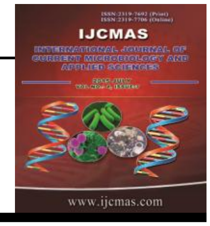
Int.J.Curr.Microbiol.App.Sci (2015) 4(8): 723-736

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 8 (2015) pp. 723-736**
http://www.ijcmas.com



Original Research Article

Lactic Acid Fermented Permeates and Mushroom Powder (*Pleurotus ostreatus* Hk 35) for Improvement of the Nutritional Value and Quality of Pan Bread

Manal Khider[1], Khaled Elbanna[2,3]*, Osama Seoudi[2] and Alaaeldien El-Fakharany[4]

[1]Department of Dairy Science, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt
[2]Deptartment of Agricultural Microbiology, Faculty of Agriculture, Fayoum University, Egypt
[3]Department of Biology, Faculty of Applied Science, Umm Al-Qura University, KSA
[4]Department of Food Science and Technology, Faculty of Agriculture, Fayoum University, Egypt
**Corresponding author*

Keywords

Pan bread, Fermented dairy permeate, Mushroom powder, Nutritional value, Bread properties, Extended shelf-life

A B S T R A C T

Malnutrition has many adverse effects on human health, especially in pre-schoolers and children of school age. In this study, fermented dairy permeate and mushroom powder were used for enhancing the quality and the nutritional values of pan bread. The highest sensory score was recorded for bread supplemented with 100 % fermented dairy permeate without mushroom powder (82.76). The bread made with 100 % fermented dairy permeate and 5 % mushroom powder had the highest protein (16.05 %), dry matter (63.23 %), and ash (1.84 %) contents. In addition, all mineral contents significantly were increased by adding fermented dairy permeate and mushroom powder. Compared to the control, the percentages in protein, calcium, phosphorus, potassium, magnesium, zinc, manganese, and iron increased by 31.99, 301.29, 102.35, 176.81, 43.94, 194.82, 90.90 and 268.49 percent, respectively. In addition, microbiological studies revealed that the total counts of spore formers and molds decreased as the percentage of fermented dairy permeate in the dough increased. From these results, it can be concluded that fermented dairy permeate and mushroom powder improve the quality and significantly increase the nutritional values of the bread via protein and mineral contents and extend the shelf-life of pan bread.

Introduction

Food supplementation defined as the addition of one or more nutrients to a food to improve its quality for the people who consume it, usually with the goal of reducing or controlling nutrient deficiency (Mahamud *et al.*, 2012). Bread is one of the most widely consumed food product in the world and it is an important staple food for many countries (Okafor *et al.*, 2012). Normal bread does not contain enough protein for human nutrition; addition of mushroom powder is an effective way to increase protein and other nutrients content in wheat bread (Okafor *et al.,* 2012; Mepba *et al.,* 2009; Agu *et al.,* 2010).

Mushroom is being widely used as food and food supplements from ancient times. It considered as one of the important food items for their significant roles in human health, nutrition and diseases (Okechukwu *et al.,* 2011; Kakon *et al.,* 2012). Mushrooms are edible fungi that contain high quality digestible protein that varied between (10–40%), carbohydrate (3–21%) and dietary fiber (3–35%) on dry weight basis depending on species. Most species contain all the essential amino acids about the same proportion as in egg. Lysine that is deficient in most grain cereals like wheat is the abundant essential amino acid in mushroom, so it will complement well with wheat flour to produce nutritionally balanced high quality bread. It is also consider as therapeutic foods and a good source of B-vitamins and vitamin C and significant amount of mineral elements (Okafor *et al.,* 2012; Aisya *et al.,* 2010; Xiaofei *et al.,* 2011; Patel *et al.,* 2012).

Permeate is an important by-product of ultra-filtration process in cheese industry. Meanwhile, substances of lower molecular weight, such as lactose and minerals, in addition to water-soluble vitamins and salts pass through the filter. Therefore, permeate can be considered as a solution of nutritious significance. It is a subject of environmental concern due to its high biological oxygen demand (Murad *et al.,* 2011; Marhamatizadeh *et al.*, 2012). A number of applications for whey-permeate have been developed in an effort to overcome the problem of its disposal. Permeate could be added as an ingredient to existing types of food supplements for moderately malnourished children. In baked products, whey permeate can offer many benefits, such as enhancing surface browning and which not only improve appearance but also imparts a pleasant caramelized flavor, moisture retention and development of a tender crumb structure. Whereas, Lactose content dictates permeates ability to improve the texture of baked products, affect their appearance and color. Permeate is also good source for electrolytes such as calcium, potassium, sodium, magnesium and phosphorus (Marhamatizadeh *et al.,* 2012; Udovicic *et al.*, 2013).

