly in bakery production of gluten- free bread leading to the best hedonic dimension for the consumer identification.

Keywords: celiac disease, gluten-free, instrumental characteristics, consumer acceptability, composite flours

1. Introduction

Foods containing gluten (prolamin and gliandin) proteins have been found to pose serious problems to celiac disease patients [1]. Coeliac disease (CD) is a systemic immune-mediated disorder which affects 1% to 6% of the global population [2, 3]. The disease primarily damages the small intestinal mucosa in response to the gliadin fraction of wheat gluten [4] and related storage proteins (prolamines) from barley, rye and oats [5]. A significant consequence of celiac disease is villous atrophy of the small intestines leading to nutrient malabsorption [2], chronic diarrhea, abdominal distension, weight loss and malnutrition [6]. Recent studies have revealed an increase in global prevalence [3] affecting people in India, the Middle East and North Africa among others.

Among the African population, there is increased prevalence of CD in North African countries including Morocco, Algeria, Tunisia, Libya Egypt and Sudan [7]. In Africa, children of the Saharawi people from Western Sahara have a 5.6% prevalence rate, the highest worldwide and five times higher than developed countries [8]. Another study also confirmed that CD was associated with nutrient malabsorption in Sudanese children [9]. The increased prevalence of CD in Africa and other developing countries is attributed to change from traditional to western diets with high wheat and barley consumption [10]. Consumption of wheat and gluten based products such as flat breads, biscuits, cookies, pasta and beer has increased incidences of celiac disease[11]. The only mandatory treatment for CD is lifelong adherence to a gluten-free diet [12, 13].

Growing interest and popularization of gluten-free products is the current trend as celiac disease patient's prevalence grows everyday [14]. Available gluten free products in the market have poor quality compared to those that have gluten [15].Gluten free bakery products have been found to be challenging due to varied defects including low quality, unstable structure, friable and dry crumb, lack of aroma and mouth feel [16, 13]. To solve these problems various technological parameters have been employed to curb these challenges and improve quality by using various starches [15], dairy products, gums and hydrocolloids [17]. Xanthan gum is one of the most effective additives in improving dough structure, while obtaining the best bread firmness and specific volume values [18]. Xantham gum contributes several positive qualities to baked products that include smoothness, air incorporation and retention and recipe tolerance to batters [19]. It also improves the volume and texture, and reduces the calorie content of GF breads [20].

Quality of a product and its related characteristics such as flavor and texture affects food purchasing and consumer decision in the market [21].One of the widely used parameter is texture analysis [22].Gluten free breads can be measured using both objective (instrumental) and subjective (sensory) tests to get the quality of bread during processing and storage [23]. Parameters observed in the texture profile analysis include hardness, adhesiveness and cohesiveness, springiness, resilience. These are widely used and compare both sensory attributes and rheological properties of various foods [22].

Descriptive sensory evaluation is a scientific discipline used in the food industry that provides a complete measure, analysis and interpretation of reactions in characterizing of food products and materials that can be perceived through senses of sight, smell, taste, touch and hearing [24].These attributes of food include appearance, odour, taste, texture, flavor and sound [25]. Sensory profile attributes include odour, appearance, texture, sound and taste based on the five senses [26]. Sensations are based on attributes which are coded according to their intensities on a sensory scale [27] referred to as hedonic scale. Sensory panelists are responsible for generating descriptors for each attribute [28]. Assessors need to understand the scale of evaluation during training and actual sensory testing [28, 29]. Assessors also come up with references and definitions used in sensory evaluation [29]. Descriptive sensory evaluation has been used in bread samples manufactured in Nordic countries and these have been reported in different publications [30, 31, 32, 33, 34, and 35]. Principal component analysis (PCA) is a method that has been used for projection and used in data reduction [36]. in verifying characteristics that have significant association [37] among instrumental and descriptive sensory texture parameters of gluten-free breads.

Gluten -free bread is very expensive to produce compared to other traditional breads [38] and studies show limited availability in the local shops and supermarkets. Consumers of gluten free bread in the market are also expectant in terms of texture, structure, flavor and overall quality [39].Therefore in producing gluten free bread on should focus mainly in producing bread with closer characteristics to wheat bread such as texture, sensory characteristics and shelf life [40]. Locally available food products such as green banana, pumpkin seed and cassava have the potential of producing gluten free bread with these characteristic if utilized in flour formulation and bread baking. There is no research on gluten free bread made from green banana, pumpkin seed and cassava composite flours so the aim of this study was to measure the instrumental texture parameters of a set of eight loaves and perform descriptive sensory evaluation using a trained panel to determine various attributes of gluten free bread.

2. Materials and methods

2.1 Materials

Three food products, cassava (*Manihote sculenta*), green banana (*Musa acuminata*) and pumpkin (*Cucurbita pepo L*) seed were purchased from the market in Eldoret, Kenya. Additional ingredients were instant dry yeast (Saf-instant®-lesaffre), Prestige margarine (Bidco Africa Ltd, Thika, Kenya), Powdered skimmed milk (“Miksi®”, Promasidor Ltd, Nairobi, Kenya), baking powder “chapa mandazi®” (Kapa oil Refineries Ltd, Nairobi, Kenya), Xanthan gum (Pradip enterprises (EA) Ltd PEL®) and eggs were available locally.

