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NUTRITIONAL AND ENERGETIC VALUE OF HARD CHEESE

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Abstract: Insufficient intake of dairy product, especially of hard cheese, in Serbia is a nutritional problem of concern. It is caused not only by income but also with low commercial availability of the product and consumer knowledge and preferences. This study assesses nutritional and microbiological parameters of hard cheese made from pasteurized cow milk. Standard chemical analyzes were performed and cheese were analyzed on the 1st, 30th and 60th days of ripening. The following microbiological indicators were monitored: *Listeria monocytogenes, coagulase-positive Staphylococci, Escherichia coli* and *Enterobacteriaceae*. Furthermore, ripened cheeses were analyzed on amino and fatty acid profile. All cheese samples presented satisfactory microbiological and nutritional characteristics for most of the assessed parameters. Ripened cheese contained on the average 29.08% milk fat, 25.29% proteins, 0.98% lactose and pH value was 5.23. The fat content on dry matter basis (FDM) and moisture in non fat substance (MNFS) were 49.11% and 55.84 %, respectively. The energy value of cheeses amounted to 366.80 kcal /1523.22 kJ. Mean values of fatty acids content (g/100 g) showed that cheese most contained saturated fatty acids, following with monounsaturated and polyunsaturated fatty acids: 66.92%, 30.13% and 2.95%, respectively. The most common essential amino acids were leucine, lysine and isoleucine. This paper confirms that hard cheese is an important source of valuable nutrients and energy and should possess priority in human diet.

Key words: hard cheese, nutritional value, microbiological quality

Introduction

The nature of dairy products has changed dramatically in recent decades, with an increased orientation towards the production of “value added products”, some of which are segmented into the “health and wellness” market (*FAO, 2013*).

At a same time, the popularity of cheese is enhanced by its healthy and positive image (*Fox et al., 2000*). Even that, cheese consumption in Serbia is very small and amounts 2.76 kg/per capita/per year (*FAOSTAT, 2013*). Cheese is a rich source of essential nutrients; in particular, proteins, bioactive peptides, amino acids, fat, fatty acids, vitamins and minerals (*Walther et al., 2008*). The role of milk and dairy products in human nutrition has been increasingly debated in recent years. Numerous studies have indicated that cheese consumption has beneficial effects on bone and dental health and is important in osteoporosis prevention (*Pampaloni et al., 2011*; *Sahni et al., 2017*). However, there is a reasonable amount of literature indicating an association between cheese intake and the incidence of some chronic disease such as cancer and cardiovascular disease (CVD), mainly owning to high content of saturated fatty acids (*Rashidinejad et al., 2017*). The relationship between dairy foods intake and various health-related CVDs is controversal and needs further assessment and confirmation (*Bonthuis et al., 2010*; *Chen et al., 2016*; *Miraghajani et al., 2017*).

The nutritional value of food depends mainly on it having the appropriate content of compounds necessary for the proper functioning of the human body. Although a particular food may provide considerable amounts of a particular nutrient, it does not always follow that the nutrient will be available for absorption and utilization. *Pampaloni et al. (2011)* stated that extra hard Parmigiano Reggiano (P-R) cheese can be considered as a "functional food" because it is easy digested with probiotic and prebiotic effect and is recommended in all feeding age groups. Cheese ripening typically involves the progressive breakdown of casein and this process, which is essential for the development of flavor and texture also increases the digestibility of cheese protein to almost 100% (*Fox et al., 2000*). The determination of free amino acids plays an important role in assessing the nutritional quality of foods (*Casella and Contursi, 2003*). If the essential amino acid index of total milk protein is assigned a value of 100, the corresponding value for cheese protein varies from 91 to 97, depending on the variety (*Fox et al., 2000*). Milk fat is highly complex, consisting of a large number of fatty acids and other lipid molecules that have various effects on human health. One portion (50 g) of full-fat cheese provides about two-thirds of the recommended daily intake of fat (*Walther et al., 2008*). Linoleic acid (omega-6) and linolenic (omega-3) acid are called the essential fatty acids and they are what the body uses to construct a variety of substances that are important to the functioning of the cardiovascular, immune, and nervous systems. These essential fatty acids are not produced in the body and must be obtained solely from the diet (*Finnegan and Gray, 1990*). Ripened cheese do not usually contain lactose, and its content in cheese is generally less than 1 g/100 g, with a few exceptions (*FAO, 2013*).