Lactic acid bacteria (LAB) occur naturally in several raw materials (e.g. milk, meat, flour, etc.) and generally used as natural starters in food fermentations; produce lactic acid as the major end product during fermentation. Their long history of safe use, led to a wide range of industrial applications i.e., flavor, texture and preservative qualities of many fermented foods such as cheese, yoghurt and breads (Holzapfel *et al.*, 2001). Formation of flavor by lactic acid bacteria based on formation of acidity, formation of flavor precursors such as amino acids and formation of volatile compounds or aroma. Lactic acid bacteria contribute to the production of safer foods by inhibiting the growth of pathogenic microbes or by removing chemicals or toxic contaminants (Ur-Rehman *et al.,* 2007; Bolourian *et al.,* 2010; Mortazavi *et al.*, 2011).

Pan bread with added mushroom powder and fermented permeate may be a convenient food format to satisfy consumer interest in the health and nutritional benefits offered by mushroom, nutrients and flavours in fermented permeate and extending the shelf life of pan bread. As far as we know, the use of mushroom and fermented permeate as a food supplements is rare. Therefore, the objectives of the present work are: (I) to develop a desirable and healthy pan bread using fermented permeate and mushroom powder as a food supplements, and (II) to evaluate the effect of different levels of lactic acid bacterial fermented permeate and mushroom powder on the chemical, physical, microbiological properties, organoleptic properties and extending the shelf life of pan bread.

Material and Methods

Material used in this study

Wheat flour (72% extraction), was obtained from Middle Egypt Flour Mills Co. at Fayoum Governorate, Egypt. Instant active dry yeast was obtained from the General Bakeries Co. Giza Governorate, Egypt. Permeate used in the bread making was obtained from Dairy Industrial Unit at Faculty of Agriculture, Fayoum University, Egypt. The major composition of fresh permeate used in bread making was determined. Lactic acid bacterial strains namely; LAB11, LAB13 and LAB107, which characterized in our previous studies, were used for preparing fermented permeate (Elbanna *et al.*, 2010).

Preparation of fermented permeate

Permeate was first diluted and the pH was adjusted to 6.4, and sterilized at 110°C for 15 minutes, then cooled inoculated with pervious strains and incubated at 32$\pm$ 2°C for fermentation.

Mushroom production and preparation

The fresh mushroom (*Pleurotus ostreatus*) was cultivated in private mushroom farm, Agriculture Recycling Unit, Faculty of Agriculture, Fayoum University, Egypt. Pure culture of *Pleurotus ostreatus*, HK35 was grown on Potato Dextrose Agar medium at 25°C for 21 days before used as inoculation.

Spawn preparation and Mushroom cultivation

Sorghum grains used as substrate for growth of fungus strain, the inoculant was prepared in polypropylene bags, each one contained 750 g of grains. The grains were washed for 2-3 times by water and surface sterilized with Clorox (1%) for 1 min, then washed several times to remove residual Clorox. The bags were autoclaved at 121º C for 20 minutes. After cooling, the grains inoculated with previously prepared 3 weeks old fungal culture strain. Aseptically 10 disks introduced into each bag and allowed to colonize sorghum grains for three weeks incubation at 27º C in absence of light. The bags containing the complete colonization of the grains surfaces by the fungal mycelia were kept at 4°C for use as inoculum, and later added to pasteurized straw at rate of 10% (w/w). Oyster mushroom was cultivated according to the method described by Pokhrel *et al.* (2013).

Mushroom powder preparation

The mushroom powder was prepared from fresh mature fruit bodies. For this, mushroom washed with cold water and dried in a thermostatically controlled oven with air fan at 60°C for 3 days, then milled to pass through a 60 mesh/inch sieve. The powder packaged in polyethylene bags at low humidity, sealed and stored in the refrigerator at 4°C until use.

Chemical composition

The major composition of mushroom powder, permeate and manufactured pan bread were determined according to the standard methods. Moisture, protein, ash and total nitrogen (macro-Kjeldahl method) were determined according to AOAC (2000). Elements content of ash was determined using Inductively Coupled Plasma (ICP) equipment (Model 6300 Duo UK, England); according to APHA, AWWA and WEF 2005. All chemical compositions expressed as dry matter basis. The pH values measured using (Kent EIL 7020) pH meter according to the method of Kosikowski (1982).

The major composition of prepared mushroom powder was 7.27% moisture, 25% protein /dm and 6.68% ash. Minerals content of mushroom powder ash was 2943.35, 893.08, 248.03, 68.17, 13.63, 4.94 and 1.36 mg/ 100g of dry matter, for K, P, Mg, Ca, Fe, Zn and Mn, respectively. The analyzed permeate contain 93.38, 6, 0.4, 0.24 % for moisture, lactose, ash, and protein (nitrogenous compounds), respectively. The pH value of the fresh permeate was 6.4. Ash permeate was also analyzed for minerals content; Ca (80 mg /100 g), K (40 mg /100 g), P (40 mg /100 g), and Zn (0.34 mg /100 g).

