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WHEY AND ITS INHIBITION OF LIVER ENZYMES

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Abstract: Whey is lately used as functional food, and whey proteins and albumens are considered to have therapeutic influence on diseases associated with oxidative stress. Whey proteins contribute to the reduction of the level of transaminases in blood, especially of alanine transaminase. In this paper we examine some components (total proteins, albumens from whey proteins, minerals: potassium (K), iron (Fe), calcium (Ca) and phosphorus (P), from whey and their inhibition effects to transaminases (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and ɣ glutamyl transferase (ɣ -GT)). The increased level of transaminases in serum is an indicator of an illness of the liver. Additionally, several samples of whey were examined by using a photometric and spectrophotometric method. The results from examination of ALT, AST and ɣ -GT *in vitro* show that whey performed inhibition on the activity of these enzymes: ALT 10.71%, AST 8.51% and ɣ -GT 18.16% in pathological serum, and in serum with normal values, whey performed inhibition on ALT 39.33%, AST 29.08% , ɣ -GT 39.59%. The examination of the whey composition shows that the proteins represented in sufficient quantity to make reduction of enzymes and of the mineral potassium (K) is the most common. From the obtained results can be concluded that whey impacts on the reduction of transaminases and performs inhibition of enzyme activity in the *in vitro* test.

Key words: whey, transaminase, whey proteins, alanine aminotransferase, liver enzyme

Introduction

Whey in its original form is a liquid comprising less than 1% protein and 93% water. Whey is a byproduct in the production of cheese, which was previously considered as waste material. Regardless of the type of treatment and duration of the processing, the whey must be previously removed from curd by filtration or

clarification procedures and then the fat is removed with centrifugal separation. The separated milk fat can be used in the cheese manufacture, other dairy desserts and the production of butter whey (*Presilski, 2004*). Separation of whey depends on the technology of producing the main product, and the quality of used milk.

Whey contains β-lactoglobulin, α-lactoalbumin, serum albumin, lactoferrin, immunoglobulins, lactoperoxidase, glycomacropeptides, lactose and minerals. The whey protein boosts the immune system, which helps the body to produce the antioxidant glutathione (*Marshall, 2004*). Because of the wide range of essential and non-essential amino acids, minerals, fats and biologically active protein, whey is often used in the treatment of various diseases.

Whey is rich in minerals: calcium/phosphorus and potassium/sodium ratios, also contains Cl, Cu, Zn, Fe, Mn and Mo in trace which are good for cell maintenance, prevention of high blood pressure, stroke and heart diseases. The healing power of whey is still being researched, but it is known that the main problem occurs when the human body reduces its ability to regenerate organs or the slowing of life processes, which normally takes years, but with intensive use of whey in the diet in all its original forms or derivatives leads to regeneration.

Enzymes are a special class of proteins that catalyze chemical reactions in biological systems, whereas in humans, animals and plants there are many metabolic processes of decomposition and synthesis (*Dzekova, 2006*). Liver enzymes which are examining liver function are: ALT-alanine aminotransferase, AST-aspartate aminotransferase, LDH-lactate dehydrogenase, γ-GT-gamma glutamyl transferase, alkaline phosphatase ALR, iron, copper, ceruloplasmin and others. In the liver, ALT catalyzes the transfer of α-amino nitrogen of the alanine to α- ketoglutarate to form pyruvate, which is used in gluconeogenesis (*Jerry Kaneko et al., 2008*).

Hepatic analyzes are indicators of liver disease, a viral liver disease; autoimmune liver disease; Toxic liver disease; hereditary liver diseases; oncological diseases of the liver (*Karlsson et al., 2009*).

The aim of this study is to prove the impact of whey of liver enzymes in human control serum with normal and abnormal values, and to present whey inhibitory activity.

Materials and Methods

Tests are made with protein-rich whey obtained in production of mixed cheese (cow's and sheep's milk) and other various types of cheese in "Ideal Shipka" Dairy – Bitola. Applied to photometric - colorimetric and nephelometric methods for proving the components of whey, minerals Ca, K, Fe and P, total protein, albums and spectrophotometric methods spectrophotometer (Screen Master) for enzymes. The inhibitory power of the whey liver enzymes ALT, AST and γ - GT was studied in vitro with HUMATROL P (serum pathological values) and

HUMATROL N (normal serum) control serum based on animal serum which was added a certain amount of whey.

