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Effect of ozonation of wheat grain on quality bread factory

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Abstract

Ozonation of wheat grain is a quick and easy process that could be realized during the storage. The ozone is in direct contact with the grain and modifies immediately the properties of the wheat. Stability and resistance of dough were increased with ozonation at 40 ppm in comparative with control sample. The results showed a gradual increase in specific volume of balady bread according to the time of ozonation. On the other hand sensory evaluation of balady bread showed ozonated wheat grain had good crust color of bread when compared with control sample. It was observed that the overall acceptability of the balady bread was affected significantly with extended exposure. Therefore overall acceptability decreased to 78.6% with ozonation at 40 ppm for 20 min.

Keywords: Wheat, Ozonation, flour, balady bread, and dough

1. Introduction

Ozonation of wheat grain is a quick and easy process that could be realized during the storage. The ozone is in direct contact with the grain and modifies immediately the properties of the wheat. However, the ozone has to go through the pericarp and the seed coat to reach the endosperm, which represents the greater proportion of the short grade flour. It is therefore expected that not all of the ozone will penetrate the endosperm and the flour that follows will have specific characteristics [6]. Ozone is a highly reactive form of oxygen where three molecules are bonded together. Generated electrically on-site where needed, it has potent antimicrobial activity and other characteristics. Interest in ozone applications for agriculture and food processing has increased in recent years. In 1997, ozone was declared a GRAS (generally recognized as safe) substance by the FDA after a Food Additive Petition containing safety and efficacy data was submitted to them.

Later, the USDA approved its use on meats and on certified organic foods [4]. There are many methods for the production of ozone gas, such as electrical discharge in oxygen, electrolysis of water, or thermal, photochemical or radiochemical methods. For industrial use ozone is generated mainly from pure oxygen or atmospheric oxygen in a corona discharge process [13]. In corona discharge, air or pure oxygen is fed into a unit that converts the oxygen to ozone using high voltage. The attractive aspect of ozone is that it decomposes rapidly (half-life of 20–50 min) to molecular oxygen without leaving a residue [11]. Several research studies have been undertaken to evaluate the effects of ozone gas on rheological properties of dough. Naito (1990) [17] treated wheat flour (medium and soft flour) with an ozone-oxygen stream (0.05 to 50 ppm ozone) at 10°C for 1 to 6 h. Physical dough testing properties showed in a farinograph test, no significant change in the consistency of both flour doughs, with an extensograph, an increase in the

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resistance to extension of both flours for the 0.5 to 50 ppm ozone treatment and a decrease in extensibility for the 0.05 to 50 ppm (soft flour) and the 5.0 to 50 ppm (medium flour) treatments. Ibanoglu (2001) [10] reported that tempering of wheat grain with ozonated water did not significantly alter the chemical, physical and rheological properties of the flours, but statistically significant reductions in the total bacterial and yeast/mould counts were attained. Desvignes et al. (2008) [5] found that application of ozone treatment 10 ppm of common wheat before milling, the required energy at breaking stage whatever the grain hardness was significantly reduced (by 10-20%) and without changes in the flour yield. The effect of ozone treatment on wheat flour, isolated wheat starch and physicochemical properties of wheat starch were determined by Sandhu et al. (2012) [22] found that chemical analysis of starch isolates indicated depolymerization of high molecular weight amylopectins; with a subsequent increase in low molecular weight starch polymers as a result of starch hydrolysis. Ozone treatment resulted in elevated levels of carboxylic groups and decreased total carbohydrate content in amylopectin fractions. Ozone gas can react with various functional groups found in lipid, protein, and carbohydrate. Ozone can react with double bonds found in carotenoid pigments, causing a reduction in yellow color and an increase in flour whiteness. Ozone could also react with double bonds associated with unsaturated fatty acids [7]. Oxidants are used in bread dough systems to whiten the flour and subsequent bread crumb and to promote disulfide bond formation between proteins, which improves dough strength and generally results in increased specific loaf volume. Ozone promotes the oxidation of sulfhydryl groups and the subsequent formation of disulfide bonds between cysteine moieties [26].