Organoleptic evaluation of pan bread

For organoleptic evaluation, samples of coded sliced bread served at room temperature (28± °C). The evaluation determined by Staff members of Food and Dairy Science departments, Faculty of Agriculture, Fayoum, Egypt. The parameters were, taste, odor, shape, crust color, crust appearance; crump color, texture and grain cell structure. The resulting data statistically was analyzed using SPSS (1999). Mean of the values, were compared with main effects by Duncan's multiple range tests (Duncan 1955) when significant F values were obtained ($P \leq 0.001$).

Pan bread experiment

To evaluate the effect of mushroom powder (0, 10%), fermented and non-fermented permeates (0, 25, 50, 75 and 100%) on the quality of pan bread, a prelaminary experiment was conducted. Due to the adverse effect of high concentration of mushroom powder on the sensory evaluation, therefore, its amount reduced from 10 to 5% in the main experiment. So, the treatments of main experiment is; T1: 60 ml water +100 g wheat flour as control, T2 (100% fermented permeate + 100 g wheat flour), T3 (50% fermented permeate +100 g wheat flour), T4 (25% fermented permeate +100 g wheat flour), T5 (95 g wheat flour + 5 g mushroom powder + 60 ml water), T6 (95 g wheat flour + 5 g mushroom powder + 100 % fermented permeate), T7 (95 g wheat flour + 5 g mushroom powder + 50% fermented permeate) and T8 (95 g wheat flour, 5 g mushroom powder +25% fermented permeate). Each previous treatment contained 1g instant active dry yeast, 1.0 g salt, 2.0 g sugar and 3.0 g shortening. All ingredients were placed in a mixing bowl (Kenwood Mixer) at 28 ± 2°C and mixing for 6 min with 60 ml water or mixed with fresh or fermented permeate. After mixing, the formulated dough rounded manually and leaved to rest for 10 min. The prepared dough (120 g) was placed in lightly greased a baking pan (5 x 9x 8cm) and fermented for 120 min in a cabinet at 30 ± 0.5°C and 85% relative humidity then baked for 20 min at 250°C in an electrical oven. The baked breads cooled for 60 min at room temperature (25 ± 2°C) and then packed in polyethylene bags for sensory evaluation and further analysis.

Pan bread weight and Specific volume

Bread loaf weight (g) was recorded after cooling for 1 h, volume of aerated loaves (cm^3) was measured by rapeseed displacement method as described by AACC (2000), and Specific volume (cm^3/g) of bread was calculated by dividing volume by weight.

Microbiological examination

Samples of different treatments were intervals taken under aseptic conditions for microbial examinations. The total counts and spore formers monitored during storage period using nutrient agar, while moulds and yeast were monitored using potato dextrose agar.

Result and Discussion

Prelaminary experiment of pan bread

Prelaminary experiment data showed that fresh and fermented permeates significantly increased the sensory evaluation of the pan bread, while using mushroom powder decreased all parameters to a certain limits, which was acceptable (data not shown). It was noticed that, bread supplemented with either fresh or fermented permeates showed the highest scores. On the other hand, bread supplemented with mushroom powder (10%) recorded the lowest scores compared to the control and other free mushroom treatments. This result might be related to the pigments and the oxidation of phenolic compounds in mushroom during baking, besides, the Maillard reaction which occurred from soluble sugars and free amino acids reaction during baking (Arendt *et al.*, 2007; Ulziijargal *et al.*, 2013). Therefor, the levle of mushroom powder was reduced from 10% to 5% in the main experiment for further work.

Main experiment of pan bread

Organoleptic properties of pan bread

Table 1 shows the effect of different levels of fermented permeate (100, 50, 25 and 0%) and mushroom powder (0 and 5%) on bread making properties. The data showed that there is a very significant difference (P<0.001) between bread treatments; in most of sensory parameters. Compared to control (78.48), the free mushroom bread supplemented with 100% fermented permeate (T2), recorded the highest total scores (82.25%), followed by bread made with 50% (T3) and 25% (T2) fermented permeates that were 80.49, 77.65%, respectively. Worth mentioning that, as fermented permeate amount increased, the scores of organoleptic increased. This may related to the metabolites compounds produced by lactic acid bacteria, which have a positive effect on the texture of bread, e.g. organic acids, exopolysaccharides and/ or enzymes. Organic acids affect the protein and starch fractions of flour. In addition, the drop in pH associated with acid production of lactic acid bacteria in fermented permeate causes an increase in the proteases and amylases activity of flour; thus leading to a softer texture (Ulziijargal *et al.*, 2013; Rizzello *et al.*, 2014). Moreover, the data presented in table 1 showed that, the free mushroom bread which contained fermented permeate had better shape, crust color, crust appearance, crump color, texture, odor, and taste, while all treatments that contained mushroom powder had low scores of all organoleptic parameters.

This may be due to more water absorbed by mushroom than did wheat flour; which affected negatively on texture and shape of the bread (Ulziijargal *et al.*, 2013). In addition, it was noticed that supplementation of pan bread with mushroom (T5, T6, T7 and T8) gave the bread dark color than the control.