Examination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) - spectrophotometric test, as recommended by the International Federation of Clinical Chemistry (IFCC), using the kinetic method (Schumann, G., et al, 2002), as recommended by the International Federation of Clinical Chemistry (IFCC). Reagent consisted of two parts, buffer / reagent and enzyme substrate are mixed in a 1: 4 ratio. The absorbance is measured at a wavelength of 340nm, optical path 1 cm at 37 ° C are placed 50 μl serum in 500 μl working reagents baked.

Examination of γ GT- gamma glutamyl transferase-colorimetric test, method is a colorimetric kinetic method standardized against the IFCC recommended method. The principle of the reaction is followed (*Schumann et al., 2010*) at a wavelength of 400-420nm.

Examination of iron with photometric colorimetric test for iron factor in clearing fat (LCF). Iron (III) is reacted with Chromeazurol B (CAB) and Cetyltrimethilammonium bromide (CTMA) and form a colored complex at absorbance maximum of 623nm. The intensity of the color is directly proportional to the concentration of iron in the sample (*Garcic, 1979*), work carefully to avoid contamination of the reagent, oily samples cause falsely high results, and distillate water must not contain iron.

Examination of calcium with photometric method. Calcium ions reacts with o-Cresolphthalein complexion in an alkaline medium and form a violet colored complex. The 570nm absorbance of this complex is proportional to the concentration of calcium in the sample (*Gitelman, 1967*)

Examination of phosphorus-UV photometric test. Phosphorus reacts with molybdate in an acidic environment and form a complex that absorbance is directly proportional to the concentration of phosphorus (*Gamst and Try, 1980*).

Examination of potassium-nephelometric method (endpoint). Potassium ions in an alkaline environment without proteins react with sodium Tetraphenylboron (TPB-Na) to form a dispersed colloidal suspension of potassium Tetraphenylboron. The resulting turbidity is proportional to the concentration of potassium in the sample (*Terri and Sesin, 1958).*

Examination of the albumen colorimetric photometric tests, BCG-method. Bromocresol green in citrate buffer with albumen forms a colored complex. The

apsorbance of this complex is proportional to the concentration of the albumen in the sample (*Rodkey, 1965; Doumas et al, 1971*).

Examination of total proteins photometric colorimetric method (method Biuret). Copper ions from proteins and peptides in an alkaline environment form violet colored complex, the 520-580nm absorbance of this complex is proportional to the concentration of protein in the sample.

Results and Discussion

I. Determination of the composition of whey

According to literature data, whey proteins and mineral substances have enormous impact on transaminases. The content of total protein and albumen from whey proteins in various types of whey obtained from different types of cheeses is shown in Table 1.

Table 1. Contents of the total protein and albumen in whey proteins

No. samples TP/ (g/L)	**W_1**	**W_2**	**W_3**	**W_4**	**W_5**
n	10	10	10	10	10
$\bar{x}$	17.6	13.7	11	9.5	12.4
SD	3.97	2.16	1.94	1.17	1.26
CV	22.60	15.78	17.66	12.40	10.20
Alb/ (g/L)					
$\bar{x}$	2.36	1.76	1.53	1.34	1.99
SD	0.95	0.47	0.33	0.27	0.15
CV	40.40	27.07	21.79	20.57	7.65

The average of total protein content in whey is 12.8 g / L, the average concentration of the albumen is 1.79 g / L, so the highest concentration of albumens is in 1 W_1=2.36 g / L, the lowest is in W_4=1.34 g / L, equivalent to the total protein content.

Minerals, along with whey proteins, give whey a high biological value. Macro and micro elements are of particular importance for the animal organism (Table 2). The content of calcium and phosphorus are different for different types of whey and have different proportional ratio. The average calcium content is 7.31 mmol / l, and it has the highest concentration in the W_3=9.47 mmol / l, while the average phosphorus content of 8.09 mmol / l, and the highest level in the W_2=9.39 mmol / l. Whey contains a certain amount of potassium with the highest concentration in W_4=26.6 mmol / l. Whey contains a very small amount of iron, or

the average amount is 10.19 μmol / l. From the data presented in Table 2, it can be seen that the highest iron content is in the W_1=11.74 μmol / l, which is also rich in protein and albumen.

