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Gassy Ozone Effect on Quality Parameters of Flaxes Made from Biologically Activated Whole Wheat Grains

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Abstract**—**The aim of the current research was to investigate the gassy ozone effect on quality parameters of flaxes made form whole biologically activated wheat grains. The research was accomplished on wheat grains variety 'Zentos'. Grains were washed, wetted; grain biological activation was performed in the climatic chamber up to 24 hours. After biological activation grains were compressed; than flaxes was dried in convective drier till constant moisture content 9±1%. Grains were treated with gassy ozone (concentration 0.0002%) for 6min. In the processed flaxes the content of α and γ tocopherol decrease by 23% and by 9%; content of B_2 and B_6 vitamins – by 11% and by 10%; elaidicacid – by 46%, oleic acid – by 29%; arginine (by 80%), glutamine (by 74%), asparagine and serine (by 68%), valine (by 62%), cysteine (by 54%) and tyrosine (by 47%).

Keywords**—**Gassy ozone, flaxes, biologically activated grains, quality parameters, treatment.

I. INTRODUCTION

WHOLE-GRAIN cereals contain a much wider range of compounds with potential antioxidant effect than do refined cereals. These include vitamin E (mainly in the germ), folates, minerals (iron, zinc) and trace elements (selenium, copper and manganese), carotenoids, phytic acid, lignin and other compounds such as betaine, choline, sulphur amino acids, alkylresorcinols and lignans found mainly in the bran fraction. Some, such as vitamin E, are considered to be direct free radical scavengers, while others act as cofactors of antioxidant enzymes (selenium, manganese and zinc), or indirect antioxidants (folates, choline and betaine) [1].

Since 1980 the *Dietary Guidelines* have been published jointly every five years by the Department of Health and Human Services (USDHHS) and the Department of Agriculture (USDA). From the beginning, these recommendations have encouraged consumption of whole-grains for a healthy diet. The 2005 *Dietary Guidelines*, however, marked a signify cant departure from past recommendations by making specific recommendations about whole-grains – which at least half of a person's daily grain intake should come from whole-grains [2].

Wheat is a member of the *Triticeae* group of cereals and indisputably one of the major food crops of the world and a foundation of human nutrition worldwide. In addition to its basic caloric value, wheat, with its high protein content, is the single most important source of plant protein in the human diet [3]. Wheat is mainly consumed as sifted white flour, although it is well known that health-promoting components, including dietary fibre, are concentrated in the bran [4].

For the increasing of grains nutritive value germination for several days or biological activation for 24 hours is necessary. During the activation time dietary fibre content has increased. The amount of vitamin B_2 increased by 54.5%, vitamin E content grown 6.5 times, and the content of niacin increased 1.3 times. Vitamin C was not found in inactivated grain samples, but during the activation process by means of biochemical reactions it was synthesized – 71.0mg/kg in wheat grain. However, total content of amino acids decreased by 17.4% in wheat grain during the grain activation time up to 24 hours [5].

There are reported incidents of food poisoning resulting from contaminated flour. Australian, European and US studies indicate that Salmonella spp., Escherichia coli, Bacillus cereus and spoilage microorganisms are present in wheat and flour at low levels [6]. At the same time during biological activation microorganisms grow is stimulated, therefore flakes from biologically activated wheat grains could not be safety as for consumption, as for other food production application. Some researchers offer to use grains treatment with ozone for insuring high quality food and for inactivation of microorganism or slowing they grow [7].

Ozone (O_3) is a strong oxidant recognized since 1997 as a generally recognized as safe substance and used in a number of applications in the food industry for destruction or detoxification of chemicals or microorganisms. These applications include the surface decontamination, storage and preservation of perishable foods as well as water or manufacturing equipment and packaging sterilization [8],[9]. Ozone in gaseous or aqueous form is reported to reduce levels of the natural microflora, as well as bacterial, fungal and mould contamination in cereals and cereal products, including spores of *Bacillus,* Coliform bacteria*, Micrococcus, Flavobacterium, Alcaligenes, Serratia, Aspergillus* and Penicillium. Ozone is not universally beneficial and in some cases may promote oxidation degradation of chemical constituents present in the grains. Surface oxidation, discoloration or development of undesirable odors may occur from excessive use of ozone. However, higher ozone

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concentrations (>50ppm) cause considerable oxidative damage to cereal grains [10].

