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CHEMICAL, PHYSICAL, BIOLOGICAL AND SENSORY EVALUATION OF SOME BAKERY PRODUCTS USING NONTRADITIONAL ADDITIVES (DDGS) MIXING WITH WHEAT FLOUR

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ABSTRACT
Two different of DDGS (brown and gold) and Egyptian hard red wheat (Masr2) local wheat cultivars were subjected to physico-chemical and biological properties. Results indicated that the Egyptian hard red wheat grains had higher total physical properties. Flour yields were about 70 % for all tested samples. A wide range of protein content (10.3 – 31.0 %) of flours was recorded. The gold DDGS flour had the highest protein content and the Egyptian hard red wheat flour was the lowest protein content. Data indicated that Mix (2) flours had more suitable properties for bread- making than the Mix (1) flours. The different tested wheat flours indicated that those made from Egyptian hard red wheat (Masr2) and Mix (2) were superior but physico-chemical and rheological characteristics as well as phytate contents of wheat, and its mixtures flour approve that DDGS flour decreased the water absorption. Composite flour containing 50% DDGS and 50% wheat flours Mix (2) showed maximum improvement in dough development and softening of dough. However sensory evaluation results showed that 50% wheat replacement with DDGS flour produced Mix (2) acceptable balady breads than the other Mix (1). The biological results also showed that feeding on bread made from a mixture of DDGs, whether brown or golden with wheat flour, improved the nutritional status of rats and reduced the level of blood fats, as well as reduced the level of bilirubin, urea and creatinine from feeding on bread made from wheat flour only. Enzymes are used for wheat flour alone, so it is recommended to add DDGS to wheat flour to make bread for human nutrition

1. INTRODUCTION

Egypt remains the world's largest wheat important. Accordingly, cereal important requirements in the next 2014/15 marketing year (July/June) are forecast at about 18.2 million tons, about 5 percent higher than the previous year and almost 10 percent higher than five-year average. Wheat imports for the just ending 2013/14 marketing year are estimated at 17.4 million tons, about 22 percent and 8 percent respectively higher than the previous year and average. Available trade data indicate that until

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March 2014, 8.2 million tons of wheat were imported. The General Authority for Supply Commodities (GASC) announced, in mid-May 2014, that Egypt's strategic reserves of wheat are enough until the end of July 2014 and that the level of reserves would increase after the expected purchase of domestic wheat from farmers which started in mid-April and should last July. The government expects to purchase 4.25 million tons of wheat from local farmers in 2014/15 (FAO, 2014). Most wheat varieties presently cultivated are grouped under the bread wheat (Triticum aestivum), which accounts for approximately 95% of world production, and durum wheat (Triticum durum), which often used for pasta production. In Egypt 10.9 million tons of different wheat varieties are milled per the year 2003. About 4,057,234 tons (37.2%) of imported wheat and 6,844,692 tons (62.8%) of local wheat were used during the season of 2003 (FAO, 2005).

Mixing two or more wheat flours may depend on the economical aspect and this could be helpful to overcome the inferior quality for one of them and to enhance the total quality of the blend. Despite the fact that different wheat varieties have been developed to suit different applications, inconsistency of wheat quality is one of the big problems of flour millers. Blending different wheat batches and adjusting the milling parameters can help to solve one problem but not all problems related to the final flour quality and this may be because the damage is too great or because no suitable raw material is available for blending. Accordingly, the use of improvers may be a solution to achieve the desired quality from affected flour. Several developing countries have encouraged the initiation of programs to evaluate the feasibility of alternative locally available flours as a substitute for wheat flour. Many efforts have been carried out to promote the use of composite flours, in which a portion of wheat flour is replaced by locally grown crops, to be used in bread, thereby decreasing the cost associated with imported wheat. Most of the research conducted on the use of composite flour for bread making purposes (Olaoye *et al.*, 2006) was devoted to studying the effects of different flour substitutions on bread making quality. Acceptability studies conducted at the Food Research Centre in Khartoum, Sudan, indicated that breads made with composite flour of 70% wheat and 30% other grains flour were acceptable (FAO, 1995).

Distillers' grains are a cereal byproduct of the distillation process. Brewer's spent grain usually refers to barley produced as a byproduct of brewing, while distillers grains are a mix of corn, rice and other grains. There are two main sources of these grains. The traditional sources were from brewers. More recently, ethanol biofuel plants are a growing source. It is created in distilleries by drying mash and is subsequently sold for a variety of purposes, usually as fodder for livestock (especially ruminants). Corn-based distillers grains from the ethanol industry are commonly sold as a high protein Wet Distillers Grains (WDG) contain primarily unfermented grain residues (protein, fiber, fat and up to 70% moisture). WDG has a shelf life of four to five days. Due to the water content, WDG transport is usually economically viable within 200 km of the ethanol production facility. Dried Distillers Grains with Soluble (DDGS) is WDG that has been dried with the concentrated thin stillage to 10-12 percent moisture. DDGS have an almost indefinite shelf life and may be shipped to any market regardless of its proximity to an ethanol plant. Drying is costly as it requires further energy input. In the USA, it is packaged and traded as a commodity product (Heuzé *et al.*, 2017).

Dry distillers grains with soluble is an excellent feed for growing Holstein steers. 10, 20 or 40% of the ration dry matter as dry distillers grains with soluble could be fed to growing Holstein steers from 425-700 lbs. without affecting feed intake or gain. Feeding wet distillers grains with soluble tended to decrease feed intake of the growing steers, but improved feed conversion. Feed cost of gain was reduced 6% when com was priced at $2.25/bu and dry distillers grains at $85/ton. At the same prices, feeding wet distillers grains reduced the cost of gain 13%. Wet or dry distillers grains can be fed to growing and finishing Holstein steers. During a 299 day feeding trial, feeding dry distillers grains at 10, 20 or 40% of ration dry matter did not affect feedlot performance or cost of gain. Steers fed 10% wet distillers grains were 4% more efficient and had 5% lower feed cost of gain. Feeding 40% of ration dry matter as wet distillers grains reduced feed intake and rate of gain with similar feed conversion and cost of gain. Feeding distillers grains to growing and finishing Holstein steers can increase profits. With com prices at $2.25/bu, there is profit from feeding 10, 20 or 40% distillers grains to growing Holstein steers if the price of distillers grains is less than $100/ton ($33/ton for wet distillers grains with 30% dry matter). When the price of distillers grains is low compared with com, there are greater profits from feeding higher levels. During the growing and finishing period with com at $2.25/bu, the price of dry distillers grains had to be less than $85/ton to profitably include it in the ration. Feeding 10 or 20% wet distillers grains to growing and finishing Holstein steers continued to be profitable with the price of the wet grains at $33/ton. Feeding 10, 20 or 40% dry distillers grains or 10 and 20% wet distillers grains did not affect carcass weight, marbling or yield grades. Steers fed 40% wet distillers grains had lighter carcasses but similar marbling and yield grades. Carcass value based on grade and yield or a marketing grid with premiums or discounts for quality and yield grades was not affected by feeding wet or dry distillers grams (USDA, 2016).

Bread is an important staple food for several countries. Wheat flour (Triticum aestivum) is more popular than other cereal grains for bread making. Its popularity has stemmed from the gluten and its mild, nutty flavor. Gluten is an essential structure-forming protein that contributes to the elastic characteristics of dough and the good appearance of bread (Abdelghafor et al., 2011). However, a number of people have celiac disease (CD) which is defined as an inflammatory response in the small intestinal mucosa exacerbated by prolamin proteins in the cereal grains i.e. wheat (gluten), rye (secalin), and barley (hordein). As a result, there has been a great interest in the development of gluten free breads. Part of this interest gets involved with the replacement of wheat flour with other flour.Among the other grain cereals, sorghum (Sorghum bicolor) is a rich source of various phytochemicals, including tannins, phenolic acids, anthocyanins, phytosterols and policosanols, the physico-chemical properties of sorghum flour are also found similar to those of wheat flour. Thus, sorghum flour is likely to have the potential to replace wheat flour for those allergic to gluten. However, the absence of gluten in sorghum flour may cause a liquid batter and baked bread with quality defects post baking poor color and crumbling texture. In the studies of (Onyango et al., 2011) , bread was made from pregelatinized cassava starch and sorghum flour. It was found that crumb firmness and chewiness declined with increasing pregelatinized starch concentration whereas crumb adhesiveness increased with increasing the starch content. In addition, enzyme combinations e.g. trans-glutaminase, alpha amylase, xylanase and protease were alternative methods to improve dough rheology, bread quality and bread shelf-life. The process of germination has been used successfully to improve the nutritional properties of legume seeds by removing several antinutrients (phytates and trypsin inhibitor), increasing oligosaccharides, and improving the digestibility of starches and proteins in legumes. In order to further expand the use of this grain, the effect of grain germination on physical and physico-chemical properties of red sorghum flour was investigated and its application to make gluten free bread was also evaluated through several aspects of physico-chemical and physical properties compared with those made from ungerminated sorghum flour and wheat flour (Elkhalifa and Bernhardt, 2010). The aim of the present study is evaluating chemical, rheological and biological properties of bread prepared from nontraditional additives (alternative flours) (DDGS) mixing with wheat flour.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Wheat and DDGS.

wheat grains (***Triticum aestivum***) cultivars (Egyptian hard red wheat) grains (Masr2) were obtained from El-Ghrbia and two different additives were obtained from the U.S.A, Distiller's Dried Grains with Solubles (Brown and Gold) DDGS which were obtained from one location (Alexandria). They were taken from three different Companies since 2017.