2.2 Flour formulations

The 3 component simplex centroid design of [41] was used to formulate seven variations of flour for the gluten- free bread preparation using cassava, green banana and pumpkin seed flours. First three were pure blends consisting of 100% green banana flour, 100% pumpkin seed flour and 100% cassava flour. Next three composite blends in the ratio 50:50 consisted of green bananas: pumpkin seed, green banana: cassava and pumpkin seed: cassava flours. Seventh variation was composited in the ratio 33:33:33 composed of blended green banana; pumpkin seed: cassava flours. An eighth variation of 100% wheat flour was added as the control. The flour formulations are shown in table 1.

Table 1: Percentage flour blends for gluten free bread preparation

Flour blends	Green Banana(GB)	Pumpkin Seed(PS)	Cassava(C)	Total %
GBF	100	0	0	100%
PSF	0	100	0	100%
CF	0	0	100	100%
GBPSF	50	50	0	100%
GBCF	50	0	50	100%
PSCF	0	50	50	100%
GBPSCF	33.33	33.33	33.33	100%
WF				100%

Pure blends: GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread) **Composites:** GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

2.3 Ingredients for bread preparation

The formulated flour blends were incorporated in the basic straight dough procedure for bread preparation [42]. For each of the flour formulations a constant amount of ingredients that included milk powder, brown sugar, xanthan gum, baking powder, egg white, and dry yeast were added in the proportions shown in table 1 for bread formulation, based on the method for gluten free bread production described by [43]. Each bread had a constant flour amounting to 200 g (32.52%) for the eight variations based on proportion on table 1. The resulting dough weight for bread amounted to 615 g (100%).

2.4 Instrumental texture analyses of gluten free bread

Texture profile Analysis (TPA) of bread made from green banana, pumpkin seed and cassava was conducted using a TA-XT plus texture analyzer (Stable Microsystems Ltd Godalming Surrey, UK) using [44] modified method 74-09 (TPA). The analyzer was fitted with a 75 mm compression platen cylinder (probe) to measure hardness, cohesiveness, chewiness, springiness and resilience. The bread was cut into 10 mm thick slices using an electric bread slicer (Ayres Jones- Mono equipment®). Rings of 30 mm diameter were punched out from two slices selected from the center of each loaf and stacked on top of each other to give a total of 20 mm thickness at the centre of the texture analyzer equipped with 50 Kg load cell. The height was set at 40 mm and force at 5 g. Pre-test speed was 1 mm/sec and test speed was 5 mm/sec when in contact with bread. The post-test speed was 5 mm/sec. The distance it moved in materials (penetration) was 10 mm/sec.

2.5 Descriptive sensory analysis Recruitment and Screening

Students and staff who normally consume bread and did not suffer from allergies or celiac disease were invited to participate on a descriptive sensory panel through advertisement on notice boards, phone calls and emails. Of the thirty eight (38) applicants who responded, 20 attended an introductory session where they were subjected to three different types of screening tests to determine their sensory acuity [45]. Before the tasting exercise the panelist filled in a consent form that informed them about the nature of the samples they would evaluate. The first test was the basic taste test to identify, sweet, sour, bitter, salty and umami tastes as described by [24], offered to panelists as filter papers of different shapes impregnated with different the taste solution. The aroma identification test was second and panelists identified pineapple, caramel, passion, banana, lemon, vanilla, chocolate and strawberry aroma. The last was an exercise to identify attributed differences relating to taste, flavor, texture and appearance among different types of bread. The final panel of 12 selected constituted five 5 men and 7 women aged between 19 to 28 years.

2.6 Training of the descriptive panel

The 12 panelists were trained in 15 sessions of 2 hours each for three consecutive weeks using the generic descriptive method described by [46]. to conduct the sensory profiling of 8 types of bread. During training the panelist were familiarized with the experimental breads and identified differences in attributes that existed among the samples with referenceto appearance, texture, flavor and aftertaste. To clarify various sensorial attributes of the bread among panelists, food items (Table 2) were used as reference samples. Panelist agreement was evaluated through several tests during training. A total of 34 descriptors were generated for gluten-free breads and the 100% wheat bread control with their definitions, reference standards to anchor the scale ends and the order of descriptors on the ballot (Table 2)

2.7 Evaluation of gluten free bread

Evaluation of gluten free breads and control was carried out over a period of three days in three sessions of 1 hour each a day following a randomized complete block design. During each session all the eight breads were randomly presented to each panelist. To avoid fatigue panelists first evaluated a set of four breads followed by a 20 minute break before evaluating a second set of 4 breads. Each sample was presented as ¼ bread showing both the crust and crumb in a transparent polyethylene zip lock type bag of 10 cm x 5 cm identified with random three digit codes [37]. arranged randomly on a white tray Figure 1.

