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FOOD IN HEALTH AND DISEASE

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DETERMINATION OF THE GLYCAEMIC INDEX OF PREPARATIONS FOR SPORTS PERFORMANCE

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Summary

Introduction: Glycaemic index (GI) of a certain quantity and type of carbohydrate affects the rate of change of glucose concentration or glucose metabolism in blood. Consumption of carbohydrates with different GI before, during and after exercise affects the athletic performance and food with a high GI is preferred.

Aims: To determine GI of two commercially available recovery preparations and accordingly assess their classification in the group of training recovery products.

Methods: Five healthy males, 21 – 26 years, full-time students, non–smokers, volunteered for the study. The main inclusion criteria were absence of diabetes, prediabetes or any other diagnosis that affects glycaemia and intensive physical activity in leisure time, measured through body composition (Tanita MC-180 analyzer) and a standardized physical activity questionnaire. GI determination for two commercially available recovery preparations was done according to ISO 26 642:2010 method.

Results: Test sample 1 had significantly lower hedonic score (4.0 ± 1.7) and a subjective feeling of satiety (50.5 ± 3.6), while Control sample had the highest scores (2.0 or 64.8 ± 9.0). Significantly higher blood glucose was determined for both test samples as compared to Control. The area under the blood glucose curve (iAUC) was significantly higher for Test sample 1 (255.9 ± 50.7) as compared to Control (78.9 ± 8.0), and Test sample 2 (127.3 ± 12.6). GI of Test sample 1 was significantly higher than the one of Test sample 2 (317.9 ± 122.4 versus 161.6 ± 14.6, $p = 0.022$).

Conclusions: Both samples belong into the category of high GI products, which is in accordance with their intended purpose. The results indicate differences in the mechanism of action; i.e. influence on glucose metabolism, probably as a result of product formulations (nutritional composition). Despite the same classification of two tested products by the manufacturer, more detailed description of mechanism of action for training recovery products should be encouraged.

Key words: controlled clinical study, recovery preparations, glycaemic index, glucose metabolism, sport performance

Introduction

The glycaemic index (GI) is a measure of the food power to raise blood glucose concentration after a meal. The GI is defined as relation of the incremental Area Under the blood glucose response Curve (iAUC) of a tested meal containing 50 g of digestible carbohydrates and the incremental area under the blood glucose response curve of the standard food, i.e. 50 g pure glucose (iAUCS) (Chlup et al., 2004).

It was first introduced thirty years ago (Jenkins et al., 1981) with the aim of identifying the physiological dimension of quality of carbohydrates (CHO) and their divisions. The concept was first developed in response to the critical and specific needs of diabetes management, later evolving towards the general interest. Short-term effects of GI food products, such as postprandial metabolic response, satiety, physical abilities, physiological functions, have been identified in a series of research as important for the long-term outcomes, such as association with the risk for cardiovascular disease, diabetes and obesity. However, GI is still under discussion and guidelines are needed in terms of food processing, dietary recommendations, target population and the public use of the GI concept through health care profession-

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als and experts in the education sector (Wolever, 2006). Research in the early 1980's demonstrated that CHO consumption could improve capacity during prolonged bouts of exercise. Since then, studies have investigated the optimal amount, type and timing of CHO to maximize endurance performance. However, it was not until the early 1990's that GI was first investigated for its potential role in optimizing sport performance.

During a low intensity workout, fats are the main source of energy for sport performance. As the intensity of exercise increases, the need for glucose increases and consequently glycogen muscle storage is being depleted. Therefore, diet rich in CHO correlates with the higher muscle glycogen storage, which improves endurance performance (Wolever, 2006). Although consumption of CHO before, during and after recovery is now generally accepted as a mean of improving endurance performance, the role of high GI and low GI foods in sport nutrition is still being debated (Donaldson et al., 2010).

The GI of a food can be influenced by the physical and chemical characteristics of the food, and although an individual's glycaemic response can be highly variable, most participants' characteristics such as age, sex, body-mass index and ethnicity are not believed to influence GI (Wolever et al., 2003). However, some evidence suggests an interrelation between GI, gender, and training status. Several studies have found a difference between trained and sedentary men (Mettler et al., 2007), whereas others found no difference in the GI using trained and sedentary women (Mettler et al., 2006). If the GI of CHO influences the rate at which CHO elicits blood glucose response, it seems plausible that consuming CHO of differing GI before, during, and after exercise will influence sport performance (Donaldson et al., 2010). Current evidence suggest that for the maximal glycogen synthesis athletes should consume around 1.2 grams of CHO per kg body mass in for of glucose of sucrose right after the training, and every hour afterward through a period of 4 to 6 hours (Spaccarotella and Andzel, 2011). This is of special importance for athletes training 2 times per day, with limited time for recovery (Donaldson et al., 2010). Sports drinks are a better option for a fast glycogen recovery (than solid foods) since they can be taken right after a training or competition when appetite is usually supressed (Spaccarotella and Andzel, 2011).

Energy and sports drinks market is one of the fastest growing, despite numerous health concerns consumers have (Canadean, 2014). Still, economic crisis had a major impact on the market position. For example, in some countries like Croatia, Bosnia and Herzegovina, Hungary, market is in decline, while in Serbia and Czech Republic market of energy and sports drinks shows strong and consistent growth (Euromonitor International, 2014). Consumers have put demands on manufacturers; they need to reignite these products with innovation: new flavours, new no-calorie sweeteners, more natural product ingredients and extracts (Canadean, 2014). More innovation in the category asks for a better labelling and a more detailed categorization of these products (Sports Dietitians Australia, 2011).

Aims

Aims of this controlled clinical study were:

1. to determine the GI of two commercially available powder products, used for the recovery of athletes after training or competition;
2. to determine sensory acceptability of tested products;
3. to determine subjective satiety; and
4. to determine accordance with the manufacturers listed purpose, i.e. recovery, and the expected level of impact in specified area of recovery for these products.

Methods

Study subjects

The main inclusion criteria were absence of a diagnosis related to elevated glycaemia (i.e. diabetes, prediabetes or any other related to impaired glycaemia) and intensive physical activity in leisure time, measured through body composition and physical activity questionnaire. Healthy males of minimum 18 years of age, who are actively involved in sports at leisure time (at least 5 days a week), came for an interview to introduce

them with the study protocol. Total of 9 subjects volunteered for the study. After informing them about the study, the study consent form was signed.

The number of subjects required for the study was determined by the power analysis method (minimum strength of 80%, with minimal glucose change for the same subject of 0.20 mmol/l). In order to satisfy the strength of the study, minimum of five subjects was required.

Subjects were asked to come for the second appointment after a minimum of 8 to 10 hours of fasting, for screening. They completed a general questionnaire on basic and socio-economic characteristics, physical activity questionnaire (Baecke et al., 1982), and anthropometric and body composition was measured by Tanita MC-180 analyzer. After analysing the results on their medical history, physical activity level and anthropometric data, five subjects were selected for the study. They were informed on the precise study protocol for their first study protocol appointment.

Test foods

Three different foods (1–3) with a known carbohydrate composition were tested:

1. Test sample 1 (Twinlab® Ultra Fuel);
2. Test sample 2 (Twinlab® Hydra Fuel);
3. Control (glucose) (Table 1).

The food was prepared professionally in the expected quality and quantity, according to manufacturer's instructions. Foods were prepared freshly, each day. Each serving contained 50 g of digestible carbohydrates. Test samples were dissolved in 300 ml of water, while glucose was dissolved in the same quantity of clear apple juice. Apple juice was selected in order to mimic the colour of other two test foods.

Table 1. Energy and nutrition profile of the tested foods (per serving)

	Test sample 1 (serving size 105.3 g)	Test sample 2 (serving size 20 g)	Control (per 100 ml)
Calories	1674 kJ/400 kcal	293 kJ/70 kcal	197 kJ/47 kcal
Total Carbohydrate	100 g	18g	11.7 g
Sugars	34 g	18g	11.5 g
Vitamin C	60 mg	30 mg	1 mg
Thiamin	1.5 mg	0.21 mg	0.02 mg
Riboflavin	1.7 mg	1.7 mg	0.02 mg
Niacin	20 mg	-	0.1 mg
Vitamin B6	2 mg	-	0.03 mg
Biotin	300 μg	-	-
Pantothenic Acid	100 mg	-	-
Magnesium	25 mg	45 mg	7 mg
Chromium	200 μg	18.8 μg	-
Sodium	60 mg	26 mg	3 mg
Potassium	100 mg	-	119 mg

Study design

Glycaemic index (GI) for the two commercially available recovery preparations was done according to ISO 26 642:2010 method (International Standards Organization, 2010). The study protocol was approved by the Ethics Committee for research on humans of the Faculty of Food Technology in Osijek.

Study subjects and test foods were randomized by an independent person, which had no contact with the study subjects or study investigators.

For every study appointment, subjects came after an 8 to 10 hours of fasting. They were given their glucometer (Bayer CONTOUR USB NEXT), lancets and strips (all Bayer). Blood samples were taken at the following time points: -5', 0', 15', 30', 45', 60', 90', 120'. Test food was giv-

en between time points 0' and 15', and subjects were asked to consume test food within 10 minutes. After consuming test food they were asked to assess how much did they liked the food, i.e. to assess their sensory acceptability by using the hedonic scale. Also, between every blood sampling, subjects were asked to fill in the form of side-effects, and the satiety questionnaire.

Statistical analysis

Postprandial blood glucose was used to calculate incremental Area Under the blood glucose response Curve (iAUC) by using the trapezoid method. Afterwards, iAUC was used to calculate GI for the two test samples, according to formula:

$$GI = \frac{\text{iAUC t}}{\text{iAUC con}} * 100$$

iAUC t – incremental Area Under the blood glucose response Curve for the test food
iAUC con – incremental Area Under the blood glucose response Curve for the standard (control)

Test foods were tested for sensory acceptability, i.e. palatability. Hedonic scale was used, ranging from score 1 ("I like it very much") to score 7 ("I extremely don't like it").
The satiety questionnaire consists of four visual analogue scales asking a subject to subjectively rate feeling of hunger, desire to eat, prospective consumption, and fullness, respectively. Extreme left point reflects the feeling of complete satiety for the concerned descriptor, except for the third scale, in the other direction. Then the rates are measured and combined at each observed time point into a total subjective appetite score using the formula:

$$\frac{(Q1 + Q2 + (100 - Q3) + Q4)}{4}$$

Data were analysed by MS Office Excel 2010 (Microsoft Corp., USA) and Statistica 12.0 (StatSoft Inc., USA). Parametric tests were used, i.e. t-test for independent and dependent variables, and Pearson's correlation test, with the level of significance p = 0.05. All data are given as average and standard deviation (± SD).

Results and discussion

Subjects' characteristics

Five healthy males, 21 – 26 years, full-time students who live alone and are childless, with an average income of 330 eur/ person, non-smokers, rarely consume alcohol (on a monthly basis) and drink an average of 2 – 2.5 litres of water per day completed the study. Only one subject was taking dietary supplements (protein shakes).
The level of physical activity was assessed through three dimensions, by using Baecke's activity questionnaire. All three dimensions were greater than for the average student population: work index 3.3 (± 0.3), sport index 4.1 (± 0.6) and leisure index 3.9 (± 0.6). Using the same questionnaire, previous study determined work index of 2.3 (± 0.5), sport index 2.9 (± 2.6) and leisure index of 2.9 (± 0.9) for student population (Banjari et al., 2011). In addition, the latest research conducted on student population showed that 25.6% of students are totally inactive while 30.3% of them play sports recreationally, seasonaly (Banjari and Ostrognjaj, 2014).
Anthropometric data and body composition results were in accordance with the reported sport participants were involved in. This especially relates to the muscle mass (66.3 ± 10.1) and whole body impedance (532.8 ± 60.6 Ω), which are in accordance to findings from other studies (Kao et al., 2011; Keogh et al., 2007).
Considering anthropometrics study subjects belong to a category of very active amateurs (Baecke et al., 1982). These findings favour the inclusion criterion; they are considered as potential users of test samples (recovery preparations) since sports they are involved in (i.e. soccer, powerlifting, Olympic weightlifting and bodybuilding) are extremely physically heavy and recovery of muscle glycogen is crucial (American Dietetic Association, 2009; Donaldson et al., 2010; Khanna et al., 2005; Wolever, 2006).

Sensory acceptability

The average consumption time for all test foods was 2.5 to 3.0 minutes, and no side-effects were

noted. For the sensory acceptability, statistically significant difference was found between Control and Test sample 1 (2.0 versus 4.0 ± 1.7; p = 0.033) (Fig. 1). Test sample 1 had the lowest preference among subjects. These results can be explained with the different amounts of powder needed to be mixed with water in order to fulfil method requirement of 50 g of available carbohydrates. The highest amount of powder was needed for Test sample 1 (154.9 g, versus 55.6 g of Test sample 2) (Table 1), which resulted in different consistency (thickened consistency). Another important feature for these preparations is their taste (Sports Dietitians Australia, 2011). Due to high amounts of powder needed to prepare test foods taste was very intense which was not considered as appealing for the subjects (Fig. 1). Therefore, we assume that the recognition and familiarity with the taste of clear apple juice which was used as a basis for Control was one of the possible reasons for the best acceptability rating (Fig. 1).

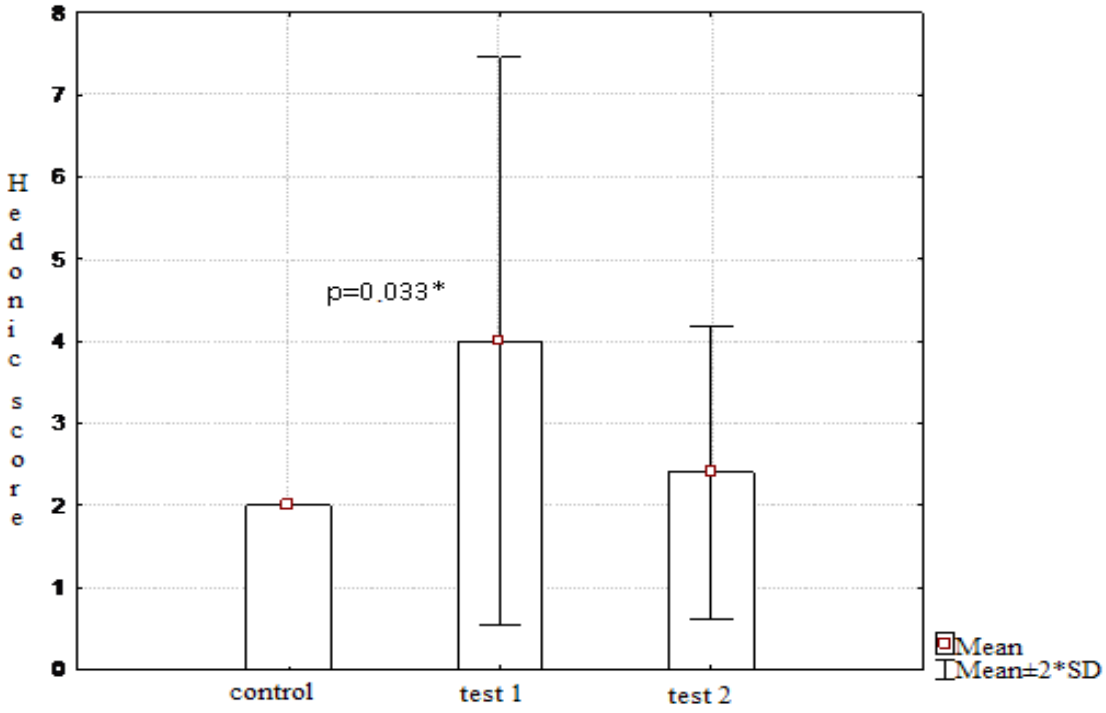

Fig. 1. Sensory acceptability of test foods expressed as hedonic score t-test for independent variables; Mean–the mean value; SD – standard deviation *indicates statistical significance between Control and Test sample1 at p <0.05

Subjective satiety

The subjective feeling of satiety is directly related to the type of consumed meal as well as its composition. It is important to point out that a meal viscosity presents a significant determinant for subjective satiety; solid food causes greater satiety than liquid food or beverage, which is directly related to the physiology and the process of digestion (Banjari et al., 2014; Guyton and Hall, 2003; Wolever, 2006). Statistically significant difference in subjective satiety was found for all three samples tested (Fig.2). Test sample 1 (50.5 ±3.6) and 2 (52.2 ±7.0) have lower subjective satiety as compared to Control (64.8 ±9.0). Moreover, Test sample 1 in 120 minute had significantly lower subjective satiety than Test sample 2 (p =0.016; Fig. 2). These results were unexpected because the change in blood glucose levels (Fig.3) indicated that at 120' blood glucose for Test sample 1 was the highest. However glucose peak was also the highest for Test sample 1, which together with the high content of B complex vitamins higher than the recommended intake observed as DRI (Institute of Medicine, Food & Nutrition Board, 2004); effectors that positively affect appetite (Banjari et al., 2014) in the product (Table 1) can be hypothesized as a possible result for the lowest subjective satiety score. Additional support

for the presented hypothesis lies in statistically significant negative correlation (-0.84) found between blood glucose response and subjective satiety score for Test sample 1 (Table 2).

Table 2. Correlation between blood glucose and subjective satiety score for test food

	Change in blood glucose concentration		
Subjective satiety score	Control	Test sample 1	Test sample 2
Control	- 0.62		
Test sample 1		- 0.84	
Test sample 2			- 0.69

Pearson's correlation test, $p < 0.05$

Control had the highest subjective satiety score (Fig.2), and when compared to Test sample 1 statistically significant difference was found in 90' ($p = 0.024$), and for Test sample 2 in 15' ($p = 0.011$) and 30' ($p = 0.032$). These results suggest difference in mechanism of action of test samples (content of CHO, combined with high content of B complex vitamins), which emphasizes the need for better labelling, and more detailed categorization of recovery preparations (Jenkins et al., 1981).

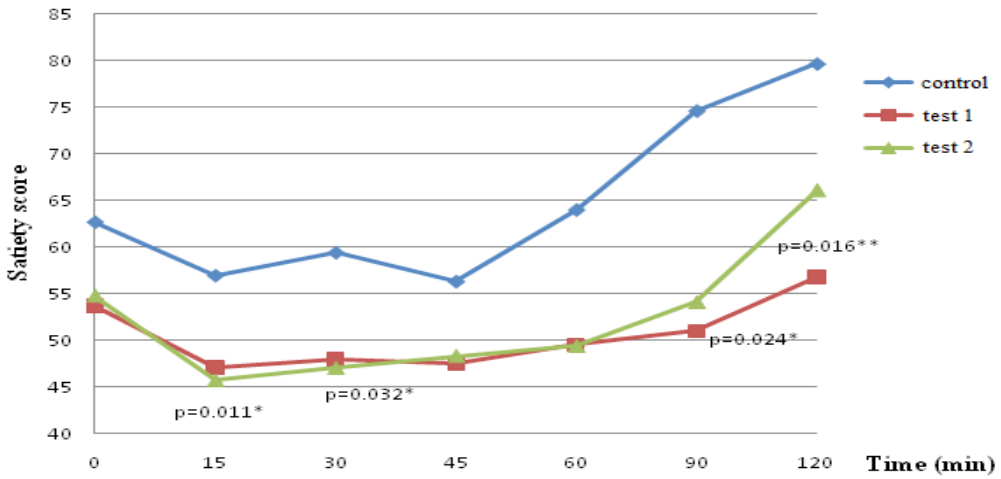

Fig. 2. Subjective satiety curves for three test samples through 120 minutes t-test for independent variables
*indicates statistical significance at $p < 0.05$; *compared to Control; **between two Test samples

Blood glucose change

The speed and the intensity of blood glucose levels increase after eating certain meals compared with the standard represents GI. In healthy subjects, a mixed meal affects the normal increase of blood glucose, which causes the secretion of insulin from pancreas in order to normalise levels of glucose back to the basic (basal) level. The amplitude of the increase in blood glucose determines the amount of secreted insulin, and is in direct relation to the number of metabolic disorders, from obesity, diabetes, metabolic syndrome, and others (Wolever, 2006). From the aspect of sports performance, the importance is even greater, especially for high intensity trainings where muscle glycogen recovery is crucial for sports performance (American College of Sports Medicine, 2011; Donaldson et al., 2010; Spaccarotella and Andzel, 2011). By comparing both test samples with Control there was a statistically significantly higher response of blood glucose. Blood glucose concentration was significantly higher from 30' to 120' for Test sample 1 when compared to Control. For Test sample 2 significantly higher concentration as compared to control was found in 45' ($p = 0.042$). When comparing two test samples, statistically significantly higher blood glucose was found for Test sample 1 in 60' ($p = 0.003$) and 90' ($p = 0.002$). Only for Test sample 1 blood glucose did not fall to baseline level (Fig. 3).

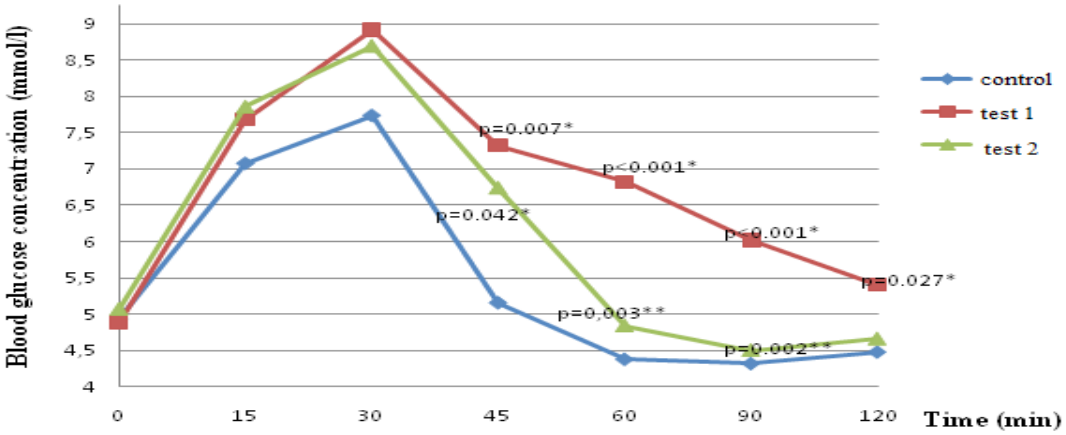

Fig. 3. Change in blood glucose levels of the test samples through 120 minutes
t-test for independent variables
*indicates statistical significance at p <0.05; *compared to Control; **between two Test samples

The results indicate the need for better labelling of such preparations (American College of Sports Medicine, 2011; Sports Dietitians Australia, 2011), since despite being classified in the same category by the manufacturer; clearly the effect on glucose metabolism is significantly different. Compensation for energy after a workout has utmost importance, and should include compensation of glycogen and elimination of accumulated lactate (American College of Sports Medicine, 2011; Donaldson et al., 2010; Guyton and Hall, 2003). Wrong choice of preparations for recovery may have significant adverse effects on exercise capacity and athletic performance, which is again most prominent in top elite sport (American College of Sports Medicine, 2011; Donaldson et al., 2010; Spaccarotella and Andzel, 2011).

Glycaemic index

GI can also be defined as a relationship of incremental or total area under the curve in response to blood glucose of tested food (iAUC, Incremental Area Under the blood glucose Curve tested for the meal) containing 50 grams of free carbohydrates and total area under the curve in response to blood glucose of standard test food (iAUCS, Incremental Area Under the blood glucose Curve for the standard meal) (Chlup et al., 2004).

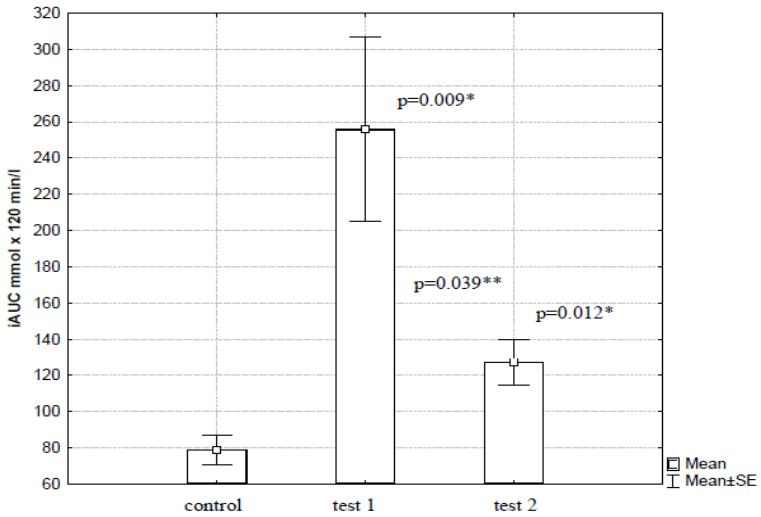

Fig. 4. The area under the blood glucose curve (iAUC) for three test samples
t-test for independent variables; Mean – the mean value; SE –standard error
*indicates statistical significance at p <0.05; *versus control; **between Test samples

Area under the curve was calculated as the sum of the areas of a trapezoid under the glucose concentration curves for the tested samples (Fig. 3) and expressed in 120 mmol x min/l. The calculated iAUC values are as follows: control 78.9±8.0; test sample 1 255.9±50.7; test sample 2 127.3±12.6 (Fig. 4).

Statistically significant difference was found between Control and Test sample 1 (p =0.009) and Test sample 2 (p =0.012). Also, significant difference was determined between Test sample 1 and 2 (p = 0.039; Fig. 4). Meal composition is essential for the normal pancreatic activity; therefore blood glucose level reflects current human need for energy. The amplitude of increase in blood glucose is determined by the amount of secreted insulin. Accordingly, various metabolic disorders lead to disturbance in insulin secretion (Wolever, 2006). It is therefore important to recognize how body reacts on certain foods through their CHO composition and GI. This is especially important for physical fitness, performance and recovery after training, especially for top athletes (American College of Sports Medicine, 2011; Donaldson et al., 2010; Spaccarotella and Andzel, 2011). GI values are susceptible to large inter- and intra-individual variability (Chlup et al., 2004; Foster-Powell et al., 2002; Wolever et al., 2003). In the European Union, the GI of a large number of foods is not determined, and also methods for determining the GI values are not standardized (Wolever et al., 2003). There is a need for standardization and systematic determination of GI of foods in order to keep pace with the advanced fields of manufacturing of novel foods and dietary supplements, as well as scientific evidence on the relationship between GI with numerous health effects (Prašek, 2004; Wolever, 2006). According to GI categories calculated values for both tested samples (Table 3) classify them in the category of high-GI and high glycaemic load (GL).

Table 3. Calculated glycaemic index for test samples

Sample	**Glycaemic index** mean ± SD	**p**
Test 1	317.9 ± 122.4	0.022*
Test 2	161.6 ± 14.6	

SD – standard deviation

t-test for independent samples; * indicates statistical significance at p <0.05

If we consider composition of test samples (Table 1), CHO content and the purpose of tested preparations, high GI was expected. However, GI of Test sample 1 was significantly higher (317.9 ± 122.4 versus 161.6 ± 14.6, p = 0.022; Table 3). These results are consistent with iAUC values (Fig. 4), but they were not expected to be that different, considering their intended purpose. The results indicate that despite the same classification of products in the group of "recovery" preparations by the manufacturer, their effect on glucose metabolism is different.

Study findings are consistent with the results of other studies (Chlup et al., 2004). Likewise, studies in the field of sports indicate that high GI foods and foods with high GL have the most beneficial effect on recovery after a long and intensive exercise, due to improvement in muscle glycogen content (American College of Sports Medicine, 2011; Sports Dietitians Australia, 2011; Wolever, 2006).

Conclusions

1. According to hedonic score, the highest acceptability had Control, followed by Test sample 2 and the lowest for Test sample 1.
2. Subjective satiety scores was the lowest for Test sample 1 (50.5 ± 3.6), when compared to Control (64.8 ± 9.0) and Test sample 2 (52.2 ± 7.0). Different time points for which the difference was determined (Test sample 1 in 90', Test sample 2 in 15' and 30') suggest difference in mechanism of action due to formulation of these two products (CHO and B complex vitamins).
3. Blood glucose curve of Test sample 1 showed the highest peak and separate glucose con-

centration at time points from 30' to 120', which did not fall to baseline level. Consequently, iAUC was the highest for Test sample 1 (255.9 ± 50.7), when compared to Control (78.9 ± 8.0) and Test sample 2 (127.3 ± 12.6).

4. Both test samples belong to a high GI and high GL category, which correlates to the intended purpose, i.e. recovery preparations. Despite same classification, GI of Test sample 1 is statistically significantly higher than the one of Test sample 2 (317.9 ± 122.4 versus 161.6 ± 14.6, $p = 0.022$).

Determined differences in formulation of the two tested recovery preparations suggest different mechanism of action on glucose metabolism, therefore changing the final outcome intended – recovery of muscle glycogen. This imposes the need for a better labelling, and more detailed classification of recovery products in order to achieve maximum impact on exercise capacity during training and competition as well as sports performance. Adding GI to the existing labels could serve as a starting point for the proposed more detailed labelling. By providing this information only, athletes and their coaches could predict the impact of specific product on muscle glycogen recovery, a crucial aspect in sports performance.

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Conflict of interest statement: None declared.

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ODREĐIVANJE GLIKEMIJSKOG INDEKSA PRIPRAVAKA ZA OPORAVAK NAKON TRENINGA

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Sažetak

Uvod: Glikemijski indeks (GI) određene količine i vrste ugljikohidrata utječe na brzinu promjene koncentracije glukoze u krvi (GUK), odnosno metabolizam glukoze. Konzumacija ugljikohidrata sa različitim GI prije, tijekom i nakon treninga utječe na sportsku izvedbu, a preferira se hrana visokog GI.

Ciljevi: Odrediti GI dva komercijalno dostupna pripravka za oporavak nakon treninga i sljedno tome procijeniti njihovu klasifikaciju u skupini pripravaka za oporavak nakon treninga.

Metode: Pet zdravih studenata, dobi 21 do 26 godina, nepušača se dobrovoljno prijavilo za istraživanje. Glavni kriteriji za uključivanje u istraživanje su bili: izostanak dijagnoze dijabetesa, preddijabetesa ili neke druge koja utječe na glikemiju, te da se ispitanici bave intenzivnom fizičkom aktivnošću u slobodno vrijeme, mjereno preko sastava tijela (Tanita MC-18 analizator sastava tijela) i standardiziranim upitnikom o fizičkoj aktivnosti. Određivanje GI dva komercijalno dostupna pripravka za oporavak nakon treninga je provedeno prema metodi ISO 26 642:2010.

Rezultati: Test uzorak 1 je imao statistički značajno najnižu hedonističku ocjenu (4,0 ± 1,7) i subjektivni osjećaj sitosti (50,5 ± 3,6), a Kontrolni uzorak najviše (2,0 odnosno 64,8 ± 9,0). Statistički značajno višu koncentraciju GUK imala su oba test uzorka u usporedbi sa kontrolom. Površina ispod krivulje (iAUC) je statistički značajno najveća za Test uzorak 1 (255,9 ± 50,7), u usporedbi s Kontrolom (78,9 ± 8,0) i Test uzorkom 2 (127,3 ± 12,6). GI Test uzorka 1 je značajno viši u odnosu na Test uzorak 2 (317,9 ± 122,4 naprema 161,6 ± 14,6, p = 0,022).

Zaključci: Oba uzorka spadaju u kategoriju visokog GI, što je u skladu s njihovom namjenom. Dobiveni rezultati upućuju na razlike u mehanizmu djelovanja; tj. na metabolizam glukoze, vjerojatno kao rezultat formulacije proizvoda (nutritivnog sastava). Unatoč istoj klasifikaciji od strane proizvođača, detaljniji mehanizam djelovanja za proizvode namijenjene oporavku nakon treninga bi trebale biti dostupne.

Ključne riječi: kontrolirano kliničko istraživanje, pripravci za oporavak nakon treninga, glikemijski indeks, metabolizam glukoze, sportska izvedba

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QUANTITATIVE DETERMINATION OF OCHRATOXIN A IN BOTTLED WINE

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Abstract

In this study work we have determined the quantity of ochratoxin A (OTA) in 54 samples of bottled wine. The methodology which we used is the method known as High Performance Liquid Chromatography with Fluorescence Detection (HPLC). Before the HPLC analysis we have done the ochratoxin A extraction through the immunoaffinity clean-up procedure by high immunoaffinity columns. Samples that are analyzed have been taken in Kosovo and are wine samples produced and bottled in Republic of Kosovo and the wine samples produced, bottled and imported in Kosovo from other Balkan and European Union (EU) countries and beyond. The aim of this research has been the analysis of ochratoxin A in bottled wine for the first time in Kosovo and determination of the risk or not by consumption of the analyzed wines by consumers. The results of all analyzed samples have been below the limit allowed by the EU for ochratoxin A i.e. 2 ng/ml and as such in the future do not pose a risk to human health.

