

## **EFFECT OF FERMENTATION AND AUTOCLAVING ON CHEMICAL AND PHYSICAL CHARACTERISTICS OF WHEAT MILLING BY-PRODUCTS AND ITS EFFECTS ON BREAD-MAKING.**

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### **ABSTRACT**

In the present study, wheat milling by-products especially with high fiber content (i.e. fine bran, coarse bran, brown shorts and white shorts) were used in replacing part of wheat flour to the production of high fiber bread. Our aim was to produce good quality wheat bread containing up to 10 - 20 % of dietary fiber (DF) by using some treatments (fermentation and autoclaving) of wheat by-products (bran-shorts) to optimizing the baking process. The breads containing 20 % wheat bran on a flour basis had low volume and poor crumb structure. Reduction of bran particle size improved crumb structure and mouth feel but the volume of the bread was not improved. Pre-fermentation of the wheat bran or shorts with yeast or with auto-fermentation improved loaf volume and crumb structure, and bread had added flavor.

### **INTRODUCTION**

Wheat is the world's most important food grain. Wheat kernel can be divided into three main parts: i) The endosperm, which forms 83% of the kernel, ii) the bran which forms 14%, and iii) the germ which forms 3%. In general, wheat is mainly consumed in bakery products manufactured from endosperm flour, which has unique technological properties for creating superior, consumer appealing product quality in terms of flavor and texture. The endosperm part of cereal kernel, however, is nutritionally inferior to the whole grain. Recent epidemiological findings have indicated a protective role of whole grain foods against several western diseases (*Jacobs et al. 1998, Liu et al. 2000, Pereira et al. 2002*). Dietary fiber has long been considered the major health protective component of grains. There is now increasing evidence also of other protective compounds, such as oligosaccharides and phytochemicals, which together with dietary fiber (DF) are concentrated in the outer layers of the grains. There is consumer interest in the health aspects of food, including functional food products with specific physiological functions of health relevance. However, good sensory properties remain a prerequisite for any successful food,

The most common source of DF in wheat baking is cereal bran, especially wheat bran. However, additions of cereal bran, especially in such amounts that health benefits can be expected, cause severe problems in the flavor and texture. Bran supplementation usually weakens the structure and baking quality of wheat dough and decreases bread volume and the elasticity of the crumb. (*Laurikainen et al. 1998*). It has been suggested that the

deleterious effects of fiber addition on the dough structure are due to the dilution of the gluten network, which in turn impairs gas retention rather than gas production (Gaillard and Gallagher 1988). According to Gan et al. (1992), the bran materials in expanded dough appear to disrupt the starch-gluten matrix and also restrict and force gas cells to expand in a particular dimension. This greatly distorts the gas cell structure and may contribute to the resultant crumb morphology, which is an important element of crumb texture. Thus, the supplementation of dietary fiber, such as wheat bran, requires changes in processing techniques for the production of baked goods with good consumer quality. Lal, et. al. (1989) found that the shorts, a wheat milling by-product distinct from bran and germ, has a specific, detrimental effect on loaf volume of bread. Shorts is a mixture of germ, aleurone and pericarp layers produced on reduction rolls. Compared to bran, shorts contains more of the aleurone layer and germ. Therefore, shorts and bran have different effects on breadmaking.

A novel method, bran sourdough, was introduced to overcome deleterious effect of bran addition in high-fiber baking. In the future, bran sourdoughs or other fermented milling fractions can be designed to produce nutritionally and technologically superior raw materials for all cereal foods, such as bread, breakfast and snack foods. Spontaneous dough fermentation starts by mixing flour with water without adding a starter culture or portion of a preceding sourdough (mother dough). The microflora of such dough depends on the microflora of the raw materials used and the prevailing hygienic conditions, and is variable in terms of kind, origin and storage conditions of the flour, as well as the technological parameters of the fermentation process applied. Degutyte- Fomins et al. (2002), found an increased solubility of beta-glucan in fermented oat bran suspension and a decreased viscosity of water soluble fraction in bran ferment. The controversial results might be due to the different acidity levels obtained and the differences in the chemical composition and enzyme activity of the preferments. Boskov-Hansen et al. (2002) found a reduced in dietary fiber content and increased in solubility of arabinoxylans during imitated rye sourdough fermentation. The mechanism for reducing the content of DF is not clear, but it is postulated to be due to the ability of microorganisms to produce extracellular enzymes that are either cell-free or cell-associated (Schwarz 2001). Exopolysaccharides (EPS.) (dextran, xanthan and levan) are produced by bacteria, which might have a positive effect on bread volume and shelf-life (Korakli et al. 2001, Tieking et al. 2003). Some of the reported benefits of sourdough on bread quality may be based on the formation of these compounds. Marttila, et. al.(2001)reported that it is possible to produce good consumer quality wheat bread containing up to 10-12% of dietary fiber, by optimizing the baking process :-

- Reduction of bran particle size improved crumb structure and mouthfeel.
- Prefermentation of the wheat bran with yeast or with yeast and lactic acid bacteria improved loaf volume, crumb structure and shelf-life. The bread had added good flavor and homogenous and elasticity of the crumb was excellent.
- Commercial baking enzymes also had a positive effect on the volume, structure and shelf-life of bread containing wheat bran.

Kati Katina (2005) reported that wheat sourdough was shown to be an efficient tool in improving bread flavor and texture. Lactic acid bacteria fermented sourdoughs were more effective in tuning bread quality compared to yeasted preferment if the appropriate conditions were utilized. Wheat bran is an important source of dietary fiber and bioactive compounds. However, addition of wheat bran in baking results in inferior bread quality. A novel method of bran sourdough was developed to pre-treat bran prior to the baking process. This pre-treatment resulted in significant improvement of bread texture due to modified starch-protein network. Sourdough thus shows promise also for production of nutritionally superior high-fiber raw materials for different cereal foods.

