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## Research Article

# New Functional Products with Chickpeas: Reception, Functional Properties

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## Abstract

**Objective:** The purpose of this study was to develop a technology of meat-vegetable pates based on mutton and poultry byproducts with addition of chickpeas for a functional nutrition of people who are prone to IDA or suffering from it. **Methodology:** The computer simulation helped to design formulations that meet the formalized Science-Based Recommendations (SBR), the nutritional adequacy of the virtual models of the pates was assessed. **Results:** Based on a comprehensive study of dynamics of water activity, pH and water-binding capacity, depending on the steeping mode, the technology for functional food pates has been worked out. The nutritional adequacy and the appropriate quality indicators of the meat-vegetable pates developed according to the SBR was defined. The medical and biological assessment of the meat-vegetable pates based on mutton and chicken by-products with the addition of a plant component distinguished by a balanced amino acid and fatty acid composition, a high animal protein content, macro and microelements, an optimal ratio of vitamin complex was carried out. The content of iron is 90 mg kg<sup>-1</sup>, zinc 181.3 mg kg<sup>-1</sup>, copper 17.1 mg kg<sup>-1</sup>, selenium 0.093 mg kg<sup>-1</sup>. Considering the recommended daily human consumption patterns of these trace elements, 100 g of the product developed satisfies the daily requirement of iron for 50-70%, zinc for 90%, copper 66%, selenium 15-20% that determines its functional healthy properties in the diet of patients with iron deficiency anemia. **Conclusion:** It has been found *in vivo* that the trace element composition of these products has a higher bioavailability, allowing to start the bodily compensatory reaction to the hematopoiesis dysfunction of iron deficiency character quickly.

**Key words:** Chickpeas, meat and vegetable pate, nutritional deficiency, functional products

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

A cause of many diseases, in particular of Iron Deficiency Anemia (IDA), is a disorder of the principles of balanced nutrition<sup>1-3</sup>. Functional products having health properties are of great importance for the IDA prevention. They make good the deficiency of vital substances, improve the body's physiological processes, increase resistance to disease and help to lead an active lifestyle<sup>4-13</sup>.

In recent times, particular attention has been paid to baby food and school meal, as well as to nutrition of pregnant and nursing women<sup>14-19</sup> with the problem of youth nutrition (from 18-29 years of age) to be disregarded. This age category represented mainly by pupils or students, military personnel has its physiological characteristics and feeding habits according to the age, the influence of intensive study, work and life. The formation of several physiological systems in the organisms in this period, primarily of the neurohumoral system, has not completed yet, so young people are very sensitive to disorder of balanced diet. Due to the regimen disorder and the defect in food quality, many students suffer from gastrointestinal diseases, hypertension, neurosis, anemia and others. Bad feeding habits of young people, as a rule, result from a limited cash budget. Therefore, an acceptable way of providing the youth diet with a right amount of essential proteins is to use cheap sources of raw material, such as by-products. It is significant that in a healthy young body, there is no hazard of retention of uric acid formed of nucleoproteins, which the by-products are so rich in.

One of the trends to create a greater variety and to increase the quality of meat products is the integrated use of raw materials of animal and plant origin<sup>20-22</sup>. Meat products containing functional ingredients with health properties are very important for the anemia prevention.

The purpose of this study was to develop a technology of meat-vegetable pates based on mutton and poultry by-products with addition of chickpeas for a functional nutrition of people who are prone to IDA or suffering from it.

## MATERIALS AND METHODS

The objects of the study were the pate samples following Formulations 1 and 2 on the basis of mutton and chicken by-products (Formulation 1: Liver, heart and lungs of mutton; Formulation 2: Liver, heart and gizzards of chicken) with addition of chickpea beans, onion, vegetable oil, pork fat, carrageenan, ascorbic acid, salt and spices before and after the heat treatment.

