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REGULAR ARTICLE

MICROBIOLOGICAL AND NUTRITIONAL QUALITY OF WARANKASHI ENRICHED BREAD

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ABSTRACT

The study was carried out to determine the microbiological and nutritional quality, organoleptic, rheological and textural effect as well as the effect on the shelf life of wheat bread enriched with West African cottage cheese (warankashi) at different substitution levels (1 %, 3 % and 5 %). The protein and fat content of wheat bread significantly increased but carbohydrate levels decreased significantly as enrichment with Warankashi increased. The amino acid profile of the wheat bread increased with increasing enrichment. The incorporation of Warankashi into wheat flour affected the rheological and textural properties of wheat flour; the rate of water absorption of the wheat flour decreased as Warankashi incorporation levels increased. Also, the dough stability time of the enriched flours was lesser than that of the wheat flour. The 3 % enrichment level had the highest dough consistency (520 BU). The extensibility of 1 % and 3 % wara bread dough were the same while that of wheat flour bread and 5 % Warankashi were the same. The 3 % wara bread dough had the highest resistance to extension. Shelf life of the bread remained unaffected by Warankashi incorporation but the rate of bacteria and fungi (yeast and mould) growth decreased significantly ($P < 0.05$) as enrichment levels increased. There was no significant difference between the organoleptic properties of wheat bread to that of the enriched breads but the 3 % Warankshi enriched bread had the highest consumer acceptability.

Keywords: Wheat bread, '*Warankashi',* shelf-life, enrichment level

INTRODUCTION

Bread is a food product that is universally accepted as a very convenient form of food that has desirability to all population rich and poor, rural and urban. Its origin dates back to the Neolithic era and is still one of the most consumed and acceptable staple in all parts of the world (**Mannay and Shadaksharaswany, 2005**). It is a good source of nutrients, such as macronutrients (carbohydrates, protein and fat) and micronutrients (minerals and vitamins that are all essential for human health. These values makes bread to be known as an essential food in human nutrition and this has lead all countries throughout the world to study the composition of the bread that consumed to improve its nutritive value. Bread has however been transformed into different types with varying characteristics depending on the innovations put into the production. All these varying attributes of bread most times detract consumers about the nutritional and wholesome quality of the bread product. This is to say that there is a need to continuously improve the nutritional and organoleptic attributes of bread (**Potter and Hotchkiss, 2006)**.

In Nigeria, bread has become the second most widely consumed non-indigenous food product after rice. **(Shittu *et.al.,* 2007)** and has become an important source of food to Nigerians. It is consumed extensively in most homes, restaurants and hotels. The most commonly consumed bread in Nigeria is white bread. This is bread made from refined whole wheat which also termed as all-purpose flour and it's known for its characteristic white color due to the removal of the wheat bran. Protein foods are usually expensive and beyond the reach of most of the populace in developing countries like Nigeria. This scarcity has greater impact on children, whose physical and mental development requires nutritionally balanced diets. Malnutrition leads to wasting, stunting and underweight so the use of acceptable traditional foods which are readily acceptable to the populace and rich in protein source is one possibility of increasing the protein component in diets, and thus reducing malnutrition. Milk proteins are ideal in that they are complete and have high essential amino acids composition. Although milk and its various derivatives form a vital human food, it also provides an excellent medium for the growth of many kinds of beneficial micro-organisms. Warakanshi is a traditional soft cheese consumed in several parts of West Africa. It originates from the Fulani cattle herdsmen from northern Nigeria, who refer to the liquid cow's milk as "Wara" and the curd-like texture of the cheese as "Kashi" (**Ogundiwin, 1978**). Warankasi is an

unripened soft cheese-like product made from fresh whole cow's milk by the application of a juice extract of Sodom apple leaf (*Calotropis procera*) or pawpaw (*Carica papaya)* (**Belewu and Aina, 2000; Fashakin and Unokiwedi, 1992**). The preferred coagulant comes from Sodom apple leaf extract (*Calotropis procera*) because the cheese made with this coagulant has a sweeter flavour and a higher protein content compared to the cheese made with the other coagulants **(Omotosho *et al*., 2011).** Warankasi is consumed in its fresh unripened state or fried. It has an average shelf life of 2 to 3 days when stored in whey at ambient temperature (approximately 28 ^{0}C) or 4 to 5 days when placed in cool well water at approximately 15 ^{0}C **(Adegoke *et al*., 1992; Umoh and Solomon, 2001; Belewu *et al*., 2005**). It is a very good source of animal protein (approximately 26 %), fat (approximately 20 %), carbohydrates (approximately 3 %), ash (approximately 2 %) and moisture (50 %) and a good source of sodium, potassium and Calcium (**Omotosho *et al*., 2011**). Studies have shown that wheat flour which is the major ingredient in bread has an inferior protein quality compared to that of other cereals and other protein sources (**Singh *et al*., 2001**). So attempts are therefore being made to enrich bakery products with high quality non wheat proteins such as eggs, milk and milk products exhibit excellent protein quality and functional characteristics. This shows there is an imbalance in the protein- calorie level in white bread. The incorporation of warankashi which is a highly proteinous milk product (cheese) which is consumed by the populace will serve as a good fortifier for the improvement of the imbalance in the protein levels in white bread and also serve as a functional supplement to the wheat protein. This study took a look at microbiological quality and effect of the incorporation of Warankashi on the nutritive value of white bread (especially in the area of protein quality) .