*Nayini and Markakis* (1983) reported that the phytate decreased and the inorganic phosphate increased, with the largest decrease in phytate occurring during first 30 min. of fermentation. Myo-Inositol 1, 2, 3, 4, 5, 6 Hexakis (dihydrogen phosphate), also known as phytic acid (PA), is nutritionally important because it may bind multivalent minerals (e.g., Zn, Fe) and render them unavailable for absorption in intestinal tract. Phytase is also present in bacteria, yeasts, and fungi. Significant decrease in PA content was observed during temper fermentation of soy beans and the noncom fermentation of peanuts. The disappearance of phytate and concurrent appearance of inorganic phosphate in yeast-raised bread was observed by several investigators.

*Fredlund et. al.*(1997) investigated the reduction of phytic acid occurred during hydrothermal treatment of whole grain wheat, rye, hulled and dehulled barley, hulled oats and naked oats were incubated with either water or acetate buffer (pH 4.8) at 55 °c for 24h with exception of oats, which were incubated at 37 °c. Phytate in wheat, rye and barley was reduced by 46-77 % when water was used and by 84 – 99% when acetate buffer was used. The phytate reduction in oats was considerably less, 8-26%, but after grinding and soaking, phytate was reduced by 72-77% in dehulled oats and by 88-94% in naked oats. Phytate is present in all cereals and contents 50-85% of the total content of phosphorus. Phytate negatively effects the bioavailability of many essential elements, such as Calcium, Iron, and Zinc.

## **MATERIALS AND METHODS**

### **Material:**

Wheat flour (72 % extraction) and other wheat by products were obtained from South Cairo Mills Co., Cairo, Egypt. Instant dry yeast (HASMAYA, made in Turkey) and salt, were purchased from the local market.

### **Methods:**

#### **\*Analytical procedures:**

Flour and other wheat by products were analyzed for moisture (method 44-15A), protein (method 46-11), lipid (method 30-10), ash (method 08-01), and crude fiber (method 32-10) by AACC (2002) procedures.

Phytic acid was determined according to the method described by *Weeler and Ferrel* (1971).

The total dietary fiber (TDF) content was analyzed by the gravimetric method of the AOAC (2005) (method 43.A14-43.A20) based on digestion of the sample with a heat-stable  $\alpha$ -amylase, protease and amyloglucosidase. The results were corrected for undigested protein (Kjeldahl  $N \times 6.25$ ) and ash (ignition at 525 °c for 8 hr) associated with the fiber. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed on fibertic apparatus using the Goering & Van Soest(1970) procedures. The NDF residue was incubated with  $\alpha$ -amylase from porcine pancreas for 18hr at 37 °c to complete the starch digestion (method 32-20, AACC). Hemicellulose was calculated as the difference between NDF and ADF. Cellulose content was estimated from the amount of ADF residue that dissolved in 72 % sulfuric acid.

**\*Samples preparation:**

1) **Fermentation:** Two types of fermentation were carried out:

a- **Natural Fermentation:** The samples were fermented according to the methods described by *Martin-Caberias, et. al.,(2004)*. Wheat-by products (coarse bran, fine bran, brown shorts and white shorts) were mixed with tap water (1 :3.5) in large plastic pans, which were covered and were allowed to ferment naturally (with only the microorganisms on the raw materials) at 37 °c for 48 hr in a fermentation units

b- **Yeast Fermentation:** The samples were fermented according to the methods described by (*Marttila et al, 2001*). Wheat-by products (coarse bran, fine bran, brown shorts and white shorts) and 1.25% instant dry yeast were mixed with tap water (1 :3.5) in large plastic pans, which were covered and were allowed to ferment at 37 °c for 16 hr in a fermentation units

2) **Autoclaving:** (as a hydrothermal treatment) was processed according to the method described by *Vijayakumart, et. Al.,(1995)*. This method was used with the fermented and non fermented wheat-by products, which were mixed with tap water 1:3.5 (w/w). The samples were autoclaved at 15 lb pressure (121 °c) for 30, 60 and 90 min. the samples were dried and powdered in Maxy Hermetic Mill Grinder, patent N: 53985 B, Italy and sieved to 60 mesh size. Phytic acid and dietary fiber contents were as described above.

3) **Bacteriological evaluation:**

Appropriate dilutions prepared from each sample were used for inoculating different nutrient and selective media. The microbial determinations were applied as follows:

**A) Total aerobic viable counts:**

Aerobic bacterial counts were estimated on glucose yeast extract nutrient agar medium as the method reported by (APHA, 1990) using pouring plate technique. Suitable plates were counted after incubation at 37C° for 48 hours.

**B) Determination of total counts of fungi**

Total counts of fungi were determined on potato dextrose agar days and the counts of fungi (cfu /g) were determined as described in American Public Health, Association (1990) and Oxoid Manual (2000). Isolation and identification of fungi.

Developed colonies on PDA medium were transferred to PDA slants and purified using the single spore technique (Hansen, 1926) and / or hyphal

tip technique (Riker and Riker, 1936). Purified isolates were identified according to their morphological and microscopical characters and confirmed by Dept., Plant Pathology institute ARC, Egypt.

#### **4) Preparation of pan bread:**

Pan bread prepared by straight dough method as described in AACC (2002). The control of pan bread recipe was used after replaced the wheat flour with untreated and yeast or auto-fermented bran (coarse +fine) and shorts (brown + white) at levels 10, 15 and 20%.

#### **5) Statistical analysis:**

Data of the sensory evaluation of cakes were analyzed by the Analysis of variance (ANOVA) and using the statistical package for the social (SPSS, Chicago);  $p < 0.05$  was considered significant. Specific differences between treatments were determined by LSD test for each attribute. Results were tested for degree of significant level at  $p < 0.05$ .

## **RESULTS AND DISCUSSION**

#### **\*Proximate chemical composition of wheat milling products:**

Different by-products i.e. fine bran, coarse bran, brown shorts and white shorts were analyzed for moisture, ash, protein, ether extract, crude fiber and carbohydrates. The obtained results are shown in Table (1).

**Table (1): A proximate chemical composition of wheat milling products.**

Materials	Moisture %	On dray basis				
		Protein %	Ether extract %	Ash %	Crude fiber %	Total Carbohydrates %
Wheat flour	10.84	11.96	0.88	0.56	0.67	85.93
Fine bran	11.48	15.44	5.75	4.1	8.5	66.21
Coarse bran	11.93	13.05	4.39	4.78	11.4	66.38
Brown shorts	9.53	17.4	6.5	3.61	5.5	66.99
White shorts	10.97	14.86	4.73	2.5	2.1	75.81

Results presented in Table (1) showed that wheat flour contained 0.56% ash, 11.96% protein, 0.88% ether extract, 0.67% crude fiber and 85.93% total carbohydrates.

