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Quality evaluation and techno-economic feasibility study of Gluten free bread prepared with Buckwheat (*Fagopyrum esculentum Moench*) and Pearl millet (*Pennisetum glaucum)*

Evaluación de calidad y estudio de viabilidad tecno-económica de pan sin gluten elaborado con Trigo Sarraceno (*Fagopyrum esculentum* Moench) y Mijo perla (*Pennisetum glaucum*)

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ABSTRACT

The gluten free breads were prepared with nutritionally rich buckwheat and pearl millet flour to replace the refined wheat flour from the diet of celiac people. The breads from five different combinations with increasing proportion of buckwheat and pearl millet flour along with control (rice flour) were analyzed for its physico-chemical, textural, microbial and sensory parameters. The mean volume and specific volume of the formulated gluten free bread samples decreased with increasing portion of rice flour (220.86-166.30 Cm^3 and 1.4-1.1 $Cm^{3/}$gm respectively). The moisture, carbohydrates and total energy decreased from (33.96-30.10% 86.24-73.85% and 408.11-362.13Kcal respectively) while proteins, fats, crude fibers and ash were increased (7.6-11.45, 1.2-3.8, 1.2-4.9 and 0.5-1.4% respectively) with the addition of buckwheat and pearl millet in the flour. The hardness of sample S00100 (93.2 g) was lowest in comparison with sample with pure buckwheat and pearl millet flour. The hardness increased with higher portion of buckwheat flour in the mixture. The chewiness (48.2 to 11.4 mJ.) and cohesiveness (1.9-0.9 g) showed decreasing trend with increasing buckwheat and pearl millet flour in the samples. The results of microbial as well as storage studies indicated that the breads are not shelf stable after 3 days of preparation. The overall organoleptic acceptability of sample with 30% buckwheat and pearl millet flour was found high (8.13). The total cost of production of the most accepted gluten free bread sample S3040 was 62.50 Rs/kg, which was found higher than control (56.25 Rs/kg).

Keywords: Gluten free bread, quality, buckwheat, pearl millet flour, techno-economic feasibility, storage study.

RESUMEN

Los panes sin gluten se prepararon con harina de trigo sarraceno y mijo perla, rica en nutrientes, para sustituir la harina de trigo refinada de la dieta de los celíacos. Se analizaron los panes de cinco combinaciones diferentes con proporción creciente de harina de trigo sarraceno y mijo perla junto con el control (harina de arroz) para determinar sus parámetros fisicoquímicos, texturales, microbianos y sensoriales. El volumen medio y el volumen específico de las muestras de pan sin gluten formuladas disminuyeron al aumentar la porción de harina de arroz (220,86-166,30 cm3 y 1,4-1,1 cm3/g, respectivamente). La humedad, los carbohidratos y la energía total disminuyeron (33,96-30,10%, 86,24-73,85% y 408,11-362,13Kcal respectivamente) mientras que las proteínas, grasas, fibras crudas y cenizas aumentaron (7,6-11,45, 1,2-3,8, 1,2-4,9 y 0,5). -1,4% respectivamente) con la adición de trigo sarraceno y mijo perla a la harina. La dureza de la muestra S00100 (93,2 g) fue la más baja en comparación con la muestra con harina pura de trigo sarraceno y mijo perla. La dureza aumentó con una mayor proporción de harina de trigo sarraceno en la mezcla. La masticabilidad (48,2 a 11,4 mJ) y la cohesividad (1,9-0,9 g) mostraron una tendencia decreciente con el aumento de la harina de trigo sarraceno y de mijo perla en las muestras. Los resultados de los estudios microbianos y de almacenamiento indicaron que los panes no son estables en almacenamiento después de 3 días de preparación. La aceptabilidad organoléptica general de la muestra con 30% de harina de trigo sarraceno y mijo perla se encontró alta (8.13). El coste total de producción de la muestra de pan sin gluten más aceptada, S3040, fue de 62,50 Rs/kg, cifra superior a la del control (56,25 Rs/kg).

Palabras clave: pan sin gluten, calidad, trigo sarraceno, harina de mijo perla, viabilidad tecnoeconómica, estudio de almacenamiento.

