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Editor Speech of IC - BTI 2019

International Conference is the 8th international interdisciplinary peer reviewed conference which publishes works of the scientists as well as practitioners in the area where UBT is active in Education, Research and Development. The UBT aims to implement an integrated strategy to establish itself as an internationally competitive, research-intensive institution, committed to the transfer of knowledge and the provision of a world-class education to the most talented students from all backgrounds. It is delivering different courses in science, management and technology. This year we celebrate the 18th Years Anniversary. The main perspective of the conference is to connect scientists and practitioners from different disciplines in the same place and make them be aware of the recent advancements in different research fields, and provide them with a unique forum to share their experiences. It is also the place to support the new academic staff for doing research and publish their work in international standard level. This conference consists of sub conferences in different fields: - Management, Business and Economics - Humanities and Social Sciences (Law, Political Sciences, Media and Communications) - Computer Science and Information Systems - Mechatronics, Robotics, Energy and Systems Engineering - Architecture, Integrated Design, Spatial Planning, Civil Engineering and Infrastructure - Life Sciences and Technologies (Medicine, Nursing, Pharmaceutical Sciences, Physcology, Dentistry, and Food Science),- Art Disciplines (Integrated Design, Music, Fashion, and Art).

This conference is the major scientific event of the UBT. It is organizing annually and always in cooperation with the partner universities from the region and Europe. In this case as partner universities are: University of Tirana – Faculty of Economics, University of Korca. As professional partners in this conference are: Kosova Association for Control, Automation and Systems Engineering (KA – CASE), Kosova Association for Modeling and Simulation (KA – SIM), Quality Kosova, Kosova Association for Management. This conference is sponsored by EUROSIM - The European Association of Simulation. We have to thank all Authors, partners, sponsors and also the conference organizing team making this event a real international scientific event. This year we have more application, participants and publication than last year.

Congratulations!

Edmond Hajrizi,

Rector of UBT and Chair of IC - BTI 2019

CONTENTS

Determination of Vitamin A and E in fodder concentrates and cow milk from region of Kumanovo, Macedonia

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Abstract. The main objective of this research was to determinate the vitamins A and E in fodder concentrate and raw cow milk from region of Kumanovo, Macedonia. As material for this research, samples of feed and samples of cow's raw milk from the cattle fed with the same food were taken. The test material was taken from a farm in the region of Kumanovo. Vitamins A and E have been examined with the methodology of extraction on HPLC – Perkin Elmer, pump: series 200LC, autosampler; ISS – 200, detector LC – 135/LC -235 C DA. The amount of vitamin A in fodder concentrate was   7.5438 mcg/100g, while the amount of vitamin E was 23.115 mcg/kg. The amount of vitamin A in raw cow milk from region of Kumanovo was 30 mcg/100g, while the amount of vitamin E was 0.86 mcg/100g.

Keywords: vitamin A, vitamin E, fodder, cow milk

Introduction

Carotenoids play a vital role in reproduction, they have antioxidant properties and regulate the immune response of both animals and humans (Biard et al. 2005). Vitamin A deficiency is a major public health problem over the world, especially in the poor countries.
It occurs mainly in young children and women of childbearing age. Inadequate intake of vitamin A is the main cause of the deficiency. The main animal sources of vitamin A are liver, eggs, milk, and milk products. Plant foods rich in provitamin A represent more than 80% of the total food intake of vitamin A because of their low cost, high availability, and diversity. Fruits, roots, tubers, and leafy vegetables are the main providers of provitamin A carotenoids. Because of their availability and affordability, green leafy vegetables are consumed largely by the poor populations, but their provitamin A activity has been proven to be less than previously assumed. Worldwide, about 250 million children are at risk of vitamin A deficiency (UNICEF, 2004). Carotenoids are called pre-vitamin forms because they can be converted into retinol (yellow and orange fruits and vegetables and dark green leaves). There are more than 600 forms of natural carotenoids and many of them have provitamin A activity, but food composition data are only available for three of them (α-carotene, β-carotene, and β-cryptoxanthin) (Van Jaarsveld et al. 2005).