Bread made from flour exposed to ozone for 4.5 min had greater specific loaf volume and whiter crumb compared to bread made with control flour. Exposure of flour to ozone for longer times (9–45min) deteriorated quality of bread Sandhu et al. (2011) [21].

2. Materials and methods

2.1. Materials: Wheat samples (*Triticum aestivum*) were obtained from the South Cairo Mills Company, Cairo, Egypt.

2. 2. Methods

*2.2.1.Production of ozone gas:*Ozone gas was produced from air using ozone generator Model OZO 6 VTTL OZO Max Ltd, Shefford, Quebec Canada. (OZO MAX LTD, shefford, Quebec, Canada) from purified extra dry oxygen feed gas. The amount of output from ozone was controlled by a monitor- controller having a plug-in sensor on board which is changed for different ranges of ozone concentration and a belt pan in the monitor-controller allows controlling the concentration in a selected range.

2.2.2. Samples treatment: The wheat grain was treated for 5, 10, 15 and 20 min at room temperature with two different ozone concentrations (20 and 40 ppm).

2.2.3. Wheat flour: The wheat samples were milled using barabender DUISBUG (type: 279002) available at Department of Bread and Dough, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. Final obtained wheat flour (82% extraction).

2.2.4. Farinograph test: The samples were tested by Brabender farinograph model No 178507 Germany HZ 50) was used to determined water absorption (%), arrival time (min), dough development time (min), dough stability (min), mixing tolerance index and degree of weakening. Three hundreds grams of tested flour samples were placed in the bowel of the apparatus and sufficient water was added so that the consistency of the dough reached the optimum form when the mixing curve centered on the 500 Brabendar units (B.U.) at the point of maximum development.

2.2.5. Extensograph test: Extensograph model No: 7724584 used for determined extensibility (E) (mm), resistance to extension (R) (B.U), Proportional number (R/E) and Dough energy (cm2).Extensograph test was carried out as follows: a normal run of the farinograph was mad in order to estimate the water absorption capacity of the flour. The dough prepared from three hundreds grams flour and six grams sodium chloride dissolved in the quantity of water (estimated by farinogeaph). The produced dough was mixed for one minute after which a sufficient salt solution was added to give a consistency of 500 Brabender line (in farinogeaph test), after 5 min of rest ,mixing was resumed and continued until reached the full development time of the farinogram.

The dough was removed from the mixer and cut into two portions, each 150 g. The dough was rounded in the extensograph rounder.

The dough ball was then carefully centered on the shaping unit and rolled into a cylindrical test pieces, this was ten clamped in a lightly greased dough holder. The tested piece was stored in humidified chamber for 45 min from the shaping operation, the stretching hook was started and stopped when the test piece was broken; dough removed after the first test, reshaped, allowed a rest period of 45 min and then stretched again. By repeating such procedure, the dough was tested at 45, 90 and 135 min. For evaluating the results of the extensograph test; the extensogram of the test period at 135 min was used

2.2.6. Preparation of Balady bread: Balady bread was prepared by mixing each 1 kg wheat flour with ingredients including 1.5% compressed yeast, 1.5%sodium chloride and water as neeted by farinograms. The mixture was well mixed in mixer (250 rpm) for 20 min. The dough was left for fermentation at 30ºC for 15 min. After fermentation, the dough was divided into 125g pieces. Each piece was molded on a wooden board previously covered with fine layer of bran and left to ferment about 15 min at the same mentioned temperature and relative humidity. The fermented dough pieces were baked at 380-400ºC for 1-2 min in electric oven. The loaves were allowed to cool at room temperature before organoleptic evaluation [16].

2.2.7. Physical properties of Balady bread: The specific volume of bread was calculated according to the AACC method 10-05.01 by dividing volume (cm^3) by weight (g).

Loaf volume was measured by rapeseed displacement immediately after removal from the oven and weighing. Loaves were placed in a container of known volume into which rapeseeds were run until the container was full. The volume of seeds displaced by the loaf was considered as the loaf volume.

Loaf Specific Volume (LSV), was calculated according to the following:

L.S.V = Loaf volume (cm^3) / Loaf weight (g) = cm^3 /g.

