PROTEINES' MODIFICATIONS STUDY IN THE PROCESS OF LACTO BACTERIAL PREPARATION OBTAINING

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Abstract

The results of wheat bran proteins' modification at different stages of the process of obtaining of lacto-bacterian preparation are shown in the present paper.
Keywords: *bran proteins, lacto-bacterian preparation.*

Introduction

Being a valuable product, the wheat bran can be utilize as a source of dietary fibers, vitamines B, mineral elements. At the same time they have a high content of proteins (14 ... 15% DM) that are balanced in essential aminoacids. Thus their main nutrients' utilization without losses represents an obvious interest for the food technology. One of possible ways is the utilization of wheat bran in the obtaining of the lacto-bacterial preparation, well-known under the name "borş acru" ("sour soup"). This preparation obtaining is a complex physico-chemical and biochemical process, that includes the nutritive substances diffusion into the liquid phase and the lactic fermentation under the influence of Lactobacilus Delbrueckii bactera. In the present work are presented the results of wheat bran proteins' modification at different stages of the process of obtaining of lacto-bacterian preparation.

Experimental

Materials. Wheat bran used in the study were received from the cereals factory in Chisinau. The indices of wheat bran chemical composition were the following, %: humidity-9,6, fats-3,69, proteines-14,0, ash-5,12, starch-21.54, cellulose-10,93. The bran was preserved at the temperature of 20°C and relative humidity of φ =60%.

Methodes. Obtaining of the lacto-bacterial preparation has included the following operations: bran scalding and cooling down till 47...49°C, sowing with the starter of Lactobacilus Delbrueckii lactic bacteria, dilution with water till the hydromodule 12:1 and fermentation during 24 ore at

T=47...49°C. Samples for analysis were extracted during fermentation after 8, 16, and 24 hours. Preparations of humid bran, extracted from the fermentation ground were dried by convection at the room temperature till the equilibrium humidity of 9,1...9,8%. The physico-chemical measurements in bran were made as follows (1,2): dry matter content – by drying in the oven at 100...105°C till constant weight, ash- by calcination in elecrical oven at 400...600°C, fats – by extraction with the mixture of etilic ether and acetone in Soxlette device, nitrogen – by Kheldal method, proteines' fractioning – by Landri and Muro method, modified in the biochemistry laboratory of "Porumbeni" association, Moldova.

Results and Discussions

Obtaining of lacto-bacterial preparation is a complex process involving physico-chemical, biochemical and microbiological changes. Changes occur inclusively in the bran nitrogen substances. Nitrogen substances are constituted by protein and extractive substances (that pass into the aqueous solutions - polipeptides, peptides, aminoacids, others). Extractive nitrogen includes hydrosoluble proteins and the nitrogen of polipeptides and free aminoacids. The results referring to the content of different forms of nitrogen in wheat bran and in the liquid phase are presented in the table 1.

Table 1: Nitrogen content in bran at different stages of technological treatment

Nr.	Sample name and treatment conditions	Nitrogen forms, % D.M.		
		Total	protein	Extractive
Solid phase				
I	Scalding	2,41	2,15	0,26
II	After 8 hours of fermentation	2,3	1,99	0,31
III	After 16 hours of fermentation	2,2	1,87	0,33
IV	After 24 hours of fermentation	1,91	1,54	0,37
Liquid phase				
1	Duration 0 hours,	0,68	0,09	0,59
2	Duration of fermentation 8 hours	0,7	0,07	0,63
3	Duration of fermentation 16 hours	0,92	0,06	0,86
4	Duration of fermentation 24 hours	1,23	0,11	1,13

Experimental data show that the total nitrogen content in solid phase (bran) is decreasing during the fermentation process, and is increasing in the liquid phase.

Protein nitrogen content to dry matter ratio in solid phase decreases from 2,15% till 1,54%, losses being of 28,4%. In the liquid phase the extractive nitrogen is accumulating from 0,5% till 1,13%; accumulation constitutes 91,5%. The obtained data confirm the published statements concerning the fact that lactic acide fermentations are accompanied by proteins' peptonization.