Vitamin E is a fat-soluble vitamin found in many foodstuffs, such as cereals, eggs, olive oils, and vegetables. Vitamin E occurs in many different forms (α-, β-, γ- and δ-tocopherols and α-, β-, γand δ-tocotrienols) and has many health benefits; it is mostly used for treating and preventing heart diseases (Pyka 2001; Zhao, 2014).

Material and methods

The main objective of this research was to determinate the vitamins A and E in fodder concentrate and cow milk in farm from region of Kumanovo, Macedonia.

As material for this research, samples of feed and samples of cow's raw milk from the cattle fed with the same food were taken. The test material was taken from a farm in the region of Kumanovo. Feed samples comprise two types of concentrates produced by "Agroinvest", feed for molar cows with at least raw protein KMK - 18%, alfalfa silage and straw. Cows were of the frieze race. Feeding was three times a day, concentrate in the morning, alfalfa and straw at the lunch, and concentrate in the evening. Milking was with the machine, three times a day in the summer period, and twice in the winter period.

Methods for the analysis of vitamin A (Retinol) and vitamin E (Tocopherol) in concentrates and milk have been examined, 2 methods of extractions and sample preparation for HPLC analysis – Perkin Elmer, pump: series 200LC, autosampler; ISS – 200, detector LC – 135/LC -235 C DA.

Extraction Method 1 (ChemElut)

Weigh 20 g of sample (approx. 0.01 g) into a 500 ml Erlenmeyer flask and add 1 g of ascorbic acid, 150 ml of ethanol (95%) and 40 ml of 50% potassium hydroxide (KOH) aqueous solution. Add the condenser to the flask and place in a water bath (approx. T = 95 ° C). Hydrolysis occurs 30 minutes after the start of the reaction. Shake the sample twice during hydrolysis. After the hydrolysis is complete, the sample is cooled to room temperature. Add 50ml of distilled water. Transfer the hydrolyzate to a 500ml volumetric flask and dilute to the ethanol mark (50%) Transfer 10.0 ml to a Chem Elut column (of 20 ml), wait approximately 10 min. The sample was eluted with 100 ml of n-hexane. Then the eluent is collected in a 500ml flask. Evaporation (evaporation to dryness) is done with some BHT granules. The sample is dissolved in n-heptane and transferred to a volumetric flask (5.0 ml). Dilute to the n-heptane mark.

Extraction method 2 (separatory funnel)

Weigh 20 g of sample (to the nearest 0,0 1 g) in a 500 ml Erlenmeyer flask and add 1 g of ascorbic acid, 150 ml of ethanol (95%) and 40 ml of 50% potassium hydroxide solution in water. Add a condenser to the flask and place on a water bath (95 0 C). The hydrolysis lasts 30 minutes from the start of the reaction. During the hydrolysis, shake the sample flask twice. After complete hydrolysis, the sample is cooled to room temperature and 50 ml of distilled water are added. Transfer the hydrolyzate into a 500 ml volumetric flask and dilute to the mark with 50% ethanol. Transfer 20 ml into a separatory funnel and dilute with 100 ml n-hexane. Shake the funnel for 1 minute, clean, wash (hexane phase, 2 times x 50ml 1M potassium hydroxide in ethanol (40%) and (2x50ml) with distilled water). Next, evaporate, evaporate to dryness in the hexane phase with a few granules of BHT and about 8ml of ethanol (99%) The sample is dissolved in n-heptane and transferred to a volumetric flask (5.0 ml). Dilute to the n-heptane mark. Analyzes are chromatographed on the apparatus used - HPLC - Perkin Elmer, pump: series 200LC, autosampler; ISS-200, Detector LC –135 / LC –235 C DA.

Vit A and Vit E tests were performed in 5 concentrate samples, as well as raw milk and packaged milk. The table shows the mean of the five samples of each assay.

Results

The milk quality depends from the health of mammary gland. Milk quality is usually depend from mastitis, milk with a low somatic cell count (SCC) and visibly normal appearance (no clots). But, in accordance with Weiss (2010), the definition of high-quality milk must be expanded. Thus, the quality of milk can also be based on the amount of antioxidants that it contains, protecting the characteristics of milk lifetime by reducing oxidation.