2.2 Methods

2.2.1 Preparation of wheat and DDGS flours

A twenty kg of each wheat sample used in this investigation was stored for 90 days at a temperature 25°c and relative humidity of less than 62% according to the methods described in USDA, (2013a) . At the end of the storage period wheat sample was cleaned mechanically to remove dirt, dockage, imparters and other strange grains by Carter Dockage Tester According to the methods described in USDA, (2014). The wheat samples were tempered to 16.5 % moisture and allowed to conditioning for 24 hours, then milled by Laboratory mill CD1 auto Chopin According to the methods described in AACC method (2000a) . The extraction rate of any flour sample was adjusted to recurred rate (72% extraction) but DDGS had milled by laboratory mill 3100 Perten According to the methods described in AACC method (2000a) for whole meal flour.

Mixture flour

(Mix 1) = 50% Egyptian wheat flour (72% extraction) + 50% Brown DDGS flour (100% extraction).

(Mix 2) = 50% Egyptian wheat flour (72% extraction) + 50% Gold DDGS flour (100% extraction).

2.2.2 Analytical methods

2.2.2.1 Physical properties

Cleanliness, dockage, shrunken and broken, foreign materials, total damaged kernels and total defects were separated and determined manually (hand picking). Test weight pound per bushel, Test weight P/B = (Kg/Hectoliter) ÷1.278 according to USDA, (2013c). A thousand kernel weight was determined by counting the kernels in a 10 g wheat sample AACC method, (2000b). Wet and dry gluten and falling number were determined according to A.O.AC., (2005).

2.2.2.2 Chemical properties

Moisture, crude protein, ash, crude fiber, fat and tannin acid were determined according to A.O.AC., (2005) and USDA, (2013b). The nitrogen free extract (N.F.E) was calculated by difference. Aflatoxin was determined according to A.O.A.C., (2005).

2.2.2.3 Rheological properties

All samples were tested by Consistgraph and alveograph. (in Regional Center for Food and Feed, Agri. Res. Center, Cairo, Egypt.) to determine the rheological properties of the different types of flour according to the methods described by AA.C.C.(2000a).

2.2.2.4 Bread processing

Different samples of flours were used to produce Balady bread according to the formula shown in Table (1).

Table (1): Balady bread formula

Type of bread	Flour	Moisture	Yeast	Salt Nacl
Balady	1000gm	14%	20gm	5gm

2.2.2.4.1 Balady bread

Wheat flour (82% extraction) from wheat flour (72% extraction +10% Fin Bran) was baked into Balady bread loaves using straight dough methods Rashaed et al. (1996). The Balady formula consists shown in Table (1). The ingredient was mixed for 20 min. after mixed with water according to Farinograph Chopin test by using Gostol-Gopan Perten Mixer and then the dough was left for 30 min dough was divided into 150gm . Pieces that were arranged on a wooden board previously sprinkled with a fine layer of bran and kept for 20 min at 30°c and 85% relative humidity. The pieces were flattened to about 20 cm diameter proofed at 30°c and 85% relative humidity for 30 min. and then baked at 400-500°c for 1-2 min. in a pilot oven in Regional Center for Food and Feed, Agri. Res. Center, Cairo, Egypt.

2.2.2.4.2 Baking mixture

Samples of wheat flour (82% extraction) were used to produce balady bread. In addition each sample of mixture wheat flour was mixed with Brown DDGS flour and Gold DDGS flour (100% extraction) by one percentage (50%) to produce two mixtures.

2.2.2.5 Sensory evaluation

Balady bread loaves were orgaolpticaly evaluated according to the method described in AACC method (2000a). The fresh sample was delivered to 10 panelists 2 hours after baking.

2.2.2.6Biological evaluation:

2.2.2.6.1 Experimental animals

Male albino rats (n=40) averaging 180 ± 5 g of BW were obtained from the animal house of the High Graduated Studies Institute, University of Alexandria, Egypt. The design of the experiments and the protocol follow the international guidelines and the ethics of the National Institutes of Health (2005). Animals received human care, and had an adequate stable diet and water ad limited. Animals stay for two weeks for acclimation to the laboratory conditions before being experimented. All laboratory biological specimens and hazardous waste were disposed of safely.

2.2.2.6.2 Experimental design:

After two weeks of acclimation, animals were classified into four equal groups' ten rats each. Group1 (control group) was fed on conventional food all over the experimental period (sucrose 50%, casein 20%,corn starch 15%, corn oil 5%,cellulose 5% ,mineral mix 3.5%,vitamin mix 1%, DL-Methionine 0.3% and Choline bitartrate 0.2%) (G1) as described by National Academy of Sciences, (1995). Group 2, 3 and 4 were fed on balady bread (Masr2) (G2), balady bread Mix1 (50% Egyptian wheat flour (72% extract), 50%Brown DDGS (100% extract)} (G3) and balady bread Mix2 {(50% Egyptian wheat flour

(72% extract), 50% Gold DDGS flour (100% extract)} (G4) respectively, for 8 weeks.

2.2.2.6.3 Body weight and organs weight:

The body weight of rats was recorded at the beginning and at the end of the experimental period. Animals were sacrificed by decapitation, and then liver, kidney, brain, heart, lung and spleen were immediately removed and weighed. Relative organ weights were calculated as g /100 g body weight.

2.2.2.6.4 Blood sample:

Blood samples were collected from the sacrificed animals in two separate tubes, one of them containing heparin. Plasma samples were obtained by centrifugation at 4000 rpm for 20 minutes, and then samples were stored at -20 ºC until used for further analyses.

2.2.2.6.5 Lipid profile:

Plasma samples were analyzed for cholesterol and triglycerides were determined according to the methods of Tietz (1995), high-density lipoprotein-cholesterol (HDL-c) was determined according to the methods of Sugiuchi et al., (1995), low-density lipoprotein-cholesterol (LDL-c) was determined by the method of Pisani et al., (1995) and very-low-density lipoprotein-cholesterol (VLDL-c) was calculated automatically by Roche /Hitachi Cobas C systems.

2.2.2.6.6 Enzymes activity and biochemical parameters:

The activities of plasma alanine transaminase (ALT) and aspartate transaminase (AST) were assayed by the method of Bergmeyer & Herder (1986). Total protein and albumin by the method of Doumas et al.,(1977), total bilirubin was measured using the method of Wahlefeld & Bergmeyer (1972).Uric acid and creatinine concentrations were measured according to the method of Lamb et al., (2006), while Glucose was analyzed according to Kunst et al.,(1984). All the aforementioned parameters were measured using commercial kits, [Bio systems S.A. (Spain), Diamond (Germany) and Randox (United Kingdom)].

2.2.2.6.7 Histological study:

Liver and Kidney specimen used for the histological study was fixed in neutral formalin for a week at room temperature, dehydrated then cleared in xylene and embedded in paraffin wax. The paraffin sections were cut at 20 microns' thickness and stained with hematoxylin and eosin for histological examination using the light microscope according to Chan et al (2002).

2.2.2.7 Statistical analysis

Data of three replicates were computed for the analysis of standard division (S.D) among the means were determined by Duncan's multiple range test using SAS programs SAS, (2011).

3. RESULTS AND DISCUSSIONS

3.1Physical and chemical properties of wheat, DDGS and their flours

Chemical composition of different wheat and sorghum kernels used in these study is given in Table (2) The moisture content of the different DDGS varieties and wheat kernels ranged from (10.7 to 11.1%) for all studied samples. Egyptian hard red wheat (Masr2) had the highest value while brown DDGS had the lowest value among all samples. As regards protein content, gold DDGS had the highest protein

(31.60%) followed by brown DDGS (28.8%), while Egyptian hard red wheat (Masr2) had the lowest protein content (10.70%). On other hand, nitrogen free extracts (NFE)% ranged from32.1% gold DDGS to 73.17% Egyptian hard red wheat (Masr2). Additionally, Egyptian hard red wheat (Masr2) had lower fat (1.50) than other samples and was lower in Ash content (1.39%) than the other samples. However, the highest ash content was observed in brown DDGS (7.9%). The results of fiber, brown DDGS had the significant highest value (6.40%) while Egyptian hard red wheat (Masr2) had the lowest value (2.14%). All of the samples were free of tannic acid which was the maximum level (3.0%) according to **USDA, (2013c).** wheat grains cultivars and two different DDGS were under detection limits (0.5 ppb), which means free from aflatoxin (B1, B2, G1 and G2) , ochratoxin , zearalenone and fumonisin. as recommended by **FAO, (2009).**

Table 2: Proximate analysis for wheat kernels and DDGS

Chemical properties	Egyptian Wheat (Masr 2)	DDGS	
		Brown	Gold
Moisture %	11.10 ±0.5	10.7±0.1	10.8±0.1
Protein%	10.70 ±0.1	28.8±1.0	31.6±1.0
Fat %	1.50 ±0.01	7.9 ±0.01	11.5±0.01
Ash%	1.39 ±0.1	7.9±0.1	7.8±0.1
Fiber%	2.14 ±0.01	6.4±0.01	6.2±0.01
Nitrogen free extracts %	73.17 ±0.01	38.3±0.01	32.1±0.01
Total caloric values%	348.98±0.01	339.5±0.01	358.3±0.01
Tannic acid%	Free	Free	Free
Ochratoxin ppb	Free	Free	Free
Zearalenone ppb	Free	Free	Free
Fumonisin ppb	Free	Free	Free
Aflatoxin ppb (B1,B2,G1,G2 &Total)	Free	Free	Free