Protein substances consists of several fractions that differ by their solubility in different environments. Visual description of protein sediments extracted with corresponding solutions and centrifugated during 10 min. at 6000 min^{-1} are presented in the tabel 2.

Table 2: Visual description of the protein sediments from bran

Sample	Extractive solution, fraction name and visual description		
	1 M NaCl albumin + globulin	80% ethanol prolamins	0,2 % NaOH glutelins
Initial	Abundent precipitate of creamy colour	Few precipitate of light creamy colour	Very few precipitate of light creamy colour
Scalded	Slightly abundent precipitate of light creamy colour	More abundent precipitate of creamy-yellow colour	More pronounced precipitate of light creamy colour
Fermentation 8 hours	Less abundent precipitate of light creamy colour	Abundent precipitate of light-yellow colour	Few precipitate of light creamy colour
Fermentation 24 hours	Few precipitate of creamy colour	Few precipitate of light yellow colour	Few precipitate of light brown colour

The results of protein fractioning at different versions of bran technological treatment by nitrogen content are presented in the tabel 3 and figure 1. In the initial sample albumins and globulins fractions are predominat. The quantities of prolamins and stroma are practically identic, and that one of glutelins is minimal. Thermical treatment (scalding) causes essential modifications in proteins structure. Because of proteins' denaturation and aggregation, a substantial part of albumins and globulins (about 50%) of their total lose the solubility, fact that conduct to a considerable stroma increase. During lactic fermentation, proteins' changes are determined both by the thermic effect and by the biochemical one.

Table 3: Nitrogen content of bran protein fractions

Proba	Nitrogen content of protein fractions, % of bran weight					Total nitrogen	Deviation from the total nitrogen
	1 M Na Cl	80 % ethanol	0,2 % Na OH	Stroma (reziduu after fractions' extraction)	Nitrogen sum of the fractions		
	Albumin + Globulin	Prolamin	Glutelin				
Initial	1,04	0,49	0,28	0,51	2,32	2,24	+ 0,08
Scalded	0,49	0,56	0,36	0,89	2,3	2,23	+ 0,07
Fermentation 8 hours	0,45	0,58	0,25	0,9	0,18	2,19	- 0,01
Fermentation 24 hours	0,43	0,38	0,24	0,01	1,96	1,93	+0,03
% out of the nitrogen sum of protein fractions							
Initial	44,8	21,1	12,1	22	100	-	-
Scalded	21,3	24,3	15.7	30,7	100	-	-
Fermentation 8 hours	20,6	26,6	11,6	41,2	100	-	-
Fermentation 24 hours	21,9	16,3	12,2	49,6	100	-	-

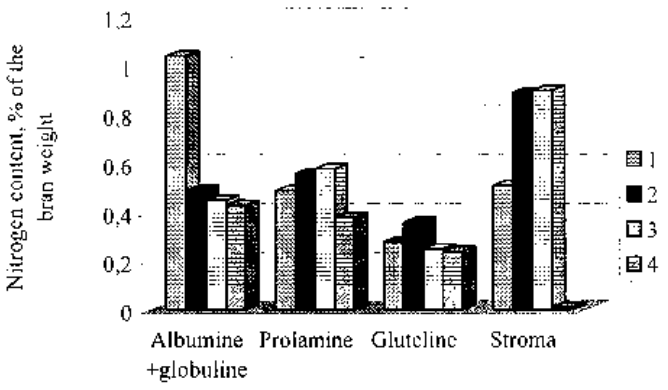

Fig. 1: Protein fractions evolution in different variants of wheat bran treatment: 1-initial; 2- scalded; 3- fermentation 8 hours; 4 – fermentation 24 hours

In the tabel 4 is presented the nitrogen content in protein fractions, including protein and extractive nitrogen at different stages of the technological process. From the tabel it follows that the albumin and

globulin content after scalding diminishes approximately twice. At the following technological stages it is noticeable a relatively slow diminish of nitrogen substances. It is evident that a sudden diminishing of the nitrogen substances content after the first operation is linked with their partial move into the ambiant milieu and partly with protein denaturation. Further reduction of nitrogen substances content is due to the fact that these are used in the quality of nutritive substrate for the developing micro-organisms.