In our research, we determined the amount of vitamin A and E of fodder concentrate, cow raw milk and packed milk, in the region of Kumanovo.

As it is shown in Table 1, the amount of vitamin A in fodder concentrate in Kumanovo is 7.5438 mcg/100 g. Mean while the amount of vitamin E in fodder concentrate is 23.115 mcg/kg.

Table 1. Amounts of vitamins A and E in fodder concentrate in region of Kumanovo, Macedonia

Vitamins	Amount (mcg/kg)
A	7.5438
E	23.115

In Table 2 it is shown the amount of vitamin A and E in raw milk from the region of Kumanovo, as well as in packed milk. The amount of vitamin A in raw cow milk from region of Kumanovo was 30 mcg at 100g, compared with the packed milk which amount (18.48) of this vitamin was drastically lower.

The amount of vitamin E in raw cow milk was 0.86 mcg at 100g, compared with the packed milk which amount (0.12) of this vitamin was drastically lower, as well.

Table 2. Comparison of amounts of vitamins A and E in raw cow milk from region of Kumanovo and package milk

Vitamins	Raw cow milk (mcg at 100 g)	Package milk (mcg at 100 g)
A	30	18.48
E	0.86 0.86	0.12 0.12

Discusion

Vitamin A is a fat-soluble vitamin involved in many important biological functions.Vitamin E is considered to act primarily as a lipid soluble antioxidant, protecting polyunsaturated fatty

acids and related substances from peroxidation and hence from rancidity (Bates & Prentice 1994).

Vitamins A (retinol) and E (tocopherol) and the carotenoids are fat-soluble micronutrients that are found in many foods, including some vegetables, fruits, meats, and animal products. Fish-liver oils, liver, egg yolks, butter, and cream are known for their higher content of vitamin A. Nuts and seeds are particularly rich sources of vitamin E (Thomas, 2006).

Vitamin A, found in foods that come from animal sources, is called preformed vitamin A. Some carotenoids found in colorful fruits and vegetables are called provitamin A; they are metabolized in the body to vitamin A. The bioconversion of carotenoids to vitamin A is different from person to person (Krinsky, 2005). Vitamin E activity is derived from at least eight naturally occurring tocopherols, the most potent of which is alphatocopherol. Other less active forms of vitamin E are plentiful in the diet, with gamma-tocopherol being the predominant form.

Our results are in accordance with results of other authors such as Mourad (2014), Michlova (2015) and Sanchez-Machado (2006).

Conclusion

Based on these researches we can conclude that amount of vitamin A is higher in raw milk cow (30 mcg/kg), while lower in fodder concentrate (7.5438 mcg/kg).

The amount of vitamin E is higher in fodder concentrate (23.115 mcg/kg), while it is lower in raw cow milk (0.86 mcg/kg).

By comparing the amount of vitamins A and E in raw milk and packaged milk, we found that their content was higher in raw milk.

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SOME DRINKS INGREDIENTS - ADVERTISING AND LABELING

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Abstract. The higher standard of life has also raised the market demand for variety of products while impacting the producers' behavior in regards to marketing. One of the ways the producers offer and present to customers their products is through label advertising. Raised awareness among customers has made possible the demand for higher quality products, but are higher quality products being offered to citizens?

This research has analyzed the food legislative framework, food product samples were analyzed, few local and international food shops were visited, and and one to one interviews were conducted with consumers.

Based on the results of the research, much remains to be desired from what is offered to the citizens by the producers, in the advertising label products they put tempting pictures for the consumer to buy the product, but not the true makeup of the product.

Keywords: product, labeling, advertising, shops.

Introduction

Regular physical activity and good nutrition contribute to the individual's good health and physical condition [1]. Fruits, vegetables, herbs, and spices that have been used alone, mixed, in the form of tea, juice, or other forms of food have been a regular part of human life. Tea is an important beverage for thousands of years and has been an important cultural component in many countries of the world. According to customs over the course of a year, Kosovars consume over 2 million kg of tea [2]. To the average American, tea is just a substitute for juice or soft drinks, where four-fifths of the tea consumed is iced tea [3]. But tea is not only valued for its good taste and world-view appearance but also for its many other benefits [4]. For a healthy body, experts advise consuming as many fruit juices that contain more vitamins A, C, E and antioxidants [5].