In our experiments it was found, that optimal grain processing parameters with ozone for maximal microbiological safety and optimal germinating power were for triticale grains – 3.92mg/m^3 8 min, for hull-les barley grain – 11.76mg/m^3 6min. Ozone concentration and treatment time not significantly influence changes in grain protein, moisture, starch and β-glycan content; however significantly influence grain germinating power; germinating power of processed triticale grains increase after three days germination by 15% and after five days germination by 7%; of processed hull-less barley grains – after three days germination by 11% and after five days germination by 9%. Ozone significantly influences grain starch microstructure, as a result starch granules swell up, its volume increase. The amount of mycotoxins before and after grain treatment with gassy ozone was under detection layer [11].

After summarizing of scientific data from literature the aim of the current research was developed as follow – to investigate the gassy ozone effect on quality parameters of flaxes made form whole biologically activated wheat grains.

II. MATERIALS AND METHODS

A. Materials

The research was accomplished on in Year 2012 from Latvia University of Agriculture research station „Peteralauki" (Latvia) harvested wheat grains variety 'Zentos'.

B. Grain Biological Activation, Flaxes Making

Grains were washed (H_2O t = +20±1^{o}C), wetted (H_2O t = +20±1^{o}C, τ = 24±1h), grain biological activation was performed in the climatic chamber at temperature +25±1°C and constant relative air humidity – 80±1% up to 24±1h [5].

After biological activation grains were compressed using electric flaker (Hawos, Germany). Than flaxes was dried in convective drier with forced air circulation at 50±1°C till constant moisture content 9±1%.

C. Flaxes Treatment with Gassy Ozone

In current research, a gassy ozone ozonizer "OZ-15G" (China) was used for grain treatment and "IKG-6M" (Russia) measure for the ozone concentration measurement. Grains were treated with gassy ozone (concentration 0.0002%) for 6min (established in previous experiments).

D. Protein Content

The total protein content was determined by Kjeldal method [12] according to the LVS ISO 8968-5:2002.

E. Amino Acids

Amino acids were determined [13] according to LVS SS-EN ISO 13903:2005.

F. Fatty Acids

Fatty acids were determined according to LVS EN ISO 5508:1995.

G. Vitamin E

The content of vitamin E was determined using liquid chromatography with high performance mass spectrometry (HPLC) [14].

H. Tocopherol

The content of tocopherol was determined [15] using standard method LVS EN 12822:2001.

I. B Group Vitamins

The content of vitamin B_2 was determined using AOAC Official Method 970.65 [16]; of vitamin B_6 [17] and vitamin B_{12} [18] using microbiological standard method.

J. Dietary Fibre

The total dietary fiber in these samples was determinate according AOAC approved method No 985.29. The experiments were carried out by using FOSS Analytical Fibertec E 1023 system providing enzymatic processing by incubation in thermostatic shaking water bath, residue filtration by Filtration Module and protein determination by Kjeldahl nitrogen equipment. The analyses were performed in three repetitions. The samples were defatted and dried with a particle size less than 0.5mm. After, each sample was enzymatically digested with α–amylase incubation at 100°C, as well as protease and amyloglucosidase incubation at 60°C. After digestion the total fiber content is precipitated by adding 95g 100/g ethanol. Later the solution was filtered and fiber was collected, dried and weighed. The protein and ash content were determined to correct any of these substances which might remain in the fiber [19].

K. Mathematical Data Processing

Data are expressed as mean ± standard deviation; for the mathematical data processing p-value at 0.05 (One Way analysis of variance, ANOVA), was used to determine the significant differences. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey's multiple comparison test the level of confidence α=0.05. The statistical analyses were performed using Microsoft Excel 2007.

