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Tracing some quality and biochemical parameters of kefir from goat milk during storage

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SUMMARY

From raw material - goat milk is prepared a fermentation product (kefir), which was characterized by main quality indicators. According to the data obtained by organoleptic evaluation, storage of kefir at 4°C is preferably up to 14 day.

After the second week, the product retains its largely useful and beneficial to the human body characteristics.

Data of physicochemical and biochemical composition resulting from goat milk fermented product (kefir) characterize it as a 100% natural product with multiple health effects.

Electrophoretic studies, which establish changes in casein and whey fractions of fresh and fermented goat milk and kefir during storage, were conducted.

The main casein fraction (α -casein and β -casein) and β -lactoglobulin and lactoalbumin of whey proteins.

Key words: kefir, quality

parameters, biochemical parameters, storage, goat milk

INTRODUCTION

Milk and dairy products are one of the most accepted and widely consumed foods. They are obtained using fermentation technology involving various lactic acid bacteria, thereby increasing their dietary potential.

Fermented milks are a particularly suitable form for absorption of nutrient substances in the milk on the one hand and to influence the health of the lactic acid bacteria on the various functions of the organism from another (Slacanac et al., 2010).

Kefir is a traditional drink, the result of two fermentations with kefir grains - lactic acid and alcohol. Kefir fungus is of irregular shape, consisting of grains ranging in size from 1 to 6 mm in diameter. They consist of casein and gelatinous colonies of microorganisms that grow in symbiosis. Kefir is considered a functional food and the benefits of its consumption are numerous (Guzel-Seydim et al., 2011).

Besides the high nutritional value of the beverage as a source of protein and calcium it is also known that kefir has antibacterial, immune-stimulating, antimutagenic and anticarcinogenic effects (Garrote et al., 1998 De Moreno et al., 2007).

Goat's milk is a food product with proven healing qualities, but the possibilities for its inclusion as a component in products with an functional purpose are limited and are not sufficiently investigated. Therefore, our interest is focused on creating a product based on goats milk fermented with symbiotic associations of lactic acid bacteria and yeast in the kefir grains.

The aim of the present

(Slacanac et al., 2010).

(Guzel-Seydim et al., 2011).

(Garrote et al., 1998; De Moreno et al., 2007).

- development is the research of the basic physicochemical parameters of kefir from goat's milk, and tracking the changes in the casein and whey fractions during storage.

MATERIAL AND METHODS

It is used goat milk and starter culture of kefir grains (2%), which was pre-activated in sterile skim milk at 25 ° C for 24 hours.

The starter culture was inoculated in pasteurized goat milk and incubated statically at 25 ° C for 24 h to obtain the fermented product to pH 4,6-4,8 and 90-100⁰T.

Organoleptic evaluation – Indicators appearance and texture, color, taste and aroma.

Physicochemical analysis – dry substance, %, active acidity () – pH-meter “Hanna” of a sample filtrate; titratable acidity – Thorner. total proteins - % (BDS 9374-82); total lipids - % (BDS 8549-74); total ash (BDS 9373-80); lactose (weight method).

energy value – 100 g product (kcal/kJ)

Biochemical research – electrophoresis in polyacrylamide gel – SDS-PAGE (by Laemmli, 1971) with gathering gel – 6.0% and dividing – 10%.

The electrophoresis runs with current intensity 30 mA and initial voltage 110V for around 3,5h. After finishing of the process the gel is colored with a solution of Coomassie brilliant blue G – 250 for 20-30 min. The coloring intensity of the obtained zones is determined on density meter “ERI - 10 “of company “Carl Zeiss – Jena “.

Statistical analysis

The results were processed using the software MS Office Excel.

(2%),

25°

24

24

25°

pH 4,6-4,8 90-100°

„Sartorius“;

pH-

9374-82);
(8549-74);
80);

(kcal/kJ)

SDS-PAGE (Laemmli, 1971)

– 6.0%

– 10%.

30 mA

110 V

3,5

blue G – 250 20-30 Coomassie brilliant

Zeiss – Jena“. “ERI – 10 “ “Carl

Office Excel.

MS

RESULTS AND DISCUSSION

The organoleptic evaluation is an important key factor for the taste receptivity of the finished product. The main indicators of lactic acid products including kefir are appearance, texture, color, taste and aroma (Meilgaard et al., 2007).

The results of the sensory assessment of samples from kefir 1,7 and 14th day are given in Table 1. The process of storage of kefir ends to the 21st day, but the organoleptic parameters abruptly change after the 14th day.

1. (n= 7)
Table 1. Organoleptic evaluation of kefir (n = 7)

Days	Appearance	Consistence	Colour	Taste	Flavour	Overall evaluation
1 day	9± 0,11	9± 0,02	9± 0,03	9± 0,01	9± 0,04	9.0± 0,02
7 day	9± 0,02	8± 0,21	9± 0,12	8± 0,02	8± 0,02	8.4± 0,12
14 day	7± 0,04	7± 0,02	8± 0,06	6± 0,11	7± 0,11	7.0± 0,10
21 day	5± 0,12	4± 0,09	5± 0,01	4± 0,02	4± 0,24	4.4± 0,13

According to this organoleptic assessment, the obtained kefir covers the normative indicators of a quality fermented product. Its appearance is homogenous and the consistency-liquid with a moderate viscosity. The colour varies from white to slightly creamy or with a yellowish hue. The product has a pure lactic acid taste and a refreshing flavor with a hint of yeast. According to the data obtained, it's desirable to keep the kefir at 4C to the 14th day. After the second week, the product retains a large portion of its useful and beneficial to the human body characteristics, but the taste indicators are reduced to 50%.

Table 2 presents the basic physicochemical parameters of kefir, after fermentation (22 hours) and in the process of storage up to 21st day.

2.

(n=5)

Table 2. Physicochemical indicators of fresh goat's milk and kefir (n =5)

Indicators	Fresh goat milk	/ Kefir () / Storage (days)							
		1 /day	7 /day	14 /day	21 /day	14 /day	21 /day	14 /day	21 /day
Dry substance %	12.21 ± 0,02	12,20± 0,04	12,21± 0,12	12,17± 0,02	10,65± 0,11				
Total protein %	3,53 ± 0,04	3,41± 0,11	3,39± 0,04	3,37± 0,04	3,09± 0,12				
Total lipids %	4,05 ± 0,01	3,47± 0,01	3,45± 0,11	3,42± 0,04	3,36± 0,02				
Lactose %	4,63 ± 0,10	2,98± 0,12	2,95± 0,01	2,96± 0,08	2,94± 0,04				
Active acidity ()	6,8 ± 0,12	4,6± 0,02	4,7± 0,15	4,6± 0,12	4,7± 0,01				
Titrateable acidity ()	19 ± 0,11	95± 0,04	95± 0,04	94± 0,01	97± 0,07				
Energy value 100 g product (kcal/ kJ)	71,12/297,57	58,47/264,64	58,08/243,01	57,77/241,71	56,02/234,39				

12.20%.
(21)
-
14- 1,55%, -
,
3,41%
(3,53%).
21
, 0,32%.
,
(0,58%),
,
(21)

The amount of dry matter in the kefir after fermentation is 12.20%. In the period of storage (to the 21st day) the dry matter content decreased to 1.55%, more sharply pronounced after the 14th day of storage. The data shows that the fermentation process does not significantly affect the dry matter content in the source material - milk.

Protein content in the kefir after fermentation is 3.41% and is close in value to the amount of protein in the starting goats milk (3.53%). In the process of storing up to the 21st day, there were no significant alterations in the values of the protein, as evident by the total amount being reduced only by 0.32%.

The data obtained also shows that the content of the total lipids after the fermentation in the kefir are lowered slightly (to 0.58%) from the starting goat milk. This is probably due to the action of the lipases produced by kefir grains, during fermentation.

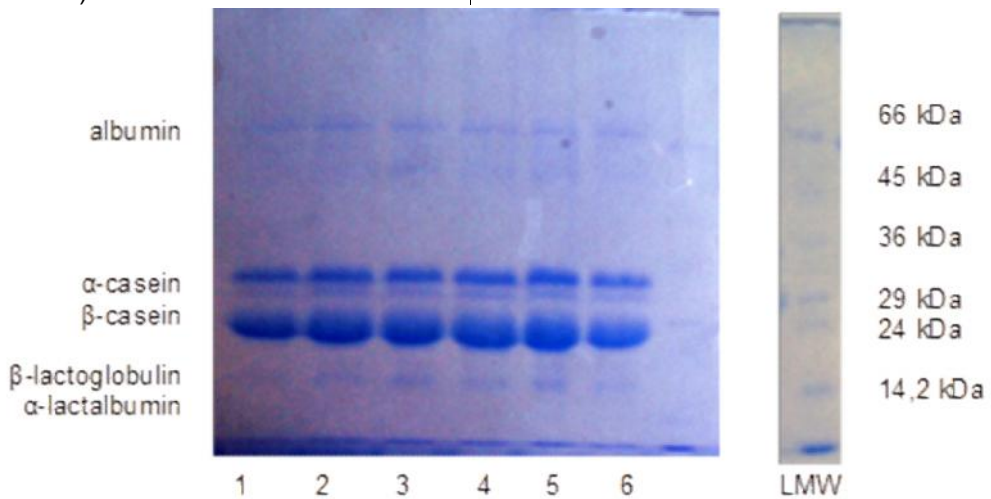
In the process of storage (to the 21st day), the amount of the total lipids

	14-			decreases, this being more expressed after 14 th day of storage and is linked to the development of molds, which are the main lipolytic agents in fermented milk. The total content of lipids during storage decreased by 0.11%.
		0,11%.		
	24	ú		The lactose is consumed by the microorganisms during fermentation and its level in the finished product after 24 hours decreased significantly (by 36%).
36%)			(
			ú	Studies during storage, however, show that the amount of lactose remains practically constant.
		2,2	(6, 8)	The active acidity of the starting milk (6, 8) decreases by 2.2 units during the fermentation process. In the next period of storage after 24 hours to the 21 st day pH values did not differ significantly. This can be explained by the fact that the population of lactic acid bacteria decreases over time, wherefore the kefir does not change its acidity.
21			24	
				Furthermore, the level of lactose remains virtually constant during storage, as much of it is absorbed by bacteria in culture. The relatively constant pH of the Kefir when stored may be due to the presence of yeast.
				In terms of titratable acidity there is a significant increase during fermentation - from 19 to 95 units, then in the course of storage to the 14 th day it holds almost constant values and increases slightly until the 21 st day.
19	95		14	
		21		
				On the basis of the chemical composition, the energy value of the output goat milk is calculated to be (71,12 kcal) and the resulting kefir (58,47 kcal). During the storage of the Kefir prior to the 21 st day a reduction is established in the value of the indicator with 15,1 kcal, which is a result of the action of microorganisms and a total reduced content of proteins, lipids and lactose.
kcal)			(71,12	
kcal).			(58,47	
	21			
			15,1 kcal,	

80%
75-
2,4%
- 2,7%
20%
(19,7%)
(28,8%),
(48,9%),
(2,6%).
(2 %
- 1, 7, 13 19
(
1).

Casein is the major protein component in the milk and makes up 75 to 80% of milk proteins. In different types of milk casein is present in different amounts. Goat's milk contains an average of 2.4% casein, bovine milk – 2.7% and sheep's milk – 5.34%. Whey proteins make up about 20% of the total protein count. They are heterogeneous, and are established in four fractions – lactoglobulin (48,9%), lactoalbumin (28.8%) of immunoglobulins (19.7%) and whey albumin (2.6%).

In the course of the experiment the electrophoretic profiles of fresh goat's milk, goat yogurt (2% lactic acid ferment) and kefir from goat's milk in different storage time were compared - 1, 7, 13 and 19 days. Milk and yogurt are used in quality control samples (Figure 1).



. 1. SDS – PAGE

1- ; 2- - 1 ; 3-
1 ; 4- - 7 ; 5 - - 14 ; 6-
- 21 ; LMW -

Fig. 1. SDS – PAGE of milk proteins
1 - fresh coat milk; 2- goat yogurt – 1 day; 3 - kefir from goat milk – 1 day ; 4 - kefir from goat milk – 7 day; 5 - kefir from goat milk – 14 day; 6 - kefir from goat milk – 21 day; LMW – protein marker

SDS - PAGE

(1) 1

(2).

25- 35 kDa,

(- -).

Greppi et al. (2008),

10 g/l - 11 g/l

-

14,2 kDa).

(

67 kDa,

87 kDa.

1 14 (3,4 5)

6 (21

)

The results of SDS - PAGE showed a similar picture of the casein and whey fractions of fresh milk (1), and yogurt after 1 day of storage (2). In all samples are recorded two major protein fractions within 25-35 kDa, corresponding to the main casein fractions (-casein and -casein).