A higher standard of living and awareness has led to increased health and beauty care, and at the same time has influenced the search for products that help in this regard, such as tea and fruit juices [3].

This demand of consumers has been used by the manufacturers to place in the market the required products as much advertising packaging with the ingredients of the products. 72% Increase in value of food & drink sector in the last decade. The food and drink sector not only maintained but strengthened its position as a leading sector of the EU economy [6].

Socio-economic factors, education, and gender can play a role in how consumers view food information and in how they use this information. Of food labelling is difficult, if not impossible, and there must be careful consideration of the kind of information that is required.

Especially as differences in how consumers use food information do not necessarily translate into how consumers want information to be presented. What is known is that consumers are mostly interested in clear, understandable, simple, comprehensive, usable, standardised and authoritative information [7].

Label is clearly helpful in allowing consumers to make an informed choice about the products they purchase. In addition, it can also minimise the chance of them being misled. Having such legislation at Community level supports the internal market which is beneficial for food business and also for consumers [7]. Labelling must not be such as to mislead the consumer 'as to the characteristics of the foodstuff and, in particular to it origin or provenance [7].

Advertising has the effect, in the past 25 years the consumption of carbonated drinks has doubled in girls and nearly tripled in boys [1].

For research have been taken some types of tea and fruit juices, food legislation has been investigated, shops with local and foreign products have been visited, designated species for research.

Based on the research results, products on the advertising label place tempting pictures for the consumer to buy the product, but not the actual composition of the product.

Materials and Methods

The research has been conducted in several directions as:
- local and EU legislation on labeling and packaging,
- consumer questionnaire was developed,
- data is collected from consumers,
- research on samples of local and imported tea and fruit juice products,
- Internet information has been used.

Samples taken for research, tea: ginger (4 samples), cranberry (6 samples), of some manufacturers and juices of some types of fruits (13 samples). The research is based on the product photo and description of the contents of the product ingredients contained in the product packaging.

The data from the questionnaires were processed using the excel statistical method and diagrams were drawn.

Results and Discussions

Results and discussion of legislation research

Foreign and local labeling and packaging legislation.

According to the legislation for a food to be placed on the market must be packaged and labeled, which must include the list of food ingredients [1].

The Directive 79/112/EC has:

Mandatory Information that must appear on food labels

The name under which the product is sold; (2) the list of ingredients; (3) the quantity of certain ingredients or categories of ingredients [7]. This is a codified version of Directive 79/112/EC to which amendments and new requirements were added through the years. The Directive sets down the mandatory information that must appear on food labels.

REGULATION (MTI) - No. 09/2013, ON LABELLING, PRESENTATION AND ADVERTISING AND FOOD PRODUCTS, Ministry of Trade and Industry Republic of Kosovo LAW NO. 04 / L-121 ON CONSUMER PROTECTION, this law regulates and

protects the fundamental rights of consumers when purchasing goods, services and other forms of free market, as well as the obligations of the seller, the producer and the supplier [8].

The seller of goods or services shall ensure that the goods he sells are labeled, containing information under article 13 [8].

The declaration is required to be affixed to any goods under article 17 [8].

The notification of the contents of the goods must be affixed to the goods or packaging in accordance with article 18 [8].

Results and discussion from consumer

The questionnaire developed for this study was distributed to 20 consumers. For each questionnaire question the data were obtained and processed in tables and diagrams.

The following is the data provided by customers through the diagrams:

Does it pay attention to the product packaging?

little 15%
no 10%
yes 75%

Do you trust the packaging photo?

not at all 10%
yes 30%
no 15%
little 45%

You buy product based on

recommendation 15%
photo 25%
conent 60%

Do you read the content of the product?

sometimes 20%

no 5%

yes 75%

Content and use in labels is in Albanian language?

yes 15%

no 5%

sometimes 80%

Content of the product match the packaging photo?

yes 20%

no 20%

somewhat 60%

Which products do you trust more?

domestic 5%

EU 45%

domestic and EU 50%

Do you believe in the tea content based on the picture?