Analysis of wheat milling by-products for ash showed that coarse bran had the highest ash content (4.78%) followed by fine bran (4.1%), brown shorts (3.61%) then white shorts (2.5%). Concerning protein content, brown shorts was the highest among wheat milling by-products in protein content (17.4%). Fine bran and white shorts had a protein content of 15.44 and 14.86 % respectively, while coarse bran was the lowest (13.05%). For fat content (ether extract), brown shorts bran contained the highest percentage of ether extract (6.5%). On the other hand, coarse bran had the lowest fat content (4.39%).

Crude fiber content of wheat milling by-products could be arranged in the following descending order: 11.4, 8.5, 5.5 and 2.1 percent for coarse bran, fine bran, brown shorts and finally white shorts, respectively.

Carbohydrate content was determined by the difference between 100 and the summation of protein, fat, ash and crude fiber. Data in Table (1) showed that fine & coarse bran and brown shorts contained the lowest level of carbohydrates (66.21%) & (66.38%) and (66.99%), respectively. These results could be due to the highest content of crude fiber). The carbohydrate content of white shorts was (75.81). All above results are found to be closely near that obtained by McIntosh, *et al* (2001) who reported that the composition of wheat aleurone flour was found to be 15.4 g of DF (as non-starch polysaccharide)/100 g, 23.6 g of protein/ 100 g, 36.5 g of starch/100 g, 6.5 g of fat/100 g, 5.1 g of moisture/100 g of and 2.36 g of phytate/100 g. The composition of wheat flour was 31.6 g of DF/100 g, 17.8 g of protein/100 g, 21.6 g of starch/100 g, 5.2 g of fat/100 g, 10.4 g of moisture/100 g of and 1.96 g of phytate/100 g.

**\*Effect of autoclaving of non-fermented and fermented wheat milling by-products on the chemical analysis:**

The non-fermented and fermented wheat-by products were autoclaved at 15 lb pressure (121 ° C) for 30, 60 and 90 min., then the samples were dried, powdered and sieved to 60 mesh size. The produced samples were analyzed for moisture, protein, lipid, ash, and crude fiber. The same samples were analyzed for Phytic acid and total dietary fiber (TDF) contents.

**1) Effect of autoclaving and type of fermentation on the chemical analysis of wheat milling by-products:**

Chemical compositions of treated wheat milling by-products are shown in Table (2). The results show that moisture, protein and fat contents of treated samples were decreased, while the ash and crude fiber contents were increased. These changes were found with respect to type of fermentation and time of autoclaving. The moisture, protein and fat contents were in the range from 6.4 to 7.4%, from 14.02 to 14.62 and from 3.07 to 5.5% for fine bran, from 5.3 to 6.6%, from 11.92 to 12.97 and from 2.21 to 4.33% for coarse bran, from 6.7 to 8.6%, from 13.99 to 15.27 and from 3.55 to 4.3% for brown shorts and from 6.3 to 7.2%, from 13.72 to 14.47 and from 2.9 to 4.07% for white shorts, respectively. Meanwhile, the moisture, protein and fat contents were 11.48%, 15.44% and 5.75% for fine bran, 11.93%, 13.05 % and 4.39% for coarse bran, 9.53%, 17.4 and 6.5% for brown shorts and 10.97%, 14.86% and 4.73% for white shorts, respectively.

For ash, it could be noticed that ash content of treated samples was in the range from 4.5 to 5.51% for fine bran, from 5.2 to 5.7% for coarse bran, from 3.46 to 4.51% for brown shorts and from 2.7 to 3.25% for white shorts. Meanwhile, it was 4.1% for fine bran, 4.78% for coarse bran, 3.61% for brown shorts and 2.5% for white shorts.

It could be noticed that crude fiber content was increased by fermentation treatments (yeast fermentation or auto-fermentation) and time of autoclaving (30, 60 or 90min.). While, crude fiber content of samples, which were autoclaved without fermentation, was decreased. The increment of crude fiber caused by yeast or auto-fermentation was in the range from 8.35 to 21.14% for fine bran, from 0.61 to 15% coarse bran, from 5.63 to 26.36% for brown shorts and from 20.95 to 54.29% for white bran.