The research study examined the following qualitative characteristics of raw materials and finished products: The mass fraction of moisture, protein, lipids, ash according to conventional techniques; the energy value by calculation; to improve the nutrient composition of the products designed, their formulations were calculated using the linear programming and the computer modeling of balance and assessment of the polycomponent food quality; the amino acid score calculation was performed using the FAO/WHO scale; the amino acid composition of the experimental samples was determined by the ion exchange chromatography on an amino acid analyzer; the fatty acid composition according to Folch on a gas chromatograph; the oxidation rate-according to the thiobarbituric acid (TBA) number. The TBA number was determined using the method of Tarladgis *et al.*<sup>23</sup>, the pH-on a stationary electronic pH-meter; the macro and trace element composition-according to the generally accepted standardized techniques; the digestibility *in vitro* to the Pokrovsky-Ertanov method in Lipatov's modification; the Water Binding Capacity (WBC)-by the Grau Hamm filter paper press method in Volovinskoy-Kelman's modification; the water activity the freezing point depression method for high-moisture materials, on the AVK-6 instrument (Russia) for objects of intermediate moisture. The results obtained were processed using the methods of mathematical statistics. The tests and analyzes were replicated three times.

The evaluation of biological value and health effect of meat and vegetable pates based on mutton and chicken by-products with added chickpeas was performed on male Wistar rats weighing 200-220 g. For the experiment, four groups of animals were formed, 12 heads each. In the first (control) group the animals were healthy. The animals in the second, third and fourth groups were deliberately diseased with iron deficiency anemia by administration of the Ethylene Diamine Tetra Acetate (EDTA).

Animals of all four groups were fed a conventional vivarium diet: Twenty five gram of complete feed and 15 g of cooked pearl barley for an animal; the diet of rats in the third and fourth groups were intervened with the test samples of pates based on mutton and chicken by-products with addition of chickpeas, respectively, in an amount of 15 g/100 g of the animal body weight per day.

The experiment was conducted for 22 days. Hematological and biochemical studies of blood were conducted before administration the article and on the 2nd, 8th and 22nd days of the experiment; the general condition and the body weight of the animals were observed throughout the experiment.

Table 1: Science-based recommendations for meat-vegetable pates

Indices	Content in 100 (g) of product	Daily maintenance (%)
Protein (g)	12-18	18-20
Fat (g)	9-12	12-15
Carbohydrates (g)	20-25	6-8
Energy value (kcal)	210-250	10-12
<b>Vitamins (mg)</b>		
Ascorbic acid	17-20	25-40
Vitamin E	0.2-0.6	10-25
<b>Minerals (mg)</b>		
Iron	7.5-9	50-75
Calcium	160-200	20-30
Phosphorus	300-500	30-40

Hemoglobin, red and white blood cells were measured in whole blood on the hematology analyzer "Hema Screen 7"; platelets were determined by a unified method adopted in clinical practice. The white blood cell count-differential was determined in Romanovsky/Giemsa-stained smears. The erythrocyte sedimentation rate was determined by the Panchenkov method.

Total protein content, serum iron, calcium, phosphorus and Total Iron Binding Capacity (TIBC) in blood serum were determined with a panel "Deacon-DS" on a biochemical analyzer "StatFax 3300".

As a result of in-depth analysis and systematization of knowledge in the field of physiological norms of adult requirement in basic macro and micronutrients, with the specific character of metabolic processes in the state of iron deficiency to be taken into account, the authors gave Science-Based Recommendations (SBR) for the composition and the ratio of nutrients in the projected functional food product for young people (18-29) who are prone to IDA or suffering from it (Table 1).

The base meat ingredients of the pates (liver, heart and lungs of mutton; liver, heart and gizzards of chicken) were selected on the ground of the analysis of their nutritional value and nutrient adequacy taking into account the SBR developed. The formulations with the highest values of nutrient and food adequacy were selected from many recipe options (Table 2).

Selection of chickpeas as a vegetable component was conditioned by its high content of protein (24-30%), balanced amino acid composition, rich in macro and microelements and vitamins. Due to the issue of genetically modified raw material, high-yielding and drought-resistant chickpea is an alternative to soy and soy products.