Table 2: Descriptive sensory attributes used by trained panel to evaluate gluten free and wheat breads

Attribute/descriptors	Definition	References	Rating scale
Appearance (crust) Surface color intensity	Color intensity of crust ranging from light brown to dark brown	White bread[1] crust(light)=0 Brown bread[2] crust(dark)=10	Not dark=0 Very dark brown=10
Evenness of surface	Degree of evenness on top surface	Bread crust(even)=0 Hard dry mandazi[3] =10	Even=0 Uneven=10
Surface shine	Light reflection on the surface	White bread(not shiny) =0 Tea scones[4](very shiny) =10	Not shiny=0 Very shiny=10
Appearance crumb Surface color intensity	Color intensity of crumb ranging from light cream to dark brown	White bread (light)=0 Brown bread(dark)=10	Light =0 Dark=10
Yellow surface color	Intensity of crust surface color associated with egg yellow	Pancake[5] (light yellow)=0 Scones(dark yellow)=10	Light yellow=0 Dark yellow=10
Roughness of top surface	The degree to which roughness could be perceived on the top surface of crumb	White bread(not rough) =0 Whole meal bread[6](very rough) =10	Not rough=0 Very rough=10
Pore size	Size of the holes on the crumb surface	White bread crumb (small) =0 Whole meal bread(big) =10	Small=0 Big=10
Pore regularity	Homogeneity of pores in the crumb	White bread (regular)=0 Hard dry mandazi (irregular) =10	Regular=0 Irregular=10
Compact	Degree of denseness of particles on top surface	White bread(not compact)=0 Hard dry mandazi (very compact) =10	Not compact=0 Very compact=10
Spongy	Extent of air pockets contained in sample	White bread(very spongy)=10 Hard dry mandazi (not spongy)=0	Not spongy=0 Very spongy=10
Fine	Degree of smallness of particles on surface perceived by sight	Brown sugar[7](not fine)=0 Icing sugar[8] (very fine)=10	Not fine=0 Very fine=10

Table 3: continued

Attribute/descriptors	Definition	References	Rating scale
Damp/moist	Perception by sight of surface water on crumb	Hard dry mandazi (not damp) =0 Stiff porridge[9] (very damp) =10	Not damp=0 Very damp=10
Aroma/smell(crumb) Stale bread aroma	Intensity of aroma associated with stale bread	Fresh baked bread(no stale bread aroma) =0 Stale bread(intense stale bread) =10	No stale bread aroma=0 Intense stale bread aroma=10
Sour milk aroma	Intensity of aroma associated with sour milk	Fresh milk[12](No sour milk aroma) =0 Sour milk[13] (intense sour milk aroma) =10	No sour milk aroma=0 Intense sour milk aroma=10
Fermented aroma	Intensity of aroma associated with fermented yeast	Pancake(no fermented aroma)=0 Fermented yeast(intense fermented aroma)=10	No fermented aroma=0 Intense fermented aroma=10
Cooked banana aroma	Intensity of aroma associated with cooked banana	White bread(no cooked banana aroma)=0 Boiled banana unsalted(intense cooked banana aroma)=10	No cooked banana aroma=0 Intense cooked banana aroma=10
Cooked cassava aroma	Intensity of aroma associated with cooked cassava	White bread(no cooked cassava aroma)=0 Boiled cassava unsalted(intense cooked cassava aroma)=10	No cooked cassava aroma=0 Intense cooked cassava aroma=10
Flavor (crumb) Sweet flavor	Fundamental taste sensation associated with sugars	Spring water without sucrose (no sweet taste)=0 5% sucrose solution in spring water (intense sweet taste)=10	No sweet taste=0 Intense sweet taste=10
Fermented maize meal flavor	Intensity of flavor associated with fermented maize meal	Stiff maize meal porridge=0 Fermented maize meal snack[15]=10	No fermented maize meal flavor=0 Intense fermented maize meal flavor=10

Table 4: continued

Attribute/descriptors	Definition	References	Rating scale
Cooked banana flavor	Intensity of flavor associated with cooked banana	White bread (no cooked banana flavor)=0 Boiled banana unsalted(intense cooked banana flavor) =10	No cooked banana flavor=0 Intense cooked banana flavor=10
Cooked cassava flavor	Intensity of flavor associated with cooked cassava	White bread (no cooked cassava flavor)=0 Boiled cassava unsalted(intense cooked cassava flavor)=10	No cooked cassava flavor=0 Intense cooked cassava flavor=10
Cooked pumpkin flavor	Intensity of flavor associated with cooked pumpkin	White bread (no cooked pumpkin flavor)=0 Boiled pumpkin unsalted(intense cooked pumpkin flavor)=10	No cooked pumpkin flavor=0 Intense cooked pumpkin flavor=10
Bland flavor	Degree of mild sensation of taste no bland taste, intense bland taste	Pancake (no bland flavor)=0 Stiff maize meal(intense bland flavor)=10	No bland flavor=0 Intense bland flavor=10
Texture (crust) Crusty texture	Noise made in the first bite of the sample between the molars(auditory assessment)	White ugali[10] (not crusty) =0 Whole meal bread (very crusty) =10	Not crusty=0 Very crusty=10
Chewy texture	Toughness of the sample perceived during mastication	Cassava bread(not chewy) =0 Pumpkin seed bread[18] (very chewy) =10	Not chewy=0 Very chewy=10
Texture (crumb) Rough texture	Degree of abrasiveness of products surface perceived by the lips and tongue during mastication	White bread(not rough)=0 Whole meal bread(very rough) =10	Not rough=0 Very rough=10
Soft texture	Amount of force required to first bite through the sample with molars	Hard dry mandazi (not soft)=0 Pancake (very soft)=10	Not soft=0 Very soft=10