According to varied sources, the molecular weight of the casein proteins in the goats milk is within known limits. According to Greppi, Roncada and Fortin (2008), casein fractions in goat's milk are in amounts of respectively 10 g/l for -casein and 11 g/l of -casein. In whey proteins are observed -lactoglobulin and -lactoalbumin (molecular weight of 14,2 kDa). A fraction with a molecular mass of about 67 kDa is also spotted, which probably corresponds to serum albumin and traces of protein with a molecular mass of about 87 kDa.

Samples from kefir from goat's milk after a period of 1 to 14 days (3,4 and 5) have a similar electrophoretic profile. In sample 6 (kefir after 21 days of storage) there can be noticed a decreased intensity of the bands of the whey proteins.

CONCLUSIONS

After analyzing the data obtained it was found that storing kefir for 14 days at a temperature of 4 ° C retains in the highest level its organoleptic qualities and flavor perception.

Changes in the physico-chemical composition of the efir and the biochemical parameters of the casein and whey fractions during storage match occuring proteolytic processes caused by the present microorganisms and yeast. The results of the analysis characterize the obtained kefir from goat's milk as a natural product with multitude health effects.

14

4°

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Study of the effects of different temperature regimes on basic physical and chemical parameters of fermented probiotic products from goat milk

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SUMMARY

The basic requirements for probiotics as nutritional and dietary product related primarily to the taste, texture, durability and microbial content.

Prolonged storage causes inevitable changes in the composition of the probiotic products. In the present study is detected the effect at three temperature regimes (5, 10 and 15°C) on the parameters – organoleptic assessment, active and titratable acidity, total protein and syneresis of fermented products of probiotic goat milk during storage.

The obtained experimental results define the optimal conditions for receiving probiotic products with good flavor receptivity, maximum reserved qualitative parameters and high biological value.

Keywords: probiotic products, physicochemical parameters, storage, goat milk

INTRODUCTION

The probiotics are contemporary forms of immune products and take up more significant place in the dietary and nutritional prophylaxis. The basic requirements to probiotics as a nutrient and dietary product are chiefly linked with the taste, consistency, durability and their microbial content (Champagne, 2005).

During prolonged storage, changes in the composition of the probiotic products are inevitable (López et al., 2014). That's why, in recent years the efforts of scientists and manufacturers are directed to studying the factors that could be used to positively affect the quality of the finished product during storage (Miteva et al., 2005).

The aim of the present study is based on the hypothesis that by fluctuation and controlling of technological indicators- temperature mode and duration of storage can be created optimal conditions for the receipt of probiotic products with good flavor receptivity, reserved quality indicators and high biological value.

MATERIAL AND METHODS

The study used fresh goat's milk with a pH – 6.70 and $t = 18^\circ$, pasteurized at $93^\circ - 95^\circ$ for 15 minutes and cooled to $45-46^\circ$. The optimal temperature for the fermentation process was $44 \pm 2^\circ$.

For receiving the probiotic products are used lyophilized strains *Lactobacillus bulgaricus* 1381, *Streptococcus thermophilus* 1374, *Lactobacillus casei* 1014 and *Bifidobacterium longum* 1714 provided by the collections of ICFT. Microorganisms are combined in three probiotic combinations containing classic starter for yogurt enriched with lactic acid bacteria strains in the following proportion:

Variante 1 – *L. bulgaricus*: *Str. thermophilus*, 1:3

pH – 6.70 ± 0.18 ,
 $93^\circ - 95^\circ$ 15
 $45-46^\circ$.
 $44 \pm 2^\circ$.

Lactobacillus bulgaricus 1381,
Streptococcus thermophilus 1374,
Lactobacillus casei 1014 *Bifidobacterium*
longum 1714,

1 – *L. bulgaricus*: *Str. thermophilus*, 1:3

2 – *L. bulgaricus*: *Str. thermophilus*: *L. casei*, 1:3:1

3 – *L. bulgaricus*: *Str. thermophilus*: *B. bifidum*, 1:3:1

– , ,
(9-) .
:
(1109-89);
() – ;
(°), (1111-
80); –
(6231-73);
(9373-80); –
(cm³),
100 cm³ .
MS Office Excel.

Variant 2 – *L. bulgaricus*: *Str. thermophilus*: *L. casei*, 1:3:1

Variant 3 – *L. bulgaricus*: *Str. thermophilus*: *B. bifidum*, 1:3:1

Organoleptic analysis is carried out on indicators – color, surface, texture and structure, taste and aroma (in a 9-ball Hedonic scale).

Physicochemical analysis: water content (BDS 1109-89); Active acidity (pH) – potentiometric; titratable acidity (°T), (BS 1111-80); total protein content – method of Kjeldahl method (BDS 6231-73); common ash (BDS 9373-80); syneresis of probiotic products-apply filtration method by measuring the quantity of separated liquid (cm³), which is defined in filter 100 ml milk.

Statistical analysis

The results were processed using the software MS Office Excel.

RESULTS AND DISCUSSION

The organoleptic evaluation is done by the 9 grade hedonic scale on indicators in taste, texture, color and aroma. The studied samples are kept for different periods of time (1, 7, 14 and 21 days) at temperatures of 5, 10 and 15° .

The results of the sensory analysis show that immediately after coagulation, the three options retain their original organoleptic characteristics. At a storage temperature of 5° and 10°, these indicators remain high until the 14th day.

A more substantial change occurs in the period after the second week, as they are edible until the 21st day of storage.

At a temperature of 15 ° organoleptic rate is decreasing after the 7th and around the 21st day of storage the flavor, aroma and texture of the variants are below the level of good flavor receptivity

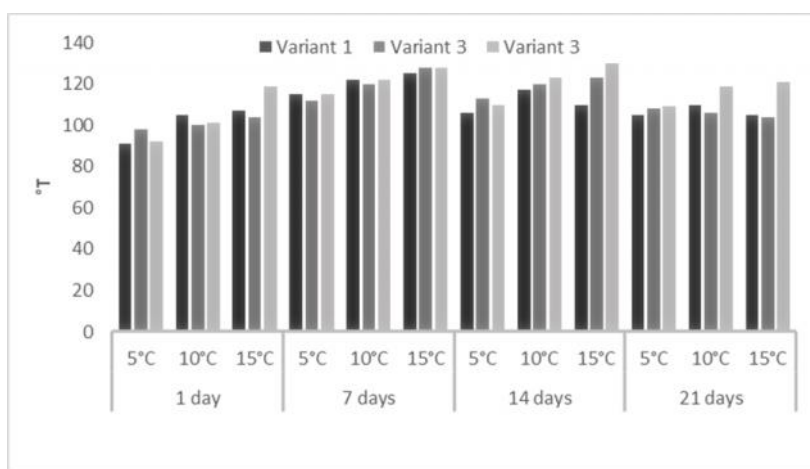
9
, ,
(1, 7, 14
21) 5, 10 15° .
,
(1).
5° 10°
14-
-
2-
21
15°
7-
21-
,
.

1.

Table 1. rganoleptic indicators of probiotic products

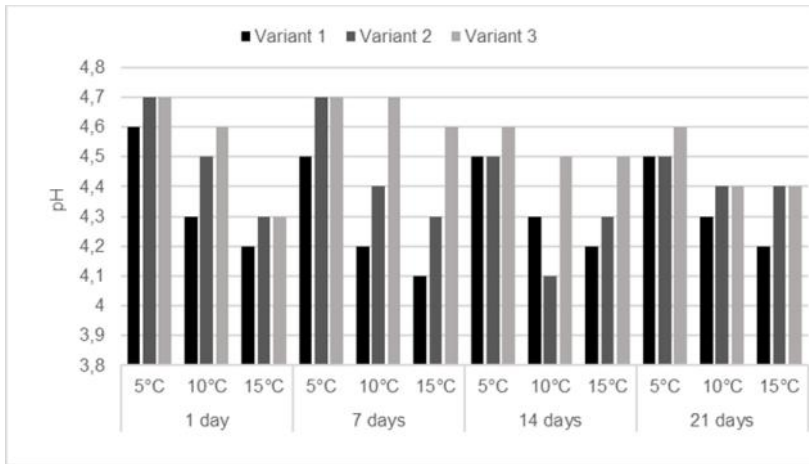
Indicators		1/Variant 1			2/Variant 2			3/Variant 3		
		5 °	10°	15 °	5 °	10°	15 °	5 °	10°	15 °
Taste	1 /day	8,20	8,00	8,40	8,40	8,20	8,20	8,80	8,80	8,80
	7 /days	7,20	7,40	7,60	7,60	7,20	6,80	8,20	8,20	8,20
	14 /days	7,00	6,20	4,80	6,80	6,00	4,60	6,80	6,60	5,20
	21 /days	6,80	5,20	3,40	6,80	5,00	2,80	6,60	6,60	3,80
Consistence	1 /day	8,60	8,40	8,40	8,60	8,80	9,00	8,60	8,60	8,60
	7 /days	7,40	7,20	4,80	7,20	7,00	5,60	7,60	7,40	6,20
	14 /days	7,20	6,40	3,40	7,00	6,20	3,40	7,00	6,80	4,00
	21 /days	5,60	4,00	2,00	5,80	4,20	2,20	5,80	5,60	2,20
Color	1 /day	8,80	9,00	9,00	9,00	9,00	9,00	9,00	9,00	9,00
	7 /days	8,60	8,60	8,60	8,80	8,60	9,00	9,00	9,00	9,00
	14 /days	7,80	7,80	7,80	8,00	8,00	8,60	8,80	8,40	8,20
	21 /days	5,80	6,80	6,60	6,80	6,80	6,60	6,60	6,40	5,80
Aroma	1 /day	8,20	8,60	7,80	8,00	8,00	8,20	8,20	8,20	7,80
	7 /days	7,60	6,40	6,20	7,80	6,60	6,20	7,80	7,80	6,80
	14 /days	6,80	4,80	4,00	7,00	5,40	3,80	7,80	7,80	3,20
	21 /days	5,60	3,60	2,20	6,00	4,00	2,00	6,80	6,40	2,00

Physicochemical studies were done for establishment of the changes in the active and titratable acidity, protein content and syneresis at temperatures of 5, 10 and 15° and storage period of 24 h to 21 days of the probiotic variants (Figure 1 and 2).



. 1.

Fig. 1. Titratable acidity of the variant samples



2.
Fig. 2. Active acidity of the variant samples

1- 7-
1 2 5°
0,4 , 1
2 - 0,3. 3
115° 5° 128°
15° , / /
- 4,6-4,7.
14-
1
2
5 10° , 15° ,
14 3 7
21-
15° , 10-

In the period from 1st to 7th day aren't any significant differences in data on total titratable acidity between the 1st and 2nd variant. At temperature of 5 ° the active acidity of the variant 1 decreases with 0,4 units, and in variant 2 – c 0,3. In variant 3 for the same reference period the total titratable acidity slightly increases: from 115 ° at 5° goes up to 128°T at 15°C and the active acidity /pH/ changes inconsiderably and remains relatively high – 4,6-4,7.

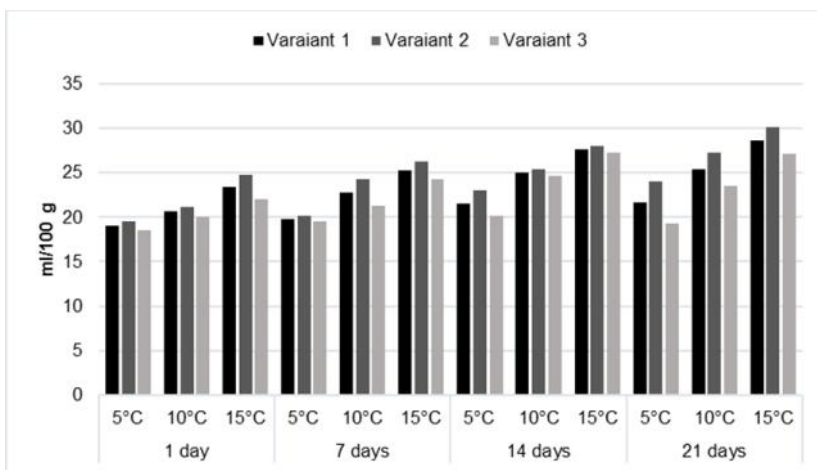
On the 14th day of storage is ascertained substantial differences between the three variants. In the first variant is observed decreasing of the acidity in approximately degree in the examined temperature regimes. In the second variant are established smaller differences in the temperatures storage of 5°C and 10°C, but at 15°C is observed reducing of the total titratable acidity and so increasing of the active acidity.