MATERIAL AND METHODS

The sourcing of the whole cow's milk and the Sodom apple leaf used in the production of warankashi was from an identified dairy farm situated in Ota. Other ingredients (flour, sugar, margarine, salt and yeast) that were used for the bread production were sourced from an open market situated in Ota.

Preparation of Warankashi

Warankashi (Wara) cheese was prepared from fresh raw cow's milk in the laboratory using the traditional method as explained by **O'Connor (1993**). A coagulant: milk ratio of 55

ml of Sodom apple plant extract (*Calotropis procera*) to 3 litres of raw milk was used. The raw fresh milk was filtered using a metal sieve to remove unwanted materials. The milk was then heated in a metal pot on a gas cooker and maintained at temperatures between 50 – 55 ^{o}C for 15 minutes. The coagulant, (Sodom apple plant extract) was being prepared by crushing eight medium sized leaves with a mortar and pestle after which it was added to 100 ml of luke warm water and stirred to enhance proper expression of the extract. After 5 minutes the juice extract was expressed into a bowl using a muslin cloth. The filtrate (55 ml) was added to a portion of the warm milk after which it was transferred to the whole milk lot. The mixture was stirred and the temperature was maintained at 50-55 ^{o}C. Coagulation began after 25-30 minutes after the addition of the coagulant and the surface scum was removed and the heating was intensified at 95-98 °C for 5 minutes to inactivate the plant enzyme and facilitate whey expulsion (Figure 1). The loose curd pieces (very soft) were poured into a metal sieve and allowed to drain. The cheese was sufficiently cooled at ambient temperature after which was put into a portion of whey stored in a plastic container in the freezer at -18 ^{o}C.

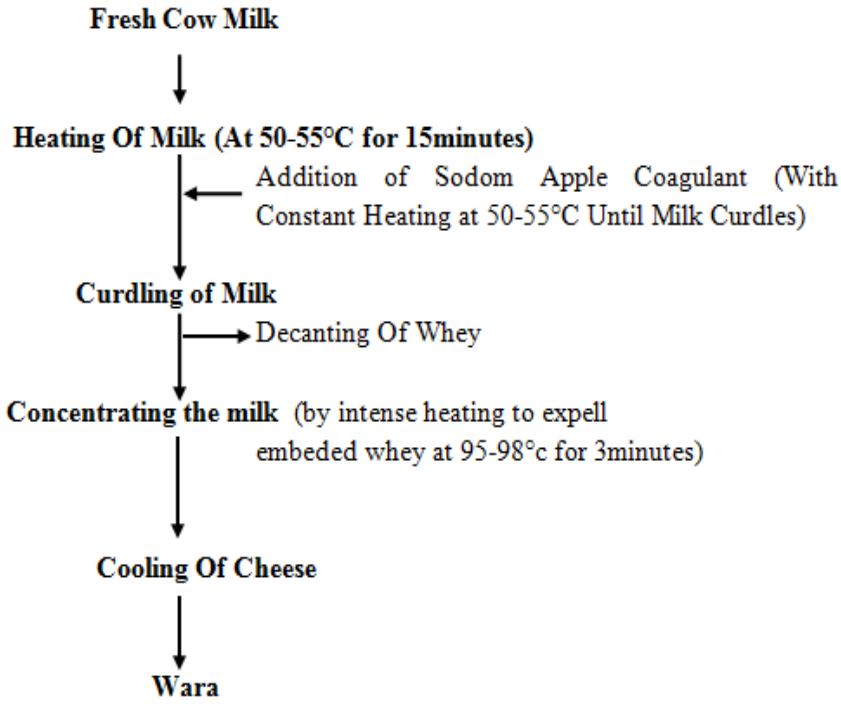

Figure 1: Flow Diagram of Warankasi Production

Preparation of Wara enriched bread samples

A straight dough process was used for the preparation of the wara enriched bread samples. Ingredients such as sugar, fat, salt and yeast were then added in appropriate proportions to each of the flour blends and the control flour. All - purpose flour (Golden penny flour) was used in the bread production. The warankashi was substituted based on flour basis into the bread dough (1 %, 3 % and 5 %). Warankashi substituted flours were mixed with bread ingredients individually in an automated mixer (3 minutes slow mixing and 12 minutes of fast mixing). The resulting dough was scaled (260 g) and then hand kneaded, shaped and panned. The dough was then subjected to proofing in a proofing chamber at 40 ^{0}C for 90 minutes. The proved bread was then subjected to baking at 190 ^{0} C for 25 minutes. The baked bread samples were then depanned and cooled at ambient temperatures and put in Ziploc bags prior to analysis.

Quantitative analysis

Each bread sample was grounded with their crusts and for analyzed for moisture content which was determined according to method 964.22 (**AOAC, 1990**); crude protein was determined using the Klejdahl method (**AOAC, 1990**); crude fat extracted in a Soxhlet extractor with hexane and quantified gravimetrically; ash according to method 923.03 (**AOAC, 1990**); also wet gluten was determined using method 10-11 (**AACC, 1984**). Crude fibre was determined. Lastly total available carbohydrates were calculated by difference.