This result is in coincidence with that reported by Eissa *et al.* (2007). However, there is no significant differences in shape and crust color of free mushroom bread treatments that contained only different levels of fermented permeate (T2, T3 and T4). While, there was a significant differences in shape and crust color scores between the treatments contained mushroom powder compared to other bread treatments.

Table.1 Organoleptic properties of pan bread as affected by fermented permeate and mushroom powder supplementation

Treatments	Shape (10)	Crust color (10)	Crust appearance (10)	Crump color (10)	Texture (10)	Grain cell structure (10)	Odor (20)	Taste (20)	Total scores (100)
T1	7.88^{ab}	7.88^{a}	7.6^{a}	7.48^{a}	7.38^{abc}	7.13^{ab}	16.63^{a}	16.5^{ab}	78.48^{ab}
T2	8.00^{ab}	8.13^{a}	8.25^{a}	8.0^{a}	8.25^{a}	7.88^{a}	17.00^{a}	17.25^{a}	82.76^{a}
T3	8.13^{a}	7.73^{a}	7.70^{a}	7.80^{a}	7.5^{ab}	7.75^{a}	16.88^{a}	17.0^{ab}	80.49^{ab}
T4	8.13^{a}	7.63^{a}	7.63^{a}	7.63^{a}	7.5^{ab}	7.0^{ab}	16.13^{a}	16.0^{ab}	77.65^{ab}
T5	6.50^{c}	6.27^{b}	5.75^{b}	6.25^{b}	6.13^{cd}	6.63^{b}	13.99^{b}	13.25^{c}	64.77^{c}
T6	6.13^{c}	6.25^{b}	5.87^{b}	6.0^{b}	5.75^{d}	7.00^{ab}	14.63^{ab}	14.00^{bc}	65.63^{c}
T7	7.13^{ab}	6.50^{b}	6.00^{b}	6.38^{b}	6.88^{bcd}	7.25^{ab}	15.13^{ab}	15.25^{ab}	70.52^{bc}
T8	6.88^{abc}	6.38^{b}	6.13^{b}	6.38^{b}	6.25^{bcd}	7.13^{ab}	14.5^{ab}	14.5^{abc}	68.15^{c}
SE±	0.39	0.35	0.33	0.31	0.41	0.37	0.87	0.95	3.002
Significance	***	***	***	***	***	***	**	***	***

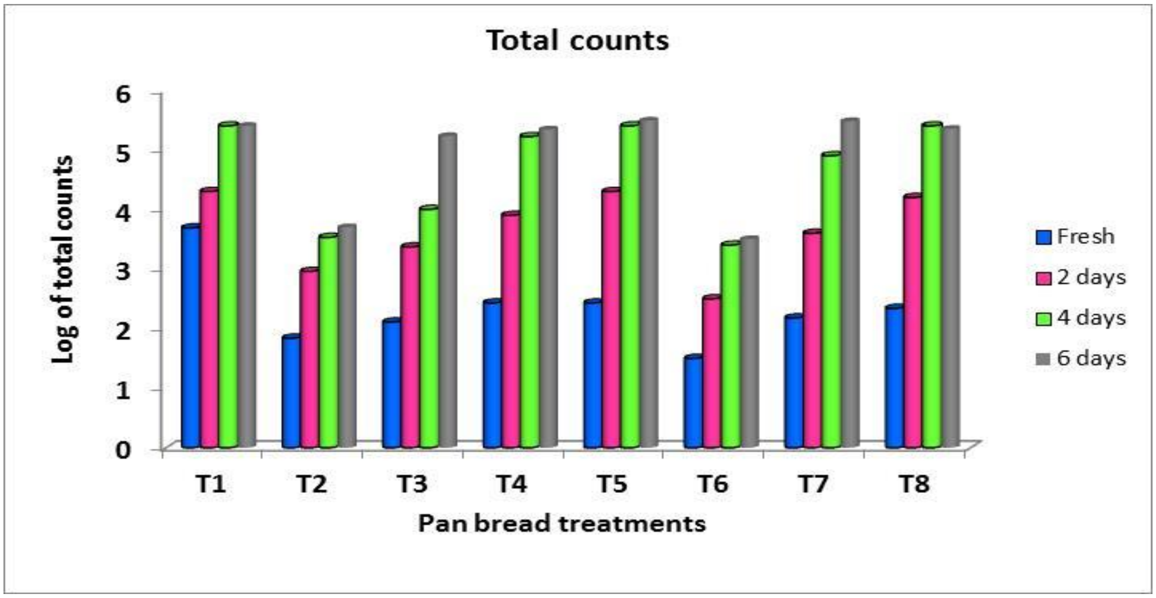
T1: 60 ml water +100 g wheat flour (control), T2: 100% fermented permeate + 100 g wheat flour, T3: 50% fermented permeate +100 g wheat flour, T4: 25% fermented permeate +100 g wheat flour, T5: 95 g wheat flour + 5 g mushroom powder + 60 ml water, T6: 95 g wheat flour + 5 g mushroom powder + 100 % fermented permeate, T7: 95 g wheat flour + 5g mushroom powder + 50% fermented permeate, T8: 95 g wheat flour + 25% fermented permeate + 5% mushroom. Means in the same column with different letters as superscript are significant different, *** ($P \leq 0.001$), ** ($P \leq 0.01$).