In the third variant through the period 7 to 14 days, the acidity slightly raises, but on the 21st day is stated decrease, which is expressed strongly at temperatures 10-15°C, in comparison with the previous 2 variants.

14-
15°
21- (Mortazavian et al., 2007).
(3).

Probably after the 14th day of storage enzymatic processes function more active at a temperature of 10-15° for the three options, which are the reason of acidity decrease on the 21st day (Mortazavian, A. M et al., 2007).

The temperature and period of storage, under the same conditions, have a direct impact on syneresis (Figure 3).



. 3.

Fig. 3. Syneresis of the variant samples after fermentation and during storage

24
1 19 ml 5°
23.4 ml 15° , 2 -
19.5 ml 24.8 ml 3 -
18.5 ml 22 ml.
10°
15° ,
2,
1 -
3.
14 ,
2, 1,
3.

The syneresis for 24h increases: in variant 1 of 19 ml at 5°C to 23,4 ml at 15°C, in variant 2 – from 19,5 ml to 22 ml and in variant 3 – from 18,5 to 22 ml. By increasing the storage temperature to 10°C and 15°C, the syneresis properties of the coagulum raises. The most pronounced syneresis is observed in the coagulum in the second variant, followed by the first variant and least pronounced in the third variant.

By augmentation of the storage period to 14 days, the syneresis of the coagulum raises more significantly in the second variant followed by the first variant.

21-

On the 21st day the syneresis decreases noticeably in the first two variants, which are accompanied by reducing the acidity. This confirms that as a result of the ongoing enzymatic processes, the hydration properties of the coagulum increase.

During the long-term storage of lactic acid products is obtained fermentative degradation of proteins (proteolysis) as a result of the action of the lactic acid bacteria enzymes. The hydrolysis of milk protein is implemented more intensively in the first hours after completion of the fermentation process and the first days of the storage period (Tripathi, M.K. et al., 2014).

(Tripathi et al., 2014).

The various types of lactic bacteria have different proteolytic activity. Lactic acid bacteria are characterized by a higher degree of hydrolysis milk protein (16-30% of casein) compared to the cocci (9-17%). When combining different strains of lactic acid bacteria the total proteolytic activity depends on the symbiotic relationship between them. The acquired results from the change of the protein during storage are presented in Table 2.

(16-30%
(9-17%).

2.

2.

=5°

Table 2. Protein content in variant samples after fermentation and during storage at T = 5° C

/ Time/days	/ Protein (g/100g)		
	1/Variant 1	2/Variant 2	3/Variant 3
1 /day	3,20±0,02	3,20±0,03	3,20±0,02
7 /days	2,72±0,01	2,50±0,01	2,40±0,02
14 /days	2,69±0,02	2,35±0,01	2,30±0,05
21 /days	2,66±0,04	2,30±0,02	2,24±0,04

1,

2,66 g/100g,

2 3,

In the first variant, in which is used a standard yogurt starter the established protein at the end of the storage period is 2,66/100g, which indicates more mild hydrolysis of casein. In the second and third variants, to which are added

<i>B.bifidum</i> ,	<i>L. casei</i>	-	additional lactic acid bacteria <i>L. casei</i> and <i>B.bifidum</i> , distinguished by higher proteolytic activity, protein content at the end of the storage period is lower.
(2,30 g/100g	2,24	At temperature of 50°C in all the three variants is observed accelerated proteolysis immediately after completion of the fermentation process and in the first few days of storage. This is better expressed in the second and third variant, where the amount of lactic acid bacteria exceeds the number of streptococci. After the seventh day of storage in the three options the rate of proteolysis slows down and almost suspended at the 21 st day.
g/100g).	5 ⁰	3	
		2	
		7-	
		21-	

CONCLUSIONS

(5, 10 15°)	(-	In the present study has been made assessment of the effects of three temperature regimes (5, 10 and 15°C) and storage period (24h to 28 days) on indicators – organoleptic evaluation,
24 h 28)	-	-	active and titratable acidity, total protein and syneresis during storage of fermented probiotic products from goat milk.
		-	The obtained experimental results determine the optimal conditions for derivation of probiotic products with good flavor receptivity, reserved quality indicators, and a high biological value.
		-	

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The electrophoretic patterns of turkey and buffalo meat

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SUMMARY

- Of all range quality indicators of
- meat, consumers define tenderness as
- one of the most important factors. In
- recent years, treatment with exogenous
- proteolytic enzymes are becoming a very
- popular method of meat tenderization.

- The aim of this study is to assesses the
- potential impact of the application of plant
- proteases bromelain and papain on the
- electrophoretic patterns of turkey and
- buffalo meat. Experiments are conducted
- with samples of raw turkey and buffalo
- meat at three variants concentrations of
- enzyme solution (50U/ml 100U/ml and
- 200 U/ml) and in three different times of
- treatment (24h, 48h, 72h). Electrophoresis
- in polyacrylamide gel (SDS-PAGE) is
- performed with the control samples and
- tenderized meat samples. In all enzyme
- treated samples establishes a change in
- the type and number of protein bands
- relative to controls.

- A cleavage of high molecular weight
- proteins is observed, which leads to

increase the fractions with higher electrophoretic mobility.

Key words: tenderization, electrophoretic patterns, turkey meat, buffalo meat

INTRODUCTION

Tenderness is considered to be one of the most important organoleptic characteristics of meat (Lawrie, 1991).

There are several factors that determine meat tenderness: sarcomere length; myofibril integrity and connective tissue integrity.

Actomyosin toughness is attributable to changes in myofibrillar proteins, whereas background toughness is due to the connective tissue (Chen et al. 2006).

The contribution of connective tissue to the secondary toughness of meat is dependent on the quantity, type and intermolecular cross-links of collagen. It is the main component of connective tissue and represents about 80% of it (Bertran et al., 1992; Light et al., 1985; Gelse et al., 2003).

The most common type I collagen is structurally heterotrimer triple-helical molecule consisting of two identical chains - $\alpha 1(I)$ chain and $\alpha 2(I)$ chain ($[\alpha 1(I)]_2[\alpha 2(I)]$). Due to the formation of cross-links between collagen molecules, meat of adult animals becomes harder.

Reducing the toughness of the meat in "post mortem" or by additional treatment with ensemble that changes occur in actomyosin complex and connective tissue is defined as tenderization.

One of the methods for tenderization is the application of exogenous proteases - of plant and microbial origin. Proteolytic enzymes derived from plants such as papain,

(Sunantha and Saroat, 2011).

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(Ionescu et al., 2008).

(Wada et al., 2002; Naveena et al., 2004).

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(Doneva et al., 2015).

e SDS–

(SDS-PAGE) (H t al., 2012; Rawdkuen and Benjakul, 2012).

bromelain, ficin, etc. have been widely used as meat tenderizers in most parts of the world (Sunantha and Saroat, 2011).

- Plant proteases affect on fibrillar proteins of the connective tissue – collagen and elastin and on the muscle fibers as well, while microbial proteases act primarily on muscle fibers and only limited on the connective tissue.

- It has been reported that thiol proteases such as papain and bromelain, successfully tenderizing beef meat by hydrolysis of connective tissue (Ionescu et al., 2008).

- Plant proteases also exhibit hydrolytic activity to myofibrillar proteins. (Wada et al. 2002; Naveena et al. 2004). Therefore, when tenderizing with proteolytic enzymes is necessary to establish the optimum amount of enzyme to achieve partial hydrolysis with minimal loss of protein (Doneva et al., 2015).

- A widely used method for studying the mechanism of tenderization and extent of hydrolytic digestion of meat samples is SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis (H t al., 2012; Rawdkuen and Benjakul, 2012).

- This study assesses the potential effects of the application of plant proteases bromelain and papain on the electrophoretic patterns of turkey and buffalo meat.

MATERIAL AND METHODS

Materials

Meat of turkey (*Meleagris gallopavo*) and buffalo (*Bubalus bubalis*) - breed Bulgarian Murrah.

Connective tissue of turkey and buffalo meat.

Enzymes – papain (Merck), bromelain (Merck).

Methods

Enzymatic processing of samples – the meat or connective tissue samples

(*Meleagris gallopavo*)

(*Bubalus bubalis*) –

–

(Merck),

(Merck).

- I (50 U/ml), II (100 U/ml), III
 (200 U/ml),
 , 0,9% NaCl,
 .
 pH 6,30.
 24, 48 72 4°C
 ,
 ,
 (SDS-PAGE)
 SDS-PAGE
 Laemmli (1970).
 - 6%
 10 %
 : Tris - , 8,5 0,1 %
 SDS.
 - 25 mA.
 0,1% Coomassie blue (30-40 min),
 ,
 24h.

200 kDa.

1

45 kDa.

ware treated with bromelain or papain with alternating enzyme concentration and duration of the process.

Enzyme solutions

Both enzyme solutions are with the following caseinolytic activity – I (50U/ml), II (100U/ml), III (200U/ml). The enzymes were dissolved in a solvent containing 0,9% NaCl, sodium hydrogen carbonate and citric acid. The active acidity of the enzyme solutions was pH 6,30.

The samples were treated with bromelain and papain solutions at 4°C for 24, 48 and 72 hrs. Alongside the samples, controls were assigned every full hour of treatment, in which the meat was placed inside enzyme-free marinade.

Polyacrylamide gel electrophoresis (SDS-PAGE).

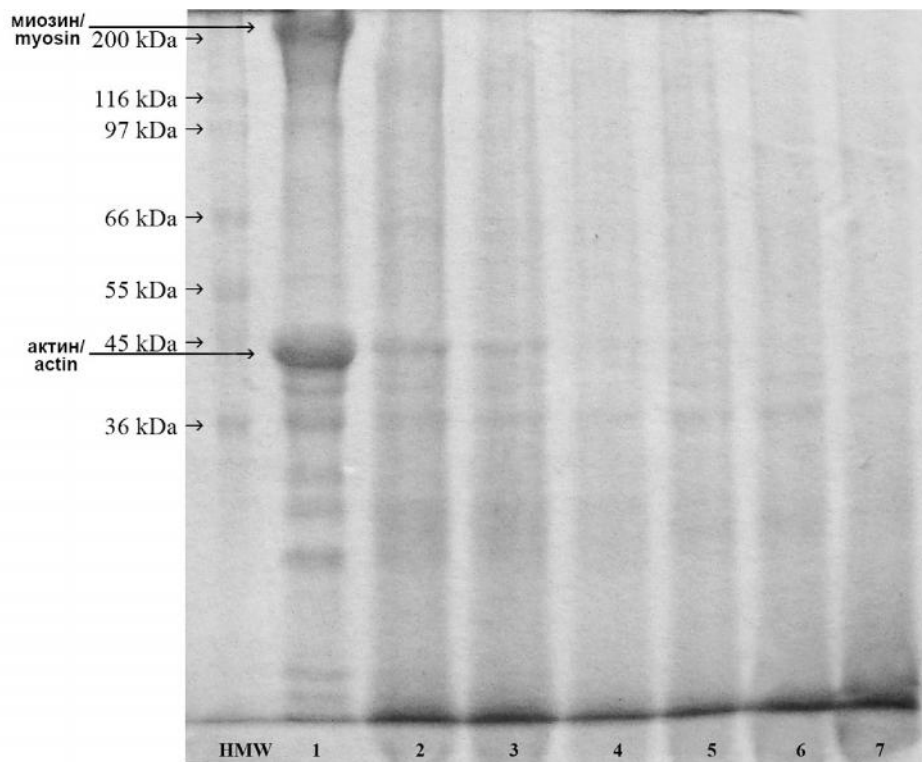
SDS-PAGE was performed with the method outlined by Laemmli (1970).

Polyacrylamide gel - 6% stacking and 10 % separating gel. Electrolyte buffer: Tris – glycine, 8,5 with 0,1 % SDS. Electrophoresis was conducted at 25 mA. The gel is then dyed with 0,1% Coomassie blue (30-40 min), whereas the gel zones lacking protein bands, was destained for 24 h.

RESULTS AND DISCUSSION

Improving meat tenderness from plant cysteine proteases is achieved by partial hydrolysis of myofibrillar proteins and disruption of muscle fibril structure. The myofibril is the main functional unit in the muscle tissue and is composed of contractile proteins - actin and myosin. Myosin is a motor protein that builds myosin filaments. Myosin heavy chain has a molecular weight of about 200 kDa. Actin is a globular protein with a molecular weight of about 45 kDa.

Figure 1 shows the result of polyacrylamide gel electrophoresis of turkey meat samples after treatment with the enzymes bromelain and papain.



