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Full Length Research Paper

Investigating the effect of storage time and growth regulator furolan on the contents of protein fraction composition and amino acids in winter wheat grain

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This investigation was carried out to study the effect of storage time and growth regulator, Furolan (the extrinsic factors) on the contents of protein fraction composition and amino acids (the intrinsic factors) of three varieties of winter wheat grains namely Batko, Diya and Krasnodar 99. The determination of mass fraction of amino acids and fractional composition of protein complex was respectively carried out by capillary electrophoresis and Osborne methods. During harvest, Furolan-treated varieties of Diya (42.9%) and Batko (41.7%) were found to differ from Furolan-treated Krasnodar 99 (48.3%) variety by their low content of albumin and globulin and the high content of alcoholic-and alkaline- soluble fractions of protein (protein gluten) due to the genetic potential of these varieties. During storage there was a decrease in the albumin and globulin contents and an increase in the protein gluten content of Krasnodar 99 variety in the control. It was observed that, during 12 months of storage the contents of cyclic amino acids in all the varieties studied decreased. Similarly, there was a decrease in monoaminomonocarboxylic amino acids (glysine, alanine, serine, threonine, valine, leucine) content of the average-ripening (Diya and Krasnodar 99) varieties.

Key words: Furolan, storage, winter wheat, protein, amino acids, gluten.

INTRODUCTION

Storing good quality grain and keeping the quality good takes excellent management by the farmer. Typically, farmers store grains for periods of three to six months. With this relatively short storage interval, changes in the bin happen very slowly. As the storage interval stretches out through the warm spring and the heat of summer, undesirable elements may establish in the bin that require quick action to avoid a possible serious situation (Helmut, 2006). In the post-harvest management of grains, storage time is a factor that requires critical attention and study.

In most countries, grains are among the most important staple foods. However they are produced on a seasonal basis and in many places there is only one harvest a year, which itself may be subject to failure. This means that in order to feed the world's population, most of the global production of maize, wheat, rice, sorghum and millet must be held in storage for periods varying from one month up to more than a year. Grain storage therefore occupies a vital place in the economies of developed and developing countries alike (web. Ref.1).

Storage of wheat at 40-60°F is optimal for most home stored grains but is usually impractical in most homes except during winter months. Freezing or sub-zero temperatures do not damage stored grains. Storage at temperatures above 60°F causes a more rapid decline in seed viability (ability to germinate) but only a slightly faster loss in food value. A moisture level over 12% encourages mold growth and chemical degradation of all grains (barley, corn, millets, oats, rice, rye, sorghum, triticale and wheat). Moisture above 12% may allow grains to start to respire causing chemical degradation. Moisture above 15% will allow molds to grow. When the moisture reaches 20% some bacteria can start to grow.