Table.2 Physical properties of pan bread as affected by fermented permeate and mushroom powder supplementation

Treatment	Volume(cm^3)	Weight (g)	Specific volume (cm^3/g)
T1	452.67^{a}	134.67^{a}	3.36^{c}
T2	391.67^{d}	134.67^{ab}	2.91^{d}
T3	530.0^{a}	134.67^{ab}	3.94^{a}
T4	492.3^{b}	135.67^{a}	3.63^{b}
T5	352.0^{g}	134.33^{b}	2.61^{g}
T6	359.67^{f}	135.33^{a}	2.66^{f}
T7	351.0^{g}	133.33^{b}	2.63^{fg}
T8	373.67^{e}	134.67^{ab}	2.78^{e}
SE $\pm$	1.093	0.441	0.011
Significances	***	NS	***

T1: 60 ml water +100 g wheat flour (control), T2: 100% fermented permeate + 100 g wheat flour, T3: 50% fermented permeate +100 g wheat flour, T4: 25% fermented permeate +100 g wheat flour, T5: 95 g wheat flour + 5 g mushroom powder + 60 ml water, T6: 95 g wheat flour + 5 g mushroom powder + 100 % fermented permeate, T7: 95 g wheat flour + 5g mushroom powder + 50% fermented permeate, T8: 95 g wheat flour + 25% fermented permeate + 5% mushroom. Means in the same column with different letters as superscript are significant different, *** ($P \leq 0.001$), ** ($P \leq 0.01$).

Fig.1 Effect of fermented permeate and mushroom powder supplementation on total counts of pan bread



T1: 60 ml water +100 g wheat flour (control), T2: 100% fermented permeate + 100 g wheat flour, T3: 50% fermented permeate +100 g wheat flour, T4: 25% fermented permeate +100 g wheat flour, T5: 95 g wheat flour + 5 g mushroom powder + 60 ml water, T6: 95 g wheat flour + 5 g mushroom powder + 100 % fermented permeate, T7: 95 g wheat flour + 5g mushroom powder + 50% fermented permeate, T8: 95 g wheat flour + 25% fermented permeate + 5% mushroom.

Regardless of bread treatments that contained mushroom powder recorded low values of organoleptic properties, but also it was acceptable to the panelists. Ulziijargal *et al.* (2013) found that incorporating 5% mushroom into the bread decreased the bread acceptability reported similar results.

Physical properties of pan bread

As shown in table 2, bread supplemented with 25% and 50% fermented permeate resulted in highly significant (P<0.001) improvement in specific volume. The data clearly indicated that pan bread made with 100 and 50% free mushroom fermented permeate recorded the highest volume which 492.33 and 530 cm^3, respectively, compared to control (452.67 cm^3) and other treatments.

Regarding the specific volume, also the bread made with 50% fermented permeate (T3) recorded the highest specific volume (3.94 cm^3/g), while addition of mushroom had negative effect on bread specific volume which was 2.61, 2.63 cm^3/g, for T5 and T7, respectively. These results are in agreement with that reported by Okafor *et al.* (2012) and Seguchi *et al.* (2001), who found that the addition of mushroom powder to bread negatively affected the height and specific volume that may be due to its proteases which decrease dough functionality and loaf volume potential (Nightingale *et al.*, 1999).

Chemical composition of pan bread

The data presented in table 3, show the effect of adding mushroom powder and fermented permeate or its mixture on chemical composition of different bread treatments. Compared to control (56.45%), supplementation of pan bread by fermented permeates and mushroom powder significantly (P<0.001) increased the dry matter content with (63.23%). The highest dry matter contents were recorded for all bread treatments contained mushroom and different levels of fermented permeate (T6, T7 and T8) which were 63.23, 63.22 and 61.45%, respectively. While free mushroom bread treatments (T2, T3 and T4) that contained only fermented, permeate showed slight increases in dry matter contents, which were, 59.37, 58.66 and 58.24%, respectively. These results indicated that supplementation of bread with mushroom powder and fermented permeate increased the dry matter content in bread. These results are in agreement with that reported by Okafor *et al.* (2012) who found that bread supplemented with 5% mushroom powder contained 68% dry matter. This increase in dry matter content of bread correlated to the protein and ash content in mushroom powder.