INTRODUCTION

Bread is a prominent food of most of the countries, prepared from white flour, yeast, sugar, fat, salt, water, etc., by a series of operations like mixing, kneading, fermentation, proofing and baking (Dewettinck et al., 2008). It is one of the oldest manmade foods consumed all over the world by all age people. The refined flour, commonly known as '*Maida*', is the premium variety of wheat flour that is used in making nearly all bakery foods and many more snack items. The bleaching process gives it the white color and makes it appealing to the eyes. Wheat (*Triticum aestivum* L.) contains 80-85% gluten that possess unique visco-elastic properties. Gluten is made up of monomeric gluten units (gliadin) which cause viscous behavior while polymeric gluten units (glutenin) are elastic (Dvořáková et al. 2013). Addition of water and kneading procedures facilitate a formation of cohesive visco-elastic dough able to hold gas produced during fermentation. That results in typical structure of bread. But in cases of celiac disease gluten must be excluded from diet because its ingestion causes serious intestinal damage (Demirkesen et al., 2010). Gluten is a protein found in wheat, barley, rye and triticale (a cross between wheat and rye). A gluten-free diet is an eating plan that except foods that enclosing gluten. A gluten-free diet is necessary for celiac disease and other medical conditions associated with gluten. Approximately 1% of total population of the world is affected with celiac disease, gluten sensitivity, Gluten ataxia and Wheat allergy (Hischenhuber et al., 2006). It is a condition in which gluten triggers immune system activity that damages the lining of the small intestine. This prevents the absorption of nutrients from food. Unfortunately the gluten-free technology involves several complications. Although the products with lack of gluten matrix are typical of worse industrial quality, short specific volume, high crumb hardness and short staling time (Moroni et al., 2009), the gluten free diet is need of the era. Hence there is a great scope to search for alternative sources that yields satisfied quality of finished products.

Buckwheat (*Fagopyrum esculentum Moench*) Known as Kuttu in Marathi, is a plant cultivated for its highly nutritious seeds (“USDA Grain Taxonomy”, 2014). Despite its name, buckwheat is not closely related to wheat. It is one of the pseudocereals, triangular seeded, domesticated in Asia. Buckwheat grows 30 to 50 inches tall, has a growing period of only 10-12 weeks, adaptable to wide range of soils and climatic conditions (Björkman, et al., 2008). It is the major source of protein with most of the essential amino acids, dietary fibers, vitamins, minerals and trace elements. It possesses higher antioxidant activity as a function of phenolic acids, flavonoids, phytic acids, carotenoids, phytosterols (Sedej et al., 2011). Pearl millet (*Pennisetum glaucum)* also known as 'Bajra' in Hindi, is the most widely grown millet of Africa and the Indian subcontinent, since prehistoric times (Fuller, 2003). It accounts for about 50% of the total world production of millets.The height of the plant ranges from 0.5 – 4 m, the grains are ellipse to oval in shape having 3–4 mm length and pale yellow, brown, grey, in color. Pearl millet is well adapted to droughts, high temperature and low soil fertility. It is a summer annual crop compatible for double cropping and rotations. It is commonly used to make flatbread and porridge. Pearl millet is good source of proteins, fiber, and minerals such as calcium and magnesium. Rich in vitamin B complex, potassium, copper, zinc and selenium. The studies conducted by the researchers showed that it has a low glycemic index (Gelaye et al., 1997). Rice (*Oryza sativa)* is the seed of the grass species It is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize. The rice is most widely consumed as staple food of half of the world's population especially in Asia and Africa. Rice is normally grown as an annual plant, although in tropical areas it can survive as a perennial. Rice flour is considered to be an appropriate substitute for wheat as it is available worldwide as it is colorless and blend taste. Rice flour is used as thickening agent for sauces, soups and gravies. They’re similar in their strength as thickeners and give a very comparable result. It has also low level of protein, sodium, fat, fiber and high amount of easily digested carbohydrates (Yano, 2010).

The aim of this study was to prepare a novel product, gluten-free bread from mixtures of buckwheat flour *(Fagopyrum esculentum Moench*) and Pearl millet (*Pennisetum glaucum)* flour and to compare textural properties of gluten-free breads.