1. SDS-PAGE

24 h: HMW – ;
 2 – III ; 3 – I ; 4 – II ; 5 – III ;
 6 – I ; 7 – II ; 8 – III

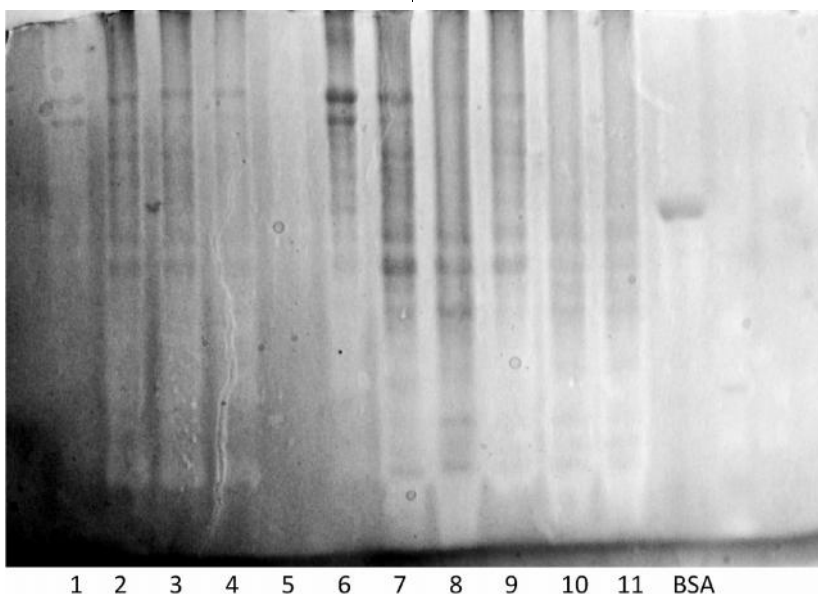
Fig. 1. SDS-PAGE of turkey meat after treatment with different concentrations of proteolytic enzymes, for 24 h: HMW – protein marker; 2 – control; 3 – variant I bromelain; 4 – variant II bromelain; 5 – variant III bromelain; 6 – variant I papain; 7 – variant II papain; 8 – variant III papain

- In the control sample, two intensive bands corresponding to the myosin and actin proteins are observed.
- At experimental variants is recorded reduction of high molecular weight proteins to a lower molecular structure, which leads to an increased number of fractions with higher electrophoretic mobility.
- In the results of the electrophoretic analysis of turkey meat differences been observed in the number of protein fractions between different variants depending both on the enzyme used and its concentration.

I – 50 U/ml).
 III – 200 U/ml,

A lower degree of hydrolysis was observed at the enzyme bromelain (variant I - 50 U/ml). At the highest concentration of both enzyme - variant III - 200 U/ml, almost complete hydrolysis of meat proteins occurs and this worsens the appearance and taste of the meat.

Collagen type I is the basic building block of connective tissue and tendons, and the main cause of the toughness of the meat. tenderizing with proteolytic enzymes purposes partial digestion of this type of tissue at the retention of muscle fibers.



2. SDS-PAGE

() 24 96 h: 1 11 -
 (1 - 24 h; 2 -
 24 h; 3 - 24 h; 4 - 24 h; 5 -
 96 h; 6 - 96 h; 7 - 96 h;
 8- 96 h; 9 - 96 h; 10 -
 96 h; 11- 96 h, BSA - -

Fig. 2. SDS-PAGE of turkey tendons samples post proteolytic enzyme treatment (bromelain or papain) for 24 and 96 h: 1 – Control 24 h; 2 – variant I bromelain 24 h; 3 – variant bromelain 24 h; 4 – variant bromelain 24 h; 5 – control 96 h; 6 – variant I bromelain 96 h; 7 – variant bromelain 96 h; 8 – variant bromelain 96 h; 9 – variant papain 96 h; 10 – variant papain 96 h; 11 – variant papain 96 h, BSA – standard – bovine serum albumin

2

- Figure 2 shows a photograph of an
- electrophoretic analysis of samples of
- turkey tendons after treatment with the
- enzymes bromelain and papain.

The control sample demonstrates
the typical visual representation of
collagen. Two high-molecule weight
fractions have been observed. They most
likely resemble the tropocollagen
molecule in collagen type I (dimers and
trimers).

- It has been proven that the
- electrophoretic mobility of the collagen
- chains is lower than that of globular
- proteins with similar molecular weights. In
- addition, collagen -2 chain has a higher
- electrophoretic mobility than collagen -1
- chain, due to a small difference (6 kDa) in
- the molecular weight between the two
- chains (Furthmayr & Timpl, 1971; Sykes
& Bailey, 1971).

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-2
-1 (Furthmayr and
Timpl, 1971),
(6 kDa)
(Sykes and Bailey,
1971).

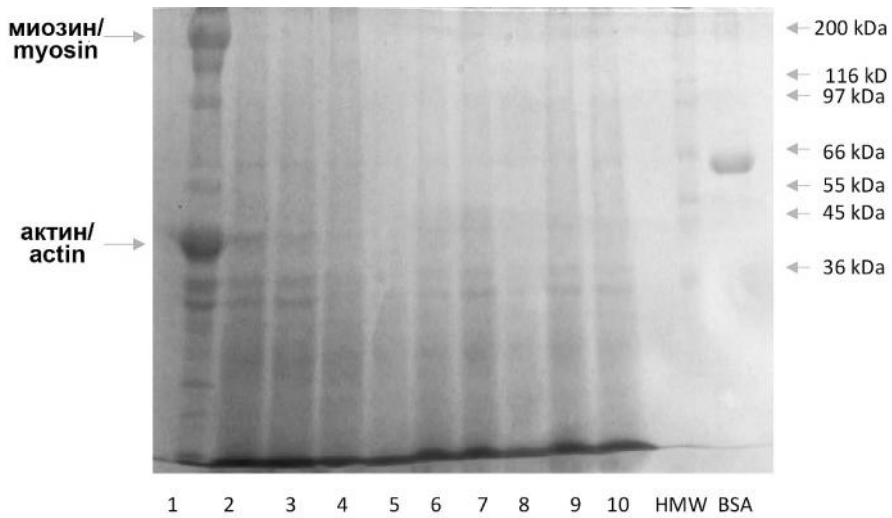
- Fractions with lower molecule
- weight are observed in the experimental
- variants. The samples treated with
- bromelain are noted to possess a smaller
- amount of fractions and a lower
- breakdown rate, whereas the papain
- samples, particularly the high-
- concentrated ones, undergo complete
- digestion of the connective tissue. Such
- intense hydrolysis leads to protein loss
- and deterioration of the organoleptic
- qualities of the turkey meat.

- Similar experiments for treatment
- with proteolytic enzymes have been
- conducted with buffalo meat. Changes in
- the toughness of buffalo meat in the
- aging and further treatment are
- determined by changes in actomyosin
- complex and connective tissue.

- The control and experimental
- samples of buffalo meat after treatment
- with an enzyme solution type marinade
- containing bromelain or papain, are
- examined by PAGE electrophoresis
(Figure 3 and 4).

PAGE

(3 4).



3. SDS-PAGE

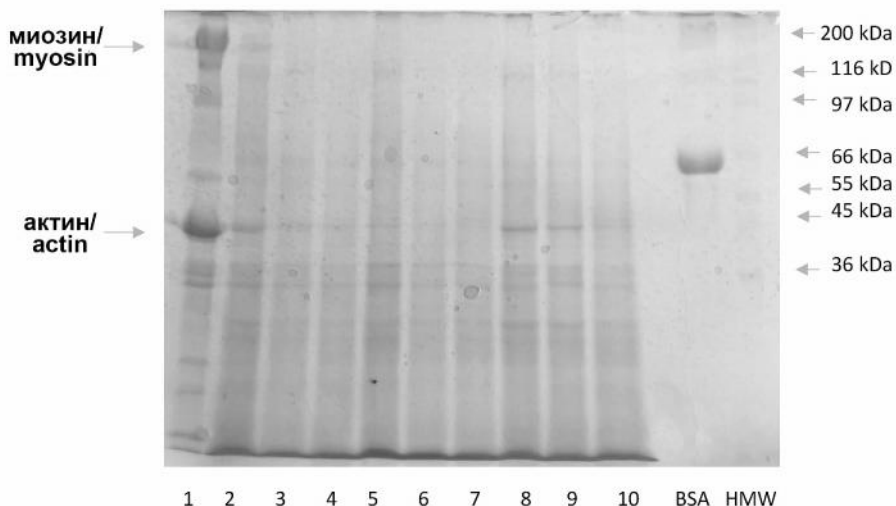
24 72 h: 1 – ; 2 –
I, 24 h; 3 – II, 24 h; 4 – III, 24 h; 5 – III, 48 h;
6 – II, 48 h; 7 – I, 48 h; 8 – III, 72 h; 9 – II, 72
h; 10 – I, 72 h; HMW – ; BSA –

Fig. 3. SDS-PAGE of buffalo meat treated with various concentrations of bromelain solution, duration of 24 to 72 h: 1 – control; 2 – variant I, 24 h; 3 – variant II, 24 h; 4 – variant III, 24 h; 5 – variant III, 48 h; 6 – variant II, 48 h; 7 – variant I, 48 h; 8 – variant III, 72 h; 9 – variant II, 72 h; 10 – variant I, 72 h; HMW – protein marker; BSA – standard – bovine serum albumin

- The rate of hydrolysis of meat proteins is contingent on several factors – enzyme type, enzyme concentration in the solution and treatment duration.

- In all variants treated with enzyme is established a change in the type and number of protein bands relative to control samples. Cleavage of high molecular weight proteins is observed. They are converted to low molecular weight, which leads to increasing the number of fractions with higher electrophoretic mobility.

- By increasing the concentration and duration of treatment is reduced intensity of bands of heavy myosin chain in the experimental variants, compared to the control. The reason is the partial breaking of myofibrillar proteins and consequently the increase of the low molecular peptides.



4. SDS-PAGE

24 72 h: 1 – ; 2 –
 I, 24 h; 3 – II, 24 h; 4– III, 24 h; 5 – I, 48 h;
 6– II, 24 h; 7 – III, 48 h; 8 – I, 72 h; 9 – II, 72
 h; 10– III, 72 h; BSA – – ; HMW –

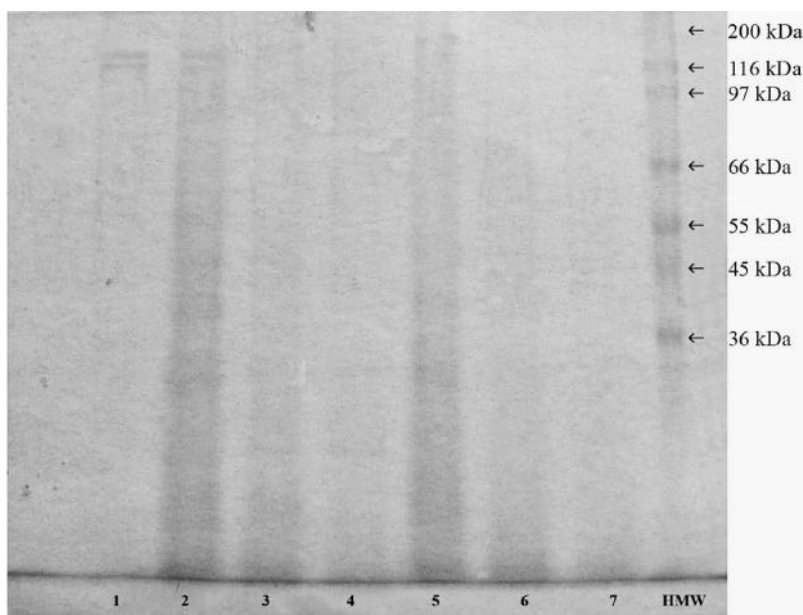
Fig. 4. SDS-PAGE of buffalo meat treated with various concentrations of papain solution, duration of 24 to 72 h: 1 – control; 2 – variant I, 24 h; 3 – variant II, 24 h; 4 – variant III, 24 h; 5 – variant I, 48 h; 6 – variant II, 24 h; 7 – variant III, 48 h; 8 – variant I, 72 h; 9 – variant II, 72 h; 10 – variant III, 72 h; BSA – standard – bovine serum albumin; HMW – protein marker

Hydrolysis of the protein fractions is more pronounced when the treatment is with the enzyme bromelain. This indicates a higher affinity for this enzyme relative to meat samples from buffalo meat compared to turkey meat. In the treatment with high concentrations of bromelain is reported pronounced activity towards myofibrillar proteins, which can lead to distortions structure of the meat and the deterioration of its sensory attributes. When processing buffalo meat with the enzyme papain it has been reported a mild hydrolysis and a higher degree preservation of organoleptic qualities of the meat after tenderization.