Key words: wine, ochratoxin A, HPLC-FD, immunoaffinity column, mycotoxin

Introduction

Ochratoxin A, N-[(3R)-(5-chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl) carbonyl]-L-phe-nylalanine, is a mycotoxin produced by certain species of *Aspergillus* and *Penicillium* filamentous fungi. OTA contaminates cereals and cereal products, coffee, beans, pork meat and meat products, milk and milk products, eggs, wine, and beer all over the world (Flajs et al, 2009). Cereals and cereal products are the main sources of OTA intake, followed by wine, grape juice and coffee. The OTA levels in wine depend on different factors such as the climate, the date of harvesting and different wine-making procedures (Arbisu et al, 2010).The *Penicillium* species that is associated with ochratoxin A production, *Penicillium verrucosum* is an important ochratoxigenic species because it is the major producer of OTA in cereals such as wheat, barley, oats and rye, in temperate and cold climates (Cabañes et al, 2010). This species is the main source of OTA contamination in cereals associated to the porcine and avian nephropathy detected in temperate and cold countries such as Denmark, Sweden, Canada or the United States (Cabañes et al, 2010). At the moment, *P. verrucosum* and *P. nordicum* are the only OTA producing species accepted in the genus *Penicillium* (Cabañes et al, 2010). *Aspergillus ochraceus* is the best known species of ochratoxin – producing *Aspergillus*. It grows at moderate temperatures and at a high water activity and is a significant source of ochratoxin A in cereals. It infects coffee beans usually during sun-drying causing contamination in green coffee (Risk Assessment Studies, http://www.cfs.gov.hk/). *Aspergillus carbonarius* is highly resistant to sunlight and survives sun-drying because of its black spores and therefore grows at high temperatures. It is associated with maturing fruits and is the source of ochratoxin A in grapes, dried vine fruits, and wine and is also another source of ochratoxin A in coffee (Risk Assessment Studies, http://www.cfs.gov.hk/). Aspergilli in vineyards varied depending on years and geographic areas: France, Greece and Israel were the areas with the highest incidence, followed by South Italy, Spain and Portugal (Oliveri et al, 2011). At normal cooking showed that OTA was only partially degraded (El Khoury et al, 2010). Moreover, this molecule can withstand steam sterilization three hours with high pressure 121 ° C, and even at 250 ° C its destruction is not complete (El Khoury et al, 2010).

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The formation and occurrence of OTA in wines represents a serious economic problem in Europe because of it high share in world vineyard areas, which represent 75% of world-wide wine production.

Mycotoxins can cause serious health problems in animals and humans known as mycotoxicosis (Marquardt et al 1992).OTA is arguably a risk factor for Balkan endemic nephropathy (BEN). BEN is a chronic tubulointerstitial kidney disease that occurs in some areas of Bosnia and Herzegovina, Bulgaria, Croatia, Romania, Serbia, and Monte Negro (Yordanova et al. 2010. International Agency for Cancer Research classifies OTA as potential carcinogenic substance for man (group 2B). Zimmerli and Dick (1995) were the first ones to report the existence of OTA in wine. The European Union Regulation (EC 123/2005) limit for OTA in wine is 2 ppb (µg/L). The purpose of this research has been the analysis for the first time of ochratoxin A in bottled wine produced in Kosovo and their comparison with imported bottled wines in Kosovo from the Balkan and European countries and beyond and defining the risk or not by consumption of these wines by consumers in the future. This research work is one of the stages in the framework of a scientific project that is implemented in Kosovo, involving the quantitative determination of ochratoxin A (OTA) from the beginning of the wine production to the final stage, which means the analysis of OTA in bottled wine. The wine samples taken for analysis are provided from the markets or wine cellars that operate in Kosovo and the selection is done spontaneously.

Material and methods

Reagents and chemicals

OTA standard (Lot No: L13092B, 10.20 µg/ml) was obtained from LGC Standards (Wesel, Germany). All chemicals were of the analytical grade and solvents for mobile phase were of the HPLC grade. A stock solution of OTA was prepared in the mobile phase (100 ng OTA/ml). The working standards for HPLC analysis were prepared by adding known amounts of the diluted stock solution to the HPLC mobile phase to give final concentrations from 0.1 to 5.0 ng OTA/ml. The working standards were freshly prepared every day.

Sampling

Wine samples were taken from supermarkets and wine cellars which are operating in Kosovo. Bottle wine samples were taken for analysis and eventual determination of the presence and quantity of OTA in wine. From total of 54 analyzed samples of bottled wine, 36 samples were produced and packed in Kosovo, 3 in Albania, 2 in Montenegro, 3 in Macedonia, 3 in Bulgaria, 3 in Italy, 1 in France, 1 in Spain, 1 in Slovenia and 1 in Australia.40 analyzed bottled wine samples were red wine while 14 samples were white wine.Years of production and the percentage of alcohol in analyzed bottled wines were different.

Extraction and clean – up

The method which we used for extraction and HPLC-FD analysis was the method which has been described by Visconti et al. (1999) for determination of ochratoxin A in wine by means of immunoaffinity column clean-up and high-performance liquid chromatography. The wine was first diluted with so-called extraction solution containing 1% polyethylene glycol (PEG 8000) and 5% sodium hydrogencarbonate, filtered and applied to an Ochra Test immunoaffinity column, Vicam Inc (USA). The column was additional washing with a washing solution containing sodium chloride (2.5%) and sodium hydrogencarbonate (0.5%) followed by water and OTA was eluted with methanol.

HPLC conditions

The OTA in eluate was quantified by reversed-phase HPLC with fluorometric detection (excitation wavelength 333 nm, emission wavelength 460 nm), column nucleodur C18 (4.6 × 250 mm), size of particles 5µm (Machenrrey – Nagel, Germany), software system ChromQuest 5.0, using acetonitrile-water-acetic acid (99:99:2) as mobile phase. The mobile phase was degassed first by sonication for 15 min in an ul-

trasonic bath. The flow rate was 1 ml/min and the injection of volume was 50 µl. Limit of detection (LOD) was 0. 05 ng/ml and limit of quantification (LOQ) was 0.1ng/ml. The retention time was 8 minute.

Results and discussion

Table 1. OTA distribution in 36 analyzed bottled wine samples by HPLC-FD produced in Kosovo

Sample code	Name of the company	Variety	Country	Year of production	Volume of bottle / ml	Content of OTA ng / ml
K1	Murati	Vranac	Kosovo		187	< LOD
K2	Stone Castle	Vranac Premium	Kosovo	2011	187	< LOD
K3	Stone Castle	Chardonnay	Kosovo	2011	187	< LOD
K4	Biopak	Vranac	Kosovo	2010	187	< LOD
K5	Stone Castle	Cabernet Sauvignon	Kosovo	2011	187	< LOD
K6	EKO	Vranac	Kosovo		187	< LOD
K7	Bodrumi i Vjeter	Chardonnay	Kosovo	2012	187	< LOD
K8	Bodrumi i Vjeter	Vranç	Kosovo	2012	187	< LOD
K9	Rahoveci	Pinot Noir	Kosovo	2006	187	< LOD
K10	Iliria	Red Wine	Kosovo	2010	750	< LOD
K11	Theranda	White Wine	Kosovo	2009	750	< LOD
K12	Suhareka	White Wine	Kosovo	2012	750	< LOD
K13	Suhareka	Chardonnay	Kosovo	2012	750	< LOD
K14	Suhareka	Italian Rhiesling	Kosovo	2012	750	< LOD
K15	EKO	Cabernet Sauvignon	Kosovo		187	< LOD
K16	Iliria	Merlot	Kosovo	2008	750	< LOD
K17	Bodrumi i Vjeter	Merlot	Kosovo	2012	187	N.D.
K18	Biopak	Chardonnay	Kosovo	2010	187	N.D.
K19	Rahoveci	Merlot	Kosovo	2006	187	N.D.
K20	Shulina	Cabernet Sauvingnon	Kosovo	2010	187	N.D.
K21	Stone Castle	Red Wine	Kosovo	2011	750	N.D.
K22	Theranda	Barrique	Kosovo	2009	750	N.D.
K23	Erenik-Pavaresia	Merlot	Kosovo	1989-2001	750	N.D.
K24	Theranda	Pinot Blanc	Kosovo	2009	750	N.D.
K25	Suhareka	Gamay Noir	Kosovo	2010	750	N.D.
K26	Theranda	Gamay Noir	Kosovo	2009	750	N.D.
K27	Theranda	Italian Rhiesling	Kosovo	2009	750	N.D.
K28	Murati	Rose	Kosovo	2011	750	N.D.
K29	Shulina	Red Wine	Kosovo	2010	750	N.D.
K30	Bodrumi i Vjeter	Vranç	Kosovo	2012	750	N.D.
K31	Suhareka	Franconia	Kosovo	2012	750	N.D.
K32	Theranda	Rhine Rhiesling	Kosovo	2009	750	N.D.
K33	Theranda	Pinot Noir	Kosovo	2009	750	N.D.
K34	EKO	Red Wine	Kosovo	2012	750	N.D.
K35	Bodrumi i Vjeter	Cabernet Sauvignon	Kosovo	2011	750	N.D.
K36	Suhareka	Red Wine	Kosovo	2010	750	N.D.

Note. LOD = limit of detection, LOQ = limit of quantification, N.D. = not detected, HPLC-FD = High Performance Liquid Chromatography with Fluorescence Detection, OTA = Ochratoxin A

Table 2. OTA distribution in 18 analyzed bottled wine samples by HPLC-FD, imported in Kosovo.

Sample code	The name of the company	Variety	Country	Year of production	Volume of bottle/ ml	Content of OTA, ng/ml
A1	Luani	Merlot	Albania	2003	187.5	1.204
A2	Luani	Cabernet Sauvignon	Albania	2011	750	0.634
A3	Luani	Riesling	Albania		750	N.D.
M1	T'GA ZA JUG	Vranec	Macedonia	2009	187	0.331
M2	T'GA ZA JUG	Red Wine	Macedonia	2012	187	0.229
M3	T'GA ZA JUG	Vranac	Macedonia	2012	187	0.203
I1	Cantine del colle	Rosso	Italy		750	0.18
I2	Cielalo	Cabernet Sauvignon	Italy	2012	750	0.17
I3	Celine Casa Bottega	Chardonnay	Italy	2009	750	N.D.
F1	Cuvee Louis XII	Chardonnay	France		750	0.09
S1	Quercus	Merlot	Slovenia	2008	200	< LOD
E1	Ash Tree Estate-Freixfenet	Shiraz-Monastrell	Spain	2011	750	< LOD
AS1	Monty's Hill	Shiraz & Cabernet Sauvignon	Australia	2012	750	N.D.
B1	Mezzek	Merlot	Bulgaria		750	< LOD
B2	Yamantiev's	Cabernet Sauvignon	Bulgaria	2012	750	< LOD
B3	Saint Ilia-Tracia	Red Wine	Bulgaria	2009	750	N.D.
MN1	Plantaze	Cabernet Sauvignon	Monte Negro	2010	750	0.06
MN2	Plantaze	Vranac Pro Corde	Monte Negro	2010	750	0.05

Note. LOD = limit of detection, LOQ = limit of quantification, N.D. = not detected, HPLC-FD = High Performance Liquid Chromatography with Fluorescence Detection, OTA = Ochratoxin A

3.1. Discussions

It was determined that the amount of OTA in all analyzed samples does not exceed the maximum level allowed by the European Union for this mycotoxin, which is 2ng/ml. From these results we can see that in most of the samples analyzed, the amount of OTA is below the detection limit (LOD) or not detected (N.D.) at all. From the results obtained (tab. 1) we can see that in 36 wine samples produced and bottled in Kosovo, the amount of ochratoxin A is < LOD in 16 of them and in 20 of them the ochatoxin A is not detected at all. The results of the wine samples from other countries that import wine in Kosovo show that in some of the Albanian and Macedonian bottled wines that we have analyzed, OTA concentration is slightly higher compared to other countries of the Balkan, Europe and beyond (tab. 2). For example if we analyze the result of the sample encoded as A1 (tab.2) we can see that the amount of OTA in this wine produced in Albania, is quite high in the bottle although within the limits allowed by EU. We can see that from three bottled wines produced in Albania in two of them we have isolated OTA (tab.2). Also from the results we can see that the bottled wines produced in Macedonia, from a total of three analyzed bottles, in three of them is isolated OTA (tab.2). Regarding the analyzed bottled wines produced in Italy, in two of them is isolated OTA, although within the limits allowed by EU. The analyzed bottled wines from other countries shown to have a minimal or no presence of ochratoxin A (tab.2.). From the results obtained we can see that OTA is isolated in bottled wines produced in different years, confirming that each year in viticulture and winery has its own specifics, which besides the technological and sanitary conditions largely influenced by climatic factors of the respective year. Also from the results we can see that the concentration of OTA above the limit of detection is shown only to samples of red wine which verifies that the risk of higher concentra-

tion of OTA is expected to be in red wines than white wines (tab.1& tab.2) Research more or less similar are also made by our colleagues who determined that in the red wines OTA amount expected to be higher compared with white grape varieties. For example a study conducted in Slovakia (Belajova et al, 2007) where the bottled wines were also involved in research, shows the greater presence of OTA in red wines than white wines. Technological progress in the making of wine, which implies first the advanced hygienic sanitary conditions as well as the standard process during all other technological stages are seen to be a key factor in the minimum concentration of OTA in bottled wine (Durguti et al, 2014). Taken into consideration the fact that many of the consumers consume wine before bottling, we recommend the Enologists who deal with scientific work to analyze the amount of OTA since the initial stages of grape processing in order to verify whether the raw wine exceed or not the limits allowed by EU for ochratoxin A. We also recommend the wine technologists (enologist), especially those from the countries where prevailing temperatures slightly higher, to increase attention to cleanliness and all other technological aspects, conditions that favor the growth of the fungi responsible for the production of OTA. This research do not shows the risk of drinking the wine that we have analyzed in the future by consumers and as such do not represent a risk for human health.

Conclusions

The importance of the obtained results lies in the fact that this type of research is performed for the first time in Kosovo (bottled wine) and as such provides harmless consumption of the wines produced and imported in Kosovo.

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PLAVA RIBA – PREDNOSTI ALI I NEKI RIZICI KONZUMIRANJA

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Revijalni rad

Sažetak
Plava riba je nutritivno izrazito vrijedna, lako probavljiva namirnica koja je izvor biološki vrijednih bjelančevina, joda i selena dok istovremeno sadrži malo masti i ima niski sadržaj kolesterola. Vrijedan je izvor višestruko nezasićenih masnih kiselina kojima se pripisuju brojni pozitivni učinci na zdravlje. Međutim uz konzumaciju plavu ribu mogu biti vezani i određeni rizici, primjerice, histaminsko trovanje. Histaminsko trovanje morskom ribom uzrokuje 5% svih oboljenja vezanih za hranu kao i 37% trovanja vezanih za ribu podrijetlom iz mora ili oceana. Ova vrsta trovanja ribom je ujedno i najčešća u svijetu. Histamin se u ribi oblikuje *post mortem* bakterijskom dekarboksilacijom esencijalne aminokiseline histidina pod djelovanjem enzima histidin- dekarboksilaze. Za nastajanje velikih količina histamina presudna je u ribljem mesu prisutnost mikroorganizma (najčešće *Morganella morganii*, Klebsiella pneumoniae i Hafnia alvei) koji imaju sposobnost tvorbe enzima histidin-dekarboksilaze. Vrste riba koje sadrže visoku razinu slobodnog histidina, pa prema tome predstavljaju i rizik za histaminsko trovanje su: inćun, haringa, skuša, lokarda, srdela, papalina i tunjevina. Period inkubacije kod histaminskog trovanja traje od 5 minuta do sat vremena, a simptomi traju od nekoliko sati do 24 sata.
U radu je uz prikaz nutritivnog značaja plave riba dan pregled dosadašnjih saznanja o nastanku histamina, mehanizmu njegove difuzije u meso ribe te nastanku i sprječavanju trovanja histaminom podrijetlom iz ribe. Uz prikaz simptoma trovanja navedeni su podaci RASFF (*Rapid Alert System for Food and Feed*) za razdoblje od 1979. do 2010 godine te su prikazane studije slučaja histaminskog trovanja u Republici Hrvatskoj.

Ključne riječi: histamin, nutritivni značaj, plava riba, RASFF, trovanje

Uvod

Svakodnevno smo svjedoci preporuka nutricionista o važnosti konzumiranja morske ribe, pogotovo plave, prvenstveno zbog obilja višestruko nezasićenih masnih kiselina kojima se pripisuju brojni pozitivni učinci na zdravlje. Međutim, uz konzumaciju plave ribe se vežu i potencijalni rizici koji mogu povećati vjerojatnost njezine zdravstvene neispravnosti te nakon konzumacije narušiti zdravlje potrošača. U potencijalne rizike koji mogu ugroziti sigurnost konzumacije plave ribe ubraja se vjerojatnost nakupljanja kontaminanata organskog podrijetla poput primjerice dioksina ili polikloriranih bifenila (Larrson i sur., 1996; Strandberg i sur., 1998) ili anorganskog podrijetla poput žive, olova i kadmija (Joiris i sur., 1999; Storelli i sur., 2002). Posebnu opasnost za zdravlje može predstavljati nastanak histamina u mesu ribe nakon izlova.
Morske ribe mogu prenositi patogene mikroorganizme na udaljena područja budući da morska voda sadrži veliki broj mikroorganizma među kojima je određeni broj patogen za čovjeka. U škrgama i utrobi riba mikroorganizmi se mogu održati duže vrijeme na životu i na taj način biti preneseni ribom na udaljena područja (Cuculić i sur., 1984, Naila i sur., 2011). Na histaminsko trovanje ribom otpada 5 % svih oboljenja vezanih za hranu kao i 37 % svih bolesti vezanih za ribu podrijetlom iz mora ili oceana. Ova vrsta trovanja ribom je ujedno i najčešća u svijetu. Najviše slučajeva trovanja je zabilježeno u SAD-u, Japanu i Ujedinjenom Kraljevstvu. Manji broj slučajeva je zabilježen u Kanadi, Novom Zelandu, Francuskoj, Njemačkoj, Švedskoj, Šri Lanki,

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Češkoj, Slovačkoj, Nizozemskoj, Australiji, Indoneziji, Južnoj Africi i Egiptu (Muscarella i sur., 2013). Histaminsko trovanje zabilježeno 1973. godine u Japanu se zbog velikog broja oboljelih (2 656 slučajeva) smatralo problemom globalnih razmjera (Singh i sur., 2012).

Zbog kratkog vremena inkubacije, burnih simptoma i stabilnosti tijekom termičke obrade, upravo je opasnost od histaminskog trovanja poseban izazov za sve koji su odgovorni za nabavu i pripremu ribe te je cilj ovog rada proširiti saznanja o ovoj uvijek aktualnoj tematici.

Nutritivni značaj plave ribe

Plava riba, kako sitna (srdela, inćun, papalina, skuša, lokarda i dr), tako i krupna (tuna, palamida i dr.) u svakodnevnoj prehrani predstavlja izvor biološki vrijednih bjelančevina. Sadrži malo masti i ima niski sadržaj kolesterola, a također je i dobar prirodni izvor joda i selena. Selen zahvaljujući svojim antioksidacijskim svojstvima smanjuje učestalost pojave karcinoma prostate, pluća i debelog crijeva (Ferro-Luiz i Branca, 1985). Ova skupina namirnica je posebno vrijedna zbog sadržaja višestruko nezasićenih masnih kiselina iz skupine n-3 masnih kiselina: EPK (eikozapentaenska kiselina; C20:5 n-3) i DHK (dokozaheksaenska kiselina; C22:6 n-3). EPK i DHK djeluju kao prekursori eikosanoida, regulatornih hormona imaju važnu ulogu u upalnim procesima, u stvaranju kolesterola, suženju i širenju krvnih žila te stimulaciji ili kočenju obrambenih mehanizama u organizmu. Višestruko nezasićenim masnim kiselinama iz skupine n-3 podrijetlom iz plave ribe pripisuju se učinci snižavanja koncentracije serumskih triglicerida, snižavanja koncentracije ukupnog kolesterola u krvi, blagog povećavanja koncentracije lipoproteina visoke gustoće (HDL), usporavanja ateroskleroze i protuupalnog djelovanja (Mozaffarian i sur., 2003; Hu i sur., 2002; Yuan i sur., 2001). Eikosapentaenska kiselina također potiče apsorpciju kalcija i njegovu pohranu u kostima čime značajno pridonosi njihovoj čvrstoći (Holub, 2002.). Količina i vrsta n-3 višestruko nezasićenih masnih kiselina ovisi o: vrsti ribe, uvjetima uzgoja, načinu ishrane i sezoni izlova.

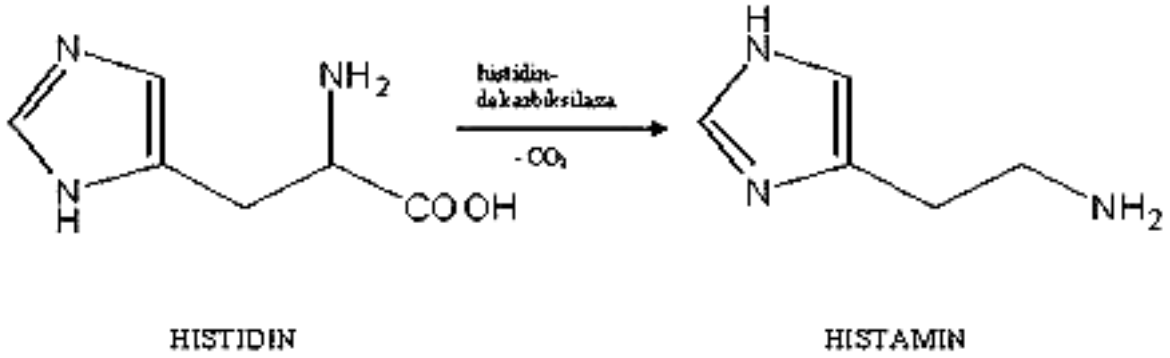
Kako se već godinama u svjetskoj znanstvenoj i stručnoj literaturi evidentiraju pozitivni učinci EPK i DHK na zdravlje, Američko udruženje za srce (AHA-*American Heart Association*) izdalo je službeni stav o preporukama za unos EPK i DHK koje se smatraju znanstveno utemeljenima i prihvaćenim širom svijeta (Kris-Etherton i sur., 2002.). Te se preporuke temelje na različitim potrebama za višestruko nezasićenim masnim kiselinama zdravih osoba, osoba koje boluju od koronarnih bolesti srca i osoba kojima je cilj prehranom sniziti koncentraciju serumskih triglicerida.

Osobe koje nemaju dijagnosticirane koronarne bolesti srca tjedno bi preventivno trebale konzumirati 2 jedinice serviranja plave ribe bogate n-3 višestrukonezasićenim masnim kiselinama, čime se osigurava oko 0,3–0,5 g/dan EPK i DHK. Također se dodatno preporučuje dnevni unos 0,8–1,1 g α-linolenske masne kiseline. Osobe koje boluju od koronarnih bolesti srca trebale bi svoj dnevni unos povećati na otprilike 1g EPK i DHK (uz unos 0,8–1,1 g/dan α-linolenske kiseline). Taj se unos može postići konzumiranjem 4 jedinice serviranja plave ribe tjedno. Osobama kojima je cilj prehranom sniziti koncentraciju serumskih triglicerida preporučuje se konzumacija 2–4 g/dan EPK i DHK (Kris-Etherton i sur., 2002.).

Potrebno je također istaknuti važnost dovoljnog unos plave ribe tijekom trudnoće i razdoblja dojenja budući da su višestruko nezasićene masne kiseline nužne za razvoj mozga i živčanog sustava djeteta, a potječu izravno iz majčine prehrane (Imhoff-Kunsch i sur., 2011; Krešić i sur., 2013). Dovoljna opskrba višestruko nezasićenim masnim kiselinama u dojenačkoj dobi također je nužna za pravilan razvoj i funkcioniranje retine oka (Makrides i sur., 2011; Bernardi i sur., 2012).

Nastajanje histamina kao rizik za sigurnost konzumacije ribe

Histamin (β-imidazol-etilamin) se u ribi oblikuje *post mortem* bakterijskom dekarboksilacijom esencijalne aminokiseline histidina (Kim i sur., 2002). Dekarboksilaciju histidina vrši enzim histidin-dekarboksilaza (Bogdanović i sur., 2009; Cuculić i sur.,1984; Leigh i sur., 2000; Taylor i sur., 2003) (Slika 1).

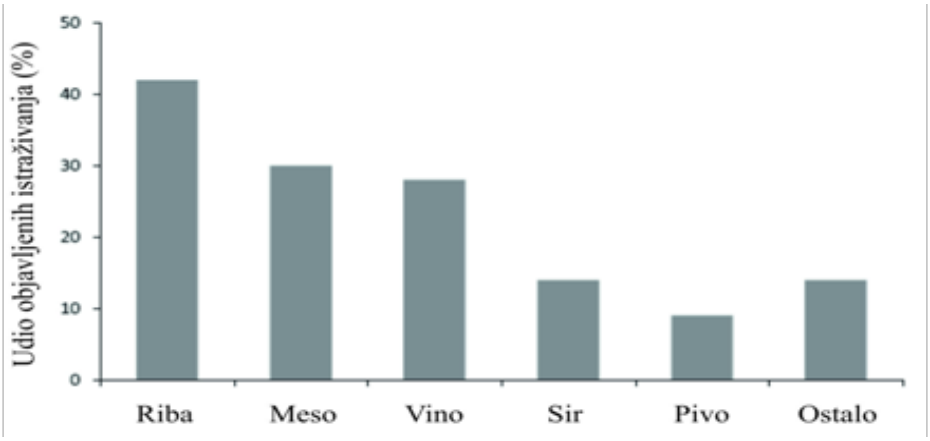


Slika 1. Dekarboksilacija slobodne aminokiseline histidina u histamin uz djelovanje enzima histidin- dekarboksilaze (Bogadanović i sur., 2009)

Fig.1. Decarboxylation of free amino acid histidine to histamine with the activity of histidine decarboxylase (Bogdanović et al., 2009)

Razine histidina variraju od 1 g/kg u haringama do 15 g/kg u mesu tunjevine. Toksični učinak histamina pojačavaju biogeni amini putrescin i kadaverin (Bogdanović i sur., 2009, Bulushi i sur., 2009, Kuley i sur., 2005, Maintz i Novak, 2007., Park i sur., 2010, Rossi i sur., 2002, Erim, 2013). Najjači proizvođači histamina su bakterije *Morganella morganii, Klebsiella pneumoniae* i *Hafnia alvei.* Prirodno su im stanište škrge i crijeva (utroba) živih riba a te bakterije ni na koji način ne ugrožavaju ribu. Međutim, nakon uginuća, obrambeni mehanizmi u organizmu ribe više ne mogu inhibirati rast bakterija i bakterije koje su naročito aktivne u nastajanju histamina se razmnožavaju i produciraju ga.

Histidin kao esencijalna aminokiselina podložna dekarboksilaciji ulazi također u sastav većine proteinskih namirnica (meso, mlijeko, sirevi, riba, bjelanjak, mahunarke) (Bender i sur., 2010; Masasaki i sur., 2004; Naile i sur., 2011) pa se osim u ribi histamin u značajnim količinama može nalaziti i u vinu, pivu, siru, kiselom kupusu, fermentiranim mesnim te sojinim proizvodima (Nosić, 2010, Lehane i sur., 2000). Za razliku od nastanka u ribi, koji je uvjetovan djelovanjem gram-negativnih bakterija, histamin u vinu i siru nastaje kao rezultat aktivnosti gram-pozitivnih bakterija (Lin i sur., 2005, McInerney i sur., 1996; Prester i sur., 2010). Histamin, putrescin, kadaverin, tiramin, triptamin, β-feniletilamin, spermin i spermidin se ubrajaju u skupinu biogenih amina i među najvažnijima su koji se mogu pojaviti u hrani (Erim,2013). Osim navedenih biogenih amina u ribi se još mogu pojaviti agmatin i serotonin (Naila i sur., 2011). Histamin je jedini biogeni amin koji je reguliran Pravilnikom o mikrobiološkim kriterijima za hranu (Nosić, 2010, Prester, 2011). Iz pregleda istraživanja biogenih amina u svijetu, provedenih u 2011. i 2012. godini vidi se da su najčešće ispitivane namirnice upravo riblji proizvodi, a slijede meso i vino (Erim, 2013) (Slika 2.)

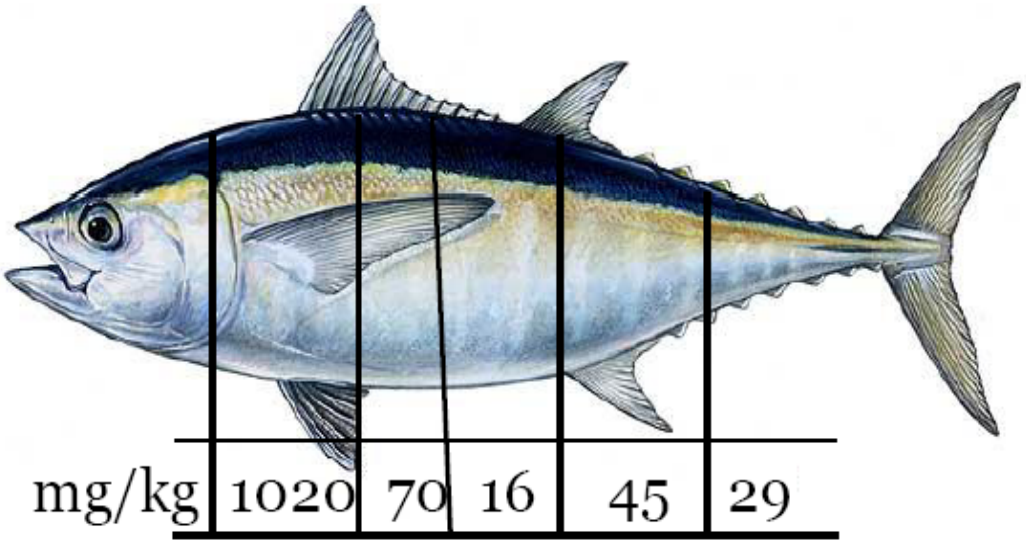


Slika 2. Distribucija istraživanja biogenih amina u različitim skupinama namirnica provedenim 2011. i 2012. godine (Erim, 2013)

Fig. 2. Distribution of researches concerning biogenic amine in various food groups conducted in 2011 and 2012 (Erim, 2013)

Istraživanja o biogenim aminima (među kojima se najčešće istražuje histamin) u hrani (najčešće u morskoj ribi) se provode s različitim ciljevima. Jedan od ciljeva je definiranje povezanosti koncentracije histamina u ribi i vrste bakterija koje ga mogu producirati (Erim, 2013), dok ostali ciljevi obuhvaćaju razvoj novih ili poboljšanje postojećih analitičkih metoda za detekciju histamina, izvještavanje o sadržaju biogenih amina u proizvodima iz različitih zemalja i regija te određivanje sadržaja biogenih amina u svrhu kontrole efikasnosti metoda razvijenih u pripremi, skladištenju i pakiranju hrane (Erim, 2013).

Inaktivacija histidin-dekarboksilaze se uglavnom odvija na temperaturi iznad 65°C. Upravo zato je koncentracija biogenih amina, prilikom kuhanja, 4-10 puta niža nego prilikom soljenja i sušenja (Bogdanović i sur., 2009). Jednom nastao enzim histidin-dekarboksilaza može nastaviti producirati histamin u ribi i u slučaju kada bakterije nisu aktivne. Taj enzim može biti aktivan i na temperaturama konzerviranja hlađenjem. Vrlo je vjerojatno da enzim ostaje stabilan i prilikom konzerviranja smrzavanjem te da se može vrlo brzo reaktivirati nakon odmrzavanja. Kada jednom nastane histamin nije podjednako raspoređen na svakom mjestu u ribi (Slika 3.).



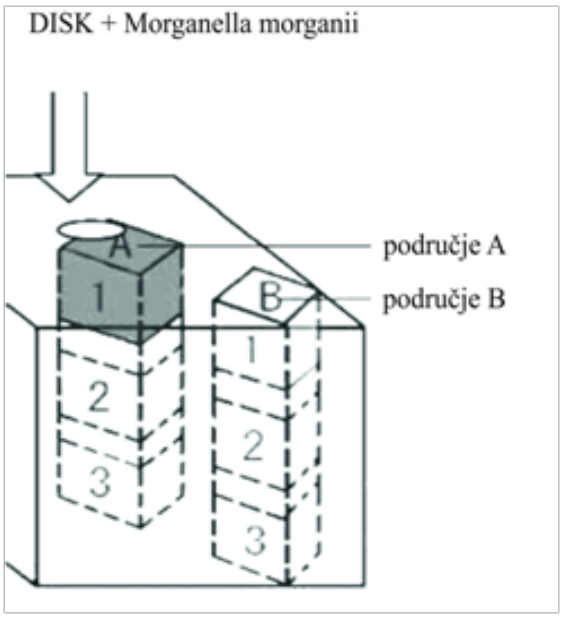
Slika 3. Raspodjela histamina u mesu ribe (Šimat, 2011)
Fig.3. Distribution of histamine in muscle of fish (Šimat, 2011)

U vrste riba koje sadrže visoku razinu *slobodnog* histidina, pa prema tome predstavljaju i rizik za histaminsko trovanje, ubrajaju se sljedeće: inćun, bonito (tunj), haringa, mlada štuka, skuša, lokarda (*Scombridae*), srdela, papalina i tunjevina. Bijela riba (npr. oslić) sadrži tek neznatne količine slobodnog histidina.

Prema nekim istraživanjima najveće se koncentracije histamina u kontaminiranom ribljem mesu s *Morganellom morganii* postižu, kada ta bakterija dođe u stacionarnu fazu rasta. Razlike u fazama rasta mikroorganizama nastaju zbog promatranja različitih vrsta mikroorganizama koji produciraju histamin kao i različitih vrsta riba - morskih ili oceanskih (Lee i sur., 2012).

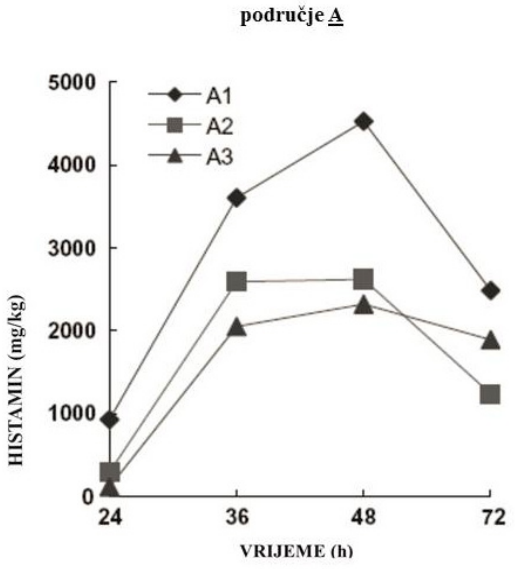
Mehanizam difuzije histamina u mesu ribe

Mehanizam difuzije histamina u mesu ribe je najviše proučavan u mesu tune (*Thunnus obesus*). Meso ribe je inokulirano bakterijom *Morganellom morganii* koja može producirati enzim histidin-dekarboksilazu i tako pretvarati aminokiselinu histidin u histamin. Shematski prikaz difuzije histamina u mesu tunja prikazan je na slikama 4. i 5. Budući da je optimalna temperatura za formiranje histamina pomoću bakterije Morganelle morganii 25°C, upravo na toj temperaturi su bili pohranjeni ispitivani uzorci.