MATERIALS AND METHODS

All the raw materials needed for the proposed research viz. buckwheat, pearl millet, rice, yeast (Saccharomyces cerevisiae), sugar, salt, were procured from local market Aurangabad. The grains were cleaned and pulverized through stone mill to fine flour. The breads were developed as per the method suggested by Valentina et al. (2017) with some modifications. The batter was prepared from mixed flour (Table 1), 1.8% dry yeast, 1.5% salt, 1.86% sugar, 0.005%, ascorbic acid. All the ingredients were mixed with warm water in a large mixing bowl and poured in bread tray lined with butter paper and allowed to set for 10 to 15 minutes, till it rises double. The baking was done for 20 minutes in a pre-heated oven at 180◦C. The breads were cooled, sliced and were packed in LDPE bags and stored at room temperature.

Table 1 Proportions of buckwheat, pearl millet and rice flours

Sample	Buckwheat flour	Pearl millet flour	Rice flour
S00100 (Pure Rice flour)	00		100
S1080	10	10	80
S2060	20	20	60
S3040	30	30	40
S4020	40	40	20
S5000 (Pure buckwheat and Pearl millet flour)	50	50	00

Evaluation of various quality characteristics of the gluten free bread

Physical parameters of gluten free bread

The physical characteristics of bread prepared with buckwheat and pearl millet flour such as mean loaf volume, specific loaf volume dough yield, % bread yield and baking loss were determined after 30 min. from the removal of loaves from oven (Araki et al., 2009; Giami et al., 2004).

Proximate composition of gluten free bread

The gluten free bread samples were evaluated for moisture, carbohydrates, protein, fat, crude fiber, ash, minerals and calories (AOAC, 2011).

Textural analysis of gluten free bread

The textural characteristics of bread were measured using Texture Pro CT V1.7 Build 28 (Brookfield Engineering Labs. Inc). The bread samples (20x20x20 mm) from the center of each bread were two times compressed by a cylindrical probe (TA3/100). Setting for textural analysis was target-4mm, hold time-0 sec, trigger load-10g, test speed- 0.50 mm/s, return speed- 0.5mm/s, number of cycles-2, recovery time-0 sec, same trigger-true, pretest trigger-2mm/s, data rate- 30 points/sec, fixture- TA-RT-KIT and load cell- 10000g. The parameters recorded were hardness (g), springiness (mm), gumminess (gm), chewiness (mJ) and cohesiveness (g).

Microbial analysis of gluten free bread

The storage life of gluten free bread was determined by microbial analysis and expressed in days. The microbial qualities of sample were determined by measuring total plate count of microorganisms (Yusufu et al., 2016). Total plate counts (TPC) of sample were determined by dissolving 1gm in 10 ml sterile saline solution and were homogenate for 1 min. The serial dilution was done by adding 1 ml of homogenate in 9 ml sterile saline, dispensed in test tubes and 0.1 ml of the dilution (10−1, 10−2, 10−3, 10-4 and 10-5) was spread on sterile petri plates containing nutrient agar media and was spread on sterile petri plates containing nutrient agar media. Yeast and mould count were estimated by using and potato dextrose agar with the help of pour plate technique and serial dilution. The numbers of microbial colonies formed on petri dish were measured using digital colony counter and expressed as CFU/g.

Sensory evaluation of gluten free bread

Organoleptic evaluation of gluten free bread was performed by a panel of 25 semi-trained panel of judges using 9-point hedonic scale with 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely for the parameters, color, appearance, taste, texture, flavor and overall acceptability (Amerine et al., 1965).

Statistical analysis

The data obtained from various experiments in triplicate for each parameter was statistically analyzed as per the methods suggested by Panse and Sukhatme (1967). Results were expressed as means with standard deviations.

Techno- economic feasibility of gluten free bread

The techno-economic feasibility of gluten free bread samples was evaluated by calculating the total cost of production per 100g of product. Considering the raw material cost, processing cost (10% raw materials cost), packaging cost and miscellaneous cost (2 % of raw material cost) and the unit cost of production was assessed and expressed in Rs. (INR) (Verma et al., 2013).