yes 15%

no 35%

little 50%

Do you believe in the juice content based on the picture?

yes 10%

no 45%

little 45%

Do you think the manufacturer is responsible to the consumer?

yes 10%
no 15%
somewhat 75%

Which teas do you use?

domestic 15%
imported 40%
different 45%

Which juices do you use for drink?

domestic 15%
imported 25%
different 60%

Results and discussion of product study

The study of Tea

Fig. 1. Types of tea

Ginger tea

Tea "Ginger Lemon & Lime" by "EMONA BRAND", producer "Capital Resources SHPK" Shqipëri for privat brand "EMONA SHPK" Kosovë,

In the picture is shown: ginger, lemon and lime, until

Ginger is the first product but it is not known as a percentage.

Tea "Ginger -Lemon" by "MILFORD", producted in Germany for "Milford Tee Austria GmbH"

In the picture is shown: ginger and lemon, until

Ginger is the first product with 65%.

Tea "Ginger Lemon Peels" by "doḡadan", producer "DOGADAN GIDA AS" Turky

In the picture is shown: ginger and lemon, until

Ginger is the first with 25%.

Tea "Ginger" by "Dea", producer "Natyra Dea Shpk" Kosovë
In the picture is shown: ginger and lemon, until
Ginger is the first product but it is not known as a percentage.

Cranberry tea
Tea "CRANBERRY" by "GOOD NATURE", producer "ALKALOID AD" Skopje-Macedonia
In the picture is shown: cranberry, until
Cranbarry is the third product and only 20%, while in the picture is presented as its sole.

Tea "CRANBERRY" by "MILFORD", produced in Germany for "Milford Tee Austria GmbH"
In the picture is shown: cranberry, until
Cranbarry is the first product but it is not known as a percentage while the picture is presented as its sole.
Tea "CRANBERRY" by "PODRAVKA", producer "Zito d.d." Lublanë-Slloveni
In the picture is shown: cranberry, until
Cranbarry is the latest product with 0.5% while the picture is presented as its sole.

Tea "CRANBERRY" by "ITALIANNO", producer "AGRISTAR LTD" Kroaci for privat brand "Viva Fresh SHPK", Kosovë
In the picture is shown: cranberry, until
Cranberry is the latest product with 1% while the picture is presented as its sole.

Tea "CRANBERRY" by "EVERGREEN", producer "UNITED BRANDS L.L.C. Kosovë.
In the picture is shown: cranberry, until
Cranbarry is the first product but it is not known as a percentage while the picture is presented as its sole.
Tea "CRANBERRY" by "EMONA", producer "Capital Resources SHPK" Shqipëri for privat brand "EMONA SHPK" Kosovë , In the picture is shown: cranberry, until
Cranbarry is the first product but it is not known as a percentage while the picture is presented as its sole.

Study of fruit juices

Fig.2. Types of juice

1. Juice "APPLE ARONIA" by "ZEN", producer "GOBEX PLUS DOOEL", R. Maqedoni
In the picture is shown: Apple and aronia, until
The ingredients in the picture are the same as the ingredients on the label.

2. Juice "Pear" by "Frutti", producer NTP" FRUTTI', Kosovë
In the picture is shown: pear, until
The pear only appears in the picture although the juice also contains apple puree.

3. Juice "Strawberry" by "Frutti", producer NTP" FRUTTI', Kosovë
In the picture is shown: strawberry, until
The strawberry only appears in the picture although the juice also contains grapefruit concentrate.

4. Juice "Sourcherry" by "Frutti", producer NTP" FRUTTI', Kosovë
In the picture is shown: sourcherry, until
Sourcherry is only in the picture and is 20% in composition.

5. Juice "RASBERRY" by "Frutomania", producer "MOEA", Kosovë
In the picture is shown: rasberry, until
Raspberry is the only one pictured in the picture, though it is only 14% of the composition, and other fruits are present in the juice.

6. Juice "SOURCHERRY" by "Frutomania", producer "MOEA", Kosovë
In the picture is shown: sourcherry, until
Sourberry is the only one pictured in the picture, which is 35% in composition, but not the apple that is 65%.