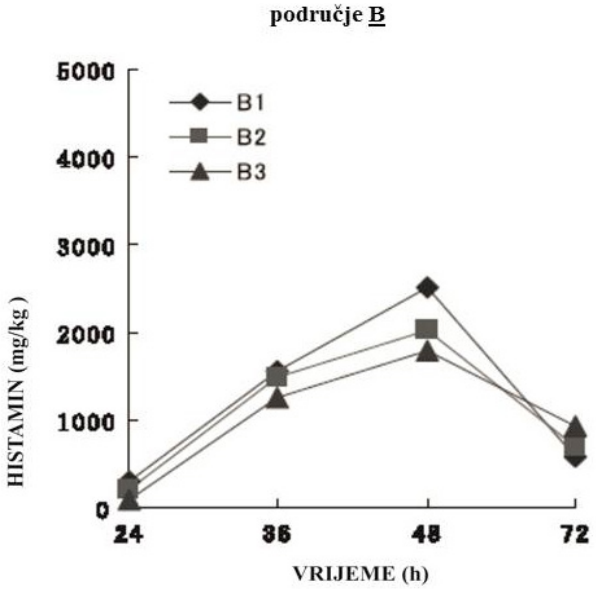
Slika 4. Shematski prikaz A- i B- područja difuzije histamina u mesu tunja (Tao i sur., 2009)
Fig.4. Diffusion of histamine in muscle of tuna fish A- and B- area (Tao et al., 2009)

Ustanovljeno je da histamin ne nastaje samo u području u kojem je inokulirana ta bakterija (područje A-1), već i vodoravno i okomito na udaljenim područjima mesa ribe (područja B-1, B-2 i B-3, kao i A-2 i A-3) (Slika 4). Kada su uzorci mesa bili pohranjeni na temperaturi 25°C, uočeno je nastajanje velike količine histamina u području A-1.



Slika 5. Formiranje histamina i difuzija u mesu *T. obesus* nacjepljenom s *M. morganii*, područje A (Tao i sur., 2009)
Fig.5. Histamine formation and diffusion in meat of *T.obesus* inoculated with M.morganii, area A (Tao et al., 2009)

Nakon 48 sati izmjerene su najviše koncentracije histamina koje su iznosile više od 4 000 mg/kg. U A-2 i A-3 području je nastajanje histamina bilo sporije. Najveća koncentracija histamina u području A-3 je bila oko 2 000 mg/kg. Obrazac promjene histamina u A-2 i A-3 području bio je isti kao u A-1 području, tj. uočljiva je najveća koncentracija nakon 48 sati (Slika 5).



Slika 6. Formiranje histamina i difuzija u mesu *T. obesus* nacjepljenom s *M. morganii*, područje B (Tao i sur., 2009)
Fig.6. Histamine formation and diffusion in meat of *T.obesus* inoculated with M.morganii, area B (Tao et al., 2009)

U udaljenom B-području je histamin nastao kasnije nego u inokuliranom A-1 području. U B- području je najviša koncentracija histamina bila 2 500 mg/kg a izmjerena je također nakon 48 sati. Dok je tijekom vremena do 48 sati uočljiv kontinuirani porast koncentracije histamina uz konstantno najvišu vrijednost u zoni B1, nakon 72 sata uočljiv je značajan pad njegove koncentracije koja je podjednaka za sva tri područja (oko 1000 mg/kg) (Slika 6).

Treba istaknuti da se bakterija (npr. *Morganella morganii*) prvo proširi po određenom području mesa ribe, a tek onda počinje pretvarati histidin u histamin u području koje je inficirala (Tao i sur., 2009).

Stav istraživača je da bi praćenje razmnožavanja bakterije *Morganella Morganii* bilo dobro implementirati u HACCP sustave tvornica ribljih proizvoda kako bi se sa sigurnošću

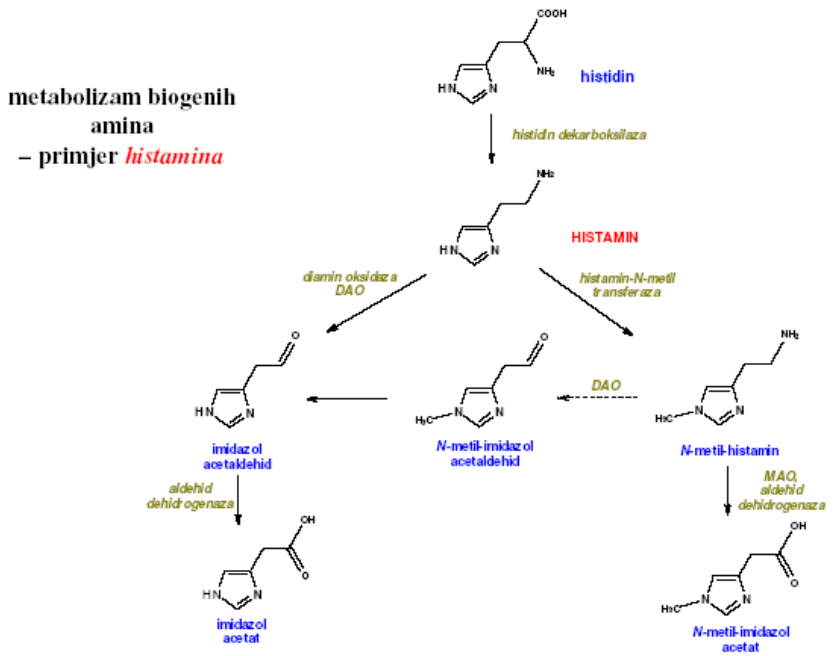
moglo nadzirati nastajanje histamina (Kim i sur., 2002). Primjećeno je da γ- ionizirajuće zračenje može smanjiti mikrobnu populaciju *Morganelle moraganii* te da se mikrobna populacija te bakterije još više smanjuje s povećanjem doza ionizirajućeg zračenja. γ- ionizirajuće zračenje od 2.5 do 3.0 kGy reducira mikrobnu populaciju Morganelle morganii toliko da se više ne može kvantificirati. Potpuno suzbijanje ove bakterije primjećeno je kada doza ionizirajućeg zračenja iznosi više od 3.5 kGy (Daisuke i sur., 2012).

Simptomi histaminskog trovanja u čovjeka

Pretpostavlja se da službene statistike ne prikazuju realan broj slučajeva histaminskog trovanja jer se često puta njegovi simptomi zamjenjuju s preosjetljivošću na morske ribe, rakove, mekušce i druge plodove mora (Singh i sur., 2012; Cuculić i sur., 1984; Lin i sur., 2012). Također se simptomi histaminskog trovanja često mogu zamijeniti s trovanjem Salmonellama (Yesudhason i sur., 2013), pa se opravdano pretpostavlja da je prijavljeni broj oboljelih samo "vrh ledene sante" (Mulić i sur., 2004).

Budući da histaminsko trovanje predstavlja skupinu simptoma i okolnosti smatra se da ne može postojati samo jedan mehanizam koji bi objašnjavao ovu vrstu toksičnog djelovanja (Bulushi i sur., 2009, McInnery i sur., 1996; Santos,1996; Shakila i sur., 2001; Shalaby,1997). Dva glavna enzima koji razgrađuju histamin su DAO (diamin-oksidaza) i HMT (histamin-N-metiltransferaza) (Bender i sur., 2010; Lehane i Olley, 2000; Naile i sur., 2012, Taylor, 1986). Studije pokazuju da su na histamin osjetljivi pojedinci kojima u sluznici tankog crijeva manjka enzim DAO što uzrokuje smanjenu razgradnju i povećanu apsorpciju histamina u gastrointestinalnom sustavu (npr. osobe sa ulceroznim kolitisom ili Chronovom bolešću). Te osobe imaju genetsko ili stečeno oštećenje enzimskih funkcija DAO-a ili HMT-a (Bender i sur., 2010).

Zdravi ljudski organizam će tolerirati određenu količinu histamina bez ikakve reakcije, a uneseni histamin će se detoksificirati u crijevima uz pomoć DAO. Kod ljudi se 68% -80 % oralno unesenog histamina apsorbira u crijevu (Naile i sur., 2012; Bender, 2010). U mozgu nema DAO-a pa je N-metilacija glavni proces koji regulira funkciju i metabolizam histamina, za razliku od dišnih puteva i želuca (Slika 7.) (Halasz i sur.,1994).Ovaj zaštitni mehanizam prestaje djelovati, ako je unos histamina i/ili drugih biogenih amina vrlo visok ili ako su navedeni enzimi blokirani drugim tvarima.



Slika 7. Metabolizam histamina (Maintz, Novak 2006)

Fig.7. Histamine metabolism (Maintz, Novak 2006)

Neki od lijekova koji mogu blokirati DAO su: kontrastna sredstva, mišićni relaksatori, narkotici, analgetici, lokalni anestetici, antihipertenzivi, antiaritmici, antidepresivi, tuberkulostatici, neki antibiotici i dr. Ima i lijekova koji mogu potaknuti oslobađanje histamina (Bender i sur., 2010; Križek i sur., 2014). Dominantni simptomi histaminskog trovanja vezani su uz kožne manifestacije (crvenilo praćeno žarenjem i znojenjem), zatim glavobolje, mučnine, pritisak u prsim uz smetnje disanja praćeno crvenilom očiju te slabošću (Bakašun i sur., 1985). Raznolikost simptoma ovisi o količini konzumiranih namirnica, tjelesnoj masi potrošača i osjetljivosti pojedinaca (Yesudhason i sur., 2012).

Period inkubacije kod histaminskog trovanja traje od 5 minuta do sat vremena, a simptomi traju od nekoliko sati do 24 sata. Liječenje se provodi uglavnom kod težih slučajeva trovanja primjenom antihistaminika (npr. dimidril) ili blokatora histaminskih receptora (npr. cimetidin) (Nosić, 2010). Kod osoba s kroničnim srčanim oboljenjima, visokim tlakom i oboljenjima organa za disanje histaminsko trovanje može dovesti do znatnog pogoršanja zdravstvenog stanja (Cuculić i sur., 1984, Boarde i sur., 2007).

Statistički podaci o histaminskim trovanjima

RASFF (*Rapid Alert System for Food and Feed*) je pristupna baza tj. svojevrsno komunikacijsko oruđe za izmjenu informacija između zemalja članica Europske Unije sa ciljem žurnog odgovaranja na prijetnje za sigurnost hrane. Portal RASFF je ustanovljen od strane Europske komisije koja ga i održava (Odjel za zdravlje i zaštitu potrošača), a svakodnevno se nadopunjava s podacima iz Unije, kao i s onima koji dolaze iz zemalja koje nisu članice Unije. Podaci koji dolaze u ovu bazu ovise o programima nadzora u nekoj državi kao i o efikasnosti državnih laboratorija koji nisu sasvim usklađeni među svim članicama RASFF-a. Preko polovica (60%) podataka u RASFF-u potječe iz Italije, Njemačke, Velike Britanije i Španjolske, a ostatak iz ostalih zemalja te od službi Europske Komisije. Sustav trenutno ima 34 zemlje članice, a Republika Hrvatska koja je postala članica 1. srpnja 2013. je u određenim korelacijama sa Sustavom još od 2009. godine. Za potrebe ovog rada izvršeno je pretraživanje RASFF baza podataka vezano za sadržaj histamina u ribi.

U podacima o histaminu od 1979. do 1994. ima 7 bilješki za plavu ribu, kako slijedi: ulovljena tuna (2), konzervirana tuna (2), sardine (2) i ostala riba (1). Za tih 7 slučajeva u RASFF sustavu nema zabilježenih rezultata za koncentracije histamina dobivene laboratorijskim analizama (u mg/kg).

U podacima o histaminu od početka 1995. do kraja 2001. godine zabilježeno je 35 slučajeva trovanja ribom i ribljim proizvodima. U bilješci od 18. prosinca 2001 su navedene velike količine histamina u inćunima s kaparima u ulju, i to redom: 1358, 1559, 1037, 1183, 1210, 1279, 1161, 1031, 954 mg/kg. U uzorkovanju od 23. svibnja 2001. godine su u konzervama tune u biljnom ulju zabilježene sljedeće koncentracije histamina: 194, 821, 259, 331 i 287 mg/kg. U bazi podataka postoji 315 bilješki za period od 1. siječnja 2002 do 31. prosinca 2010., a u osam slučajeva je koncentracija histamina bila iznad 4000 mg/kg. Podaci iz RASFF baze podataka pokazuju da mogu postojati velike razlike u analizi histamina između uzoraka koji su uzeti iz iste šarže. U jednom slučaju je raspon rezultata u 9 uzetih uzoraka bio od 12 mg/kg do 1660 mg/kg. U drugom slučaju je uzeto 11 uzoraka a raspon rezultata bio je od 50 do 500 mg/kg. Najveća zabilježena koncentracija histamina u RASFF bazi je 10g/kg, a odnosila se na tunj filete u biljnom ulju (Leuschner i sur., 2013). Od 1. siječnja do 31. prosinca 2010. histamin je zabilježen u 39 slučajeva ribe i ribljih proizvoda (koncentracija je bila veća od 200 mg/kg). Od toga su se 22 slučaja odnosila na tunu, 6 na sardine, 4 na inćune, 2 na skuše, 1 slučaj na marinirane haringe dok u ostalim slučajevima ribe nisu specificirane.

RASFF sustav šalje „Upozorenje“ (*Alert*), za čitavo područje koje pokriva, kada hrana (u ovom slučaju riba i proizvodi od ribe) predstavlja visok rizik za tržište. Prema godišnjim izvještajima “Upozorenje” je poslano u 2013. godini 77 puta, u 2012. godini 63 puta, u 2011. godini 95 puta i u 2010. godini 111 puta. RASFF sustav šalje i „Zabranu prelaska granice“ (*Border rejection notification*), za čitavo područje koje pokriva, kada hrana (u ovom slučaju riba i proizvodi od

ribe) predstavlja visok rizik za zdravlje ljudi ali i životinja. Prema godišnjim izvještajima „Zabrane prelaska granice“ su poslane u 2013. godini 86 puta, u 2012. godini 166 puta, u 2011. godini 217 puta i u 2010 godini 183 puta (Rapid Alert System for Food and Feed, 2010, 2011, 2012, 2013).

U Hrvatskoj je u razdoblju od 1.1.1992. do 31.12.2001.godine histaminsko trovanje hranom uzrokovalo 4 epidemije od kojih su tri (75 %) nastale nakon konzumacije plave ribe (srdela, inćuni, tuna). Epidemija koja je izbila 1998. godine u Rijeci nastala je u transportnih radnika koji su prenosili riblje brašno (Mulić i sur., 2004). Inćuni u ulju u konzervama (50 g) koji su uzorkovani od hrvatskih proizvođača, u periodu od svibnja 2009 do rujna 2011. sadržavali su prosječnu vrijednost histamina 6.5 ppm. (Koral i sur., 2013).

Studije slučaja histaminskog trovanja u Hrvatskoj

Trovanje plavicom

Strani turisti su dobili ribu od poznanika koji su je kupili na tržnici. Jedan dio su konzumirali, a ostatak držali dva dana u hladnjaku. Nakon toga su ribu poklonili turistima koji su je zatim jedan dan držali na sobnoj temperaturi, a potom pržili. Hospitalizirani bolesnici bili su mlađe osobe, jedan 20, a drugi 22 godine. Simptomi histaminskog trovanja su se počeli pojavljivati jedan sat nakon obroka. Osobe koje su bile u pratnji oboljelih izjavile su kako su oboljeli po licu i vratu bili “crveni kao rak”. Na liječenje su primljeni četiri sata nakon početka tegoba tako da su promjene na koži bile malo uočljivije. Žalili su se na klonulost i umor, te grčeve u trbuhu i mučninu. Uz uobičajeni način liječenja stanje se brzo popravilo tako da su slijedećeg dana otpušteni na kućnu njegu (Cuculić i sur., 1984).

Trovanje smrznutom tunjevinom iz hladnjaka restorana

Nakon obroka pržene tunjevine u jednom restoranu su oboljele tri osobe, a simptomi su se javili unutar jednog sata po obroku. Očitovali su se crvenilom kože, osipom, žarenjem i znojenjem kože, lupanjem srca, slabošću, umorom, žarenjem u ustima i crvenilom očiju. Jedan od njih imao je pritisak u prsima, a uz bolove i grčeve u trbuhu praćene mučninom javio se i proljev. Svi su pri konzumiranju imali peckav okus u ustima i pri jelu su pomišljali da se radi o nekom začinu. Uzorci ribe su dostavljeni inspekciji koja je utvrdila povišenu koncentraciju histamina (Cuculić i sur., 1984).

Slučaj iz tvornice za preradu ribe

Opis simptoma kod inspektora nakon što je konzumirao ribu iz limenki kojima je zabranjeno puštanje u prodaju: “Nakon probanja i kada se riba pojede ostaje u ustima, ždrijelu i jednjaku kao i u želucu neki ružan okus i osjećaj žarenja. Nakon dva sata javio mi se osjećaj mučnine i glavobolje, a poslije podne i povraćanje. Moram napomenuti da sam žućni bolesnik pa je kod mene reakcija vjerojatno jača nego kod zdravog organizma. Polazim od pretpostavke da veliki broj ljudi uzima riblju konzervu kao laganu, čak dijetalnu hranu, pa se bojim da bi konkretno ovakva konzerva mogla dovesti do neželjenih posljedica po potrošača” (Cuculić i sur.,1984).

Sprječavanje histaminskog trovanja

Optimalna temperatura za mikrobiološku fazu nastajanja histamina je oko 20°C, a ubrzano nastajanje histamina je primijećeno u mesu tunjevine pri 25 °C (Daisuke, 2014). Histamin se odlikuje velikom termorezistencijom, tj. prema nekim autorima izdržava čak i temperaturu od 200°C čime predstavlja veliki problem u industriji ribljih konzervi jer ga ne mogu uništiti temperature sterilizacije (Bogdanović i sur., 2009).

Ako se riba ne drži na temperaturama hlađenja (ili nižim) duže od 16 sati dolazi do nastajanja visokih koncentracija histamina. Bakterije proizvođači histamina, se za vrijeme od 24 sata mogu dovoljno razmnožiti da stvore toksičnu koncentraciju histamina (Naila i sur., 2011). Jedina učinkovita metoda sprječavanja nastajanja histamina u morskoj ribi je skladištenje ribe na temperaturi nižoj ili jednakoj 4,4 °C u svakom trenutku od ulova do potrošnje (CDC, 2008).

Brzo hlađenje ribe odmah nakon izlovljavanja

je najvažniji element u strategiji prevencije kvarenja ribe, a samim tim i stvaranja biogenih amina u njoj. Ovo se posebno odnosi na ribu koja je izložena višim temperaturama vode ili zraka, kao i na velike tune koje zadržavaju toplinu u tkivima i poslije smrti (Smajlović i sur., 2008). Osim brzog hlađenja ribe nakon ulova, važno je i postupanje s ribom u trgovinama na veliko i malo, gdje se savjetuje čuvati na temperaturama hlađenja ili smrzavanja. Međutim ni to ne može dati odgovarajuće rezultate ako kupac, kao važna karika u lancu sigurnosti hrane, brzo ne transportira ribu svojoj kući i ne uskladišti ju na odgovarajuće nisku temperaturu dok ju termički ne obradi i konzumira (Silva i sur., 2011).

S ciljem suzbijanja nastajanja histamina se na suvremeno opremljenim ribarskim brodovima, nakon ulova, s ribom treba postupati na ispravan način. Nakon što iskrvari ribu treba eviscerirati, isprati s filtriranom i ohlađenom morskom vodom te ukloniti višak vode. Ribe treba pokriti s pamučnim pokrivačem kako bi se zaštitile od direktnog kontakta sa ledom i skladištiti u rashladnim komorama s ledom izrađenim od klorirane vode u omjeru jedan dio leda naprama jednom djelu vode. Sve se to treba napraviti do 10 minuta nakon ulova (Oliveira i sur., 2012.).

Prema FDA preporukama, za sprječavanje pojave rasta patogenih bakterija u proizvodima ribarstva se predlaže održavanje pH vrijednost između 4,0 i 5,0 ovisno o bakterijskim vrstama. FDA također sugerira da pH treba biti ispod 4,6 ako se želi izbjeći nastajanje bakterije *Clostridium botulinim* u ribljim proizvodima pakiranim u vakuum vrećicama ili u limenkama (Koral i sur., 2013).

Postupak soljenja ne može jamčiti nisku razinu histamina kao niti ostalih biogenih amina iako je opaženo blago opadanje udjela histamina tijekom procesa zrenja slanih inćuna, što je objašnjeno difuzijom histamina u salamuru (Bogdanović i sur., 2009; Koral i sur., 2013).

Promjene na ribi nisu dobro mjerilo za procjenu toksične količine histamina, već je nužna laboratorijska analiza (Bogdanović i sur., 2009, Bulushi i sur.,2009). U analizu se uzima 600 do 800 grama mesa za svaki od 9 uzoraka ribe, stavlja u sterilne vrećice i drži u ledu sve dok se ne dostavi u nadležni laboratorij na analizu (Oliveira i sur., 2012). Prema Pravilniku o mikrobiološkim kriterijima za hranu (2008) iz svake serije treba uzeti 9 uzoraka koji moraju udovoljavati sljedećim uvjetima: dva uzorka mogu sadržavati više od 100 mg/kg, a manje od 200 mg/kg histamina, a niti jedan uzorak ne smije sadržavati više od 200 mg/kg histamina (Nosić, 2010; Muscarella 2013)

Zaključak

Plava riba je zbog svog nutritivnog sastava namirnica koju se savjetuje konzumirati tjedno u količini od 2-3 jedinice serviranja, a budući da je riba iz te skupine najčešće rizična za nastajanje histamina i pojavu histaminskog trovanja, nužno je ulagati napore u zdravstveno prosvjećivanje stanovništva o prednostima ali i rizicima vezanim uz konzumaciju plave ribe. Histamin je od 1973. godine postao globalni problem. Od tada su učinjeni mnogi pomaci, no oni su, čini se, u pojedinim područjima svijeta ipak nedovoljni jer se slučajevi histaminskog trovanja još uvijek javljaju, a često puta su i medijski popraćeni te mogu prerasti u javnozdravstveni problem. Budući da je histamin termostabilan, jedina učinkovita metoda sprječavanja histaminskog trovanja je skladištenje ribe na temperaturi nižoj ili jednakoj 4,4 °C u svakom trenutku od ulova do potrošnje. Vrijedan doprinos povećanju protočnosti informacija vezanih uz sigurnost hrane pruža RASFF sustav.

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OILY FISH CONSUMPTION- BENEFITS AND SOME HEALTH RISKS

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Review

Summary
Consumption of oily fish is highly recommended nowadays due to its nutritional composition characterissed with proteins of high biological value, natural sources of selenium and iodione and low amount of fats and cholesterol. This food is valuable source of long-chain polyunsaturated fatty acids (from ω-3 classes) whose consumption is related to numeorous health-benefts. However, despite positive effects of cinsumption there are also some health-risks i.e. histamine posioning. Histamine fish poisoning causes 5% of food related illnesses and 37% of fish related food poisoning , and it is the most common fish-caused poisonong worldwide. Histamine in fish is formed post mortem due to the bacterial activity (*Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei*) which convert aminoacid histidine into histamine. The fish species which contain high amounts of histidine and are dangerorus due to the histamine formation are anchovy, herring, mackerel, sardines, sprat and tuna. Incubation perod is from 5 minutes to 24 hours while the symptoms lasts several hours to one day. Beside nutritional benefits of oily fish consumption this review provides an overview of one of the helath risk of consumption, namely histamine poisoning, about formation and diffusion mechanism of histamine in muscle of fish and also about prevention of histamine fish poisoning. Notifications concerning histamine in fish products, from RASFF (Rapid Alert System for Food and Feed) database (1979- 2010) are discussed. The case studies of histamine fish poisonongs in Croatia are also described.

Key words: histamine, nutritive value, oily fish, RASFF, poisoning

Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2015) 4 (1) 28-33

DETERMINATION OF CALCIUM CONTENT IN DIETARY SUPPLEMENTS

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Summary

Calcium is a macro element that is very important for the human body: its content and circulation in the body is large, it serves as the electrolyte, it has a building role and participates in the process of metabolism. The European Union, the World Health Organization (WHO) and the Ministry of Food and Drug (Food and Drug Administration, FDA) gave the RDA (Recommended Dietary Allowances,) for this macro element. The absorption and bioavailability of the calcium may vary depending on a number of factors, and because all of the foregoing it is consumed by means of different supplements.

The aim of this study was to determine the content of calcium in the various diet products using the volumetric analytical method of analysis. Supplements that were analyzed are divided into two groups. The first group consists supplements in which the calcium is present in the form of different chemical compounds, and the second group consists of supplements of a number of different manufacturers in which the calcium is in the form of calcium carbonate.

Calcium content, obtained by applying the method above, which ranged from 95.11% to 99.80% compared to the theoretical value. Results were analyzed using the t-test, while not producing a statistically significant difference.

Keywords: calcium, supplements, volumetric analysis method

Introduction

In the chemical analysis different methods of qualitative and quantitative analytical chemistry are used (Harris, 1987). According to the type of physical size, which in the final analysis is the measurement methods of quantitative chemical analysis and it is divided into two main groups: classical methods of analysis and instrumental methods of analysis. Classical methods include gravimetric and volumetric methods of analysis, while the instrumental methods of analysis based on the measurement of physical quantities that are directly related to the amount of determined substances, such as: conductometry, potentiometry, photoelectric photometry, spectrophotometry, etc. (Vindakijević and Sladojević, 2005). Group of methods determining the volume of solution of known concentration of the substance, which came in response to the tested ingredient in foods, called volumetric methods (volumetric titration). The best known are the neutralization reaction, the redox and complexometric titration, which can be used for determining the content of certain vitamins and minerals in food (Grujić et al. 2007).

Calcium (Ca) is a metal that is of all of the mineral matter is the most present in a human body (Hass, 1992; Grujić and Miletić, 2006). Of the total body mass 1.5-2.0% is calcium. Most calcium is incorporated into bone (98%), about 1% is incorporated in the teeth, where it is in the form of $(PO_4)_2 \cdot Ca(OH)_2$ $Ca_5(PO_4)_3(OH)$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$. Bones contain 150 mg Ca /g of dry matter (Bronner, 1994; Otten et al., 2006). The remaining 1% of the total calcium is in the tissues and body fluids, or in soft tissues is 35 mg Ca /g of dry matter. A man weighing 70 kg in the body is about 1.54 kg of calcium.

Calcium plays several important roles in the human organism. The main role of calcium can be represented by the following: Calcium is a component of bones and teeth, calcium regulates the contraction and relaxation of muscles, calcium regulates the functioning of the nerve tissue, the calcium is responsible for the clotting of blood and takes part in the regulation of blood pressure

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and the signal transmission. Magnesium, together with calcium has a role in the functioning of the blood, the work of the nerves and muscles, in particular the regulation of muscle contraction and heart rate and conduction of nervous impulse. Calcium is excreted in the urine and feces (Grujić and Miletić, 2006).

The total amount of calcium consumed is not absorbed. Only 20-50% of the entered calcium is absorbed. The amount of calcium absorbed depends on the type of food, food composition and physiological state of the individual. In order to be able to absorb calcium from the food the presence of vitamin D, of phosphorus, and magnesium is necessary. Together with calcium in the bones and teeth phosphorus is embedded. Vitamin D is required to make the calcium (and phosphorus) absorbed in the digestive tract. Together with some hormones, vitamin D regulates calcium levels in the blood. The balance of calcium in the blood is essential for life, especially for the heart condition. The normal state of the blood calcium is about 10 mg /100 cm3 of blood. The calcium in the blood is in the ion form of Ca^{2+} (5.5 mg), attached to a protein (about 4.0 mg) or linked to the phosphate and citrate (approximately 0.5 mg) (Grujić and Miletić, 2006).

The efficiency of absorption of calcium from most food is about the same. Absorption of calcium may be less if the used foods are rich in oxalic acid (spinach, sweet potatoes and beans) and foods rich in phytic acid (unleavened bread, raw beans, seeds, nuts, soy isolate, and others) (Otten et al., 2006). Some organic acids (oxalic acid, phytic and others) with Ca^{2+} ions form the salt hardly soluble and thus hinder its absorption (Kerstetter et al., 2005). Efficiency of calcium absorption depends on the age of the individual and the greater the younger the person. Certain differences in the absorption of macro elements were observed due to differences in gender of the person, which is probably a consequence of the state of hormones. Absorption calcium in men, irrespective of age, is greater than for women of the same age (Hope et al., 1992; Bygrave and Benedetti, 1993).

Absorption of calcium is in inverse proportion to the amount entered calcium; if the input decreases the extent of absorption efficiency increases. However, the increase in absorption at extremely low calcium intake was not able to fully cover losses due to insufficient intake through food (Otten et al., 2006).

Foods in which the ratio of Ca:P is in the range of from 2:1 to 1:2 allow optimum absorption of Ca. If this relationship is different will be disrupted calcium intake (Allen, 1982; Anderson, 1991).

The main sources of calcium in the human diet are milk, cheese, meat, fish, vegetables and cereals, and its deficiency can lead to various disorders and diseases (osteoporosis, rickets, neurological disorders) (Szefer and Nriagu , 2007).

Chronic calcium deficiency can occur due to insufficient intake or poor absorption in the gut. Then there is its resorption from bone. In this way the health of the bone is endangered. Chronic calcium deficiency is regarded as one of the causes of the reduction of bone mass, osteoporosis and frequent bone fractures. In the United States annually more than 1.5 million bone fractures associated with osteoporosis happen (Otten et al., 2006).

Since absorption of calcium is dependent on many factors, the question of how much of the macro elements should be determined as a reference input. In setting the RDA for calcium Food and Nutrition Board of the National Academy of Sciences USA is estimated that 12-50% of the initial amount of calcium is available for use. RDA is set on a four-fold higher dose (800 mg Ca / day) so that this element was sufficient for the needs of the organism.

However, there are several categories of the population which can not provide optimal amounts of all necessary nutrients through the food. In addition, there are numerous situations in which people have the need for increased intake of certain nutrients. In the category of the population that has a need for a greater intake of vitamins and minerals are athletes, the elderly, pregnant women, menopausal women, people who do heavy physical work or those who are under great mental effort, and patients, convalescents, etc. (Gómez et al., 2011; Zofková et al., 2013; Fanian et al., 2013).

In these situations, the use of supplements can help, not only to avoid disease of deficient diet, but also to supplement the daily diet, and their

use is to improve the health of consumers (Anon, 2014).

The chemical forms of calcium whose use is permitted in the manufacture of nutritional supplements are: calcium-acetate, calcium L-ascorbate, calcium-bisglicinat, calcium-carbonate, calcium-chloride, calcium-citrate-malate, calcium citrate, calcium-gluconate, calcium-glycerophosphate, calcium-lactate, calcium-pyruvate, calcium salt of phosphoric acid, calcium-succinate, calcium L-lisinat, calcium-maltat, calcium-oxide, calcium L-pidolate, calcium L-treonat, calcium-hydroxide, calcium-sulfate (Anon, 2014).

Materials and Methods

Samples used in the process of volumetric determining are:

- "Calcium - gluconate ampoules, 1000mg/10ml",
- "Calcium-citrate, supplement",
- "Eunova-Multi-Vitalstoffe Langzeit 50%",
- "Calcium Complex 600",
- "Calcium with lemon flavor",
- "Effervescent tablets of calcium with orange flavor",
- "Calcimed Hermes 500 mg, effervescent tablets",
- "Calcium plus, 20 effervescent tablets with calcium and vitamins" and
- "Calcium, Vitamin D3".

Analyzed samples were obtained in the free market in Bosnia and Herzegovina and Serbia.

"Calcium-gluconate ampoules, 1000mg/10ml" is the supplement in which calcium is bound in the form of calcium-gluconate for injection ($C_{12}H_{22}CaO_{14}\cdot H_2O$). Calcium-gluconate for injection contains from 99.0% to 101.0% of calcium-D-gluconate, monohydrate. White, crystalline, or granular powder, little soluble in water, and easily soluble in hot water (Pharmacopoes Jugoslavica, 2000).

Data from the packaging - Manufacturer: Monico Spa; Country of origin: Italy; Importer for BiH: Pharmacy Medicus, Prijedor; Shelf life: 04.2015., Series: 12DA109.

"Calcium citrate, supplement" is a composition in which the calcium is bonded in the form of calcium citrate ($C_{12}H_{10}Ca_3O_{14}$), a colorless or white crystalline powder of organic tricarboxylic acids.

Data from the packaging - Manufacturer: Natural Wealth Nutrition Corp., Bohemia, NY; Country of origin: United States; Importer for BiH: M and D Company, Čitluk; Shelf life: 10.2016., Series PB 751001B.

"Eunova-Multi-Vitalstoffe Langzeit 50%" is a supplement in which the calcium is bonded in the form of a chemical compound of calcium-hydrogenphosphate ($CaHPO_4$), and calcium-D-pantothenate ($C_{18}H_{32}CaN_2O_{10}$). Anhydrous calcium-hydrogenphosphate contains from 98.0% to 101.0% $CaHPO_4$, calculated on the dry substance. It occurs as a white, crystalline powder or as colorless crystals, almost insoluble in water and alcohol. It dissolves in dilute hydrochloric acid and dilute nitric acid (Pharmacopoes Jugoslavica, 2000). Calcium-D-pantothenate is a white, almost odorless, slightly hygroscopic powder. It is easily soluble in water, soluble in glycerol, slightly soluble in ethanol and practically insoluble in ether and chloroform (The Chemical Company, 2005).