Moisture Content

The Moisture Content was determined using procedure described by **AOAC, (1990)** was used**.** The moisture content of each sample was determined by weighing 5 g of the sample into an aluminium moisture can. The sample was then dried to constant weight at 105±2 °C.

$$\text{Moisture content} = \frac{\text{Weight of can} - \text{weight of empty can}}{\text{Weight of sample}} \times 100$$

Crude Protein

The Protein Content was determined using a Foss Tecator^{Tm} protein digestor and KJECTEC 2200 distillation apparatus (Kjeldahl method) according to the procedure of **AOAC, (1990)**. Concentrated H_2SO_4 (12 cm^3) and 2 tablets of catalyst were put into a Kjeldahl digestion flask containing 5 g of the sample. The flask was placed in the digestor in a fume cupboard and switched on and digestion was done for 45 minutes to obtain a clear colourless solution. The digest was distilled with 4 % boric acid, 20 % Sodium hydroxide solutions were automatically metered into it in the KJECTEC 2200 distillation equipment until distillation was completed. 0.1 M HCl 0.1M HCl was used to titrate the distillate until a violet colour formation indicating the end point. A blank was run under the same condition as with the sample. Total nitrogen content was then calculated according to the formula:

$$\text{Crude Protein} = \frac{(\text{Titre of sample} - \text{blank}) \times 0.01 \times 14.007}{10 \times \text{weight of sample}} \times 6.25$$

Crude Fat Content

Crude fat extracted in a Soxhlet extractor with hexane and quantified gravimetrically. 1 g of sample was weighed into an extraction thimble and then stopped with grease-free cotton. Before extraction commenced the round bottom cans was dried, cooled and weighed. The thimble was placed in extraction chamber and 80 ml hexane was added to extract the fat. The extraction was carried out at 155 ^{0} C lasted for 1 hour 40 minutes after which the fat collected in the bottom cans were cooled in a desiccator.

$$\text{Crude Fat} = \frac{\text{Weight of can + fat} - \text{Weight of empty can}}{\text{Weight of sample}} \times 100$$

Ash Content

Two grams of samples were weighed into well incinerated crucibles and then ashed in a muffle furnace at 600 ^{0}C for 3 hours. The ash content was calculated as

$$\text{Ash Content} = \frac{\text{Weight of crucible + Ash} - \text{Weight of empty crucible}}{\text{Weight of sample}} \times 100$$

Crude Fibre

Two grams of the sample was transferred into 1 litre conical flask litre. 100 ml of sulphuric acid (0.255 M) was heated to boiling and then introduced into the conical flask containing the sample. The contents were then boiled for 30 minutes and ensuring that the level of the acid was maintained by addition of distilled water. After 30 minutes, the contents were then filtered through a muslin cloth held in a funnel. The residue was rinsed thoroughly until its washing was no longer acidic to litmus. The residue was then transferred into a conical flask. 100 ml of sodium hydroxide (0.313 M) was then brought to boil and then introduced into the conical flask containing the sample. The contents were then boiled for 30 minutes and ensuring that the level of the acid was maintained by addition of distilled water. After 30 minutes, the contents were then filtered through a muslin cloth held in a funnel. The residue was rinsed thoroughly until its washing was no longer alkali. The residue was then introduced into an already dried crucible and ashed at $600\ ^0C \pm 200\ ^0C$.

$$\text{Crude Fibre} = \frac{\text{Final Weight of Crucible} - \text{Initial weight of crucible}}{\text{Weight of Sample}} \times 100$$

Wet Gluten

A weighed sample (25 g) was transferred into a clean dry mixing bowl and 15 ml of water was added. The contents were formed into a stiff dough ball. The dough ball was dipped into water for half an hour and then washed by hand under tap water until free from starch. The wet gluten thus obtained was weighed and its weight expressed as a percentage of the original flour sample (25 g).

$$\text{Wet Gluten} = \frac{\text{Weight of Gluten}}{\text{Initial Weight of Sample}} \times 100$$

Sensory evaluation

The Multiple Comparism Test method will be used for the sensory evaluation of the produced bread samples. A panel of 20 judges will be used in carrying out the evaluation. Samples will be coded with three digit random numbers and presented in random order. A 9-point hedonic scale rating crumb texture, crust texture, crust colour, appearance, flavour, taste and overall acceptability was used ; with 1 meaning extremely dislike and 9 extremely like. White bread without Wara substitution was used as the reference material.

Dough rheological testing

Farinograph

Farinograph Testing was carried out on control (All-purpose wheat flour) and enriched flour blends (0 %, 1 %, 3 %, 5 %) with the use of a Brabender - Farinograph®-E (AACC 54-21 / ICC 115/1 /ISO 5530-1) (**AACC, 2000**). The dough development time (DDT) was time for the dough to reach maximum consistency (peak); stability was the time that the top portion of the curve is above the 500 BU line; mixing tolerance index (MTI) is the drop in BU from the top of the curve at DDT to the top of the curve 5 minutes after DDT.