## RESULTS AND DISCUSSION

More recently studies have considered the overall diet and combinations of foods eaten, through the analysis of

dietary patterns and practices. This includes the development and validation of dietary assessment tools to assess iron-related dietary patterns<sup>4</sup>. The protein utility and minimum score indices of the pate formulations designed confirmed their high biological value. The formulations contained significant amount of polyunsaturated fatty acids (linoleic, linolenic and arachidonic acid), the ratio w6/w3 was 10:1. The analysis of the fatty acid composition for the ratio of the SFA, MSFA and PUFA  $R_{Li} = 1...3$  sums and of the SFA, MSFA and PUFA sums with account of the balance of linoleic, linolenic and arachidonic acids  $R_{Li} = 1...6$  proved high values of the fatty acid balance coefficients.

For the functional food production, high performance technologies that ensure maximum retention of nutritional and biological value of the feedstock are required. As discussed by Gibson *et al.*<sup>15</sup> to reduce the risk of deficiency, the content and bioavailability of iron should be enhanced in diets by consumption of fortified cereals and milk, by consumption of leavened whole grains, by soaking dried legumes before cooking and discarding the soaking water and by replacing tea and coffee at meals with vitamin C-rich drinks, fruit or vegetables. The iron food fortification is considered more cost effective and economically more attractive than iron supplementation<sup>11</sup>. The flow diagram developed (see the Appendix for a complete understanding) involves operations of preparing the chickpea beans, these are 5-6 h of soaking in cold water at  $18 \pm 3^\circ\text{C}$  in a ratio of 1:6, chopping in a cutter and the preparation of protein-fat composition based on the fine cut chickpea, pork fat, structuring compositions, ascorbic acid and carrageenan. To obtain best structural and mechanical characteristics of the finished pates, it was necessary to improve the moisture content of the vegetable material, bringing its value as near toward the moisture values of the recipe components of animal origin as possible. To determine the technological parameters of the chickpea beans preparation, the indices of pH, WRC and water activity  $a_w$  of the by-product samples were investigated (Table 3).

Table 3 shows that the WRC of the by-products closely correlated with the pH: The higher the pH is, the higher the WRC. Similar results were observed by Gorlov *et al.*<sup>20</sup>. The water activity of mutton and chicken liver is lower than in other types of by-products because of a large content of the extractives.

The analysis of the data obtained showed that a sharp increase in the water activity values took place in the first 2 h of the chickpea bean soaking. With the course of soaking time, the pH went down, with the water activity to be growing. Within 3-7 h, the water activity increased moderately. After 6 h of soaking, the pH went down from 6.73-6.45, with

Table 2: Indicators of dietary adequacy and nutrient balance of the pate formulations

Ingredients and indices	Formulation 1 (Mutton by-products)	Formulation 2 (Chicken by-products)
<b>Ingredient ratio (%)</b>		
Chickpea (after soaking)	35.0	33.0
Liver	26.4	27.4
Heart	8.0	8.0
Gizzards	-	4.0
Lung	4.0	-
Sunflower-seed oil	5.4	-
Olive oil	-	6.6
Water (broth)	13.6	13.4
Onion fresh chopped	2.6	2.6
Pork fat	5.0	5.0
<b>Indices of chemical composition, mass fraction (%)</b>		
Protein	15.67	15.15
Fat	9.31	9.22
Carbohydrates	21.31	21.19
<b>Amino acid balance</b>		
Min. score, unit fraction ( $C_{min}$ )	0.959	0.764
Utility coefficient, unit fraction (s)	0.786	0.689
Comparable redundancy coefficient, in 100 g of protein (U)	9.414	12.427
<b>Fatty acid balance</b>		
Fatty acid balance coefficient, unit $l = 1...3$	0.91	0.88
Fraction ( $R_{Li}$ ) $l = 1...6$	0.69	0.76