2.2.8. Organoleptic evaluation of balady bread: Balady brad samples were evaluated for the following characteristics: taste (20), crumb distribution (15), odor (10), appearance (10), crust color (9), roundness (6), separation of layers (5) and overall acceptability (10) [16]. A score card and a description sheet were prepared to describe the excellent criteria of each of those characteristics and collect panelist's scores of their acceptability. Two hours after baking loaves (three-digit-codes) were randomized and evaluated by 12 persons.

Statistical analysis: All data were statistically analyzed using the General Linear Model Procedure of the SPSS var 18. The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio [16]. All statements of significance were based on probability of $P < 0.05$.

3. Results and Discussion

3.1. Effect of ozone treatment on farinogram parameters: Data in **Table (1)** show the farinograph parameters from wheat flour dough (82 % extraction) after ozonation wheat grain, i.e. water absorption, arrival time, dough development time, dough stability and dough weakening.

The water absorption of wheat flour dough was 59% in control sample and all treatments. But arrival time was 1.5 min with sample control and treatment at 20 and 40 ppm for 5 and 10 min respectively. Increased arrival time to 2.0 min with ozonation at 20 and 40 ppm for 15 and 20 min respectively. Also, development time increased gradually to 3.0 min after ozonation at 20 ppm for 20 min. While decreased to 2.5 with 40 ppm after 20 min.

Dough stability of control and ozonation at 20 ppm for 5 min were similar 9.5 min but the data showed a trend toward an initial increase in stability with 10 and 15 min exposure to ozone; however, further treatment of wheat grain with ozone gas (20 min) decreased dough stability to 8.5 min.

Dough stability with ozonation at 40 ppm were increase comparative with control to 12.5 min with 5 and 10 min exposure to ozone gas while 15 and 20 min exposure stability was 10.5 min . These results are in agreement with those obtained by Ibanoglu, 2001 and Sandhu *et al*., 2011 [10,21].

These results indicate that a short exposure to ozone gas might increase dough strength, while an extended treatment will decreese dough strength Fig (1).

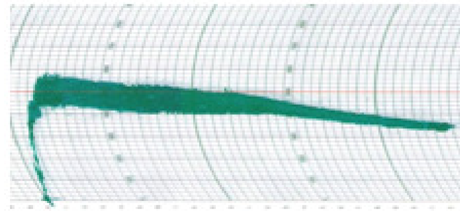
Wheat grain proteins can be classified on the basis of their solubility in different solvents: albumins (soluble in water), globulins (salt), and prolamins [gliadins (alcohol) and glutenins (dilute acid or alkali)].

Wheat prolamins have been classified into two groups, the gliadins and glutenins, according to their solubility in aqueous/alcohol solutions. Gliadin was a mixture of monomeric polypeptides and glutenins consist of polypeptides aggregated by disulphide bonds. The gliadin and glutenin constitute up to 80 to 85% of the total flour protein, and confer elasticity and extensibility properties that are essential for functionality of wheat flours [23].

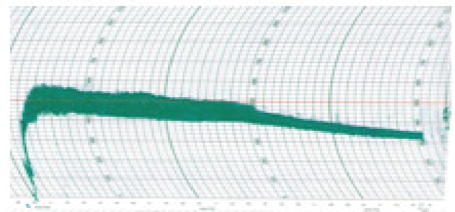
Ozone promotes the oxidation of sulfhydryl groups and the subsequent formation of disulfide bonds between cysteine moieties. An increase in unextractable polymeric proteins in ozone-treated soft wheat flour, which was attributed to an increase in disulfide bonding between protein subunits [3].

Disulfide bonds play a major role in determining dough properties. Exposure to oxidants can increase dough strength by the oxidation of sulfhydryl groups to disulfide bonds [27]. High dose and short exposure to ozone might improve dough strength by promoting disulfide bond formation. Unextractable polymeric proteins have been positively correlated with dough strength[8].