The protein and albumen from whey protein, as well as relative proportions of various minerals seen from Figure 1, the units of measurement from all parameters are converted into mg / dL.

Statistical analysis of the results made in Microsoft Office Excel by using the test ANOVA for comparison of the multiple modalities of one factor (*Ott and Longnecker, 2001*).

The results obtained for the total protein and albumin from different whey is done comparing the exactly defined values of the normal and pathological serum expressed in percentages.

The elements: Ca, P, K, Fe, total protein and albumen in whey proteins have different values, and from that variable is found the composition of whey according to the composition of milk and the applied technological process of preparation of cheese.

Table 2. Ca, P, K, Fe contents of various whey types

No. of sample Ca/ (mmol/l)	**W_1**	**W_2**	**W_3**	**W_4**	**W_5**
n	10	10	10	10	10
$\bar{x}$	7.87	5.842	9.469	6.205	7.178
SD	0.34	0.55	1.12	0.08	0.06
CV	4.36	9.50	11.83	1.44	0.951
P/ (mmol/l)					
$\bar{x}$	7.056	9.395	8.065	7.769	8.186
SD	0.30	1.92	1.31	0.09	0.07
CV	4.32	20.52	16.32	1.20	0.85
K/ (mmol/l)					
$\bar{x}$	24.042	25.34	25.975	26.6	25.691
SD	1.54	1.33	1.30	0.85	0.44
CV	6.42	5.28	5.01	3.21	1,74
Fe/ (μmol/l)					
$\bar{x}$	11.74	9.202	9.99	10.05	9.999
SD	0.89	1.54	0.24	0.94	0.32
CV	7.59	16.83	2.42	9.39	3.29

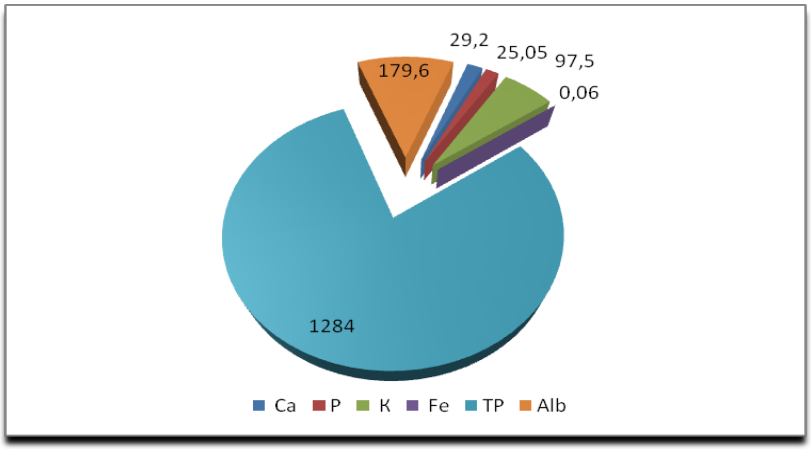

Figure 1. The proportions of surveyed substances in the whey (mg / dL)

II. *In vitro* tests for ALT, AST and ɣ-GT

In vitro test was followed by the effects of whey on the transaminase with adding whey into pathological and normal serum. Inhibition of whey on Pathological and Normal serum (HUMATROL P and N) are shown in Tables 3 and 4, where it can be concluded that whey inhibit the enzymatic activity of ALT, AST and ɣ-GT.