$l = 1...3$  is balance of  $\Sigma SFA$ ,  $\Sigma MUFA$ ,  $\Sigma PUFA$ ,  $l = 1...6$  is balance of  $\Sigma SFA$ ,  $\Sigma MUFA$ ,  $\Sigma PUFA$  of linoleic, linolenic and arachidonic amino acids

Table 3: pH, WRC and  $a_w$  values in the test by-product samples

Byproduct samples	Indices					
	pH		WRC (total moisture)		$a_w$	
	M	S	M	S	M	S
<b>Mutton</b>						
Liver	6.07	0.01	92.27	0.55	0.9759	0.0019
Heart	5.36	0.05	90.64	0.75	0.9867	0.0019
Lung	6.43	0.02	85.06	1.00	0.9860	0.0033
<b>Chicken</b>						
Liver	5.93	0.03	87.36	1.93	0.9763	0.0028
Heart	5.61	0.05	93.42	1.14	0.9767	0.0049
Gizzard	5.57	0.04	89.07	1.06	0.9853	0.0031

M: Average value of the index and S: Standard Deviation

Table 4: Changes in pH and water activity  $a_w$  values depending on the chickpea soaking time of duration

Soaking time (h)	Indices			
	pH		$a_w$	
	M	S	M	S
Prior to soaking	-	-	0.4764	0.0018
1	6.73	0.03	0.9446	0.0037
2	6.58	0.04	0.9567	0.0035
3	6.52	0.02	0.9744	0.0006
6	6.45	0.02	0.9763	0.0032
9	6.37	0.03	0.9803	0.0004
12	6.30	0.02	0.9857	0.0004

the water activity to grow from 0.4764-0.9763, approaching the water activity values of the base meat components (Table 4).

During the soaking, the mass of chickpea beans was measured and the kinetics of moisture content changes was studied (Fig. 1a, b).

The graphs showed that after 3 h of soaking, the chickpea mass doubled, while their moisture content increased from 13.8-58.1%; after 5 h, the mass changes were insignificant and the moisture values reached 58.9%. Further on, the bean mass and the moisture varied slightly. So, the necessary and sufficient soaking time for the chickpea beans was 5-6 h, because at this stage, the pH and water activity  $a_w$  values were comparable to those of the meat raw material that was favorable for the best structural, mechanical and technological parameters of the pates and the mass of soaked beans reached its maximum value in this period.

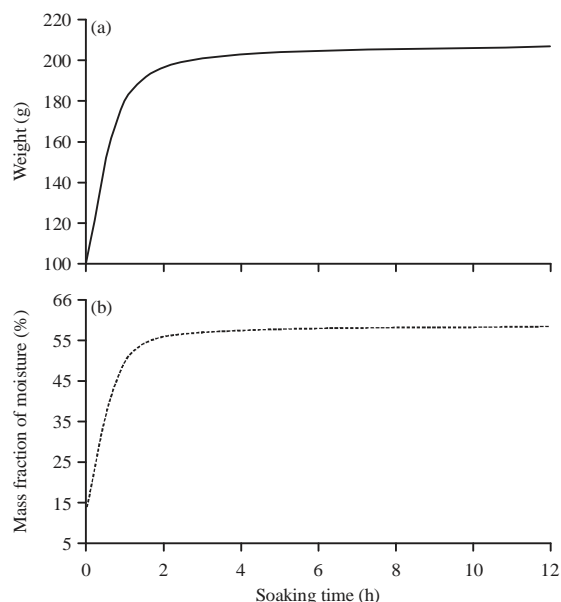


Fig. 1(a-b): (a) Change in weight of chickpea during the soaking and (b) Kinetics of moisture content change in chickpea beans during the soaking

Table 5: Chemical composition and energy value of the test pate samples

Indice	SBR	Formulation 1		Formulation 2	
		M	S	M	S
Moisture (%)	-	47.75	1.81	47.08	1.79
Protein (%)	12-18	15.50	0.03	15.36	0.03
Fat (%)	9-12	9.53	0.05	8.85	0.04
Carbohydrates (%)	20-25	23.62	0.05	23.31	0.05
Ash (%)	-	2.60	0.01	2.40	0.01
Energy value (kcal)	225-250	242.30	-	234.30	-