Table 4: Protein and extractive (non-protein) nitrogen content in protein fractions obtained from different technological versions of bran treatment

Denumirea	Nitrogen content in protein fractions, % of weight								
	Albumins + globulins			Prolamins			glutelins		
	N fract.	Nprot.	Nextr.	N fract.	Nprot.	Nextr.	N fract.	Nprot.	Nextr.
Initial material	1,04	0,8	0,24	0,49	0.42	0.07	0.28	0.26	0,02
Scalded	0,49	0,34	0,15	0,56	0.46	0.1	0.33	0.33	0,03
Fermentation 8 hours	0,45	0,31	0,14	0,58	0.48	0.1	0.25	0.23	0,02
Fermentation 24 hours	0,43	0,31	0,12	0,38	0.33	0.05	0.24	0.23	0,01
In % from the fraction sum									
Initial material	100	76,9	23,1	100	885.7	14.3	100	92.8	7,2
Scalded	100	69,4	30,6	100	82.1	17.9	100	91.7	8,3
Fermentation 8 hours	100	68,9	31,1	100	82.8	17.2	100	92	8
Fermentation 24 hours	100	72,1	27,9	100	86.8	13.2	100	95.8	4,2

For prolamins is characteristic their gradual accumulation after scalding and during the fermentation process in the first 8-10 hours and the diminish of this fraction quantity at the end of the fermentation. The glutamins quantity increase after scalding and then occur a gradual reduction at the end of fermentation.

Stroma's proteines accumulate gradually and at the end of fermentation their quantity increases more than twice. The increase of this fraction is linked with protein denaturation and with micro-organisms development and the accummulation of microbial protein, while the reduction of prolamins and glutenins–with their partial hydrolisis. For all studied variants is characteristic the reduction of extractive nitrogen

Proteins modification in the process of lacto-bacterial preparate obtaining are determined both by the thermic effect and by the biochemical and microbiological ones.

The increase of active acidity in the milieu promotes proteins' hydration and solubilisation firstly of the gliadin residues. It occurs also proteins' depolimerization under the influence of proteolitic enzymes, which activity increase along with the increase of milieu's acidity. All these conduct to the increase in the content of extractive nitrogen content in protein fractions.

As mentioned, during the obtaining of lacto-bacterial preparate an exchange of nitrogen substances occur between the solid and liquid phases (figure 2).