RESULTS AND DISCUSSIONS

Physical parameters of gluten free bread

It was observed that as the buckwheat flour and pearl millet flour decreases in bread, the mean volume, specific volume, dough yield and bread yield also decreased with increased baking loss. Addition of buckwheat flour to dough increases its water-absorbing ability. This might be due to presence of protein, containing 80% albumin and globulin, has a higher water-retaining capacity (Vang et al., 2010). The buckwheat starch granules are mainly polygonal and less spherical in shape with rough surfaces (Juan, et al., 2009) which results in high stabilizing and thickening properties. The mean volume and specific volume of the formulated gluten free bread samples decreased with increasing portion of rice flour (Table 2). This might be due to the hydrophobic nature of rice proteins which resist swelling in water (Kadan et al., 2001). The increasing rice flour in the bread is thus associated with the poor quality. These low values of specific volume are typical of gluten free breads, which is in agreement with Brites et al. (2010).

Table 2 Physical parameters of gluten free bread

Samples	Mean Volume (Cm^3)	Specific volume (Cm^3/gm)	Dough yield	% Bread yield	Baking loss
S00100 (Pure Rice flour)	166.30±0.01	1.1±0.04	169.5±0.02	144.2±0.01	15.2±0.01
S1080	175.65±0.02	1.1±0.03	171.6±0.01	147.6±0.02	13.4±0.02
S2060	201.10±0.01	1.2±0.01	173.4±0.02	152.8±0.03	13.0±0.01
S3040	209.62±0.06	1.2±0.02	175.1±0.04	153.5±0.01	12.1±0.04
S4020	212.20±0.01	1.3±0.02	177.0±0.03	156.3±0.03	11.2±0.02
S5000 (Pure buckwheat and Pearl millet flour)	220.86±0.05	1.4±0.01	178.2±0.01	158.2±0.04	10.8±0.01

Proximate composition of gluten free bread

The proximate composition of gluten free bread samples along with control presented in table 3 depicted that the moisture content of all the samples made was in the range of 30.10 to 33.96 %. A slight increase in moisture was observed with respect to decrease in buckwheat and pearl millet percentage in the bread. This might be due to retrogradation of the rice starches that binds the water molecules to form elastic structure. The carbohydrates, proteins and fats increased significantly with respect to increase in buckwheat flour and pearl millet flour in the samples. The highest value of carbohydrate, protein and fat content for control samples were 73.85, 11.45 and 3.8 % respectively. These data is in general agreement with those presented earlier by Mahmoud (1971). The crude fiber and ash present in buckwheat and pearl millet were much high than in rice flour. Gradual incorporation of the same increased crude fiber and ash content of the bread samples. These results were compared with those reported by Wall and Ross, (1970). The highest values of fibers and ash were observed for sample S5000 (Pure buckwheat and Pearl millet flour) (4.9 and 1.4 % respectively). The energy value was lowest for the bread with highest buckwheat and pearl millet flour (360.70 Kcal) and was highest for the bread made from rice flour (408.11 Kcal).

Textural analysis of gluten free bread

The crumb of gluten-free products observed to be wet after baking and stuck together, but after cooling it becomes dry (Torbica et al., 2010) this might be due to retrogradation of starch in absence of gluten network (Guarda et al., 2004). The increasing amount of buckwheat flour in the mixture had deteriorating effect on the crumb hardness. The hardness of sample S00100 (93.2 g) was lowest in comparison with sample with pure buckwheat and pearl millet flour. The hardness increased with higher portion of buckwheat flour in the mixture. The highest value of crumb hardness reached the sample containing pure buckwheat and pearl millet flour (Table 4). Springiness is the ability of a material to return to its original shape after removal of the force. . If springiness is high, it requires more mastication energy in the mouth. The elasticity of the sample expressed as springiness ranged from 3.6 to 4.2 mm. All of the samples showed little differences in elasticity of the bread crumb. Addition of buckwheat flour and pearl millet flour decreased the elasticity of the samples. This might be due to lack of thickening and gluten network. Gumminess is correlated with hardness and cohesiveness. It was found lowest for sample S5000 (212.1 gm) and highest for S00100 (Pure Rice flour) (406.2 gm). The higher value of gumminess is always desirable in order to avoid the disintegration of the product during mastication. The chewiness of the gluten free breads found decreased with increase in buckwheat and pearl millet flour in the composition from 48.2 to 11.4 mJ. Whereas the cohesiveness showed same trend and the highest cohesiveness was observed for sample S1080 (1.90 g). As the cohesiveness is correlated with the internal resistance of food structure, more cohesiveness is desired to obtain quality product.