Table 6: Nutrient balance indices of the test samples

Indices	Formulation 1	Formulation 2
<b>Amino acid balance</b>		
Min. score, unit fraction ( $C_{min}$ )	0.90	0.73
Utility coefficient, unit fraction (s)	0.71	0.70
Comparable redundancy coefficient, in 100 g of protein (U)	8.94	11.56
<b>Fatty acid balance</b>		
$\omega_6/\omega_3$ ratio	9.14	9.37
Fatty acid balance I = 1...3	0.90	0.82
coefficient, unit fraction ( $R_{II}$ ) I = 1...6	0.70	0.74

The results of the test sample studies have shown that the chemical composition of the pates was comparable to the chemical composition designed by computer simulation and corresponded to the formalized SBR (Table 5).

The amino acid composition of protein in the test samples is shown in Fig. 2. The histogram analysis shows that the amino acid composition of protein in the test samples was comparable with the standard.

The analysis of the balance markers of the pates developed for the amino acid and fatty acid composition (Table 6) confirmed them to be up to the quality of the virtual models designed and the nutrient value to be adequate to the specific diets of young people who are prone to anemia or suffering from it. The  $\omega_6/\omega_3$  ratio of the fatty acids was close to the ratio designed and made 9.14 and 9.37.

The studies of the microelement composition of the product developed showed that the content of iron was 90 mg kg<sup>-1</sup>, zinc 181.3 mg kg<sup>-1</sup>, copper 17.1 mg kg<sup>-1</sup>, selenium 0.093 mg kg<sup>-1</sup>. Taking into account the daily maintenance of micronutrient human consumption, 100 g of the meat-vegetable pates with chickpea satisfies daily requirement of iron for 50-70%, zinc for 90%, copper 66%, selenium 15-20%. The content of iron, copper, zinc and their ratio in the product (1:0;2:2) allowed recommending the pates designed for the patients suffering from the iron deficiency anemia. The ratio of calcium and phosphorus was 1:2.5, which was close to the optimal formula of balanced nutrition.

Control of microbial contamination and general determination of the presence of pathogenic bacteria and bacterial toxins was an obligatory stage of the finished product study. Table 7 shows the results of testing of microbiological stability and hygienic safety of the pates developed after 33 days of storage at 4±2°C. The data in the tables shows the absence of microbial contamination in the pates developed, indicating the correct choice of the heat treatment modes that ensured a high quality of the product within 30 days of storage at 4±2°C.

To assess the stability of the pate properties and to determine the allowable storage period, the dynamics of the lipid oxidation was established. The study evaluated

Table 7: Results of microbiological tests of meat-containing pates during the storage

Indicator name	Norm	Analysis	
		Formulation 1	Formulation 2
Quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), (CFU 0.1 g <sup>-1</sup> )	1 × 10 <sup>3</sup>	7	ND
CGB (Escherichia coli group bacteria), in 1.0 g	-	ND	ND
Pathogenic microorganisms (including salmonella), in 25.0 g	-	ND	ND
Sulphite-reducing clostridia, in 0.1 g	-	ND	ND
<i>Staphylococcus aureus</i> , in 0.1 g	-	ND	ND