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The result is spoiled grain unfit for use (web.ref. 2).
By definition plant growth regulator is any substance or mixture of substances intended, through physiological action, for accelerating or retarding the rate of growth or maturation or for otherwise altering the behavior of ornamental or crop plants or the produce thereof, but not including substances intended as plant nutrients, trace elements, nutritional chemicals, plant inoculants, or soil amendments. The use of plant growth regulators in agricultural production within the United States began in the 1930s. The first discovery and use of plant growth regulators was with acetylene and ethylene, which enhanced flower production in pineapple. Subsequently, use of plant growth regulators has grown exponentially to become a major component of agricultural commodity production (web ref. 3).
In this study the plant growth regulator, Furolan containing 99.98% of active substance and synthesized in PNIL NIIHGS KubGTU (Koslina et al, 1997; Nenco et al, 1995; Nenco et al, 2005) was used to investigate the influence of the growth regulator on intrinsic factors of winter wheat grain. The name of the active substance of Furolan is, according to ISO – 2- (1, 3-dioksolanyl-2) furan and by IUPAC – (2-furil) - 1, 3 – dioksolan. Furolan – is a colorless liquid with weak characteristic acetylene smell, containing 98.89% of active substance, 2 (1, 3 dioksolanyl-2) furan, soluble in water and adequately soluble in organic solvents. Furolan could be classified as average toxic compound (LD50 = 511-626 mg/kg). The amino acids formed as a result of intensive proteolysis of reserved proteins in germinating seeds and synthesized from organic acids formed during oxidation are used in the formation of protein components in cells of germinating sprouts. The reserved substances in the endosperm and germ cells serve as source of nitrogen for germinating seeds (Bonner, 1976; Badovskaya, 1975). The break-down of proteins begins soon after the soaking of seeds. Thus, the appearance of free amino acids in germs cells occurs in the very early period of germination (12 – 24hrs) than in the endosperm which is not unconnected to the early hydration of germ cells and hence, early mobilization of enzymes (Pchelnikova et al, 1973).
Amino acids can be formed not only from protein proteolysis, but also from products of break-down oxidization of soluble carbohydrates which are much more in the germ cells than in endosperm. Nitrogenous substances are transported much later from endosperm to the germ cells and the amino acids content in endosperm and seed-coat reaches a maximum only on the third − the fourth day, but this content increases noticeably in the germinal axis from second day of germination. Thus, follows significant presence in the amount of glutamic acids and asparagine and small − aromatic acids (Popov and Yanishlieva, 1976; Palamarchuk and Veselova, 1971). Mobilization of the seed reserve is dependent upon growth rate of the axial part of the sprouts which is caused by the level of activity of proteolytic enzymes in the endosperm reaching the maximum value by the fourth day of germination (Peshkova, 1972; Sibulko, 1976). Products of proteolysis act on the sprout, ensuring intensive course of growth processes (Maksimov, 1958).
The aim of this study is to investigate the effect of these important extrinsic factors; storage time and growth regulator, Furolan on these equally significant intrinsic factors; proteins and amino acids in the cultivation and post-harvest management of winter wheat.

MATERIALS AND METHODS

Determination of the Amino Acids Content in Winter Wheat Grain

The technique of measuring mass fraction of amino acids in samples was carried out by capillary electrophoresis method using the system of capillary electrophoresis "Drops" M-04-38-2004, St-Petersburg developed by the Ural scientific research institute of metrology.
After sorting out the spoiled grains, the weed impurity was then removed. The grain samples were collected during the phase of full ripeness and ground in a centrifugal mill. From each prepared portion of grains, two samples of 100 ± 0.2 mg were taken and added 10cm^3 6M a hydrochloric acid and allowed to hydrolyze at temp. 110 °C for 14 hours. Then 50 microlitre (0.05 ml) was taken and dried in current of dry air.
The dry deposit was moistened with 50 microlitre of water and to every weighing bottle was added 100 microlitre 0.1M solution of sodium carbonate, mixed and added 300 microlitre of isopropanol solution of phenylisothiocyanate and then mixed again until solution primarily formed a white deposit. At the end of the reaction the weighing bottles were left closed for 35 minutes at room temperature and then solution allowed to evaporate to dryness in a stream of warm air. The dried remains were dissolved in 500 microlitre of water and used for the analysis. The solutions were then centrifuged before analysis.
Three different regimes were used in the determination of amino acids. Each standardization was carried out separately. After which, a "Multichrome" program was created.
Standardization system of capillary electrophoresis was carried out using a single standard for two measurements. For this purpose, standardized mixture №1, №2, №3 of the three solutions were each prepared for analysis (Table 1).
Thereafter, the electrophoregrams obtained was described according to the user's guide of the program "Multichrome". The program was used to calculate the average value of standardized coefficient and the root-mean-square deviation (RMSD) of the sample.

Table 1. Recommended parameters of intake and analysis of standardized solutions.