Regarding the content of protein in bread treatments; the data in table 3 showed that the protein content of bread was highly significant (P<0.001) increased by addition of both mushroom powder and fermented permeates. The highest protein content was recorded for the treatment supplemented with 100% fermented permeate and 5% mushroom powder (16.05%) (T6), while the lowest value was for control (12.16%). Other free mushroom treatments (T2, T3 and T4) that contained only fermented permeate had slightly increased in protein content. The high protein content in bread treatment containing mushroom is due to the high protein content in mushroom and biomass of lactic acid bacteria grown in fermented permeate. These results were in accordance with that reported by Eissa *et al.* (2007) who found that the protein content in balady bread supplemented with 5% mushroom flours was 7.50 %, compared to control, which was 5.67%. In addition, Okafor *et al.* (2012) found that the protein content in free mushroom and bread supplemented with 5% mushroom powder was 7.96 and 9.68, respectively. In this context, it was reported that the protein content and ash in mushroom powder were approximately 20-35 and 7.5%, respectively (Mahamud, 2012; Kakon *et al.,* 2012).

The data presented in table 3 also showed the effects of adding mushroom powder, fermented permeate and its mixture's on ash content of bread. From these data, it was clearly noticed that the ash content increased by supplementation of bread with fermented permeate and mushroom powder. The highest ash values were recorded for all treatments that contained mushroom and fermented permeate (T6, T7 and T8) which were 1.84, 1.84 and 1.83%, respectively, while the lowest ash value was for control (1.45%). Similar results were reported by Mahamoud *et al.* (2012) who found that the ash value of bread contained 5% mushroom was 1.82%.

The pH values of pan bread treatments (Table 3) were very significantly (P<0.001) decreased by adding fermented permeate; whereas the lowest value (5.5) was recorded for bread made by 100% fermented permeate (T2), while the highest pH value (6.2) was recorded for bread supplemented with only mushroom (T5), followed by control treatment (6.05). The drop in pH was resulted from lactic acid produced by lactic acid bacteria in fermented permeate.

Comparing to control, generally all minerals content were significantly (P<0.001) increased (Table 3) by adding fermented permeate and mushroom powder or its mixtures. The highest values of Ca, P, K, Mg, Zn, Mn and Fe were recorded for the bread made by 100% fermented permeate and 5% mushroom (T6) which were 148.44, 250.50, 416.60, 80.77, 1.71, 1.68 and 16.14 mg/100 gdm, respectively. While, the lowest values of these elements were recorded for control, which were 36.99, 123.79, 150.50, 56.11, 0.58, 0.88 and 4.38 mg/100 gdm, respectively. Compared to control, the increasing percentage in protein, calcium, phosphorus, potassium, Magnesium, zinc, manganese and iron was 31.99, 301.29, 102.35, 176.81, 43.94, 194.82, 90.90 and 268.49%, respectively. The increases in minerals content in all bread treatments which contained mushroom powder and fermented permeate was related to the high mineral contents in mushroom and permeate (Mahamoud *et al.,* 2012; Okafor *et al.,* 2012; Kakon *et al.,* 2012; Murad *et al.,* 2011; Udovicic *et al.,* 2013). It was reported that mushrooms are not only sources of nutrients but have also been reported as therapeutic foods which act as metabolic activators, prevent/control intoxication and microbial/viral infections, help in immune-balancing and immunomodulation, as antioxidants with rejuvenating and energy boosting properties, useful in preventing diabetic, cancer and heart diseases (Okafor *et al.,* 2012; Aisya *et al.,* 2010; Patel *et al.,* 2012).

Nokes *et al.* (1998) reported that the micronutrient deficiencies are widespread in many developing countries and are common among young person's hailing from low socioeconomic groups. Deficiency is associated with significantly poorer performance on psychomotor and mental development scales and behavioral ratings in infants, lower scores on cognitive function tests and lower educational achievement tests in preschool and school age children.

Microbiological examination of pan bread

In all bread treatments, total counts, spore formers and moulds enumerated during the storage period (Fig. 1, 2 and 3). Generally, the counts of all examined microorganisms were decreased as the percentage of fermented permeate increased.