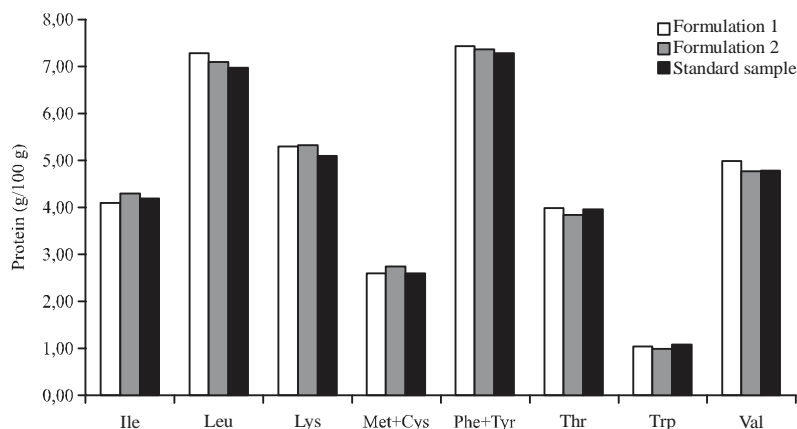


Fig. 2: Amino acid composition of protein in the test samples and the standard sample

Table 8: Change in chemical indices of the pates during the storage

Samples	Storage period (days)	Acid-degree value (mg KOH)		Peroxide value (%)		Thiobarbituric value (mg kg <sup>-1</sup> )	
		M	S	M	S	M	S
Formulation 1	0	0	-	-	-	-	-
	15	1.41	0.11	-	-	0.23	0.10
	30	1.90	0.12	0.28	0.02	0.31	0.01
Formulation 2	0	0	-	0	-	0	-
	15	1.53	0.23	-	-	0.28	0.11
	30	2.01	0.15	0.30	0.06	0.33	0.10

Table 9: *In vitro* digestibility of protein components of meat-vegetable pates for a functional nutrition, mg of tyrosine/g of protein

Sample name	Digestibility by pepsin		Digestibility trypsin		Total digestibility	
	M	S	M	S	M	S
Control sample	5.41	0.46	5.82	0.57	11.23	1.04
Mutton by-product pate with chickpea	5.20	0.48	5.91	0.58	11.15	1.06
Chicken by-product pate with chickpea	6.01	0.50	6.40	0.55	12.43	1.05

As a test sample, a beef liver pate with soybean was taken

changes in acid-degree, peroxide and thiobarbituric values during the storage (Table 8).

The analysis showed that in the initial stages of storage (15 days at  $4 \pm 2^\circ\text{C}$ ), the oxidative processes in the pates were very low, as evidenced by the peroxide value. At further storage there was a gradual increase in the oxidation rate of the lipid fraction. After 30 days of storage at  $4 \pm 2^\circ\text{C}$ , there was registered an increase in peroxides and thiobarbituric values from 0.33-0.31 mg kg<sup>-1</sup>, respectively.

The enzymatic method for determining the biological value of dietary protein *in vitro* is one of the main parameters of metabolic adequacy of the protein components of the meat-based products. So, the meat and vegetable pate samples were exposed to acidic and alkaline proteases, followed by a comparative assessment of the digestibility of the samples under study.

This study (Table 9) showed that the digestibility of the pates based on mutton and chicken by-products with addition of chickpeas was as good as the control.

Analysis of the microstructure features of the samples showed that the pate was a sufficiently dense homogeneous mass with the inclusion of fine particles of connective tissue, chickpea and by-product fragments forming a fine protein mass. This mass gave greater density and homogeneity to the pate, helping to improve the organoleptic characteristics of the finished product. In general, the content of identified particles of animal origin was sufficiently high (Fig. 3a). Apart from the components of animal origin in the composition of minced product mass, there were found numerous cellular complexes of chickpea origin (Fig. 3b).

These plant complexes were uniformly distributed in the pate mass. The farce openness was low; the number of vacuoles and pores was insignificant.

Kolosova *et al.*<sup>7</sup> found, that reducing the amount of iron in the body leads to breaking of the hemoglobin formation, reduction in the rate of synthesis, development of hypochromic anemia and trophic disorders in organs and