III. RESULTS AND DISCUSSION

According to the literature,treatments with ozone significantly influence not only microbiological quality, but also chemical composition of grains [11].

Proteins, fiber and total sugar content of flakes made from biologically activated grains before and after treatment with ozone was not changed (see Table I) significantly (p>0.05) and this statement match with other researchers' conclusions [7], [20].

In a three-year study carried out at a rural site in Switzerland, spring wheat was exposed to different levels of ozone (O_3) in open-top-field chambers from the two-leaf stage until harvest. Grain recovered from the different treatments was analyzed for minerals (Ca, Mg, K, and P), starch, protein, amino acids and α-tocopherol, in order to investigate the effect of O_3 on grain composition.

TABLE I
PROTEINS, FIBER AND TOTAL SUGAR OF FLAKES MADE FROM WHOLE BIOLOGICALLY ACTIVATED WHEAT GRAINS

Parameter	Value,% in dry matter	
	Flakes before treatment with ozone	Flakes after treatment with ozone
Proteins	11.61±0.05	11.51±0.05
Insoluble fiber	1.99±0.01	1.99±0.01
Total sugar	2.50±0.01	2.20±0.01

As a result no effect of ozone on the content of α-tocopherol and on the essential amino acid index of the protein was observed. It is concluded that compositional changes in wheat grain in response to ozone are minor, and that ambient ozone is not likely to cause important changes [20].

TABLE II
TOCOPHEROL CONTENT IN FLAKES MADE FROM BIOLOGICALLY ACTIVATED WHOLE WHEAT

Sample	α-tocopherol mg/kg	γ-tocopherol, mg/kg
Before treatment with ozone	8.71±0.44	4.75±0.24
After treatment with ozone	6.68±0.33	4.30±0.22

Content of α-tocopherol significantly decrease ($p<0.05$) by 23% in wheat flakes after treatment with ozone γ-tocopherol decrease too, but insignificantly (by 9%). These negative changes can be explained with fact, that α and γ-tocopherol becomes unstable in higher temperature and after treatment with oxygen [21]. Stability of tocopherol and tocotrienol extracted from unsaponifiable fraction (of grains under various temperature and oxygen condition), flakes was treated with ozone, as results vitamins are oxidized.

Biologically activated grainsare sources of B group vitamins [5], therefore, content of theses vitamins before and after treatment with ozone was determined in produced flakes too (see Table III).

TABLE III
AMOUNT OF VITAMINS B_2, B_6, B_{12} IN WHEAT GRAINS AND IN FLAKES MADE FROM BIOLOGICALLY ACTIVATED WHOLE WHEAT GRAINS

Sample	B_2, mg/100g	B_6, mg/100g	B_{12},μg/100g
Wheat grains	0.45±0.025	0.14±0.010	0.02±0.0001
Flakes before treatment with ozone	0.61±0.001	0.40±0.020	0.02±0.0001
Flakes after treatment with ozone	0.54±0.025	0.36±0.010	0.02±0.0001

In wheat highest was B_2 vitamin content – 0.45mg/100g, but lowest B_{12} vitamin content – 0.02mg/100g, according to the literature [5] content of B_2 vitamin can range from 0.35 to 0.43mg/100g and B_6 vitamin from 0.15 to 0.19mg/100g. After biological activation of grains, the content of vitamin B_2 and B_6 significantly increase ($p<0.05$). The content of vitamin B_2 increases by 26%, but of B_6 – by 65%. These data is according to the other research results, where authors found, that during grains biological activation content of B group vitamins increase [5].

After treatment with ozone content of vitamin B_2 and B_6 decrease, accordingly by 11% and by 10%, these results can be explained, with knowledge about vitamin stability, still it is known, that B group vitamin is very sensitive to light and oxygen, and during storage lost some of its value [5].