**Table (2): Effect of two types of fermentation and autoclaving on the chemical analysis of wheat milling products**

Treatments	Time	Moisture %	On dray basis					Total Carbohydrates %
			Protein %	Ether extract %	Ash %	Crude fiber %		
Fin bran	Autoclaving	30 min.	6.4	14.32	3.07	4.50	8.71	69.40
		60 min.	6.7	14.27	3.23	4.65	8.60	69.25
		90 min.	6.6	14.37	3.41	4.60	8.53	69.09
	Yeast fermentation and autoclaving	30 min.	6.9	14.32	4.02	5.20	9.55	66.91
		60 min.	7.2	14.21	3.89	5.40	9.33	67.17
		90 min.	7.4	14.02	4.40	5.30	9.21	67.07
	Auto-fermentation and autoclaving	30 min.	7.1	14.62	4.97	5.20	10.32	64.91
		60 min.	6.8	14.52	5.50	5.31	10.30	64.37
		90 min.	6.6	14.52	5.32	5.51	10.22	64.43
Coarse bran	Autoclaving	30 min.	5.5	13.57	2.21	5.20	11.39	67.63
		60 min.	5.3	13.17	2.37	5.30	11.06	67.57
		90 min.	5.8	13.02	2.25	5.2	11.01	68.52
	Yeast fermentation and autoclaving	30 min.	6.1	13.47	3.30	5.3	11.90	66.92
		60 min.	6.3	12.97	3.79	5.4	11.84	66.00
		90 min.	6.5	13.52	3.90	5.3	11.47	65.81
	Auto-fermentation and autoclaving	30 min.	6.4	13.76	3.92	5.50	13.11	63.71
		60 min.	6.4	13.91	4.11	5.55	12.85	63.58
		90 min.	6.6	13.97	4.33	5.70	12.64	63.36
Brown shorts	Autoclaving	30 min.	6.7	15.27	3.72	3.56	5.20	72.25
		60 min.	6.9	15.17	3.55	3.60	5.02	72.66
		90 min.	7.3	14.51	3.60	3.46	5.14	72.29
	Yeast fermentation and autoclaving	30 min.	7.7	14.55	4.21	4.40	6.45	70.39
		60 min.	7.7	14.02	4.13	4.10	6.17	71.58
		90 min.	8.0	14.22	4.30	4.23	5.81	71.44
	Auto-fermentation and autoclaving	30 min.	7.8	13.22	4.21	4.45	6.95	71.17
		60 min.	7.9	13.26	4.17	4.51	6.42	71.64
		90 min.	8.2	13.27	4.22	4.35	6.43	71.73
White shorts	Autoclaving	30 min.	7.2	14.12	3.15	2.7	1.98	78.05
		60 min.	7.1	14.47	2.9	2.75	1.88	78.00
		90 min.	6.8	14.02	3.11	2.80	1.75	78.32
	Yeast fermentation and autoclaving	30 min.	7.0	13.72	3.73	3.06	2.99	76.50
		60 min.	6.6	14.06	3.65	3.0	2.76	76.53
		90 min.	6.3	14.02	3.75	3.11	2.54	76.58
	Auto-fermentation and autoclaving	30 min.	6.9	14.0	3.83	3.17	3.24	75.76
		60 min.	6.5	14.21	4.01	3.25	3.00	75.53
		90 min.	6.5	14.07	4.07	3.22	2.99	75.65

A negative relationship could be noticed between type of fermentation and total carbohydrate content. This could be attributed to the increase in utilization of carbohydrate content associated with microorganism activation. These results in agree with (Martínez-Anaya 2003) who reported that the main factor regulating acidification is the amount of fermentable carbohydrates. White flours have very low quantities of free sugars, about 1.55.1.84% (sucrose, maltose, glucose, glucose, fructose and oligosaccharides) but the endogenous  $\alpha$ - amylase activity, started during mixing, increases initial maltose levels by ten- to fifteen-fold. The Alfa-amylase activity of wheat flour depends on the extraction rate and quality of

flour; whole-meal flour and especially the bran fraction having the highest enzyme activity.

**2) Phytic acid content of wheat milling products:**

Phytic acid was considered as the major constituent in bran and whole wheat responsible for di-and trivalent mineral deficiency disorders in animals (Reinhold *et al.*, 1973).

Data tabulated in Table (3) indicated that wheat flour has lower phytic acid content (0.46%) in comparison to different wheat milling by products. It could be noticed that coarse bran has the highest acid content (1.37%) followed by brown shorts (1.36%) and fine bran (1.35%), while, white shorts was the lowest in phytic acid content (1.34 %).

From the same Table, it could be noticed that phytic acid content was decreased by treatments (fermentation and autoclaving). Auto-fermentation and autoclaving was the most effective method in lowering phytic acid level, followed by Yeast-fermentation and autoclaving. A negative relationship could be observed between phytic acid % and autoclaving time (30, 60, and 90 min.). The present results are in agreement with those reported by *Harland and Harland (1980)* who found that in experiments phytate content was reduced by doubling the yeast in each recipe and extending the fermentation time. *Marttila, (2001)* found that pre-fermentation of wheat bran with yeast or with yeast and lactic acid bacteria improved the loaf volume, crumb structure and shelf life of bread supplemented with bran.

**Table (3): Effect of two types of fermentation and autoclaving on phytic acid content of wheat milling by products.**

Wheat milling products	Without treatment	Autoclaving			Yeast-fermentation and autoclaving			Auto-fermentation and autoclaving		
		30 min.	60 min.	90 min.	30 min.	60 min.	90 min.	30 min.	60 min.	90 min.
Wheat flour (72% ext.)	0.46	-	-	-	-	-	-	-	-	-
Fin bran	1.35	1.32	1.24	1.18	1.15	1.12	1.11	1.13	1.07	0.99
Coarse bran	1.37	1.35	1.20	1.18	1.16	1.14	1.13	1.30	1.22	1.19
Brown shorts	1.36	1.32	1.25	1.17	1.23	1.15	1.12	1.25	1.14	1.09
White shorts	1.33	1.30	1.20	1.22	1.27	1.26	1.18	1.18	1.10	1.05

**3) Dietary fiber content of wheat milling products:**

Dietary fiber is an important food component that consists of plant materials which is resistant to hydrolysis by the endogenous enzymes of the mammalian digestive tract (Schneeman, 1989). Dietary fiber includes all non-digestible, fermentable polysaccharides, whether they are polyglucoses such as cellulose, hemicelluloses or beta-glucans (the mainstay of dietary fiber), or polyfructoses such as inulin, or heteropolymers such as arabinoxylans and arabinogalactans, or analogous carbohydrates. Although lignin and associated plant substances."-Waxes, cutin, and suberin ( indigestible fatty acid derivatives) are not polysaccharides, they are intricately tied to the dietary fiber polysaccharides in foods and increase the resistance to digestion. For many dietary fibers, the large molecular size of the cellulose gives fiber its "fibrous" appearance, while beta-glucans provide the gummy,



gelatinous nature characteristic of soluble dietary fibers. Therefore, all by-products used were analyzed for dietary fiber content and the results are shown in Table (4). The results in table (4) show that coarse bran has the highest values of neutral detergent fiber (NDF). acid detergent fiber (ADF), acid detergent lignin (ADL). insoluble ash, cellulose and lignin, meanwhile fine bran has the highest values of hemi-cellulose.

