Influence of Ozonation and Storage of Wheat Grains on Chemical Composition and Rheological Properties

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ABSTRACT

Ozonation of wheat grains is considered as a quick and easy process that could be realized during the storage. The ozone is in direct contact with the grain and may modify the properties of the wheat. In the present study the effect of ozonation local wheat (LW) and red Roman wheat (RRW**)** at 80 ppm with exposure time 30 and 60 min before storage for 3 and 6 months on chemical composition, rheological properties of wheat flour and content of amino acids were carried out. The obtained **r**esults indicated that ozonated wheat grains did not significantly alter the chemical composition of LW and RRW after ozonation. On the other hand stability and resistance of dough were increased with ozonation for 60 min in comparison with control sample. In general, the results indicated that increments in the amount of amino acids with increasing time of exposure to ozone gas.

Keywords: Ozonation, Local wheat, Red Roman Wheat and amino acids.

Introduction

Around the world, there has been an over-reliance on the use of synthetic pesticides like methyl bromide and phosphine in stored grain protection that has produced many important problems such as development of resistance by stored product pests, concerns with the environment (ozone depletion), and non-desirable side effects on nontarget organisms such as humans and animals. These side effects have raised public concern about the routine use and safety of pesticides including the phase out of methyl bromide under the Montreal Protocol **(Kells *et al.*, 2001).** Therefore, there is an increasing demand worldwide for the use of alternatives for pest control such as temperature extremes, modified atmospheres, heat treatment of empty structures, physical exclusion, non-chemical protectants, biological controls, and the application of ozone to control stored product pests. Ozone gas is a powerful oxidant capable of reaction with numerous chemical groups. Ozone gas is a powerful oxidising agent with a demonstrated ability to reduce populations of bacteria and fungi in a diversity of use situations **(Kim *et al.*, 1999**). Ozone gas can inhibit growth of *Aspergillus flavus* and degradation aflatoxin B_1 in wheat grains during storage (**El-Desouky *et al.*, 2012a,b).** This powerful oxidant makes it possible to destroy many micro-organisms and organic molecules. Ozone was approved as a generally recognized as safe (GRAS) in 1997 by food and drug administration (FDA), this reagent is more and more used for food treatment as grain **(Guzel-Seydim *et al.*, 2004).** Ozone offers unique advantages for food grain processing with minimal or desired effects on the physicochemical properties. Hence ozone treatment is a potential greener alternative to conventional fumigants. Ozone gas is biologically safe and does not produce any secondary compounds that may cause toxicity **(El-Desouky *et al.*, 2017)**. 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. Therefore expected that not all of the ozone will penetrate the endosperm and the flour that follows will have specific characteristics. The main aims of the present is in a trial to study are to evaluation of evaluate the ozonation effect on chemical composition of wheat grains and rheological properties of dough.

Materials and Methods

1. Materials:

Red Roman wheat (RRW) samples were obtained from the General Company for Silos and Storage (GCSS), Giza Governorate, Egypt. Local wheat (LW) samples were obtained from the General Shoun of Wheat.

1.1. 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. Methods:

2.1. 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 was 72% extraction.

2.2. Samples treatment:

Wheat grains were transferred into a 1000 ml flask and the sample flask was plugged with a silicone stopper with 2 holes in it. One hole was for the ozone line and the other was for tubing connected to the ozone destruct unit. The wheat grain was treated at 80ppm for 30 and 60 min at room temperature.

2.3. Determination of ash, crude fat, crude protein:

Ash, crude fat content and crude protein of wheat samples were determined according to AOAC official methods **(AOAC, 2000).**

2.4. Total carbohydrates:

Total carbohydrate of wheat grain was calculated from the equations described in **Samati and Rajagopal (1996):**

Total Carbohydrates (%) = 100 − (% protein + % fat + % ash)

2.5. Rheological properties:

2.5.1. Farinograph test:

The samples were tested by Brabender farinograph APH model No 177511 Germany) 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. Different parameters were obtained from the farinograms i.e. (Water absorption (%), arrival time (min), dough development time (min), dough stability (min), mixing tolerance index and weakening of dough (B.U.)