Table 5: continued

Attribute/descriptors	Definition	References	Rating scale
Crumbly texture	Ease with which the sample is broken into smaller particles when chewed	Pancake(not crumbly)=0 Rich cake=10	Not crumbly=0 Very crumbly=10
Slimy texture	Degree to which a sample slides over the tongue during mastication	Hard dry mandazi (not slimy)=0 Jute mallow(very slimy)	Not slimy=0 Very slimy=10
Plastic texture	Degree to which the sample retains shape and does not return	Hard dry mandazi (not plastic)=0 Stiff porridge(very plastic)=10	Not plastic =0 Very plastic=10
Damp texture	Perception of surface water on crumb felt by touching with the finger	Hard dry mandazi (not damp) =0 Stiff porridge (very damp) =10	Not damp=0 Very damp=10
After taste (crumb) Fermented aftertaste	Intensity of flavor associated with fermented yeast	Pancake(no fermented taste)=0 Fermented yeast (intense fermented taste)=10	No fermented taste=0 Intense fermented taste=10
Gritty(grainy) residue in mouth	Degree to which mouth contains small particles after all of the sample has been swallowed	Pancake(not gritty)=0 Roasted fermented maize meal flour (very gritty)=10	Not gritty=0 Very gritty=10
Fibrous after taste	Degree to which the mouth contains fiber like particles after the sample has been swallowed	Pancake(not fibrous)=0 Pumpkin seed bread(very fibrous) =10	Not fibrous=0 Very fibrous=10

White bread [1], brown bread [2], tea scones [4], whole meal bread [6] and rich cake [14], Hard dry mandazi [3] (kaangumu), Pancake [5], Brown sugar [7] and sucrose [11], Icing sugar [8], White ugali [10] and stiff porridge [9]. Fresh milk [12] and sour milk [13], Fermented maize meal flour [15].

Fig 1: Tray set up for descriptive sensory evaluation of gluten free bread

Panelists assessed the samples seated in individual stations where they could not see each other. Each panelist was provided with a plastic tumbler filled with distilled water and carrots slices for cleansing the palate before and between tasting of samples [37], a serviette and toothpick. Additionally the panelists received a ballot for assessment, a list of descriptors with definitions, a pencil and a rubber. Reference samples were available throughout the evaluation sessions. Sensory evaluation was done in the research room which was well ventilated and lit for evaluation to take place at ambient temperature.

Using the 34 descriptors (Table 2) each of the 8 bread samples were rated for appearance, aroma, flavor, texture and aftertaste on a (0-10) scale. Responses were entered manually on the ballot.

2.8 Data analysis
Textural and Descriptive sensory analysis, mean scores by panelists for the sensory parameters were determined by two-way analysis of variance ANOVA with samples as fixed effects and panelists as random effects and means were compared by Least Significant Difference(LSD), the software used was Statistica Version 8.0 (Statsoft, Tulsa, OK). A correlation matrix with bread samples in rows and descriptors in columns was used to perform Principle Component Analysis (PCA) of the significant sensory attributes obtained from means across panelists.

Results and Discussions
3.1 Instrumental Texture Analysis
The instrumental texture characteristics of gluten free breads made from GBF, PSF, CF and their composites is shown in Table 3. Hardness of breads ranged from 4.31 N (WB) to 11.07 N (GBB). Green banana bread was significantly different in terms of hardness and chewiness from WB and other gluten free breads. In texture profiling hardness refers to the force required to deform the product to given distance i.e force to compress between molars, bite through with incisors, compress between tongue and palate [47, 48]. According to [49] bread hardness is related to moisture content, moisture migration and its redistribution. It is also possible that higher hardness in GBB may be due to incomplete gelatinization of starch, lack of gluten matrix and less expansion of gas cells [50].
Springiness or elasticity indicates the elastic recovery that occurs when the compressive force is removed [51]. The values for springiness obtained in this study ranged between 0.77 (PSCB) and 0.94 (WB). Wheat bread again had significantly higher springiness than all the other breads. This may be explained by the absence of gluten which reduced the ability of the gluten free bread batter to hold gases, leading to elastic reduction in breads [52]. Springiness in gluten free breads is affected by moisture content, moisture redistribution and retrogradation of starch {49, 53}. Higher values of springiness have been reported by [54] to be more desirable on bread freshness and elasticity.

Table 6: Instrumental Texture characteristics of gluten free breads made from green banana, pumpkin seed, cassava and their composites

	Bread	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience
1	GBB(pure)	11.07^{a}	0.82^{cbd}	0.62^{ed}	5.52^{a}	0.30^{cd}
2	PSB(pure)	9.26^{ba}	0.78^{d}	0.59^{e}	4.10^{ba}	0.26^{d}
3	CB(pure)	5.37^{bc}	0.88^{b}	0.73^{b}	3.31^{b}	0.36^{bc}
4	WB (Control)	4.31^{c}	0.94^{a}	0.81^{a}	3.24^{b}	0.47^{a}
5	GBPSB(binary)	7.38^{bac}	0.82^{cd}	0.62^{ed}	3.71^{ba}	0.29^{cd}
6	GBCB(binary)	6.96^{bac}	0.83^{cb}	0.70^{cb}	4.04^{ba}	0.41^{ba}
7	PSCB(binary)	6.77^{bac}	0.77^{d}	0.69^{cb}	2.53^{b}	0.34^{bcd}
8	GBPSCB(centroid)	8.87 ^{bac}	0.80^{cd}	0.66^{cd}	3.99^{ba}	0.30^{cd}