TABLE IV
FATTY ACIDS CONTENT (% FROM TOTAL FATTY ACIDS CONTENT) IN FLAKES MADE FROM BIOLOGICALLY ACTIVATED WHOLE WHEAT GRAINS

Fatty acids	Before treatment with ozone	After treatment with ozone
Palmitic acid C 16:0	22.47±1.10	22.89±1.10
Stearic acid C18:0	2.83±0.10	1.00±0.05
Oleic acid C18:1n9c	14.78±0.10	10.43±0.50
Elaidic acid C18:1n9t	1.72±0.10	0.93±0.05
Linoleic acid C18:2n6c	54.06±2.70	52.64±2.60
α-linoleic acid C18:3n3	3.69±0.20	3.73±0.20
Gadoleic acid C20:1n9	0.95±0.05	0.81±0.05

Comparing grains before biological activation and flakes after treatment with ozone still content of vitamins B_2 and B_6 was higher accordingly by 17% and by 61% times, but content of B_{12} was the same in all analyzed samples (Table III).

The content of fatty acids decreased in all analysed samples after treatment with ozone, except palmitic acid, linoleic acid andα-linoleic acid (see Table IV). Content of these fatty acids changed the margin of error. Decrease content of fatty acids evaluated negatively, treatment regime (concentration of ozone and time) significantly ($p<0.05$).

TABLE V
THE CONTENT OF AMINO ACIDS INFLAXES MADE FORM BIOLOGICALLY ACTIVATED WHOLE WHEAT GRAINS MG/100G

Amino acids	Flakes before treatment with ozone	Flakes after treatment with ozone
Valine	3.34±0.16	1.27±0.05
Leucine	6.76±0.30	4.50±0.21
Isoleucine	3.24±0.15	2.23±0.11
Phenylalanine	4.28±0.15	3.27±0.12
Cysteine	2.24±0.10	1.02±0.05
Lysine	2.25±0.10	2.07±0.10
Agrinine	4.01±0.16	0.82±0.03
Histidine	2.84±0.10	1.97±0.09
Asparagine	4.57±0.21	1.45±0.08
Serine	4.51±0.21	1.46±0.08
Glutamine	13.52±0.62	3.50±0.17
Glycine	2.73±0.10	2.62±0.11
Threonine	2.40±0.10	2.31±0.11
Alanine	3.34±0.15	2.22±0.11
Proline	0.62±0.30	0.57±0.25
Tyrosine	2.15±0.10	1.14±0.49
Methionine	1.04±0.05	1.02±0.05

Greatest decrease was detected in elaidic acid and oleic acid concentrations; accordingly it was by 46% and by 29% lowerscomparing to samples before treatment. This decrease can be evaluated negatively still it is known, that these fatty acids are very important in grain and grains' products. Such results don't coincide with other researcher results, where was detected, that ozone have no influence on fatty acids concentration [7].

After research results, it can be concluded, that ozone is very strong oxidant, as result fatty acids concentration significantly decrease.

Content of individual amino acids significantly decrease ($p<0.05$) after treatment with ozone (see Table V).Greater decrease was detected in a case with arginine (by 80%), glutamine (by 74%), asparagine and serine (by 68%), valine (by 62%), cysteine (by 54%) and tyrosine (by 47%).

Decrease concentration of amino acids evaluated negatively, still it is known that essential acids are very important nutritionally.

IV. Conclusions

Content of α and γ tocopherol in flakes made from biologically activated whole wheat grains decrease by 23% and by 9% after treatment with ozone.

Content of B_2 and B_6 vitamin in flakes from biologically activated wheat grains decrease by 11% and by 10% after treatment with ozone, but content of vitamin B_{12} remained unchanged.Concentration of fatty acids in flakes made from biologically activated wheat grains decrease; ofelaidic acid – by 46%, and oleic acid – by 29%.

Content of individual amino acids significantly decrease ($p<0.05$) after treatment with ozone. Greater decrease was detected in a case with arginine (by 80%), glutamine (by 74%), asparagine and serine (by 68%), valine (by 62%), cysteine (by 54%) and tyrosine (by 47%).

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