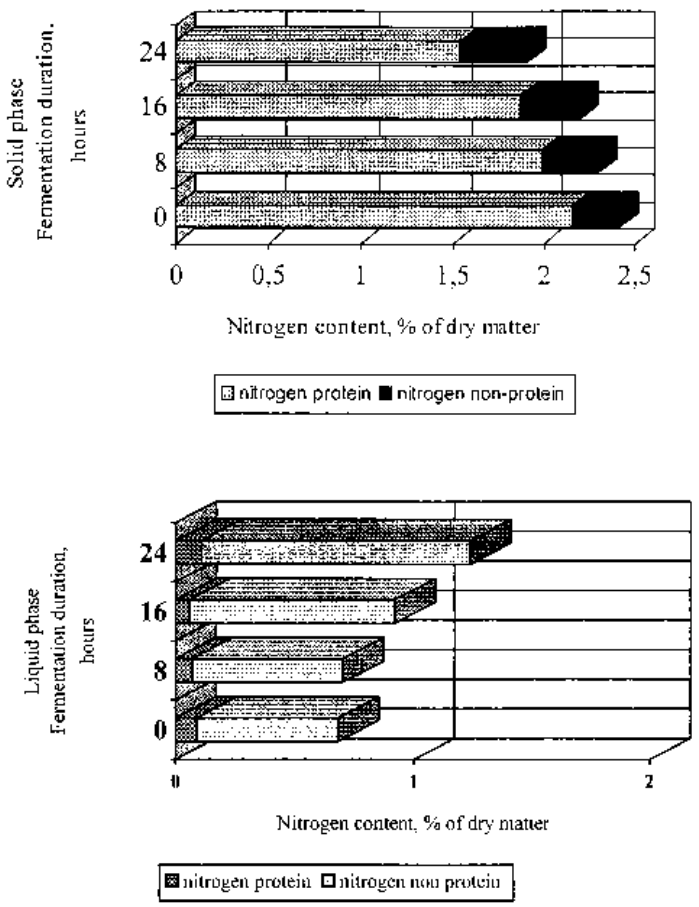

Fig. 2: Nitrogen substances evolution during fermentation process

Meanwhile the biological value of the lacto-bacterial preparate will be determined greatly by the amino acids content. In the whole protein were identified 16 amino acids. Data concerning the amino acids composition in the whole protein and in the protein fractions at different stages of the process are presented in the tabel 5.

Table 5: Proteinogenic amino acids content in different tehnological variants of wheat bran treatment and in protein fractions obtained with different solvents, sedimented with tricloracetic acid and acetone

Title	Amino acid															
	Acid asparti c	*Treoni-ne*	*Serine*	*Acid gluta -mic*	*Prolin e*	*Glicine*	*Alanine*	*Valine*	*Methionine*	*Izoleu-cine*	*Leuci-ne*	*Tirosi-ne*	*Phenyl-alanine*	*Histidi-ne*	*Lysine*	*Argini-ne*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Initial weight and technological variants, % out of the weight of dry bran after oil extraction																
Initial	1,19	0,58	0,46	2,7	0,73	0,62	0,75	0,94	0,35	0,46	1,03	0,43	0,78	0,49	0,73	1,04
Scalded	0,81	0,36	0,45	3,54	1,64	0,77	0,61	0,61	0,21	0,44	0,94	0,32	0,68	0,47	0,58	0,91
F 8 h.	0,84	0,48	0,6	3,76	0,77	0,8	0,75	0,86	0,15	0,46	1,05	0,37	0,69	0,49	0,62	0,92
F 24 h.	0,88	0,36	0,48	2,32	0,61	0,74	0,81	0,82	0,08	0,46	1,01	00,31	0,72	0,41	0,69	0,93
Protein fractions, % of protein Albumins + globulins (1M NaCl + Triclor acetic acid–5%), % from the protein fraction weight																
Initial	7,90	3,45	3,25	16,35	4,00	4,20	6,65	8,60	2,64	3,75	7,85	2,90	4,15	2,80	6,40	9,75