Data from the packaging - Manufacturer: Hemofarm; Country of origin: Serbia; Shelf life: 02.2015., Series: 990320.

"Calcium Complex 600" is a supplement in which calcium occurs as calcium-citrate ($C_{12}H_{10}Ca_3O_{14}$) and calcium-glycerophosphate ($C_3H_7CaO_6P$).

Data from the packaging - Manufacturer: Biofar, Nahterre; Country of origin: EU, France; Importer for BiH: Pharma Swiss, Serbia; Shelf life: 09.2015., Series: L2259/7.

"Calcium with lemon flavor", "Effervescent tablets of calcium with orange flavor", "Calcimed Hermes 500 mg, effervescent tablets", "Calcium plus, 20 effervescent tablets with calcium and vitamins" and "Calcium, Vitamin D3" are supplements in which the calcium is bonded in the form of calcium-carbonate ($CaCO_3$). Calcium-carbonate contains from 98.5% to 100.5% of $CaCO_3$, calculated relative to the dry substance. It is white powder, virtually insoluble in water (Pharmacopoes Jugoslavica, 2000).

Data from the packaging:

"Calcium with lemon flavor" - Manufacturer: Sunlife GmbH, Horelhof, Germany; Country of

origin: EU, Germany; Importer for BiH: Dugi Commerce, Široki brijeg, Interpromet; Shelf life: 06.2016., Series: L3163/5.
"Effervescent tablets of calcium with orange flavor" - Manufacturer: dm-drogeriemarkt, Karlsruhe, Germany; Country of origin: EU, Germany; Importer for BiH: dm drogeriemark, Ilidža; Shelf life: 01.2016., Series: L4028.
"Calcimed Hermes 500 mg, effervescent tablets" - Manufacturer: Hermes Arzeneimiittel, Munich; Country of origin: EU, Germany; Importer for BiH: Oktal Pharma, Ilidža; Shelf life: 10.2016., Series: 3108351.
"Calcium plus, 20 effervescent tablets with calcium and vitamins" - Manufacturer: Multivita, Vršac, Serbia; Country of origin: Serbia; Importer for BiH: ATACO, Mostar; Shelf life: 01.2016., Series: B304832.
"Calcium, Vitamin D3" - Manufacturer: Bifar, Nanterre- France; Country of origin: EU, France; Importer for BiH: Blagoleks, Bijeljina; Shelf life: 10.2016., Series: L3296/5.
Determination of calcium in these diet products was performed using standard complexometric analysis methods (Rajaković et al., 2000).

Results and discussion

Complexometric calcium determination is based on the reaction:

$$Ca^{2+}(aq) + H_2Y^{2-}(aq) + 2H_2O \leftrightarrow CaY^{2-}(aq) + 2H_3O^{+}(aq),$$
$$CaY^{2-}(aq) \leftrightarrow Ca^{2+}(aq) + Y^{4-}(aq)$$

For the complexometric determination of calcium is used as an indicator murexide. The sensitivity of this reaction is high. Titration with the murexide is done in a very alkaline environment (pH = 11-13), and the change in color of the indicator from red-purple to blue-violet is very easy to see. In a insufficient alkaline environment transition color is not sharp, and in a overly alkaline results are too low (Rajaković et al., 2000). For each analyzed sample is made five solutions of the calcium salt, at least five titrations were done, and from the volume of spent Complexone III middle values were calculated (Table 1 and 2).

Table 1. Content of calcium in dietary supplements in which the calcium is bonded in the form of different chemical compound

Dietary supplement	The mass of the analyzed sample (g)	Volumes of spent reagents (ml)	Calcium content (mg)
Calcium-gluconate ampoules, 1000mg/10ml	0.0186	4.60	183.60
Calcium-citrate, supplement	1.0245	4.87	194.41
Eunova-Multi-Vitalstoffe Langzeit 50%	0.5287	3.00	119.00
Calcium Complex 600	5.4852	14.33	572.00
Calcium, Vitamin D_3	4.4917	12.30	491.00

Table 2. Content of calcium in dietary supplements in which the calcium is bonded in the form of calcium-carbonate

Dietary supplement	The mass of the analyzed sample (g)	Volumes of spent reagents (ml)	Calcium content (mg)
Calcium with lemon flavor	3.9928	12.03	481.00
Effervescent tablets of calcium with orange flavor	4.1162	9.53	380.43
Calcimed Hermes 500 mg, effervescent tablets	3.8928	12.50	499.00
Calcium plus, 20 effervescent tablets with calcium and vitamins	3.7517	6.07	243.00
Calcium, Vitamin D3	4.4917	12.30	491.00

The results of the tests indicate no significant deviation between the values that are listed in the products and the value obtained by applying the used titrimetric methods of analysis (Figure 1 and 2). Statistical calculation of the t-test ($t=1.2087<t_{(4\ i\ 0.05)}=2.78$) it was found that the obtained difference in the values of the content of calcium, bound in the form of different chemical compounds, and the theoretical values of the content of calcium is not statistically significant. The value of t-test ($t=1.0995<t_{(4\ i\ 0.05)}=2.78$) for calcium in the form of calcium-carbonate also showed a statistically not substantively difference ($P>0.95$).

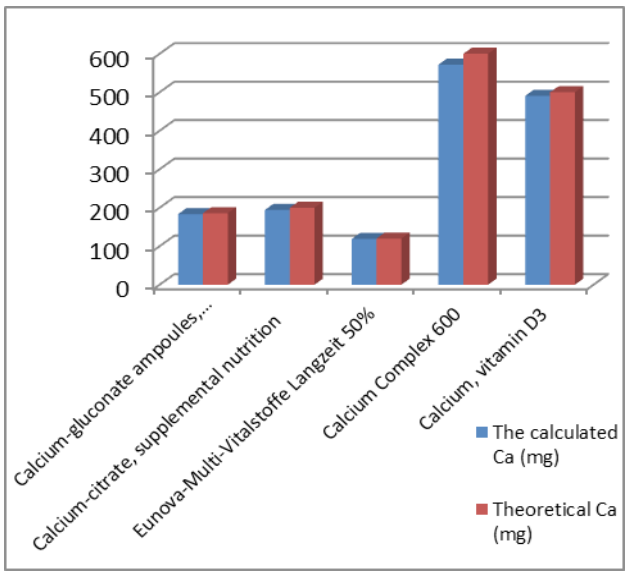

Figure 1. Calculated and the theoretically amount of calcium in supplements in which the calcium is bonded in the form of different chemical compounds

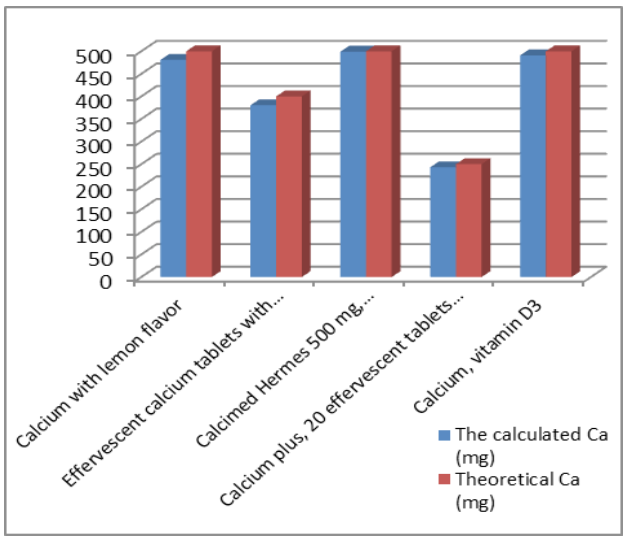

Figure 2. Calculate and the theoretical amount of calcium in supplements in which the calcium is bound in the form of calcium-carbonate

In the samples in which the content has been demonstrated that calcium is bonded in the form of different chemical compounds in the supplements, the difference ranging from 0.83% to 4.70%. With different samples of dietary supplements in which calcium is bound in the form of calcium- carbonate, the difference ranged from 0.20% to 4.80%. Recovery values of all tested dietary supplements are shown in Table 3. They were obtained as the ratio of the calculated mass of the tested calcium and theoretical mass of calcium analyzed sample multiplied by 100%. From the results it can be seen that the content of calcium, obtained by applying the methods above ranged from 95.11% to 99.80% compared to the theoretical value.

Table 3. Recovery values for all analyzed dietary supplements

Dietary supplements	**Recovery value (%)**
Calcium-gluconate ampoules, 1000mg/10ml	98.70
Calcium-citrate, supplement	97.20
Eunova-Multi-Vitalstoffe Langzeit 50%	99.17
Calcium Complex 600	95.30
Calcium, Vitamin D3	98.20
Calcium with lemon flavor	96.20
Effervescent tablets of calcium with orange flavor	95.11
Calcimed Hermes 500 mg, effervescent tablets	99.80
Calcium plus, 20 effervescent tablets with calcium and vitamins	97.20

Conclusion

Volumetry as a method of quantitative chemical analysis is in the classical methods analysis, which is still widely used in the determination of mineral matter.

In this paper we compared the results of the calcium content in the two groups of dietary supplements, while there was no statistically significant difference between the calculated and theoretical values. On that basis, it can be concluded that the volumetric analysis method suitable for the determination of calcium content in the tested pharmaceutical substances.

Deviations of the experimental data obtained in relation to the theoretical value, which is related to the amount of calcium bound in the form of different chemical compounds in the dietary supplements, amounted 0.83% to 4.70%.
The deviation obtained during the determination of calcium in the form of calcium- carbonate is of similar values, and ranges from 0.20% to 4.80%.

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MISCONCEPTIONS ABOUT NUTRITIONAL SUPPLEMENTS AND MODERN DISEASES

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Summary

It is very important to know all the requisite information on nutritional supplements before taking them. It is known that minerals and vitamins are necessary for optimal health. However, many of us just don't know what kind of minerals and vitamins to take which creates a lot of confusion, apathy, and frustration for a lot of people. Namely, numerous companies in the world are producing multi minerals and vitamins and promoting them as necessary for good health. But, at the same time are sabotaging the health of millions of people who are unsuspecting buyers of harmful and synthetic vitamins and minerals. The fact is that most mineral and vitamin brands on the market are synthetic, i.e. made from oxide minerals and other chemical substances. These products contain harmful substances, for example: preservatives, fillers, binders, coal tar, bitumen, gelatin, waste products etc. Consequently, these products cannot be digested by the human body and therefore cannot be assimilated. Moreover, they do not have a synergism due to being industrial-made and thus cannot find their way out of the body and thus get lodged in the tissues of the body causing disease or enhancing present diseases. Multi complexes are manufactured by pharmaceutical and food companies, and are not organic which is the state all digestible foods must be in for the body to break them down in order to absorb their nutrients. For those who are unsure, this article will provide some information to determine if vitamin tablets or multi mineral complexes are food or imitations.

Keywords: supplements, vitamins, minerals, diseases, nutrition.

Introduction

Nutritional supplements, and above all the vitamins and minerals, can be purchased today in supermarket or local drugstore, as well as at health food stores (Fig. 1). Whether the vitamins and minerals in the health food stores in some way better or were higher quality? Or it can be assumed that the ones in the supermarket or drugstore just as good? More than 95% of the vitamins, minerals and antioxidants that you can buy at "health food" stores and close to 100% of those sold in other stores are now made by the same few pharmaceutical and chemical companies who supply them to most all the vitamin and mineral companies. They are no longer the nutritional supplements they once were but are, more accurately, "nutraceuticals". In such a way, the drug companies are quiet taken over the nutrition business. Thus, for example, people tend to assume that vitamin C from one brand is pretty much the same as vitamin C from another brand. And in many cases they're right since the vitamin C came from the same original supplier. But what most people do not realize is that these vitamins and minerals are not true and complete vitamins and minerals as would be supplied by good organically-grown whole foods. In fact, they are chemical synthetics made from such things as coal tar, petroleum products, animal by-products, ground rocks, stones, shells and metal (Thiel, 2014; Hui, 1992). And not only do they not supply the benefits of the real vitamins and minerals but they can actually be quite harmful when taken over time (Chong, 2005). Especially in the mega doses so often recommended. Real vitamins only require very minute doses to be effective. Do you sometimes ask yourself, how

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many people suffer from rickets or scurvy? Probably none. What about cancer, heart disease, high cholesterol and diabetes? Probably a lot. The latter are all diseases that arise from having too much! Multi-vitamin won't help much there.

Figure 1. The vitamins & supplements department at a foods market (http://zoolies.us/natural-food-market. Retrieved: October 2014)

For a long time, public media present us daily interesting marketing propaganda in the field of health and nutrition. There are some rather disturbing marketing trends that are geared towards human health and their diet. Namely, print and television advertisements show athletes, pretty girls and attractive women while nibbling candy bar that has been fortified with a bunch of synthetic vitamins and minerals, as well as a whole host of other artificial additives and preservatives. Smiling children have fortified juices, cereal bars, and even gummy candies and chewing gum. What these ad campaigns don't show is how these products are obtained and how they are processed that foods may contain harmful ingredients like hydrogenated oils, synthetic vitamins, and neurotoxins. Specific nutrients that are shown to be beneficial in clinical studies are isolated, often in synthetic form, and heralded as new weapon against cancer, heart disease, old age, etc (Pietrzik, 1996). Even in the field of alternative health we find this same sort of reductionism going on. Herbal compounds are isolated, extracted and ingested in inappropriate quantities, without the synergy that the whole plant provides. However, there is something to be said for using plants, their fruits, and foods in their whole forms and for cultivating a relationship with the different energies offered by the natural products around us. It's very hard to improve on a diet of wild foods and herbs. Well-nourished bodies and minds enjoy balanced hormones and hearty immune systems. Daily input of nourishing products, such as fruits and vegetables, are a wonderful way to add extra nutrients to your diet. Unlike synthetic pills, natural products provide essential nutrients in a highly assimilable form.

Real or synthetic

Multivitamins are good for preventing conditions that arise from severe deficiency, but their helpfulness seems to taper off pretty quickly when the body is in a high calorie, insulin-rich physiological state that most people with a western diet find themselves in (Pietrzik, 1996). This isn't meant to demonize them, but to help people understand that taking one doesn't help the body exert the metabolic control needed to prevent diseases of convenience. Namely, Americans are spending more than $17 billion a year on supplements for health and wellness. Strangely enough, the rates of some forms of chronic disease have not changed, while the rates of others have actually increased. There are a number of reasons for these poor statistics and many things remain a mystery. One thing seems fairly clear, however. Most supplements aren't helping very much (Chong, 2005). An article

suggests that individuals frequently exceed safe nutrient levels when they take vitamin supplements (Troppman et al., 2002).

The truth behind whole-food supplements

Namely, whole food supplements are what their name suggests. Supplements made from concentrated whole foods (Fig. 2). The vitamins found within these supplements are not isolated. They are highly complex structures that combine a variety of enzymes, coenzymes, antioxidants, trace elements, activators and many other unknown or undiscovered factors all working together synergistically, to enable this vitamin complex to do its job in your body. Nutrients from within this complex cannot be taken apart or isolated from the whole, and then be expected to do the same job in the body as the whole complex is designed to do.

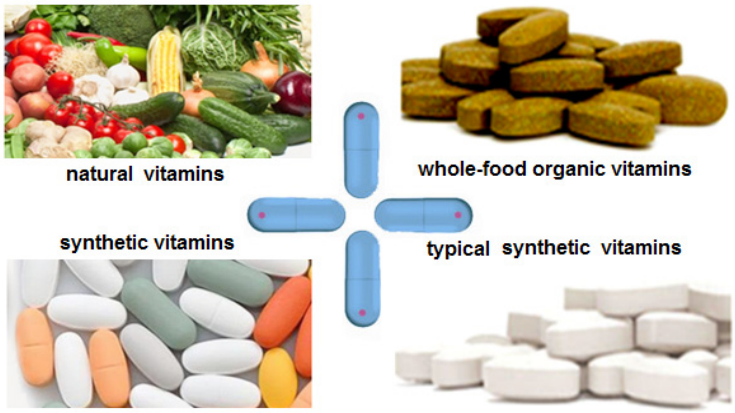

Figure 2. The whole-food vitamins product compared to a traditional synthetic brand (http://blog.healthkismet.com/vitamins. Retrieved: October 2014)

The perfect example of this difference can be understood on the example of an automobile (Chong, 2005). An automobile is a wonderfully designed complex machine that needs all of its parts to be present and in place to function properly. Wheels are certainly an important part of the whole, but you could never isolate them from the rest of the car, call them a car or expect them to function like a car. They need the engine, body and everything else. The same analogy applies to the vitamin C (ascorbic acid) or vitamin E (delta tocopherol) you can find on most health food store shelves. They are parts of an entire complex that serve a purpose when part of the whole (Fig. 3). However, they cannot do the job of the entire complex by themselves. With similar logic in place, one can analyze what a typical multivitamin truly is. The automobile equivalent of creating a multivitamin would be going to a junk yard, finding all of the separate parts you would need to make up an entire automobile, throwing them together in a heap (or capsule in terms of the multivitamin) and expecting that heap to drive like a car.

Figure 3. Typical example of analogy between functioning a car engine and organic vitamin C (http://www.advancedhealth07.com/nutrition. Retrieved: June 2014)

Isolated nutrients or synthetic nutrients are not natural, in that they are never found by themselves in nature. Taking these isolated nutrients, especially at the ultra-high doses found in formulas today, is more like taking a drug. Studies show the body treats these isolated and synthetic nutrients like xenobiotics (foreign substances). By the same token, food-based supplements are never treated like this by your body. For example, your urine will never turn florescent yellow, no matter how much meat (a good source of B vitamins) you eat. This sort of rapid excretion happens only with foreign substances in your body. Not only are isolated nutrients treated like drugs or other chemicals by your body. Like drugs, they can create problems for you too. Nature does not produce any nutrient in an isolated form. The nutrients in foods are blended together in a specific way and work best in that format. For an isolated nutrient to work properly in the body, it needs all the other parts that are naturally present in the food too. If the parts are not all there from the start, they are taken from the body's stored supply. This is why isolated nutrients often work for a little while, then seem to stop working. Once your body's store of the extra nutrients is used up, the isolated nutrient you're taking doesn't work as well anymore. Worse yet, a deficiency in these extra nutrients can be created in your body. And, because most nutrients are isolated from the foods they come in - using a wide array of potentially nasty solvents and other chemicals - taking high amounts of these products can also expose you to these potentially toxic chemicals, if care is not taken to remove them. With the burden we are already facing from the high number of chemicals in our environment, why would anyone want to add more?

The various parts of a natural vitamin complex work together in a synergistic manner. Synergy means that the whole is greater than the sum of its parts. Nutritionist Judith De Cava (1997) puts it best: "Separating the group of compounds (in a vitamin complex) converts it from a physiological, biochemical, active micronutrient into a disabled, debilitated chemical of little or no value to living cells. The synergy is gone." In other words, the automobile, in its original form, will drive better than a pile of its individual parts. Most people don't follow this logic when examining a nutritional supplement. Supplement makers typically try to stuff as much as possible in a capsule, telling us that the more we take, the better it is for us. This is simply not the case. As you now know, it is not necessarily the amount of a nutrient you ingest that is important, but its form and how much is bioavailable that counts the most. In fact, remembering that ingesting single nutrients can actually create imbalances in the body, logic would dictate the higher the level of a single nutrient that you take in, the quicker this imbalance will occur. What all of this means: The potency of a supplement has much more to do with synergy than with actual nutrient levels. It is a combined effect of all the parts of the food, rather than the chemical effect of a single part, that is most important.

Whole-food or synthetic supplements

In the previous chapter was already discussed the importance of taking natural supplements to support body and immune system. But which supplements do you take, and how do you know which supplements are the best ones? For example, Americans spend billions of dollars every year on supplements, and it's very important to understand that not all supplements are created equal. Sometimes, there are vast differences between products. What is the difference between whole food supplements and synthetic supplements (isolated or fractionated supplements)? A great example would be just to simplify look at almost any multivitamin. When you scan the nutritional content fact and ingredient label, you'll see quite an assortment of vitamins (A, C, E, etc.). Here is a portion of a label for a common multivitamin-multimineral supplement (Table 1). For this product say that replaces key nutrients that may be depleted through the stress of intense physical activity. Maximum formula with key ingredients (important vitamins and minerals) to keep you healthy and energized: physical energy (Cr, Fe, biotin, vitamins B_6 and B_{12}), mental energy (vitamins B_6 and B_{12}, thiamin, riboflavin, niacin), natural defense and immunity (Se, Zn, vitamins C and A), stress of physical activity (Se, vitamins A, C, and E), joints and bones (Ca, Mg, Zn, vitamins A, C, and D). However, although this product manufactured in USA, the statement has not been

evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

Table 1. Example of a multivitamin/multimineral supplement with amount per tablet and ingredient label (http://www.naturemade.com/multivitamins)

Vitamin	Value	Daily	Component	Value	Daily
A (as beta-Carotene)	4000 IU	50 %	Thiamin (as Thiamin mononitrate)	1.5 mg	88 %
C (as Ascorbic acid)	100 mg	167 %	Riboflavin	1.7 mg	85 %
D3 (as Cholecalciferol)	400 IU	100 %	Niacin (as Niacinamide)	18 mg	90 %
E (as dl-alpha-Tocopheryl acetate)	11 IU	37 %	Folic acid	800 µg	100 %
B6 (as Pyridoxine HCl)	2.6 mg	104 %	Calcium (as CaCO3)	250 mg	19 %
B12 (as Cyanocobalamin)	4 µg	50 %	Iron (as Ferrous fumarate)	27 mg	150 %

Ingredients
$Ca_3(PO_4)_2$, $CaHPO_4$, MgO, KCl, $CaCO_3$, ZnO, $MnSO_4$, $CuSO_4$, SiO_2, TiO_2, $Na_2B_4O_7$, $NaVO_3$, KI, Na_2O_4Se, $NiSO_4$, $SnCl_2$, Na_2MoO_4, $CrCl_3$, Na_2SiO_3.
Ascorbic acid, dl-alpha-Tocopheryl acetate, beta-Carotene, Vitamin A acetate, Niacinamide, Riboflavin, D-Calcium pantothenate, Pyridoxine hydrochloride, Thiamine mononitrate, Folic acid, Phytonadione, Biotin, Ergocalciferol, Cyanocobalamin, Ferrous fumarate, Acacia.
Glucose, Cellulose, Dextrin, Dextrose, Hypromellose, Corn starch, Croscarmellose sodium, Gelatin, Magnesium stearate, Stearic acid, Lecithin, Crospovidone, Polyethylene glycol, FD&C Red No. 40 lake, FD&C Blue No. 2 lake.

Namely, there are a lot of vitamins listed on this label (Table 1). Look at the items listed under "Ingredients". Those are "isolated" vitamins and other chemicals. There are no foods or herbal ingredients listed only partial vitamins and other chemicals. Nature intended for us to consume food in its whole form because all the vitamins, minerals, antioxidants and enzymes are bound together in one package and work synergistically to deliver the nutrition your body needs. Synthetic supplements give isolated or fractionated pieces of the whole. It is simply not the same, you're not getting the full benefit nature intended. The other problem is, by taking isolated vitamins, sometimes we are getting massive doses of some vitamins, but not enough of others. This imbalance this can cause health problems too. Aren't we trying to get healthier – rather than cause more problems?

Multivitamin and mineral supplements

Vitamins and minerals, and also enzymes, work closely together as co-factors for each other's efficacy (Grujić et al., 2014). If one part is missing, or in the wrong form or the wrong amount, entire chains of metabolic processes will not proceed normally. Vitamins and minerals are not functionally separable. They make each other work. Example: vitamin D is necessary for the body to absorb calcium. Copper is necessary for vitamin C activity. And so on. Mineral deficiencies can cause vitamin deficiencies, and vice versa. So that is the other prime difference between whole food vitamins and synthetics: whole food vitamins contain within them many essential trace minerals necessary for their synergistic operation. Synthetic vitamins contain no trace minerals, relying on, and depleting, the body's own mineral reserves.

Figure 4. Nutritional and synthetic supplements in a variety of dosage forms (http://marchildebrand.com/nutrition/; https://www.goodchinow.com/supplements/)

Significance of natural minerals

Minerals must come from land, respectively natural foods (fruits, vegetables, plants, seeds, nuts, grains). Minerals derived from natural and living substances such as food supra are organic. Minerals derived from non-living sources, i.e. metallic substances or synthetic metal salts are inorganic, i.e. non-living (Fig. 5). Most people are unwisely and unknowingly consuming inorganic minerals and vitamins by multi mixture products today. When using minerals for yourself you must know what to look for. A good mineral brand will list all organic sources of the minerals. These organic sources should be derived from food (fruit, plant, vegetable sources) exclusively. Any brand that lists any of the 102 minerals with a term following the mineral is synthetic. For example, nature made calcium, but not calcium gluconate. Nature made iron, but not iron ferrous. Nature did make oxide minerals, but not for purposes of human consumption. You must remember that nature has produced two states of every mineral: organic and inorganic, or phosphate and oxide. Humans require organic or phosphate minerals. Also, nature does not give us anything mono-structured. A mono-structured element or mineral by itself or isolated (from other elements) is unstable and will sabotage your health before ultimately killing you. For example, as humans, we need and require oxygen, but oxygen by itself and not balanced with nitrogen is a poison. Therefore, nature balanced oxygen with nitrogen. Similarly, water is required for life, but nature balanced the water with hydrogen and oxygen. Water like the air (oxygen) we breathe is balanced. Therefore, your minerals and vitamins must also be balanced. The land can convert inorganic elements (minerals) into organic elements by humic acid. Humic acid improves the absorption of all vitamins and minerals, especially those derived from organic sources.

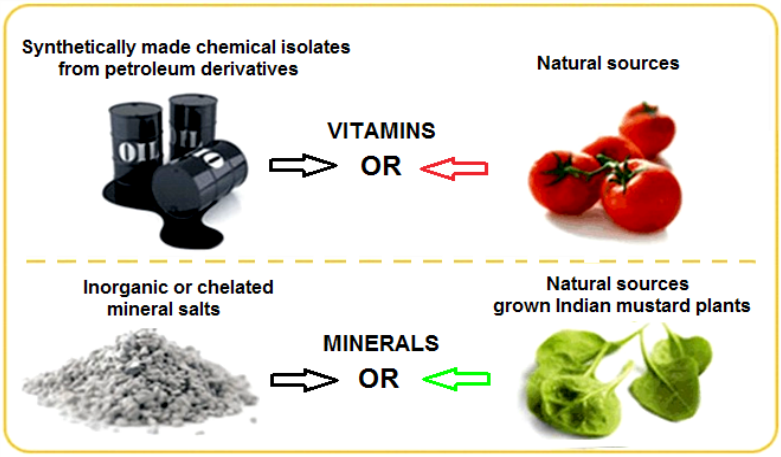

Figure 5. The origin of the vitamins and minerals that are part of the supplements (http://mymannapages.com. Retrieved: June 2014)

Calcium is one of the most common supplements. Researchers have managed to finagle a study that even shows calcium supplements aren't useful for boosting the bone density of elderly patients. People are being told that calcium is absolutely worthless if you don't digest it and absorb it. Calcium needs to have an acidic environment in order to be broken down and assimilated by digestive system. If you don't have acid in your stomach, then you can't absorb the calcium. And if you don't have vitamin D in small intestine, you can't absorb the calcium, either. There are a lot of senior citizens out there who are spending small fortunes on calcium supplements and antacid tablets, but they're not getting any sunshine. And that means they're not getting enough vitamin D. With a vitamin D deficiency, they can't even absorb the calcium. It's either going right through their bodies or actually contributing to the buildup of calcification in their kidneys. They'll probably end up with kidney stones.

Significance of food-based vitamins

It is known that a good diet should supply all necessary nutrients. However, the true story builds a much more complex picture. Namely, even natural sources of vitamin A in unnatural quantities produce problems for species with particularly high susceptibility to vitamin A toxicity, like metabolic osteopathy (Polizopoulou et al., 2005). For precursors to vitamin A, the carotenoids, as many as nine factors influence their bioavailability when ingested in food (Van het Hof et al., 2000). The carotenoid lycopene, which lowers prostate cancer risk, illustrates the integrity of the plant substrate when carotenoids are ingested in food. Cooking improves lycopene bioavailability because heating and homogenizing lycopene-rich tomatoes into paste disrupts the fibrous plant cell walls, releasing the lycopene for. Partnering carotenoids with certain fats maximizes their intestinal and lymphatic uptake. Humans eating salads with either fat-free or full-fat dressing demonstrated significantly different plasma levels, with those ingesting vegetables dressed in the full-fat version enjoying higher amounts of circulating carotenoids (Brown et al., 2004).

Members of the water-soluble vitamin B class have a reputation of being safe in any amount. Nutritionists have long-advocated vitamin B9, or folate, for cancer prevention. However, a concern erupted in the 1940s that is now receiving more attention: Too much supplementation with folic acid (the synthetic form of folate) appears to promote tumorigenesis. This springs from its role as a key cofactor in nucleotide synthesis. Large amounts of folic acid facilitate cellular proliferation, causing growth in neoplastic foci. This finding has provoked calls in the human food-processing industry for curtailing mandatory folic acid fortification (Mason, 2009). The lower amounts of folate ordinarily found in food are still viewed as cancer-preventive.

Many nonhuman species manufacture vitamin C. While they rely less on dietary sources, research testing their true capacity shows it falls far short of previous assumptions. Aging hepatocytes lose some ability to synthesize and recycle vitamin C, producing a conditionally essential status for vitamin C in geriatric dogs and cats. Health status may further influence vitamin C requirements. A study showed that healthy dogs supplemented with vitamin C produced no improvement in antioxidative capacity or serum IgA and IgG concentrations (Hesta et al., 2009). In contrast, dogs with untreated lymphoma exhibited significant alterations in their antioxidant levels compared to healthy control dogs. After remission, dogs with lymphoma had significantly lower levels of vitamin C, raising questions about the potential need for post-chemotherapy supplementation with dietary antioxidants. Finally, Vitamin C supplementation, along with vitamins B and E, aminocaproic acid and N-acetylcysteine, disappointingly failed to forestall deterioration in the neurological status of dogs with suspected degenerative myelopathy (Hesta et al., 2009).

In the wild, carnivores obtain vitamin D by consuming the body fat, blood and liver of their prey; depending on the contents of homemade diets, dogs and cats may not be receiving enough vitamin D (How et al., 1994). They cannot meet their needs simply through sun exposure, as dogs and cats lack the ability to cutaneously synthesize D3 in adequate amounts. Problems such as “rubber jaw syndrome,” or secondary hyperparathyroidism, have arisen in young dogs. On the other hand, overnutrition with supplemented Vitamin D, calcium, and calories in puppy diets has been linked to predisposition for canine elbow dysplasia (Janutta et al., 2008).

The situation is similar with the vitamin E. On a supplement label, natural vitamin E is listed as d-alpha-tocopherol, d-alpha tocopheryl acetate, or d-alpha tocopheryl succinate. In contrast, synthetic forms of vitamin E are labeled with a dl- prefix. There is little difference between the natural and a synthetic form of vitamin E, but natural is better (Challem, 2000). Natural vitamin E refers to eight chemically different compounds obtained from plants: four tocopherols and four trienols (Fig. 6). Alpha-tocopherol is the most biologically active form of vitamin E, and its natural form consists of one isomer. In contrast, synthetic vitamin E (i.e. synthetic alpha-tocopherol) consists of eight stereoisomeric forms of alpha-tocopherol, with only one (about 12 %

of the synthetic molecule) equal to the naturally occurring stereoisomeric form (Yu et al., 2008). The other seven isomers range in potency from 21 to 90 % of natural d-alpha-tocopherol. This may appear to be arcane nutritional chemistry, but it is key to understanding how the body absorbs natural and synthetic supplements differently. Molecular structure determines how the body uses vitamin E. Researchers have found that natural vitamin E assimilates far better than synthetic versions. Specific binding and transport proteins produced in the liver select the natural d-alpha form of vitamin E and largely ignore all other forms (Traber, 1998).

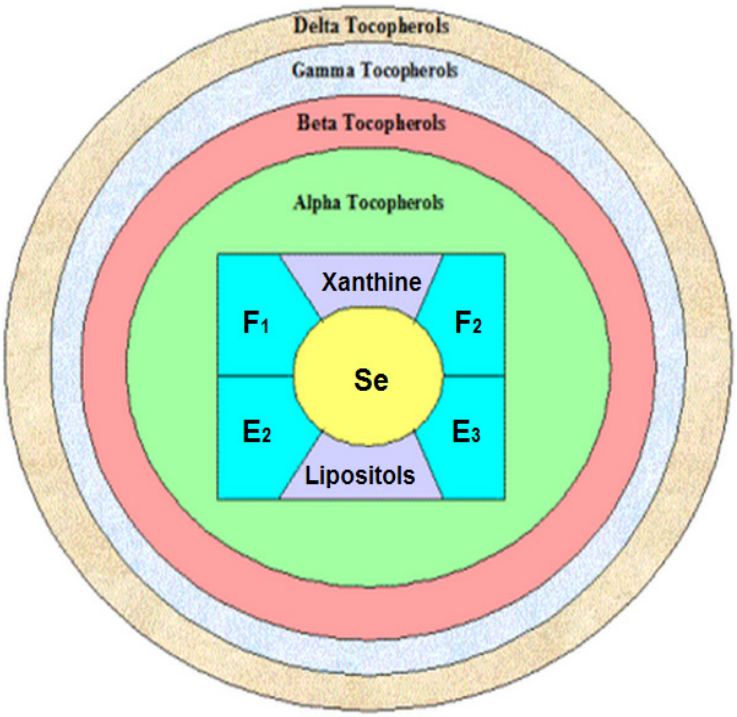

Figure 6. Functional architecture of vitamin E complex
(http://alternativehealthatlanta.com/vitamins-minerals. Retrieved: June 2014)

Generally, the health-supporting and cancer-preventive superiority of either natural or synthetic vitamin E remains unclear (Blatt et al., 2004). Studies exploring species-specific differences have further muddied the waters, with some indicating better results with the synthetic and others with the natural form (Weiss et al., 2009). Attention is turning to the formerly neglected half of the natural vitamin E family, the tocotrienols. Tocotrienols confer neuroprotective benefits and possibly more antioxidant properties than alpha-tocopherol. Tocotrienols distribute more widely throughout the fatty layers of cell membranes and tocotrienol, not tocopherol, suppressed growth of human breast cancer cells (Sen et al., 2007). Clearly, questions remain about when and how much vitamin supplementations small animals require, and whether or not pet food diets can meet their needs. However, veterinarians can convey to clients facts based on the information that is available, fulfilling their role of protecting animal health and welfare.