2.5.2. Extensograph test:

Extensograph model No: 178525 was used for determination the parameters of extensibility (E) (mm), resistance to extension (R) (B.U), proportional number (R/E) and dough energy (cm^2).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 was removed after the first test, reshaped, allowed a rest period of 45 min and then was 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 45; 90 and 135 min were used to measure the follwing data. dough extensibility (E) (mm); dough resistance to extension (R) (B.U.) and proportional number (R/E).

2.6. Amino acids evaluation:

Amino acids contents in RRW and LW after and before ozonation at 80 ppm with exposure time 30and 60 min were determined to illustrate the effect of these treatments on the profile of the amino acid content. The determination of amino acid contents were carried out according to **Moore *et al.* (1958)** using Bechman Amino Acid Analyzer Model 121.

Results and Discussion

1. **Effect of ozone gas on chemical composition of local wheat (LW) and Red Roman Wheat (RRW):**

The data presented in **Table (1)** show the chemical composition of LW**.** The results show that the control sample of wheat grain contained 12.6. % moisture, 2.4 ash, 1.8% fat, 11.9 % protein, 2.3% crude fiber and 81.6% total carbohydrates. The obtained results are in agreement with those reported by **(Selim, 2000 and Hussein *et al.*, 2010).** The achieved results indicated that ozonated wheat grain did not significantly alter the chemical of the wheat flour. The accomplished results arein agreement with other studies (**Ibanoglu, 2002; Paul 2005 and Sandhu *et al.*, 2011)**. The obtained results of moisture content were in agreement with values obtained by **Ibanoglu (2002)** who reported that the ozonated tempering had no effect on moisture content. The protein values of ozonated and non-ozonated wheat ranged from 11.9 to 12.3%. These results were in agreement with that reported by **Ibanoglu** (**200**2) who concluded that the ozonated wheat grains did not cause any chemical or physical changes. The effect of ozone is considered to be restricted to the outer surface of exposed material. Thus, even though flour is exposed to ozone, only the functional groups exposed on the surface of the flour particle would be subjected to oxidation by ozone. Also, flour particles on the exposed surface of a flour container or silo would be more subject to ozone oxidation than particles in the interior of the silo or container **(Sandhu *et al.*, 2012).** The ozone is indirect 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 Therefore expected that not all of the ozone will penetrate the endosperm and the flour that follows will have specific characteristics **(Dubois *et al.*, 2006)**

Table 1. Chemical composition of local wheat (LW) after ozonation at 80 ppm. (Dry weight basis)

Treatment	Chemical composition of local wheat					
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	Carbo-hydrates* (%)
Control	**12.6**	**2.4**	**1.8**	**11.9**	**2.3**	**81.6**
ozonation for 30min	**12.4**	**2.4**	**1.8**	**12.0**	**2.3**	**81.5**
ozonation for 60min	**12.3**	**2.4**	**1.8**	**12.3**	**2.3**	**81.2**

*Carbohydrate by difference.

The data presented in **Table (2)** show the chemical composition of RRW**.** The results show that the control sample of wheat grain was contained 12.5% moisture, 2.3% ash, 1. % fat, 11.7% protein, 2.5% crude fiber and 81.8% total carbohydrates**.** Also the results indicate that no significant effect on chemical composition of RRW after ozonation at 80ppm for 30 and 60 min. The obtained results are in agreement with that reported by **Ibanoglu** (**200**2) and Sandhu ***et al.* (2012).**

Table 2. Chemical composition of Red Roman Wheat (RRW) after ozonation at 80 ppm . (dry weight basis)

Treatment Wheat control	Chemical composition of local wheat					
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	Carbo-hydrates* (%)
Control	**12.5**	**2.3**	**1.7**	**11.7**	**2.5**	**81.8**
ozonation for 30min	**12.5**	**2.3**	**1.8**	**11.7**	**2.3**	**81.9**
ozonation for 60min	**12.3**	**2.3**	**1.8**	**11.9**	**2.3**	**81.7**

*Carbohydrate by difference.