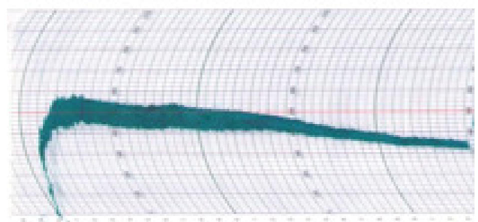
Ozone treatment increased the amount of unextractable polymeric proteins. An increase in unextractable polymeric proteins would improve dough strength and thus increase the dough development time [21]. Amino acids could be oxidized by ozone. In fact, tyrosine is oxidised to dityrosine by ozone. Some authors discovered dityrosine in wheat flour, dough, and bread. These authors suggested that dityrosine could be a new kind of stabilizing cross-link in the wheat gluten structure in addition to disulphide bonds [24]. Storage of grains in ozone rich atmospheres does not influence the rheological properties of grains. For example Mendez *et al*. (2003) [14] investigated the efficacy of ozone to control pests for stored wheat and rice. They reported that ozone treatment does not significantly change the bread-making properties of hard wheat, including tolerance of the dough to overmixing, absorption of water, dough weight, and proof height.



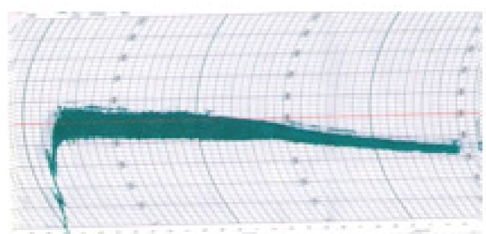
(A)The farinogram in sample ozonated at 20ppm for 5 min



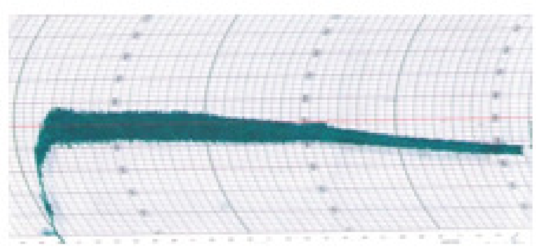
(B) The farinogram in sample ozonated at 20ppm for 10 min



(C) The farinogram in sample ozonated at 20ppm for 15 min



(D) The farinogram in sample ozonated at 20ppm for 20 min



(E) The farinogram in sample ozonated at 40ppm for 5 min.

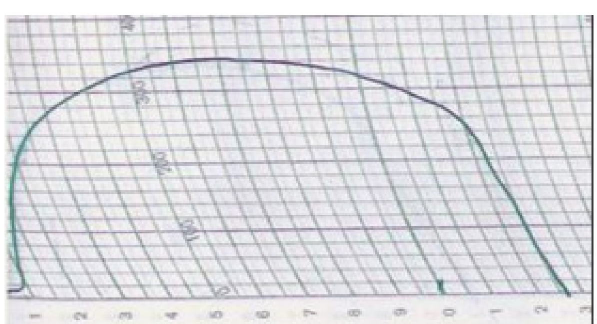
3.2. Effect of ozone treatment on extensograph parameters: Results presented in **Table (2)** and illustrated in **Fig. (2)** Showed effect of ozonation by different treatment on extensograph parameters. Ozonation at 20 ppm extensibility was 120,115,110 and 90 with 5, 10, 15 and 20 min respectively. No change with 5 min in comparing with control sample was 120 min. Extensibility decreased gradually with ozonation at 40 ppm to 100, 100, 75 and 65 min for 5, 10, 15 and 20 min respectively**.** Data show that, resistance to extension (R) of control sample was 320 B.U. No change in resistance between ozonation at 20 ppm for four times with control sample. But increased the resistance to 360 B.U., with ozonation at 40 ppm for 5, 10 and 15 and decrease to 340 B.U with ozonation for 20min.