Microorganisms play essentials role in the manufacture and ripening of cheese. Cheeses are currently considered to be one of the safest foods consumed, however, pathogenic bacteria that can be transmitted by dairy products, including cheese, are important to the dairy industry (*Little et al., 2008*). Pasteurization will eliminate *L. monocytogenes*, but cheeses made from raw milk could be contaminated by milk-borne *L. monocytogenes*. In research *Little et al*. (2008) raw or thermized milk cheeses were of unsatisfactory quality due to levels of *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes*, whereas pasteurized milk cheeses were of unsatisfactory quality due to *S. aureus* and *E. coli*. The control of spoilage yeasts and moulds has been traditionally done by chemical additives, but the application of new antifungal protective cultures is very promising, especially for the cheese industry. It has also been recently shown that naturally established cheese microflora can efficiently prevent the growth of pathogenic or spoilage microorganisms (*Grattepanche et al*., *2008*). Analysis of nutrition survey data in research *Paulin et al. (2015)* indicates that hard and semi-hard types of cheese present a lower risk for exposure to *L. monocytogenes* due to low water activity and low pH.

Quality control of dairy products is particularly important for public health and safety. The quality of cheese is influenced by its composition, especially moisture content, NaCl concentration, pH, moisture in nonfat substances (MNFS), and percentage fat in dry matter (FDM) (*Fox et al., 2000*). Considering these aspects, our study focused on investigation of chemical parameters during cheese ripening, including determination of amino and fatty acid profile of mature cheese. The other objective was to evaluate the microbiological safety of hard type cheese during ripening.

Material and methods

Cheese samples were produced from cow pasteurized milk. Samples (n=3) were analyzed on chemical composition on the 1st, 30th and 60th days of ripening. The composition of cheese was determined by standard methods. Total protein of cheese was determined by measuring total nitrogen in the cheeses using the Kjeldahl method. Dry matter was measured by drying the sample to a constant weight. Fat content was determined according to Van Gulik (IDF, 2008) and pH was measured with a pH meter, WTW, Type pH inoLab 720. To determine fatty acids (FA) in cheese samples, gas chromatograph (GC) with FID detectors was used, while for detection of amino acids (AA) in cheese was used HPLC method with fluorescence detection. Total and soluble nitrogen were determined according to Kjeldahl standard method, while the coefficient of maturity was measured by Kjeldahl / Kjeldahl-Van Slyke standard method. The energy value was determined in accordance with *Serbian Regulations (2013)*. Furthermore, the following microbiological indicators were tested and used methods for discovering and counting *Listeria monocytogenes* (SRPS EN ISO 11290-1:2009) and *coagulase-positive Staphylococci* (SRPS EN ISO 6888-1:2009). *Escherichia coli* (SRPS ISO 16649-2:2009) and *Enterobacteriaceae* (SRPS ISO 21528- 2: 2009) were investigated as hygienic parameters. Data were statistically processed using Microsoft Excel 10 and have been used methods of descriptive statistic. Results were presented as mean ± standard deviation.

Results and discussion

Chemical composition

The results of the chemical analyses of hard-type cheese during ripening are summarized in Table 1.The obtained values presented as the means of three replicates ± standard deviation. During 60 days of ripening total content of fat in dry matter (FDM) increased from 47.04 % to 49.11 % which was supported by the increased of a dry matter content. In opposite, moisture content continually decreased, from 50.32 % to 39.58 %. According to *Serbian Regulations (2014)* the mature cheese in this study was classified as a high-fat hard cheese, since its FDM and moisture content in non fat substance (MNFS) were 49.11% and 55.84 %, respectively. This was in accordance with the results obtained previously from *Popović-Vranješ et al. (2016),* while some others authors found higher values of fat, protein, FDM and pH (Livanjski hard cheese, industrially produced of cow's milk) (*Matić et al.,2014*).