Table 3 Proximate composition of gluten free bread

Samples	Moisture (g)	Carbohydrate (g)	Protein (g)	Fat (g)	Crude fiber (g)	Ash (g)	Energy (Kcal)
S00100 (Pure Rice flour)	33.96±0.01	86.24±0.02	7.6±0.02	1.2±0.03	1.2±0.03	0.5±0.04	408.11±0.01
S1080	33.78±0.02	84.22±0.01	8.12±0.01	1.6±0.02	1.8±0.04	1.1±0.01	388.12±0.02
S2060	33.16±0.01	81.23±0.02	8.98±0.01	2.1±0.02	2.5±0.01	1.2±0.03	380.52±0.03
S3040	32.65±0.03	76.31±0.03	10.32±0.03	2.8±0.01	3.2±0.02	1.3±0.03	375.12±0.01
S4020	31.74±0.01	74.22±0.01	10.65±0.01	3.2±0.05	4.1±0.02	1.4±0.02	369.85±0.02
S5000 (Pure buckwheat, Pearl millet flour)	30.10±0.02	73.85±0.02	11.45±0.01	3.8±0.02	4.9±0.03	1.4±0.01	362.13±0.04

Table 4 Textural analysis of gluten free bread

Samples	Hardness (g)	Springiness (mm)	Gumminess (gm)	Chewiness (mJ)	Cohesiveness (g)
S00100 (Pure Rice flour)	93.2±0.01	4.2±0.01	406.2±0.02	37.2	1.6
S1080	99.3±0.02	4.2±0.02	395.2±0.01	48.2	1.9
S2060	108.5±0.01	4.1±0.02	320.3±0.05	41.5	1.6
S3040	122.4±0.03	4.0±0.01	287.4±0.01	36.2	1.3
S4020	137.1±0.01	3.9±0.02	245.2±0.01	25.0	1.1
S5000 (Pure buckwheat, Pearl millet flour)	145.4±0.04	3.6±0.01	212.1±0.03	11.4	0.9

Microbial analysis of gluten free bread

The different formulations of gluten free breads were packed in LDPE bags and stored at room temperature on shelves and checked for microbial analysis after 2 days interval. The bakery products stored at room temperature may leads to undesirable microbial growth, resulting in spoilage (Jariyawaranugoon, 2013). The higher values of shelf-stability means lower freshness of the breads and vice versa. The microbial analysis of buckwheat and pearl millet flour based gluten free bread during storage presented in table 5 resulted that all the breads packed in LDPE packets did not show any growth on 1st day of storage at ambient temperature. On 3rd day, the visible growth was observed for the samples with buckwheat and pearl millet flour. The increasing number of colonies were observed as the buckwheat and pearl millet flour increased in the composition (1×10^3 to 3.2×10^3 cfu/g). The buckwheat and pearl millet gluten free breads provides an excellent growth medium for variety of microorganisms, as it provides rich nutrients for microorganisms, high moisture and neutral pH (Siriken et al., 2009). After 3 days of storage, fungal growth was observed for samples S4020 and S5000 (Pure buckwheat and Pearl millet flour). At the end of 3rd day, visible growth was observed for control sample. The reason behind this is, the room temperature (25°C ± 2°C) is the optimum temperature for growth of microorganism. Furthermore, it is also due to the presence of oxygen which allows growth of aerobic microorganisms (Jariyawaranugoon, 2013).