7. Juice "PEACH" by "Frutomania", producer "MOEA", Kosovë
In the picture is shown: peach, until
Peach is the only one pictured in the picture, which is 40% in composition, but not the apple that is 60%.

8. Juice "PEACH" by "Pfanner", producer "Hermann Pfanner Getränke GmbH", Austria
In the picture is shown: peach, until
Peach is the only one pictured in the picture, which is 25%, although it also contains concentrated apple juice

9. Juice "Multifrutti A+C+E" by "Pfanner", producer "Hermann Pfanner Getränke GmbH", Austria
In the picture is shown: apple, orange, lemon, carrot until
In the picture of the juice "Multifrutti A + C + E", no pears appear although the juice also contains concentrated pear juice.

10. Juice "STRAWBERRY APPLE GRAPE" by "Fructal", producer "NECTAR" Serbi.
In the picture is shown: strawberry under it writes STRAWBERRY APPLE GRAPE), until
Strawberry is the only one pictured in the picture, which is 30%, although it also contains apple pure 11%, and grape juice concentrate4%.

Juice "STRAWBERRY" by "Jaffa Champion plus", producer "FLUIDI' Kosovë
In the picture is shown: strawberry, until
Strawberries only appear in the picture even though they are apple and aronia.

Juice "Sour Cherry" by "Jaffa Champion plus", producer "FLUIDI' Kosovë
In the picture is shown: sour cherry, until
Sour Cherry only figures in the picture and is only 12%.

Juice "Sour Cherry" by "Tango Life Vishnje", producer "FLUIDI' Kosovë
In the picture is shown: sour cherry, until
Sour Cherry only figures in the picture and is only 10%.

Conclusions

Tea, in addition to being consumed as a habit, is now widely consumed for aesthetic and health reasons, while juices are consumed as refreshments and health although considering the composition of certain types of liquids it is questionable how useful they are.

The average expense for tea is 8.5 €/month and for fruit juices 22 €/month.

All foods that have more than one ingredient must list all ingredients in descending order of volume, should include certain additives that some people may be allergic to, also in non-alcoholic beverages containing fruit juices must indicate the percentage of volume that constitutes the fruit juice. [1]. According to our legislation non-harmonization of product appearance with ingredients does not violate the law, because nowhere does it say that the promotional photo of the product must match the ingredients listed on the label, but is this a kind of misleading of the producer to the consumer, I think yes, it is precisely the manufacturer through the photo that makes the product's advertising very appealing and on the other hand the product often contains very little of that ingredient or contains other types not reflected in the photo.

For research, several types of tea and fruit juices have been obtained, where the packaging image and product composition do not match, often a product is found in the photo, while more products are in the composition, or the photo product is the latest in composition and with a very small percentage. Although the product label should be legible by law, in many products it is difficult to read even through the magnifier.

Based on the research results, a lot must be improved from what is offered to consumers by manufacturers, products on the advertising label place tempting pictures for the consumer to buy the product, but not always the true composition of the product.

This whole study focuses on raising consumer awareness of the product they are buying, reading the content of the product, and buying reliable products as well as seeking better quality products.

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A Research of Some Pathogen Microorganisms in Traditional Sharri Cheese

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Abstract. Sharri cheese is a traditional hard, fatty and salty type of cheese produced in Sharri, Gora, Opoja and Shtirpca towns in the Sharr Mountains region. Sharri cheese is produced by processing sheep milk, cow milk or their mixture. The aim of this study was to determine the microbiological quality of traditionally and industrially produced Sharri cheese. In total 58 samples of cheese were examined. Staphylococcus aureus was found in 57 samples. Escherichia coli were determined in 34 samples and the number of E. coli were found between 1×10^1 CFU/g to 4.5×10^6 CFU/g. According to our results, Sharri cheese can be risky in terms of public health as it contains several foodborne pathogens.