Extensograph

Extensograph Testing was carried out on control (All-purpose wheat flour) and enriched flour blends (0 %, 1 %, 3 %, 5 %) with the use of a Brabender- Extensograph®-E (AACC 54-10 / ISO 5530-2 /ICC 114/1) (AACC, 2000). A Brabender - Farinograph-E was used to mix the dough for 6 minutes after which the dough was subjected to proving at for 45 minutes after which the dough was stretched until rupture in the Extensograph®-E. This procedure was repeated twice after which a graph was plotted showing the exerted force as a function of the stretching length (time). The following parameters were determined from the graph:

1. Water absorption (%).
2. Energy (Area under the curve) (cm3).
3. Resistance to Extenison(BU).
4. Externsibility(mm).

5. Maximum (BU)
6. Ratio number.
7. Ratio number (Max.).

Textural analysis

Bread firmness

Bread firmness was measured on freshly baked bread loaves using a TVT-300XP texture analyzer which has a cylinder probe with a 1 kg load cell. The weighted probe which was positioned vertically over the surface of the test sample (six centre slices from the bread loaves) was allowed to fall unto the sample and the depth of penetration after a fixed period of time was determined. The bread macro software provided by the texture analyzer was used to collect the data and the results were presented in terms of hardness.

Physical measurement on bread

The loaf weight, volume, specific volume, density and height were determined with Tex-volume instrument BVM-L370.

Shelf life studies on bread samples

Physical analysis

The bread samples (duplicated) were stored under ambient temperature (26 ^{0}C - 33 ^{o}C) and refrigeration temperature (3 ^{0}C ± 2) and observed for 7 days. Bread samples were analysed for apparent spoilage by visual observations for mould growth. Visual analysis for presence of mold growth was carried out on the samples stored in each storage condition.

Microbial analysis

Total mesophilic (total viable count and fungi count (yeast and mould count) was carried out on the bread samples for eight days (analysis was carried out on a day interval i.e 0, 2, 4, 6 and 8th day) to determine the microbial load of the samples as described by **APHA**,

(1992). Bread samples were prepared by mashing and mixing in peptone water. Sub-samples were diluted decimally and spread plated. 0.1 millilitre aliquots were spread on nutrient agar (Oxoid) and incubated at 30° C for 48 hrs. The yeast and mould counts were determined by plating one millilitre of the aliquot on potato dextrose agar (Oxoid) and incubating the plates at 30° C for 48 hrs. Observed colonies were subcultured to obtain pure cultures which were subsequently isolated and identified using morphological characteristics, spore formation and production of fruiting bodies after incubation for 5 - 7 days.

Essential amino acids profile analysis

Eleven essential amino acids (valine, isoleusine, leucine, lysine, tryptophan, methionine + cystine and Phenylalanin + threonine (considered as nine) were obtained by ninhydrin colorimetric method of analysis. The extract was suitably diluted to 1ml of this was added 0.5 ml cyanide acetate buffer and 0.5 ml of 3 % ninhydrin solution in methyl cello solve. The mixture was heated for 15 minutes in 100 ^{o} C water bath. Thereafter, 5ml isopropyl alcohol water mixture as added and shaken vigorously. After cooling, the colour was read in a colorimeter at 570 nm. The concentration of amino acids was calculated from a standard graph based on known concentration of various amino acids.

RESULTS AND DISCUSSION

The formulation and proximate analysis of the control and the wara enriched bread samples are presented in Table 1, 2. It was observed that the protein content of wheat flour/wara enriched flour significant increased significantly ($P > 0.05$). However, there was no significant difference within the enriched flours ($P < 0.05$). There was significant increase ($P < 0.05$) in the moisture content as substitution levels increased. But It was discovered that the higher the enrichment level the higher the moisture content of the compared to that of the bread. The fat content of the fortified breads had slight increase; on the other hand there was a slight decrease in the carbohydrate content of the enriched breads compared to that made from of wheat flour. There was a decrease in the crude fibre of the enriched breads as compared to that of wheat. The ash content of the fortified breads compared to that of wheat bread had no significant change ($P \leq 0.05$) (Table 2).

Effect of Ware enrichment on farinograph parameters

The effect of wara enrichment on the rheological properties of wheat flour is summarised in Table 3. The Farinograph water absorption, dough stability time, dough development time and time to breakdown for the used wheat flour (control) were 58.90 %, 9. 40 minutes, 2.40 minutes and 6.50 minutes respectively (Table 3). Compared with the control, water absorption decreased by addition of Wara as a function of increasing protein content in the dough (Table 3). It was reported that gluten which is wheat dough protein and Wara (casein) are water insoluble (**Anton *et al*., 2008)**. Therefore, the lower water absorption of the blends could be related to the poor water absorption of the protein in Wara (casein). The dough development time increased in the 1 to 5 % substitution levels but was lower than that of wheat flour. The dough consistencies of the wara enriched flours were within tolerable limits (480 to 520 BU). The 1 % and 5 % (485 BU) enriched flours had the same dough consistency values but were lower than that of the 3 % which had the highest consistency value (520 BU). Dough Stability (DS) is given by the time from when the Farinograph trace touches the 500 BU line up to the break time. Dough stability decreased in the wara enriched dough compared to wheat flour (Table 3). This was due to the casein protein has an effect on wheat dough viscoelastic properties (**Zadow, 1981**). Therefore, by the addition of whey protein the dough rheological characteristics are negatively affected. This was also in line with **Zadow (1981)** who reported that addition of whey protein concentrate in the preparation of the bread resulted in a weaker and less elastic dough. He further opined that the weakening of the wheat flour dough was due to interference of whey protein concentrate sulphydryl groups in the normal sulphydryl/disulphide interchange reactions occurring during wheat flour dough development. Stability to mechanical agitation of the wara enriched flours were lower than that of wheat flour. The 5 % Wara enriched flour had the next highest stability followed by 1 % and then 3 %. The 5 % Wara enriched flour had the highest farinograph quality number of 75.