These results agree with those obtained by [17] who found ozonation of soft to medium wheat flour is reported to cause an increase in the resistance to extension of wheat flours and a decrease in extensibility**.**

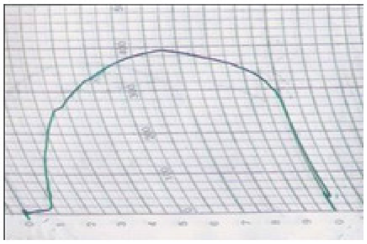
Ozonation of wheat grain led to flour with higher force and tenacity and lower extensibility than the control. It might be a competition between a protein polymerization/de-polymerization probably due to oxidation by ozone Violleau *et al.* (2012) [25]**.** Oxidising agents are used to improve the bread making capacity of flour. The use oxidants such as acetone peroxide and chlorine dioxide [9] would decrease the extensibility of the dough while maximum resistance and energy values would be increased giving the dough more strength at the expense of extensibility.

Table 1. Effect of ozonation wheat grain on farinogram parameters in wheat flourdough (82% extraction).

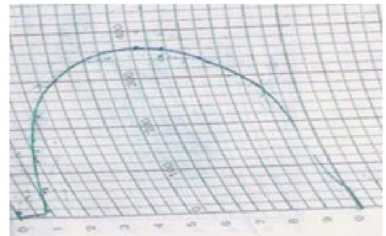
Samples	Water absorption %	Arrival time (min)	Development time (min)	Stability time (min)	Mixing tolerance index (B.U)	Degree of weakening (B.U)
Control (untrated)	59.0	1.5	2.0	9.5	30	160
wheat grain ozonated at 20 ppm for 5min	59.0	1.5	2.0	9.5	30	160
wheat grain ozonated at 20 ppm for 10 min	59.0	1.5	2.5	12.5	20	160
wheat grain ozonated at 20 ppm for 15min	59.0	2.0	3.0	10.5	30	160
wheat grain ozonated at 20 ppm for 20min	59.0	2.0	3.0	8.5	20	160
wheat grain ozonated at 40 ppm for 5min	59.0	1.5	2.5	12.5	20	160
wheat grain ozonated at 40 ppm for 10min	59.0	1.5	2.5	12.5	20	160
wheat grain ozonated at 40 ppm for 15min	59.0	2.0	3.0	10.5	20	160
wheat grain ozonated at 40 ppm for 20min	59.0	2.0	2.5	10.5	30	160



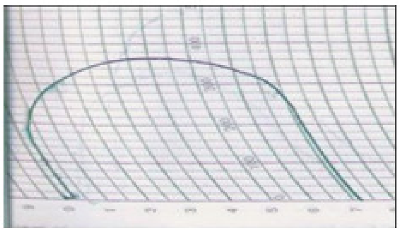
(A) The extensogram in control sample



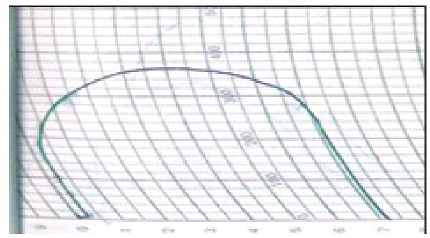
(B) The extensogram in sample ozonated at 40ppm for 5 min



(C) The extensogram in sample ozonated at 40ppm for 10 min



(D) The extensogram in sample ozonated at 40ppm for 15 min



(E) The extensogram in sample ozonated at 40ppm for 20 min

Figure 2. Comparison of extensograms of dough after ozonation wheat grain.

Table 2. Effect of ozonation on extensogram parameters.

Samples	Extensibility (E) (m.m)	Resistance to extension (R) (B.U.):	Proportional number (R/E)	Dough energy (cm^2):
Control (untrated)	120	320	2.7	54
wheat grain ozonated at 20 ppm for 5min	120	320	2.7	45
wheat grain ozonated at 20 ppm for 10 min	115	320	2.8	45
wheat grain ozonated at 20 ppm for 15min	110	320	2.9	42
wheat grain ozonated at 20 ppm for 20min	90	320	3.5	40
wheat grain ozonated at 40 ppm for 5min	110	360	3.3	45
wheat grain ozonated at 40 ppm for 10min	100	360	3.6	45
wheat grain ozonated at 40 ppm for 15min	80	360	4.8	39
wheat grain ozonated at 40 ppm for 20min	75	340	5.2	26