Problem of synthetic vitamins

What occurs with all synthetic vitamins? If a person has in body enough reserves of the missing parts of a particular vitamin when combined with the vitamin isolate from the synthetic they will experience some initial relief. But as soon as those reserves are used up, the synthetic will no longer work, the symptoms will return and the person will now experience the often unpleasant effects of vitamin imbalance and deficiency. The body treats them as toxins, leading to the "expensive urine" of excess vitamin intake referred to frequently, since the human system via the urinary tract attempts to rid itself of the major

quantity of such foreign chemicals (De Cava, 1997). De Cava points out that vitamin B1, as synthetic thiamine (thiamine HCl) will initially allay fatigue but will eventually cause fatigue by the buildup of pyruvic acid. This leads to the vicious cycle of thinking more and more Thiamine is needed, resulting in more and more fatigue along with other accumulated complaints. Also, natural food-source vitamins are enzymatically alive, and man-made synthetic vitamins are dead chemicals. True whole food vitamins don't leech the body of their missing co-factors and don't cause a vitamin imbalance leading to the return and increase of symptoms. True vitamins also don't need mega doses to create their effect. A high number of milligrams is often an indication of a synthetic source.

A study in the of some 30,000 Finnish subjects showed conclusively that synthetic vitamin A had no antioxidant effect whatsoever (NEJM study group, 1994). A true antioxidant helps to protect heart muscle, lungs, and artery surfaces from breaking down prematurely. In this study, the subjects who received the synthetic beta-carotene actually had an 8% higher incidence of fatal heart attacks, strokes, and lung cancer than those who got the placebo (sugar pill). Stands to reason: the synthetic brought no vitamin activity to the tissues that needed it. As a dead, purified chemical introduced into the body, the synthetic further stressed the immune system, the liver, and the kidneys which all had to try to break down this odd chemical and remove it from the body. It would be bad enough if they were harmless, but synthetic vitamins actually have a net negative effect.

Identification of synthetic vitamins

Step 1. Search for words listed in the ingredients that begin with "dl." When a word contains "dl" in the prefix, it is an indication that the vitamin is synthetic. As an example, "dl-alpha-tocopherol acetate" and "dl-alpha-tocopherol" are synthetic forms of vitamin E.

Step 2. Find words that end with "ate" or "ide" in the list of ingredients. These words indicate that the manufacturer used synthetic materials for increasing the vitamin's potency and stability. Some words to look for include nitrate, acetate, sodium ascorbate, sodium benzoate, chloride, hydrochloride, silicon dioxide and titanium dioxide.

Step 3. Find the synthetic form of the vitamin listed under the ingredient list. Natural vitamins come from natural food sources. If you see the vitamin listed as the vitamin itself, such as "vitamin D," then it is sure to be the synthetic version. Look for food sources such as "citrus" instead of "vitamin C" or "parsley" instead of "vitamin K."

Step 4. Identify the words "natural" on the vitamin bottle. If the bottle says, "100 percent natural" the vitamin supplement does not contain synthetics. On the other hand, a label that says, "natural," might have at least some synthetic components. According to Earl Mindell's, only 10 % of the product must come from natural food sources in order for a company to claim "natural" on the product's label. If the product label does not say "100 percent animal-based" or "100 percent plant-based," the supplement is synthetic.

Step 5. Look for the vitamin potency listed on the product's label. According to the Organic Consumers Association, if the vitamin supplement has a high or otherwise unnatural potency, the product is synthetic. For example, a product that provides 1,000 percent of vitamin C is unusually high. This is ten times the amount you need daily, and an amount that even a healthy diet - consisting of natural, whole-food sources - cannot provide.

Labels. The new nutritional disclosure labels actually make it more difficult to determine what healthy ingredients are and what are not (Fig. 7). The old labels (still on many products), tell you more about what is really in the product. As a general rule of thumb, vitamins listed in the ingredient section of the label, it is synthetic or fractionated, otherwise it would be listed as the food. Now on the new labeling, you have to look under the "Nutrition facts" section as well (where it lists what percentage of the daily value of basic nutrients is contained in the product). Where the vitamins are listed, look where it displays parenthesis (for example: vitamin A (as beta-carotene)". The word "as" generally denotes a synthetic or fractionated source, unless it sounds like a food. The ingredient label (since it is supposed to be food that you are eating) should sound like a food. So instead of the label saying, for example, "vitamin C (as ascorbic acid)", it should say "vitamin C (from acerola cherries)".

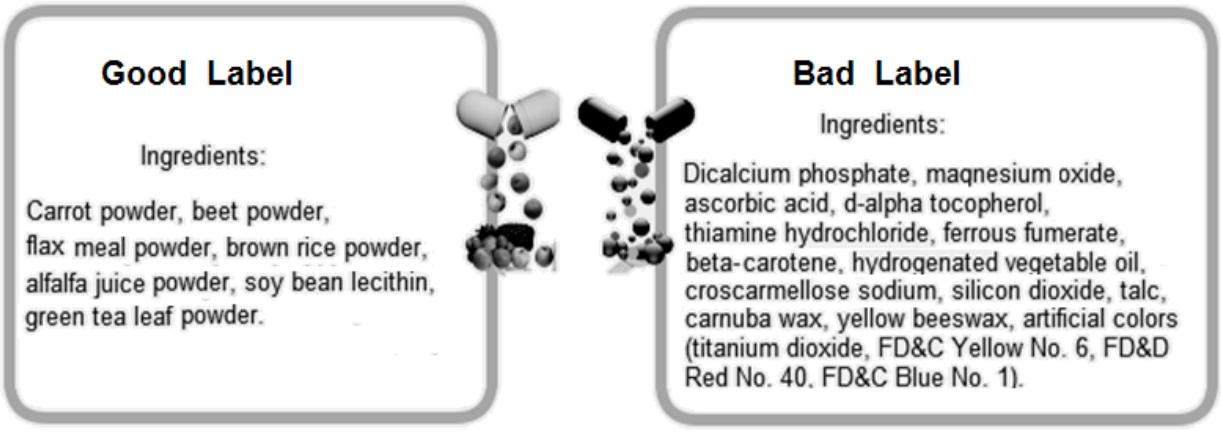

Figure 7. Samples of good and bad label for ingredients section (http://www.vitalhealthcda.com/whole-food-supplements. Retrieved: June 2014)

Synthetic and isolated nutritional supplements

According to science, vitamins are organic substances that are essential in small amounts for the health, growth, reproduction, and maintenance of one or more animal species. Vitamins must be included in the diet since they cannot be synthesized at all or in sufficient quantity in the body. Each vitamin performs a specific function; hence one cannot replace another. Vitamins originate primarily in plant tissues. Isolated non-food vitamins (call. natural or USP or pharmaceutical grade) are not naturally "included in the diet", do not necessarily "originate primarily in plant tissues", and cannot fully replace all natural vitamin activities (Ensminger, 1994). Synthetic vitamins were originally developed because they cost less. Most vitamins in supplements are petroleum extracts, coal tar derivatives, and chemically processed sugar (plus sometimes industrially processed fish oils), with other acids and industrial chemicals (such as formaldehyde) used to process them (Table 2).

Table 2. Composition of food and non-food vitamins (Hui, 1992; Thiel, 2014)

Vitamin	Food nutrient	Natural vitamin analogue and some process chemicals
Vitamin A Beta-carotene	Carrots	Methanol, benzene, petroleum esters; acetylene; refined oils
Vitamin B-1	Nutritional yeast, rice bran	Coal tar derivatives, hydrochloric acid; acetonitrile with ammonia
Vitamin B-2	Nutritional yeast, rice bran	Synthetically produced with 2N acetic acid
Vitamin B-3	Nutritional yeast, rice bran	Coal tar derivatives, 3-cyanopyridine; ammonia and acid
Vitamin B-5	Nutritional yeast, rice bran	Condensing isobutyraldehyde with formaldehyde
Vitamin B-6	Nutritional yeast, rice bran	Petroleum ester & hydrochloric acid with formaldehyde
Vitamin B-8	Rice	Phytin hydrolyzed with calcium hydroxide and sulfuric acid
Vitamin B-9	Broccoli, rice bran	Processed with petroleum derivatives and acids; acetylene
Vitamin B-12	Nutritional yeast	Cobalamins reacted with cyanide
Vitamin 'B-x'	PABA Nutritional yeast	Coal tar oxidized with nitric acid (from ammonia)
Choline	Nutritional yeast, rice bran	Ethylene and ammonia with HCl or tartaric acid
Vitamin C	Acerola cherries, citrus fruits	Hydrogenated sugar processed with acetone
Vitamin D	Nutritional yeast	Irradiated animal fat/cattle brains or solvently extracted
Vitamin E	Rice, vegetable oils	Trimethylhydroquinone with isophytol; refined oils
Vitamin H	Nutritional yeast, rice bran	Biosynthetically produced
Vitamin K	Cabbage	Coal tar derivative; produced with p-allelic-nickel

Non-food vitamins should be considered as vitamin analogues (artificial imitations), and not as true vitamins for humans. One of the best ways to recognize whether or not a vitamin supplement contains natural vitamins as found in food is to know the chemical differences between food and non-food vitamins (sometimes called USP vitamins). As shown in Table 3, the chemical forms of food and synthetic nutrients are normally different.

Table 3. Chemical form of food and non-food vitamins (Hui 1992, Thiel 2014)

Primary chemical vitamin form in food	**Vitamin analogue chemical form (called natural)**
Vitamin A/Beta-carotene; retinyl esters; mixed carotenoids	Vitamin A acetate; vitamin A palmitate; beta-carotene (isolated)
Vitamin B-1; thiamin pyrophosphate (food)	Thiamin mononitrate; thiamin hydrochloride; thiamin HCL
Vitamin B-2; riboflavin, multiple forms (food)	Riboflavin (isolated); USP vitamin B2
Vitamin B-3; niacinamide (food)	Niacin (isolated); niacinamide (isolated)
Vitamin B-5; pantothenate (food)	Pantothenic acid; calcium pantothenate; panthenol
Vitamin B-6; 5'0 (beta-D) pyridoxine	Pyridoxine hydrochloride; pyridoxine HCL
Vitamin B-9; folate	Folic acid
Vitamin B-12; methylcobalamin; deoxy-adenosylcobalamin	Cyanocobalamin; hydroxycobalamin
Choline (food); phosphatidyl choline (food)	Choline chloride; choline bitartrate
Vitamin C; ascorbate (food); dehy-droascorbate	Ascorbic acid; most mineral ascorbates (i.e. sodium ascorbate)
Vitamin D; mixed forms, primarily D3 (food)	Vitamin D1 (isolated); Vitamin D2 (isolated); Vitamin D3 (isolated) ; Vita-min D4; ergosterol (isolated); cholecalciferol (isolated); lumisterol
Vitamin E; RRR-alpha-tocopherol (food)	Vitamin E acetate; Mixed tocopherols; all-rac-alpha-tocopherol; d-l--al-pha-tocopherol; d-alpha-tocopherol (isolated); dl-alpha-tocopheryl acetate; all acetate forms
Vitamin H; biotin	All non-yeast or non-rice vegetarian biotin forms
Vitamin K; phylloquinone (food)	Vitamin K3; menadione; phytonadione; naphthoquinone; dihydro-vitamin K1

The belief that body cannot recognize whether a vitamin in the bloodstream came from natural products or from laboratories is quite misconception for several reasons (Whitney et al., 1987). First, it seems to assume that the process of getting the amount of the vitamin into the bloodstream is the same, which is frequently not the case (Shils, 1999). Secondly, particle size is an important factor in nutrient absorption even though particle size is not detected by chemical assessment. Thirdly, the food factors that influence the absorption of nutrients relate not only to the nature of the nutrients themselves, but also their interaction with each other and with the non absorbable components of food (Jenkins et al., 1994). Fourthly, the physic-chemical form of a nutrient is a major factor in bioavailability, and food and non-food vitamins are not normally in the same form (Macrae et al., 1993). Fifthly, most non-food vitamins are crystalline in structure (Budvari, 1996).

Food vitamins are in the physic-chemical forms which the body recognizes, contain food factors that affect bioavailability, appear to have smaller particle sizes and generally are not crystalline in structure (Fig. 8). This does not mean that non-food vitamins do not have any value (they clearly do), but it is important to understand that natural food complex vitamins have actually been shown to be better than isolated, non-food, vitamins.

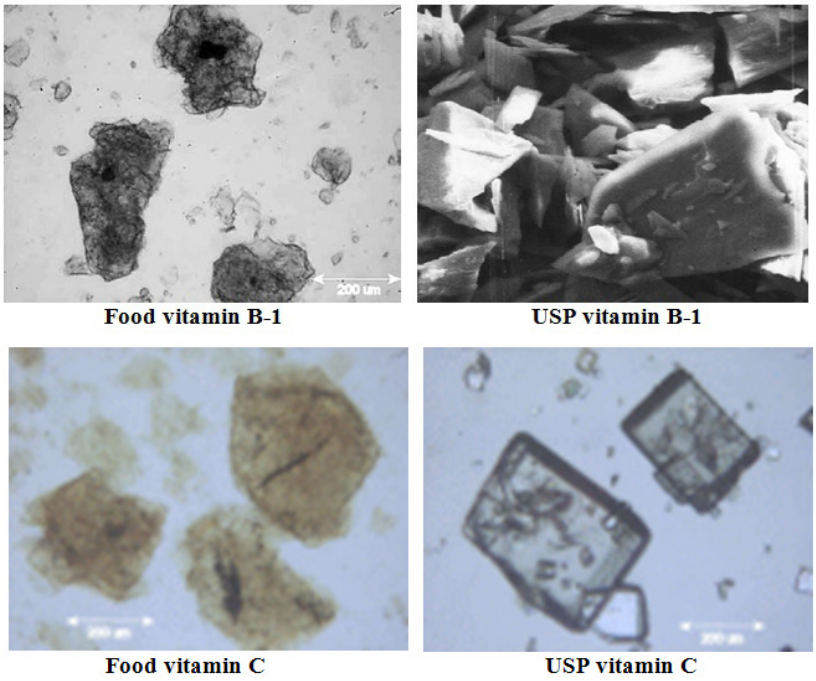

Figure 8. Electronic photos and structural differences of food vitamins and isolated USP vitamins (Thiel, 2014)

Conclusion

Science cannot create life. Only life can create life. Obviously, there is a difference. Supplements food-based, isolated or synthetic, has detracted from the most important part of health and healing. The basics of proper diet, exercise, detoxification, structure, mental/emotional and spiritual health must all be in order for true healing to occur. No supplement will work on its own if these foundations are not in place. However, even when these foundations are in place, or if the situation is acute enough to necessitate a more immediate treatment response, supplement support may still be needed for a while. You may also want to take one or more food-based supplements to ensure you are getting an adequate array of nutrients in your diet. When these situations arise, it is recommend food-based supplements be your first choice. How to known whether or not a supplement good choice? For starters, make sure it has the following characteristics: it is as close as possible to its natural form. The utmost care has been taken in all phases of its production, from growing its ingredients, to manufacturing, testing for potency and quality control. Select from companies that have a long track record of providing high quality products that produce good clinical results.

Most vitamins sold are not food. They are synthetically processed petroleum and/or hydrogenated sugar extracts, even if they say “natural” on the label. They are not in the same chemical form or structural form as real vitamins are in foods; thus they are not natural for the human body. True natural food vitamins are superior to synthetic ones. Food vitamins are functionally superior to non-food vitamins as they tend to be preferentially absorbed and/or retained by the body. Isolated, non-food vitamins, even when not chemically different are only fractionated nutrients.

Studies cited throughout this paper suggest that the bioavailability of food vitamins is better than that of most isolated USP vitamins, that they may have better effects on maintaining aspects of human health beyond traditional vitamin deficiency syndromes, and at least some seem to be preferentially retained by the human body. It is not always clear if these advantages are due to the physiochemical form of the vitamin, with the other food constituents that are naturally found with them, or some combination. Regardless, it seems logical to conclude that for purposes of maintaining normal health, natural vitamins are superior to synthetic ones. Unlike some synthetic

vitamins, no natural vitamin has been found to not perform all of its natural functions. The truth is that only foods, or supplements composed of 100% foods, can be counted on as not containing non-food vitamin analogues. How do you know if the vitamins on your kitchen counter are from whole foods or if they are synthetic? If the list of ingredients includes an actual vitamin like vitamin C" rather than an actual food that contains natural vitamin C like "acerola cherry powder", you can bet that it is a synthetic vitamin. If you choose to use nutritional supplements, it is in your best interest to use only those products that list actual foods as their ingredients rather than synthetic and isolated vitamins. While some synthetic and isolated vitamins have been shown to provide minimal health benefits, on the whole, most of them cause more harm than good and you are far better off spending your money on whole foods. It is important to note that the principles in this article are just as relevant and applicable to minerals and mineral supplements.

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Kod pisanja knjiga, ili poglavlja u knjigama, paziti da se napiše Grad, te da se napiše na isto mjesto!
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PHYSICOCHEMICAL PROPERTIES OF STIFF DOUGH "AMALA" PREPARED FROM PLANTAIN (*MUSA PARADISCA*) FLOUR AND MORINGA *(MORINGA OLEIFERA)* LEAF POWDER

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Summary
Plantains are a good source of resistant starch and are currently being used in the dietary management of diabetes when consumed in the unripe stage. They can be used in making *amala*, a stiff dough commonly consumed in some parts of Africa including Nigeria. The addition of fortificant to foods may affect product composition and functionality; therefore this study investigated the effect of *Moringa oleifera* leaf powder at varying concentrations (0, 0.5, 1.0, 1.5, 2.0 and 2.5 %) on pasting and functional properties of the fortified plantain flour. The proximate composition, mineral content and sensory properties of *amala* prepared from the fortified plantain flour were also determined. Protein and carbohydrate were the major components of plantain flour and *Moringa oleifera* leaf powder. Generally, water absorption capacity, bulk densities, swelling power and pasting properties of the fortified plantain flour decreased with increasing concentration of *Moringa oleifera* leaf powder. *Moringa oleifera* leaf powder seems to reduce hydration and swelling power of plantain flour. The protein contents of *amala* prepared from the fortified plantain flour significantly increased from 3.52 to 10.36%. Ash and fat contents of the *amala* also increased from 1.71-2.93% and 1.82 to 2.37% respectively. Similarly, the calcium, magnesium, potassium, sodium and iron contents of the *amala* also increased following the addition of *Moringa oleifera* leaf powder. The use of *Moringa oleifera* leaf powder thus has the potential to combat protein-energy malnutrition and micronutrient deficiencies in developing countries.

Keywords: *Amala*, *Moringa oleifera*, plantain flour, pasting, functional

Introduction

Plantain (*Musa paradisiacae*) is an important dietary source of carbohydrate in many parts of Africa, Asia and South America (Robinson, 1996; Falade and Olugbuyi, 2010). It is consumed mainly for its vitamins and minerals contents.
Plantains are usually harvested at a matured but unripe stage and ripens within 2-7 days (Falade and Olugbuyi, 2010). The high moisture content of plantain predisposes it to spoilage; hence it is dried to increase its shelf life. Plantain may be processed by frying, grilling, boiling and drying at different stages of maturity (Falade and Olugbuyi, 2010). Drying of plantain and subsequent milling into flour seems to be the most effective way of utilizing. The incorporation of plantain flour into foods has been encouraged due to its relatively high resistant starch content which has been recommended for dietary management of *diabetes mellitus* and other related disease (Eleazu et al. 2010; Eleazu et al. 2013).
Plantain flour can make good stiff dough called *amala* either singly or in combination with yam flour (Abulude and Ojediran, 2006). *Amala* is regarded as a starchy gel or stiff dough traditionally prepared from yam (Dioscorea spp) flour (Awoyale et al., 2010; Abiodun and Akinoso, 2014). It is prepared by reconstituting yam flour in boiling water until a dark smooth paste is formed (Karim et al., 2013). A similar paste with whiter appearance can however, be prepared from fermented cassava flour (Karim et al., 2013). *Amala* is majorly consumed in the South Western part of Nigeria (Abiodun and Akinoso, 2014) and some part of Ghana where it is called Kokonte (Jimoh and Olatidoye, 2009). According to Awoyale et al. (2010), *amala* contains

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majorly carbohydrates and as a result does not provide adequate nutrients especially among rural dwellers. Efforts are therefore geared towards improving the nutritional value of such staples through the incorporation of legumes and other protein-rich plant foods. For example, supplementing yam flour with 35% distillers spent grain was reported to increase the protein content of yam by over 100% (Awoyale et al., 2010). Similarly, Jimoh and Olatidoye (2009) reported an increase in protein content from 3.16 to 18.21% for yam flour fortified with 30% soybean flour.

Consumers' awareness of the relationship between food, health and nutrition has spurred the need to develop foods with functional ingredients especially from plant materials such as *Moringa oleifera*. The leave of Moringa has been used as an alternative food source to combat malnutrition, especially among children and infants (Anwar et al., 2007). It contains substantial amounts of proteins (19-29%) (Jongrungruangchok et al., 2010) vitamins and minerals (Jongrungruangchok et al., 2010; Hekmat et al., 2015). These nutrients are known to scavenge free radicals when combined with a balanced diet and may have immunosuppressive effects (Danmalam et al., 2001). In Africa, the use of *Moringa Oleifera* as a food fortificant is on the increase. Many studies have reported the use of the leaves and flower in food applications such as in making soups (Babayeju et al., 2014), fortifying weaning foods (Arise et al., 2014), and in enriching yoghurt (Hekmat et al., 2015). Recently, the fortification of yam flour with *Moringa Oleifera* Leaf Powder (MOLP) at 2.5% level was reported to be sufficient to improve the proximate and mineral composition of yam flour without having any negative influence on the sensory properties (Karim et al., 2013). The addition of fortificant to foods may affect product composition and functionality. For instance, Jimoh and Olatidoye (2009), reported an increase in protein, ash and fat contents of yam flour fortified with soybean flour. However, the peak viscosity, water absorption capacity and swelling index of the fortified yam flour decreased significantly with increasing concentration of soybean flour. The use of *Moringa Oleifera* Leaf Powder to improve the nutritional composition of plantain flour has not been reported. Therefore, this study investigates the functional, pasting, proximate and sensory properties of *amala* prepared from plantain flour and *Moringa oleifera* leaf powder.

Materials and methods

Plant materials

Freshly harvested plantains were obtained from a farm in Ilorin, Nigeria. Dried *Moringa oleifera* leaves were obtained from University of Ilorin Agricultural commercial farms Ilorin, Nigeria.

Plantain flour

Plantation flour was prepared following the methods of Falade and Olugbuyi, (2010). Briefly, the fingers were removed, rinsed in clean water and peeled manually with knives. Peeled plantain were cut into slices, steamed for 15 min to inactivate enzymes and dried at 60^{o}C for 24 h in a hot air oven (D-37520, Thermo Fischer Scientific, South Africa). Dried plantain slices were milled in a Warring blender (HGBTWTS3, Torrington USA) and sieved (Sieve aperture size: 350 μm) into flour, sealed and used immediately for analysis.

Plantain flour-moringa leaf formulation

Moringa oleifera leaf powder (MOLP) was added to plantain flour in concentrations ranging from 0, 0.5, 1, 1.5, 2 and 2.5 % on dry weight basis. These levels of incorporation was adopted based on our previous studies on yam flour fortified with MOLP (Karim et al., 2013). A 2.5% level of MOLP to yam flour was found acceptable for making *amala*. Plantain flour without MOLP (0%) served as the control. Each sample contained 200 g of the plantain flour packaged in polythene bags.

Functional properties of raw and fortified plantain flour

Water absorption capacity

The water absorption capacity (WAC) of raw and fortified plantain flour was determined as described by Oyeyinka et al. (2013) with few modifications. Briefly, one gram of each sample was weighed into a dry, clean centrifuge tube. Water

(10 mL) was poured into the tube and properly mixed by vortexing. The suspension was allowed to stand for 30 min and centrifuged (Centrifuge Model: Ependorf 5810R, Germany) at 3,500×g for 30 min. Supernatant was discarded and the tube with its content reweighed. Gain in weight expressed, as a percentage of water bound, was calculated as the WAC of the sample.

Loose and Packed Bulk Density

A measuring cylinder (100 mL) was filled with flour to mark (100 mL), and the content weighed. The packed bulk density was also obtained by following the same procedure but tapping the side of the measuring cylinder several times until the flour volume was constant. Bulk density was calculated as the ratio of the bulk weight and the volume of the container (g/mL) (Oyeyinka et al., 2013).

Swelling power

Swelling power of raw and fortified plantain flour was determined by methods described by Madruga et al.(2014) with slight modification. Briefly, flour samples (0.1 g starch in 10 ml of distilled water) were stirred and placed in a water bath for 30 min at 90^{o}C with constant stirring. The suspension was centrifuged (Centrifuge model: Ependorf 5810R, Germany) at 3400×g for 20 min and the supernatant discarded. Swelling power was obtained by weighing the residue after centrifugation and dividing by original weight of flour on dry weight basis.

Pasting

Pasting characteristics of the plantain flours fortified with MOLP were determined using a Rapid Visco Analyser (Model RVA 3D; Newport Scientific, Narrabeen, NSW, Australia). Plantain flour (3 g) was weighed into a dried empty canister and 25 mL of distilled water was added. The mixture was thoroughly stirred, and the canister was fitted into the RVA as recommended. The slurry was heated from 50 to 95^{o}C with a holding time of 2 min followed by cooling to 50^{o}C with 2 min holding time. The rate of heating and cooling was at a constant rate of 11.25^{o}C min^{-1}. Peak viscosity, trough, breakdown, final viscosity, set back, peak time and pasting temperature were read from the pasting profile with the aid of Thermocline for Windows Software connected to a computer (Falade and Olugbuyi, 2010).

Preparation of amala

Amala was prepared by the method of Karim et al., (2013). Briefly, plantain flour was poured into boiling water with continuous stirring until a homogenous paste was formed. The paste was covered and left on the fire for about 5 min to cook. It was further stirred, packed and wrapped with thin labeled polythene wraps.

Proximate composition

Moisture, ash and fat contents of *Moringa oleifera*, plantain flour and *amala* were determined using standard methods (AOAC, 2000) The protein content was determined by kjeldahl method (N x 6.25) and total carbohydrate was calculated by difference.

Mineral composition

Amala sample were digested as described in Amonsou et al. (2014) and the mineral content analysed using Inductively Coupled Plasma Mass Spectrometry (ICP)-Mass spectrometer (Perkin-Elmer). Samples (0.5 g) were acid digested using 65% nitric acid. Digested samples solutions were then quantified against standard solutions of known concentrations.

Sensory evaluation

A 9- point hedonic preference scale and a multiple comparison test were used to assess the acceptability of *amala* made from plantain flour fortified with MOLP using 50 trained panelists. Panelists were selected from student of the Department of Home Economics and Food Science, University of Ilorin, Nigeria. The selected students were those accustomed to eating *amala*. Prior to the sensory analysis, they were screened with respect to their interest and ability to differentiate food sensory properties. The samples were evaluated for colour, aroma, mouldability, consistency, mouth feel and overall acceptability.

Statistical analysis

All experiments were conducted in duplicate. Data were analysed using analysis of variance (ANOVA) and means were compared using Fischer's Least Significant Difference Test ($p<0.05$).

Results and discussion

Proximate and mineral composition of *Moringa oleifera* leaves and plantain flour

Protein and carbohydrate were the major components of *Moringa oleifera* leaves and plantain flour (Table 1). The protein (24.55%) and ash (6.52%) contents of *Moringa oleifera* leaves were higher than those of plantain flour (protein: 3.54%, ash: 1.76%). Similar composition have been reported for *Moringa oleifera* leaves (Jongrungruangchok et al., 2010) and plantain flour (Akubor et al., 2003; Zakpaa et al., 2010).

The calcium and potassium contents of Moringaoleifera leaves and plantain flour were very high whiletheir sodium and iron contents were low (Table 2). Plantain flour had lower (approx. 5 times) magnesium content compared to the Moringaoleifera leaves.. The potassium content of plantain flour was however higher than for *Moringa oleifera* leaves. Sodium and iron contents of both *Moringa oleifera* leaves and plantain flour were very low. However, the iron content of *Moringa oleifera* leaves was substantially higher than for plantain flour.

Table 1. Proximate composition of Moringa oleifera leaves and plantain flour (%)[1]

Samples	Moisture	Protein	Fats	Ash	Carbohydrate
Moringa leaves	10.11±0.02	24.55±0.02	2.48±0.01	6.52±0.01	56.34±0.01
Plantain flour	6.01±0.04	3.54±0.02	1.86±0.01	1.76±0.01	80.07±0.01

[1]Mean ± SD. Values are expressed on dry weight basis

Table 2. Mineral composition of *Moringa oleifera* leaves and plantain flour (mg /100 g)[1]

Samples	Calcium	Potassium	Magnesium	Sodium	Iron
Moringa leaves	2480.31±0.02	1782.15±0.01	448.23±0.01	26.52±0.01	26.34±0.01
Plantain flour	193.06±0.04	4658.36±0.02	94.43±0.01	46.20±0.01	2.40±0.01

[1]Mean ± SD.Values are expressed on dry weight basis

Functional properties of plantain flour fortified with MOLP

The water absorption capacity (WAC) of the plantain flour decreased from 1.11 to 0.77 g water/ g flour with increasing concentration of Moringa oleifera leaf powder (MOLP) (Figure 1). Although the WAC of the fortified plantain flour decreased, the decrease was not very substantial. Variation in the WAC of flours has been suggested to depend on differences in the granule structure and the degrees of availability of the water binding sites among the flours (Wootton and Bamunuarachchi, 1978). The added MOLP thus, may have partially blocked the sites available for water binding thereby reducing the amounts of water absorbed by the plantain flour. Akubo et al. (2003) also reported a reduction in water absorption capacity of plantain flour following the addition of cowpea (Vigna unguiculata) flour.

The loose and packed bulk densities of plantain flour did not vary significantly ($p\leq.05$) when MOLP was added (Figure 1). The loose bulk density varied from 0.53 to 0.54 g/mL, while packed bulk density varied from 0.72 to 0.78 g/mL. Expectedly, the packed bulk density of both fortified plantain flour and the control were higher than their loose bulk density. The slight decrease in loose and packed bulk densities of the fortified plantain flours suggest that the requirements for packaging these flours will not change significantly, but rather it will allow the use of a more economical package (Osundahunsi and Aworh, 2002). The LBD and PBD of the fortified plantain flour is within the range reported by previous authors for plantain flour (Falade and Olugbuyi, 2010; Falade and Oyeyinka, 2014).

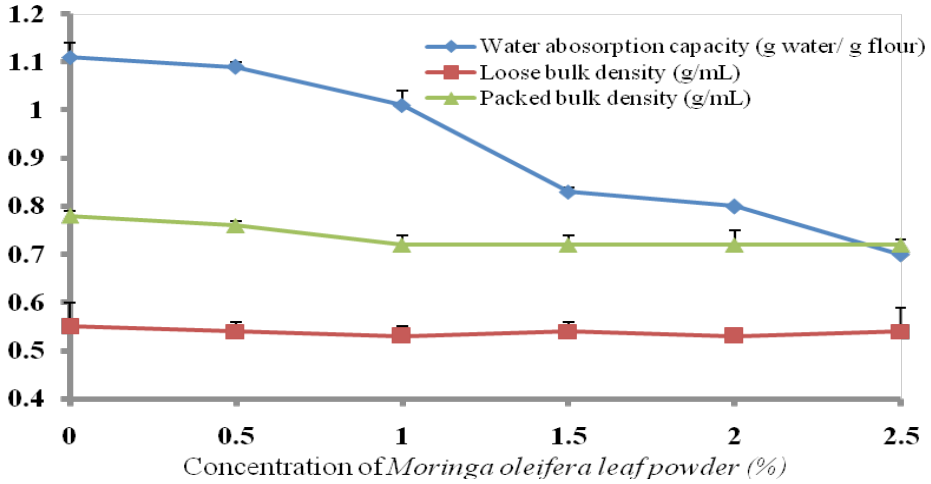


Figure 1. Water absorption capacity and bulk densities of plantain flour fortified with MOLP

The swelling ability of plantain flour in water was not significantly ($p<0.05$) affected by MOLP (Figure 3). A reduction from 6.78 to 6.70 g water per g flour was recorded following the addition of MOLP. MOLP may possibly have covered starch granule surface slightly restricting hydration and swelling. Swelling of flour is an important phenomenon that aids paste formation. Similar reduction in swelling power following the addition of soybean to plantain flour has been reported (Abioye et al., 2011).