Keywords: Sharri cheese, microbiology, Staphylococcus aureus, Escherichia coli

Introduction

In order for a person to live a healthy and long life, he must have adequate and balanced nutrition throughout his life. Adequate and balanced nutrition increases people's productivity and success rate in society. It is sufficient for a person to consume various nutrients (proteins, fat, carbohydrates, vitamins, minerals and water) in foods continuously and regularly throughout his life [4].
With unbalanced and malnutrition high blood pressure, weight gain or deficiency, growth-developmental retardation, decreased human productivity and diseases such as cardiovascular disorders, muscle / bone loss and so on are emerging. Diseases such as physical and mental disorders have various problems during a person's life [6].
The milk group is a food group containing most nutrients and provides a large portion of the daily need. Dairy and cheese products contain many important vitamins and minerals such as calcium, phosphorus, magnesium, proteins, vitamins A, D, E, K, B vitamins [3]. There are different types of cheeses in the world where they can be classified according to: type of milk used during cheese production (cow, sheep, buffalo, coat etc.), fat percentage (full fat, half fat, fat cheese, double cream, cream, low fat), consistency of cheese (very strong, strong, half strong, half soft, soft, fresh cheese), fermentation type, surface (hard, soft, spicy, molds), interior (holes, molds) [5].
Milk and milk products create an excellent environment for pathogenic microorganisms. Milk and dairy products can be important sources of foodborne diseases. One of the most important foodborne intoxication in world is caused by Staphylococcus aureus. The illness comes from taking the enterotoxins formed in food. The disease can appear within 2-4 hours by taking 1-10μg of enterotoxin in the body through food. S. aureus can be found in the human body, in the animal body, at food processing sites. Toxins formation can occur when the milk is taken from the diseased animal with mastitis, pasteurized milk is contaminated and stored under inappropriate temperature conditions [2].

Another type of foodborne intoxication is caused by Escherichia coli. This type of bacteria forms hemorrhagic colitis which causes diarrhea, hemolytic, uremic syndrome, and death. Minimal infection dose is known to be 10-100 cfu. The source of this bacterium may be unpasteurized products but also pasteurized dairy products that have been contaminated by various causes such as unhygienic conditions of production, personnel hygienic conditions [2]. Sharri cheese is a type of cheese produced traditionally Strpce, Opoje, Dragas villages in Sharri mountain region. Production places usually are placed above 2000m and the cheese is produced on summer months. After the milking from sheeps is finished they add the rennet and coagulate the milk without pasteurizing it. Besides traditionally produced cheese, there are some industrial producers in region that produce Sharri cheese with pasteurized cow milk instead of sheep milk [7] [13]. The old processing methods are still used in traditional cheese production. The microbiological quality of the cheese depends on several factors such as: unpasteurized raw material, lack of hygienic conditions in the production sites, lack of standardization of the cheese process where it is influenced by the individual processor. In previous years (Rysha et al) research has shown that 75% of raw milk samples do not comply with EU legislation, while cheese samples produced from raw milk do not comply with EU legislation [12]. The aim of this study was to determine the microbiological quality of traditionally and industrially produced Sharri cheese.

Materials and Methods

58 cheese samples from traditional and industrial producers were taken in sterile conditions from different regions and were transported in cold chain. 30 samples were from traditionally producers and 28 samples were from industrially producers. Analyzes were conducted at Istanbul University- Cerrahpasa, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology. Sharri cheese samples were taken in spring time from May to June 2019 and analyzes were performed in June 2019.

Manufacture of Sharri Cheese

Traditionally produced Sharri cheese is made from fresh milk taken after the milking of sheep's is finished. Rennet is added and the coagulation period lasts about 60 minutes. After the curd is cut, whey is drained and is filtered with a piece of cloth about 10-12 hours. The oval cheese up to 10kg matures on wooden boards for 2 weeks. Cheese is broken by hands and put on brine containing approximately 10% salt. Cheese is ready to eat after 45 days [7].

Industrially produced cheese is made after the milk is filtered and pasteurized. Culture and rennet is added and the coagulation period lasts about 45 minutes. After the curd is cut, whey is drained by pumps and oval cheese is formed with a piece of cloth. Oval cheese maturates in cold rooms maximum 8°C for 5-7 days. After that cheese is broken by hands and put in brine containing approximately 7-10% salt. Cheese is ready to eat because of pasteurization process on raw milk.