To establish the effects of the used enzyme solutions on the connective tissue in the meat, an experiment is performed with treatment of samples of

buffalo tendons. Collagen type I in buffalo tendons is attached to strong fibers, which are essential for the toughness of the meat. Treatment with proteolytic enzymes leads to disorderly and disintegration of the structural elements of collagen as loosens and breaks the intermolecular bonds.

Figure 5 shows a picture of polyacrylamide gel electrophoresis of samples from bovine tendon after treatment with the enzymes bromelain and papain.

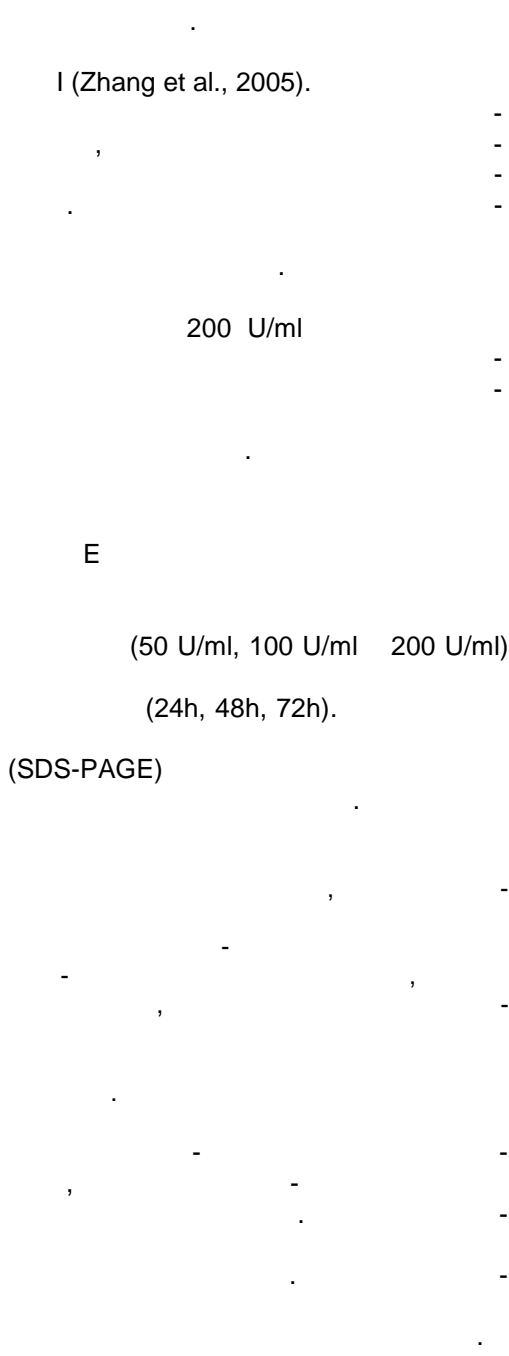


5. SDS-PAGE

1- control; 2 - variant I bromelain; 3 - variant II bromelain; 4 - variant III bromelain; 5 - variant I papain; 6 - variant II papain; 7 - variant III papain; HMW - protein marker

Fig. 5. SDS-PAGE of samples from bovine tendons after treatment with different concentrations of proteolytic enzymes duration of 96 h: 1 – control; 2 – variant I bromelain; 3 – variant II bromelain; 4 – variant III bromelain; 5 – variant I papain; 6 – variant II, papain; 7 – variant III, papain; HMW – protein marker

In the control sample is observed a weak fraction (about 200 kDa) and two bands, which correspond to the separated polypeptide chains of the triple helix of tropocollagen. This is a



characteristic electrophoretic pattern of collagen type I (Zhang et al., 2005).

- In experimental variants is found
- that both plant proteases hydrolyze
- collagen proteins. The correlation
- between the degree of hydrolysis and the
- concentration of the enzyme is observed.
- Almost complete digestion of the collagen
- chains is observed after treatment with
- enzyme concentrations of 200 U/ml and
- respectively a larger number of fractions
- are measured compared to the control
- sample.

CONCLUSIONS

Experiments are conducted with samples of raw turkey and buffalo meat at three variants concentrations of enzyme solution (50U/ml 100U/ml and 200 U/ml) and in three different times of treatment (24h, 48h, 72h). Electrophoresis in polyacrylamide gel (SDS-PAGE) is performed with the control samples and tenderized meat samples.

Electrophoretic analysis of samples of turkey meat and tendons after enzymatic hydrolysis showed that the samples treated with bromelain present a smaller number of fractions with a lesser degree of degradation, whereas papain, especially at high concentration, and long-term treatment has almost complete breaking the protein fractions.

In samples from buffalo meat papain is more suitable for tenderization because it is of lower affinity against the meat samples. Both experimented proteases hydrolyzed collagen proteins. There is correlation between the degree of hydrolysis and the concentration of the enzyme.

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Establishment of radioprotective effect of lyophilized foods in experimental animals exposed to radiation stress

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SUMMARY

The health of the human organism depends on the substances accepted by food – except nutrients and elements we take toxins, carcinogens and other harmful substances. A balanced and healthy diet enhances the body's resistance and helps to more quickly overcome the disease.

The authors present scientific results related to the development of lyophilized foods based on turkey and buffalo meat for specialized nutrition. The new food recipes include vegetable, fruit and cereal components.

The research used additional nutrients in order to enrich the knowledge for radioprotective diets.

(Miteva et al., 2008).

- problem and the gained effect depends on multitude of factors. Hence, there is a
- need for targeted experimental studies and clinical trials, consistent with the sophisticated pathogenesis of radiation damage (Miteva et al., 2008). The development of anti-radiation foods is one
- of the trends in reducing of radioactive damage.

- In radiology long ago established the basic of damage produced by external and internal radiation of radionuclides.
- There are many powerful chemical resources developed for prevention of radiodamage, as well as for decorporation of radioisotopes taken by various ways.

(Hadjiiski et al.,1993; Miteva et al., 2005).

- These agents, however, are usually highly toxic, what excludes large-scaled and repetitive application to humans. This directs our efforts to the study of biological radioprotection and especially to the special feeding as a possible factor for lessening the incorporation of radionucleotides and the extent of radiodamage (Hadjiiski et al.,1993; Miteva et al., 2005).

The purpose of this study is to establish the radioprotective effect of new freeze-dried meat-based foods by using a biological experiment conducted on test animals (mice).

MATERIAL AND METHODS

Specialized food

- We have developed prescription formulas for production of freeze-dried meat-based foods for clinical nutrition. The composition of the novel foods includes sources of building and energy ingredients as well as physiologically active substances of various origin, namely buffalo and turkey meat,
- vegetable and grain components, oligo- and polysaccharides, ascorbic acid, natural antioxidants, lecithins, etc.

The freeze-drying is carried out in a sublimation system "Hochvakuum-TG –

"Hochvakuum-TG -16.50"

$10^{-1}-10^{-2}$ mmHg.

^{137}Cs 2,25 Gy
1,78 Gy/min.

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Phoenix NCC -1211.

2. -

- 16.50" by contact heating of plates and residual pressure in the range of 10-1-10-2 (mmHg).

The radiation is applied like gamma rays with dose of 2,25 Gy from source ^{137}Cs like an external beam with over the whole bodies of the tested groups of mice. The dose power produced by the source is 1,78 Gy/min.

Biological experiment

- Series of four biological experiments are done in order to study the effects of each used specialized food, namely 2 with turkey meat and 2 with buffalo meat (respectively, with grain-vegetable and grain-fruit ingredients).

The animals from all groups are placed in same conditions with unlimited access to food and water throughout the day.

The experiments are carried out with white mice BDF males, divided into 3 groups of 5 pieces per group:

- I-st – irradiated animals; the control fed with vivarium meal (compressed yeast and water),
- II-nd – irradiated animals treated with the curative feeding (after irradiation),
- III-rd – irradiated animals treated with a prophylactic feeding (before and after irradiation).

Tested indicators:

- 1. Hematological analyses of peripheral blood: leukocytes by haematological analyzer Phoenix NCC-1211.
- 2. Survival – monitoring the weight change and the survival of tested animals.

Statistical Methods:

A mathematical model, based on the method of weighting factors, is developed for evaluation of the proposed special foods in order to choose the most appropriate of them for use in clinically ill patients after radiotherapy.

RESULTS AND DISCUSSION

1 2, - | The results of the measurements of
- | the test values are presented in Tables 1
. | and 2, expressed as a percentage relative
| to the control group in dynamics by days
| of measurement.

1.

Table 1. Weight of irradiated mice at different feeding modes

Kind of food	Feeding mode	/ Reference indicator									Min
		(%) / Weight (% in relation to the control)									
		1	2	3	4	5	6	7	8	9	
		1 date	2 date	3 date	4 date	5 date	6 date	7 date	8 date	9 date	min
		T ₁	2	3	4	5	6	7	8	9	
1 Turkey meat food with grain-vegetable ingredients	- Prophylactic	94,26	100,9	99,59	102,84						94,26
	Curative	82,62	97,42	99,43	102,43						82,62
	Control	93,13	93,71	95,46	94,69						93,13
	Proph./contr. (%)	101,21	107,67	104,33	108,61						101,21
	Cur./contr. (%)	88,71	103,96	104,16	108,17						88,71
2 Turkey meat food with grain-fruit ingredients	- Prophylactic	88,36	98,87	101,85	99,47	101,19	99,59	103,20	103,52	105,15	88,36
	Curative	97,42	87,56	97,67	104,94	102,00	102,42	104,13	106,36	103,88	87,56
	Control	92,57	96,29	94,01	95,54	95,26	93,35	94,71	94,57	97,53	92,57
	Proph./contr. (%)	95,45	102,68	108,34	104,11	106,23	106,68	108,96	109,46	107,81	95,45
	Cur./contr. (%)	105,24	90,93	103,89	109,84	107,08	109,72	109,95	112,47	106,51	94,59
3 Buffalo meat food with grain-vegetable ingredients	o Prophylactic	94,77	97,38	101,20	101,34	105,16	116,46				94,77
	- Curative	101,29	104,61	106,91	108,92	109,78	111,95				101,29
	Control	92,87	103,60	103,20	102,96	98,88	102,80				92,87
	Proph./contr. (%)	102,15	94,00	98,06	98,43	106,35	113,29				102,15
	Cur./contr. (%)	109,07	100,97	103,59	105,79	111,02	108,90				109,07
4 Buffalo meat food with grain-fruit ingredients	- Prophylactic	103,09	101,97	102,32	110,21	112,30	114,85				101,97
	Curative	108,29	107,09	114,96	113,08	123,50	120,60				107,09
	Control	92,87	103,60	103,20	102,96	98,88	102,80				92,87
	Proph./contr. (%)	111,00	98,43	99,15	107,04	113,57	111,72				109,80
	Cur./contr. (%)	116,60	103,37	111,40	109,83	124,90	117,32				115,31

2.

Table 2. Leukocytes in the blood of irradiated mice with different feeding modes

Kind of food	Feeding mode	/ Reference indicator				
		Leukocytes in the blood (% in relation to the control)				
		1	2	3	Min	
		L ₁	L ₂	L ₃	L _{min}	
		1 date	2 date	3 date		
		L ₁	L ₂	L ₃		
1	Turkey meat food with grain-vegetable ingredients	- Prophylactic	32,15	56,54		32,15
		Curative	34,96	51,17		34,96
		Control	46,22	29,65		29,65
		./ . (%) Proph./contr. (%)	69,56	190,69		108,43
		./ . (%) Cur./contr. (%)	75,64	172,58		117,91
2	Turkey meat food with grain-fruit ingredients	- Prophylactic	62,18	101,93	106,45	62,18
		Curative	62,54	103,13	123,75	62,54
		Control	62,27	102,56	104,53	62,27
		./ . (%) Proph./contr. (%)	99,86	99,39	101,84	99,86
		./ . (%) Cur./contr. (%)	100,43	94,13	118,39	100,43
3	Buffalo meat with grain-vegetable ingredients	o	58,93	84,07		58,93
		- Prophylactic	58,93	84,07		58,93
		Curative	56,43	75,90		56,43
		Control	59,70	76,61		59,70
		./ . (%) Proph./contr. (%)	98,71	109,74		98,71
./ . (%) Cur./contr. (%)	94,52	99,07		94,52		
4	Buffalo meat with grain-fruit ingredients	- Prophylactic	61,64	86,52		61,64
		Curative	69,32	69,57		69,32
		Control	64,71	67,61		64,71
		./ . (%) Proph./contr. (%)	95,26	127,97		95,26

- By the aid of this model, we
 - examine possible radioprotective effects
 - caused by the created meat-based food
 - concentrates. We simulate the feeding of
 - patients undergoing radiation therapy
 - with the feeding of test animals exposed
 - to whole body low dose gamma radiation
 - and fed with the developed special foods.