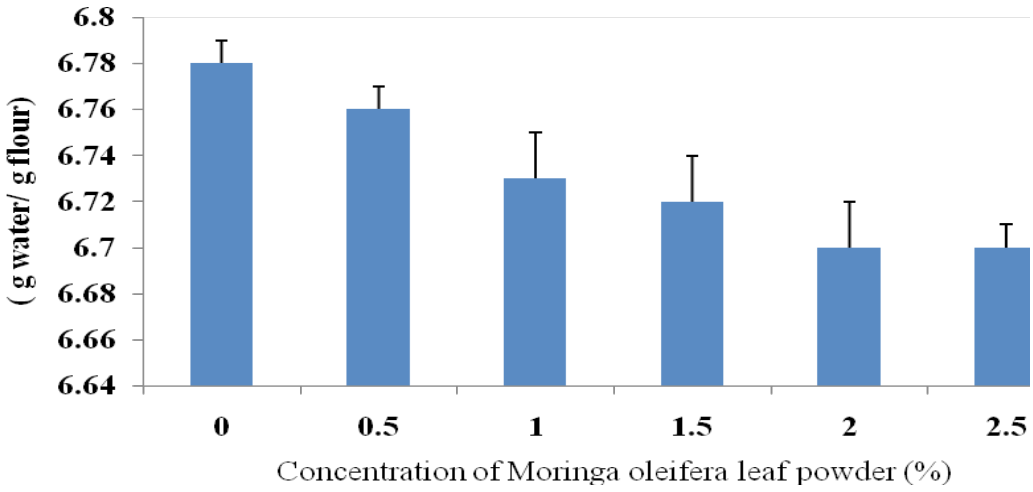
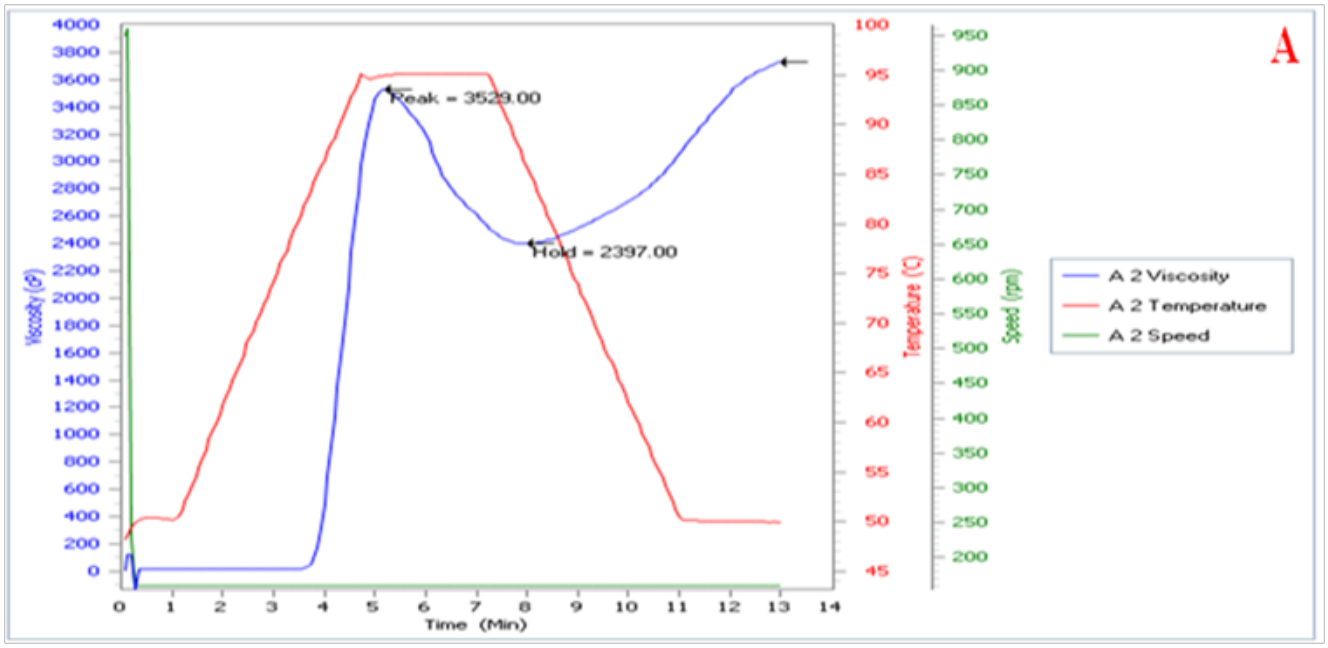


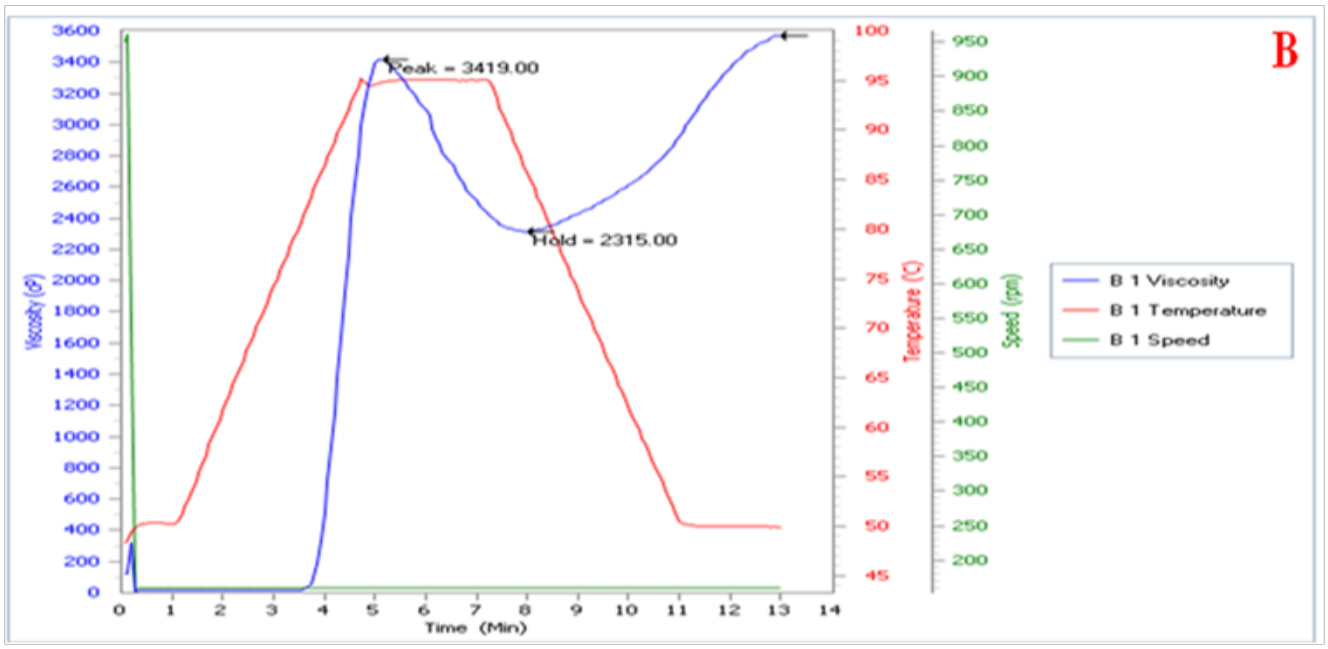
Figure 2. Swelling power of plantain flour fortified with MOLP

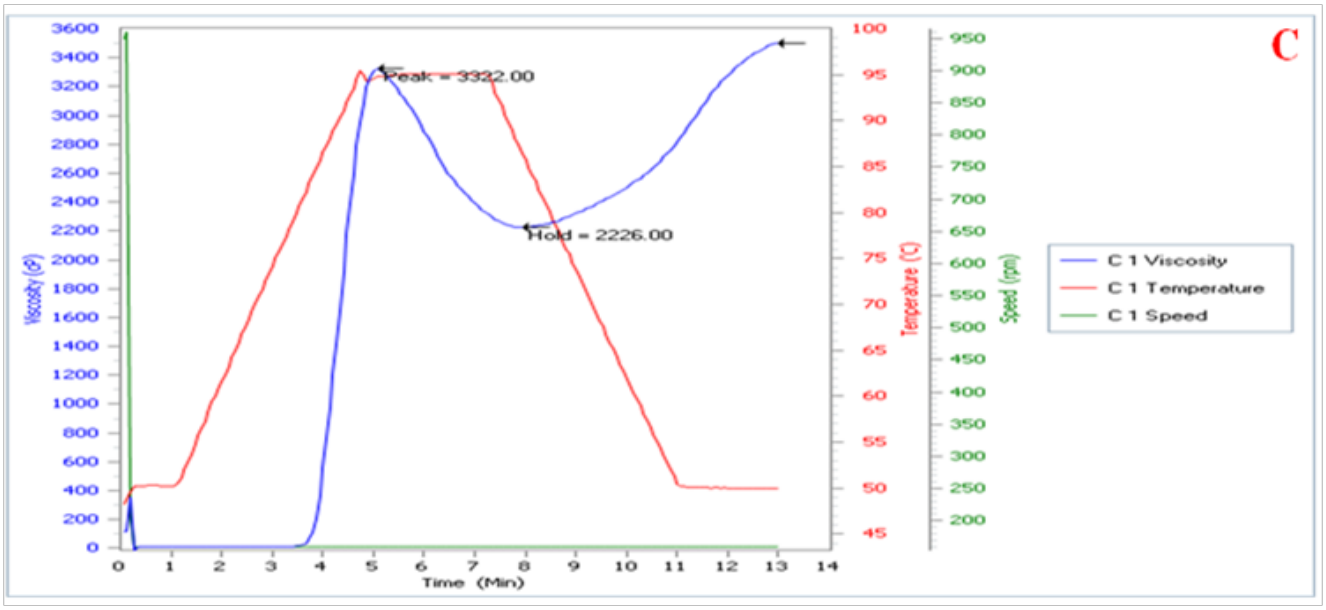
Pasting properties of plantain flour fortified with MOLP

Plantain flour without MOLP (A) had higher peak (3551.00 RVU), trough (2425.50 RVU), breakdown (1125.50 RVU) and final (3771.00 RVU) viscosities compared to the fortified plantain flours (Figure 3). A slight, but progressive decrease in these viscosities was observed with increasing concentration of MOLP. The slight reduction in peak viscosities of plantain flour confirms the minimal change in swelling behaviour (Figure 2) and water absorption capacities (Figure 1). The addition of MOLP did not significantly change the pasting properties of the plantain flour. With the addition of 2.5% MOLP, the peak viscosity of the plantain flour decreased by approximately 8%. Jimoh and Olatidoye (2009) reported a higher decrease (approx. 17%) in peak viscosity for yam flour fortified with 10% soybean flour. Similarly, approximately 19% decrease in peak viscosity was reported for plantain flour fortified with soybean flour (Abioye et al., 2011). The higher decrease in peak viscosity reported by the above authors may be attributed to the higher concentration of the fortificant (soybean) used.

The slight reduction in the final viscosities of the fortified plantain flour, in this study may be advantageous in the preparation of the stiff dough 'amala', since the energy required to create shear may be reduced. Set back viscosity which measures tendency for syneresis of starch upon cooling also decreased with the addition of MOLP. It seems that MOLP interacted with the starch component of the plantain flour during pasting causing a slight decrease in the set back value. High set back value has been associated with higher tendency for retrogradation especially during storage. MOLP fortified amala, thus, will be more stable than the unfortified sample especially when the product is not consume immediately after preparation. Although, the time to peak and pasting temperature also decreased with increasing concentration of MOLP, the decrease was not very significant. Higher pasting temperatures have been attributed to the presence of resistant starch (Maninder et al., 2007).



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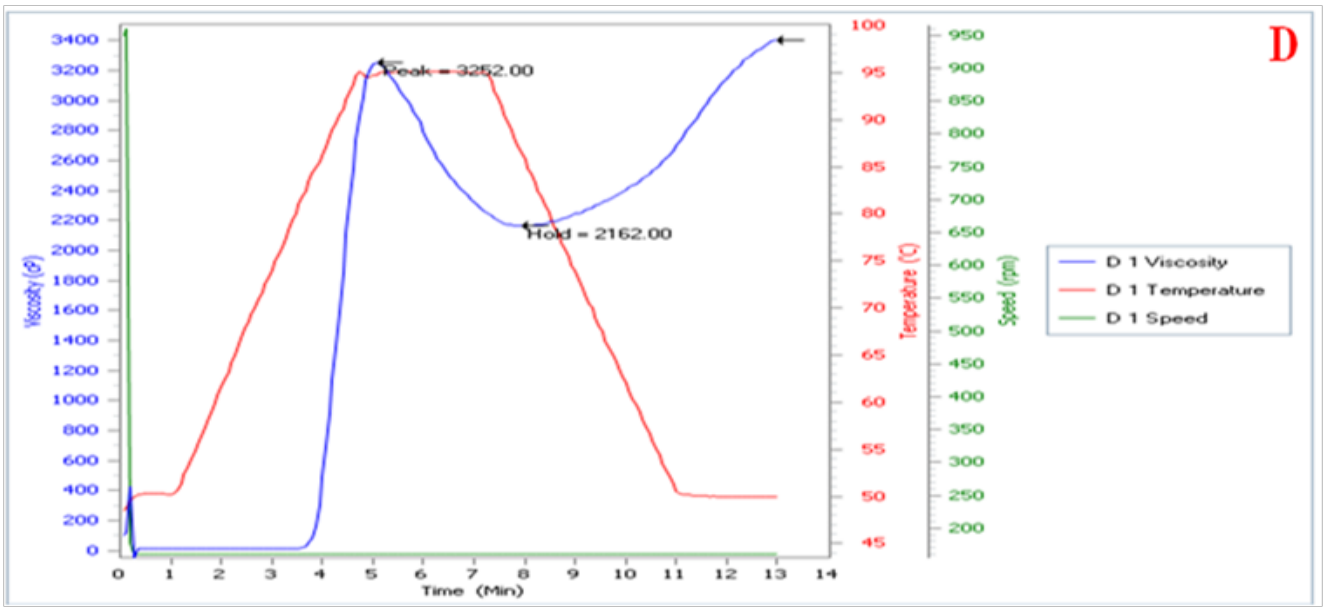
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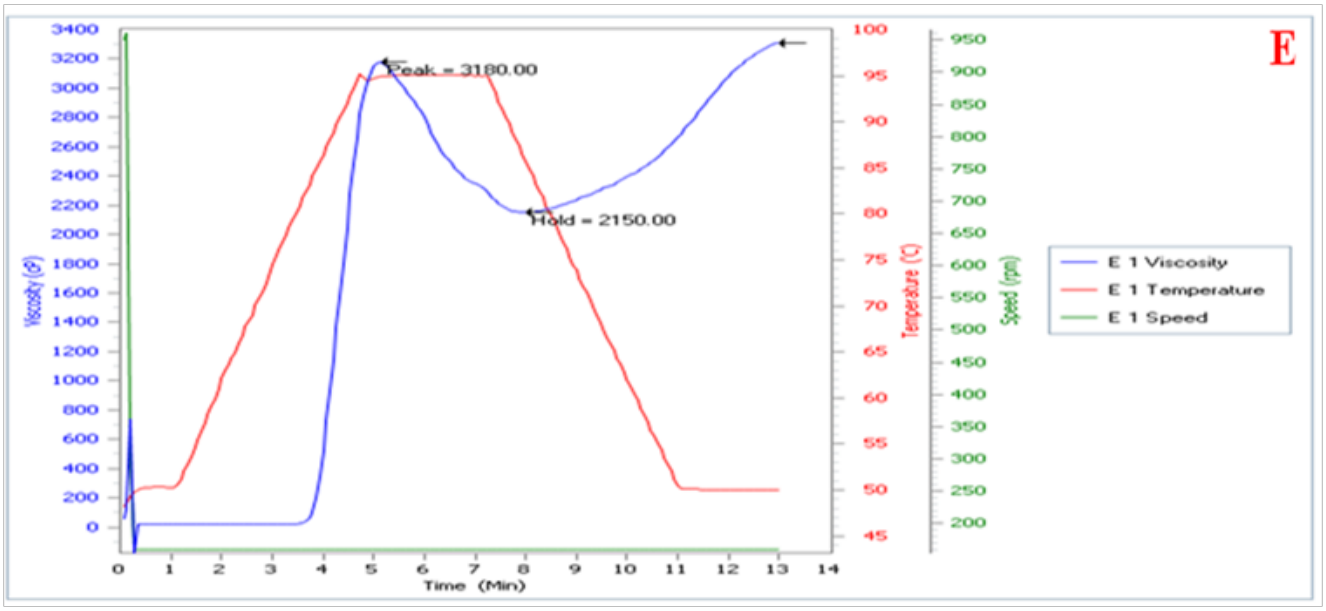
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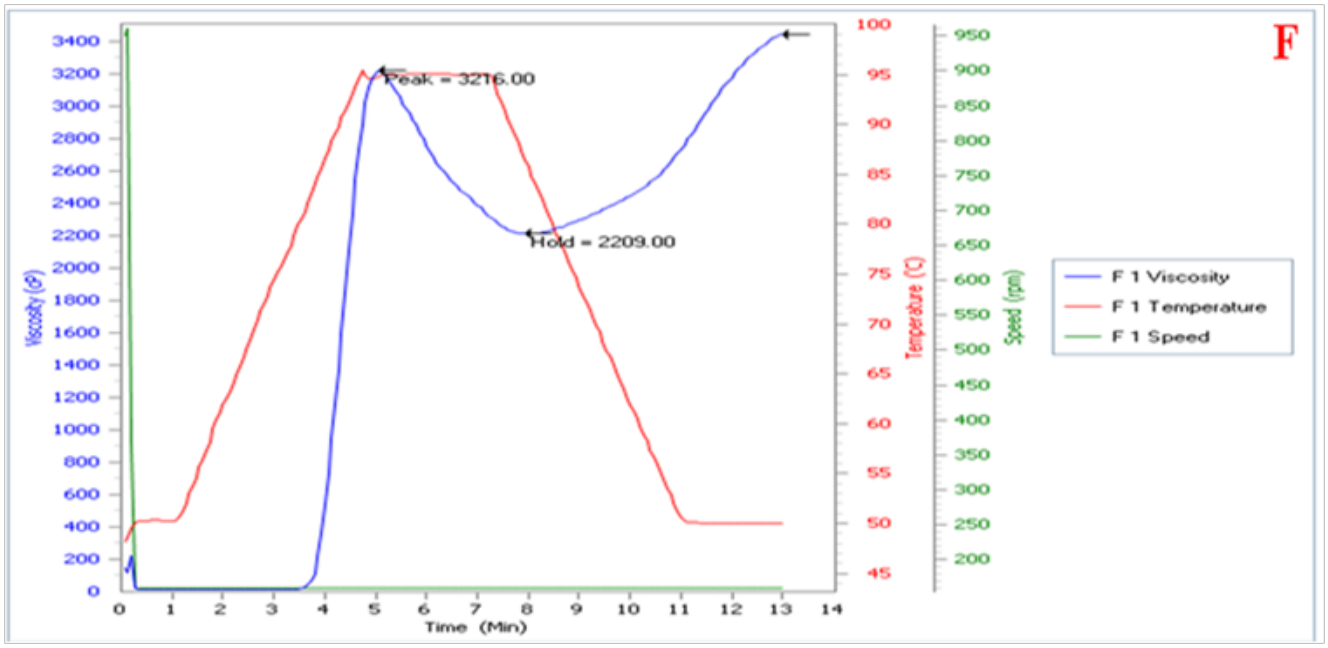

Figure 3. Pasting curves of plantain flour fortified with MOLP

A= Plantain flour amala without MOLP
B= Plantain flour amala fortified with 0.5% MOLP
C= Plantain flour amala fortified with 1.0% MOLP
D= Plantain flour amala fortified with 1.5% MOLP
E= Plantain flour amala fortified with 2.0% MOLP
F = Plantain flour amala fortified with 2.5% MOLP

Proximate composition of amala prepared from plantain flour fortified with MOLP

Stiff dough (amala) prepared from plantain flour fortified with MOLP had higher protein (6.64-10.36%), ash (2.11-2.93) and fat (1.95-2.37%) contents compared to amala without MOLP (Table 3). However, the moisture and carbohydrate content of amala prepared from fortified plantain flour were lower than the control. Amala fortified with MOLP at 2.5% showed significantly higher protein (approx 3 times) and ash (approx. 1.7 times) contents compared to the control. The increase in protein of the amala following the addition of MOLP may be associated with the higher protein content of Moringa oleifera leaves (Table 1). Previous studies by Karim et al. (2013) similarly reported increase in protein content of amala prepared from yam flour fortified with MOLP. However, the protein content of plantain amala fortified with 2.5% MOLP in this study was higher (1.6 times) compared to those reported for MOLP fortified yam flour amala (Karim et al., 2013). The differences in protein content may be attributed to higher protein content of plantain flour compared to yam flour.

Table 3. Proximate composition of amala prepared from plantain flour fortified with MOLP (%)

Samples	Moisture	Protein	Fats	Ash	Carbohydrate
$PFAM_{0}$	$74.29^{ab}\pm0.00$	$3.52^{a}\pm0.03$	$1.82^{a}\pm0.04$	$1.71^{a}\pm0.00$	$18.66^{d}\pm0.01$
$PFAM_{0.5}$	$74.26^{ab}\pm0.10$	$6.64^{b}\pm0.02$	$1.95^{a}\pm0.01$	$2.11^{b}\pm0.00$	$15.04^{bc}\pm0.03$
$PFAM_{1.0}$	$72.29^{a}\pm0.02$	$8.51^{c}\pm0.00$	$2.12^{b}\pm0.04$	$2.71^{bc}\pm0.02$	$14.37^{b}\pm0.03$
$PFAM_{1.5}$	$72.14^{a}\pm0.04$	$8.71^{d}\pm0.10$	$2.02^{b}\pm0.04$	$2.79^{bc}\pm0.04$	$14.34^{b}\pm0.02$
$PFAM_{2.0}$	$70.29^{a}\pm0.05$	$10.21^{e}\pm0.02$	$2.06^{b}\pm0.20$	$2.71^{bc}\pm0.04$	$14.73^{b}\pm0.03$
$PFAM_{2.5}$	$70.36^{a}\pm0.04$	$10.36^{e}\pm0.04$	$2.37^{c}\pm0.02$	$2.93^{bc}\pm0.04$	$13.98^{a}\pm0.06$

Mean ± SD. Mean with different superscript along a column are significantly different ($p<0.05$).
PFAM0= Plantain flour amala without MOLP
PFAM0.5= Plantain flour amala fortified with 0.5% MOLP
PFAM1.0= Plantain flour amala fortified with 1.0% MOLP
PFAM1.5= Plantain flour amala fortified with 1.5% MOLP
PFAM2.0= Plantain flour amala fortified with 2.0% MOLP
PFAM2.5 = Plantain flour amala fortified with 2.5% MOLP

Mineral composition of amala prepared from plantain flour fortified with MOLP

Generally, the mineral content of the amala increased with increase in MOLP, which may be attributed to high contents of these minerals in the leaves (Table 2). Calcium and potassium were the major minerals in the prepared amala, while magnesium, sodium and iron were relatively low (Table 4). Calcium contents of the amala increased from 190.03 to 254.42 mg/100 g, magnesium, from 4612.10 to 4915.10 mg/100 g, potassium from 94.06 to 132.04 mg/100 g, sodium from 45.11 to 54.12 mg/100 g and iron contents increased from 2.43 to 3.29 mg/100 g. Previous studies on amala prepared from yam flour similarly reported increase in mineral content following the addition of MOLP (Karim et al., 2013). However, the increase in mineral composition in this study was much higher than those reported by these authors, possibly due to the higher mineral content of the plantain flour.

Table 4. Mineral contents of amala prepared from plantain flour fortified with MOLP (mg/100 g)

Samples	Calcium	Potassium	Magnesium	Sodium	Iron
PFAM0	190.03^{a}±0.03	4612.10^{a}±0.02	94.06^{a}±0.23	45.11^{a}±0.14	2.43^{a}±0.02
PFAM0.5	204.12^{b}±0.06	4672.02^{a}±0.00	102.71^{b}±0.12	46.71^{a}±0.23	2.67^{a}±0.05
PFAM1.0	224.20^{b}±0.14	4742.12^{a}±0.01	105.04^{b}±0.11	52.71^{b}±0.14	2.72^{a}±0.04
PFAM1.5	245.13^{b}±0.03	4743.02^{a}±0.12	123.36^{c}±0.22	50.79^{b}±0.24	2.86^{bc}±0.12
PFAM2.0	248.29^{b}±0.12	4812.21^{ab}±0.14	129.33^{c}±0.32	53.71^{b}±0.02	2.88^{bc}±0.03
PFAM2.5	254.42^{b}±0.12	4945.10^{ab}±0.03	132.04^{cd}±0.12	54.12^{b}±0.20	3.29^{c}±0.04

Mean ± SD. Mean with different superscript along a column are significantly different ($p<0.05$).
PFAM0= Plantain flour amala without MOLP
PFAM0.5= Plantain flour amala fortified with 0.5% MOLP
PFAM1.0= Plantain flour amala fortified with 1.0% MOLP
PFAM1.5= Plantain flour amala fortified with 1.5% MOLP
PFAM2.0= Plantain flour amala fortified with 2.0% MOLP
PFAM2.5 = Plantain flour amala fortified with 2.5% MOLP

Sensory properties of amala prepared from plantain flour fortified with MOLP

Amala prepared from the unfortified plantain flour, which served as the control had the highest rating for colour, aroma, mouldability, consistency, mouthfeel and overall acceptability compared to the fortified amala (Table 5). With the exception of amala prepared from plantain flour with 2.5% MOLP, the ratings for overall acceptability of the fortified samples were comparable to the control. The colour rating for amala with 2.5% MOLP was very low. This may be attributed to the colouration of the amala by chlorophyll from the MOLP which may have masked the colour of the amala as previously reported (Karim et al., 2013). Karim et al. (2013) reported that 2.5% level of MOLP to yam flour was sufficient for making amala with acceptable qualities. Our finding agree with previous reports by Karim et al. (2013) who suggested the use of MOLP at 2.5% as sufficient to improve the proximate and mineral composition of yam flour amala without having significant effect on the sensory properties.

Table 5. Mean sensory scores of amala prepared from plantain flour fortified with MOLP

Samples	Colour	Aroma	Mouldability	Consistency	Mouth feel	Overall Acceptability
PFAM0	7.29c±0.01	7.86d±0.02	6.86c±0.03	6.71c±0.04	7.43c±0.02	7.64bc±0.02
PFAM0.5	5.36b±0.12	6.64c±0.00	5.71bc±0.02	5.71b±0.03	6.50bc±0.05	6.89b±0.01
PFAM1.0	5.29b±0.01	5.71b±0.01	6.00c±0.01	5.71b±0.04	5.86b±0.04	6.93b±0.02
PFAM1.5	5.93b±0.03	5.71b±0.12	5.36bc±0.02	5.79b±0.04	5.00b±0.12	6.09b±0.03
PFAM2.0	5.29b±0.04	5.21b±0.14	5.36bc±0.02	5.71b±0.02	6.36cbc±0.03	6.07b±0.04
PFAM2.5	3.36a±0.06	4.36a±0.03	4.57a±0.02	3.93a±0.00	4.21a±0.04	5.90a±0.05

Mean ± SD. Mean with different superscript along a column are significantly different ($p<0.05$).

PFAM0= Plantain flour amala without MOLP
PFAM0.5= Plantain flour amala fortified with 0.5% MOLP
PFAM1.0= Plantain flour amala fortified with 1.0% MOLP
PFAM1.5= Plantain flour amala fortified with 1.5% MOLP
PFAM2.0= Plantain flour amala fortified with 2.0% MOLP
PFAM2.5 = Plantain flour amala fortified with 2.5% MOLP

Conclusions

The addition of Moringa oleifera leaf powder to plantain flour improves the nutritional quality of stiff dough without substantial changes in the functional properties. The use of Moringa oleifera leaf powder thus has the potential to combat protein-energy malnutrition and micronutrient deficiencies in developing countries. However, the effect of Moringa oleifera leaf powder on digestibility may be a subject of further research. Further, the effect of the Moringa leaf on storage stability of the flours may also be investigated.

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Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2015) 4 (1) 59-64

THE IMPORTANCE OF PATIENTS KNOWLEDGE AND THEIR FAMILIES ABOUT THE RISK, SYMPTOMS, TREATMENT AND PREVENTION OF TYPE 2 DIABETES

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Summary

Introduction: *Diabetes mellitus* is the most common metabolic disease in the world. There is very important influence of patients knowledge and their families knowledge about type 2 diabetes to the risk reduction, treatment and prevention of disease.

The aim: The aim of the research was to estimate the connection between the knowledge of patients and their families about the risk factors, symptoms, treatment and prevention of type 2 diabetes and occurrence of diabetes.

Participians and methods: The study was conducted by randomly interviewing 102 volunteers in Tuzla. The main instrument of the research was self-designed questionnaire on risk, symptoms and the treatment of diabetes. We measured values of glucose in blood for all volunteers.

Results: The results of this study suggest that patients and their family members are not informed enough about the risks, symptoms and treatment of type 2 diabetes.

Conclusion: There was a significant connection between the number of correct answers with the knowledge of diabetes.

Keywords: diabetes mellitus, risk factors, symptoms, treatment and prevention, insulin

Introduction

Diabetes mellitus (DM) is a medical condition defined by high level of glucose in blood (Ryde'n L. 2013). It is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia when metabolism of charbohydrates, proteins and fats is deranged, and is a result of the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced, or both. It can lead to serious health complications, even premature death, but people suffering from this disease can control it and decrease the risk relating the complications. There are many causes of diabetes. First of all, genetic predisposition has a significant role, following constitution, different pancreas anomalies, different inflammations, tumors, obesity, etc. (Imamovic Kuluglic, 2008).

The meaning of the word *diabetes mellitus* (sugar diabetes) suggests high level of sugar in blood and urin in people who do not treat diabetes. Diabetes sets on when the pancreas do not produce enough insulin, and so the level of sugar, i.e. glucose in blood is uncontrolable. With the absence of insulin, glucose is stacked in blood causing various biochemical damage (Barnard 1981).

Classification of *diabetes mellitus* is based on recommendation of World Health Organisation (WHO) and American Diabetes Association (ADA). Glycated hemoglobin A1c (HbA1c) is recommended as a test fot determining *diabetes mellitus* although there are some doubts relating its sensitivity in determination of diabetes. HbA1c values > 6,5% do not exclude diabetes. There are four types of diabetes, and they are: *Diabetes mellitus* type 1 (T1DM), type 2 (T2DM), gestational *diabetes mellitus* and other specific

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types of diabetes. (Ryde'n L. and others, 2013).
Diabetes type 1 is characterized by lack of insulin due to destructiopn of beta cells of the pancreas, which progresses to absolute lack of insulin. Typically, T1DM is common in young, skinny people who suffer from polyuria, increased thirst, weight loss, with slight tendency to ketosis. However, T1DM can appear in any age. In another form, latent auto-immune diabetes of adults (LADA) develops over a longer period of time. People with auto-antibodies to beta cells of the pancreas such as proteins glutamic acid decarboxylase, inhibitors of protein tyrosine phosphatase, insulinoma-associated auto-antibodies, or zinc transporter auto-antibodies will probably cause an accute attack or a slow progressive insulin addiction (Lars Ryde'n and others, 2013). Patients suffering from this type of diabetes are obligated to inject themselves with a daily dose of insulin in order to prevent comatose state. (Insel and Roth, 2004).
Diabetes type 2 is characterized by a combination of insulin resistance, weakness of beta cells. Obesity and sedentary life style are the major risk factors causing T2DM. Insulin resistance and stage one low production of insulin cause postprandial hypoglycemia as an early stage of T2DM. This stage is followed by relapse, and in the second stage appears persistent hyperglycemia. T2DM usually develops after middle age and includes more than 90% of the adult population suffering from diabetes. It does not show any specific symptoms for many years which explains the fact that half of the cases of T2DM are not diagnosed in time. Hypoclycemic screening is very useful in cardiovascular risk assessment as well as determining the right diagnosis on time. Early detection of T2DM reduces the risks of cardiovascular diseases (Griffin and others, 2011). The most common symptoms of T2DM are: excessive thirst, frequent urination, blurry vision, recurrent infections, slow healing cuts, irritability, prickling sensation, loss of sense in arms and legs. With T2DM the pancreas usually produces some or enough insulin, but there is a significant insulin resistance due to cell disorder in the pancreas, liver and the muscles. Insulin resistance is a pathological condition in which cells fail to respond to the normal actions of the hormone insulin. The body produces insulin, but the cells in the body become resistant to insulin and are unable to use it effectively. Insulin resistance plays an important role in pathophysiology of T2DM as well as lifestyle factors and genetics. More than 90% of people with T2DM is obese, and have elevated plasma levels of free fatty acids (FFA) which are known to cause peripheral (muscle) insulin resistance. T2DM can be controlled by balanced diet, weight loss and regular physical activity. If that is not enough, patients should take some oral antidiabetic medications or insulin shots. Around 1/3 of the patients with T2DM have to inject themselves with the insulin shots, while the rest of the mentioned patients use some kind of oral medication which lead to increase of insulin production or cell stimulation to absorb glucose (Insel and Roth, 2004).
There is also the third type of diabetes which occurs in 2-4 % of all pregnancies. This type of diabetes, also known as gestational diabetes, may improve or disappear completely after delivery. However, after pregnancy half of the women with gestational diabetes are found to have diabetes mellitus, most commonly type 2 (Insel and Roth, 2004). Big Canadian research has shown that probability of developing *diabetes mellitus* after gestational diabetes is 4% in period of 9 months after delivery, and 19% in period of 9 years after delivery (Lars Ryde'n and others, 2013).
Healthy and balanced diet is very important for preventing any complications related to diabetes mellitus. We can distinguish two types of diet in diabetes patients, standard one during the illness, and acidosis diet. Standard diet includes information about weight, height, and age of a patient. If the weight is desirable, a diet of 25 calories per kilogram of patient's weight is determined. Obese patients should try to reduce weight gradually. Acidosis diet consists of giving insulin, fluids and carbohydrates in dietary measures. In extreme cases, such as shock, blood transfusion is required (Imamovic, Kuluglic, 2008).
There are many tests used to diagnose diabetes, such as: determining the level of glucose in blood, the oral glucose tolerance test (OGTT), the intravenous glucose tolerance test, determining ketone bodies in urine, determining insulin levels, C-peptides, and others. DETECT 2 study

has analyzed the results of 44 000 people in 9 studies in 5 countries. It is concluded that HbA1c > 6,5 % (48 mmol/L) and values of fasting blood glucose test > 6,5 mmol/L (117 mg/dl) is diagnostic parameter for diabetes mellitus, and for values of HbA1c 6,0-6,5 %, it is necessary to do fasting blood glucose in order to determine a diagnosis. The value of fasting blood glucose test to diagnose *diabetes mellitus* is 7,0 mmol (126 mg/dl) in venous plasma (which is recommended). It is also necessary to determine the values of glucose 2 hours after OGTT is done. Definite diagnosis of the disease is following values: 11,1 mmol (200mg/dl) in venous plasma, 9,4 mmol (169 mg/dl) in venous blood and 10,3 mmol (185 mg/dl) in capillary blood (WHO, 2006/2011; ADA 2003/2012).