Scalded	8,36	1,11	1,15	13,69	3,32	2,22	6,66	10,4	71,7	44,62	8,51	2,59	5,55	2,22	4,81	9,25
F 8 h.	8,51	2,88	3,45	20,58	3,33	5,52	5,29	5,75	2,15	3,22	6,67	2,65	8,28	3,68	4,71	9,66
F 24 h.	11,11	1,14	1,71	19,38	3,69	3,42	6,84	9,12	1,12	3,99	7,98	1,57	6,84	3,42	5,27	9,83
Prolamins (80% ethanol +acetone+ vaccum), % from the protein fraction weight																
Initial	6,05	1,20	2,08	0,2	9,3	3,40	2,90	3,80	0,10	2,80	6,30	2,78	6,03	2,13	2,18	3,58
Scalded	2,22	1,74	3,05	43,44	11,52	2,16	2,58	3,84	0,60	3,18	6,66	2,22	4,08	1,92	1,50	3,32
F 8 h.	4,79	0,98	3,10	45,22	7,24	3,08	2,59	3,29	0,54	2,84	6,26	1,61	5,64	2,27	1,61	2,21
F 24 h.	4,56	1,54	2,42	36,30	4,83	5,06	5,50	6,61	0,42	3,74	7,41	1,98	4,40	3,08	1,38	8,14
Glutelins (0,2% NaOH + tricloracetic acid –5%), % from the protein fraction weight																
Initial	4,56	2,85	3,99	37,05	3,42	6,27	3,42	6,31	0,57	2,85	6,27	1,71	2,28	1,82	3,89	8,55
Scalded	3,64	2,08	3,25	37,31	9,36	4,16	4,03	5,07	0,43	3,38	7,54	3,51	3,77	2,60	2,21	3,69
F 8 h.	5,18	2,66	3,50	32,38	6,05	4,68	5,01	5,86	0,42	3,92	8,12	3,50	4,02	2,49	3,52	3,84
F 24 h.	6,81	2,67	3,48	25,61	3,74	5,44	6,29	7,14	0,38	4,42	8,33	2,55	4,59	3,74	5,95	5,78
Stroma (residue after protein fractions extraction – proteines linked with nucleic acids and lipids - membrans), % of bran dry weight																
Initial	0,31	0,13	0,12	0,41	0,22	0,22	0,23	0,20	0,05	0,13	0,28	0,09	0,12	0,1	0,17	0,28
Scalded	0,46	0,20	0,22	0,75	0,37	0,38	0,32	0,32	0,06	0,18	0,40	0,14	0,20	0,18	0,28	0,42
F 8 h.	0,54	0,25	0,28	0,94	0,48	0,47	0,40	0,38	0,05	0,21	0,49	0,17	0,34	0,31	0,30	0,51
F 24 h.	0,61	0,14	0,21	0,94	0,38	0,49	0,40	0,39	0,05	0,22	0,51	0,15	0,31	0,29	0,30	0,50