**Table (4): Effect of fermentation and autoclaving on dietary fiber content of wheat milling by products.**

Treatments	Time	NDF* %	ADF* %	ADL* %	Insoluble ash %	Hemi-cellulose %	Cellulose %	Lignin %	
<b>Fin bran</b>	--	52.32	16.9	7.01	5.23	35.42	9.89	1.78	
<b>Coarse bran</b>	--	89.00	71.91	36.11	14.47	17.09	35.80	21.64	
<b>Brown shorts</b>	--	46.00	17.23	8.44	6.87	28.77	8.79	1.57	
<b>White shorts</b>	--	32.12	20.77	12.75	10.12	11.35	8.02	2.63	
<b>Fin bran</b>	Autoclaving	30 min.	50.30	14.80	6.68	4.38	35.50	8.12	2.30
		60 min.	49.30	16.08	7.48	4.35	33.22	8.60	3.13
		90 min.	48.11	16.11	7.95	3.84	32.00	8.16	4.11
	Yeast fermentation and autoclaving	30 min.	47.18	12.19	3.08	0.11	34.99	9.11	2.97
		60 min.	46.82	11.68	2.99	0.58	34.58	8.69	2.41
		90 min.	45.45	7.72	5.90	3.18	37.73	1.80	2.72
	Auto-fermentation and autoclaving	30 min.	44.88	12.98	4.29	0.63	31.90	8.69	4.26
		60 min.	43.00	12.05	3.64	0.36	30.95	8.41	3.28
		90 min.	40.85	11.98	3.65	0.68	28.87	8.33	3.17
<b>Coarse bran</b>	Autoclaving	30 min.	86.03	68.86	35.13	13.11	17.17	33.73	22.02
		60 min.	76.86	67.05	34.90	10.19	9.81	32.15	24.71
		90 min.	68.41	61.29	34.7	7.92	7.12	30.59	26.78
	Yeast fermentation and autoclaving	30 min.	72.87	50.24	30.40	10.42	22.63	19.84	19.98
		60 min.	71.47	43.23	26.47	6.05	28.24	16.76	20.42
		90 min.	70.22	38.01	25.41	2.40	32.21	12.60	23.01
	Auto-fermentation and autoclaving	30 min.	62.30	27.18	17.05	6.12	35.12	10.13	11.93
		60 min.	60.12	23.96	15.17	3.16	36.16	8.79	12.01
		90 min.	56.36	19.1	14.37	1.96	37.26	4.73	12.41
<b>Brown shorts</b>	Autoclaving	30 min.	32.81	11.21	2.55	1.15	21.60	8.66	1.40
		60 min.	30.26	10.66	4.24	1.49	19.60	6.42	2.75
		90 min.	28.68	9.81	5.62	2.36	18.58	4.19	3.26
	Yeast fermentation and autoclaving	30 min.	28.39	10.64	7.34	4.41	17.75	3.30	2.93
		60 min.	27.82	8.99	5.46	2.33	18.83	3.53	3.13
		90 min.	26.29	7.39	3.6	0.42	18.90	3.59	3.18
	Auto-fermentation and autoclaving	30 min.	26.18	8.03	4.70	2.69	18.15	3.33	2.01
		60 min.	24.72	6.45	3.43	1.23	18.27	3.02	2.20
		90 min.	22.64	4.13	2.48	0.22	18.51	1.65	2.26
<b>White shorts</b>	Autoclaving	30 min.	21.64	14.03	8.78	6.17	7.61	5.25	2.61
		60 min.	21.00	13.63	8.09	5.21	7.37	5.54	2.88
		90 min.	19.26	13.04	11.73	6.84	6.22	1.31	4.89
	Yeast fermentation and autoclaving	30 min.	16.52	10.32	7.03	5.51	6.20	3.29	1.52
		60 min.	14.70	9.35	6.09	4.29	5.35	3.26	1.80
		90 min.	13.08	7.22	5.02	3.04	5.86	2.20	1.98
	Auto-fermentation and autoclaving	30 min.	14.40	9.07	6.19	4.49	5.33	2.88	1.70
		60 min.	12.5	7.95	5.23	3.36	4.55	2.72	1.87
		90 min.	10.62	6.82	4.21	2.20	3.80	2.61	2.01

\*NDF= Neutral Detergent Fiber

\*ADF= Acid Detergent Fiber

\* ADL= Acid Detergent Lignin

According to NDF content, wheat milling by products could be arranged in descending order as follows: coarse bran (89.0%), fine bran (52.32%), brown shorts (46.0%) and white shorts (32.12%). For ADF fine bran and brown shorts have almost the same content of ADF (16.9 and 17.23%) whereas, other fiber sources were higher (71.91 % for coarse bran and 20.77% for white shorts). Concerning the hemi-cellulose content, fine bran has the highest source in hemi-cellulose content (35.42%) followed by brown shorts (28.79%), coarse bran (17.09%) and finally white shorts (11.35%).

These results are in agreement with those obtained by Prosky *et al.*, (1988) who found that wheat bran contained average TDF of 43.87%.

Generally, the percentage of crude fiber content was lower than percentage of dietary fiber DF. This may be due to method of estimation, since the crude fiber method gives an inaccurate estimation of cellulose and lignin contents in foods, and does not estimate non cellulose polysaccharides (Schneeman, 1989).

From table (4), it could be also noticed that dietary fiber content was affected by all used treatments. auto-fermentation was the most effective treatment, followed by yeast fermentation and finally time of autoclaving (30, 60 or 90min.). Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), insoluble ash and cellulose decreased by fermentation treatments (yeast fermentation or auto-fermentation) and time of autoclaving (30, 60 or 90min.). While, lignin and hemi-cellulose content of samples was increased. These results are in agreement with those obtained by *Boskov-Hansen et al. (2002)* who found a reduce in dietary fiber content and increase in solubility of arabinoxylans during imitated rye sourdough fermentation. The mechanism of reducing the content of DF is not clear, but it is postulated to be due to the ability of microorganisms to produce extracellular enzymes that are either cell-free or cell-associated (*Schwarz 2001*).

#### **4) Microbial population changes during yeast or auto-fermentation:**

Results) concerning microbial counts of the prepared fermented dough are given in table (5). LAB and yeast populations increased from  $3 \times 10^{-2}$  to  $105 \times 10^5$  and from N.D(not detectable) to  $60 \times 10^4$  for fine bran, from  $50 \times 10^{-4}$  to  $150 \times 10^4$  and from  $2 \times 10^{-3}$  to  $156 \times 10^4$  for coarse bran, from  $1 \times 10^{-3}$  to  $117 \times 10^4$  and from N.D to  $102 \times 10^4$  for brown shorts and from  $3 \times 10^{-3}$  to  $98 \times 10^4$  and from N.D to  $95 \times 10^4$  for white shorts, respectively, during yeast or auto-fermentation,. Hence, a high level of yeasts was found throughout the yeast fermentation.