Table 3. Impact of whey on Pathological serum

Transaminases	**ALT/(U/L)**	**AST/(U/L)**	**γ - GT/(U/L)**
Pathological serum-1	203.5	145.181	129.991
Whey 1	181.56	143.69	102.34
Pathological serum-2	186.0	165.368	120.153
Whey 2	167.43	148.56	101.04
Pathological serum-3	179.4	151.151	118.443
Whey **3**	158.86	130.11	98.06

Table 4. Impact of whey on Normal serum

Transaminases	ALT/(U/L)	AST/(U/L)	γ - GT/(U/L)
Normal serum-1	50.4	46.7	48.3
Whey-1	31.46	39.2	29.0
Normal serum-2	48.3	45.4	45.4
Whey-2	28.42	26.1	27.6

Calculation of inhibitory activity of the whey on enzymes, the percentage of inhibition of the whey on transferases is calculated by bellow mathematical formula (*Kaiser et al., 2007*).

$$\% \text{ Inhibition} = [(\text{normal activity} - \text{inhibition}) / (\text{normal activity})] \cdot 100\ \%$$

The resulting values show that whey inhibits the pathological serum ALT 10.71%, of AST 8.51% and γ-GT 18.16%. In serum with normal values whey also inhibits ALT 39.33%, of AST 29.08% and γ-GT 39.59%, Figure 2.

Whey appears as an inhibitor of the activity of ALT, AST and γ-GT, it can be seen in Table 5, where their activity is expressed as a percentage, and notes that in both (pathological and normal serum) reduces activity around 0.2%.

Table 5. Enzyme activity (%)

Type of sample	ALT/ %	AST/ %	γ- GT/ %
P	1.89	1.53	1.22
P + W	1.69	1.41	1.0
N	0.49	0.46	0.47
N + W	0.29	0.33	0.28

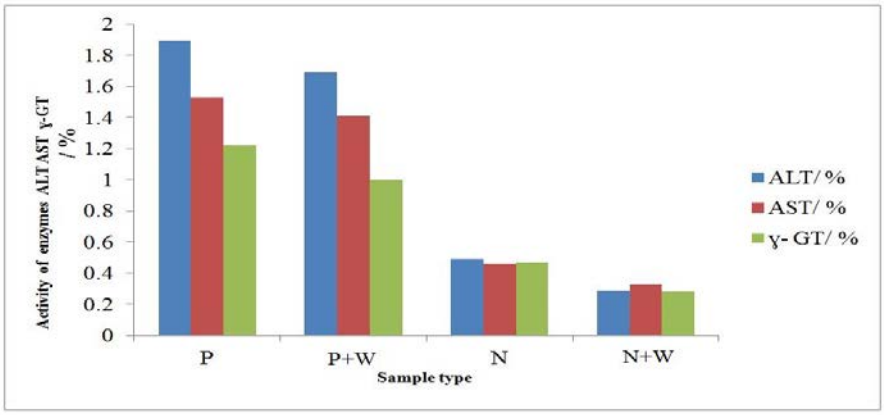

Figure 2. Chart of the enzyme activity of pathological and normal serum with and without of whey.

Most whey (92%) is obtained in the manufacture of various types of cheese. First the fat content of milk is standardized between 2.5% (40% fat cheese) and 3.5% (whole fat cheese). Starter culture provides the desired characteristics of the cheese, and rennet (or a replacement) produces gelatin of casein, including milk fat globules from the milk. This milky gel forms for about 30 minutes at 30° C and then cut into cubes. They will precipitate in curd, leaving the whey as the clear liquid lies above a sediment as precipitate i.e. supernatant (*de Wit, 2001*).

Whey consists of several proteins, including beta-lactoglobulin, alpha-lactalbumin, serum albumin (BSA) and Glukomakropeptides (GMP). Whey proteins contain all the essential amino acids in higher concentrations compared with some vegetables that are sources of protein such as soy, corn and wheat (*Walzem et al., 2002*). Leucine has been identified as the key amino acid in the metabolism of proteins (Anthony J, et al., 2001). Whey protein is not susceptible to the action of acids or enzymes, and during coagulation remains unchanged and after the removal of casein lump transferred in whey. Therefore, there's a similar amount of protein in sweet and sour whey (*Presilski, 2004*).