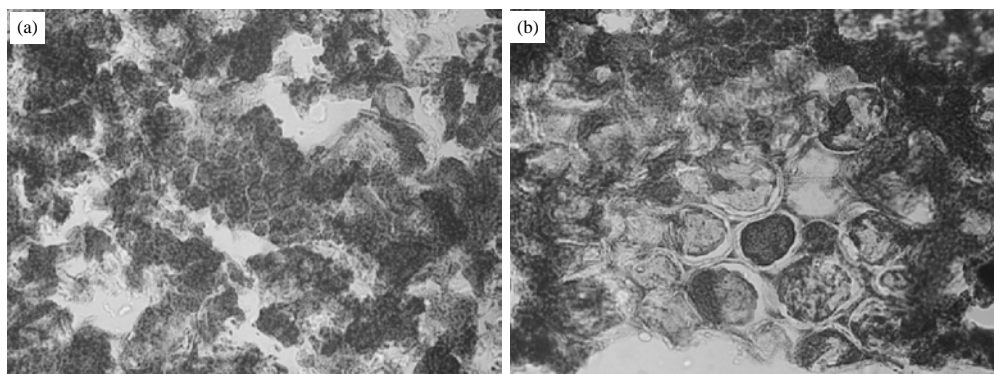


Fig.3(a-b): Microstructure features of the meat-vegetable pate, (a) Uniform distribution of the particles of animal origin and (b) Cellular complexes of vegetable origin

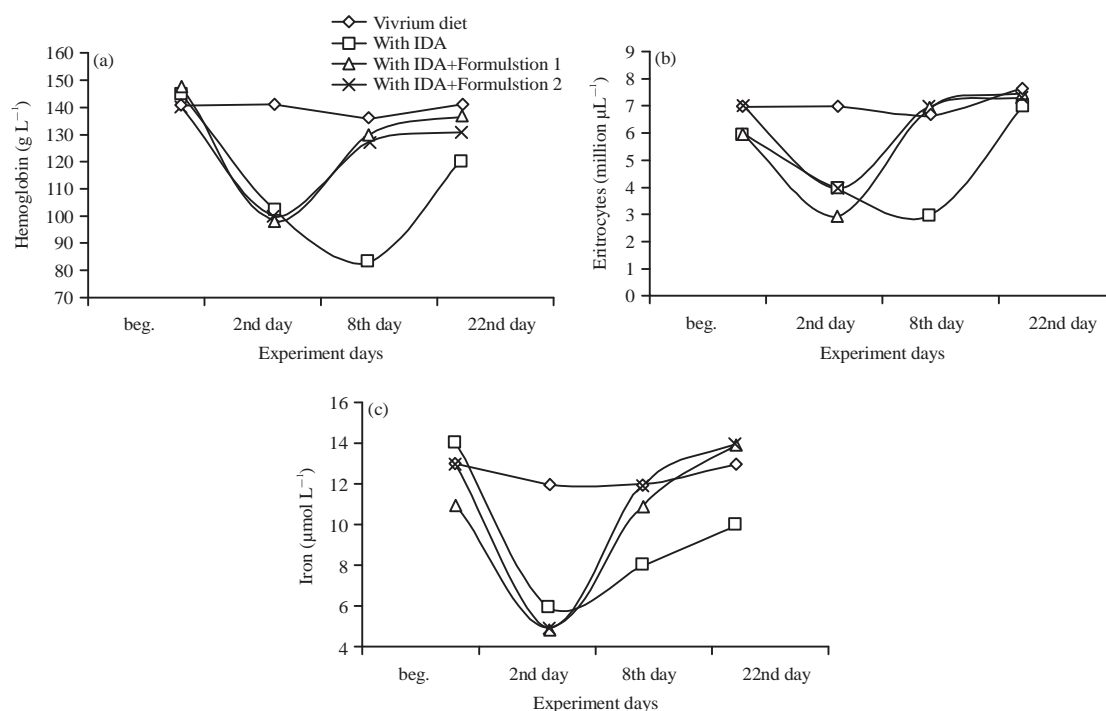


Fig. 4(a-c): Dynamics of individual hematological blood parameters of laboratory animal

tissues. In this regard, to identify the health effect of the meat-vegetable pates, the dynamics of blood indices of laboratory rats was studied (Fig. 4). Similar results were observed by Kyyaly *et al.*<sup>8</sup> So, at the end of the experiment, the hemoglobin content in blood of the rats in Groups 3 and 4 was higher than that in Group 2 by 13.9% ( $p < 0.01$ ) and 8.9% ( $p < 0.05$ ); iron content by 40.5% ( $p < 0.001$ ) and 37.9% ( $p < 0.001$ ), respectively. In comparison with Group 1 (control), the iron content in Groups 3 and 4 was higher by 10.2% ( $p < 0.05$ ) and 8.1% ( $p < 0.05$ ), respectively.