The mean value of physical properties of Egyptian hard red wheat (Masr 2) kernel cultivars are presented in Table (3). Moisture content was 11.1%. It can be concluded that the test weight was 60.5 pounds per bushel. More ever the foreign material was 0.19%, either shrunken and broken kernels was (0.32%). For damage kernels that contest of heat damage and total damage, Egyptian hard red wheat (Masr 2) have total damage kernels percentage (3.7%) but it hasn't heat damage. More ever from the same table, it could be noticed that samples are free from insects and ok odor. The Egyptian stander no. 1601/1986 and it's a modification on 23/4/2002 has obligation that the dockage % (first separated from sample) not exceed 1%, foreign material % does not exceed 1%, total damage kernels % (heat damage, sprout damage, insect damage and mold damage kernels) not exceed than 4%. However that difference between wheat samples, all wheat samples had grade one according to **USAD, (2013c).**

Table 3: Grading of wheat kernel cultivars

Grading requirements		Egyptian Wheat (Masr 2)
Moisture %		11.10±0.5
Test weight p/b		60.50±0.01
Foreign Material %		0.19±0.01
Shrunken &Broken kernels,%		0.32±0.01
Damage Kernels %	Heat Damage	0.0
	Total Damage	3.70±1.0
Odor		Ok
Insect		Free
Grade		2

p/b= Pound per Bushel (American unit)

Results in Table (4) showed that 1000 kernels of wheat was 50.0 gm. for addition the colour in all samples, wheat is red whereas DDGS are brown and gold. Additionally, it showed that wet, dry gluten, hydration ratio and gluten index were (27.0%), (8.4%), (221%) and (87.2%) respectively for wheat and free to the two different DDGS. A falling number indicted enzyme activity of Alfa amylase. In case of falling number, Egyptian hard red wheat (Masr 2) has highest falling number (431 sec.) and lowest enzyme activity. It can be concluded that Egyptian hard red wheat (Masr 2) has the good quality for physical properties according to (ES, 2004) and (ES, 2006).

Table 4: physical properties of wheat kernels and two different DDGS

Physical properties	Egyptian Wheat (Masr 2)	DDGS	
		Brown	Gold
Weigh per 1000 kernels gm	49.0±0.1	*	*
Hardness%	62 ±1.0	*	*
Colour	Red	Brown	Gold
Wet gluten %	27.0±0.1	Free	Free
Dry gluten %	8.40±0.1	Free	Free
Hydration ratio	221±0.1	Free	Free
Gluten index %	87.20±0.1	Free	Free
Falling Number in Sec.	431.0±1.0	245±1.0	264±1.0

* = Not detected

Results of Table (5) showed that the flour yield was different slightly among test samples and ranged from 70.14 to 100%. So data present indicated that Egyptian hard red wheat (Masr2) had flour yield (70.14%) while (brown and gold) DDGS had flour yield (100%) respectively. On the other hand Egyptian hard red wheat (Masr 2) had coarse bran (15.35%). However fin bran (5.41%) and semolina (9.1%). However, these differences may be partly attributed due to different growing and environmental conditions that prevailed during growing periods **(Randhawa *et al.*, 2002)**.

Table 5: Extraction of different flour obtained from wheat kernels and two different DDGS

Flour	Egyptian Wheat (Masr 2)	DDGS	
		Brown	Gold
Coarse Bran%	15.35	*	*
Fin Bran %	5.41	*	*
Semolina %	9.10	*	*
Flour yield %	70.14	100.0	100.0

* = Not detected

Chemical composition of flour prepared from different DDGS, wheat and mixtures is showing in Table (6). Results indicated that chemical compositions of flour are different in all investigated samples. Moisture content is ranged from 10.7% brown DDGS flour to 14.1% (Egyptian hard red wheat flour) while (gold DDGS) flour contains the highest protein (31.6%) and lower nitrogen free extract (32.1%). However it had the highest fat content compared with other studied samples. On other hand, the Egyptian hard red wheat flour had the lowest ash content.

Table 6: Proximate analysis of different flour obtained from wheat kernels, two different DDGS and it's mixtures

Chemical properties	Egyptian Wheat (Masr 2)	DDGS		Mix 1	Mix 2
		Brown	Gold		
Moisture%	14.1	10.7±0.1	10.8±0.1	12.3±0.1	12.4±0.1
Protein%	10.30±0.1	28.8±1.0	31.6±1.0	19.4±1.0	20.6±0.1
Fat %	1.0±0.1	7.9 ±0.01	11.5±0.01	4.4±1.0	6.22±0.01
Ash%	0.50±0.01	7.9±0.1	7.8±0.1	4.1±0.01	4.0±0.1
Fiber%	0.15±0.01	6.4±0.01	6.2±0.01	10.4±0.01	10.2±0.01
NFE%	73.95 ±0.1	38.3±0.01	32.1±0.01	49.4±0.01	46.58±0.1
Total caloric values%	346.0±0.01	339.5±0.01	358.3±0.01	314.8±0.01	324.7±0.01

The data in Table (7) showed that the highest starch damage was in brown DDGS flour (11.2%) while Egyptian hard red wheat (Masr 2) flour was the lowest (7.0%). The rheological properties of wheat flour dough were tested by alveograph and consistgraph. The results of the wet and the dry gluten and hydration ratio of different flour samples are given in Table (7). The Egyptian hard red wheat (Masr 2) flour showed protein content of 10.30% have wet , dry gluten and hydration ratio than other samples 30.1, 11.30 and 163 % respectively. Additionally, all samples investigated good characteristics to production of bread except the brown DDGS flour and gold DDGS flour, while Egyptian hard red Wheat (Masr 2) flour can be used to produce pasta and bread. The same table reviewed that the falling number values were ranged from 245 to 454 sec. Egyptian hard red Wheat (Masr 2) flour had the highest value (454 sec.) and the brown flour had lower values (324 sec.). Economic European community recommended that the falling number of flour should exceed 230sec **Milatovie and Mondelli, (1991)**. Egyptian standard no. 1419/2006 of white flour for the production of bread has the following requirement: protein content not less than 10.2% Ash content not exceed than 0.9% And the falling number showed exceed than 200 Sec. Also, Egyptian standard no. 1649/2004 for durum wheat has obligation that protein content of durum wheat not less than 10.5% and ash content not exceed than 1.3%. From the same Table (7) it can be concluded that the percentage of sediment ranged from 35.0 to 59.0%. Gold DDGS flour was the highest sediment ratio. It could be also seen that the wheat had the highest value of whiteness colour for flour colour (Egyptian hard red Wheat (Masr 1) flour) 38.3% than the brown DDGS flour which is less in whiteness. Starches damaged are ranged from 7.0 to 11.2%. Brown DDGS flour had the highest value while Egyptian hard red Wheat (Masr 1) flour had the lowest value.

Table 7: physicochemical properties of different flour obtained from wheat kernels, two different DDGS and it's mixtures

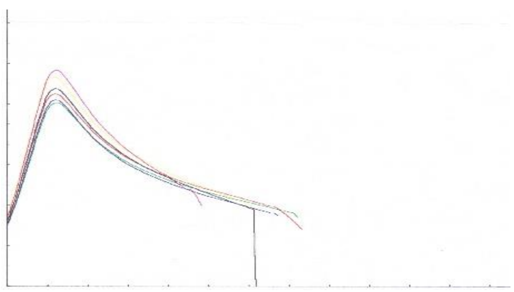
Physicochemical properties		Egyptian Wheat (Masr 2)	DDGS		Mix 1	Mix 2
			Brown	Gold		
Starch damage %		7.00	11.2	10.9	8.9	8.5
Gluten quantity	Wet%	30.10	Free	Free	15.0	15.1
	Dry%	11.30	Free	Free	5.20	5.60
	Hydration ratio	163.0	Free	Free	81.0	81.2
	Index%	94.10	Free	Free	45.10	46.5
Protein sediment %		35.0	55.0	59.0	45.0	47.0
Falling Number Sec.		454.0±1.0	245±1.0	264±1.0	350±1.0	361±1.0
flour Colour %	White	38.30	14.0	20.50	23.80	30.70
	Yellow	14.40	35.90	21.90	25.20	15.50

3.2 Rheological properties from wheat kernels, two different DDGS and it's mixtures flour samples

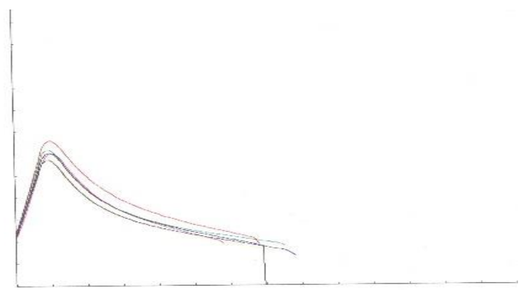
Consistgraph studies were conducted to determine water absorption of wheat, two different DDGS and it's mixtures flour (Table 8). The highest water absorption (57.0%) was observed in Egyptian hard red Wheat (Masr 2) flour followed by (Mix2) flour (51.50%) while brown DDGS flour had the lowest water absorption (47.2%). Water absorption is considered to be an important characteristic of flour. Stronger wheat flours have the ability to absorb and retain more water as compared to weak flours. Higher water absorption is required for good bread characteristics which remain soft for a longer time. Results in (table 8) for different wheat flour varieties were comparable to the earlier findings of **(Raman et al.,2009), (Rehman *et al*., 2001)** and **(Huma (2004).** Results in (Table 8) and Fig (1) showed that the Tenacity (P) values were highly different between all cultivars which ranged from 19 mm H2O to 139mm H2O, Egyptian hard red wheat (Masr 2) flour (139 mm H2O) had the highest value while (Mix 1) flour (19 mm H2O) was the lowest. For L, a value of 100 mm is generally regarded as good, but for some applications like biscuit making, it is the minimum accepted so that the Egyptian hard red wheat (Masr 2) flour (54mm H2O) was the highest value while (Mix2) flour (9.0 mm H2O) was the lowest value. G can be interpreted in the same way as L which ranged between (6.7 ml) to (16.4 ml). The P/L value is increasingly used in the wheat trade. A value of 0.50 corresponds either to resistant and very extensible dough or dough that is less resistant and only moderately extensible (the most common case). A value of 1.50 corresponds to a very strong and moderately extensible dough. The milling industry requires balanced wheat, i.e. with a P/L in the 0.50–0.80 range so that the (Mix2) flour (2.78%) had the highest value while Egyptian hard red wheat (Masr 2) flour (1.57%) was the lowest. Baking strength (W) showed that the Egyptian hard red wheat (Masr 2) flour (277 jol) had the highest value while (Mix 1) flour (9.0 jol) was the lowest. The different alveograph curve measurements give information about the strength and extensibility of dough. The P values of standard wheat range from 60 to 80 mm H2O and of very good quality wheat from 80 to 100 mm H2O; the values for extra strong wheats are higher than 100 mm H2O. W is the most widely used characteristic because it summarizes all the others. The very different shapes of the curves from 'extreme' individuals indicate the great variation in dough strength and extensibility present in the core collection. The relationships between grain characteristics, flour and dough properties and from results in Table 4, 6, 7 and 8.