Whey acts as an antioxidant and detoxifies, due to its participation in the synthesis of glutathione (GSH) which is an intracellular antioxidant. Whey is rich in cysteine which is combined with glutamate and glycine to form glutathione. GSH containing thiol (sulfhydryl) group serves as an active reducing agent in preventing oxidation and tissue damage. Carried out by direct conjugation, it detoxifies endogenous and exogenous toxins, including toxic metals, petroleum distillates, lipid peroxides, bilirubin and prostaglandins. Riboflavin, niacinamid and glutathione reductase are essential cofactors in the reduction of glutathione (*Marz, 2010*). Whey lately is used as a supplement for lowering blood pressure, as

antihypertensive peptides are isolated from the primary sequence of the bovine lactoglobuline β- (*Michael et al., 2005, Tomovska et al., 2006)*.

From minerals, iron is present at least 10.19 μmol / l which in hemoglobin is as metaloenzymes, lactoperoxidaze, catalaze, and participates in the transmission of oxygen. Lactoferrin can prevent some unwanted bacteria to connect with iron, thus inhibiting their growth in the gut (*Fox, 2000*). Potassium, with the average amount of 25.5 mmol / l, is an important element for the optimal functioning of cells, tissues and organs. It regulates the activity of the heart, participate in the construction of proteins and metabolism of carbohydrates.

Hepatic analysis: ALT, AST and ɣ -GT *in vitro* show that the whey inhibits the activity of these enzymes of ALT 10.71%, AST 8,51% and ɣ -GT 18.16% in serum P, and ALT 39.33%, AST 29.08% and ɣ -GT 39.59% in serum N. By measuring the ALT, AST and ɣ -GT, it can be detected the disruption of liver cells and monitoring of the clinical progress (*Center, 2007*).

Conclusion

In the 21st century whey is still an enigma and is insufficiently used, it's interesting for researchers, production and marketing.

The use of whey in normal and pathological control serum values indicates that whey has reduction reaction of the transaminases meaning inhibit enzyme activity in *vitro test* of ALT, AST and γ -GT.

The content of total protein, albumen whey protein and minerals Fe, K, Ca and P was determined in samples of whey, and concluded that from minerals mostly present is potassium (K) then calcium (Ca), while iron (Fe) is present in a very small amounts.

Ingredients that actually impact on inhibitory activity of transaminases we can't prove, but we explored some components such as total protein, albumen of whey proteins and mineral elements (Ca, P, K, and Fe) present in whey and possible the impact of whey on enzymatic activity inhibition.

Whey proteins contribute to the reduction of the level of transaminases in blood, especially of alanine transaminases, and it's very important for an organism that increased level of transaminases in serum is as an indicator of illness of the liver.

Surutka i uticaj na inhibiciju enzima jetre

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Rezime

Surutka se u poslednje vreme koristi kao funkcionalna hrana, a smatra se da proteini i belančevine surutke imaju terapeutski uticaj na bolesti povezane sa oksidativnim stresom. Proteini surutke doprinose smanjenju nivoa transaminaza u krvi, posebno alanin transaminaze. U ovom radu razmatraju se neke komponente (ukupni proteini, belančevine iz proteina surutke, minerali: Kalijum (K), gvožđe (Fe), kalcijum (Ca) i fosfor (P) iz surutke i njihov efekat inhibicije transaminaze (alanin aminotransferaza (ALT), aspartat aminotransferaze (AST) i ɣ glutamil transferaza (ɣ -GT)). Povećan nivo transaminaza u serumu je pokazatelj bolesti jetre. Osim toga, nekoliko uzoraka surutke su ispitivani korišćenjem fotometrijske i spektrofotometrijske metoda. Rezultati ispitivanja ALT, AST i ɣ -GT *in vitro* pokazuju da surutka inhibira aktivnost ovih enzima: ALT 10,71%, AST 8,51% i ɣ -GT 18,16% u patološkom serumu, a u serumu sa normalnim vrednostima, surutka inhibira ALT 39,33%, AST 29,08%, ɣ -GT 39,59%. Ispitivanje sastava surutke pokazuje da su proteini zastupljeni u dovoljnoj količini da dovedu do smanjenja enzima i kalijuma (K) koji je najčešći. Iz dobijenih rezultata može se zaključiti da postoji uticaj surutke na smanjenje transaminaza i inhibiciju aktivnosti enzima u *in vitro* testu.

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