Table 1. Chemical and physical composition of the hard cheese during ripening (*Mean* ± SD)

Samples	Dry matter (%)	Moisture (%)	Protein (%)	Fat (%)	Lactose (%)	FDM (%)	MNFS (%)	pH
Cheese 1stday	49.68 ± 1.73	50.32 ± 1.73	19.29 ± 0.66	23.37 ± 0.95	2.03 ± 0.08	47.04 ± 0.62	65.55 ± 1.52	5.37 ± 0.14
Cheese 30th days	56.31 ± 1.84	43.69 ± 1.84	23.69 ± 1.14	27.11 ± 0.56	1.04 ± 0.16	48.14 ± 2.50	59.94 ± 2.96	5.17 ± 0.06
Cheese 60th days	60.42 ± 0.98	39.58 ± 0.53	25.29 ± 0.56	29.08 ± 1.44	0.98 ± 0.06	49.11 ± 2.45	55.84 ± 1.80	5.23 ± 0.04

FDM - fat in dry matter; MNFS - moisture in non fat solids; SD- standard deviation

In the experiment, pH value changes little during ripening and in the second month of ripening slightly increased up to 5.23. The amino acids released by proteolysis reaction cause a slight increase in pH during cheese ripening (*Waagner-Nielsen., 1993; Fox et al., 2000*). The pH of cheese is influenced by two major factors: how much acid is formed and how much calcium phosphate, or other buffers, remain accessible in the cheese. The less calcium phosphate remains in the curd leading to less buffering in the cheese and to lower pH. The pH and acid content also play a major role in flavor perception. For example, the pH of 1- day Cheddar cheese may range from 5.3 to 4.9 (*Fox et al., 2004)*. Hard cheese contain small amount of lactose and therefore suitable for the nutrition of lactose-intolerant individuals. In the current study, estimated mean value of lactosa decreased from 2.03% to 0.98%. Contrary, *Pampaloni et al*. (2011) reported total absence of lactose in extra-hard P-R cheese.

Table 2 shows the effects of ripening on total and soluble N, coefficient of ripening and energy value. In monitored samples, coefficient of ripening increased during the cheese ripening from 8.95 to 13.73. Total N increased during first month of ripening and after that slightly decreased (3.80, 4.13 and 4.08%, respectively).The content of soluble N compounds reflect the "width" of ripening (*Fox et al., 2004*). Cheeses are different depending on the production technology and ripening conditions and they also differ from each other due to the extent of proteolysis and other changes that occur during the ripening period. Different content of soluble N in cheese (a widely-used proteolysis index) occurs because of the difference in moisture content, pH value, the ripening duration and curd drying temperature *(Popović-Vranješ et al., 2017*). Regarding to *Fosnerič,* (*1967*) the amount of soluble nitrogen compounds in hard cheese (Cheddar, Emmentaler, etc.) is up to 20-25%.

Table 2. Total and soluble N, coefficient of ripening and energy value of hard cheese during ripening (*Mean* ± SD)

Samples	Total N	Soluble N	Coefficient of ripening	Energy value kcal/KJ
Cheese 1st day	3.80 ± 0.11	0.34 ± 0.04	8.95 ± 1.12	295.61± 10.45/ 1227.13 ± 43.25
Cheese 30th days	4.13 ± 0.05	0.51 ± 0.04	12.35 ± 0.86	342.91 ± 6.26/ 1423.48 ± 31.60
Cheese 60th days	4.08 ± 0.07	0.56 ± 0.05	13.73 ± 1.06	366.80 ± 12.02/ 1523.22 ± 49.90

Hard cheese is a particularly good source of energy. The cheese 60 days old possessed an energy value of 366.80 kcal/ 1523.22 KJ. *Pampaloni et al.* (2011) reported that reduced water content of the P-R cheese (30% approximately) and the presence of as many as 70% nutrients, first of all protein and fat, caused the high energy value, equal to 388 kcal per 100 grams of product. Comparing with others cheese types, approximate energy value for Cheddar is 412 kcal/100g, Gruyere 409 kcal/100g and Cottage cheese only 98/100g kcal *(Fox et al., 2000)*.