Standardized Solution	№1	№2
Defined amino acid	Ala, Arg, Cys-Cys (in accordance to the peak of Cysteine) Val, His, Gly, Ile, Leu, Lys, Met, Pro, Tyr, Thr, Ser,Phe	Asp, Glu, Cys-Cys(in accordance to the peak of Cysteinic acid)
Analysis:		
Wave length	254nm	254nm
Force	+8kV	+14kV
Pressure	0 millibar	0 millibar
Temperature	25° C	25°C
Time	35 min	35 min
Table Buffer solution	–	Diluted twice with distilled water

Standardization was considered acceptable, if the value of RMSD did not exceed 5% for alanine, arginine, valine, glycine, isoleucine and leucine, lysine, methionine, proline, threoline and serine, and 10% - for histine, tyrosine, phenylalanine, asparagines, glutamine and cysteinic acids. Otherwise the standardized mixture was further analyzed until the specified value of RMSD was obtained. Unsatisfactory standardized points were rejected in the event that square deviation of the peak and simultaneous time of exit of the peak of a given component exceeded 5% of the average values.

Of the 18 identified amino acids 15 exited after the peak of the osmotic stream in the seventh minute; thereafter the relatively close group of 14 peaks (leucine and isoleucine formed a combined peak) exited in the course of 3 - 4 minutes. Glutamic acid exited in another 10-15 minutes and 2 minutes later exited the peak of asparaginic acids. Thus, overall time for a single analysis was about 30 minutes. It must be noted that acid hydrolysis of poorly kept cysteine introduced pre-oxidized to cysteinic acid (meaning the exit time was more than for asparaginic acids).

In the preparation of table buffer solution; 0.1135 g of β-cyclodextrin was placed in a flask of 25 cm^3 capacity to which was added 7.5 cm^3 of reserved solution, mixed and added 10-12 cm^3 of water to dissolve cyclodextrin. The resulting solution was heated up in a warm water-bath as required. After the complete dissolution of macro-cyclic contents of the flask, it was made up to the water mark.

Concentration of the leading electrolyte was - 30 mM; β-cyclodextrin - 4 mM, pH value of buffer was 7.7 - 7.8.

The period of storage of table buffer solution in polyethylene containers was two weeks. Before using the table buffer solution, it was filtered through cellulose-acetate filter (after discarding the first portion), to degas by centrifugation. In the preparation of reserved buffer solution: into a measuring flask of 100cm^3 were introduced 40.5cm^3 solution of 0.2 mol/dm^3 sodium hydrophosphate and 9.5 cm3 solution of 0.2 mol/dm^3 sodium dehydrophosphate, mixed and made up to watermark. Period of storage of reserved buffer solution was two months.

Determination of Fractional Composition of Protein Complex by Osborne Method

Batches of grains 50.0 ± 0.1 in weight were set aside from all the samples for analysis. Dry grains were cleared of weed impurity and spoiled grains and ground in laboratory mill. For determination of the protein fractions in the general protein complex of ripened wheat grain, two equal batches of 1g each were weighed with a marginal error of ± 0.001g.

Soluble proteins were extracted from freshly-prepared toster with fivefold volume of 1M NaCl in phosphate buffer (pH 7.0) overnight at temperature of 4 ˚C.

Supernatants were separated by centrifugation at 6000g

Table 2. Dynamics of fractional composition of varieties of winter wheat grains during storage, % to the general protein content.

Variety	Fraction	During Harvest			7 mths duration		
		Control	Furolan	± Control	Control	Furolan	± Control
Batko	Albumin+ Globulin	45.7	41.7	- 4.0	41.8	37.1	- 4.7
	Prolamin	23.9	24.6	0.7	22.1	27.8	5.7
	Gluten	30.4	33.7	3.3	36.1	35.1	- 1.0
Diya	Albumin+ Globulin	44.8	42.9	-1.9	39.9	37.0	-2.9
	Prolamin	22.9	23.2	0.3	23.8	27.6	3.8
	Gluten	32.3	33.8	1.5	36.3	35.4	-0.9
Krasnodar 99	Albumin+ Globulin	51.0	48.3	-2.7	42.8	48.7	5.9
	Prolamin	19.8	22.3	2.5	23.8	18.9	-4.9
	Gluten	29.2	29.4	0.2	33.4	32.4	-1.0
$LSD_{0.95}$							
	2.5				3.4		

in 15 minutes on ice. The deposit was washed 4 times in tenfold volume of cooling reagent.