Amino acids evolution from the solid phase in the process of lactic fermentation are presented in the figures 3-7.

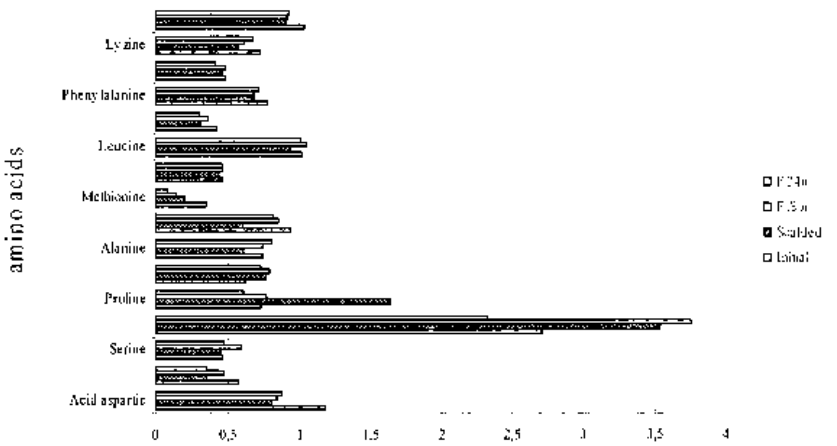

Fig. 3: Proteic amino acids evolution in the bran during lactic fermentation

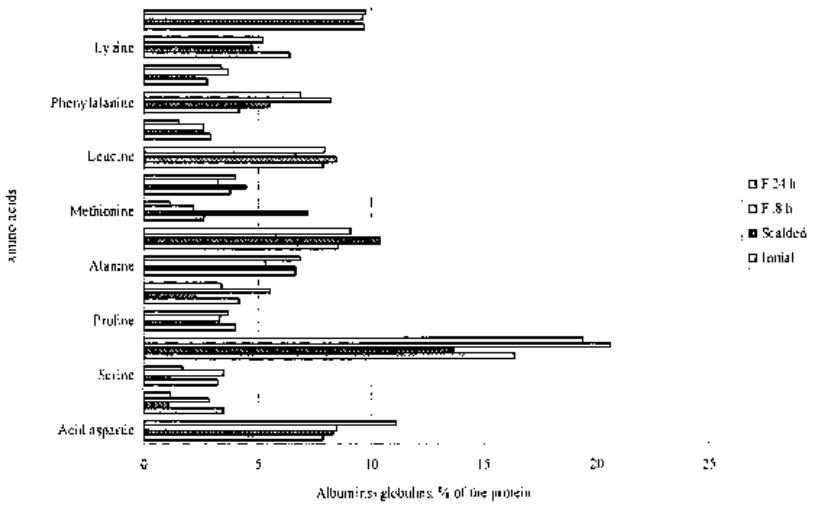

Fig. 4: Proteic amino acids evolution in bran during the lactic fermentation

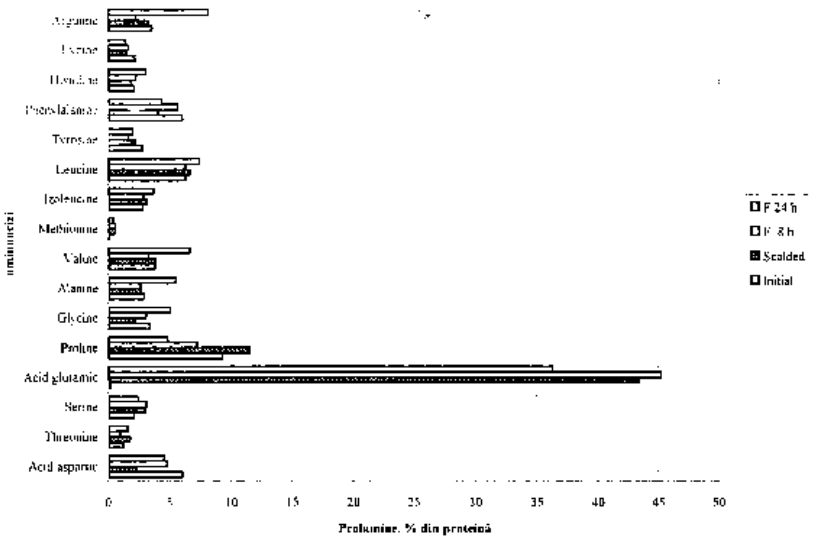

Fig. 5: Proteic amino acids evolution in bran during lactic fermentation

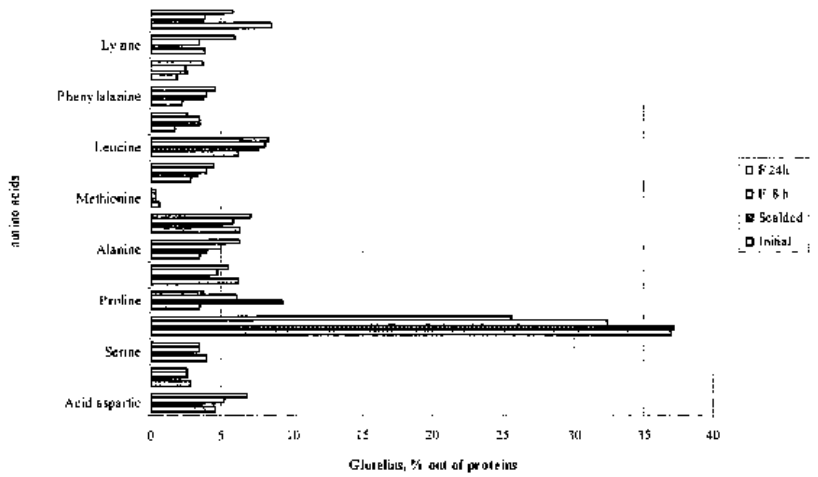

Fig. 6: Proteic amino acids evolution in bran during lactic fermentation

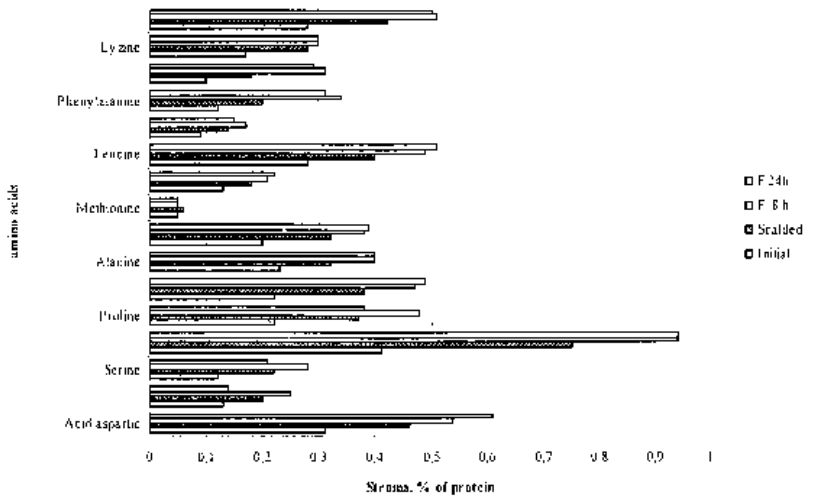

Fig. 7: Proteic amino acids evolution in bran during lactiv fermentation

Conclusions

Analysis results demonstrate that the amino acids content evolution is different and depends on their nature. The modification of the amino acids originated from all protein fractions are practically adequate to respective fraction's evolution (the amino acids content from albumins, globulins, prolamins, glutelins decreases and the content of amino acids from stroma increases). The values of these modifications are comparable with the modification of protein fraction.

It was determined the free amino acids content in the liquid phase. Data concerning the evolution of the free amino acids content at different stages of technological treatment are presented in the table 6. These data show that the sum of the free amino acids increases from 134,5 mg% in the initial sample till 983,9 mg% after 24 hours of fermentation. The content of the asparaginic