Values are means± standard deviation. Values with the same superscript letters on the same column are significantly different at (P<0.05) as assessed by Least significant difference

Pure blends: GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread)
Composites: GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)
Cohesiveness depicts the strength of internal bonds and characterizes the extent to which a material can be deformed before it raptures [55]. Bread with higher cohesiveness is considered more desirable since it forms a bolus rather than disintegrates during mastication while bread lows in cohesiveness will easily crumble. Wheat bread was the most cohesive with a mean value of 0.81while the least cohesive was 100% PSB with 0.58, indicating that pumpkin seed bread will easily crumble. The crumbly texture of bread with high pumpkin seed content may be attributed to presence of large grainy/coarse particles associated with whole meal flours due to retainment of bran during milling [56, 57].
Notably, among gluten free bread blends, CB and its composites, GBCB, PSCB and GBPSCB depicted more cohesiveness with a mean of 0.73, 0.70, 0.69 and 0.66, respectively. This is consistent with the findings of [58] who suggested that cassava starch forms more cohesive gels due to high concentration of amylopectin to amylose resulting into a more cohesive food product. More cohesive products retain more gas and produce a higher bread specific volume [59].
Chewiness is the product of firmness, cohesiveness and springiness and is defined as energy required masticating solid food to a simple soluble product ready for swallowing [59]. or the hardness behavior of bread [60]. Bread samples had chewiness values ranging from 2.52-5.52 N. Green banana bread was the most chewy (5.52 N) while WB was the least (2.52 N). Majority of gluten free breads have been reported to record chewiness values of 2.33 to 5.77 N [61]. Lower chewiness is associated with products that easily break in the mouth such as biscuits [61].
Resilience values showed that WB had the highest elasticity value of 0.48 while PSB was the least with 0.26. It has been suggested that reduced resilience and springiness is due to loss of elasticity [55] this is, attributed to use of xanthan gum instead of gluten, lowering the dough's ability to hold gases in gluten free bread [62]. The more resilient and elastic characteristics of WB may be explained by interaction between starch and gluten in the dough, causing the dough to be more elastic thus forming a continuous sponge structure of bread after heating [63].

3.2 Descriptive sensory evaluation
Descriptive profiling of the 9 types of bread scored by a trained sensory panel yielded 34 attributes (Table 2). Analysis of variance (ANOVA) F-values were significant for 24 attributes at p≤0.05 between the bread types (Table 4). The data were further analyzed by Principle Component Analysis (PCA) a multivariate data analysis model to summarize the variation in the bread attributes. The first two principle components explained 86% of the total variation in bread samples shown in Figure 2. Principle component (PC1) expressed 57% of the total variation and separated

wheat bread to the right from gluten free breads to the left (Figure 2). Wheat bread was characterized by big and regular pores, shiny and spongy surface, crumbly, hard and chewy texture and sweet flavor. In contrast, the gluten free breads had damp/plastic/ compact/ slimy texture, compact and moist appearance and fermented aroma Figure 2. The second Principal Component (PC2) added 29% to the explanation of variation and separated all bread types with pumpkin seed on the top side of the plot from those without (i.e. banana and cassava breads) on the bottom side of the plot. The breads with pumpkin seed were characterized by crumb and crust roughness, fibrous, crusty and gritty residues which were depicted both in texture and aftertaste and were negatively correlated with even surface crust, fine appearance and soft texture of cassava bread. The existence of visible and regular pores in WB may be attributed to glutenin and gliadin which when hydrated is responsible for the elastic and cohesive properties of gluten [64, 65].Gluten influences gas retention during dough expansion resulting in the formation of pores creating a spongy crumb, shiny surface and crumbly texture [65, 66]. Open cell structured porous food materials consisting of pores that form an interconnected network is comparatively softer than closed cell networks [67]. Additionally nutrients in wheat flour together with other ingredients such as fat, sugar, minerals, starch and protein results in the final characteristics of taste and texture of WB such as crumbly texture [68]. Lack of gluten matrix in the gluten free bread resulted in damp /moist, compact and plastic appearance and texture due to closeness of the pores and high moisture content. Excessive moisture in gluten free breads as explained earlier is attributed to slower hydration (high moisture content in the batter) and less air entrapment in bread thus leading to heaviness in Bread [69]. The sweet flavor in WB may also be attributed to the use of sugar and yeast through the process of fermentation resulted to formation of 3 methyl-1-butanol the major volatile compound resulting in flavor and aroma in bread crust and crumb [70]. Due to use of lipid these results to formation of volatile compounds originating from lipid oxidation and maillard reaction resulting to formation of 2 acetyl-pyroline, 4 hydroxy-2,5 Dimethyl-3(2H) furanone responsible for flavor and aroma in bread [71, 72]. However 2-E-nonenal has been identified from maillard reaction causing strong aroma in WB [71].

Cassava bread in the same quadrant with wheat bread figure 2a is associated with evenness of the surface, fine appearance and soft texture. This may be attributed to cassava flour having binding characteristics similar to those of wheat such as being crunchy and crumby texture [73].Current innovations in gluten-free product development have proved that cassava flour possesses positive bakery characteristics such as acceptable texture, mild taste and fineness, giving it higher advantage in replacing wheat. [74] studied effect of partial replacement of wheat flour with high quality cassava flour on chemical composition, antioxidant activity, sensory quality and microbial quality of bread concluded that substitution of wheat flour with varying levels of cassava flour(for example 10%,20%,30% and 40%) produced bread with acceptable sensory attributes similar to those of 100% wheat.