Effect of Wara enrichment on the extensograph parameters

The Extensograph energy, resistance to extension, extensibility, maximum, ratio number, ratio number maximum for the wheat flour (control) and Wara enriched flours were summarized in Table 4. It was discovered that at a proving time of 45 minutes, the energy of the wara enriched doughs had decreased compared to that of the all wheat flour dough but the

energies of each of the wara enriched dough had a slight increase. The extensibility of the wara enriched doughs were lower than that of the wheat flour dough. The resistance to extension of the wheat flour + 3% wara was higher than the rest of the sample.

Effect of Wara enrichment on the textural properties of the bread samples

Bread firmness was expressed as hardness. It was generally observed that as the levels of enrichment of wara increased the lesser the hardness of loaves compared to that of the control. Also, the weight of the samples remained unchanged as enrichment levels increased. It was also observed that the 1 % wara enriched bread had a higher dough volume (100.5 ml) compared to the rest of the bread samples which were not significantly different for each other. The density of the bread loaves remained unchanged. The springiness and cohesiveness were not significant difference but the bread of that of 3 % wara incorporated bread had a lower springiness and cohesiveness (0.87 and 0.70 respectively) (Table 5).

Effect of Wara enrichment on the shelf life of bread

Effect of physical spoilage

It was discovered that the different wara incorporation levels did not have any effect on the shelf-life of the bread samples. All the bread samples including the control samples stored under ambient temperature began spoilage from the third day of storage. Whereas, the was no spoilage recorded up to the seventh day for those stored in refrigeration condition (Table 6) which is similar to the result obtained by **Divya *et al.* (2009**).

Effect on the total viable count and fungi (yeast and mould) count of bread samples

It was discovered that the rate of microbial growth decreased as the enrichment level increased. The control had the highest bacteria and fungi growth compared to the rest of the samples (Tables 7 and 8). There was significant difference ($P < 0.05$) in both the total viable count and yeast and mould count of the bread samples stored at ambient and refrigeration temperatures (Tables 9 and 10). The 5 % wara enriched bread had a lesser bacteria and fungi growth compared to that of 3 % which was lesser than that of 1 % wara enrichment levels. This is in line with the results of **Divya *et al.* (2009)** who observed a decrease in the yeast and

mold count of the incorporation of Indian cheese whey (paneer) in bread making. Also, **Yousif *et al.* (1998)** observed that use of concentrated whey retarded staling and improved the keeping quality of French-type bread.

Effect of Wara enrichment on the sensory characteristics of bread

Based on the response of the panellist there was no significant difference ($P < 0.05$) in the organoleptic properties of the enriched bread samples compared to the control (wheat bread) (Table 11). However, the 3 % Wara enriched bread sample had the highest overall acceptability score of 7.55 followed by that of the 5 % enriched bread which had a score of 7.50 followed by that of the control which had a sore of 7.30 and then the least score of 7.15 which was for 1 % Wara enriched bread.

Effect wara enrichment on the protein quality of bread

Effect of wara enrichment levels on essential amino acid profile of bread

There was significant increase with each essential amino acid profile of wheat bread as enrichment levels increased (Table 12). This finding was backed by the fact that the milk proteins casein had an adequate supply of all the essential amino acids with the possible inclusion of sulphur-containing amino acids such as methionine and cysteine (**FAO/WHO, 1991**). Therefore, the increase in lysine, improved it's limiting attributes in wheat flour. It also improved the sulphur containing amino acids; methionine and cystine present in bread as well as improving the level of histidine.

Effect of Wara enrichment on the nutritive value of wheat bread

Table 1 Formulation for Wheat and Wara Enriched Wheat bread

	ENRICH (%)	Wara (g)	WF (g)	Yeast (g)	Fat (g)	Sugar (g)	Salt (g)	Improver (g)
WF	**0**	**0**	**1000**	**4**	**400**	**136**	**18**	**1**
WFW1	**1**	**10**	**1000**	**4**	**400**	**136**	**18**	**1**
WFW3	**3**	**30**	**1000**	**4**	**400**	**136**	**18**	**1**
WFW5	**5**	**50**	**1000**	**4**	**400**	**136**	**18**	**1**

The amount of water added was determined based on the water absorption values obtained from the farinograph.