4

The input data of the mathematical model are results from the 4 biological experiments on tested animals, which are fed therapeutically and prophylactically with specialized food based on meat-grain-vegetable and meat-grain-fruit components. In the course of the experiments, we measured the changes in weight and blood leukocytes indicators.

In state of radiation stress, there are typical symptoms, namely weight loss and changes in blood count characterized by a decrease in the values of leukocytes, which worsen the living status of organism.

This is the reason to assume that the highest results, obtained from measuring of the tested indicators, are more favorable in achieving the objective. We seek the maximum of the target function that in general terms is:

$$i = f(t_1, \dots, t_n, l_1, \dots, l_n) \quad [1],$$

t_1, \dots, t_n

l_1, \dots, l_n

where X_i is the type of the used feeding mode,

t_1, \dots, t_n are the results obtained from the measurement of body weight of the test objects at different dates of measurement,

l_1, \dots, l_n are the values of leukocytes in the blood of test objects at different dates of measurement.

In order to assess the actual ongoing changes in the physiological status of organisms, we impose certain constraints over the model variables:

1.

1. We assume that the concentration of leukocytes in the blood is more important for the life status, in comparison to the other index – the weight of the mice. Therefore, this indicator will contribute twice as much weight to the final expression of the target function.

2.

2. The lowest values of the results obtained in a respective date are

50%

100.

$$X_i = \frac{1}{3} \left[\frac{Ti_{\min} + \left(\frac{Ti_1 + \dots + Ti_n}{n} \right)}{2} \right] + \frac{2}{3} \left[\frac{Li_{\min} + \left(\frac{Li_1 + \dots + Li_n}{n} \right)}{2} \right] \quad [2],$$

X_i

Ti_1, \dots, Ti_n

Li_1, \dots, Li_n

Ti_{\min} Li_{\min}

3

99,73 113,96,

8

113,96
112,33

the most life-threatening. So, these results will take part with 50% weight in the final form of the target function as much as the results from all other dates of measurement.

The values of each parameter are normalized as a percentage of the control group, fed by the usual manner. The comparison is made not only between the foods additives used in various biological experiments, but also with the control group, which result is assumed to be 100.

In so imposed parameters, the objective function yields the following final form:

where X_i they used different diet regimes.

Ti_1, \dots, Ti_n are the values obtained for the indicator weight at the appropriate diet,

Li_1, \dots, Li_n are the values obtained for the indicator leukocytes in the blood at the appropriate diet,

Ti_{\min} Li_{\min} the minimum values of the corresponding indicator.

In Table 3 are presented the results of the objective function value in descending order.

Complex assessment of comparative studies of specialized foods used ranged between 99.73 and 113.96, in 7 of 8 cases higher than the control group fed with regular food.

The highest complex assessment was obtained in the specialized feed of turkey meat on cereal-vegetable base, 113.96 in prophylactic meals and 112.33 in curative meals. As a complete assessment of the different types of

- specialized foods, according to the results, turkey meat has a slight predominance over the buffalo.
 - The added cereal-vegetable additive gives better results than the cereal-fruit base
- 3.

Table 3. Evaluation of different kind of foods and feeding modes in irradiated mice

Kind of food and Feeding mode	Rating
1. Turkey meat food with grain-vegetable ingredients accepted preventive supplement all the time	113,96
2. Turkey meat food with grain-vegetable ingredients accepted curative supplement after irradiation	112,33
3. Buffalo meat food with grain-fruit ingredients accepted curative supplement after irradiation	108,91
4. Buffalo meat food with grain-fruit ingredients accepted preventive supplement all the time	105,06
5. Turkey meat food with grain-fruit ingredients accepted curative supplement after irradiation	101,71
6. Buffalo meat food with grain-vegetable ingredients accepted preventive supplement all the time	101,68
7. Turkey meat with grain-fruit ingredients accepted preventive supplement all the time	100,24
8. Control group fed with yeast and water	100,00
9. Buffalo meat food with grain-vegetable ingredients accepted curative supplement after irradiation	99,73

CONCLUSIONS

1. - 1. The positive effects of feeding with the developed specialized meat based foods on the total life status of test animals, exposed to low dose radiation stress, is proven.
2. - 2. There are no significant differences between the groups of test animals fed with specialized prophylactic additives throughout the study period and those fed therapeutically after irradiation.
3. - 3. According to the applied mathematical model, the developed food of turkey meat with grain-vegetable components gives the best result. This food could be proposed for usage in restorative nutrition of patients undergoing radiotherapy.
4. - 4. Proven is the radioprotective effect of new meat products over test animals. The positive results gained in our study can be employed in ascertaining the regularities of the process of irradiation and the reception of food by the patients.

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Specialized meat-based foods for reconvalescent nutrition

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SUMMARY

Dietary nutrition marked a milestone in the complex treatment of patients in stationary conditions. Because undiagnosed malnutrition significant proportion of hospitalized patients are exposed to additional risk.

In this study are presented four variants meat-based foods for reconvalescent nutrition that are characterized by standard physicochemical and microbiological methods. The developed products are freeze-dried and tested in experimental animals. Based on the conducted analyzes and biological experiments most beneficial effect was observed in animals fed a concentrate of turkey meat included grain and vegetable corrigents.

The received data suggest the authors to recommend new products for reconvalescent nutrition of patients in a state of malnutrition due health, physical or mental reasons.

Key words: specialized foods,

lyophilization, reconvalescent nutrition, meat, biological experiment

INTRODUCTION

Modern consumers are becoming more informed about the strong link between diet and health status and define the food not only as a means of providing the necessary nutrients, but also as a way to prevent many diseases and maintaining a high quality of life.

In response to consumer expectations in the last decade meat producers have directed their efforts in creating a healthy and functional meat products.

The creation of specialized foods must meet a number of specific requirements: high digestibility of protein; biological value, content of minerals, vitamins and iron in absorbable form after the heat treatment and lyophilization (Doneva, 2005, Conte et al., 2011).

Targeted usage of some foods, as well as foods with special ingredients, provides a number of options to reduce damage to the body subjected to a number of stress conditions such as illness, exercise, malnutrition and the like. (Fernández et al., 2005; Mc Afee et al., 2010).

Current trend in the manufacture of meat products is their modification by adding various beneficial health components (vegetable extracts, fibers, etc.) and limit the harmful ones.

Ensuring the safety of the meat products to potential industrial applications can be achieved by the use of additives that increase the functional qualities but after a study of the interaction between them (Schönfeldt et al., 2008).

The main purpose of this study is to creation of specialized meat-based foods

(Doneva, 2005; Conte et al., 2011).

(Fernández et al., 2005; Mc Afee et al., 2010).

(Schönfeldt et al., 2008).

(Schönfeldt et

al., 2008).

- for reconvalescent nutrition of patients in a state of malnutrition due to healthful, physical or mental reasons.
-
- For establishing the most appropriate nutritional formula is carried out a biological experiment with animals.

MATERIAL AND METHODS

- Physico-chemical studies of newly created freeze-dried meat foods were made on the following indicators: residual moisture content after lyophilization (BDS 1109-89), protein content (%) – Kjeldahl method (BS 9374: 1982); fat (%) – (ISO 1444: 1996); total ash (%) – (ISO 1841-1: 1996); active acidity (pH), titratable acidity– titrimetrically (°T); determination of NaCl (%) – method Moor; determination of vitamin C (%) – method of Muri; determination of reducing sugars– method of Schoorl

- Microbiological studies – Have been conducted according BDS 6835-81
- Conducting a biological experiment– Biological experiments for evaluating the specialized meats were conducted on clinically healthy experimental white mice BDF lines weighing 20-22g. The effects were evaluated against a control set groups receiving conventional foods.

- Blood leukocyte counts were performed by a Phoenix NCC-1211 haematological analyzer. Trace is the weight loss of the test animals from the 2nd to the 16th day.

- Lyophilization – was carried out by sublimation installation company – "Hochvakuum-TG – 16.50" by contact heating of the plates and the residual pressure in the sublimator in the range of 10^{-1} - 10^{-2} mmHg.

1109-89;
 9374:1982);
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 20-22 g.
 Phoenix NCC -1211.
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 -
 - "Hochvakuum-TG – 16.50"
 10^{-1} - 10^{-2} mmHg.

RESULTS AND DISCUSSION

Food concentrates from turkey and buffalo meat with cereal, fruit, grain and vegetable ingredients have been prepared. Prescription formulas include:

variant 1 – lyophilized food with turkey meat of grain vegetable base (turkey meat, rice, cauliflower, zucchini, red bell pepper, tahini, xanthan);

variant 2 – lyophilized food with turkey meat of cereal fruit base (turkey meat, chick peas, carrots, prunes, raisins, pumpkin seed, xanthan);

variant 3 – a lyophilized food with buffalo meat vegetable-based (buffalo meat, buckwheat, sunflower seeds, zucchini, peppers, carrageenan);

variant 4 – lyophilized food with buffalo meat of cereal fruit-based (buffalo meat, prunes, raisins, carrots, linseed oil, carrageenan).

At formulating of the recipes of foods is expected unification nutritional, protective and healing properties of raw materials with proven health effect.

The new products are lyophilized and characterized by standard physiochemical indicators, the results of which are presented in Table 1.

1.

Table 1. Physico-chemical indicators of lyophilized test samples

/ Indicators	Variant 1	Variant 2	Variant 3	Variant 4
/ lipids (%)	1.68	2.07	7.44	9.56
/ Total proteins (%)	17.50	17.76	18.85	19.69
/ Total ash (%)	1.59	3.34	3.56	3.36
/ Humidity (%)	0.59	3.34	3.97	4.49
/Active acidity ()	5.82	5.87	5.77	5.55
() / Total acidity	1.3464	0.6768	1.420	1.346
NaCl (%)	0.101	0.118	0.123	0.127
/ Vit. (%)	12.020	4.840	8.862	4.074
/ Reducing sugars(%)	0.370	2.940	0.580	1.640
/100 g	409.75/1	393.38/164	417.82/174	427.53/178
Energy value/100 g product (kcal/ kJ	714.41	4.73	8.14	8.78

<p>0,59 4,49%.</p>	<p>- From the data obtained, the specialized meat food are feed concentrates with a residual moisture content of 0.59 to 4.49%. With respect to the active acidity (pH) no significant changes in the values of the test samples – they are in the range – 5.5-5.87, in the standard norms for meat.</p>
<p>()</p>	<p>- Exactly pH affects largely on enzymatic and biochemical activity in meat products, which is important for the safety of the same. The data for the salt content showed slightly higher values for raw meat – 0.323-0.635% at a rate of 0:12 to 12:27% in 100 g product, while in prepared foods they are in the standard norms – 0.12% (at a rate of 0:12 to 12:27% in 100 g product).</p>
<p>– 0.323-0.635% 0.27% 100</p>	<p>- The contents of total lipids decreases, which is due both to the imported herbal supplements in food products and the included hydrocolloids – carrageenan and xanthan. The higher content of vitamin C in the lyophilized foods is determined by the added nutrients.</p>
<p>– 0.12% (0.12-0.27%)</p>	<p>- The content of total protein is also within an acceptable range – 18-20%. Eminent positive characteristics in the qualitative composition of the new functional foods are supplemented by relatively high energy content per 100g product as the main carrier of energy is carbohydrate-protein complex.</p>
<p>18-20%.</p>	<p>- Indicators of total aerobic mesophilic aerobic and psychrophilic microorganisms contents of molds and yeasts is in a admissible norms to BDS 6835-81. The lack of pathogenic forms proves the purity and suitability for use of novel foods.</p>
<p>100g</p>	<p>- The inclusion of additives of plant origin with proven beneficial effects on human health, increases the biological value of the specialized meat-based foods. Included nuts and oils from different plants contain significant</p>
<p>6835-81.</p>	<p>-</p>
<p></p>	<p>-</p>
<p></p>	<p>-</p>
<p></p>	<p>-</p>

amounts of soluble enzymes: A, E, and vitamin C that give the food antioxidant properties.

These properties are related to the prevention of cardiovascular diseases, leading to the regulation of blood pressure, reduction in body lipids.

The conducted biological experiment is in order to study the effects of 4 food variants on the number of leukocytes in the blood and the change in body mass of the test specimens. The goal is to establish the most appropriate of them for restorative nutrition of malnourished patients.

The experiments are carried out with white mice BDF males, divided into 5 groups of 5 pieces per group:

I group – the control – mice, fed with vivarium meal (compressed yeast and water),

II group – mice, fed with functional turkey meat food with grain-vegetable ingredients.

III group – mice, fed with functional turkey meat food with grain-fruit ingredients.

IV group – mice, fed with functional buffalo meat food with grain-vegetable ingredients.