Table 5 Microbial analysis of gluten free bread

Samples	Microbial Quality Parameters (CFU/g)	Storage Period (Days)				
		0	1	2	3	4
S00100 (Pure Rice flour)	Total viable count	Nil	Nil	Nil	Nil	2.9×10^4
	Total fungal count	Nil	Nil	Nil	Nil	3×10^3
S1080	Total viable count	Nil	Nil	1×10^3	1.3×10^3	2.5×10^4
	Total fungal count	Nil	Nil	Nil	Nil	1.5×10^3
S2060	Total viable count	Nil	Nil	1.3×10^3	1.5×10^3	1.5×10^4
	Total fungal count	Nil	Nil	Nil	Nil	1.7×10^3
S3040	Total viable count	Nil	Nil	2.4×10^3	2.6×10^3	2×10^4
	Total fungal count	Nil	Nil	Nil	2×10^2	1.5×10^3
S4020	Total viable count	Nil	Nil	3×10^3	3.5×10^3	2×10^5
	Total fungal count	Nil	Nil	Nil	2.2×10^3	2×10^3
S5000 (Pure buckwheat and Pearl millet flour)	Total viable count	Nil	Nil	3.2×10^3	3.6×10^3	2.2×10^5
	Total fungal count	Nil	Nil	Nil	2.4×10^4	2.2×10^5

The total viable count and fungal count of buckwheat and pearl millet flour breads on 4th day of storage showed significant increase from (2.5×10^4 to 2.2×10^5 cfu/g) and (1.5×10^3 To 2.2×10^5 cfu/g) respectively. This indicates that the gluten free breads are not shelf stable after 3 days of preparation and pose a potential public health risk if subjected to high temperature during storage, distribution and marketing. After 4th day the sample was deteriorated. According to Good Manufacturing Practice (GMP) guidelines, the maximum limit of fungal contamination expected immediately following production of food, for bakery products is 2 log10 CFU (Carl, 2014). Whereas the highest acceptable level of total viable count for ready to eat foods is < 4 log10 CFU, while ≥ 5 log10 CFU is considered unsatisfactory (Food Standards Australia New Zealand, 2015). Similar results were observed for the study of ragi cakes by Chaudhari et al. (2017) where mold growth was observed on 6th day of storage. Essra et al. (2019) Formulated gluten free cakes from quinoa and chia flour and found that cakes can be consumed safely up to 8 days when preserved at cold (4°C), room (20°C) or warm (37°C) temperatures.

Shelf life of study of gluten free bread

The storage temperature, atmospheric humidity and composition of food affect the storage life of the gluten free breads. The formulated gluten free bread samples were packed in LDPE bags and stored at ambient temperature on shelves and observed for moisture, water activity and alcoholic acidity on first and third day of preparation and presented in table 6. During storage rapid loss of flavor and changes in bread texture. This brings the increased hardness and fracturability, decreased elasticity and solubility occur. That causes dry and hard structure of bread. This is mainly due to starch retrogradation. The breads with high level of moisture are highly susceptibility to microbial spoilage (Moroni et al., 2011). The moisture content of pure rice flour sample was highest (32.5 %) among all gluten free breads. Whereas the least moisture was observed for sample S5000 (Pure buckwheat and Pearl millet flour) (28.13%). The difference in the moisture content might be due to presence of high fiber content of buckwheat flour and pearl millet flour as compared to rice flour. The moisture loss in bread is mainly due to absorption of water by the dietary fibers. The significant decrease in moisture content of gluten free breads was observed during storage. This is the effect of transfer of moisture from crumb to crust as a function of starch recrystallization during storage (Pateras 1998). Studies showed that in absence of glutn network, water migrates from crumb to crust leading to moisture loss and thus initiates faster ageing of the bread (Sciarini et al., 2010).

The water activity of buckwheat and pearl millet bread was lower as compared to rice bread. On 3rd day of storage, the water activity decreased significantly. It might be due to nature of pearl millet starch, more prone to retrogradation during storage. The similar findings were also reported those by Kadan et al. (2001) where rice based gluten free bread found more prone to retrograde the starch during storage than wheat bread. The alcoholic acidity decides the age of the bread. On first day of storage, it ranged from 0.04 to 0.06 and increased gradually with increasing percentage of buckwheat and pearl millet flour in the gluten free bread. Again on storage it increased as compared to control. After 3rd day, the alcoholic acidity increased to 0.1 for sample S5000 (Pure buckwheat and Pearl millet flour). This might be the function of chemical changes in foods during storage. The acid phosphatase, amino acids and free fatty acids increase due to the enzymatic hydrolysis of proteins and fat, respectively. All these are soluble in strong alcohol. Thus, it is always expected to have low alcoholic acidity. Thus form the above study, it can be concluded that the gluten free breads made with buckwheat and pearl millet flour can be safely stored up to 3 days at room temperature.