From the obtained results (Tables 1 and 2), it has concluded that there are slight differentiation between the chemical composition of local wheat (LW) and red Roman wheat (RRW). And also, the ozonation treatment had no or did not effect on both types of wheat.

3.2. Effect of ozone treatment on farinogram parameters:

Data in **Tables (3 and 4)** show the farinograph parameters from wheat flour dough (72 % extraction) after ozonation wheat grain at 80 ppm, i.e. water absorption, arrival time, dough development time, dough stability and dough weakening. In case LW the results indicated that the water absorption ranged from 64 to 66% in control sample and different treatment. But arrival time was 1. to 2.5 min with sample control and treatment.. While there were increments in mixing tolerance index and degree of weakening. RRW grains ozonated at 80 ppm for 30 and 60 min the water absorption ranged from 62 to 65 % in control sample and treatment as shown in Table (4), respectively. Arrival time was increased to 3.5 min with ozonation sample and stored for 3months compared with control sample (2.5 min). Development time was 2.5 min in control samples and ozonated for 30 and 60 min stored for 6 months. The obtained results are in agreement with those obtained by **(Ibanoglu, 2002 and Sandhu *et al.*, 2011)**. 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 (**Gupta *et al.*, 1993 and El-Desouky *et al.*, 2013).** 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 **(Sandhu *et al.*, 2011)**. Amino acids could be oxidized by ozone. In fact, tyrosine is oxidised to dityrosine by ozone. **Takasaki *et al.* (2005)** suggested that dityrosine could be a new kind of stabilizing cross-link in the wheat gluten structure in addition to disulphide bonds**.** On the other hand **Mendez** *et al.* (**2003)** 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. **Hoseney (1994)** reported that the formation of new disulphide bonds in the presence of an oxidizing agent would increase the strength of the dough with high stability and low degree of softening. Similarly, any cleavage of the disulphide bond would result in the weakness of the dough giving low stability and high degree of softening. So the storage of grains in ozone rich atmospheres does not influence the rheological properties of grains. For example **Mendez *et. al.* (2003)** 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 over mixing, absorption of water, dough weight, and proof height.

Table 3. Farinogram parameters of local wheat (LW) ozonated at 80ppm which stored for 3 and 6 months.

Farinogram parameters	Control	LW stored for 3 months	LW stored for 6 months	LW stored for 3 months and ozonation for 30 min	LW stored for 3 months and ozonation for 60 min	LW stored for 6 months and ozonation for 30 min	LW stored for 6 months and ozonation for 60 min
Water absorption (%)	**64**	**66**	**65**	**66**	**65**	**65**	**64**
Arrival time (min)	**2.5**	**2.0**	**1.5**	**1.0**	**1.5**	**2.0**	**1.0**
Development time (min)	**3.0**	**2.5**	**2.0**	**1.5**	**2.5**	**2.5**	**1.5**
Stability time (min)	**9.5**	**14.50**	**9.5**	**4.5**	**5.5**	**4.0**	**12.0**
Mixing tolerance index (B.U)	**10**	**20**	**40**	**60**	**50**	**60**	**60**
Degree of weakening (B.U)	**60**	**30**	**70**	**10**	**40**	**60**	**50**

Table 4. Farinogram parameters of **Red Roman Wheat (RRW)** ozonated at 80ppm which stored for 3 and 6 months.