V group – mice, fed with functional buffalo meat food with grain-fruit ingredients.

The animals of the experimental groups were fed with the indicated feed for 16 days 3 g daily.

The results of measurements of body weight and leukocytes in the blood of test animals during the experiment are presented in Table 2 and 3.

Data are expressed as a percentage relative to the control group which was assumed to be 100%.

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Data are expressed as a percentage relative to the control group which was assumed to be 100%.

2.

Table 2. Dynamics of changes in body weight during the experiment

Kind of food	Relative weight change in measuring days (% in relation to the control)							
	2 day	4 day	6 day	8 day	10 day	12 day	14 day	16 day
1. Turkey meat food with grain-vegetable ingredients	104,20	105,03	107,76	103,95	109,54	110,68	113,31	111,74
2. Turkey meat food with grain-fruit ingredients	105,87	107,23	108,04	108,73	111,72	108,50	119,71	123,10
3. Buffalo meat food with grain-vegetable ingredients	86,98	88,84	91,46	89,89	92,75	93,44	92,60	103,95
4. Buffalo meat food with grain-fruit ingredients	94,08	91,51	90,63	87,58	91,87	89,92	97,23	108,41

The results obtained show that the use of turkey meat-based food has a positive effect on the change in body mass values for foods on cereal-vegetable and cereal- fruit-base.

Over the entire study period, higher values were observed compared to the control group fed in the usual way, with differences increasing over time.

During feeding with buffalo meat-based food the opposite trend is noticed - we find lower values of this indicator almost during the entire investigation period for both types of enhancers used.

Over time, differences in control group decrease and at the end of the study period in both types of buffalo meat-based food we found higher values relative to the control group.

Lower blood leukocyte levels in the comparator control group are most likely due to a deficiency of B₁₂ vitamins and some trace elements - mainly copper and zinc - as a consequence of the diet and dietary supplements used in experimental groups, which have a higher nutritional value.

3.

Table 3. Dynamics of change of leukocytes in the blood

Kind of food	Relative leukocytes in the blood change in measuring days (% in relation to the control)		
	6 day	12 day	18 day
1. Turkey meat food with grain-vegetable ingredients	114,52	117,66	129,34
2. Turkey meat food with grain-fruit ingredients	116,13	111,91	112,34
3. Buffalo meat food with grain-vegetable ingredients	118,21	121,09	132,58
4. Buffalo meat food with grain-fruit ingredients	124,72	121,20	132,35

In order to determine which food is best for healthy eating we made a comprehensive assessment the four types of food, assuming that the higher values of corresponding indicator are more favorable to the health of the studied objects. The reduced amount of leukocytes leads to a decrease in the immune system and susceptibility to various diseases. This practically means a decrease in the number of cells that fight the disease. The condition is called leucopenia, which varies depending on the age and gender of the individual.

This statement has a positive impact on the body's lifespan. Since we believe that the reduced amount of leukocytes in the blood is more life-threatening, we assume that the weighting of this indicator will be twice the weight of the weight change in the composite assessment. After these clarifications, the formula for the assessment of the types of food takes the following form:

$$X_i = \frac{1}{3} \left(\frac{T_{i1} + \dots + T_{in}}{N} \right) + \frac{2}{3} \left(\frac{L_{i1} + \dots + L_{in}}{N} \right)$$

X_i
 $T_{i1} \dots T_{in}$

$L_{i1} \dots L_{in}$

where X_i are the different kind of foods.
 $T_{i1} \dots T_{in}$ are the values obtained for the indicator weight of the respective food.

$L_{i1} \dots L_{in}$ are the values obtained for

- | | | |
|----|---|----------|
| 1. | - | - 116,43 |
| 2. | - | - 115,36 |
| 3. | - | - 113,47 |
| 4. | - | - 112,84 |

the indicator leukocytes in the blood of the respective food.

The data we use are from Table 1 and 2 above in the text. After substituting in the formula we get the following results arranged in descending order:

1. Turkey meat food with grain-vegetable ingredients - 116.43
2. Buffalo meat food with grain-fruit ingredients - 115.36
3. Buffalo meat food with grain-vegetable ingredients - 113.47
4. Turkey meat food with grain-fruit ingredients -112.84

CONCLUSIONS

1. Using a special food from turkey meat have a positive effect on body weight increase of the studied mice.
2. It has been found an increase in the values of the leukocytes in the blood of all experimental groups compared with the control group, which is a statistically higher rank of proof.
3. The highest comprehensive evaluation of the investigated indicators is the turkey meat food with grain - vegetable additives.

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Influence of season and stage of lactation on the physicochemical and fatty acid composition of ewe's milk in Karakachan breed rearing in the region of the Middle Rhodopes

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SUMMARY

The aim of this study was to trace the dynamics of the composition of meadow grass, physicochemical and microbiological indicators of ewe's milk and tracking fatty acid spectrum in various plant and animal materials.

Conducted research on the fatty acid composition of grass associations in the region of the Middle Rhodopes depending on the season shows that with increasing vegetation is changing the concentration of the main groups of fatty acids in which stepwise increases the amount of saturated by 14% and

the taste and sensory characteristics of the dairy products obtained (Pirreda et al., 2002; Cabiddu et al., 2003b; Pirisi et al., 2004).

Obtaining new data on the mountainous areas with different geological structure, altitude and botanical composition of pasture vegetation as well as the differences in the feeding regime of lactating sheep are an important condition for clarifying the transfer of fatty acids and the production of milk and dairy products enriched with various biologically active and anticarcinogenic substances (-3, -6, C18:1trans11 and CLA). The research of a number of scientists focused not only on the diet of ruminants but also on the biochemical changes in the fatty acid fractions of sheep milk as well as their importance for the human nutrition (Tsvetkova and Angelow, 2013, Mihailova and Odjakova, 2006).

The composition of ewe's milk varies widely and depends primarily on the ration, the lactation period, the season, the breed, the geographic region, etc. (Fegeros et al 1995, Kafedjiev and Mihailova, 1998). With advance of lactation, the milk fat content of sheep milk is growing significantly than cow's milk (Petrova et al., 1998, Petrova et al., 1999). In depend on the breed, its composition varies widely: lipids 6-11%, protein 4-7%, dry matter 17-21% and lactose 4-6% (Dario et al., 1995; Path, 1995; Simos Et al., 1996).

The factors mentioned above focus on the attention of researchers as well as consumers and producers, especially those related to human nutrition. Their importance is enhanced especially in identifying the quality of dairy products (Cabiddu et al., 2003a; Mihailova et al., 2004, Mihailova, 2006) and the relation between quality and the conditions of their production. In order to obtain high quality

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Milkoscan 133 , A/S Foss Electric, Denmark

COMBIFOSS-5000 BACTOSCAN-FC.

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NaHSO₄·H₂O.

(FAME)

Shimadzu-2010 (Kioto, Japan)

- of animal products (milk and dairy products) with improved technological indicators, it is necessary to know not only the diet (composition of diets) but also the physicochemical parameter of the milk.

- The aim of the present study is to investigate the dynamic of the botanical composition of meadow vegetation, the physicochemical and microbiological characteristics of w's milk and the traceability of the fatty acid spectrum in the plant associations and the raw materials of animal origin.

MATERIAL AND METHODS

- Physico-chemical and microbiological analysis of we's milk

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The bulk milk samples of 50 ewes were collected during the pasture period May-July (01.05. - 01.06. - 01.07.) and have been investigated for the main physico-chemical (protein, fat, lactose, FPD, SNF and DM) and microbiological parameters (TNM, Somatic cell, CFU). The analysis was performed using Milkoscan 133 B, A/S Foss Electric, Denmark and automatic analyzer COMBIFOSS-5000 and BACTOSCAN-FC.

Fatty acids analyses

The extraction of total lipids in plant samples was performed by Bligh & Dyer (1959) using chloroform and methanol in a ratio 1: 2 followed by methylation with acetyl chloride. The extraction of total lipids in the milk was carried out by Roese-Gottlieb (1973), by means of diethyl ether and petroleum ether and subsequent methylation with sodium methylate (CH₃ONa, Merck, Darmstadt) and dehydration with NaHSO₄·H₂O.

Fatty acid methyl esters (FAME) were analyzed using a Shimadzu-2010 gas chromatograph (Kioto, Japan) equipped with flame ionization detector and an

(AOC-2010i).
 CP7420 (100 m x 0.25 mm i.d., 0.2 m, Varian Inc., Palo Alto, CA).
 make-up - .
 80°C/min, 15 min,
 170°C 12°C/min 20
 186°C 19 4°C/min 220°C
 4°C/min .

automatic injection system (AOC-2010i).

The analysis was performed on capillary column CP7420 (100 m x 0.25 mm i.d., 0.2 m, Varian Inc., Palo Alto, CA). Hydrogen is used as carrier gas, and as make-up gas – nitrogen. Four-step furnace mode is programmed – the initial column temperature is 80°C/min, maintained for 15 minutes, then increased by 12°C/min to 170°C and maintained for 20 minutes, followed by a new 4°C/min increase to 186°C for 19 minutes and up to 220°C with 4°C/min until the process is complete.

RESULTS AND DISCUSSION

The data from the botanical composition of the natural pastures and meadows at different altitudes are presented in Table 1 and show that the grass associations are predominant in *Poaceae* family.

1.
 , %
Table 1. Botanical composition of natural meadow vegetation and pasture grass, %

Altitude, m	Plots	Poaceae	Fabaceae	Various herbaceous
1100 m / Meadow	1	73.68	5.26	21.06
	2	50.00	8.82	41.18
	3	33.77	23.38	42.85
1530 m / Grass	1	94.00	2.20	3.80
	2	86.49	2.70	10.81
	3	95.12	2.44	2.44

33.77% 73.68%.

86.49% 95.12%.

Their relative share in the meadow grass ranges from 33.77% to 73.68%. The share of *Poaceae* family in the natural pasture grass is definitely higher and ranges from 86.49% to 95.12%.

Among the predominant *Poaceae* family in the natural meadow grasses are *Bromus inermis* and *Festuca rubra*, while from the natural meadow pastures are

5.26% 23.53%
0% 2.70%

42.85% 21.06%

(2).

Poa pretensis and *Agrosis alba*.

The percentage of legumes represented by *Trifolium pratense* and *Trifolium repens*, *Lotus corniculatus* and *Vicia sativa* is comparatively small and fluctuated between 5.26% and 23.53% for meadow vegetation and 0% and 2.70% respectively for pasture grass. The share of other plant species in the grassland from natural meadows ranges from 21.06% to 42.85%.

The season and altitude affect not only the protein level but also the composition of fatty acids spectrum in the plant associations of the experimental sites (Table 2).

2.

), g/100g

Table 2. Influence of season and altitude on the fatty acid composition of pasture grass in the Middle Rhodope Mountain (area Kainadina), g/100 g fat

Group fatty acids	Pasture grass May		Pasture grass July		p	%
	x	sd	x	sd		
SFA	22,70	2,93	25,89	6,81	>0,05	114
MUFA	6,83	3,51	12,17	6,16	>0,05	178
PUFA	70,33	0,52	61,88	0,63	<0,001	88
C-18:1trans-FA	0,03	0,01	0,06	0,07	>0,05	200
C-18:1cis-FA	6,46	3,52	11,52	5,93	>0,05	178
n-3	49,78	4,30	33,83	6,27	>0,05	68
n-6	20,51	3,82	28,05	6,90	>0,05	137
n-6/ n-3	0,42	0,11	0,86	0,36	>0,05	205
Branched FA	0,20	0,06	0,08	0,02	>0,05	40

(g/100g)

The fatty acid profile (g/100g fat) of meadow grass shows that in depend on the season and with advance of vegetation, the concentration of the major fatty acid groups changes. The amount of saturated acids increased by 14% and monounsaturated fatty acids by 78%

3.

()

(01.05 - 01.07.)

Table 3. Physicochemical composition of ewe's milk (Karakachan breed) during the grazing period (01.05 - 01.07.)

		Fat %	Protein %	Lactose %	T ^o FP (-) ^o	DM %	SNF %
01.05.	x	7,86	6,65	4,67	0,57	19,85	12,44
	sd	0,01	0,02	0,05	0,05	0,02	0,02
01.06.	x	7,94	5,91	4,62	0,57	19,17	11,65
	sd	1,07	0,08	0,04	0,05	0,95	0,13
01.07.	x	7,85	6,05	4,54	0,58	19,13	11,70
	sd	0,12	0,01	0,05	0,05	0,11	0,01

10³ 7.85 10³.