These values are similar to those reported in literature (Röcken and Voysey, 1992). whole grain cereals and 100% extraction rye flour may contain  $104.106 \text{CFU}$  of unspecified bacteria per gram; in which  $102.103 \text{CFU-g}$  belongs to genus *Lactobacillus* (Salovaara 2004). The dominating microbes in spontaneously fermented doughs are homofermentative lactobacilli and pediococci, which are found both in wheat and rye sourdoughs at the level of  $3 \times 10^{8.3} \times 10^9 \text{CFU-g}$ . Various yeast strains have also been isolated from spontaneous fermentations such as *S. cerevisiae* and *Pichia satoui* (Beech and Davenport 1971). Sourdough fermentation is based on lactic acid and alcoholic fermentation depending on the composition of microflora and fermentation conditions. (Brummer and Lorenz 1991).

**Table (5): Microbial population changes during yeast or auto-fermentation of wheat milling by products.**

Type of fermentation		P.C	L.A	Yeast	Mold
Fin bran	Untreated raw material	5×10 <sup>-3</sup>	3×10 <sup>-2</sup>	N.D	N.D
	Yeast fermentation	16×10 <sup>-3</sup>	35×10 <sup>2</sup>	60×10 <sup>4</sup>	2×10 <sup>-4</sup>
	Auto-fermentation	60×10 <sup>-3</sup>	105×10 <sup>5</sup>	45×10 <sup>3</sup>	7×10 <sup>-3</sup>
Coarse bran	Untreated raw material	35×10 <sup>-3</sup>	50×10 <sup>-4</sup>	2×10 <sup>-3</sup>	8×10 <sup>-2</sup>
	Yeast fermentation	15×10 <sup>-2</sup>	45×10 <sup>-1</sup>	156×10 <sup>4</sup>	10×10 <sup>-2</sup>
	Auto-fermentation	71×10 <sup>-3</sup>	150×10 <sup>4</sup>	98×10 <sup>4</sup>	22×10 <sup>-2</sup>
Brown shorts	Untreated raw material	3×10 <sup>-3</sup>	1×10 <sup>-3</sup>	N.D	N.D
	Yeast fermentation	13×10 <sup>-3</sup>	66×10 <sup>-2</sup>	102×10 <sup>4</sup>	1×10 <sup>-3</sup>
	Auto-fermentation	45×10 <sup>-3</sup>	117×10 <sup>4</sup>	65×10 <sup>4</sup>	4×10 <sup>-3</sup>
White shorts	Untreated raw material	11×10 <sup>-3</sup>	3×10 <sup>-3</sup>	N.D	N.D
	Yeast fermentation	3×10 <sup>-2</sup>	75×10 <sup>-3</sup>	95×10 <sup>4</sup>	6×10 <sup>-4</sup>
	Auto-fermentation	35×10 <sup>-3</sup>	98×10 <sup>4</sup>	43×10 <sup>4</sup>	5×10 <sup>-3</sup>

\*P.C= proteolytic bacteria      \* L.A =lactic acid bacteria      \*N.D= not detected

**5) Sensory characteristics of pan bread:**

Sensory evaluation of pan bread prepared with varying levels 10, 15 and 20% of untreated or treated (yeast or auto-fermentation) bran (coarse & fine) or shorts (brown & white), are presented in Table (6) and illustrated in Fig (1).

**Table (6): Effect of addition of 10, 15 or 20% untreated and yeast or auto-fermented Y.F &A.F bran (fine +coarse) and shorts on pan bread sensory characteristics**