Farinogram parameters	Control	RRW stored for 3 months	RRW stored for 6 months	RRW stored for 3 months and ozonation for 30 min	RRW stored for 3 months and ozonation for 60 min	RRW stored for 6 months and ozonation for 30 min	RRW stored for 6 months and ozonation for 60 min
Water absorption (%)	**62**	**64**	**62**	**64**	**65**	**65**	**64**
Arrival time (min)	**1.5**	**1.0**	**2.0**	**2.5**	**2.5**	**2.0**	**2.0**
Development time(min)	**2.5**	**2.0**	**2.5**	**3.5**	**3.0**	**2.5**	**2.5**
Stability time (min)	**11.5**	**10.0**	**11.0**	**11.5**	**10.5**	**3.0**	**3.0**
Mixing tolerance index (B.U)	**10**	**20**	**20**	**20**	**20**	**70**	**50**
Degree of weakening (B.U)	**50**	**80**	**50**	**60**	**50**	**70**	**70**

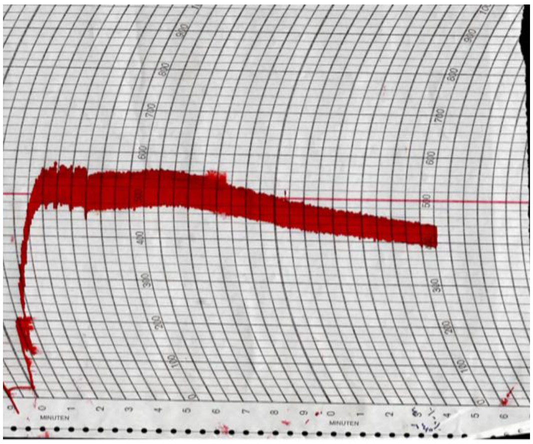

Fig (1) The farinogram in control sample of RRW.

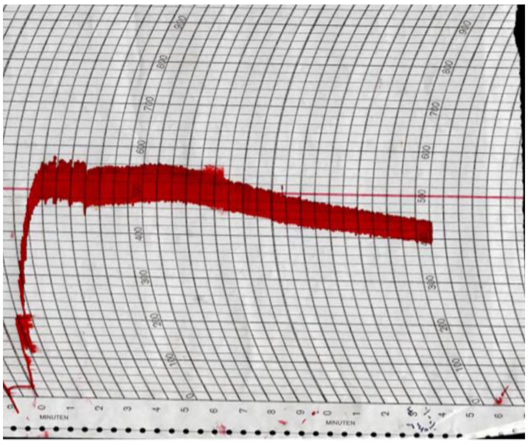

Fig (2)The farinogram in RRW sample ozonated at 80ppm for 60 min

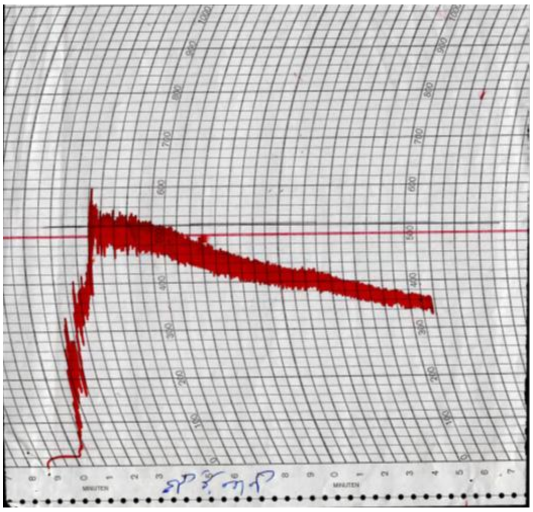

Fig (3) The farinogram in control sample of LW.