Fatty acid profile

One of the main factors affecting cheese quality is the fatty acid profile. Based on the results, main determined FA were palmitic (C16:0) and oleic acid (C18:1) with 27.99 and 25.51 %, respectively, followed by, stearic (C18:0) and myristic (C14:0) acids, 11.76 and 9.13% respectively. Saturated fatty acid amounted 66.92%, monounsaturated 30.13 % and polyunsaturated 2.95% (Table 3.).
These results support findings from several authors. *Walther et al.* (*2008*) reported that saturated fatty acids accounted of the total fatty acid content in analyzed cheese and the most common saturated FA is palmitic acid (16:0), in second place myristic acid (14:0) and in third place stearic acid (18:0). All other saturated FA are less present. FA composition of cheese fat is roughly proportional to that of the milk used in its production so the FA composition reflects the composition of milk fat, with a ratio of saturated fatty acids and unsaturated 3:1 (*Pampaloni et al., 2011*). Cheese fat (except in mold cheese, fat does not change during ripening), has an average content of 600 g · kg−1 fat of saturated fatty acids (SFA), 235 g · kg−1 fat of monounsaturated fatty acids (MUFA) and 46 g · kg−1 fat of polyunsaturated fatty acids (PUFA) (*Walther et al., 2008*). In addition, *Fox et al.* (*2000*) recommended that 50 g of Cheddar cheese provides 17 g fat, in which approximately 66% of the fatty acids are saturated, 30% are monounsaturated, and 4% are polyunsaturated.

Table 3. Fatty acid profile of hard cheese (60 days old, g/100g)

Parameter	Butyric C4:0	Capric C8:0	Caprinic C10:0	Lauric C12:0	Myristic C14:0	Palmitic C16:0	Stearic C18:0	Oleic C18:1	Linolec C18:2	Linolenic C18:3	Arachidic C20:0
Mean ± SD	0.94 ± 0.10	1.16 ±0.19	2.96 ± 0.63	2.48 ± 0.18	9.13 ± 0.33	27.99 ±0.35	11.76 ±0.07	25.51 ±0.38	1.80 ± 0.39	0.70 ± 0.39	0.25 ± 0.11
Fatty acid content expressed as % in weight of total fatty acids: SSCFA 2.48 % * SFA 66.92% ; MUFA 30.13 %; PUFA 2.95 % SMCFA 17.20 % SLCFA 47.24 %											

SSCFA- saturated short-chain fatty acids (C4:0, C8:0)
SMCFA-saturated medium-chain fatty acids (C10:0, C12:0, C14:0)
SLCFA-saturated long-chain fatty acids (C16:0, C18:0, C20:0)
*SFA-(sum) saturated fatty acids
MUFA-monounsaturated fatty acids (C18:1)
PUFA-polyunsaturated fatty acids (C18:2, C18:3)

Amino acids profile

Free amino acid composition of cheese was evaluated in order to determine the quantity and the ratios of particular amino acids which significantly influence the texture and organoleptic properties of cheese as well as its digestibility and easy assimilation. Among essential amino acids, leucine, lysine, isoleucine and valine, were more concentrated in cheese samples and among non-essential, aspartic and glutamic acid were dominant. Regarding to the ratio of essential in relation to non-essential amino acids, it amounted 44.45 / 55.55 % (Table 4.).

Table 4. Amino acids profile of hard cheese (60days old, g/100g)

Amino acids			
Essential	*Mean* ± SD	Non-essential	*Mean* ± SD
THR	0.885 ± 0.24	ASP	3.593 ± 0.96
VAL	1.897 ± 0.16	GLU	8.323 ± 0.63
MET	1.220 ± 0.28	SER	1.589 ± 0.06
PHE	1.074 ± 0.19	GLY	0.927 ± 0.16
ISO	2.815 ± 0.75	ALA	0.841 ± 0.02
LEU	3.384 ± 0.02	TYR	2.589 ± 1.06
LYS	3.018 ± 0.68		
Ratio of essential / non-essential AA (%)		44.45/55.55	

In hard cheese large part of free amino acids are essential (leucine, valine, isoleucine, lysine) and also the level of non-essential is very high which effectively reduces the metabolic energy expended on biosynthetic reactions. For example, except for metionine+cystine, 50 g of Grana Padano and P-R cheese are enough to meet the daily requirements of the other essential amino acids (*Fox et al., 2004)*. Long-ripened cheese may be differentiated from young cheese by the content of glutamic acid, glycine, serine and threonine, while cheese produced from raw or pasteurized milk can be differentiated by the concentration of asparagine and glutamine (*Frau et al., 1997*). Branched amino acids (leucine, isoleucine, valine) are necessary in the muscle cells to promote protein synthesis and they are metabolized to generate energy in muscles rather than in liver (*Poltronieri et al., 2012*). The amino acid composition is ideal for the absorption due to profound changes that protein fraction undergoes during the long maturation period, which contributes to the separation of the milk casein into compounds of molecular weight smaller and smaller and finally into free amino acids (about 25% of total nitrogen) (*Pampaloni et al., 2011*).