Table 8: Rheological properties of different flour obtained from wheat kernels, two different DDGS and it's mixtures

Rheological properties		Egyptian Wheat (Masr 2)	DDGS		Mix 1	Mix 2
			Brown	Gold		
Consistgraph	Water absorption %	57.0	47.2	48.5	49.5	51.50
Alveograph test	Tenacity mm H2o (p)	139	*	*	19.0	25.0
	Expandability mm (L)	54	*	*	10.0	9.0
	Swelling ml (G)	16.4	*	*	7.0	6.70
	Baking strength Jol (w)	277	*	*	9.0	61.0
	Configuration rate % (P/L)	1.57	*	*	1.9	2.78



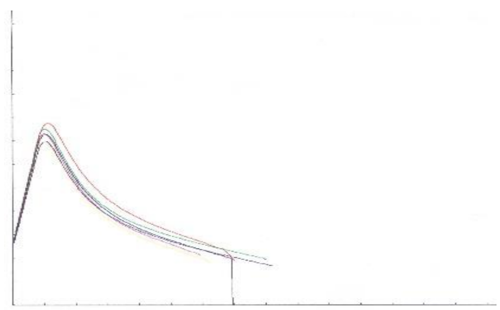
Egyptian wheat flour (Masr2)



(Mix 1) flour

No graph test

(Brown & gold) DDGS flour



(Mix 2) flour

Fig. (1): Effect of wheat flour and it's mixtures on Alveograph test

3.3 Physical properties of Balady bread made from wheat kernels, two different DDGS and its mixtures flours

Data of (physical properties) in Table (9) showed that Egyptian balady bread Masr2 had the golden yellow colour 50.0 while (Mix 1) balady bread had some browning with golden yellow colour 60.20. On the other hand, the (Mix 2) balady bread had golden red colour 42.78 which is good ability to panelists. Additionally the weight after baking for among of balady bread were ranged between 128 to 130 gm. Which the Egyptian balady bread Masr2 had heaviest weight 130 gm followed by (Mix 2) balady bread 129 gm while (Mix 1) balady bread had less weight 128 gm. In the other side the volume after baking is different because the Egyptian balady bread Masr2 had a highest volume 883 cm^3 followed by (Mix2) balady bread 350cm^3 while (Mix 1) balady bread had lowest volume 270 cm^3, so the specific volume is related to the volume too because the Egyptian balady bread Masr2 had highest volume 6.79 cm^3/g followed by (Mix2) balady bread 2.71 cm^3/g while (Mix 1) balady bread had lowest volume 2.11 cm^3/g, however loaf volume for Egyptian balady bread Masr2 had lowest loaf volume 0.14 g/cm^3 and more air inside it, then followed by (Mix2) balady bread 0.37 g/cm^3 while (Mix 1) balady bread had highest loaf volume 0.47 g/cm^3 and less air inside it.

Table 9: Physical properties of Balady Bread made from wheat kernels, two different DDGS and mixtures flours

Balady Bread	Crust colour		Weight after baking gm	Volume after baking	Specific volume cm3/g	Loaf volume g/cm3
	White	yellow				
Egyptian Wheat (Masr 2)	-30.88	50.00	130.0	883	6.79	0.14
DDGS	*	*	*	*	*	*
Mix 1	-20.50	60.20	128.0	270	2.11	0.47
Mix 2	-25.68	42.78	129.0	350	2.71	0.37

***** = Not detected

3.4 Sensory evaluation

Data in Table (10) showed the Sensory evaluation of Balady Bread made from different wheat and mixture flour. It can be noticed that Egyptian balady bread Masr2 had the highest total scores than the (Mix 2) balady bread 79.0 and 75.5% respectively until the lowest one is (Mix 1) balady bread 69.5%. Bread prepared from different wheat cultivars and mixture flour were subjected to sensory evaluation for crust colour, crust characteristic, crumb colour, taste and flavour, grain and texture and chewing each their mean scores were calculated (Table 10). Highest mean score for crust colour (8.0) was obtained by Egyptian balady bread Masr2 whereas (Mix 1) balady bread got the lowest score (6.5). The low score of (Mix 1) balady bread may be due to high fiber and ash content, which affect the colour of bread since consumers prefer creamy colour and not dark brown bread. In case of taste and flovour, Egyptian balady bread Masr2 was at the top (15.0) followed by (Mix1) and (Mix 2) balady bread (14.0). Maximum crust characteristic score (8.0) was attained by Egyptian balady bread Masr2 while (Mix 1) balady bread received the minimum score (7.0). (Mix 1) balady bread obtained the least score (15.0) for crumb colour whereas Egyptian Masr2 and (Mix2) balady bread received the highest score (16.0). The differences in colour, taste and flavour of all the bread were attributed to the differences in hardness/softness of wheat grains and other factors like wheat varieties and milling characteristics of wheat. For grain and texture, highest mean score (16.0) was obtained by Egyptian balady bread Masr2 followed by (Mix2) balady bread (15.0). As regards chewing, Egyptian balady bread Masr2 got the maximum score (16.0) and (Mix 2) balady bread obtained the minimum score (14.0). A wheat aroma and taste are desirable with a non-sticky, soft chewing feel in mouth**.** With respect to overall acceptability of chapattis, highest score (79.0) was obtained by Egyptian balady bread Masr2 and thus regarded as more acceptable than other mixture flour while lowest score (69.0) was obtained by (Mix 1) balady bread thus considered least acceptable. This results are parallel with the results obtained by **Rabie, (1992), (Dhaliwal et al., 1996)** and **(Farooq et al., 2001).**

Table 10: Sensory evaluation of Balady Bread made from wheat kernels, two different DDGS and mixtures flours

Balady Bread	Crust Colour 10	Crust characteristics 10	Crumb colour 20	Grain and texture 20	Taste and flavor 20	Chewing 20	Total scores 100
Egyptian Wheat (Masr 2)	8.0[a]	8.0[a]	16.0[a]	16.0[a]	15.0[a]	16.0[a]	79.0
Mix 1	6.5[ab]	7.0[a]	15.0[ab]	13.0[a]	14.0[a]	14.0[a]	69.5
Mix 2	8.0[a]	7.5[a]	16.0[a]	15.0[a]	14.0[a]	15.0[a]	75.5

3.5 Biological evaluation:

This is the first study to evaluate the biological effect of some bakery products made by mixing DDGS (brown, gold) with wheat flour as bread for human nutrition.
Rats fed on Balady bread from wheat and two different DDGS mixtures flours (brown, gold and wheat flour) for 8 weeks then sacrificed, and plasma sample were taken for different analysis.

3.5.1Body weight gain and relative organs weight:

Table (11) showed that there was a significant difference in body weight gain in control group and the other groups fed on balady bread and two different DDGS mixture flours. Mix1caused significant increase in body weight gain (4.4±1.6 g) than control group (2±1.1g), Egyptian wheat (Masr2) (-1±0.1g) and Mix2 (-11±1.1g) while Mix2 bread caused significant decrease in body weight gain than control group, Egyptian bread (Masr2) and Mix1. For the relative weight it was found that there were no significant differences in relative organs weight among 4 groups except for liver it was found that there was a significant increase in relative liver weight for Egyptian bread Masr2, Mix1 and Mix2 than control group.