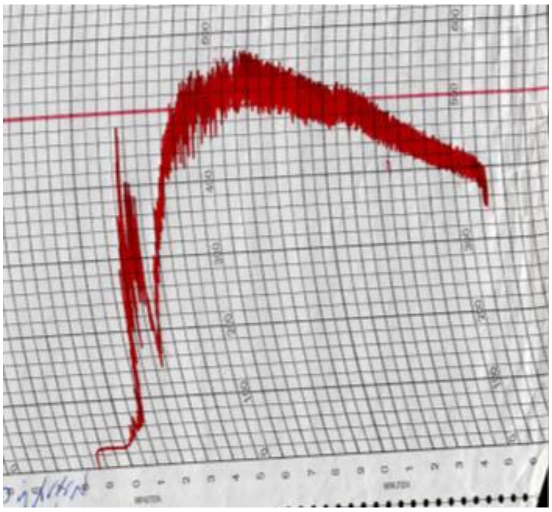

Fig (4)The farinogram in LW sample ozonated at 80ppm for 60 min

3.3. Effect of ozone treatment on extensograph parameters:

Results presented in **Tables (5)** showed the effect of ozonation at 80ppm for 30 and 60 min with storage period 3 and 6 months on extensograph parameters for both types of wheat (LWand RRW). Extensibility values in control LW were 560, 440 and 420 mm after fermentation for 45, 90 and 135 min, respectively. The results indicated that increments in extensibility after ozonation to 620, 700 and 440 mm with LW stored 3 and 6 months, respectively treated by ozone gas for 30 min .In addition the resistance increased to 580 B.U., with samples stored for 6 months after ozonated for 60 min comparing with control which equaled to 470 B.U. The accomplished results are agreement with those obtained by **(Naito, 1990)** who found ozonation of soft to medium wheat flour caused 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 may be due to oxidation by ozone **Violleau *et al.* (2012).** Whereas the intermolecular SH groups of wheat flour were decreased by about 30% by ozone treatment at 50 ppm for 1 hr, but intermolecular S–S bonds were increased by about 5% by the same treatment. (**Dubois *et al.*, 2006**).

Results in Table (6) showed the effect of ozonation at 80 ppm for 30 and 6 min with storage period 3 and 6 months on exensograph parameters for RRW. Extensibility values forcontrol were 490, 400 and 380 mm after three period fermentation i.e. 45, 90 and 135 min, respectively. The obtained results indicated there were decrements in extensibility as the period fermentation increased in control sample. The values of extensibility were increased (590 and 640 mm) after the storage for 3 and 6 months, respectively in untreated samples also this parameter was increased in case of treated sample with ozonaion (80 ppm) for 30 min and stored for three months which reached to 640 mm. On the other hands, RRW samples stored for 3, 6, 6 months andozonated for 60, 30, 60 in, the extensibility values were decreased, these values were 380, 400, 420 mm for 45 mn period fermentation.

The obtained results there were decrements in extensibility as the period of fermentation decreased (90, 135 min), these results had the same trend of control sample.

The obtained results cited in Table (6) indicated that the resistance of control sample equaled to 470B.U. While these values decreased and then increased i.e. the resistance parameter had the same trend of extensibility parameter, respectively.

The obtained are in agreement with that reported by **Hoseney (1994), Takasaki *et al.* (2005) and Sandhu *et al.* (2011)**.

From the results in Tables (3 and 5) it concluded that the application of ozonation treatment for local wheat at 80 ppm for 30, 60 min and storage period for 6 months gave the best for farinograph results and extensograph parameters.

On the other hand, from the Tables (4 and 6) it has been observed that the application of ozonation treatment for red Roman wheat at 80 ppm for 30 and 60 min and storage period for three months gave the best results of farinograph and extensograph parameters.

Table 5. Extensogram parameters of LW ozonated at 80 ppm.

Treatment samples	**Extensibility (E) (m.m) after three period fermentation**			**Resistance to extension (R) (B.U.):**	**Proportional number (R/E)**
	45 min	90 min	135 min		
Control	560	440	420	470	2.5
LW stored for 3 months	360	300	260	315	2.5
LW stored for 6 months	300	240	180	245	1.8
LW stored for 3 months and ozonation for 30 min	430	360	300	360	2.0
LW stored for3 months and ozonation for 60 min	620	520	460	405	2.4
LW stored for 6 months and ozonation for 30 min	700	600	560	520	3.2
LW stored for 6 months and ozonation for 60 min	740	640	620	580	3.7

Table 6. Extensogram parameters of RRW ozonated at 80ppm.