Salts of the first extracts were separated before hand to obtain the alcoholic- (gliadin) fractions from the deposit. The residue was filled with the cooled distilled water, mixed and immediately, in order to prevent the loss of gliadin, centrifugalized at 6000g for 10 minutes on ice. After that the deposit was flushed with two-fold volume 70% ethanol.

Extraction was conducted for 3 hrs periodically and slowly stirred at a room temperature. Supernatant containing gliadin, was separated by centrifugation at 6000 g in 15 minutes at 18 ˚C temperature. To complete the separation of gliadin, the deposit was washed not less than four times in fivefold volume of 70 % ethanol.

Gluten was extracted from the remaining deposit in triple volume 0.05 normal NaOH in three (3) hours on ice. The extract was separated with fourfold centrifugation on ice at 6000g for 30 minutes (Kotiyashkina, 1968).

The content of protein in the analyzed fractions was determined by Spectro-photometric method.

Calculation was according to the formula:

$(E\ 280.1.45 - E260.0.74) .V/m$

Where,

E 280 – Extraction at wavelength of 280 nanometers,

E 260 - Extraction at wavelength of 260 nanometers,

V - Volume of protein extract, ml,

m – Mass of batch, g.

RESULTS AND DISCUSSION

Results of determination of protein fraction composition in winter wheat grain during seven months of storage are presented in Table 2.

Effect of Furolan on Protein Fraction Composition in Varieties of winter wheat grains.

Protein complex of wheat grain could be expressed as hydro-, saline-, alcoholic-and alkaline soluble fractions. Results of determination of protein fraction composition in winter wheat grain during seven months storage are presented in Table 2.

During harvest, Diya and Batko varieties differed from Krasnodar 99 variety by their low content of albumin and globulin and the high content of alcoholic-and alkaline-soluble fractions of protein (protein gluten), due to the genetic potential of these varieties.

Application of Furolan resulted in an increase in gliadin content in Krasnodar 99 variety and gluten in Batko variety; in Diya's variety the tendency of this index to increase was observed. During storage, there was decrease in the contents of albumin and globulin and increase in gluten content in the control of Batko and Diya varieties. In their experimental variants (Batko and Diya) there was an increase in the content of gliadin frac-

Table 2. Dynamics of fractional composition of varieties of winter wheat grains during storage, % to the general protein content.

Variety	Fraction	During Harvest			7 mths duration		
		Control	Furolan	± Control	Control	Furolan	± Control
Batko	Albumin+ Globulin	45.7	41.7	- 4.0	41.8	37.1	- 4.7
	Prolamin	23.9	24.6	0.7	22.1	27.8	5.7
	Gluten	30.4	33.7	3.3	36.1	35.1	- 1.0
Diya	Albumin+ Globulin	44.8	42.9	-1.9	39.9	37.0	-2.9
	Prolamin	22.9	23.2	0.3	23.8	27.6	3.8
	Gluten	32.3	33.8	1.5	36.3	35.4	-0.9
Krasnodar 99	Albumin+ Globulin	51.0	48.3	-2.7	42.8	48.7	5.9
	Prolamin	19.8	22.3	2.5	23.8	18.9	-4.9
	Gluten	29.2	29.4	0.2	33.4	32.4	-1.0
$LSD_{0.95}$							
	2.5				3.4		

Table 3. Amino acid content of winter wheat grain of Batko variety, mic.g/g.

Aminoacid	Full ripeness			12 mths Storage		
	Control	Experiment	± Control, %	Control	Experiment	± Control, %
Arginine	3.37	4.04	19.88	3.60	4.20	16.72
Lysine	1.97	2.43	23.35	2.33	2.87	23.13
Phenylalanine	1.04	1.41	35.58	1.21	1.14	6.04
Histidine	7.75	9.55	23.23	5.82	6.16	5.86
Leucine	5.50	6.72	22.18	6.41	7.17	11.74
Methionine	2.07	2.27	9.66	1.92	2.55	32.93
Valine	2.56	3.14	22.66	2.80	2.88	2.75
Proline	11.15	13.09	17.4	12.63	13.17	4.28
Threonine	6.98	8.36	19.77	7.26	7.61	4.79
Serine	3.02	3.59	18.87	3.08	3.34	8.62
Alanine	7.54	9.07	20.29	8.11	9.08	11.90
Glycine	4.18	4.86	16.27	4.55	4.93	8.40

Table 3. Cont.