Legend: WF: Hard Wheat Flour, WFW1: Wheat Flour + 1 % Wara, WFW3: Wheat Flour + 3 % Wara, WFW5: Wheat Flour + 5 % Wara

Table 2 Effect of Wara on the Proximate Composition of Bread

SAMPLES	PROTEIN (%)	CARBOHYDRATES (%)	ASH (%)	CRUDE FIBRE (%)	MOISTURE CONTENT (%)	FAT (%)
WF	8.17±0.73^{a}	57.77±1.58^{b}	2.36±0.11^{a}	1.05	27.92±0.66^{a}	2.74±0.09^{a}
WFW1	9.29±0.04^{ab}	55.65±0.55$^{a\,b}$	2.50^{a}	0.01	29.00±0.20^{a}	3.60±0.39
WFW3	9.83±0.14^{b}	54.66±1.39$^{a\,b}$	2.52±0.20^{a}	0.02	29.33±0.45^{a}	3.64±0.61^{a}
WFW5	10.39^{b}	51.26±1.67^{a}	3.45±0.99^{a}	0.03	29.45±0.63^{a}	5.42±0.06^{b}
WARA	34.20	10.50±0.12	1.71±0.03	0.03	39.82±0.01	14.74

Legend: Mean ± standard error, WF: Wheat Flour, WFW1: Wheat Flour+ 1 % Wara, WFW3: Wheat Flour+ 3 % Wara, WFW5: Wheat Flour + 5 % Wara

Table 3 Farinograph Parameters for Wheat Flour and Wara Enriched Flour Blends

FARINOGRAPH TREATMENTS	WF	WFW1	WFW3	WFW5
Water absorption(corrected for 500FU)	58.90 %	57.70 %	55.50 %	54.40 %
Water absorption (corrected for 14 %)	56.90	56.10	54.70	54.40
Development Time (min)	2.40	1.80	2.0	3.50
Stability (min)	9.40	4.10	3.4	7.70
Consistency (FU)	517	485	520	486
Tolerance Index (MTI) (FU)	30	51	58	37
Time to breakdown (mm)	6.50	5.0	4.0	7.50
Farinograph Quality Number	65	50	40	75
Moisture Content	12.30%	12.60 %	13.30 %	14.00 %

Legend: WF: Wheat Flour, WFW1: Wheat Flour+ 1 % Wara, WFW3: Wheat Flour+ 3 % Wara, WFW5: Wheat Flour + 5 % Wara

Table 4 Extensograph Parameters for Wheat Flour and Wara Enriched Flour Blends

EXTENSOGRAPH TREATMENTS	WF	WFW 1	WFW3	WFW5
Water absorption (%)	57.5	56.5	54.5	53.8
Proving Time (min)	45	45	45	45
Energy (cm^2)	140	123	131	138
Resistance to Extension (BU)	452	455	507	472
Extensibility (mm)	166	150	150	165
Maximum(BU)	700	662	760	732
Ratio Number	2.7	3	3.4	2.9
Ratio Number (Max.)	4.2	4.4	5.1	4.5

Legend: WF: Wheat Flour, WFW1: Wheat Flour + 1 % Wara, WFW3: Wheat Flour + 3 % Wara, WFW5: Wheat Flour + 5 % Wara

Table 5 Effect of Wara on the Textural Properties of Wheat Bread

	Weight	Volume	Specific volume	Density	Height	Total hardness	Springness	Cohessive
	(g)	(ml)	(ml/g)	(g/ml)	(mm)			
WF	221.0	950.90	4.35	0.20	11.00	258.00	0.87	0.71
WFW1	220.50	100.50	4.35	0.20	11.00	236.00	0.87	0.71
WFW3	220.50	951.60	4.30	0.20	10.00	233.00	0.85	0.70
WFW5	220.00	949.30	4.40	0.20	11.00	229.00	0.87	0.71

Legend: WF: Wheat Flour, WFW1: Wheat Flour + 1 % Wara, WFW3: Wheat Flour + 3 % Wara, WFW5: Wheat Flour + 5 % Wara

Table 6 Apparent Spoilage (Visual Observation of Mould Growth) At Ambient (32 ± 3 ^{0}c) and Refrigeration (-3± 2 ^{0}C) Temperatures

STORAGE DAYS	1		2		3		4		5		6		7	
SAMPLES	**RT**	**RF**	**RT**	**RF**	**RT**	**RF**	**RT**	**RF**	**RT**	**RF**	**RT**	**RF**	**RT**	**RF**
WF	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**
WFW1	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**
WFW3	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**
WFW5	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**	**+VE**	**NIL**

Legend: WF: Wheat Flour, WFW1: Wheat Flour + 1 % Wara, WFW3: Wheat Flour + 3 %Wara, WFW5: Wheat Flour + 5 %Wara, RT: Visual spoilage recorded in room temperature, RF: Visual spoilage recorded in refrigerated temperature, NIL: Not present

Table 7 Daily Total Viable Count Results of Bread Samples Stored Under Ambient and Refrigeration Temperatures (cfu/g)