Table (11): Body weight gain and relative weight for experimental animals fed on control diet and Balady Bread made from wheat kernels, two different DDGS mixtures flours

	Control G1	Masr2 G2	Mix1G3	Mix2 G4
Initial weight	202±8.5[a]	192±6.6[a]	196±6.4[a]	189±5.8[a]
Final weight	204±9.6[a]	191.8±6.7 [ab]	200.4±8.0 [ab]	178±6.7 [b]
Body weight gain	2±1.1[b]	- 1±0.1[c]	4.4±1.6[a]	-11±1.1[d]
Liver	2.95±0.07[c]	3.7±0.10[a]	3.4±0.08 [b]	3.5±0.15 [ab]
Kidney	0.8±0.05 [b]	0.89±0.04 [b]	1.02±0.016[a]	1.06±0.04[a]
Heart	0.31±0.07[a]	0.31±0.01[a]	0.32±0.01[a]	0.31±0.01[a]
Lung	0.84±0.01 [b]	0.93±0.02[a]	0.91±0.02[a]	0.85±0.02 [b]
Brain	0.79±0.01[a]	0.81±0.04[a]	0.84±0.03[a]	0.85±0.02[a]
Spleen	0.4±0.03[a]	0.39±0.02[a]	0.37±0.03[a]	0.42±0.02[a]

3.5.2 Lipid profile:

Table (12) describe the lipid profile for experimental animals and it was noticed that Egyptian bread (Masr 2) caused a significant increase in the levels of Total lipid, T.G, Cho., LDL and VLDL (452.6±6.1, 144.8±4.6, 93.0±0.94, 42.0 and 42.0 ±1.7mg/dl) respectively than control diet (358.2, 73.6, 78.4, 19.7 and 19.7 mg/dl) respectively, while, it caused a significant decrease in HDL (22.0 mg/dl) than control group (44.0 mg/dl).Mixing DDGS with wheat flour significantly decreased the levels of total lipid ,TG, Cho., LDL and VLDL than Egyptian bread (Masr2) and also lower than control group, on the other hand, the level of HDL was increase for animals fed on bread prepared from mixing DDGS with wheat flour. The best effect on lipid profile was found in the group of rat fed on Mix 2 (50%wheat flour+50% Gold DDGS) it enhance lipid profile than control group who fed on normal diet.

Table 12: Lipid profile for experimental animals fed on control diet and Balady Bread made from wheat kernels, two different DDGS mixtures flours

Lipid profile	Control G1	Masr2 G2	Mix1 G3	Mix2 G4
TL mg/dl	358±1.2[b]	452±6.1[a]	353.4±3.3 [b]	294.2±4.7[c]
TG mg/dl	73.6±2.5 [c]	144.8±4.6[a]	94.0±1.97 [b]	59.8±1.3 [d]
Choles mg/dl	78.4±1.65 [b]	93.0±0.94[a]	57.2±0.85 [c]	90.4±2.2[a]
HDL mg/dl	44.0±1.2[a]	22.0±0.7 [c]	38.8±0.97 [b]	39.6±1.3 [b]
LDL mg/dl	19.7±2.1 [b]	42.0±1.7[a]	4.4±0.7 [c]	38.8±1.1[a]
VLDL mg/dl	14.7±0.49 [c]	28.96±0.92[a]	18.8±0.39 [b]	11.96±0.26 [d]

3.5.3 Biochemical parameters

Biochemical parameters for experimental animals summarized in table (13), it was noticed that Balady Egyptian bread (Masr2), Mix1 and Mix2 caused a significant decrease in total protein, albumin and globulin than control group (7.1± 0.07, 3.6± 0.08 and 3.5± 0.09 mg/ml), respectively. The most decrease was found with mix2 (6.1± 0.07, 2.9± 0.07 and 3.2± 0.13 mg/ml), respectively. On the other hand it was found that Egyptian bread (Masr2) caused significant increase in total bilirubin, Urea and creatinine (0.298± 0.012, 62.0± 0.8 and 1.07± 0.06 mg/ml) respectively than control group (0.036± 0.007, 44.0± 0.7 and 0.7± 0.045) respectively, while mixing wheat flour with DDGS flour (brown and gold) caused a significant decrease in total bilirubin, urea and creatinine especially with 50% gold

DDGS (0.028± 0.002, 54.8± 1.36 and 0.52± 0.039) respectively than Egyptian bread (Masr2) it also lower than control group especially in total bilirubin and creatinine. For glucose level, it was noticed that there was no significant change in glucose levels between treatment groups and control group except with Mix1 that causes a significant decrease in glucose level(49.0± 1.6) than control group (60.4± 1.3) Egyptian wheat (60.2± 1.2) and Mix2 (61.0± 1.9).

Table (13): Biochemical analysis for experimental animals fed on control diet and Balady Bread made from wheat flour, two different DDGS mixtures flours

Biochemical parameters	Control G1	Masr2 G2	Mix1 G2	Mix2 G4
TP	7.1±0.07^{a}	6.5±0.1 ^{b}	6.7±0.13 ^{b}	6.1±0.07 ^{c}
Albumin	3.6±0.08^{a}	3.3±0.07 ^{b}	3.3±0.08 ^{b}	2.9±0.07 ^{c}
Globulin	3.5±0.09^{a}	3.2±0.14 ^{b}	3.3±0.07 ^{b}	3.2±0.13 ^{c}
Total bilirubin	0.036±0.007^{c}	0.298±0.012^{a}	0.160±0.006^{b}	0.028±0.002^{c}
Urea	44.0±0.7 ^{c}	62.0±0.8^{a}	54.4±0.83 ^{b}	54.8±1.36 ^{b}
Creat	0.7±0.045 ^{b}	1.07±0.06^{a}	0.69±0.045^{a}	0.52±0.039 ^{c}
Glucose	60.4±1.3^{a}	60.2±1.2^{a}	49.0±1.6 ^{b}	61.0±1.9^{a}

3.5.4 Enzymes activity:

Table (14) describe the activity of the enzyme for experimental animals, it was found that Egyptian bread (Masr 2) caused a significant increase in all enzymes activity (AST, ALT, ALP) (85.0± 1.9, 61.4± 0.83, 64.0± 1.5), respectively than control group (46.2± 0.7, 28.6± 0.85, 34.0± 1.15) ,respectively, while mixing DDGS flours (brown, gold) with wheat flour significantly decrease enzymes activity than Egyptian bread (Masr2) but it still higher than control group. The best effect was found with Mix1 (50.2± 0.83, 35.2± 1.14 and 38.8± 0.97mg/ml), respectively.

Table (14): Enzymes activity in experimental animals fed on control diet and Balady Bread made from wheat flour, two different DDGS mixtures flours

Enzymes	Control G1	Masr2 G2	Mix1 G3	Mix4 G4
AST	46.2±.7 ^{c}	85.0±1.9^{a}	50.2±0.83 ^{c}	63.2±2.01 ^{b}
ALT	28.6±0.85 ^{d}	61.4±0.83^{a}	35.2±1.14 ^{c}	45.2±1.65 ^{b}
AlP	34.0±1.15 ^{c}	64.0±1.5^{a}	38.8±0.97 ^{b}	36.4±1.15 ^{bc}

3.5.5 HISTOLOGICAL OBSERVATIONS:-

3.5.5.1 The liver:-

3.5.5.1.1 Liver of control group:

Examination of liver of control group showed that the structural unit of the liver is the hepatic lobule which is made up of radiating plates, cords, or strands of hepatocytes forming a network around central vein **(Fig.2)**. The liver strands are alternating with narrow blood sinusoids also radially extending along the liver lobules the centrolobular vein has a generally a circular outline, being limited by a thin coat consists of layer of endothelial cells supported by a space population of collagen fibers. The sinusoids are narrow blood spaces with irregular boundaries composed essentially of only single layer fenestrated endothelial cells in addition to large irregular shape cells of the mononuclear type, the *von kupffer* cells which are known to be actively phagocytic cells. The portal veins are comparatively wide in size, whereas the hepatic artery branches are much narrower and are normally devoid of any blood cells. The bile ductules appear round and bounded by alayer of cuboidal cells encircled by a thin sheath of connective tissue.

3.5.5.1.2 Liver of balady Masr2, Mixture 1and 2 treated rats

Rat liver sections in balady Masr2, Mixture 1and 2 treated rats (G2-G4) groups showed normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in-between the hepatic cords with a fine arrangement of Kupffer cells **(Fig. 3-5)**. No histological alterations were observed.

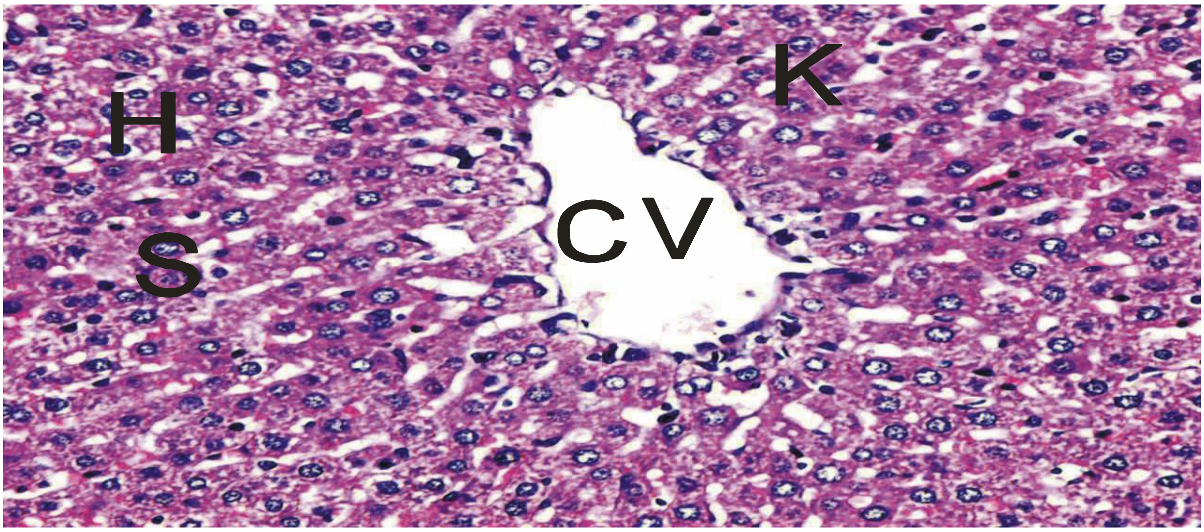


Fig.(2): Section of liver of a control group (G1) showed normal large polygonal cells with prominent round nuclei and central vein (CV), hepatocytes (H) and few spaced hepatic sinusoids (S) arranged in-between the hepatic cords with fine arrangement of Kupffer cells (K), (H&E X200).