Glutamine	22.96	28.13	22.52	35.93	37.58	4.59
Asparagine	6.35	7.31	15.12	9.07	10.91	20.36
Tyrosine	1.10	1.13	2.73	0.45	0.44	1.12
Cysteine	0.20	0.23	15.0	0.29	0.35	18.97
Tryptophan	1.59	1.76	10.69	0.86	0.92	7.59
Total	89.32	107.07	19.87	106.31	115.29	8.45

Table 4. Amino acid content of winter wheat grain of Diya variety, mic.g/g.

Aminoacid	**Full ripeness**			**12 mths Storage**		
	Control	**Experiment**	**± Control, %**	**Control**	**Experiment**	**± Control, %**
Arginine	3.60	4.20	16.7	4.05	5.11	26.17
Lysine	2.33	2.87	23.2	1.80	3.26	81.11
Phenylalanine	1.21	1.14	-6.0	1.19	1.37	15.13
Histidine	5.82	6.16	5.8	4.70	7.61	61.91
Leucine	6.41	7.17	11.9	5.33	8.88	66.60
Methionine	1.92	2.55	32.8	3.54	3.79	7.06
Valine	2.80	2.88	2.9	1.83	3.34	82.51
Proline	12.63	13.17	4.3	12.46	17.31	38.92
Threonine	7.26	7.61	4.8	7.49	10.23	36.58
Serine	3.08	3.34	8.4	3,19	4.47	40.13
Alanine	8.11	9.08	12.0	7.72	11.28	46.11
Glycine	4.55	4.93	8.4	4.23	6.30	48.94
Glutamine	35.93	37.58	4.6	27.26	33.70	23.62
Asparagine	9.07	10.91	20.3	6.55	8.18	24.88
Tyrosine	0.45	0.44	-2.0	0.68	-	-98.53
Cysteine	0.29	0.35	20.7	0.13	0.38	192.38
Tryptophan	0.86	0.92	7.0	-	0.51	100.00
Total	106.31	115.29	8.4	92.11	125.71	36.48

tions of protein in comparison to the control by 5.0 and 3.5% respectively and correspondingly, there was a decrease in the content of alkaline-, hydro-and saline soluble fractions.

In the control variant of Krasnodar 99 variety during storage there was a decrease in the contents of albumin and globulin while an increase in the content of protein gluten was observed. In the experimental variant of Krasnodar 99 variety during storage there was an increase in the content of alkaline soluble fractions of protein, as well as the content of the albumin and globulin in comparison to the control.

Table 5. Amino acid content of winter wheat grain of Krasnodar 99 variety, mic.g/g.

Aminoacid	Full ripeness			12 mths Storage		
	Control	Experiment	± Control,%	Control	Experiment	± Control,%
Arginine	2.73	2.80	2.56	2.99	4.08	36.45
Lysine	1.96	2.84	44.9	2.05	1.99	-2.90
Phenylalanine	1.02	1.16	13.73	0.81	1.32	62.96
Histidine	4.84	5.48	13.22	4.68	5.10	8.97
Leucine	5.07	5.40	6.51	5.38	4.85	-9.90
Methionine	1.68	1.99	18.45	3.20	3.37	5.31
Valine	2.21	2.46	11.31	2.09	1.61	-23.00
Proline	10.16	11.26	10.83	10.19	12.12	18.94
Threonine	5.43	7.91	45.67	6.34	7.07	11.51
Serine	2.54	2.99	17.72	2.66	3.45	29.70
Alanine	6.51	8.28	27.19	7.25	8.33	14.90
Glycine	3.61	4.22	16.90	3.59	4.72	31.48
Glutamine	26.22	28.37	8.20	28.93	33.19	14.73
Asparagine	7.33	7.74	5.59	8.94	9.21	3.02
Tyrosine	0.93	1.14	22.58	-	0.72	71.00
Cysteine	0.22	0.26	18.18	-	0.39	38.00
Tryptophan	1.08	1.12	3.70	0.41	-	-97.60
Total	87.89	95.42	8.57	89.52	101.52	13.40