Table 3. Effect of ozonated wheat grain on physical characteristics (baking quality) of balady bread

Samples	Laof Weight (g)	Loaf Volume (cm^3)	Loaf Specific volume (cm^3/g)
Control (untrated)	118.57±0.23	282.5±0.28	2.38
ozonation at 20 ppm for 5min	111.0±0.50	262.42±0.22	2.36
Ozonation at 20 ppm for 10 min	111.93±0.06	299.87±0.58	2.68
ozonation at 20 ppm for 15 min	113.77±0.14	320.58±0.36	2.82
ozonation at 20 ppm for 20 min	114.33±0.44	303.83±0.60	2.66
ozonation at 40 ppm for 5 min	112.76±0.14	285.72±0.63	2.53
ozonation at 40 ppm for 10 min	123.56±0.38	310.68±0.51	2.51
ozonation at 40 ppm for 15 min	114.83±0.17	304.87±0.58	2.65
ozonation at 40 ppm for 20min	116.56±0.38	311.6±0.086	2.67

Table 4. Sensory evaluation of balady bread made from ozonated wheat grain (scores mean values ± SE)

Characteristics*	Treated samples								
	Control (untreated)	Ozonated at 20 ppm for 5min	Ozonated at 20 ppm for 10min	Ozonated at 20 ppm for 15min	Ozonated at 20 ppm for 20min	Ozonated at 40 ppm for 5min	Ozonated at 40 ppm for 10min	Ozonated at 40 ppm for 15min	Ozonated at 40 ppm for 20min
Taste (20)	18.6±0.0.14^{a}	17.6±0.15^{b}	17.4±0.25^{bc}	16.6±0.24^{cd}	16.2±0.37^{d}	17.8±0.20^{ab}	17.6±0.24^{b}	16.4±0.40^{d}	16.0±0.316^{d}
Crumb distribution (15)	14.06±0.33^{a}	14.16±0.34^{a}	14.26±0.22^{a}	14.16±0.21^{a}	14.22±0.24^{a}	13.86±0.99^{a}	13.94±0.08^{a}	13.96±0.13^{a}	13.88±0.25^{a}
Odor(10)	9.2±0.37^{a}	8.4±0.40^{ab}	7.8±0.20^{b}	6.4±0.24^{c}	6.2±0.12^{c}	8.5±0.22^{ab}	7.7±0.20^{b}	6.4±0.25^{c}	5.8±0.41^{c}
Appearance (10)	8.8±0.37^{a}	8.5±0.22^{a}	8.6±0.23^{a}	8.4±0.18^{a}	8.3±0.20^{ab}	8.6±0.24^{a}	8.4±0.25^{a}	8.4±0.24^{a}	7.6±0.24^{b}
Crust color (9)	7.7±0.30^{a}	7.8±0.2^{ab}	8.7±0.20^{c}	8.4±0.18^{abc}	8.4±0.17^{abc}	8.6±0.24^{c}	8.5±0.22^{bc}	8.6±0.25^{c}	8.3±0.20^{abc}
Roundness (6)	5.95±0.02^{a}	5.86±0.09^{a}	5.82±0.11^{a}	5.84±0.10^{a}	5.94±0.06^{a}	5.88±0.07^{a}	5.96±0.04^{a}	5.94±0.06	5.88±0.08^{a}
Separation of layers (5)	4.9±0.06^{a}	4.88±0.08^{a}	4.92±0.08^{a}	4.8±0.13^{a}	4.58±0.19^{a}	4.81±0.14^{a}	4.95±0.03^{a}	4.62±0.16^{a}	4.64±0.18^{a}
Overall acceptability (10)	95.4±2.04^{a}	95.2±1.78^{a}	94.6±0.81^{a}	94.2±1.15^{a}	93.8±0.37^{a}	94.8±0.20^{a}	94.4±0.67^{a}	85.0±1.34^{b}	78.6±0.51^{c}

Any two values not followed by the same letters are significantly different at 5% level.
* Score system, according **to Mousa *et al*. (1979)**.