Pumpkin seed had the greatest influence on the descriptive sensory properties of gluten free breads (Figure 2b). This could be attributed to fiber that was present in the husk [75, 76], giving the rough, fibrous, crusty and gritty texture and aftertaste and affecting the chewing process. Pyrazins and aldehydes formed during roasting of pumpkin seeds have been shown to contribute to desirable roasted flavor, sweetness and chewy texture in pumpkin seed bread [76, 77, 78].

Green banana bread was located on the same quadrant with PSB and was associated with fermented, damp compact and slimy texture and appearance (Figure 2b). This could be attributed to GBB having higher moisture content diluting the protein network making bread to have lower specific volume resulting in hardness in bread due to closed structure of the dough [79].

Table 7: Mean scores for sensory attributes of gluten free blends as evaluated by a trained descriptive sensory panel (n=12)

Attributes	GBB 100%	PSB 100%	CB 100%	WB 100%	GBPSB 50:50%	GBCB 50:50%	PSCB 50:50%	GBPSCB 33.3:33:3:33.3	F values
Surface color crust	$4.94^{cde} \pm 2.6$	$6.18^{ef}\pm2.5$	$1.55^{a}\pm1.7$	$3.79^{bc}\pm3.3$	$6.64^{f}\pm2.5$	$5.45^{def}\pm2.9$	$3.52^{b}\pm2.7$	$4.30^{bcd}\pm2.6$	*12.86
Even surface crust	$5.91^{c} \pm 2.2$	$3.55^{ab}\pm2.2$	$4.70^{b}\pm2.5$	$6.88^{c}\pm2.7$	$4.58^{ab}\pm2.3$	$4.61^{ab}\pm2.5$	$3.52^{a}\pm2.3$	$3.55^{ab}\pm1.9$	*8.57
Surface shine crust	$2.68^{a} \pm 2.2$	$2.18^{a}\pm1.9$	$2.67^{a}\pm2.4$	$6.03^{b}\pm2.7$	$3.00^{a}\pm2.7$	$2.70^{a}\pm2.3$	$2.36^{a}\pm2.3$	$2.45^{a}\pm2.0$	*9.19
Surface color intensity crumb	$6.68^{d} \pm 2.3$	$6.42^{cd}\pm1.9$	$1.45^{a}\pm1.0$	$2.06^{a}\pm1.5$	$6.42^{cd}\pm2.0$	$5.58^{cb}\pm2.2$	$5.15^{b}\pm2.2$	$5.79^{cdb}\pm2.2$	*33.88
Rough crumb top surface	$3.39^{a}\pm1.9$	$6.64^{d}\pm1.9$	$3.30^{a}\pm2.2$	$4.36^{ab}\pm3.1$	$6.21^{cd}\pm2.0$	$4.21^{a}\pm2.4$	$5.76^{cd}\pm2.1$	$5.45^{cd}\pm2.2$	*10.37
Pore size crumb	$2.03^{a}\pm1.9$	$4.79^{bc}\pm2.1$	$4.15^{bc}\pm2.0$	$6.33^{d}\pm2.6$	$3.88^{b}\pm2.0$	$4.27^{bc}\pm2.2$	$5.06^{c}\pm2.1$	$4.76^{bc}\pm2.1$	*10.53
Pore regularity	$3.70^{a}\pm1.7$	$4.55^{cba}\pm1.8$	$4.12^{ba}\pm2.1$	$5.09^{ca}\pm2.2$	$4.58^{cba}\pm2.0$	$5.00^{cb}\pm2.0$	$4.70^{cb}\pm2.0$	$5.36^{c}\pm1.6$	*2.44
Compact Appearance	$6.45^{d}\pm2.0$	$4.27^{bc}\pm1.6$	$4.12^{bc}\pm2.0$	$2.85^{a}\pm1.8$	$4.18^{bc}\pm2.1$	$4.85^{c}\pm1.7$	$3.58^{ba}\pm1.6$	$4.30^{bc\pm}1.83$	*10.30
Spongy surface	$2.45^{a}\pm1.9$	$4.06^{bc}\pm2.5$	$5.33^{d}\pm2.4$	$7.64^{e}\pm2.4$	$3.61^{b}\pm1.9$	$4.58^{bcd}\pm2.1$	$4.40^{bcd}\pm2.6$	$4.88^{cd}\pm2.0$	*14.23
Fine appearance	$5.70^{c}\pm2.5$	$3.64^{a}\pm2.2$	$5.45^{cb}\pm2.1$	$5.70^{c}\pm3.1$	$3.67^{a}\pm2.1$	$4.30^{a}\pm2.0$	$3.48^{a}\pm2.2$	$4.55^{ab}\pm2.1$	*5.29
Damp/moist appearance	$7.12^{c}\pm2.2$	$6.00^{bc}\pm2.2$	$6.00^{bc}\pm2.3$	$3.27^{a}\pm2.6$	$6.30^{bc}\pm2.1$	$6.18^{bc}\pm2.1$	$5.36^{ba}\pm2.3$	$5.24^{b}\pm2.5$	*7.81
Stale bread aroma	$4.79^{b}\pm2.7$	$5.24^{b}\pm2.5$	$4.67^{b}\pm2.8$	$2.15^{a}\pm2.7$	$4.88^{b}\pm2.6$	$4.64^{b}\pm2.7$	$4.39^{b}\pm2.9$	$4.52^{b}\pm2.8$	*3.81
Sour milk aroma	$3.52^{a}\pm3.2$	$3.64^{a}\pm3.2$	$4.00^{a}\pm3.2$	$2.73^{a}\pm3.1$	$3.36^{a}\pm3.0$	$3.48^{a}\pm2.9$	$3.94^{a}\pm3.2$	$3.73^{a}\pm3.2$	0.52ns
Fermented aroma	$5.12^{b}\pm2.9$	$5.58^{b}\pm2.9$	$4.79^{b}\pm3.0$	$2.52^{a}\pm2.9$	$4.82^{b}\pm3.0$	$4.76^{b}\pm2.8$	$5.00^{b}\pm2.7$	$4.61^{b}\pm3.0$	*3.20
Cooked banana aroma	$3.97^{b}\pm2.3$	$2.82^{a}\pm2.0$	$3.40^{ab}\pm2.4$	$3.09^{ab}\pm2.3$	$3.61^{ab}\pm2.2$	$3.61^{ab}\pm2.3$	$3.45^{ab}\pm2.4$	$2.70^{a}\pm2.3$	*1.15ns
Cooked cassava aroma	$3.48^{ab}\pm2.0$	$3.27^{ab}\pm2.0$	$4.30^{b}\pm2.4$	$3.03^{a}\pm2.2$	$3.33^{ab}\pm2.2$	$3.33^{ab}\pm2.4$	$3.91^{ab}\pm2.5$	$2.73^{ab}\pm2.2$	*1.05ns
Sweet flavor	$3.18^{a}\pm1.6$	$3.33^{a}\pm2.2$	$3.21^{a}\pm1.7$	$5.76^{b}\pm2.3$	$2.76^{a}\pm1.5$	$3.45^{a}\pm1.7$	$3.27^{a}\pm2.2$	$3.24^{a}\pm2.0$	*7.38