Earlier studies show that, Furolan promotes the redistribution of gliadin fractions and increases the contents of the ω-gliadin fraction, a characteristic feature of which is its ability to form intermolecular interactions due to the hydrogen and hydrophobic bonds. This property of gliadin protein ensured its leading role in the formation of gluten (Konarev, 1980). Consequently, the increase in the contents of gliadin fraction of protein in the experimental variants of Batko and Diya varieties during storage promoted the strengthening of gluten.

Results of determination of amino acid fractions of protein are presented in Tables 3 – 5.

Effect of Storage Time on Amino Acids Contents in Wheat Grain

It is known that gluten fraction of protein of wheat grain is made up of proteins which contains large quantities of amino acids such as asparagine, glutamine and amides, while in gliadin - they include cysteine and methionine (Nenco, 2005). In Batko variety, Furolan increased the contents of monoaminomonocarboxylic acids (glysine, alanine, serine, threonine, valine, leucine) - and diaminomonocarboxylic acids (arginine and lysine), glutamic acids (which conformed with increase in reserve proteins especially gliadin) and phenylalanine - the precursor in synthesis of the phenolic compounds, which turned out to influence the growth of plants and conferred stability in the plants to damage by phytopathogens.

During storage the contents of cyclic amino acids (tyrosine, tryptophan, histidine) decreased in winter wheat grains of Batko variety of both the experimental and control. Thus, increase in the contents of cysteine and methionine was in conformity with the strengthening of gluten in the experimental grain (Nenco, 2006).

Increase in the asparagine content of the experimental variant was of great importance, as it was the chain link in protein and carbohydrate exchange taking part in the synthesis of purine and pyrimidine bases and liasing between carbohydrate and nucleic acid exchange.

During storage the cyclic and monoaminodicarboxylic amino acids content in winter wheat grains of Diya's variety decreased both in the experimental and control. Thus, the experimental variant surpassed the control in contents of diaminomonocarboxylic, cyclic amino acids and cysteine. The increase in the contents of latter amino acid promoted the strengthening of gluten.

In the Krasnodar 99 variety during storage the content of monoaminomonocarboxylic and cyclic amino acids decreased while methionine (in the control and experimental variants) and cysteine (in the experimental variant) increased, promoting the strengthening of gluten. Furolan promoted an increase in the contents of monoaminomonocarboxylic (glycine, serine, cysteine) and cyclic (phenylalanine) amino acids in the grains of this variety.

Thus, during 12 months of storage the contents of cyclic amino acids in the grains of all varieties studied decreased; in the average-ripening varieties (Diya and Krasnodar 99) – there was decrease in monoaminomonocarboxylic amino acids as it was with the variety with lowest potential for protein synthesis.

In all the experimental varieties during storage there was increase in the presence of sulphur-containing amino acids of cysteine and in the average – ripening varieties - of methionine, which strengthened gluten in Krasnodar 99 variety - in the control and experiment, which apparently explained the specificity of these varieties.

The increase in the contents of cysteine in the grains of Batko and Diya varieties and also cysteine and methionine, glutamine - in Krasnodar 99 variety conformed with the changes in protein fraction composition: increase in the gliadin content in first two varieties (Batko and Diya) and gluten – in the latter (Krasnodar 99 variety).

CONCLUSION

Furolan, as earlier established, promotes the redistribution of gliadin fractions and increases the contents of the ω-gliadin fraction, characteristic feature of which is its ability to form intermolecular interactions due to the hydrogen and hydrophobic bonds. This property of gliadin protein ensures its leading role in formation of gluten, a significant and major chemical component of winter wheat. Furolan is thus recommended in the cultivation of winter wheat.

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