Microbiological quality

The results of the microbiological analyses of cheeses during ripening showed that foodborne pathogens *Escherichia coli*, *Listeria monocytogenes*, *Enterobacteriaceae* and *coagulase-positive Staphylococci*, were not detected in any of the tested samples. This result indicates the good microbiological quality of raw milk, proper milk-handling and manufacturing practices.

In many studies, the microbiological safety of dairy products was considered. *Fox et al.* (*2000*) suggested that in modern factories, where enclosed vats and other equipment is used, the level of contamination from the environment is very low. Most of foodborne outbreaks of *E coli* O157:H7 have been associated with the consumption of foods contaminated with cattle feces and enterohemorrhagic *Escherichia coli* 0157:H7 is relatively an acid tolerant microorganism (*ICMSF, 2006*). *Enterobacteriaceae* and coliforms, microorganisms in many cases showed postpasteurization contamination of the cheese from the environment. The impact of factors on the fate of pathogens during cheese manufacture varies significantly between cheesemaking processes and cheese types. Compliance with microbiological criteria at the end of cheese production (final product) doesn't guarantee that microbiological hazards are excluded. *Listeria monocytogenes* is a ubiquitous bacteria and secondary contamination of products is possible under poor hygienic conditions. Despite the fact that the growth of the pathogen is limited in semi-hard and hard cheeses by low water activity, the secondary (surface) contamination could result in hazardous products (*Vrdoljak et al., 2016*).

Conclusion

Results of this study showed that investigated hard cheese belonged to the group of hard, full fat cheese and could provide a wide range of essential nutrients to the diet. Particularly, it is a good source of high-quality protein, amino acids, fat, fatty acids and energy essential for growth and maintenance of various body functions. In addition, cheese is very suitable source of protein for people who are not eat meat and considered to be one of the main food groups important in a healthy balanced diet. The unique nutrients and important bioactive compounds of hard cheese make it a product of added value. The greatest impact on the quality of the final product had variables connected to microbiological quality. In our investigation, microbiological safety is achieved by focusing on the prevention, adhering to the good hygiene practice and due to good control of ripening conditions.

Nutritivna i energetska vrednost tvrdog sira

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Rezime

Nedovoljna konzumacija mlečnih proizvoda, posebno tvrdog sira, u Srbiji predstavlja nutritivni razlog za zabrinutost. Osim slabe kupovne moći, ovo je prouzrokovano i lošom komercijalnom dostupnošću proizvoda ali i znanjem i afinitetom potrošača. Osnovni cilj ovog istraživanja je bio da se determinišu nutritivni i mikrobiološki parametri tvrdog sira proizvedenog od pasterizovanog mleka. Standardnim hemijskim analizima sir je ispitivan prvog, 30-og i 60-og dana zrenja. Mikrobiološki parametri koji su utvrdjivani su: *Listeria monocytogenes*, koagulaza pozitivnih *Staphylococci*, *Escherichia coli* i *Enterobacteriaceae*. Osim toga, utvrdjivan je profil amino i masnih kiselina kod uzoraka sira nakon 60 dana zrenja. Rezultati su pokazali da su svi ispitivani uzorci pokazali zadovoljavajuće mikrobiološke i nutritivne karakteristike za većinu ocenjivanih parametara. Zreo sir je raspolagao sa prosečno 29.08% mlečne masti, 25.29% proteina, 0.98% laktoze i pH vrednošću od 5,23. Udeo mlečne masti u suvoj materiji sira i sadržaj vode u bezmasnoj materiji sira su posedovali prosečne vrednosti od 49.11% i 55.84%, pojedinačno. Energetska vrednost je iznosila 366.80 kcal / 1523 KJ. Srednje vrednosti sadržaja masnih kiselina su pokazale da je tvrdi sir raspolagao najviše sa zasićenim masnim kiselinama, zatim mononezasićenim i najmanje polinezasićenim sa pojedinačnim udelima od: 66.92%, 30.13% i 2.95%. U pogledu esencijalnih aminokiselina sir je najviše raspolagao sa leucinom, lizinom i izoleucinom. Ovim istraživanjem potvrdjena je činjenica da tvrdi sir predstavlja bogat izvor hranljivh sastojaka i energije i treba da poseduje prioritet u ljudskoj ishrani.

Ključne reči: tvrdi sir, nutritivna vrednost, mikrobiološki kvalitet

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