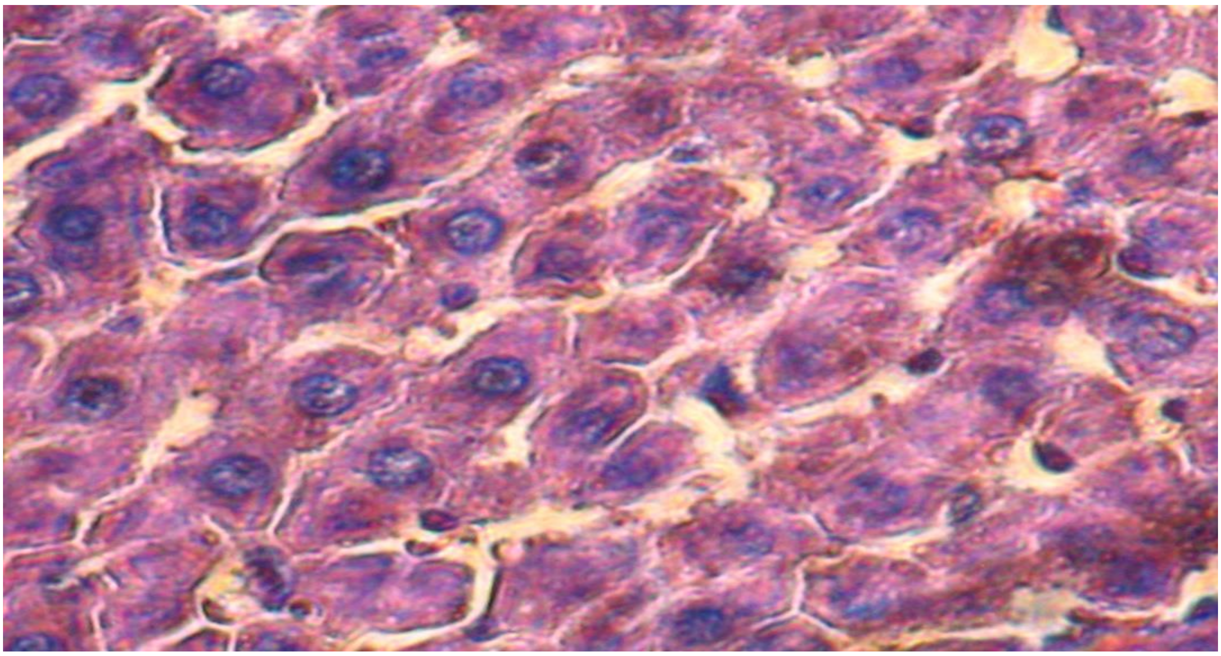


Fig. (3): Liver section of balady Masr2 group (G2) showed normal structure of liver with normal hepatocytes with central nuclei (H&E.,X400).

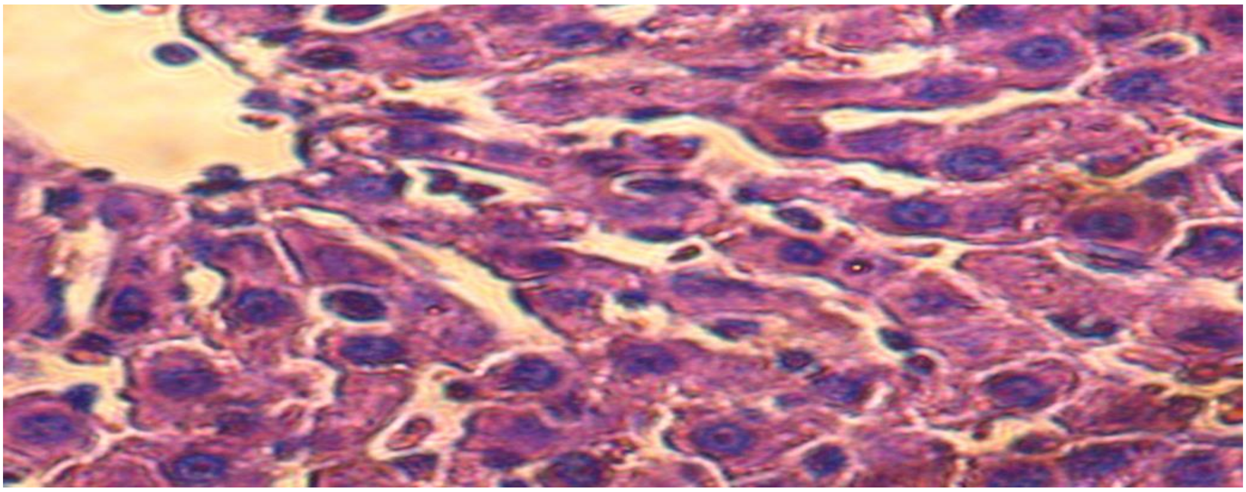


Fig.(4): Section of liver of Mix 1 group (G3) showed central vein, hepatocytes (H) and few spaced hepatic sinusoids arranged in-between the hepatic cords with fine arrangement of Kupffer cells ((H&E.,X400).

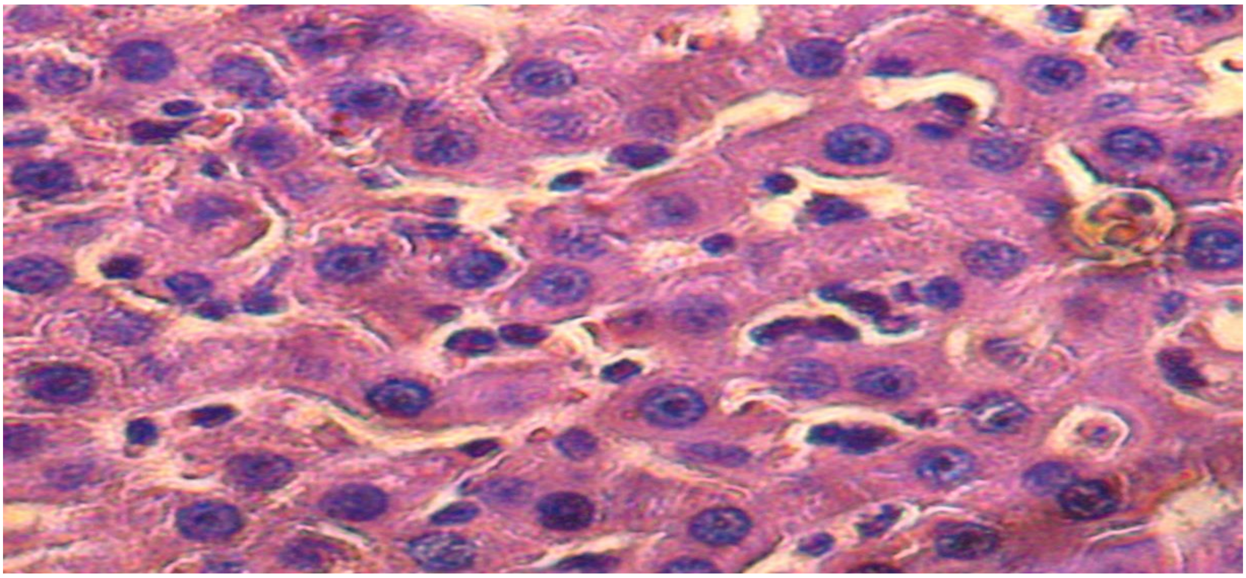


Fig.(5): Liver section of Mix 2 group (G4) showed normal structure of liver with normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in-between the hepatic cords with fine arrangement of Kupffer cells (H&E.,X400).

3.5.5.2 The kidney: -

3.5.5.2.1 Kidney of control group:

The kidney of control rats (G1) is a bean-shaped compound tubular gland consisting of a large number of uriniferous tubules that constitute the major part of the renal tissue. It is covered by a firm connective tissue-capsule composed of collagenous fibers and few elastic fibers. The kidney is differentiated into two regions; an outer cortex and an inner medulla **(Fig. 6).** The cortex consists of Malpighian corpuscles and both proximal and distal convoluted tubules while the medulla consists mainly of the descending and ascending limbs of Henle's loop. However, the collection tubules are located in both the cortical and medullary regions **(Fig. 6)**.

The Malpighian corpuscles consist of tuft of blood capillaries, the glomerulus and Bowman's capsule. The latter is double walled cup formed of two layers of simple squamous epithelium, an outer parietal layer and an inner wisceral layer or glomerular epithelium consists of a single layer of epithelial cells, which closely invest the glomerulus and dips down the lobules of the glomerular capillaries. In addition, the glomerular capillaries possess certain cells known as mesangial cells. The glomerulus consists of a number of capillary loops of an afferent and efferent arteriole separated by reticular connective tissue fibers. The proximal convoluted tubules are the longest segments of the nephron and occupy the major part of the cortex, each has definite basement membrane. They are lined by a single layer of short columnar cell boundaries, deeply stained eosinophilic cytoplasm and large round basely located nuclei. The free ends of the cells, towards the lumen, are provided with a peculiar brush border. The distal convoluted tubules are shorter since they are less convoluted than the proximal ones. They are lined by cuboidal cells that possess distinct cells boundaries and contain homogenous cytoplasm, which stain less intensely with acidic dyes compared with that of the proximal convoluted tubules, and conspicuous spherical centrally located nuclei. The collecting tubules are long straight and extending in a radial direction, throughout the medullary rays and in the medulla. They are lined with cuboidal cells with distinct cell boundaries, homogenous cytoplasm and round darkly stained nuclei **(Fig. 6).**

3.5.5.2.2 Kidney of balady Masr2, Mixture 1and 2 treated rats:

Rat kidney sections in balady Masr2, Mixture 1and 2 treated rats (G2-G4) groups showed no histological alterations and kidney sections in the three groups have normal histological structures of the glomeruli and renal tubules in the cortical and medullary portions. The glomerulus surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes (Figures 7-9).