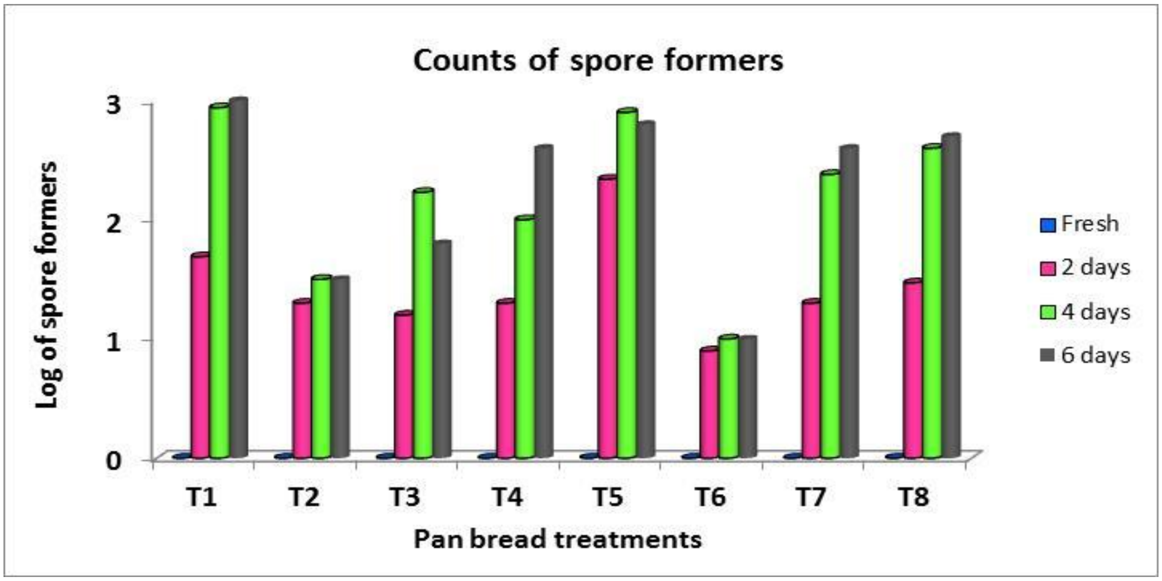
Table.3 Chemical composition (%) and Elements content (mg/100gdm) of pan bread as affected by fermented permeate and mushroom powder supplementation

Chemical composition	Bread Treatments										Increasing (%)*
	T1	T2	T3	T4	T5	T6	T7	T8	SE±	Sig.	
DM	56.45^{f}	59.37^{c}	58.66^{d}	58.24^{e}	59.35^{c}	63.23^{a}	63.22^{a}	61.45^{b}	0.007	***	12.01
Protein/dm	12.16^{h}	13.78^{e}	13.59^{f}	12.94^{g}	15.12^{d}	16.05^{a}	15.799^{b}	15.698^{c}	0.007	***	31.99
Ash/dm	1.45^{f}	1.77^{c}	1.62^{d}	1.49^{e}	1.79^{b}	1.84^{a}	1.84^{a}	1.83^{a}	0.003	***	26.89
Ca	36.99^{h}	61.85^{e}	59.93^{f}	57.267^{g}	69.87^{d}	148.44^{a}	94.56^{b}	82.05^{c}	0.291	***	301.29
P	123.79^{h}	169.68^{e}	159.59^{f}	136.85^{g}	193.19^{d}	250.50^{a}	207.97^{b}	204.61^{c}	0.361	***	102.35
K	150.50^{h}	271.63^{e}	197.61^{f}	179.82^{g}	311.7^{d}	416.60^{a}	370.39^{b}	334.08^{c}	1.004	***	176.81
Mg	56.11^{h}	68.24^{e}	63.07^{f}	58.15^{g}	72.65^{d}	80.77^{a}	77.73^{b}	77.05^{c}	0.166	***	43.94
Zn	0.58^{h}	1.21^{e}	0.99^{f}	0.88^{g}	1.40^{d}	1.71^{a}	1.65^{b}	1.58^{c}	0.004	***	194.82
Mn	0.88^{g}	1.20^{e}	1.20^{e}	0.95^{f}	1.26^{d}	1.68^{a}	1.53^{b}	1.49^{c}	0.004	***	90.90
Fe	4.38^{g}	9.28^{e}	5.24^{f}	4.94^{f}	9.52^{d}	16.14^{a}	11.49^{b}	10.62^{c}	0.036	***	268.49
pH	6.05^{b}	5.54^{f}	5.73^{d}	5.83^{c}	6.2^{a}	5.64^{e}	5.73^{d}	5.85^{c}	0.029	***	-

T1: 60 ml water +100 g wheat flour (control), T2: 100% fermented permeate + 100 g wheat flour, T3: 50% fermented permeate +100 g wheat flour, T4: 25% fermented permeate +100 g wheat flour, T5: 95 g wheat flour + 5 g mushroom powder + 60 ml water, T6: 95 g wheat flour + 5 g mushroom powder + 100 % fermented permeate, T7: 95 g wheat flour + 5g mushroom powder + 50% fermented permeate, T8: 95 g wheat flour + 25% fermented permeate + 5% mushroom. Means in the same column with different letters as superscript are significant different, *** (P≤0.001), ** (P≤ 0.01). The increase percentage in all nutrients was calculated using the following equation: .