Treatment samples	Extensibility (E) (m.m) after three period fermentation			Resistance to extension (R) (B.U.):	Proportional number (R/E)
	45 min	90 min	135 min		
Control	490	400	380	420	2
RRW stored for 3 months	590	520	500	530	3
RRW stored for 6 months	640	480	460	520	2.8
RRW stored for 3 months and ozonation for 30min	640	410	400	480	2.7
RRW stored for3 months and ozonation for 60min	380	300	220	330	1.8
RRW stored for6 months and ozonation for 30min	400	280	240	370	1.8
RRW stored for6 months and ozonation for 60min	420	340	280	380	2.4

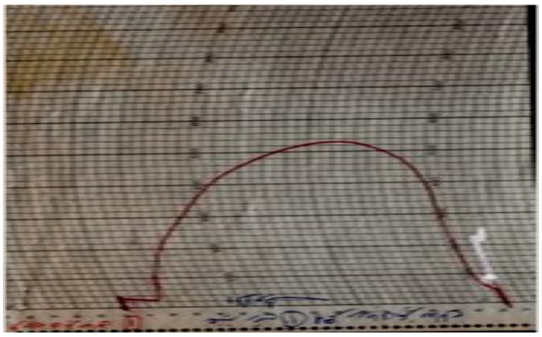

Fig. (5): The extensogram in control sample of RRW

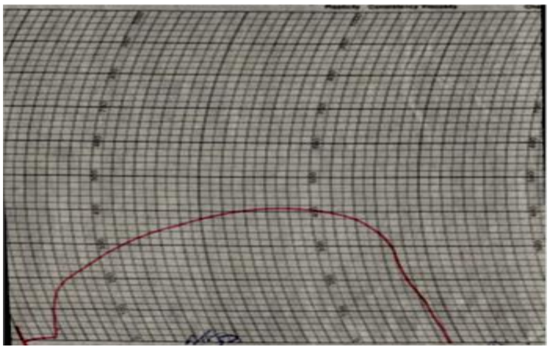

Fig. (6): The extensogram in ozonated sample RRW

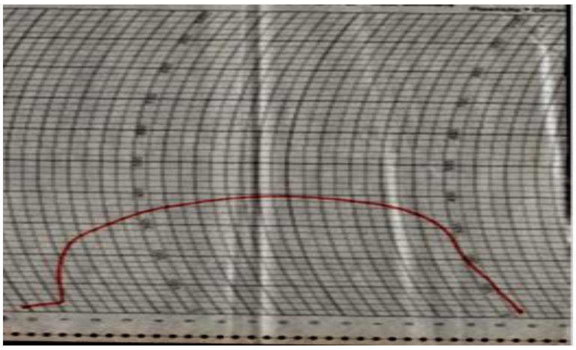

Fig. (7): The extensogram in control sample of LW

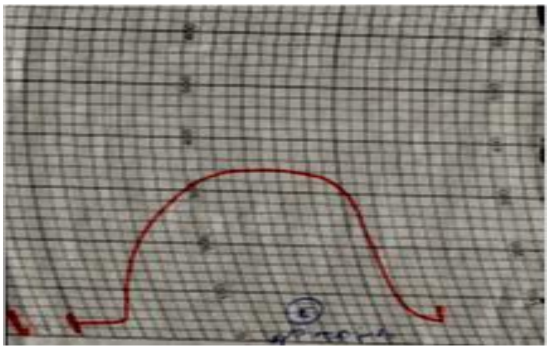

Fig. (8): the extensogram in ozonated sample of LW

3. 4. Evaluation of ozonation effect on content of amino acid in wheat grains:

Results in Tables **(7 and 8)** show of amino acids content in RRW and LW, after and before ozonation at 80 ppm with exposure time 30 and 60 min. Generally, the results indicated that increase in the amount of amino acids with increasing time of exposure to ozone gas. In case of RRW and LW the content of aspartic acid increased to 0.67 and 0.72 g/100g after exposure time 30 and 60 min, respectively if compared with control sample 0.61g/100g. Glutamic acid reached to 3.41 g/100g after exposure 60 min. While the control sample values 3.03 g/100g. Tyrosine acid after ozonation at 80ppm for 30and 60min amounted to 0.43 and 0.46 g/100g compared with control sample 0.38 g/100g. The content of cysteine amino acid in control samples 0.30 increased to 0.44g/100g in sample ozonation for 60min. Local wheat (LW) content of amino acid was presented in **Table (8)**, all amino acids were increased with increased exposure to ozone gas, for example, Tyrosine evaluated to 0.48g/100g, as well as lysine, proline and cysteine were 0.35, 1.32 and 0.47 g/100g, respectively. In the presence of organic compounds, ozone reacts in a variety of complex reactions due to the formation of various reactive species**.** Ozone reacts with proteins and causes the oxidation of the polypeptide backbone of the protein, peptide bond cleavage, protein– protein cross-linking and a range of amino acid side chain modifications **(Kelly and Mudway, 2003).** Although all amino acids are potential targets for oxidation by reactive oxygen, the major aromatic amino acids tyrosine, tryptophan, phenylalanine, the sulphur containing amino acids cysteine, methionine as well as the aliphatic amino acids arginine, lysine, proline and histidine appear especially sensitive to oxidation. The sequence of amino acids that are condensed in a chain by amide bonds, representing main chain backbone of proteins exhibits negligible or very limited chain scission during ozonation (**Uzun *et al.*, 2012).** The C-H and S-H bonds of alkanes, alkenes, amines and sulfhydryl compounds are attacked by ozone. Amino acids may be attacked directly at the primary amine nitrogen atom or on the R group **(Mustafa 1990; Adachi 2001**).

The obtained results are in agreement with that obtained by **Naitoh (1992), Erdman (1997) and Sandhu *et al.* (2011)**.

Table 7. Effect of ozone treatment on the amino acid content of Red Roman Wheat (RRW):

Amino acid (g/100 g. sample)	Exposure time (min)		
	0	30	60
Aspartic	0.61	0.67	0.72
Therionine	0.31	0.32	0.33
Serine	0.43	0.43	0.44
Glutamic	3.03	3.39	3.41
Glycine	0.43	0.47	0.47
Alanine	0.46	0.46	0.46
Valine	0.54	0.51	0.52
Isoleucine	0.35	0.38	0.41
Leucine	0.71	0.76	0.77
Tyrosine	0.38	0.43	0.46
Phenylalanine	0.53	0.56	0.58
Hisitidine	0.26	0.28	0.32
Lysine	0.31	0.34	0.35
Arginine	0.53	0.59	0.62
Proline	0.93	1.15	1.21
Cysteine	0.30	0.39	0.44
methionine	0.36	0.22	0.24

Table 8. Effect of ozone treatment on the amino acid content of Local wheat (LW).

Amino acid (g/100 g. sample)	Exposure time (min)		
	0	30	60
Aspartic	0.71	0.77	0.79
Therionine	0.31	0.32	0.33
Serine	0.43	0.43	0.44
Glutamic	3.33	3.41	3.52
Glycine	0.45	0.47	0.5
Alanine	0.46	0.46	0.46
Valine	0.54	0.51	0.52
Isoleucine	0.35	0.38	0.41
Leucine	0.71	0.76	0.77
Tyrosine	0.35	0.43	0.48
Phenylalanine	0.53	0.56	0.58
Hisitidine	0.23	0.25	0.28
Lysine	0.31	0.34	0.35
Arginine	0.53	0.59	0.62
Proline	0.97	1.25	1.32
Cysteine	0.35	0.39	0.47
methionine	0.33	0.30	0.33

Conclusion

The obtained results of the present study, it has been concluded that the application of ozonated wheat grain did not significantly alter the chemical composition of LW and RRW after ozonation at 80 ppm for 30 and 60 min. In addition to that the achieved the results indicated that increaments in extensibility**,** resistance and amount of amino acids with increasing time of exposure to ozone gas has been occurred.

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