According the global assessment for year 2011, International Diabetes Federation has shown that 52 million Europeans aged 20 to 79 have diabetes and that number will have increased to more than 64 million by the year 2030. 281 million of male and 317 million of female diabetes patients died in 2011. around the world, mostly from cardiovascular diseases. Costs of treating diabetes in Europe were about 75 billion Euros in 2011. and it is estimated that the costs will increase to 90 billion Euros in 2030. (Lars Ryde'n and others, 2013)

According to the data gathered by Association of diabetologists of Federation of Bosnia and Herzegovina, it is estimated that more than 200,000 people suffer from some type of diabetes. The number of T2DM patients is increasing significantly. At least half of the T2DM patients do not have proper diagnosis and are not aware of their illness. In 2010. in Federation of Bosnia and Herzegovina there are more than 50,000 registered people who suffer from diabetes. In Republic of Srpska, there are about 60,000 registered patients, of which 15,000 receive insulin therapy. The number of young people and children who suffer from T2DM, which usually affects older adults, is in significant increase in Federation of Bosnia and Herzegovina.

The aim of this paper is to assess the connection between knowledge of basic risks, symptoms, treatment and prevention of diabetes and family relations of T2DM patients in Tuzla Canton.

Participians and methods

A cross-sectional research has been conducted. The sample of population from primary care practice is provided by stratified sampling where random units have been divided into two groups depending on the values of glucose in blood. Research tool has been a self-designed questionnaire about risk factors, symptoms and treatment of T2DM patients. The questionnaire consists of 12 questions and is divided into 3 parts. The first part consists of questions about general characteristics and types of diabetes (definition of diabetes, type of diabetes T1DM, T2DM, gestational diabetes, diabetes insipidus). The second part consisted of knowledge questions about symptoms of diabetes (itchy skin, extensive thirst, frequent urination, frequent infections, and impairment of vision, kidneys, nerves). The third part of the questionnaire consisted of knowledge questions about risk factors (genetic predisposition, infection, obesity, old age, pregnancy). There have been 2-4 options given for each question, but only one is correct answer. At the end of the questionnaire, there has been a question about family relations with the patient. Immediate family members are spouse, parents, children, brothers and sisters. The given options are following: I suffer from diabetes, my immediate family member suffers from diabetes, no one in my family suffers from diabetes, and I do not know anyone suffering from diabetes. The connection between correct answers in the questionnaire and family relations of *diabetes mellitus* type 2 patients is assessed through these optional answers that were given.

This cross-sectional research has been conducted in Tuzla. During filling out the questionnaire, the subjects have had their capillary blood taken before the meal and the value of fasting blood glucose test has been determined. Experimental group consisted of subjects who did not have high values of fasting blood glucose test. The subjects of this research consented to participate in this study. Control group consisted of volunteers who had higher values of fasting blood glucose test, above 6,7 mmol/l. The research included people of both sexes, age between 20 and 65 (table 1).

Table 1. Structure of volonteers by gender and age
Tabela 1. Struktura ispitanika po spolu i godinama

Gender	Age					
	20-30	30-40	40-50	50-60	60-65	Total
Female	6	9	24	17	4	60
Male	4	6	11	19	2	42
Total	10	15	35	36	6	102

People excluded from this research are under 20 years of age and older illiterate people. There were 102 volunteers who participated in this study, of which 50% of subjects were in experimental group, and 50% of them were in control group. There has not been a single form of education relating this study of the included subjects before they joined the research. Completion of the questionnaire has been according to ethical code and terms of anonymity.

Results

The highest number of test subjects answered with 60% correct answers. It has been recorded that 24,5% of the test subjects answered less than 50% of the questions correctly. It has also been recorded that 24,5% of the test subjects answered above 50% of the questions correctly (picture 1).

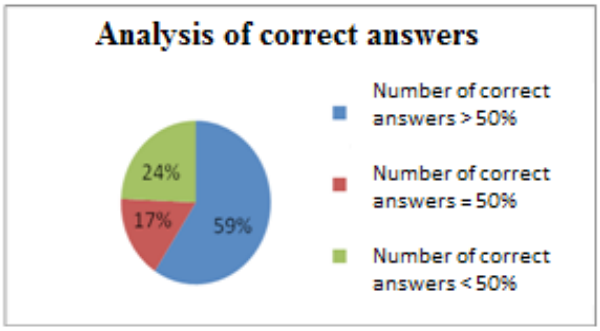

Figure 1. Assessment of correct answers in relation to the total number of participants testing
Slika 1. Procjena tačnih odgovora u odnosu na ukupan broj ispitanika

In this questionnaire, there was also a question about type of relationship that test subjects have with DM patients. 2/3 of the total number of test subjects said they knew a DM patient, or they themselves were DM patient. 29,4% of the test subjects did not know anyone suffering from diabetes while 9,8% of the test subjects were non-committal (picture 2).

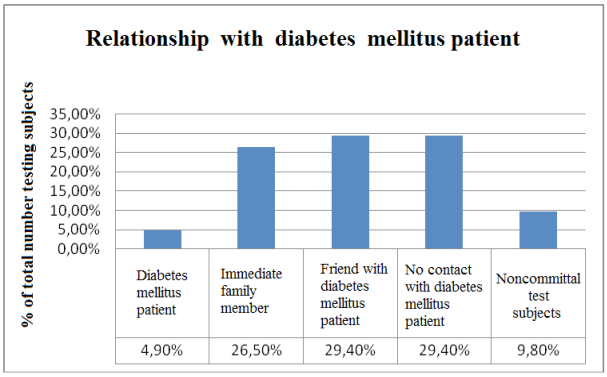

Figure 2. Knowing people with diabetes
Slika 2. Poznavanje oboljelih od dijabetesa

A significant connection has been established between the number of correct answers and the relationship with people suffering from diabetes. Three out of five test subjects who were in fact DM patients scored less than 33,3% correct answers, and seven out of twenty-seven test subjects who have a family member suffering from DM scored less than 50% correct answers (picture 3).

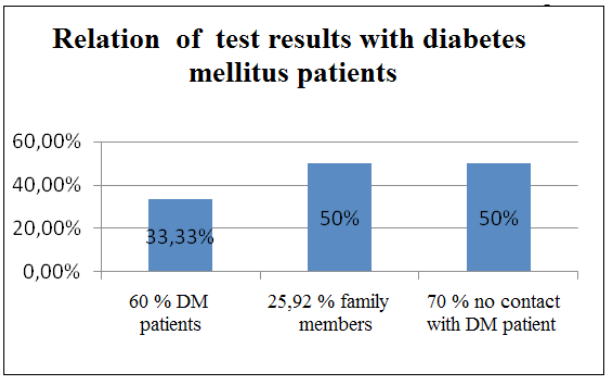

Figure 3. Relation of test results with being a *diabetes mellitus* patient or knowing a DM patients
Slika 3. Veza rezultata odgovora ispitanika sa poznavanjem osoba oboljelih od dijabetesa

It is concluded that patients and their family members need to be educated additionally about the illness in order to reduce cases of complications relating diabetes.
It is very important to emphasize the fact that 65% of the family members had the value of fasting blood glucose test above 6,7 mmol/l, and that they did not have a diagnosis established earlier (table 2).

Table 2. Data of the experimental and control group of volonteers
Tabela 2. Podaci o ekperimentalnoj i kontrolnoj skupini ispitanika

Connection with DM patient	Value of fasting blood glucose		number of test subjects
	>6,7 mmol/l	<6,7 mmol/l	
DM patient	22	6	28
Immediate family member	22	12	34
No contact with DM patient	3	27	30
Noncommittal	4	6	10
Total	51	51	102

Conclusion

Although *Diabetes mellitus* is one of the most common types of endocrine disease in the world, diabetes patients are still not educated enough about their disease. This education is conducted through individual doctor-patient conversation, work in small groups, leaflets and brochures, lectures and association of diabetologists in Bosnia and Herzegovina. Prevention of diabetes involves change of lifestyle, reducing body weight, daily physical activity and healthy diet. Inadequate diet, lack of physical activity, obesity, insufficient education relating risks, symptoms, prevention and treatment of diabetes can lead to heavy diabetes complications.

The results of the research show that diabetes patients and members of their family are not informed enough about risks, symptoms and treatment of this disease. The results of a research on how much diabetes patients know about their illness obtained in Heath centre Zajecaj show that they are well informed about their illness. Despite all that, great number of test subjects do not have fasting glycemia regulated, belong to the group of obese people and do not have five daily meals (Jovanovic i Bogdanovic-Zivkov, 2005).

Education of the diabetes patients and especially their family members promptly should be primary in diabetes prevention in order to prevent and treat any possible complications.

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ZNAČAJ ZNANJA BOLESNIKA I ČLANOVA NJIHOVIH PORODICA O RIZICIMA, SIMPTOMIMA, LIJEČENJU I PREVENCIJI DIJABETESA TIPA II

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Sažetak

Uvod: Diabetes mellitus je jedno od najčešćih endokrinoloških oboljenja u svijetu. Veliki je uticaj znanja bolesnika i članova njihovih porodica o dijabetesu tipa 2 na smanjenje rizika, liječenje i prevenciju bolesti.
Cilj: Cilj istraživanja je procijeniti povezanost znanja bolesnika i članova njihovih porodica o osnovnim rizicima, simptomima, liječenju i prevenciji dijabetesa tipa 2.
Metode i ispitanici: Studija je provedena anketiranjem 102 dobrovoljca u Tuzli metodom slučaja. Instrument za istraživanje je bio samodizajnirani upitnik o rizicima, simptomima i liječenju oboljelih od dijabetesa. Mjerene su vrijednosti glukoze na tašte kod svih ispitanika.
Rezultati: Rezultati istraživanja ukazuju da bolesnici od dijabetesa tipa 2 i članovi njihovih porodica nisu dovoljno educirani o rizicima, simptomima i liječenju šećerne bolesti.
Zaključak: Utvrđena je značajna veza između broja tačnih odgovora sa poznanstvom oboljelih od dijabetesa.

Ključne riječi: diabetes mellitus, faktori rizika, simptomi, liječenje i prevencija, inzulin

Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2015) 4 (1) 65-70

PREHRANA KAO UZROK POJAVE MIGRENE I METODE NJENOG LIJEČENJA

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Stručni rad

Sažetak

Migrena je funkcijska vaskularna paroksizmalna glavobolja koja se javlja periodično i obično zahvaća polovicu glave (hemikranija).Mnogim osobama koje boluju od migrene poznato je da određene prehrambene namirnice pogoršavaju migrenoznu glavobolju ili ju pak ublažavaju. Kako je svaka osoba individua za sebe, tako je i individualan utjecaj namirnica na glavobolju pojedinca. Postoji više vrsta namirnica i dodataka prehrani koje pozitivno utječu na pojavu migrene, ali i na njeno ublažavanje. Liječenje migrene može podrazumijevati istodobnu primjenu više terapeutskih metoda: terapija lijekovima, promjena načina života i prehrane, suportivnu psihoterapiju, kao i primjenu alternativnih metoda liječenja.
Cilj ovog rada je prikazati pozitivne i negativne učinke prehrane i prehrambenih dodataka, na pojavu i jačinu migrene kao i terapijske metode liječenja migrene.

Ključne riječi: migrena, pozitivni učinci, negativni učinci, prehrana, terapija

Uvod

Migrena se često definira kao kronična, epizodna, primarna glavobolja čiji simptomi u pravilu traju do 72 sata, a mogu biti vrlo izraženi (Merck i sur.,2014.). Bol je najčešće jednostrana te pulsirajuća, pogoršava se s fizičkom aktivnošću i praćena je popratnim simptomima (npr. mučnina, osjetljivost na svijetlo, zvuk i mirise). Smatra se da migrenska glavobolja nastaje kao posljedica složenih promjena na mozgu i krvnim žilama. Kod osoba sklonih migreni (smatra se da je migrena jednim dijelom nasljedna) postoji povećana senzibilizacija određenih dijelova mozga. Kada se zbog utjecaja vanjskih ili unutarnjih faktora aktiviraju određeni živci i dijelovi mozga (hipotalamus, vidni dio moždane kore, živac trigeminus, krvne žile u mozgu), počinje cijeli slijed događaja u mozgu koji u konačnici dovodi do migrene (Demarin, Vuković, 2005.).Međunarodni tim znanstvenika, na čelu s dr. Zameel Caderom sa sveučilišta u Oxfordu, već neko vrijeme tvrdi, da bi snažna bol koja se javlja kod migrene mogla biti genetski uvjetovana. Proučavajući DNK uzorke 110 osoba koje pate od migrene kao i njihovih obitelji, tim znanstvenika identificirao je mutaciju na genu TRESK koja je povezana s osjetljivošću na bol. Kod ljudi koji nose tu genetsku mutaciju, centri za bol u mozgu se lakše aktiviraju i uzrokuju migrenu (Lafrenière i sur.,2010.). Osim predisponirajućih čimbenika i određene namirnice uzrokuju i/ili pogoršavaju glavobolju, pogotovo neke kao što su čokolada, jaki sirevi, pivo, crno vino i šampanjac, mesni proizvodi poput kobasica, suhomesnatih proizvoda, hamburgera te vrhnje, lješnjaci i njihovi proizvodi, gazirana pića, banane, citrusi, kisela hrana i jogurt. Također, postoje namirnice koje pomažu pri smanjenju ili potpunom nestanku simptoma migrene. Najčešće sadrže visok udio zasićenih masti životinjskog podrijetla te ljekovite biljke koje svojim djelovanjem utječu na širenje krvnih žila u mozgu. Migrena je prisutna kod 15-20% osoba (kod svake šeste žene, odnosno svakog petnaestog muškarca, odnosno kod 12-20% žena i 3-6%muškaraca). Obično se javlja između 20. i 40. godine života, ali se može javiti kako u djetinjstvu tako i u starijoj životnoj dobi. Prema podacima Svjetske zdravstvene organizacije (WHO) od nje pati više od 300 milijuna ljudi (Doyle, 2013.). Neki ljudi imaju napad 1 do 2 puta godišnje, dok neki mogu imati do ne-

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koliko napada mjesečno ili čak tjedno. Kod manjeg broja žena migrene su isključivo vezane uz menstruacijski ciklus (migrena se javlja 2-3 dana prije ili za vrijeme menstruacije), ali najveći broj žena ima napadaje i izvan tog razdoblja. Kada se migrena javlja više od 15 puta mjesečno naziva se, kroničnom migrenom. Za vrijeme trudnoće migrene najčešće prestaju ili su puno rjeđe. Napadaji mogu biti uzrokovani i drugim provokativnim čimbenicima kao što su: psihički stres, tjelesni napor, meteorološki uvjeti i nagle promjene vremena. U prošlosti se smatralo da od migrene boluju ljudi koji su iznimno inteligentni ili emotivno osjetljivi, međutim to nikada nije znanstveno dokazano.Mnoge važne povijesne ličnosti kao što su Karl Marx, Sigmund Freud i Alfred Nobel patili su upravo od migrene (Demarin, 2007.). U nastavku će se rad osvrnuti na pozitivne i negativne učinke prehrambenih proizvoda na pojavu migrenoznih napada te metode njenog liječenja.

Prehrana i njeni dodaci kao uzrok pojave migrene

Različite namirnice imaju različiti utjecaj na ljudski organizam, pa tako postoje i namirnice koje imaju negativan utjecaj na glavobolje tj. potiču nastanak migrene. Osim pojedinih namirnica, veliku ulogu u izazivanju migrene ima i svakodnevna prehrana. S obzirom da je svaki pojedinac individua i da svatko ne reagira isto na određenu namirnicu tj. određeni sastav namirnice, širok je spektar namirnica koje uzrokuju ubrzanje pojave napada migrene. Najčešće negativan utjecaj imaju namirnice bogate vazoaminima, tj. kemijskim spojevima koji uzrokuju suženje krvnih žila, a time i pojavu migrene. Jedan od glavnih amina je tiramin. Namirnice koje sadrže tiramin, a nalaze se u svakodnevnoj prehrani su: fermentirani sirevi, crveno vino, kvasac, crno grožđe, pivo, čokolada, agrumi, kiseli kupus, soja sos, avokado i konzervansi. Poseban utjecaj na pojavu napada migrene ima konzumiranje alkoholnih napitaka uslijed čega dolazi do povećanog priljeva krvi u mozak. To se posebice odnosi na crno vino, pivo, viski i šampanjac. Osim tiramina, negativan utjecaj imaju i nitrati i nitriti koji se mogu naći u namirnicama biljnog i životinjskog podrijetla, a ima ih u celeru, cikli, špinatu, radiću i zelenoj salati. Dodatciprehrani koji mogu potaknuti glavobolju su također aspartam (umjetno sladilo), tetrazin (daje žutu boju hrani), sumporni dioksid (za sušenje voća), sol i natrijev benzoat (konzervans)(Demarin, 2007.). Dehidracija također može uzrokovati migrenu. Omega 6 masne kiseline dodatno potiču upale i bolove. Nalaze se u nekim biljnim uljima kao što su kukuruzno i suncokretovo ulje. Međutim, ne bi trebalo izbjegavati sve masti osobito ne one koje sadrže velik udio omega 3 masnih kiselina s obzirom da upravo one djeluju vrlo povoljno na smanjenje migrenoznih napada. Omega 3 masne kiseline nalaze se u ribljem mesu, plodovima mora te orašastim plodovima. Kao što postoji velik broj namirnica koje pogoršavaju migrenu, tako postoje i namirnice koje ublažavaju, ili čak u potpunosti smiruju i prekidaju pojavu napada migrene. Pokusi provedeni na američkim sveučilištima pokazali su da konzumiranje ribljeg ulja zaustavlja migrenozne glavobolje kod oko 65% ispitanika s teškim migrenama upola smanjujući broj napada. Pokazalo se da smanjivanjekoličine zasićenih masti životinjskog porijekla u prehrani može ponekad spriječiti migrene, obzirom da zasićene masti potiču stvaranje spojeva srodnih hormonima koji izazivaju procese što mogu dovesti do migrena (Mulaosmanović-Zalihić, 2009.). Jedno od istraživanjepokazalo je da ograničeno uzimanje masnoća dnevno, ne više od 20 grama, smanjuje učestalost, intenzitet te trajanje migrene (Mulaosmanović-Zalihić, 2009). U navedenom istraživanju 54 osobe podložne migrenama, kroz 12 tjedana uzimale su hranu s malo masnoća (samo su 10 do 15% kalorija dobivale iz njih). Pri tome 94% ispitanika doživjelo je najmanje 40% manje glavobolja. Kod migrenoznih napada koje su doživjeli u vrijeme provođenja ovog istraživanja i zadesila, bolovi su bili 66% slabiji, a glavobolja bi trajala približno 70% kraće(Mulaosmanović-Zalihić, 2009).

Metode liječenja migrene

Liječenje migrene može podrazumijevati istodobnu primjenu više terapeutskih metoda: terapiju lijekovima, promjenu načina života i prehrane, suportivnu psihoterapiju, te primjenu alternativnih metoda liječenja. Važno je ukloniti čim-

benike koji predstavljaju svojevrsne''okidače'' migrenskog napadaja.

Farmakološka terapija

Farmakološka se terapija sastoji od akutne terapije u migrenskom napadaju i profilakse tj. nastojanja prorjeđivanja učestalosti i sprječavanja nastanka migrenskih napadaja. Za bolesnike s jednim ili dva migrenskih napada mjesečno smatra se da je dovoljna akutna terapija, a za one s 3 ili više potrebno je uvesti profilaktičku terapiju. Klinički pristup u terapiji migrene prema individualnim potrebama bolesnika naziva se, stratificiranim pristupom, nasuprot postupnom pristupu, odnosno metodi pokušaja i pogrješaka (Jančuljak, 2012.).

Promjena načina života

Kako je migrena najčešće uzrokovana načinom života, na nju se može utjecati promjenama u prehrani, tjelesnoj aktivnosti, disanju i psihičkoj stabilnosti. Promjena načina života podrazumijeva prestanak pušenja, poticanje tjelesne aktivnosti, promjenu načina prehrane, redovito spavanje i odmaranje, smanjenje pretjeranog konzumiranja alkohola i kofeina te smanjenje prekomjerne tjelesne težine.

Promjena načina prehrane

Promjena načina prehrane treba se temeljiti na smanjenju unosa masnih kiselina, posebno zasićenih masnih kiselina i kolesterola, te namirnica koje sadrže tiramin. Važno je paziti da se u svakodnevnoj prehrani nalazi hrana bogata proteinima te voda kako nebi došlo do dehidracije. Također, poteškoće se javljaju i kod ne konzumiranja hrane u periodu dužem od 16 sati. Tada dolazi do promjena u nivou serotonina i norepinefrina i širenje krvnih žila u mozgu. Ukoliko se nakon dužeg neunošenja hrane unese obrok bogat ugljikohidratima, neizbježna je pojava migrene zbog povećanja lučenja inzulina i smanjenja razine šećera u krvi.
Također, neki stručnjaci govore o ljekovitim pripravcima temeljenima na lopuhu prije svega u obliku čaja. U istraživanju provedenu na 245 osoba, objavljenu u američkom časopisu Neurology, 68% ispitanika koji su uzimali lopuh u obliku proizvoda Petadolexa doživjelo pad broja migrena za najmanje 50% — što je bolji rezultat od onih koje postižu neki lijekovi za sprečavanje migrena, a koji inače predstavljaju standard u njezinu liječenju (AAN, 2012.). Napadaj migrene, izazvan promjenom vremena ili fenom, uklanja često već jedna šalica stolisnikova čaja pije li se veoma vruć u gutljajima. Nakon redovitog pijenja čaja migrena može i posve nestati (Treben, 2008.). Cvijet vratić iz roda suncokreta sadrži partenolide koji se uspješno bore sa sužavanjem krvnih žila u glavi tipičnim za napadaj migrene, što je pokazala studija provedena na Sveučilištu Maryland (Dobson, 2013.). Također, sadrži i druge sastojke koji imaju protuupalno djelovanje te blokiraju otpuštanje serotonina i prostaglandina za koje se vjeruje da doprinose pojavi migrene. Jedu se svježe ubrane ili sjeckaju u salatu, a lišće se može spremati i sušiti te u jelo dodavati kao začin. Prema različitim istraživanjima korištenje cvijeta vratića uvelike ublažava napade, njihov se broj značajno smanjuje (Treben, 2008.). Vrlo je važno paziti da se u svakodnevnoj prehrani nalazi svježa hrana bogata proteinima kao što je piletina, puretina, jaja, orašasti plodovi, banane i grah. Biljke koje su djelotvorne protiv migrene su lovor, jaglac i stolisnik jer korištenje njihovih pripravaka širi krvne žile, potiče prodiranje krvi u organe i pozitivno utječe na perifernu cirkulaciju. Tu je također i kora vrbe koja sadrži male količine salicina, kemijske supstance slične aspirinu, koja ublažuje bol (Treben, 2008.).Konzumacija đumbira u prehrani također ima brojne pozitivne učinke na cjelokupni metabolizam.

Suportivna psihoterapija

Suportivna (podupiruća) psihoterapija specifičan je oblik psihoterapije pri kojoj je glavna komponenta liječenja terapeutovo pružanje potpore pacijentu (Kozarić Kovačić, Frančišković, 2014.). Ona integrira i psihodinamske, kognitivno-bihevioralne i interpersonalne konceptualne modele i tehnike. Ubraja se u površne psihoterapijske metode kojima je cilj da terapeut pobudi pacijentove zdrave i adaptivne oblike ponašan-

ja u svrhu reduciranja intrapsihičkih konflikata koji proizvode simptome mentalnih poremećaja (Kozarić Kovačić, Frančišković, 2014.). Suportivna psihoterapija predstavlja još jednu dimenziju načina liječenja migrene. Ciljevi suportivne psihoterapije su olakšavanje tretmana liječenja: smanjivanje i uklanjanje boli, omogućavanje kontrole neželjenih kontraindikacija lijekova (mučnina i povraćanje, neutropenija…), poboljšavanje općeg stanje pacijenta, smanjivanje osjećaj umora i sl. Još jedan od ciljeva suportivne terapije je jačanje već postojećih sposobnosti za rješavanje svakodnevnih životnih poteškoća, pri čemu je pažnja fokusirana na psihijatrovo optimiziranje obiteljskog, radnog i socijalnog funkcioniranja. Primjena suportivne psihoterapije je od podjednakog značaja kako za bolesnike kod kojih je tek otkrivena migrena tako i za one koji se već duže vremena liječe (Kozarić Kovačić, Frančišković, 2014.).

Alternativne metode

Aromaterapija

Aromaterapija (alternativna grana medicine koja koristi ljekovita svojstva eteričnih ulja) je sigurno jedan od najefikasnijih prirodnih odgovora na migrenu. Sljedeće biljke i njihova eterična ulja pomažu u rješavanju boli koju uzrokuje migrena: lavanda, eukaliptus, rimska kamilica, ulje grejpa, sandalovina, paprena metvica ili pepermint, zelena metvica. Najpopularnija tehnika primjene ulja protiv glavobolje je masiranje sljepoočica, čela i eventualno poleđine vrata s nekim od spomenutih ulja ili s uljnom smjesom (Podnar, 2013.).
'Biofeedback'
Metoda poputbiofeedbacka, ima neke prednosti u liječenju migrene. 'Biofeedback' je tehnika liječenja kojom se osobe treniraju kako poboljšati svoje zdravlje kontroliranjem tjelesnih unutrašnjih procesa koji su inace automatski i nevoljni, kao npr. puls, krvni tlak, napetost mišica, tjelesna temperatura. 'Biofeedback' je tehnika kod koje ljudi uče promijeniti način na koje njihovo tijelo reagira na fizičke simptome. Ovo je vrlo učinkovita metoda terapije za mnoga stanja, ali se primarno se koristi za liječenje migrenske glavobolje, kronične boli i inkontinencije (Mudrovčić, 2007.).

Akupunktura

Njemački su znanstvenici ustanovili kako akupunktura može pomoći osobama koje boluju od migrena, a učinci ovog tretmana su dugotrajni. Istraživači Medicinskog centra Charité Sveučilišta u Berlinu proučavali su 500 osoba s migrenama koje su podijelili u dvije skupine.
Ispitanici prve skupine prošli su tradicionalne tretmane akupunkturom, kroz razdoblje od četiri tjedana, dok su akupunkturne iglice nasumce raspoređene po tijelu ispitanika druge skupine (Horvat, 2012.). Prije početka istraživanja ispitanici su prosječno pretrpjeli napad migrene šest puta na mjesec. Nakon tretmana iglicama (propisnih i onih fingiranih), svi su ispitanici osjetili poboljšanje– izjavili su kako su u sljedećih mjesec dana imali samo tri migrene, a one su također bile slabijeg intenziteta. No, ovaj dobrobitan učinak pokazao se dugotrajnijim jedino kod ispitanika koji su zaista bili podvrgnuti tretmanu akupunkture. Ovi su ispitanicii dalje imali prosječno manje napadaja migrenatri mjeseca nakon tretmana, za razliku od ispitanika koji su bili podvrgnuti lažnom tretmanu (Horvat, 2012.).

Masaža glave

Masaža glave jedna je od najstarijih terapija u liječenju migrena, a najbolje djeluje ako se primjeni brzo nakon početnih simptoma. Dijeli se na masažu vrata i krvnih žila (kreće se od dna vrata sve do same baze glave, gdje se masira kružnim pokretima), masažu područja sljepoočnice (pokreti trebaju biti nešto nježniji, sporiji i kružni), masažu za bol iznad očiju (pritiskanje točki oko nosa i kod korjena obrva), čeonu glavobolju (ravni pokreti povlačenja prstiju od obrva do vrha čela).

Zaključak

Rezultati istraživanja pokazuju da migrena češće pogađa žene nego muškarce i češće od nje pate odrasli u odnosu na djecu. Zahvaća između 15 i 20% sveukupne populacije, uglavnom između 20-e i 40-e godine života. Također, dokazano je

da određene vrste namirnica i dodataka prehrani mogu pozitivno, odnosno negativno djelovati na samu pojavu migrene, te na njen intenzitet. Najčešće negativan utjecaj na samu migrenu imajunamirnice bogate vazoaminima, a jedan od glavnih amina je tiramin. Kao što postoji velik broj namirnica koje pospješuju pojavu migrene te njezin intenzitet, tako postoje i namirnice koje ublažavaju ili u potpunosti sprječavaju pojavu napada migrene, npr. namirnice bogate omega 3 masnim kiselinama.

Premda je migrena česta primarna glavobolja, nije dovoljno dobro dijagnosticirana, niti dovoljno dobro liječena; stoga je prijeko potrebno ukazati pacijentima da postoje specifični pristupi za sprječavanje i ublažavanje migrenskog napadaja. Ne postoji lijek za migrenu, ali promjena načina života (kao npr. poticanje na tjelesnu aktivnost, redovno uzimanje obroka, dovoljno sati sna te promjena načina prehrane) može pomoći u smanjenju intenziteta i učestalosti pojave migrene. Osim toga, tretman se usredotočuje na izbjegavanje tvari koje mogu pospješiti pojavu napada migrene. Potrebno je i identificiranje lijekova koji sprječavaju ili smanjuju intenzitet napada i lijekova koji smanjuju izrazito jaku bol kod teškog napada migrene.

Na ublažavanje migrene pozitivno djeluju lijekovi, prije svega analgetici, tealternativne metode među kojima se posebno ističu aromaterapija, akupunktura, biofeedbackte, masaža glave. Također, pacijentima se, koji pate od migrene, preporuča se promjena stila načina života te osobito prehrane.

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NUTRITION AS THE CAUSE OF MIGRAINE AND ITS TREATMENT METHODS

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Professional paper

Abstract

Migraine is afunctionalvascularparoxysmalheadachethat occursperiodicallyandusuallyaffectshalf of thehead known ashemicrania.

Manypeople who suffer frommigraineknow thatcertainfoods can soften or make migraine headache even worse. Each person isan individualfor himself and so is theindividualimpactof foodonthe veryheadachesufferer. There are several typesof foodsand dietary supplementsthathave a positive impacton the occurrence ofmigraine, but alsoonits treatment. The treatment ofmigrainemay include the use ofmultipletherapeutic methods at the same time: drug therapy, lifestyle changesanddiet, supportive psychotherapy, andimplementation of alternativemethodsof treatment.

The aim of thispaper is to presentpositive and negativeeffects ofdietandnutritional supplementson the incidenceand severity ofmigraine, and therapeuticmethodsof treatingmigraine.

Keywords: migraine, positive effects, negative effects, nutrition, therapy

Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2015) 4 (1) 71-80

UTICAJ VISINE TEMPERATURE I REŽIMA TOPLOTNE OBRADE NA PROMIJENU TEHNOLOŠKIH OSOBINA MESA

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Pregledni rad

Sažetak
Meso je veoma bitna namirnica u ishrani ljudi zbog svojih nutritivno vrijednih sastojaka. Gotovo svi proizvodi od mesa se na neki način toplotno obrađuju kako bi se postigle željene senzorne i nutritivne osobine, kao i zadovoljili kriterijumi po pitanju mikrobiološke stabilnosti i bezbijednosti proizvoda. Najčešća su dva tipa toplotne obrade u industrijskim uslovima, a to su toplotna obrada kuvanjem na atmosferskom pritisku ili vakumu i suva toplotna obrada pečenjem. Stoga ovaj rad ima za cilj dati kratak pregled najnovijih dostignuća u pogledu uticaja tehnoloških parametara tokom toplotne obrade u prvom planu visine temperature u centru uzorka i razlučitih postupaka toplotne obrade (kuvanjem i pečenjem) na promjene na proteinima, boji mesa, reološkim osobinama mesa, pH mesa, sposobnosti vezivanja vode, hemijskim osobinama i senzornim osobinama mesa.

Ključne riječi: meso, kuvanje mesa, pečenje mesa, boja mesa, stanje proteina

Uvod

Da bi odgovorila zahtjevimanastalim usljed promjene načina života ljudi, industrija prerade mesa je povećela proizvodnju toplotnopoluobrađenih i toplotno potpuno obrađenih proizvoda od mesa. Na primjer, sedamdesetih godina 70% od ukupne proizvodnje mesa svinja u SAD se prodavalo kao svježe meso, a svega 30% prerađeno u proizvode. Situacija se danas potpuno promijenila u korist prerađenog mesa (Toldrá i sar., 2010). Više autora u svijetu je tokom proteklih godina ispitivalo uticaj toplotne obrade mesa na strukturne promjene proteina u mesu (Paul, 1963; Bouton i sar., 1981; Martens i sar., 1982;Bertola i sar., 1994; Dumoulin i sar., 1998). Bramblett i sar., (1959); Marshall i sar., (1960); Penfield i Meyer (1975) vjerovali su da niže temperature tokom toplotne obrade daju proizvode sa manjim gubicima vode. Davey i Gilbert (1974) pronašli su da tekstura mesa varira u zavisnosti od temperature u rasponu od 40°C do 75°C. Machlik i Draudt (1963) proučavali su zavisnost tvrdoće mesa od temperature i otkrili da opada u temperaturnom intervalu od 58°C do 60°C, a raste od 65°C do 75°C.Martens i sar., (1982); Findlay i sar.,(1986); Bertola i sar.,(1994) proučavali su zavisnost teksture od toplotno indukovane denaturacije proteina. Dokazano je takođe, da temperatura u značajnoj mjeri utiče i na rastvorljivost proteina (Bouton i Harris, 1972). Takođe je poznato da i rastvorljivost kolagena tokom zagrijevanjamože da ima značajan uticaj na promjenu teksture mesa (Zayas i Naewbanij,1986). Toplotno indukovane promjene u proteinima imaju značajan uticaj i na sposobnost vezivanja vode u mesu(Bouton i Harris, 1972;Bertola i sar., 1994), što za rezulta daje gubitak vode tokom toplotne obrade. Ove promjene u sadržaju vode utiču na sočnost, skupljanje mesa i tvrdoću (Offer i sar., 1984). Takođe, Promeyrat i sar., (2010) pokazali su da sa povećanjem temperature tokom toplotne obrade dolazi do smanjenja rastvorljivosti proteinskih frakcija, a sa druge strane povećanja rastvorljivosti aminokiselinskih frakcija u proteinskom ekstraktu (osnovne aminokiseline, aromatske aminokiseline, cistein i td) što je u saglasnosti sa ranijim istraživanjima (Haak i sar., 2006; Astruc i sar., 2007; Santé-Lhoutellier i sar., 2008; Gatellier i sar., 2009).