(4)

7.90

In the course of lactation insignificantly decreased DM and SNF in ewe's milk. Microbiological assessment of the bulk milk samples show that the somatic cells content in raw ewe's milk (Table 4) was in the norm and a low values at 01. June and at 01. July, respectively 7.90×10^3 and 7.85×10^3 have been established.

4.

/

(01.05 - 01.07.)

Table 4. Microbiological characteristics of sheep milk / Karakachanian breed / during the grassing period (01.05. - 01.07.)

Period		Somatic cells x 10 ³	TNM x 10 ³	CFU x 10 ³
01.05.	x	356	514	200
	sd	62	188	62
01.06.	x	7,90	5,90	4,60
	sd	1,1	0,1	0,04
01.07.	x	7,85	6,10	4,50
	sd	0,1	0,01	0,1

1000 10³.300 10³200 10³

Allowable values for this indicator are in the range of 300×10^3 to 1000×10^3 . The CFU level data was within the allowable range and varied from 200×10^3 to 4.50×10^3 . The results obtained of

4.50 10³.

the microbiological studies are indicative of the extremely low level of microorganisms in the milk, which would allow its direct use in the production of dairy products such as yoghurt, cheese and yellow cheese.

500.000 somatic cell/ml (EU 92/46 EU Regulation 953/2004).

Regarding somatic cell content in ewe's milk, there are no strict requirements for cow milk. During the transit period is foreseen, with the upper limit not exceeding <500,000 somatic cell/ml (According to EU Directive 92/46 and EU Regulation 953/2004).

5. 66.95 65.54 g/100g

The fatty acid composition of ewe's milk is presented in Table 5. Saturated fatty acids range from 66.95 to 65.54 g/100g fat. The total content of saturated fatty acids in the milk varied within very narrow limits. Similar conclusions can be made for the content of monounsaturated and polyunsaturated fatty acids in the milk samples. The observed differences are statistically insignificant.

5. , g/100g

Table 5. Fatty acid composition of raw ewe's milk during the grazing period (g/100g fat)

FA-profile	May		June		July	
	x	sd	x	sd	x	sd
/ SFA	66,74	0,14	66,95	0,19	65,54	0,02
/ MUFA	25,28	0,15	25,66	0,16	26,15	0,03
/ PUFA	7,98	0,02	7,39	0,03	8,31	0,05
C-18:1trans-FA	7,55	0,10	5,74	0,11	5,75	0,06
C-18:1tr11	4,21	0,02	3,14	0,03	2,75	0,04
CLA	3,49	0,03	2,99	0,02	3,00	0,04
C-16:0/C-18:1cis9	1,41	0,01	1,33	0,01	1,35	0,01
C-16:0/C-18:1 total	0,97	0,01	1,02	0,01	1,05	0,01
n-3	1,78	0,05	1,83	0,00	2,29	0,04
n-6	3,43	0,02	3,13	0,01	3,72	0,07
n-6/ n-3	1,93	0,06	1,71	0,01	1,62	0,06
MCT (C-10>C-14)	23,28	0,01	19,84	0,18	18,02	0,02
SCT (C-4>C-8)	10,23	0,10	8,95	0,12	8,54	0,07
CLA 9c,11t	2,60	0,08	2,39	0,01	2,33	0,01

8:0, 10:0 12:0)
12.26 g/100g (6:0, 18.96 6).

The amount of some short- and medium chain fatty acids (C6:0, C8:0, C10:0 and C12:0) decreased in the course of lactation from 18.96 to 12.26 g/100g of fat (Table 6).

6.

(g/100g)

Table 6. Influence of the season on the content of saturated fatty acids in ewe's milk (g/100g fat)

SFA	May		June		July	
	x	sd	x	sd	x	sd
C-4:0	3,80	0,06	3,78	0,03	4,09	0,01
C-6:0	3,33	0,04	2,83	0,06	2,57	0,02
C-8:0	3,03	0,01	2,30	0,04	1,87	0,02
C-10:0	8,43	0,02	6,36	0,08	5,03	0,03
C-12:0	4,17	0,004	3,20	0,04	2,79	0,01
C-14:0	9,82	0,03	9,49	0,06	9,37	0,01
C-15iso	0,29	0,005	0,41	0,001	0,43	0,003
C-15aiso	0,64	0,002	0,70	0,001	0,70	0,01
C-15:0	1,13	0,005	1,29	0,01	1,47	0,005
C-16iso	0,27	0,004	0,30	0,001	0,30	0,01
C-16:0	21,35	0,07	23,18	0,02	23,73	0,04
C-17iso	0,39	0,003	0,52	0,003	0,51	0,004
C-17aiso	0,49	0,002	0,52	0,003	0,51	0,001
C-17:0	0,69	0,003	0,79	0,001	0,85	0,002
C-18:0	7,90	0,002	10,11	0,11	10,14	0,04
C-20:0	0,20	0,002	0,28	0,01	0,32	0,002
C-22:0	0,14	0,002	0,17	0,003	0,16	0,003

16:0 18:0
(-14:0)
(MUFA)

These changes are due to an increase in C4:0 content and a decrease of caproic, caprylic and capric acid content, which is statistically significant.

Myristic acid (C-14:0) in the milk tends to a slight decrease of 1-2%.

A detailed examination of the distribution of monounsaturated fatty acids (MUFA) in the milk during the different sub-periods shows that their total content increased insignificantly

25.28 26.15 g/100g | from 25.28 to 26.15 g/100g fat at the end
 . | of the period. These changes are due to
 (7). | changes in the concentrations of
 | vaccenic and oleic acids (Table 7).

7.

(g/100g)

Table 7. Influence of the season on the content of monounsaturated fatty acids in ewe's milk (g/100g fat)

MUFA	May		June		July	
	x	sd	x	sd	x	sd
C-10:1	0,28	0,002	0,23	0,004	0,23	0,001
C-14:1	0,19	0,000	0,19	0,003	0,22	0,001
C-16:1	0,99	0,003	1,03	0,005	1,14	0,02
C-18:1tr4	0,07	0,003	0,06	0,001	0,05	0,02
C-18:1tr5	0,03	0,001	0,03	0,001	0,07	0,02
C-18:1tr6/7	0,26	0,018	0,21	0,026	0,25	0,05
C-18:1tr9	0,33	0,005	0,26	0,010	0,28	0,02
C-18:1tr10	0,45	0,005	0,30	0,014	0,31	0,01
C-18:1tr11	4,21	0,025	3,14	0,028	2,75	0,04
C-18:1tr12	0,51	0,002	0,36	0,013	0,41	0,02
C-18:1tr13	0,67	0,063	0,47	0,019	0,53	0,06
C-18:1tr15	0,41	0,004	0,34	0,008	0,40	0,02
C-18:1tr16	0,67	0,038	0,59	0,005	0,70	0,01
C-18:1cis9	15,16	0,025	17,48	0,116	17,63	0,07
C-18:1cis11	0,45	0,001	0,47	0,007	0,51	0,02
C-18:1cis12	0,17	0,008	0,15	0,038	0,20	0,06
C-18:1cis13	0,21	0,030	0,17	0,007	0,24	0,02
C-18:1cis15	0,18	0,002	0,14	0,003	0,18	0,03

MUFA -
 cis- trans-
 (18:1). -
 18:1 -
 7.55 5.75 g/100g ,
 - 18:1 -
 16.17 18.76 g/100g .
 18:1cis9 -
 15.16 17.63 g/100g .
 trans- -

The MUFA profile is the richest in the spectrum of cis- and trans-isomers of the oleic acid (C18:1). The trans-isomers of C18:1 in the milk decreased from 7.55 to 5.75 g/100g fat, while the cis-isomers of C18:1 increased from 16.17 to 18.76 g/100g fat. Oleic acid C18:1cis9 increased from 15.16 to 17.63 g/100g fat. Scientifically, the trans-fatty acid content of the milk samples during the lactation period is of interest. Their concentration

10
 18:1 trans11,
 4.21 2.75 g/100g
 trans-
 18:1t11 " ",
 (PUFA) -
 7.98 g/100g
 7.39 g/100g
 8.31 g/100g
 (C-18:2cis9,12; C-18:2trans9,12)
 (0.17-0.13),
 1.40
 1.85 g/100g (8).
 (CLA)
 (8).
 3.49 3.00 g/100g
 cis9,trans11- trans9, cis11
 3 -6)

in the 10 isomers studied decreased stepwise from May to July. A particular place in this regard is the vaccenic acid (C18:1trans11), which appears as a substrate for the synthesis of conjugated linoleic acids in the mammary gland by the action of the 9-desaturase enzyme. It decreased reliably in the milk from 4.21 to 2.75 g /100g fat. All trans-isomers except C-18:1t11 are considered as "undesirable", due to the presence of a carcinogenic effect.

Polyunsaturated fatty acids (PUFA) occupy the lowest share of major fatty acid groups. Their amount in the ewe's milk ranges from 7.98 g/100g fat in May to 7.39 g/100g fat in June. At the end of the investigated period the highest values of 8.31 g/100 g fat were found.

The linoleic acid isomers (C-18: 2cis9,12, C-18: 2trans9,12) decreased during the lactation, the -linolenic acid was relatively low, over the considered period (0.17-0.13) and -linolenic acid was relatively constant and increased during the lactation period from 1.40 to 1.85 g /100g fat (Table 8).

The content of conjugated fatty acids (CLA) in the studied milk samples is influenced by the season, respectively by the quality of meadow grass (Table 8). Their amount ranges from 3.49 to 3.00 g/100g fat. In terms of nutrition, only the configurations - cis9, trans11- and trans9,cis11 - which are important as functional nutritional component for prevention of colon and stomach cancer.

Unsaturated long-chain fatty acids occupy an important place in the human nutrition in the treatment of coronary and cardiovascular diseases (omega-3 and omega-6 fatty acids).

8.

(g/100g)

Table 8. Influence of the season on the content of polyunsaturated fatty acids in ewe's milk (g/100g fat)

PUFA	May		June		July	
	x	sd	x	sd	x	sd
C-18:2tr9,12	0,78	0,096	0,61	0,003	0,72	0,014
C-18:2cis9;12	1,59	0,009	1,60	0,003	1,99	0,001
C-18:3cis6,9,12 ()	0,17	0,003	0,15	0,002	0,13	0,003
C-18:3cis9,12,15 ()	1,40	0,050	1,42	0,001	1,85	0,003
C-18:4 c6,9,12,15	0,04	0,002	0,04	0,003	0,04	0,000
CLA-Z	0,02	0,001	0,02	0,000	0,03	0,007
CLA 9c,11t/8t,10c	2,60	0,078	2,39	0,002	2,33	0,001
CLA 11c,13t	0,04	0,007	0,03	0,009	0,02	0,008
CLA 10t,12c/11c,13t	0,05	0,012	0,01	0,003	0,01	0,001
CLA-11t,13c	0,12	0,007	0,12	0,000	0,13	0,003
CLAc9c11	0,11	0,009	0,10	0,009	0,12	0,038
CLAt11t13	0,05	0,054	0,08	0,005	0,08	0,003
CLAt9t11	0,46	0,011	0,20	0,004	0,24	0,003
C-20:4n6	0,13	0,005	0,13	0,003	0,15	0,002
C-20:5n3	0,11	0,002	0,12	0,003	0,13	0,004
C-22:5n3	0,16	0,01	0,17	0,011	0,17	0,021
C-22:6n3	0,06	0,007	0,08	0,021	0,10	0,017

g/100g

1.78 2.29

3.43 3.72 g/100g

1.93 1.62,

<5

-3

Sheep milk is poor in omega-3 fatty acids. Their concentration increased from May to July from 1.78 to 2.29 g/100g fat. Similar dependencies were found in omega-6 fatty acids – from 3.43 to 3.72 g/100g fat. The ratio between two groups of fatty acids ranges from 1.93 to 1.62, indicating that the tested milk is a low risk factor for human health (raw materials and natural foods whose factor is <5 have a low risk factor).

CONCLUSIONS

The study on the fatty acid composition of the grass associations in the region of the Middle Rhodopes show that with advance of vegetation, the concentration of the major fatty acid

78% (SFA MUFA) (PUFA).
 (C18:2cis9,12) -
 CLA ()
 ()
 3, -6)
 CLA, -
)

groups changes, resulting in a stepwise increase in the amount of saturated and monounsaturated fatty acids by 14% resp. 78% (SFA and MUFA) compared to polyunsaturated (PUFA). Dynamic changes in the linoleic (C18: 2 cis9,12) and alpha-linolenic acid, which are a substrate for CLA synthesis (anticancerogenic activity) in the rumen of ruminants, have been followed. Analyzed milk had a high biological value (high content of CLA, -3, -6 and vaccenic acid). Sheep's milk from the Karakachan breed is an important raw material for the production of dairy products with an increased biological value and foods with anticancerogenic action.

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