Table 6 Shelf life of study of gluten free bread

Treatments	Parameters					
	Moisture %		Water Activity		Alcoholic Acidity	
	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
S00100 (Pure Rice flour)	32.5±0.02	30.76±0.02	0.9±0.02	0.86±0.02	0.04±0.02	0.07±0.02
S1080	30.65±0.01	29.32±0.01	0.96±0.01	0.92±0.01	0.06±0.01	0.07±0.01
S2060	30.26±0.01	29.28±0.01	0.95±0.01	0.90±0.01	0.07±0.01	0.08±0.01
S3040	30.22±0.01	29.18±0.01	0.94±0.01	0.9±0.01	0.06±0.01	0.07±0.01
S4020	29.16±0.02	28.35±0.02	0.93±0.02	0.89±0.02	0.06±0.02	0.08±0.02
S5000 (Pure buckwheat and Pearl millet flour)	28.13±0.01	27.28±0.01	0.91±0.01	0.89±0.01	0.06±0.01	0.1±0.01

Sensory evaluation of gluten free bread

The sensory evaluation of gluten free bread samples prepared from buckwheat and pearl millet flour was done with a panel of 25 semi trained members. The control rice bread had the highest value (8.8) in terms of overall acceptability while the bread from buckwheat and pearl millet (sample -S5000) had least overall acceptability value of 7.12. As the percentage of substitution increases, overall acceptability decreased. The buckwheat and pearl millet flour incorporated bread samples had darker color with increasing its fortification. The sensory scores for color for the samples ranged from 6.7 to 8.5. As pert the study of Samuel (2016), significant change in color was detected for Sorghum and wheat flour cookies at 35% level of replacement, and this became penetrating at 50% level. The breads with buckwheat and pearl millet flour showed typical grainy flavor and taste. As a result of this, the panelists selected sample S3040 with 30% buckwheat and pearl millet flour in terms of the taste parameters. The sample S1080 was found higher loaf compared to other samples. Also, the crumb texture of S1080 was as good as control. On an average, the overall acceptability of sample with 30% buckwheat and pearl millet flour was found high scores (8.13). Similar results were found by Perten (1983), where the bread made with 30% Sorghum flour and 70% wheat flour was acceptable and was appraised as excellent by panel.

Techno- economic feasibility of gluten free bread

All direct and indirect costs contribute towards the total cost of the product. The cost of production of most accepted gluten free bread prepared with addition buckwheat and pearl millet flour was studied. The direct cost consists of cost of raw materials and the indirect cost consists of salary of working personnel's, rent, lighting, telephone expenses, fuel and power cost, transportation cost, etc. The cost of all the raw materials per kg viz. buckwheat flour (100/-), pearl millet flour (40/-), rice flour (32/-), yeast (300/-), sugar (40/-), salt (20/-) and fat (70/-) was added with the processing cost and miscellaneous costs. Thus the total cost of production of the most accepted gluten free bread sample S3040 was 62.50 Rs/kg, which was found higher than control (56.25 Rs/kg). The above findings were compared with the observations of Garg (2010) where the total production cost of bread in Meerut Region in 2008-09 was 19.75 Rs/kg. Thus, from above findings it is clear that although the addition of buckwheat and pearl millet flour increases in the composition, the cost of production was high than the control but can be commercially viable.

CONCLUSION

It was found that incorporation of buckwheat and pearl millet flour to rice flour in the different ratios, significantly affected the final quality of gluten free bread. It was determined that higher amounts of buckwheat flour enhanced the parameters of baking test – mean bread volume, specific volume of bread, dough and bread yield. Also the textural parameters are impaired while the proximate study showed better results for protein, fats and crude fibers over rice breads. The microbial and storage studies indicated shelf life of these gluten free breads up to 3 days at room temperature. Hence as per the organoleptic study, the sample with 30% buckwheat and pearl millet flour rated high and was techno-economically feasible. Further, the commercialization of this gluten free breads along with animal studies should be focused.

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