Cilj ovoga rada je da se da kratak pregled uticajatemperatura i različitih postupaka toplotne obrade na stanje miofibrilarnih proteina, reološka i fizičko-hemijska svojstva toplotno obrađenog mesa.

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Tipovi toplotne obrade mesa

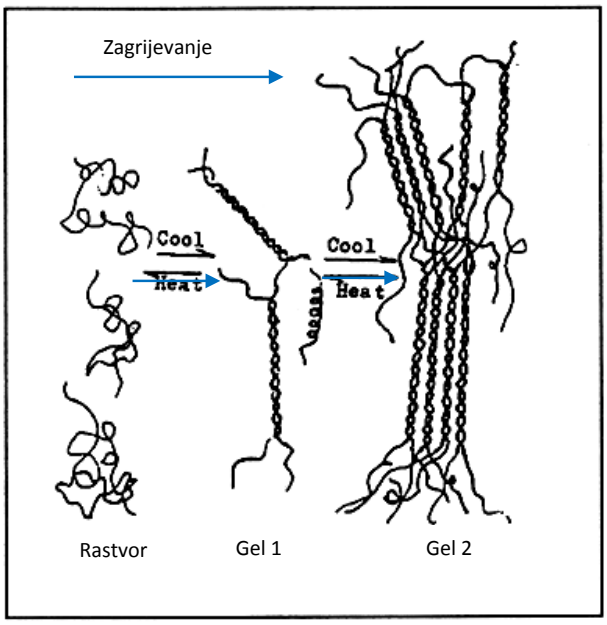
Mnogi procesi koriste autoklave za kuvanje pod povećanim pritiskom ili uređaje za kuvanje na vodenoj pari pod atmosferskim pritiskom (vodena kupatila). Ovi uređaji mogu da imaju veoma složenu kompijutersku kontrolu vođenja procesa. Za manje pogone ovi uređaji su šaržnog tipa, dok veći pogoni posjeduju uređaje kontinualnog tipa. Proizvodi u ovim uređajima se uglavnom potapaju u vruću vodu ili se toplotno obrađuju vodenom parom iznad tečne faze. Brzina prenosa toplote zavisi od režima toplotne obrade koji se primjenjuje. Da bi se postigla željena temperatura proizvoda tokom toplotne obrade, uređaj u kome se provodi toplotna obrada mora obezbjediti veću temperaturu nego što je ona u proizvodu. Prema Boles-u i Swan-u (2002a) proizvod se brže toplotno obrađuje i brže se dostiže željena temperatura u centru proizvoda ako se toplotno obrađuju na konstantnoj temperaturi tokom toplotne obrade, nego kada se temperatura povećava postepeno za određenu vrijednost Δt (Drummond i Sun, 2006). U novije vrijeme u industrijskim uslovima široko je rasprostranjena metoda suve toplotne obrade putem mikrotalasa. Meso obrađeno toplotnom obradom pečenjem putem mikrotalasa zahtjeva mnogo manje vremena za postizanje završne temperature toplotne obrade. Zbog prenosa toplote radijacijom mnogo je ujednačeniji proizvod po presjeku bez izražene karakteristične braonkaste boje, koja se pojavljuje kod uzoraka mesa obrađenih konvektivnim načinom prenosa toplote u konvekcionim pećima (Zhang, 2006).
Kontaktna toplotna obrada spada u grupu suve toplotne obrade i susreće se najčešće kod proizvoda obrađenih na roštilju. Oroszvári i sar., (2005b) pokazali su da veće temperature tokom toplotne obrade smanjuju vrijeme zadržavanja proizvoda i ubrzavaju postizanje željene temperature u centru proizvoda. Pored temperature kod proizvoda obrađenih na roštilju sadržaj vode u uzorku mesa igra ključnu ulogu za brzinu dostizanja željene temperature (Toldrá, 2010).

Kalo toplotne obrade

Kalo toplotne obrade je veoma važan parametar tokom obrade svježeg mesa. Pri ovom procesu sadržaj vlage u toplotno obrađenom proizvodu se značajno smanjuje. Kalo toplotne obrade nastaje usljed gubitka vlage u obliku tečnosti i u obliku pare. Iznad 70°C kalo toplotne obrade se značajno povećava. Kalo toplotne obrade isparavanjem može se značajno umanjiti ukoliko se relativna vlažnost u peći poveća i temperatura u peći održava ispod 65°C. Boles i Swan (2002b) utvrdili su da sa povećanjem postmortema mesa povećava se i kalo toplotne obrade, s druge strane ukoliko se održava nešto veća vrijednost pH mesa tokom skladištenja u frižideru kalo toplotne obrade se smanjuje (Toldrá, 2010).

Ponašanje proteina mesa tokom toplotne obrade

Konformacione promjene na proteinima koje se dešavaju tokom toplotne obrade obično se nazivaju denaturacija proteina. Temperatura toplotne obrade na kojoj se ove promjene dešavaju obično se naziva temperatura denaturacije i ona najčešće predstavlja predmet istraživanja. Pored denaturacije tokom toplotne obrade dolazi i do protein – protein interakcija, što za rezultat daje agregaciju (geliranje) proteina. Ove promjene na proteinima se najčešće prate mjerenjem rastvorljivosti i molekulskog sastava nastalog ekstrakta putem elektroforetskih metoda (Thornberg, 2005).
Mehanizam geliranja proteina prikazan je na sljedećoj slici 1.



Slika 1. Mehanizam geliranja proteina (FAO, 2011)

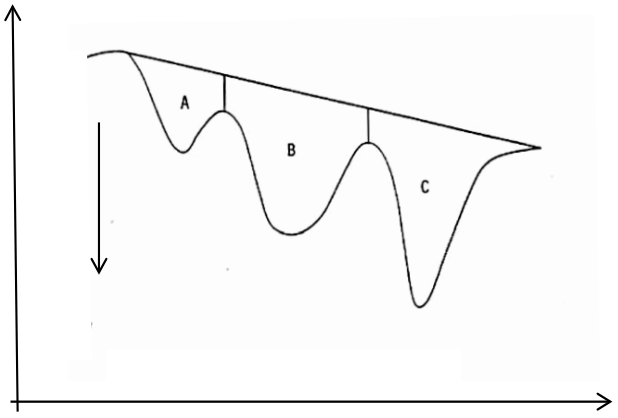
Sarkoplazmatski proteini

Većina sarkoplazmatskih proteina (mišićni proteini rastvorljivi u vodi ili rastvoračima niske jonske jačine) grade gelove uglavnom između 40˚C i 60˚C. Davey i Gilbert (1974) otkrili su da se ovaj temperaturni interval agregacije može proširiti čak i do 90˚C. Oni su takođe prvi ukazali na to da sarkoplazmatski proteini mogu imati ulogu u promijeni konzistentnosti kuvanog mesa na takav način da toplotno indukovane promjene na sarkoplazmatskim proteinima mogu formirati gel između strukturnih elemenata mesa i na taj način ih povezati u jednu stabilnu strukturnu cijelinu. Reološkim mjerenjima nježnosti mesa Thornberg (2005) je potvrdio ovu tvrdnju. Još jedan zanimljiv aspekt sarkoplazmatskih proteina je efekat smekšavanja koji se pojavljuje uz niže temperature i duže vrijeme zagrijevanja (0,1°C/min) (Wu i sar., 2007). Paulsen i sar., (2006) su pokazali da agregiranje može ostati aktivno u mesu na temperaturama toplotne obrade manjim od 60°C, dok brže zagrijevanje i nešto veće temperature od 70°C do 80°C na kraju toplotne obrade ga inaktiviraju. Prema istim autorima potrebno je oko 6h laganog zagrijevanja uzorka mesa da bi imali značajan efekat omekšavanja i niže vrijednosti na WB testu presijecanja. Međutim, ovako dugo vrijeme toplotne obrade uzrokuje i veliki kalo negdje između 30% i 35% (Thornberg, 2005).

Miofibrilarni proteini

Proučavajući toplotno indukovane promijene u sekundarnoj strukturi miozina Morita i Yasui (1991) su pratili promjene u sadržaju heliksa i površinsku hidrofobnost lakog mero-miozina LMM (light mero-myosin) pri pH 6 i 0,6 M KCl. LMM heliksni sadržaj počinje da pada već pri 30°C i dostiže minimum na 70°C. Uporedo zagrijevajući do 65°C raste površinska hidrofobnost, nakon koje počinje naglo da pada. Smanjenje površinske hidrofobnosti na višim temperaturama ukazuje na to da dio proteinski hidrofobnih rezidua učestvuju u protein – protein interakcijama, što za rezultat ima formiranje mreže agregata, odnosno stvaranje gela (Paulsen i sar.,2006).

Tipična kriva (dijagram 1) prenosa toplote kroz mišić sastoji se od tri osnovne zone A, B i C.



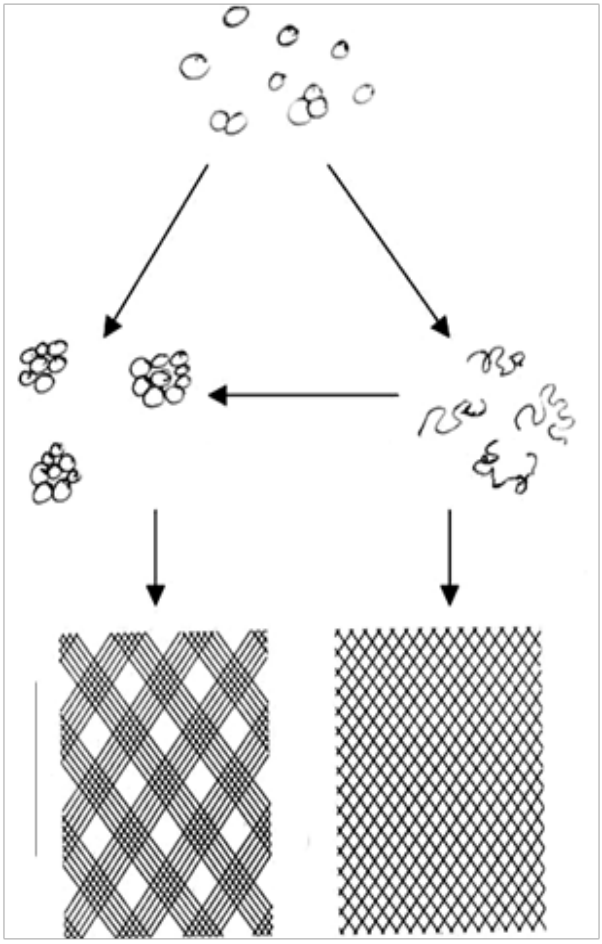
Dijagram 1. Toplotna kriva za mišić sastavljena iz tri zone: A miozinska, B sarkoplazmatska i kolagena i C aktinska (Findlay i sar., 1989)

Maksimum prvog prelaza nalazi se između 54°C i 58°C i posljedica je promijena na miozinu. Drugi maksimum nalazi se između 65°C i 67°C i vezan je za promjene na kolagenu i sarkoplazmatskim proteinima i treći pik posljedica je denaturacije aktina i leži između 80°C i 83°C. U zoni B pored kolagena i sarkoplazmatskih proteina u istom temperaturnom intervalu izolovani su još i aktomiozin, miozin i njihove rezidue u prelaznim oblicima. Proučavajući toplotnu denaturaciju titina iz mesa svinja i goveda potvrđeno je da se njegov denaturacioni pik kreće između 78,4°C i 75,6°C bez obzira na vrstu mesa (Pospiech i sar., 2002).

Prema Xiong i Brekke (1990a, 1990b) hidrofobnost proteina rastvorljivih u slanim rastvorima (miofibrilarni proteini) ne zavisi direktno od tipa mišića iz koga potiču, već samo od načina i tipa protein – protein interakcija i formiranja gela. Protein – protein interakcije kod miofibrilarnih proteina počinju već u rasponu temperatura od 36°C do 40°C. Ovom procesu prethodi prvo ekspandovanje i razmotavanje dugih molekula globularnih proteina na temperaturama od 30°C do 32°C. Intenzivnije geliranje miofibilarnih proteina otpočinje već u rasponu temperatura od 45°C do 50°C, što se može zapaziti intenzivnijim povećanjem gustine rastvora proteina. Na ove procese ne utiče stanje post – rigor ili pre – rigor u kome se meso nalazi. Shodno tome, denaturacija miofibrilarnih proteina u rastvori-

ma uglavnom rezultuje formiranjem gela, zato što miozin ima visoku moć agregiranja već pri veoma malim koncentarcijama od 0,5% (Hermansson i Langton, 1988). Sarkoplazmatski proteini za razliku od miofibrilarnih poređenja radi, moraju da se nalaze u koncentraciji od minimum 3% da bi proces agregiranja počeo. Miofibrilarni proteini, prema tome, grade visoko kompaktne i stabilne gelove koji imaju visoku moć vezivanja vode i dobre reološke osobine što je opet uzrokovano uslovima pod kojima se gel formira (jonska jačina, pH, vrijeme zagrijevanja i td) (Sharp i Offer, 1992; Thornberg, 2005).

Tipovi formiranja gela tokom toplotne obrade globularnih proteina prikazani su na sljedećoj slici 2.



Slika 2. Različiti tipovi formiranja gela tokom toplotne obrade globularnih proteina sa različitim stepenom agregiranja (Hermansson, 1982)

Ukoliko se zagrijeva čist rastvor miozina, gel koji se pri tom procesu nagrađuje, kao posledica umrežavanja dugih miozinskih lanaca, dostiže maksimum svoje čvrstoće na temperaturi od 45°C i pH rastvora od 5,5 ili na 60°C pri pH rastvora 6. Takođe, zapaženo je da ukoliko je aktin prisutan u čistom rastvoru miozina srazmjerno već pri malim koncentracijama povećava se i čvrstoća gela pri istim temperaturama i pH vrijednostima. Jonska jačina rastvora i pH su važni faktori koji određuju da li će miozin da se nalazi u monomernom obliku ili povezan u obliku filamenata. Pri jonskoj jačini većoj od 0,3 i pH neutralno, miozinski molekuli su dispergovani u rastvoru kao monomeri formirajući grubu mrežu sa velikim porama. Na nižim jonskim jačinama rastvora miozinski molekuli se nalaze vezani u filamentu, nalik prirodno debelim filamentima u mišiću. Što su filamenti duži to se tokom zagrijevanja formira čvršći gel. Takav gel se sastoji od fine i uniformne mreže sa malim porama (Sharp i Offer, 1992).

Formiranje miozinskog gela tokom zagrijevanja odvija se iz dva koraka u toku dva zasebna temperaturna intervala. Prvi dio reakcije pojavljuje se na temperaturama između 30˚C i 50˚C, a drugi iznad 50˚C. Prvi korak podrazumijeva agregaciju gdje se veze ostvaruju na nivou globularnih glava miozina. U drugom koraku dešavaju se promijene na nivou heliks strukture miozina što dovodi do formiranja mreže gdje hidrofobne grupe preuzimaju primarnu ulogu u vezama na nivou protein – protein interakcije (Lrinczy, 2009).

Micklander i sar., (2002) godine proučavali su uticaj dužine vremena zagrijevanja na odgovarajućim konstantnim vrijednostima temperatura na proces geliranja čistog rastvora miozina. Nakon zagrijevanja rastvora miozina 30 min na 30˚C miozin – miozin interakcije se ne pojavljuju. Nakon zadržavanja istog vremena od 30 min i povećanja temperature na 35˚C miozin – miozin interakcije otpočinju, rastvor postaje gušći sa izraženijim optičkim svojstvima, ali je još uvijek dominantan miozin u prirodnom obliku sa dvije glave. Prve forme agregata sastoje se iz dvije molekule miozina, agregirane dimerizacijom preko globularnih glava (Thornberg, 2005). Zagrijevanjem na 40˚C sav miozin u prirodnom obliku iščezava, a preko 50˚C proces agregiranja se značajno ubrzava i pojedinačne miozinske repove već je teško uočiti. Između 50˚C i 60˚C ukoliko se rastvor miozina zadržava 30 min dolazi do nagrađivanja velikih globularnih agre-

gata. Iznad ovih temperatura nije moguće uočiti prisustvo pojedinačnih miozinskih repova u rastvoru. Daljim zagrijevanjem iznad 60˚C dolazi do formiranja heliksa preko miozinskih hidrofobnih grupa (Aalhus i sar.,2009).

Proteini vezivnog tkiva

Na temperaturama između 53˚C i 63˚C dolazi do denaturacije kolagena na način da prvo dolazi do kidanja vodoničnih veza i pucanja fibrilarne strukture, a zatim kontrakcije kolagenog molekula. Na temperaturama između 60˚C i 70˚C slobodna kolagena vlakna se skupljaju do jedne četvrtine svoje prvobitne dužine (Christensen i sar., 2000). Ukoliko nisu stabilizovana toplotno otpornim međumolekulskim vezama daljim zagrijevanjem će se rastvoriti i formiraće gel. Prisustvo toplotno otpornih međumolekulskih veza znači da one opstaju na odgovarajućim temperaturama i zadržavaju matriks strukturu kolagena. Kod mlađih životinja, epimizijum se sastoji uglavnom od toplotno labilnih međumolekulskih veza, perimizijum je mješavina toplotno labilnih i toplotno stabilnih veza i endomizijum čine toplotno stabilne unakrsne veze (Andersson i sar., 2000). Kako životinja stari smanjuje se količina toplotno labilnih na račun toplotno stabilnih veza. Veći nivo toplotno stabilnih unakrsnih veza dovodi do razvoja veće napetosti u vezivnom tkivu tokom toplotne obrade. Tokom toplotne obrade na 60˚C epimizijum ne pokazuje nikakve promijene, dok perimizalni i endomizalni kolagen počinju da se agregiraji na 80˚C i prelaze u gel. Ovako formirani gelovi pokazuju različitu toplotnu stabilnost. Tip 1 (epimizalni je mnogo lakše rastvorljiv već pri nižim temperarurama za razliku od tipa 3 (endomizalnog). (Aktas i Kaya, 2001).
Wu i Bertram (2007) proučavali su uticaj temperature toplotne obrade, pH i starosti životinje na toplotno indukovane promijene i skupljanje kolagena. Zagrijevanjem kolagena vlakna se smanjuju na vrijednost nešto manju ispod 0,7 od prvobitne dužine, što je u saglasnosti sa istraživanjima Christensena i sar., (2000). Ovo je ujedno i donja granica ispod koje kolagen postaje amorfan i gumenolik na sobnoj temperaturi. Najbolju toplotno indukovanu stabilnost kolagena vlakna pokazala su na pH vrijednostima između 5 i 6. Žilavost kolagena povećavala je se 3 do 4 puta sa povećanjem starosti životinja između 30 mjeseci i 11 godina. Ovo povećanje žilavosti djelimično se može umanjiti toplotnim tretmanom pri nešto nižim pH vrijednostima (Lepetit, 2009).

Uticaj toplotne obrade na promijenu hemijskog sastava mesa

U temperaturnom intervalu od 50˚C do 100˚C dešavaju se značajne promjene na različitim proteinima (miozin, sarkoplazmatski proteini, aktin, proteini vezivnog tkiva). Kao posljedica navedenih promjena (denaturacija, koagulacija, hidroliza) dolazi do promjene odnosa između proteina i vode koja se nalazi u mesu, što uzrokuje kidanje veza lanci proteina-molekule vode i otpuštanja vode iz uzorka. Što su promjene na proteinima veće, veći broj molekula vode će biti otpušten i intenzivniji je gubitak vode tokom toplotne obrade. Zajedno sa vodom iz mesa se tokom toplotne obrade gube i druge materije rastvorljive u vodi (rastvorljivi proteini, mineralne materije i vitamini) (Kazemi i sar., 2009).
Oroszvári i sar., (2005b); Meinert i sar., (2007); Van der Sman; (2007); Leo i sar., (2009) navode da gubitak vode iz mesa uzrokuje povećanje sadržaja suve materije i sastojaka koji čine suvu materiju (proteini, masti, mineralne materije). Na različitim temperaturama toplotne obrade odvijaju se različite promjene na različitim proteinima, što uslovljava količinu otpuštene vode, a time i sadržaj ostalih materija. Brže promjene tokom toplotne obrade su u uzorcima koji se obrađuju toplotnom obradom pečenjem. Toplota u tom slučaju prije dostiže određenu visinu usljed većeg gradijenta temperatura u peći i uzorku. Ovako visoka temperaturna razlika, a s obzirom na činjenicu da meso ima nizak koeficijent provođenja toplote, izaziva pojavu ne uniformne raspodjele temperaturnih intervala po aksijalnom pravcu posmatrano od površine ka cenru uzorka mesa, tako da dolazi do pojave većeg broja intervala sa višim temperaturama od one koja se želi postići u centru. Kod kuvanih uzoraka manji je temperaturni gradijent, pa je i manji broj ovih intervala. Sve ovo za rezultat daje veći gubitak vode kod uzoraka obrađenih toplotnom obradom

pečenjem nego kod onih obrađenih toplotnom obradom kuvanjem (Amézquita, 2004).
Promjena pH, aw i SVV vrijednosti
Boles i sar., (2002a); Thornberg (2005) navode da u toku toplotne obrade mesa dolazi do denaturacije i hidrolize proteina, što u krajnjem slučaju daje blago povećanje pH vrijednosti, stim da je ono intenzivnije kod uzoraka obrađenih toplotnom obradom kuvanjem.
Prema Thornberg (2005) i Toldrá (2010) sa povećanjem temperature tokom toplotne obrade dolazi do povećanog lučenja tečnosti kako u vidu tečne faze tako i isparavanjem. Ovo za krajnji rezultat ima smanjenje vlage u uzorku sa povećanjem temperature toplotene obrade. Smanjenje vlage u uzorku ima za direktnu posljedicu smanjenje i aktivnosti vode sa povećanjem temperature tokom topotne obrade uzorka mesa.
Offer i sar., (1984); Barbieri i Rivaldi (2008) navode da sposobnost vezivanja vode u mesu (SVV) direktno zavisi od promijena na proteinima tokom toplotne obrade. Između 60°C i 80°C dolazi do raspadanje miofibrilarne strukture i povećanog lučenja tečne faze. To je i ujedno interval u kome dolazi do povećanja plastičnsoti (SVV cm2). U tom intervalu dolazi do povećanje vrijednosti (SVV cm2) sve do 61°C. Nakon 61°C počinje lagano da opada.

Promijena boje tokom toplotne obrade

Toplotna obrada mesa ima značajan uticaj na promjenu boje svježeg mesa putem denaturacije i promjena na mioglobinu. Prije toplotne obrade meso je crvenkaste boje, da bi nakon toplotne obrade u zavisnosti od režima toplotne obrade poprimilo svijetlu kod toplotne obrade kuvanjem do tamnobraon kod toplotne obrade pečenjem i prženjem (Toldrá, 2010).
Nicola, (2006); Aalhus i sar., (2009); Toldrá, (2010) navode da sa povećanjem temperature toplotne obrade dolazi do promijena na mioglobinu i denaturacije mioglobina. Kao rezultat ovih promijena dolazi do promijena boje tokom toplotne obrade u spektru od svijetlocrvene do braon u zavisnosti od režima toplotne obrade, brzine obrade i visine temperature tokom toplotne obrade. Gotovo svi proteini se denaturišu tokom toplotne obrade mesa, što za posljedicu ima dramatične promjene u boji mesa. Mioglobin se denaturiše na negdje oko 60˚C (Godsell, 2000). Promjena boje mesa tokom toplotne obrade zavisi od stepena denaturacije tri forme mioglobina. Metmioglobin je broan globin hemihromogen, dok su oksimioglobin i deoksimioglobin forme crvenog globin hromogena, koji se lako može oksidisati do hemihromogena. Veliki broj faktora, kao što su (režim toplotne obrade, brzina zagrijevanja, način pakovanja i čuvanja mesa, pH itd) utiču na postojanost boje svježeg mesa ili intenziviranje braonkaste boje toplotno obrađenog mesa. Stoga, od velike važnosti je pravilna kontrola temperature tokom toplotne obrade mesa (Aalhus i sar., 2009).

Tekstura mesa

Tekstura označava fizička svojstva mesa koja se percepiraju čulima vida, dodira i sluha, kao i prilikom žvakanja (Fjelkner - Modig, 1986). Taktilna predstava teksture mesa, do koje se dolazi čulom dodira i prilikom žvakanja, odnosi se na čvrstoću, tj. tvrdoću i mekoću mesa (konzistencija). Takođe zvuk koji se emituje prilikom grižen-ja i žvakanja na neki način je pokazatelj teksture (Guo, 2006). Tekstura mesa je u bliskoj vezi sa starošću, vrstom, polom, rasom i uhranjenošću životinje, odnosno činiocima koji uslovljavaju građu mišićnog tkiva, razvijenost i osobine vezivnog tkiva i količinu intramuskularnog masnog tkiva, kao i njihovu povezanost u mesu. Tekstura mesa zavisi od intenziteta postmortalnih promjena (Murphy i sar., 2001). Skeletna struktura odmah poslije klanja životinje je mekoelastična, a već nekoliko sati poslije toga ona gubi elastičnost i postaje čvršća, što je posljedica, u prvom redu, razvoja postmortalnog rigora. Što je kontrakcija mišića snažnija, meso postaje čvršće. Meso dobija čvršću konzistenciju i prilikom hlađenja usled očvršćavanja intermuskularne i intramuskularne masti. Meso se razmekšava tek za vrijeme zrenja, kao rezultat strukturnih promjena u miofibrilima, kolagenu itd. Poznato je da sposobnost vezanja vode mesa, bilo da je u vezi sa pojavom postmortalnog rigora, bilo brzinom postmnortalne glikolize, znatno utiče na strukturu i teksturu mesa (Vuković, 2006).
Visina temperatura tokom toplotne obrade mesa

u značajnoj mjeri utiče na nježnost proizvoda koji potiču od mesa starijih životinja. Povećanje udjela termostabilnih unakrsnih veza kolagena kod starijih životinja ima značajan utizaj na nježnost mesa. Nježnost mesa se lagano povećava sa povećanjem temperature toplotne obrade do između 60°C i 70°C, kada počinje da stagnira ili lagano da pada. Tvrdoća mesa značajno se mijenja sa povećanjem temperature tokom toplotne obrade, kao i sa promijenom režima toplotne obrade. U pravilu svi proizvodi obrađeni suvom toplotnom obradom imaju veću tvrdoću za istu vrijednost temperature proizvoda obrađenog vlažnom toplotnom obradom (Toldrá, 2010).

Kako navode Bouton i Harris, (1981) promjene u ponašanju reoloških svojstava sa porastom temperature u direktnoj su vezi sa promjenama na proteinima (miofibrilarnim i proteinima vezivnog tkiva). Zagrijevanjem dolazi do omekšavanja vezivnog tkiva uzrokovanog želiranjem kolagena i povećanjem žilavosti mišićnih vlakana prouzrokovanih toplotnom koagulacijom miofibrilarnih proteina. Zayas i Naewbanij, (1986) navode da vrijednost parametara koji definišu teksturu mesa rastu sa raspadanjem miofibrila, a smanjuju sa povećanjem rastvorljivosti kolagena. Skraćenje lanaca miofibrilarnih proteina može uzrokovati povećanje tvrdoće toplotno obrađenog mesa. Murphy i sar., (2000); Tornberg, (2005); Toldrá, (2010) navode da toplotna obrada značajno utiče na teksturu mesa. Ovaj uticaj ogleda se u promijeni maksimuma sile za dato svojstvo koje se posmatra. U rasponu temperatura od 50˚C do 60˚C maksimum sile za posmatrano svojstvo teksture može se povećati i do 150%. Povećanjem temperature od 60˚C do 80˚C maksimum sile može da ostane konstantan ili da opada sa povećanjem temperature i do 14%. (Murphy i sar., 2000).

Prekursori koji doprinose promijeni senzornih osobina tokom toplotne obrade

Prekursori pržene arome u hrani su obično povezana sa prisutnim heterocikličnim jedinjenjima kao što su pirazini, tiazoli i oksazoli. Mnogi alkalni pirazini su pronađeni u isparenjima mesa, a mogu se svrstati u dvije klase bicikličnih jedinjenja, 6,7 - dihidro - 5 (H) - ciklopentapirazine i pirolopirazine. Alkilno supstituisani tiazoli, uopšteno, imaju niži mirisni prag od pirazina, i oni su pronađeni u nižim koncentracijama u mesu. Obije klase jedinjenja se povećavaju primjetno sa povećanjem jačine temperature i u dobro isprženom mesu, pirazini su glavna jedinjenja isparljivih materija (Birch, 1994; Farmer i sar., 2009).

Bitna karakteristika isparljivih komponenti iz kuvanog mesa je da se većina pojavljuje u malim količinama, ali te količine izazivaju moćne arome i važni su doprinosioci arome kuvanog mesa. Poređenje kuvanog i prženog govođeg mesa pokazuje da su veće količine alifatičnih tiola, sulfida i disulfida zastupljene u kuvanom mesu. Heterociklična jedinjenja sa jednim, dva ili tri atoma sumpora u petočlanim ili šestočlanim prstenovima (kao što su tiofeni, tioaleni i td.) su mnogo više zastupljeni u kuvanom nego u prženom mesu (Birch, 1993; Meinert i Tikk, 2009).

Prema American Meat Science Association (Research Guidlenes, 1995) najbolje senzorne osobine imaju uzorci obrađeni toplotnom obradom u rasponu temperatura od 61˚C do 81˚C, što je interval u kome dolazi do najintenzivnijih toplotno indukovanih promijena na proteinima i raspadanju miofibrila. Ispod ovog intervala uzorci su po presjeku nedovoljno pečeni – kuvani sa ne zadovoljavajućom aromom i prevelikom žilavošću. Iznad ovog temperaturnog intervala uzorci su previše suvi i tvrdi, što je posebno izraženo kod toplotne obrade pečenjem. Takođe, iznad ovog intervala kod uzoraka obrađenih toplotnom obradom pečenjem pojavljujese i velika zagorelost površine uzoraka, posebno na 100˚C.Toplotna obrada mesa je završena postizanjem temperature u centru uzorka od minimum 71°. Na ovaj način smatra se da je uzorak mikrobiološki ispravan nakon toplotne obrade American Meat Science Association (Research Guidlenes, 1995).

Zaključak

1. Kao što se moglo vidjeti iz pregleda literature najintenzivnije promijene dešavaju se na proteinima tokom oba postupka toplotne obrade kako pečenjem tako i kuvanjem. Proteinima tokom toplotne obrade uglavnom drastično pad rastvorljivost sa povećanjem tempera-

ture u centru uzorka, a što za krajnji rezultat ima opet povećanje koncentracije aminokiselinskih frakcija u ekstraktu. Intenzivnije je smanjenje koncentracija u ekstraktima kod uzoraka obrađenih toplotnom obradom kuvanjem, nego kod onih obrađenih toplotnom obradom pečenjem.

2. Usled povećanja temperature tokom toplotne obrade dolazi do intenzivnijeg gubitka tečne faze a time i do povećanja sadržaja ukupnih proteina, pepela, masti i ostalih hemijskih parametara kvaliteta. Boja se intenzivnije razvija kod uzoraka obrađenih toplotnom obradom pečenjem, nego kod onih obrađenih toplotnom obradom kuvanjem, što je pogotovo izraženo na višim temperaturama. pH vrijednost se blago povećava sa povećanjem temperature tokom toplotne obrade. Aktivnost vode za razliku od pH intenzivno se smanjuje sa porastom temperature u centru uzorka. Sposobnost vezivanja vode SVV povećava se do temperature od oko 60˚C, a zatim opada.
3. Tvrdoće i čvrstoće sa povećanjem temperature tokom toplotne obrade se povećavaju s tim da je intenzitet porasta ovih parametara značajno veći kod uzoraka obrađenih toplotnom obradom pečenjem nego kod onih obrađenih toplotnom obradom kuvanjem. Intenzitet porasta ovih parametara značajno stagnira u rasponu temperatura između 60˚C i 80˚C.

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INFLUENCE OF TEMPERATURE AND HEAT TREATMENT PROCEDURE ON THE CHANGE OF TECHNOLOGICAL PROPERTIES OF MEAT

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Summary
Meat is a very important food in the diet of people, due to its valuable nutritional ingredients. Almost all of the meat products are somehow heat treated to achieve the desired sensory and nutritional properties, as well as meet the criteria in terms of microbial stability and safety of the product. The most common are the two types of heat treatment in industrial conditions, such as heat treatment by cooking at atmospheric pressure or vacuum and dry heat treatment by roasting. Therefore, goal of this paper is to give a brief overview of the latest developments, regarding the influence of technological parameters during thermal processing of meat. Temperature level in the center of the sample and different thermal treatment procedures (cooking and roasting) were monitored in terms of impact on the protein's structure changes, meat color, rheological properties of meat, meat pH, water holding capacity, chemical properties and organoleptic characteristics of meat.

Keywords: meat, meat cooking, roasting, meat color, state of the protein

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