Treatments + 60 min. autoclaving time		Addition %	Appearance 10	Crust color 10	Texture 15	Grain 15	Taste 15	Odor 15	Crumb color 10	Volume 10	Overall acceptability 100
Control wheat flour 72%ex,		-	9.4 <sup>a</sup>	9.4 <sup>a</sup>	14.3 <sup>a</sup>	14.2 <sup>a</sup>	14.2 <sup>a</sup>	14.1 <sup>a</sup>	9.6 <sup>a</sup>	9.4 <sup>a</sup>	94.5 <sup>a</sup>
Fin+ coarse bran	Untreated	10	4.1 <sup>f</sup>	6.8 <sup>f</sup>	7.0 <sup>h</sup>	7.0 <sup>j</sup>	7.0 <sup>f</sup>	8.4 <sup>f</sup>	4.9 <sup>g</sup>	4.0	49.2 <sup>h</sup>
		15	3.6 <sup>f</sup>	4.0 <sup>h</sup>	8.0 <sup>g</sup>	6.2 <sup>k</sup>	4.8 <sup>h</sup>	5.1 <sup>h</sup>	3.8 <sup>hi</sup>	5.4 <sup>d</sup>	41.4 <sup>i</sup>
		20	3.5 <sup>f</sup>	3.8 <sup>h</sup>	7.3 <sup>h</sup>	6.0 <sup>k</sup>	4.7 <sup>h</sup>	5.0 <sup>h</sup>	3.6 <sup>i</sup>	4.7 <sup>e</sup>	38.4 <sup>i</sup>
	Yeast fermentation	10	6.4 <sup>cde</sup>	7.0 <sup>ef</sup>	12.0 <sup>c</sup>	11.2 <sup>de</sup>	9.7 <sup>de</sup>	10.0 <sup>de</sup>	6.8 <sup>ef</sup>	7.6 <sup>ab</sup>	70.7 <sup>f</sup>
		15	6.0 <sup>cd</sup>	7.2 <sup>ef</sup>	8.9 <sup>f</sup>	10.8 <sup>e</sup>	8.7 <sup>e</sup>	10.0 <sup>de</sup>	6.8 <sup>ef</sup>	4.9 <sup>e</sup>	63.3 <sup>g</sup>
		20	6.5 <sup>cd</sup>	7.0 <sup>ef</sup>	7.5 <sup>h</sup>	8.5 <sup>h</sup>	8.5 <sup>e</sup>	10.0 <sup>de</sup>	5.5 <sup>g</sup>	5.0 <sup>ef</sup>	60.5 <sup>g</sup>
	Auto-fermentation	10	8.5 <sup>ab</sup>	8.5 <sup>bc</sup>	11.5 <sup>d</sup>	11.5 <sup>de</sup>	13.5 <sup>a</sup>	13.5 <sup>a</sup>	9.0 <sup>ab</sup>	9.0 <sup>ab</sup>	85.0 <sup>bc</sup>
		15	8.0 <sup>ab</sup>	8.0 <sup>bcd</sup>	11.5 <sup>d</sup>	10.5 <sup>f</sup>	12.5 <sup>a</sup>	12.0 <sup>bc</sup>	8.5 <sup>bc</sup>	8.0 <sup>ab</sup>	81.0 <sup>cd</sup>
		20	6.8 <sup>bc</sup>	6.5 <sup>f</sup>	10.0 <sup>e</sup>	11.7 <sup>d</sup>	10.6 <sup>d</sup>	10.4 <sup>d</sup>	6.7 <sup>ef</sup>	6.4 <sup>bcd</sup>	69.2 <sup>f</sup>
Shorts	Untreated	10	5.1 <sup>def</sup>	5.4 <sup>g</sup>	7.5 <sup>h</sup>	10.1 <sup>f</sup>	8.3 <sup>ef</sup>	8.97 <sup>ef</sup>	5.4 <sup>g</sup>	4.4 <sup>e</sup>	55.2 <sup>h</sup>
		15	4.6 <sup>ef</sup>	4.5 <sup>h</sup>	10.2 <sup>e</sup>	7.8 <sup>i</sup>	6.2 <sup>gh</sup>	6.2 <sup>g</sup>	4.3 <sup>hi</sup>	6.9 <sup>bc</sup>	50.8 <sup>h</sup>
		20	4.6 <sup>ef</sup>	4.4 <sup>h</sup>	9.4 <sup>i</sup>	7.9 <sup>j</sup>	6.0 <sup>gh</sup>	6.2 <sup>g</sup>	3.9 <sup>hi</sup>	5.9 <sup>bcd</sup>	47.4 <sup>h</sup>
	Yeast fermentation	10	7.3 <sup>bc</sup>	8.4 <sup>bc</sup>	12.1 <sup>c</sup>	12.5 <sup>c</sup>	12.3 <sup>bc</sup>	12.0 <sup>c</sup>	7.9 <sup>cd</sup>	7.4 <sup>bc</sup>	80.4 <sup>d</sup>
		15	7.3 <sup>bc</sup>	8.1 <sup>bcd</sup>	11.4 <sup>d</sup>	12.3 <sup>c</sup>	10.8 <sup>cd</sup>	12.1 <sup>bc</sup>	7.7 <sup>d</sup>	6.9 <sup>bcd</sup>	76.6 <sup>e</sup>
		20	5.6 <sup>de</sup>	7.5 <sup>def</sup>	9.7 <sup>e</sup>	9.2 <sup>g</sup>	9.2 <sup>de</sup>	10.1 <sup>d</sup>	6.4 <sup>f</sup>	5.5 <sup>cd</sup>	63.7 <sup>g</sup>
	Auto-fermentation	10	8.3 <sup>ab</sup>	8.8 <sup>ab</sup>	13.0 <sup>b</sup>	13.4 <sup>b</sup>	12.7 <sup>ab</sup>	13.1 <sup>b</sup>	8.8 <sup>b</sup>	8.3 <sup>ab</sup>	86.48 <sup>b</sup>
		15	8.0 <sup>ab</sup>	7.8 <sup>cde</sup>	12.2 <sup>c</sup>	13.0 <sup>b</sup>	12.6 <sup>ab</sup>	12.3 <sup>bc</sup>	7.8 <sup>d</sup>	8.0 <sup>ab</sup>	81.7 <sup>cd</sup>
		20	6.6 <sup>cd</sup>	7.2 <sup>ef</sup>	12.4 <sup>c</sup>	11.7 <sup>d</sup>	10.3 <sup>d</sup>	10.0 <sup>de</sup>	7.3 <sup>de</sup>	8.2 <sup>ab</sup>	73.7 <sup>ef</sup>
L.S.D 0.05			1.84	0.85	0.54	0.53	1.61	1.05	0.62	1.92	4.43