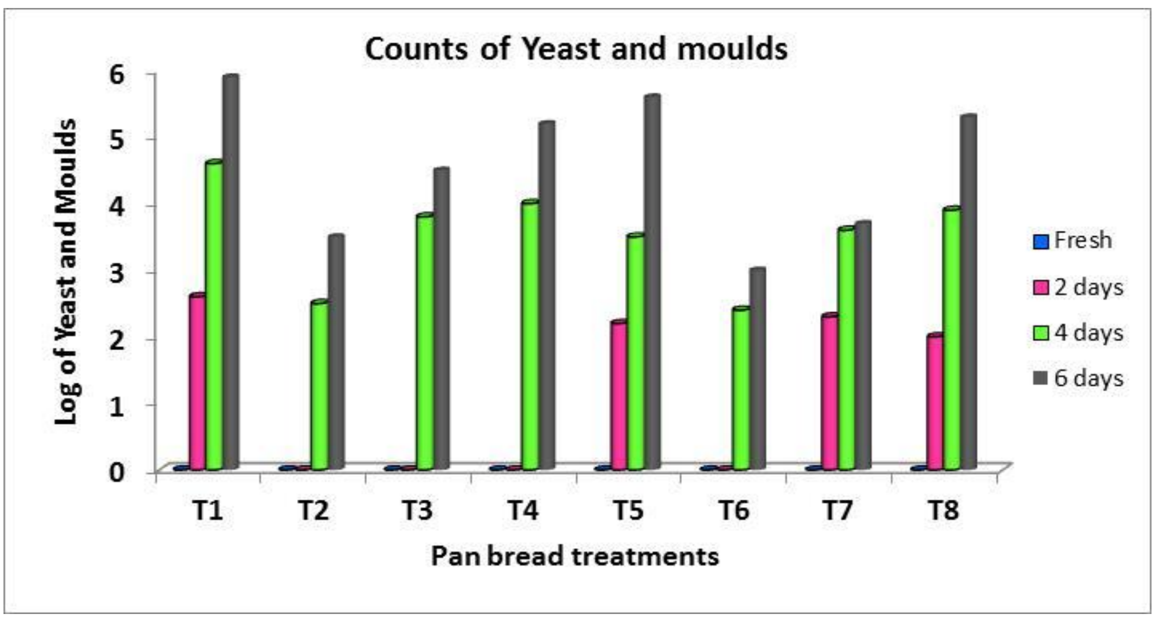
$$\text{Increasing (\%)} = \frac{\text{Bread treatment supplemented with mushroom and 100\% permeate (T6)} - \text{Control (T1)}}{\text{Control (T1)}} x100$$

Fig.2 Effect of fermented permeate and mushroom powder supplementation on spore formers of pan bread



T1: 60 ml water +100 g wheat flour (control), T2: 100% fermented permeate + 100 g wheat flour, T3: 50% fermented permeate +100 g wheat flour, T4: 25% fermented permeate +100 g wheat flour, T5: 95 g wheat flour + 5 g mushroom powder + 60 ml water, T6: 95 g wheat flour + 5 g mushroom powder + 100 % fermented permeate, T7: 95 g wheat flour + 5g mushroom powder + 50% fermented permeate, T8: 95 g wheat flour + 25% fermented permeate + 5% mushroom.

Fig.3 Effect of fermented permeate and mushroom powder supplementation on yeast and moulds of pan bread



T1: 60 ml water +100 g wheat flour (control), T2: 100% fermented permeate + 100 g wheat flour, T3: 50% fermented permeate +100 g wheat flour, T4: 25% fermented permeate +100 g wheat flour, T5: 95 g wheat flour + 5 g mushroom powder + 60 ml water, T6: 95 g wheat flour + 5 g mushroom powder + 100 % fermented permeate, T7: 95 g wheat flour + 5g mushroom powder + 50% fermented permeate, T8: 95 g wheat flour + 25% fermented permeate + 5% mushroom.

The log of total counts was increased during storage period in all treatments and the counts of microorganisms became nearly stable by the end of storage (6 days). It was noticed that bread made with 100% fermented permeate and 5% mushroom (T6) or made with 100 % fermented permeate without mushroom (T2) recorded the lowest log of total counts (Fig. 1) which were 3.5 and 3.7, respectively. Similar observation was noticed for spore formers and moulds, whereas the lowest log counts were recorded for T6 and T2 which were 1.0, 1.5, 3.0 and 3.5 (Fig. 2 and 3), respectively. These results indicate that, the decreasing in microbial counts, spore formers and moulds may be due to the ability of lactic acid bacteria to produce a variety of antimicrobial agents, including organic acids, and bacteriocins (Cintas *et al.*, 2001; Deegan *et al.*, 2006; Khanian *et al.*, 2014; Dalie *et al.*, 2010). In this context, it was reported that these bacteria are well recognized for their extensive use in food processing, such as in dairy and meat fermented products for improving shelf life, texture, and organoleptic properties. Additionally, they play an essential role in food preservation (Katina, 2005). Mortazavi, and Sadeghi (2011) reported that the inhibition and control of the spoilage microorganisms in bread maybe due to low pH value and antimicrobial activity produced by lactic acid bacteria. Also, Katina (2005) found that *Bacillus* spp. caused bacterial spoilage and potential risk of the bread when present at level 10^5 CFU/g in breadcrumb.

From these results, it could be concluded that the supplementation of pan bread with fermented permeate and mushroom powder improved the quality and significantly increased the nutritional value of the bread via protein and mineral contents and extendind the shelf-life of pan bread.

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