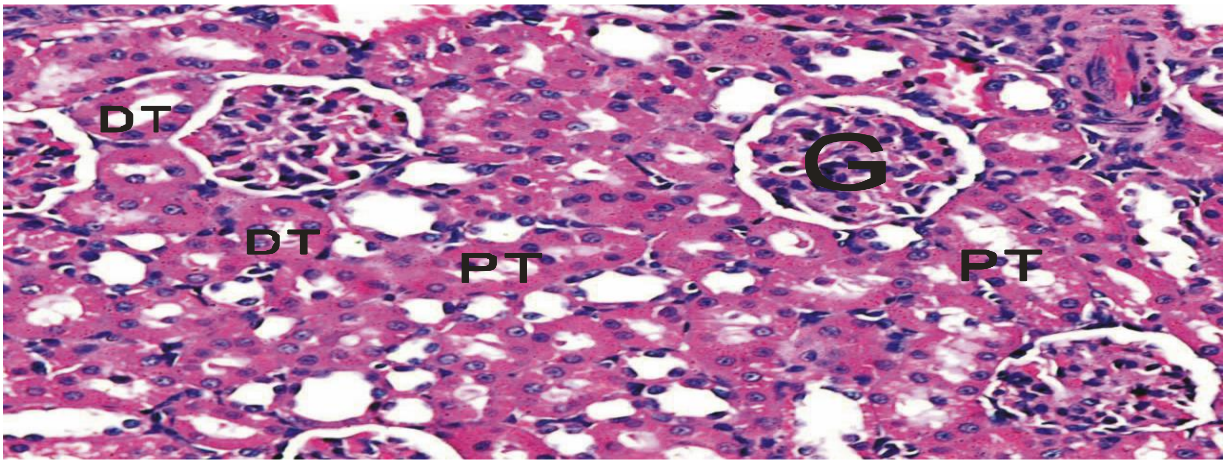


Fig. 6: Photomicrograph of rat kidney sections stained by H&E. Section of kidney of control group (G1) showed normal structure of the cortex and medulla, glomeruli (G), proximal convoluted tubules (PT) and distal convoluted tubules (DT), (H&E., X200).

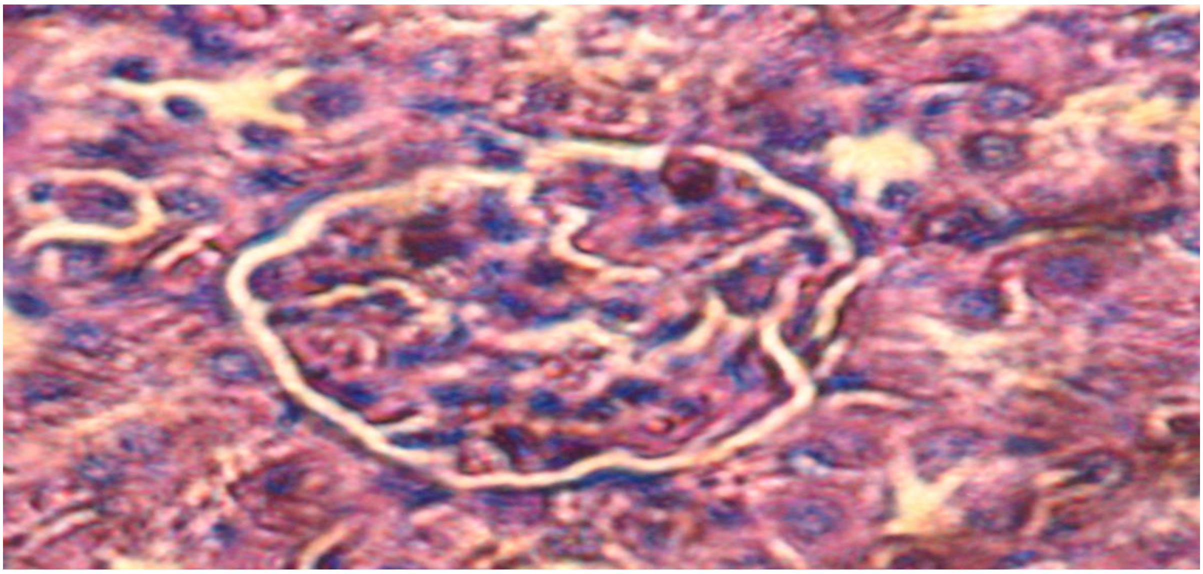


Fig. 7: Photomicrograph of rat kidney sections stained by H&E. Section of kidney of balady Masr2 group (G2) showed normal structure of the cortex and medulla, glomeruli and renal tubules (H&E., X400).

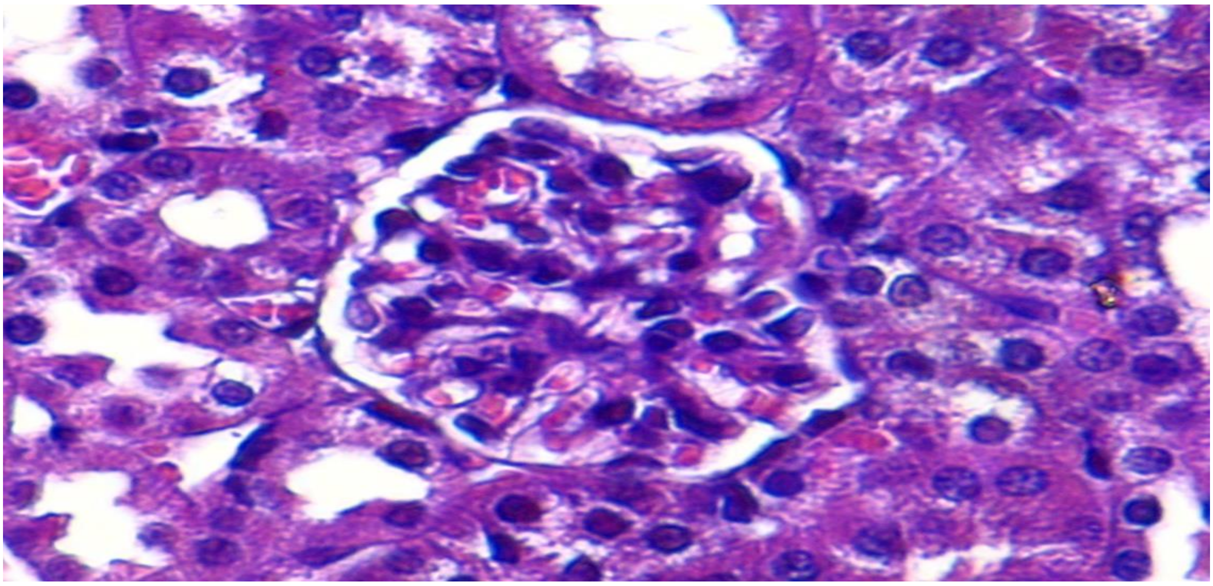


Fig. 8: Photomicrograph of rat kidney sections stained by H&E. kidney section in treated rats with Mix 1 (G3) showed normal histological structures of the glomeruli and renal tubules (H&E., X400).

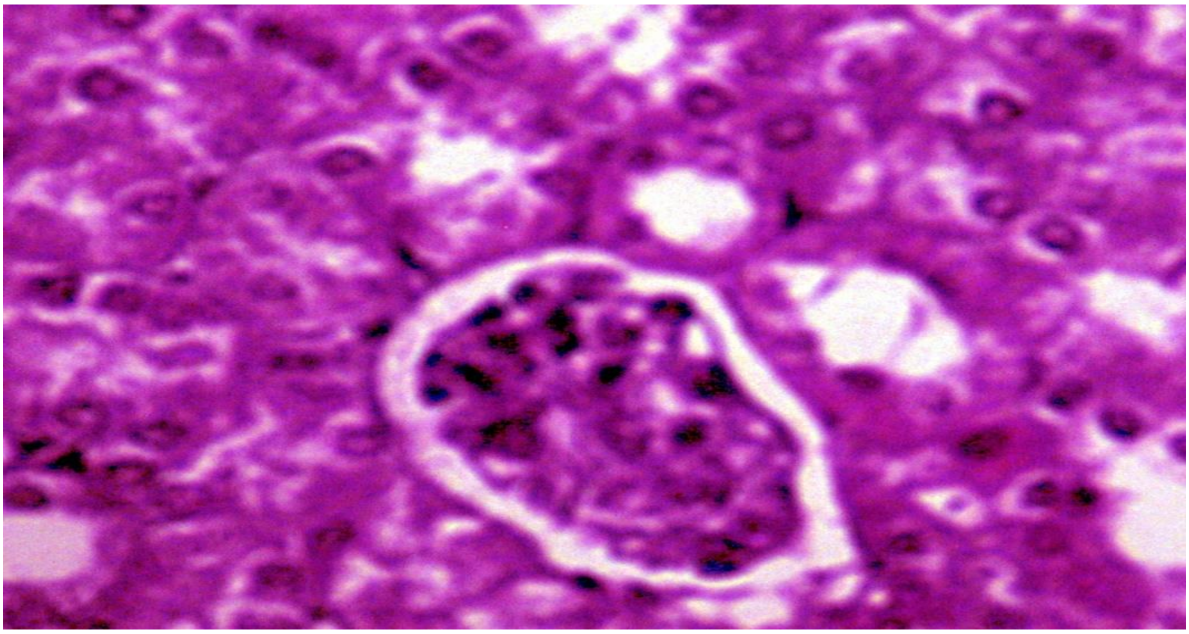


Figure 9: Photomicrograph of rat kidney sections **stained** by H&E. kidney section in treated rats with Mix 2 (G4) showed normal histological structures of the glomeruli and renal tubules (H&E., X400).