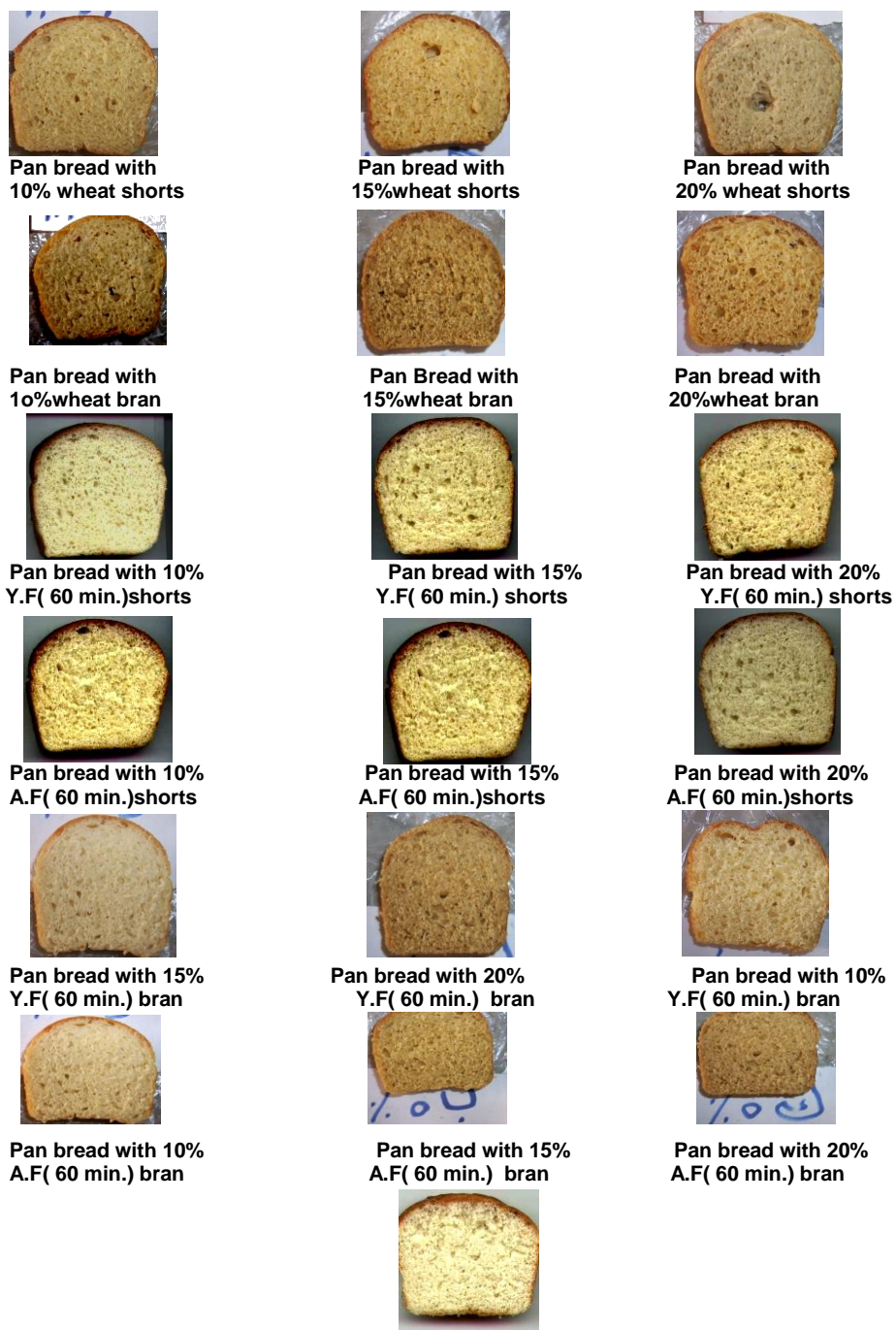


Fig. (1): Effect of addition of 10, 15 or 20% untreated and yeast or auto-fermented Y.F &A.F bran (fine +coarse) and shorts on pan bread sensory characteristics

Results showed that all high fiber substituted pan bread were significantly ( $P < 0.05$ ) lower than pan bread control in all sensory characteristics i.e. appearance, crust color, grains, texture, taste, odor, crumb color, volume and overall acceptability.

For appearance, it could be noticed that pan bread containing auto fermented bran or shorts at level 10% were nearest to control sample (8.5), (8.3), and (9.4), respectively. The lowest score of appearance was that obtained by pan bread containing untreated bran at level of 20% with score value of (3.5) then pan bread containing 15% untreated bran with score value of (3.6). There are no significant differences ( $P > 0.05$ ) between control and samples prepared with substitution of wheat flour with 10 and 15 % auto-fermented bran or shorts.

Concerning crust color, control sample had the highest score (9.4), followed by 10% auto-fermented shorts (8.8), then 10% auto-fermented bran (8.5) and 15% auto-fermented shorts (8.4). The lowest score of crust color was that obtained by pan bread containing 15% untreated bran with score value of (3.8). The same trend was observed with other characters (texture, grain property, taste, odor, crumb color and volume).

In general, replacement of wheat flour with untreated bran or shorts at 10, 15 and 20 % levels caused a significant deterioration in pan bread samples properties, while addition of treated (yeast or auto-fermentation) bran (coarse & fine ) or shorts (brown & white), improved bread samples properties. Control sample had the highest score of overall acceptability, followed by 10% auto-fermented shorts. There was no significant difference in overall acceptability between pan bread containing auto-fermented bran or shorts at 10% replacement level, also between pan bread containing 10% auto-fermented bran and that containing 15 % of auto-fermented bran or shorts.

The above mentioned results are in agreement with those obtained by Marttila, *et al* (2001); who mentioned that pre-fermentation of the wheat bran with yeast or with yeast and lactic acid bacteria improved crumb structure and elasticity of the bread. The bread crumb was softer and had a much slower staling rate than the crumb of the control bread.

#### **Conclusions:**

Wheat bran is an important source of dietary fiber and bioactive compounds. However, addition of wheat bran in baking results in inferior bread quality. Pre-treatment resulted in significant improvement of bread texture due to modified starch-protein network. A novel method of bran sourdough (autofermentation) was developed to pre-treat bran prior to the baking process. Sourdough thus shows promise also for production of nutritionally superior high-fiber raw materials for different cereal foods.

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تأثير استخدام التخمير والمعاملة الحرارية باللاوتوكلاف على الخصائص الكيماوية والطبيعية للمنتجات الثانوية لطحن القمح وتأثير ذلك على إنتاج الخبز  
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تم في هذه الدراسة استخدام نواتج طحن القمح المرتفعة في محتواها من الألياف الغذائية (الردة بنوعها الناعمة والخشنة والسن الأحمر والأبيض) لاستبدال جزء من دقيق القمح في إنتاج الخبز العالي في محتواها من الألياف الغذائية. وكان الهدف الوصول إلي خبز عالي الجودة يحتوي ١٠ - ٢٠% من الردة أو السنون وذلك باستخدام بعض المعاملات مثل التخمير والمعاملة الحرارية الرطبة باللاوتوكلاف لتحسين عملية الإنتاج. الخبز الناتج من استخدام ٢٠% نسبة استبدال كانت له صفات غير مقبولة من حيث انخفاض الحجم والقوام الردي للبابة وحتى مع تقليل حجم حبيبات الردة أو السن تحسن القوام والشعور بالفم إلا إن الحجم لم يتحسن. وقد أظهرت النتائج أن استخدام التخمير (سواء بخميرة الخباز أو بالتخمير الذاتي) للردة والسن قبل استخدامها في إنتاج الخبز ادي إلي تحسين جودة الخبز الناتج من حيث الحجم والقوام بالإضافة إلي النكهة.

قام بتحكيم البحث

كلية الزراعة - جامعة المنصورة  
كلية الزراعة بمشتهر - جامعة بنها

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