On completing of the experiment, the autopsy study of the digestive, blood circulation and hemopoietic systems, pancreas, liver, the respiratory and urinary systems of the test animals in all groups found no evidence of any pathological or inflammatory processes.

Similar results were obtained by Kobayashi *et al.*<sup>6</sup> So, Kobayashi *et al.*<sup>6</sup> found, that egg white protein was useful for recovery from IDA and one of the efficacious components was ovalbumin, while egg yolk protein delayed recovery of IDA, i.e., bioavailability of dietary iron varied depending on the source of dietary protein. Furthermore, as discussed by

Liao *et al.*<sup>22</sup> in legume seeds more than 90% of iron is stored in the form of ferritin in amyloplasts, which could be used as novel, utilizable, plant-based forms of iron for populations with a low iron status.

## CONCLUSION

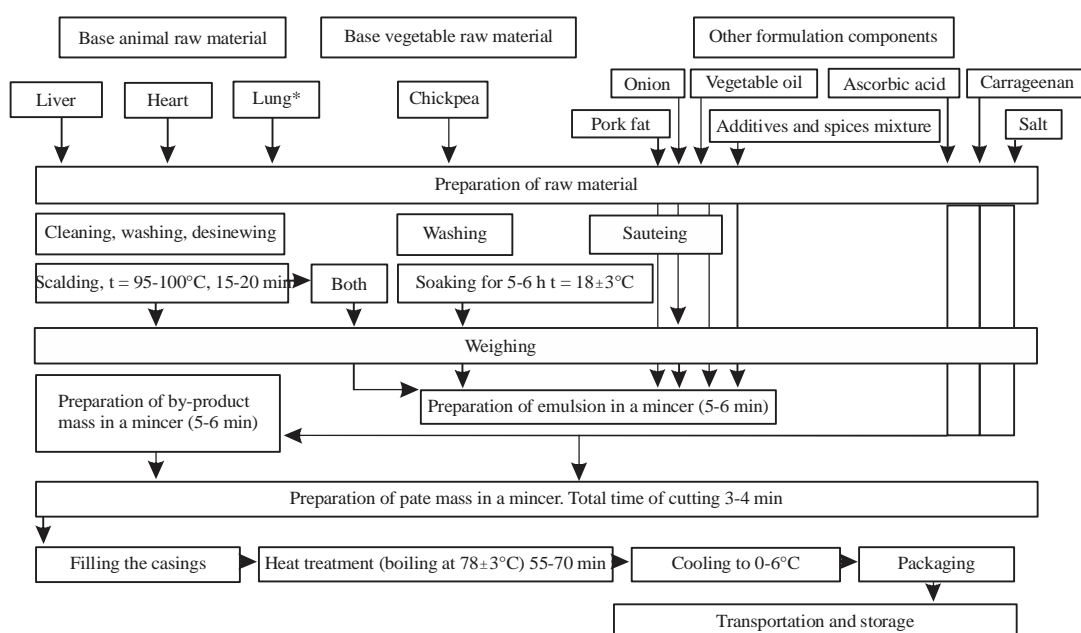
Thus, *in vivo* experiments have confirmed that the pates designed can be used for the preventive care of the iron deficiency hematopoiesis. The trace element composition of

these products has a high bioavailability, allowing starting the bodily compensatory reaction to the hematopoiesis dysfunction of iron deficiency character quickly.

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## Appendix



Process chart of meat and vegetable pates (\*: For Formulation 1, in Formulation 2: Chicken gizzards)

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