Values are means± standard deviations. Values in a row followed by different letter notations ($^{a-e)}$ are significantly different at $p \leq 0.05$,* $p \leq 0.05$ ns= not significant

Table 8: Continued

Attributes	GBB 100%	PSB 100%	CB 100%	WB 100%	GBPSB 50:50%	GBCB 50:50%	PSCB 50:50%	GBPSCB 33.3:33:3:33.3	F value
Fermented maize flavor	5.03^{b}±2.6	4.82^{b}±2.7	4.06^{b}±2.8	1.97^{a}±1.9	5.00^{b}±2.8	4.33^{b}±2.5	4.36^{b}±2.8	4.27^{b}± 2.9	*4.40
Cooked banana flavor	3.94^{a}±2.5	3.00^{a}±2.2	3.64^{a}±2.3	3.18^{a}±2.3	3.42^{a}±2.5	3.74^{a}±2.2	3.61^{a}±2.4	3.09^{a}±2.3	0.64ns
Cooked cassava flavor	3.94^{a}±2.2	3.64^{a}±2.4	4.00^{a}±2.2	3.36^{a}±2.6	3.09^{a}±2.3	3.45^{a} ±2.2	3.82^{a}±2.4	3.76^{a}±2.1	0.58ns
Cooked pumpkin flavor	2.82^{cbb}±2.4	3.87^{c}±2.5	2.33^{b}±2.0	2.79^{cbb}±2.7	3.82^{c}±2.5	2.45^{bb}±2.0	3.52^{cb}±2.0	3.54^{cb}±2.4	*2.19
Bland flavor	2.88^{ab}±2.9	3.42^{ab}±2.9	3.64^{b}±3.0	2.12^{a}±2.9	3.33^{ab}±2.9	3.21^{ab}±2.8	2.94^{ab}±2.8	3.52^{ab}±3.0	0.89ns
Crusty texture crumb	2.79^{cab}±2.2	4.58^{d}±2.8	2.27^{a}±1.9	2.55^{ab}±2.7	3.88^{cd}±2.8	3.27^{cab}±2.5	3.97^{cd}±2.6	3.64^{cbd}±2.8	*3.10
Chewy texture crumb	4.18^{bc}±2.9	7.09^{e}±2.6	4.00^{b}±2.7	2.00^{a}±2.8	6.21^{de}±2.6	4.24^{bc}±2.6	6.00^{de}±2.2	5.33^{cd}±2.9	*11.64
Rough texture crust	3.33^{ba}±2.2	6.03^{d}±2.5	4.21^{cb}±2.6	2.30^{a}±2.7	5.00^{cd}±2.0	4.18^{cb}±2.2	5.64^{d}±2.3	5.03^{cd}±2.5	*8.39
Soft texture	5.76^{d}±2.3	3.12^{a}±2.3	6.15^{d}±2.5	6.39^{d}±3.2	4.36^{bc}±2.2	5.54^{dc}±2.5	3.73^{ba}±2.4	4.48^{bc}±2.4	*7.20
Crumbly texture	3.61^{bc}±2.7	4.58^{c}±2.6	2.27^{a}±1.6	7.76^{d}±2.4	3.76^{bc}±2.6	3.09^{ba}±2.2	3.36^{ba}±2.4	3.82^{bc}±2.7	*14.66
Compact texture	6.15^{c}±2.6	4.18^{b}±2.2	4.24^{b}±2.4	1.30^{a}±1.4	4.73^{b}±2.4	4.94^{b}±2.4	3.82^{b}±2.3	4.09^{b}±2.4	*11.58
Slimy texture	3.82^{c}±2.4	2.58^{ba}±2.0	5.00^{d}±2.9	2.24^{a}±2.4	3.55^{cb}±2.2	4.30^{cd}±2.7	3.58^{cb}±2.3	3.67^{cb}±1.8	*4.38
Plastic texture	6.27^{b}±2.3	5.82^{b}±2.6	6.12^{b}±2.5	3.30^{a}±3.0	5.12^{b}±2.5	5.27^{b}±2.4	5.27^{b}±2.4	5.30^{b}±2.4	*4.27
Damp texture	6.27^{b}±2.5	6.09^{b}±2.4	5.91^{b}±2.7	2.42^{a}±2.3	5.42^{b}±2.4	5.76^{b}±2.2	5.58^{b}±2.2	5.30^{b}±2.4	*8.33
Fermented after taste	5.21^{d}±2.6	5.12^{cd}±2.4	3.64^{b}±2.6	1.40^{a}±1.4	4.85^{cd}±2.6	3.94^{cb}±2.6	4.27^{cdb}±2.5	4.18^{cdb}±2.6	*7.95
Gritty residue in mouth	2.76^{a}±2.1	7.70^{c}±1.8	1.94^{a}±2.2	1.70^{a}±2.6	6.12^{b}±2.3	2.76^{a}±2.6	5.94^{b}±2.5	5.12^{b}±2.6	*28.66
Fibrous aftertaste	2.24^{a}±1.7	7.73^{d}±2.0	1.76^{a}±1.9	1.61^{a}±2.5	6.61^{c}±2.5	2.36^{a}±2.0	6.12^{bc}±2.5	5.18^{b}±2.7	*38.52