3.3. Quality of Balady bread made from ozonated wheat grain:

Data in table (3) show the effect of ozonation wheat grain on the loaf weight, loaf volume and specific volume of balady bread. The control balady bread which prepared from untreated wheat grain showed a specific volume of 2.38 cm^3/g. The data showed a gradual increase in specific volume according to the time of ozonation when comperd with control bread. The greatest specific loaf volume occurred when bread was made from wheat grain was exposed to ozone for 15 and 20 min.

The increase in specific loaf volume might be caused by changes in the structure of proteins due to over exposure of flour proteins to ozone. Over-oxidation of protein would make the dough too strong and able to expand during baking**.** It is also hypothesized that excess formation of disulfide bonds at longer treatment times, with the accompanying loss of sulfhydryl groups, may hinder disulfide bond interchange and thus lead to more rapid breakdown of dough [2]. Oxidants, by facilitating the formation of disulfide bonds between glutenin subunits, can improve dough strength by making dough more elastic, thus enhancing its gas retention capacity [1].

High molecular weight glutenin subunits present in flour greatly determine the loaf volume of bread. These glutenin subunits consist of sulfur-containing amino acids (esp. cysteine), which are covalently linked through disulfide bonds. Higher amount of disulfide bond formation increases dough strength and improves gas holding capacity of dough, thus increasing the loaf volume of bread [19]. Lipids (particularly polar lipids) have a positive effect on dough formation and bread loaf volume during bread making **[12]**. Polar lipids are mostly found in bound form complexing with proteins and starch [19].

Fatty acids, particularly unsaturated fatty acids, are reported to strengthen the gluten by way of oxidation of the S–H group through enzyme-coupled reactions. Linoleic acid C_{18}: 2 is the predominant unsaturated fatty acid found in flour lipid. Oxidation of fatty acids may affect its bonding with protein (gluten) and thus affect loaf volume of the bread [20].

Ozone, because of to its high oxidation reduction potential (2.07 V), can react with various functional groups found in lipid, protein, and carbohydrate. Ozone can react with double bonds found in carotenoid pigments, causing a reduction in yellow color and an increase in flour whiteness.

Ozone could also react with double bonds associated with unsaturated fatty acids, forming free radicals associated with the onset of rancidity [7]**.**

Oxidants are used in bread dough systems to whiten the flour and subsequent bread crumb and to promote disulfide bond formation between. Proteins, which improves dough strength and generally results in increased specific loaf volume. Ozone promotes the oxidation of sulfhydryl groups and the subsequent formation of disulfide bonds between cysteine moieties [26].

Data in table (4) shows the sensory evaluation of balady bread produced from wheat flour 82% extraction which obtained after ozonated wheat grain.

Ozone treatment did not affect crumb distribution, roundness and separation of layers. The results showed significant differences in taste between the samples as compared with control samples. This is probably due to the odor of ozone gas, which had a significant effect with treatments where there was a decrease score of odor bread, which in turn played an important role in the general acceptance of the bread product. Ozonated wheat grain had good crust color of bread when compared with control samples. While observed that the overall acceptability of the balady bread was affected significantly with extended exposure of wheat grain to ozonation at 40 ppm for 15 and 20 min**.**

Crust quality was assessed by visual inspection of bread. Bread crust color changed from white to golden yellow and then brown as ozonation time of the wheat grain increased. Crust color of bread made from wheat flour treated at 40 ppm for 20 min appeared dark brown**.** Maillard and caramelization reactions are the two reactions which cause browning of the bread crust, with Maillard type browning being the primary reaction. Maillard reaction is the chemical reaction between amino acids and reducing sugars generally in the presence of heat [15]. The reaction depends upon type of reagent, temperature, water activity and pH, and is promoted under alkaline conditions.The average pH of the control flour was 5.6. Flour pH decreased to 4.5 after exposure of wheat to ozone. The decrease in pH is attributed to the oxidation of hydroxyl groups to carboxyl groups by ozone. It is postulated that the decrease in pH of flour, oxidation of amino groups and/or chemical changes in reducing sugars might have reduced the Maillard reaction and the associated browning of bread crust [21]**.**

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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