	WF (cfu/g)		**WFW1 (cfu/g)**		**WFW3 (cfu/g)**		**WFW5 (cfu/g)**	
	AT	**RF**	**AT**	**RF**	**AT**	**RF**	**AT**	**RF**
DAY 0	2.6×10^4	NIL	NIL	NIL	NIL	NIL	NIL	NIL
	2.8×10^4	NIL	NIL	NIL	NIL	NIL	NIL	NIL
DAY 2	4.1×10^6	9×10^5	2.1×10^6	5×10^5	5×10^5	NIL	NIL	NIL
	3.8×10^6	7×10^5	2.4×10^6	3×10^5	2×10^5	NIL	NIL	NIL
DAY 4	3.8×10^7	1.8×10^7	1.8×10^7	9×10^6	1.1×10^7	2×10^6	1×10^6	2×10^5
	3.5×10^7	1.6×10^7	1.6×10^7	1.1×10^7	9×10^7	3×10^6	1×10^6	1×10^5
DAY6	17.8×10^7	3.6×10^7	9.4×10^7	2.8×10^7	7.5×10^7	1.1×10^6	2.4×10^7	5×10^6
	18.2×10^7	3.9×10^7	8.9×10^7	3.2×10^7	7.2×10^7	1.5×10^6	2.7×10^7	7×10^6
DAY8	9.2×10^8	6.7×10^7	21.6×10^7	4.9×10^7	14.9×10^7	7.7×10^6	7.4×10^7	1.9×10^6
	8.8×10^8	6.4×10^7	22.4×10^7	5.3×10^7	15.4×10^7	7.1×10^6	8.0×10^7	2.3×10^6

Legend: WF: Wheat Flour, WFW1: Wheat Flour + 1 % Wara , WFW3: Wheat Flour+ 3 % Wara, WFW5: Wheat Flour + 5 % Wara, AT- Ambient Temperature, RF- Refrigeration Temperature, NIL : Not present

Table 8: Daily Fungi Count (Yeast And Mould) Results of Bread Samples Stored Under Ambient and Refrigeration Temperatures (cfu/g)

	WF (cfu/g)		**WFW1 (cfu/g)**		**WFW3 (cfu/g)**		**WFW5 (cfu/g)**	
	AT	**RF**	**AT**	**RF**	**AT**	**RF**	**AT**	**RF**
DAY 0	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
DAY 2	1.8×10^{6} 2.2×10^{6}	1×10^{5} 1×10^{5}	8×10^{5} 5×10^{5}	NIL NIL	3×10^{5} 4×10^{5}	NIL NIL	1×10^{5} NIL	NIL NIL
DAY 4	3.9×10^{6} 4.2×10^{6}	4×10^{5} 5×10^{5}	1.2×10^{6} 1.8×10^{6}	1×10^{5} 2×10^{5}	6×10^{5} 9×10^{5}	1×10^{5} NIL	3×10^{5} 2×10^{5}	NIL NIL
DAY6	8.0×10^{7} 8.3×10^{7}	1.8×10^{7} 1.5×10^{7}	5.5×10^{7} 6.9×10^{7}	9×10^{6} 1.0×10^{7}	2.6×10^{7} 3.3×10^{7}	4×10^{5} 3×10^{5}	1.4×10^{7} 1.1×10^{7}	1×10^{5} 2×10^{5}
DAY8	15.5×10^{7} 14.9×10^{7}	3.9×10^{7} 4.4×10^{7}	9.4×10^{7} 9.9×10^{7}	3.0×10^{7} 2.5×10^{7}	5.1×10^{7} 4.8×10^{7}	3.6×10^{6} 3.9×10^{6}	3.3×10^{7} 3.7×10^{7}	2.3×10^{6} 2.8×10^{6}

Legend: WF: Wheat Flour, WFW1: Wheat Flour+ 1 % Wara, WFW3: Wheat Flour+ 3 % Wara, WFW5: Wheat Flour + 5 % Wara, AT- Ambient Temperature, RF- Refrigeration Temperature, NIL: Not present

Table 9 Daily Total Viable Count of Bread Samples Stored Under Ambient and Refrigeration Temperatures (Statistical Analysis)

	WF (cfu/g)		**WFW1 (cfu/g)**		**WFW3 (cfu/g)**		**WFW5 (cfu/g)**	
	AT	**RF**	**AT**	**RF**	**AT**	**RF**	**AT**	**RF**
DAY 0	4.44 ± 0.02^{c}	0	3.00^{b}	0	0	0	0	0
DAY 2	6.60 ± 0.02^{c}	5.90 ± 0.05^{c}	6.35 ± 0.03^{c}	5.59 ± 0.16^{b}	5.50 ± 0.20^{b}	0	0	0
DAY 4	7.56 ± 0.02	7.23 ± 0.03^{b}	7.23 ± 0.05^{c}	7.00 ± 0.05^{b}	7.00 ± 0.05^{b}	5.39 ± 0.09^{a}	6.00^{a}	5.15 ± 0.15^{a}
DAY6	7.27 ± 0.04^{c}	7.58 ±0.02^{d}	7.02 ± 0.03^{b}	7.48 ± 0.04^{c}	6.84 ± 0.06^{b}	6.11 ±0.70^{b}	6.54 ± 0.08^{a}	5.78 ± 0.08^{a}
DAY8	8.95 ± 0.01^{d}	7.82 ± 0.01^{c}	8.34 ± 0.01^{c}	7.71 ± 0.02^{b}	8.18 ± 0.01^{b}	7.86 ± 0.01^{c}	7.88 ± 0.02^{a}	6.32 ± 0.04^{a}