acid, leucine, lysine, arginin, glutamine, serine in the liquid phase increases more than 10 times; that one of the phenilalanine, izoleucine – aproximativly by 7…8 times. Less is the increase of the proline, methionine content (≈2…3 ori). For the proteinogenic amino acids there are no essential changes during the fermentation process. The total content of the

proteinogenic amino acids is 92,82% in the initial sample and 93,80% in the sample after 24 hours of fermentation (tabel 6.).

Table 6: Amino acids content in liquid phase

Name	Amino acids															
	Acid aspartic	Threonine	Serine	Acid glutamic	Pro-line	Gly-cine	Alani-ne	Valine	Methio-nine	Izoleu-cine	Leu-cine	Tyro-sine	Phenyl-alanine	Histi-dine	Lyzine	Argi-nine
Free amino acids, mg%, in the bran dry matter																
Initial	9,5	5,5	5,9	16,6	6,8	6,0	9,0	8,8	2,1	5,7	8,9	7,0	7,7	11,1	12,1	17,7
Ferm 8 hours	53,8	16,5	32,4	56,4	2,0	12,0	36,0	44,0	2,4	28,5	66,8	14,1	25,1	37,0	28,4	55,4
Ferm.16 hours	72,8	19,3	44,2	66,4	2,8	15,3	49,5	61,6	4,3	39,9	111,2	35	46,2	44,1	109,6	88,2
Ferm 24 hours	108,8	35,8	74,4	82,9	5,6	22,4	53,2	42,4	6,3	41,2	116,9	53,5	71,6	60,3	102,3	118,5
Proteinogenic amino acids, % protein																
Initial	2,64	5,1	2,52	31,14	8,28	0,96	4,5	0,54	0,24	4,2	7,86	2,52	3,84	4,2	7,32	6,96
Ferm 8 hours	6,29	7,69	2,72	27,37	5,23	4,08	5,44	1,76	1,02	3,57	6,46	2,38	3,4	3,57	4,76	4,08
Ferm 16 hours	7,75	8,22	4,5	23,71	10,1	4,38	2,93	3,33	0,89	2,31	4,73	1,93	3,15	3,32	4,03	6,21
Ferm.24 hours	5,46	6,83	3,74	22,89	11,9	3,45	3,92	3,98	0,81	2,83	4,86	3,75	4,82	4,77	3,71	5,53

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