4. CONCLUSIONS

Data indicated that wheat and (Mix 2) flours had more suitable properties for bread- making than the (Mix 1) flours. (Mix 2) flour was low cost than wheat flour which is more suitable flour to us than the other mixtures. From the different tested flours indicated that those made from Egyptian hard red Wheat (Masr 2) flour and (Mix2) flours were superior. From the previous results of biological experimental we can concluded that fed on bread making from mixing DDGs flour either from brown or gold can enhance health status of animals than fed on bread from Egyptian wheat only (Masr 2). So we can advised that its healthier to add DDGs flour to wheat flour to make bakery for human.

Conflict of interests

The authors declare no conflict of interest.

REFERENCES

1. AACC (2000a). American association of cereal chemists, Approved method of the AACC 10th ed., vol. 1, AACC, St Paul, MN.
2. AACC (2000b). American association of cereal chemists, Approved method of the AACC 10th ed., vol. 2, AACC, St Paul, MN.
3. Abdelghafor, O. R.F., Mustafa, A.I., Ibrahim, A.M.H. & Krishnan, P.G. (2011). Quality of Bread from Composite Flour of Sorghum and Hard White Winter Wheat," *Advance Journal of Food Science and Technology*, 3, (1), 9-15.
4. A.O.A.C. (2005). Association of Official Analytical Chemists. *Official Methods of Analysis*. 18th Ed. Published by A.O.A.C. W. Horwitz. North Frederick, U.S.A.

5. Bergmeyer, H., Horder M, & Rej, R. (1986). Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3.IFCC Method for alanine amino transferase. *Journal of Clinical Chemistry and Biochemistry*, *24*:481-495.
6. Chan, K. & Lowe, J. (2002). *Techniques in neuropathology*. In Bancroft JD, Gamble M, eds. Theory and practice of histological techniques. (5th ed., chapter18).New York, Edinburgh, London, Madrid, Melbourne, San Francisco, Tokyo: Churchill Livingstone; 374–5.
7. Dhaliwal, Y.S., Hatcher, D.W., Sekhon, K.S. & Kruger, J.E. (1996). Methodology for preparation and testing of chapattis produced from different classes of Canadian wheat. *Food Res. Int.*, *29*: 163-168.
8. Doumas, B.T., Watson, W.A. & Biggs, H.G. (1977). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinical Chemistry. Acta*, *31*:87-96.
9. Elkhalifa, A. E. O. & Bernhardt, R. (2010). Influence of grain germination on functional properties of sorghum flour; *Food Chemistry*, *121*, 387-392.
10. ES (1986). Egyptian Standard of wheat grains. *Egyptian Organization for Standardization and Quality Control*, No. 1601, and its modification No. 2/2002. Arab Republic of Egypt.
11. ES (2004). Egyptian Standard of durum wheat. *Egyptian Organization for Standardization and Quality Control*, No. 1649. Arab Republic of Egypt.
12. ES (2006). Egyptian Standard of white flour for production of bread. *Egyptian Organization for Standardization and Quality Control*, No. 1419. Arab Republic of Egypt.
13. FAO (1995). *Food and Agricultural Organization Food and Agricultural Organization, Sorghum and millet in human nutrition.* FAO Food and Nutrition Series No. 27. ISBN 92-5-103381-1.
14. FAO (2005). *Food and Agriculture Organization.* FAO Statistics Database. Accessed on: Feb. 14, 2005; Available at: http:// a pps.fao.org/faostatl.
15. FAO (2009). *Food and Agricultural Organization*. Food and Agriculture Bulletin on Food Security http://www.fao.org/docrep/x5030E/ X15032E06.htm.
16. FAO (2014). *Food and Agriculture Organization*. FAO Statistics Database. Accessed on: April, 2014; Available at: http:// a pps.fao.org/faostatl.
17. Farooq, Z., Rehman, S. & Bilal, M.Q. (2001). Suitability of wheat varieties/lines for the production of leavened flat bread (naan). *J. Res. Sci.*, *12*: 171-179.
18. Heuzé V., Tran G., Sauvant D., Noblet J., Lessire M. & Lebas F. (2017). *Wheat distillers grain.* Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/4265
19. Huma, N. (2004). Fortification of whole-wheat flour with iron for the production of unleavened flat bread (Chapattis). Ph.D. Thesis, Dept. Food Technol. Univ. Agric. Faisalabad.
20. Kunst, A., Draeger, B., & Ziegenhom, J. (1984). *In: Bergmmeyer. Methods of Enzymatic analysis*, 3rd ed. Volume VI, Metabolites1: Carbohydrates 163-172.
21. Lamb, E., Newman, D. & Price, C. (2006). *Kidney function tests In: Burtis CA*, A shwood, E.R., Bruns, D. E. & Tietz. Text book of clinical chemistry and molecular diagnostics.4th ed. St. Louis, MO: Elsevier Saunders, 797-835.
22. Milatovie, L. & Mondelli, G. (1991). *Pasta Technology Today*. Ed. by Chiriotti-Poinerolo (To) - Italy.

23. National Institutes of Health (NIH). *Guidelines for rodent transportation* (12/09/15) and for Survival Bleeding of Mice and Rats (08/12/15).NIH-ARAC Guidelines [online] 2005. Jan 12. Available from: http://www.oacu.od.nih.gov/ARAC/Bleeding. [Accessed on 2010 Mar 09].
24. Olaoye, O.A., Onilude, A.A. & Idowu, O.A. (2006). Quality characteristics of bread produced from composite flours of wheat, plantain and soybeans. *Afr. J. Biotechnol., 5*, 1102-1106.
25. Onyango, C., Mutungi, C., Unbehend, G. & Lindhauer, M. G. (2011). Rheological and textural properties of sorghum-based formulations modified with variable amounts of native or pregelatinised cassava starch, *LWT - Food Science and Technology*, *44*, 687-693.
26. Pisani, T., Gebski, C. & Leary, E. (1995). Accurate Direct Determination of Low-Density Lipoprotein Cholesterol Using an Immunoseparation Reagent and Enzymatic Cholesterol Assay. *Archives of Pathology and Laboratory Medicine*, *119 (12)*:1127-1135.
27. Rabie, Samir Mohamed Hussein , (1992) Soft wheat quality factors that influence the quality characteristics of Egyptian Balady bread, Ph.D., Theses Michigan State University, 244 pages; AAT 9233925
28. Raman, R.; Allen, H.; Diffey, S.; Raman, H.; Martin, P. and McKelvie, K. (2009). Localisation of quantitative trait loci for quality attributes in a doubled haploid population of wheat (Triticum aestivum L.). NRC Research Press, genome 52: 701–715.
29. Randhawa, M.A., F.M. Anjum & Butt, M.S. (2002). Physico-chemical and milling properties of new spring wheat's grown in Punjab and Sind for the production of pizza. *Int. J. Agric. Biol., 4*: 482-484.
30. Rashed, M.M., Atia, A.A. & Hassein, A.A. (1996). Effect of wheat flour and yeast on balady bread characterisitics. *Egypt. J. Food Sci., 24(1)*:81-92.
31. Rehman, S., Nazir,Y., Hussain S. & Huma, N. (2001). Study on the evaluation of wheat varieties of Sindh Province for the production of ring doughnuts. *JAPS, 3:* 135-138.
32. SAS (2011). SAS / Stat. *User's Guide: statistics, system for windows*, version 4.10 (releasa 6.12 TS level 0020), SAS Inst., Inc. Cary, North Carolina, USA.
33. Sugiuchi, H., Okabe, H. & Irie, T. (1995). Direct Measurement of High Density Lipoprotein Cholesterol in Serum with Polyethylene Glycol Modified Enzymes and Sulfated α-Cyclodextrin. *Clinical Chemistry, 41*:717-723.
34. Tietz N. (1995). *Clinical Guid to Laboratory Tests*, 3rd ed. Philadelphia PA: WB Saunders Company. 130 -131.
35. U.S. Department of Agriculture, (2013a). *GRAIN INSPECTION HANDBOOK I. Grain Inspection,* Packers and Stockyards Administration, Federal Grain Inspection Service Probe Sampling, Washington, D.C. 20090-6454.
36. U.S. Department of Agriculture, (2013b). *MOISTURE HANDBOOK Grain Inspection*, Packers and Stockyards Administration 1400 Independence Ave., S. W. Washington, D.C. 20250-3600.
37. U.S. Department of Agriculture, (2013c). *GRAIN INSPECTION HANDBOOK II Grain Inspection,* Packers and Stockyards Administration, Federal Grain Inspection Service.
38. U.S. Department of Agriculture, (2014). *EQUIPMENT HANDBOOK. Grain Inspection,* Packers and Stockyards Administration, 1400 Independence Ave., S.W. Washington, D.C. 20250-3600.
39. U.S. Department of Agriculture, (2016). *The 3rd Edition of the DDGS User Handbook Distiller's Dried Grains with Solubles (DDGS),* Distillers Grains Technology Council.

40. Wahlefeld, A. & Bergmeyer, H. (1972). Methods of Enzymatic Analysis. *Scand. Journal of Clinical Laboratory Investigation*, 29 (126): Abstract 11&12.