Legend: Mean of the log of duplicate samples ± standard error, WF: Wheat Flour, WFW1: Wheat Flour+ 1 % Wara, WFW3: Wheat Flour+ 3 % Wara, WFW5: Wheat Flour + 5 % Wara, AT- Ambient Temperature, RF- Refrigeration Temperature

Table 10: Daily Fungi Count (Yeast and Mould) of Bread Samples Stored Under Ambient and Refrigeration Temperatures (Statistical Analysis)

	WF (cfu/g)		**WFW1 (cfu/g)**		**WFW3 (cfu/g)**		**WFW5 (cfu/g)**	
	AT	**RF**	**AT**	**RF**	**AT**	**RF**	**AT**	**RF**
DAY 0	0	0	0	0	0	0	0	0
DAY 2	6.30 ± 0.04 [a]	5.00	5.80 ± 0.10 [a]	0	5.54 ±0.06 [a]	0	2.50 ± 2.50 [a]	0
DAY 4	6.61 ± 0.02 [d]	5.65 ± 0.05 [ab]	6.17 ± 0.09 [c]	5.15 ± 0.15 [ab]	5.87 ± 0.09 [b]	2.65 ± 2.65 [a]	5.39 ± 0.09 [a]	0
DAY6	7.91 ± 0.01 [c]	7.22 ± 0.04 [c]	7.79 [c]	6.98[c]	7.47 ± 0.06 [b]	5.54 ± 0.07 [b]	7.10 ± 0.06 [a]	5.15 ± 0.15 [a]
DAY8	8.18 ± 0.01 [d]	7.61 ± 0.03 [d]	7.98 ± 0.01 [c]	7.44 ± 0.04 [c]	7.70 ± 0.02 [b]	6.58 ± 0.02 [b]	7.55 ± 0.03 [a]	6.41 ± 0.05 [a]

Legend: Mean of the log of duplicate samples ± standard error, WF: Wheat Flour, WFW1: Wheat Flour + 1 % Wara, WFW3: Wheat Flour + 3 % Wara, WFW5: Wheat Flour + 5 % Wara, AT- Ambient Temperature, RF- Refrigeration Temperature

Table 11 Effect of Wara Enrichment on the Organoleptic Properties of Wheat Bread

Samples	Crust Colour	Appearance	Aroma	Crust texture	Crumb texture	Taste	Overall Acceptability
WF	6.65± 0.34	7.00±0.40	7.30 ±0.37	7.15± 0.30	6.60±0.31	6.95 ± 0.37	7.30±0.26
WFW1	7.25± 0.30	7.05±0.39	7.45±0.34	7.25± 0.24	6.85± 0.32	7.10 ± 0.38	7.15±0.36
WFW3	7.65± 0.25	7.40±0.28	7.15±0.23	6.90± 0.36	6.80± 0.34	7.50 ± 0.25	7.55±0.21
WFW5	7.10± 0.34	7.20±0.36	7.00±0.26	7.20± 0.20	7.25± 0.18	7.40 ± 0.18	7.50±0.18

Legend: Mean± standard error, WF: Wheat Flour, WFW1: Wheat Flour+ 1 % Wara, WFW3: Wheat Flour+ 3 % Wara, WFW5: Wheat Flour + 5 % Wara

Table 12 Effect of wara enrichment on the amino acid profile of wheat bread samples

Essential amino acids (g/16N)	WF	WFW1	WFW3	WFW5
Lysine	2.74	2.86	3.12	3.43
Valine	4.58	4.61	4.70	4.88
Isoleucine	4.23	4.57	4.69	4.83
Leucine	7.34	7.86	7.94	8.17
Tryptophan	1.28	1.47	1.58	2.1
Histidine	2.21	2.38	2.46	2.63
Threonine	3.37	3.51	3.74	3.82
Methionine + Cystiene	4.45	4.66	5.03	5.54
Phenylalanine+ Tryosine	9.46	10.12	10.59	10.86

Legend: WF: Wheat Flour, WFW1: Wheat Flour + 1 % Wara, WFW3: Wheat Flour + 3 % Wara, WFW5: Wheat Flour + 5 % Wara

CONCLUSION

The use of Warankashi in the enrichment of wheat bread had effect on the nutritional status of wheat bread with the protein and fat content of bread increased significantly ($P > 0.05$) with increasing enrichment levels. Also, the amino acid profile of wheat bread increased with increasing enrichment levels. Furthermore, the amino acid profile of the bread increased with increasing enrichment levels. The rheological characteristics of wheat bread were affected as water absorption rate decreased with increasing enrichment levels. The sensory characteristics of the wheat bread were not significantly different ($P < 0.05$). However, that of the 3 % Warankashi substituted bread had the highest overall acceptability. The shelf life of bread was unchanged but the levels of bacteria and fungi (yeast and mould) growth reduced significantly ($P < 0.05$) as enrichment levels increased. In conclusion, it was discovered that enriching wheat flour beyond 5 % substitution level, will be detrimental to the production of acceptable bread by the consumers as confirmed from these studies. From our findings in this study, we recommend higher enrichment levels beyond the 5 % composite Warankashi substitution in wheat bread to further confirm or otherwise, counter our findings on the 3 % substitution as the best substitution level for consumer acceptability.

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