Values are means ± standard deviations. Values in a row followed by different letter notations ($^{a-e}$) are significantly different at p≤0.05,* p≤0.05 ns= not significant

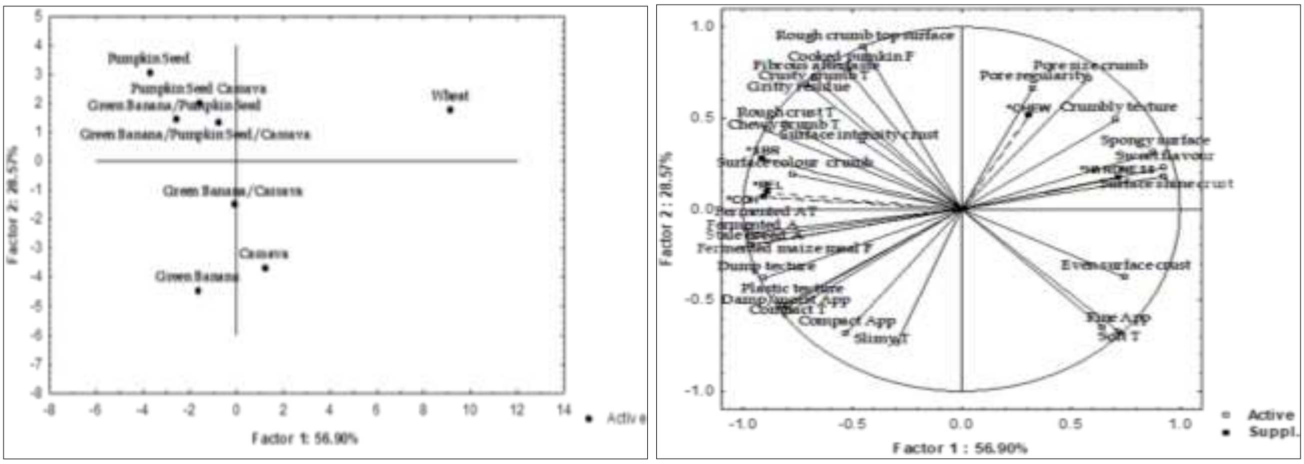

Fig 2: Principle component analysis (correlation matrix) of variation of gluten free bread blends in relation to control. (a) Plot for the first two principle components of gluten free breads (b) plot for the loading projections for the different significant sensory attribute *A- Aroma, APP-Appearance, AT- Aftertaste, CHEW-Chewiness, COH-Cohesiveness F-Flavor REL-Resilience SBR-Springiness, T-Texture

3. Conclusions

Compositing flours decreased hardness, improved cohesiveness, chewiness, springiness and resilience. Compositing flours with pumpkin seed imparted positive sensory characteristics such as crusty and chewy crumb, brown color of crumb and crust, texture of crumb, increased springiness, cohesiveness and resilience and reduced hardness and dense texture which are negative characteristics.

The role of PCA and the correlation matrix between instrumental and descriptive sensory analysis results were effective showing that instrumental hardness parameter of gluten free bread crumb and crust can be used to predict the final textural quality attributes of the gluten free bread loaves.

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