Microbiological analyses

Determination of *E. coli*

The method used for the identification of *E. coli* was ISO 16649-2-- Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of β-glucuronidase- positive *Escherichia coli*—Part 2: Colony-count technique 4°C using 5-bromo4-chloro-3-indolyl β-D-glucuronide.

Determination of *S. aureus*

The method used for the identification of S. aureus was ISO 6888-1—Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of coagulase- positive staphylococci (Staphylococcus aureus and other species- Part 1: Technique using Baird- Parker agar medium.

Results and Discussion

In total 58 samples of traditionally and industrially produced Sharri cheese were analyzed. All of the cheese samples have undergone heat treatment. Shown in Table 1 at the end of the microbiological analysis of *E. coli*, according to EU legislation limits for *E. coli* that can be found on the cheeses that are made from milk that are undergone heat treatment are 100 CFU/g to the maximum of 1000 CFU/g. The results of our analyses show that 16 samples of traditionally produced cheese are under 100 CFU/g, 3 samples are within limits 100 CFU/g to 1000 CFU/g while 11 samples containing more than 1000 CFU/g do not complain with the EU regulation 2073/2005. From industrial producers according to EU legislation limits for *E. coli* that can be found on the cheeses that are made from milk that are undergone heat treatment are 100 CFU/g to the maximum 1000 CFU/g. The results shown on Table 1 of our analyses show that 15 samples of traditionally produced cheese are under 100 CFU/g, 6 samples are within limits 100 CFU/g to 1000 CFU/g while 7 samples containing more than 1000 CFU/g do not complain with the EU regulation 2073/2005.

Table 1: The result of the analysis of E. coli in Sharri cheese

	<100 cfu/g	100 cfu/g to 1000 cfu/g	>1000 cfu/g
Traditional	16 samples	3 samples	11 samples
Industrial	15 samples	6 samples	7 samples

Shown in Table 2 at the end of the microbiological analysis of S. aureus, according to EU legislation limits for S. aureus that can be found on the cheeses that are made from milk that are undergone heat treatment are 100 cfu/g to the maximum 1000 cfu/g. The results of our analyses show that all of the 58 samples of traditionally and industrially produced cheese are above the limit 1000 cfu/g so these cheese samples do not complain with the EU regulation 2073/2005.

Table 2: The result of the analysis of S. aureus in Sharri cheese

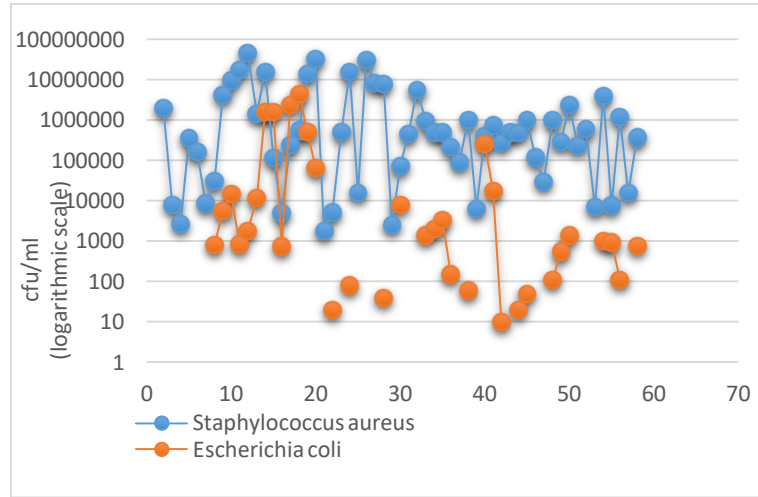|	<100 cfu/g	100 cfu/g to 1000 cfu/g	>1000 cfu/g
Traditional	-	-	30 samples
Industrial	-	-	28 samples

Figure 1: Presence of E. coli and S. aureus count in Sharri cheese.

This study has shown that at the end of the microbiological analysis of E. coli, according to EU legislation, from traditional producers only 10% of the samples are within the limit while 36% of the samples do not comply with (EU regulation 2073/2005). From industrial producers 21% of the samples are within the limit while 25% of the samples do not comply with (EU regulation 2073/2005).

At the end of the microbiological analysis of S. aureus, in the traditional and industrial method all cheese samples are out of the permissible limit (EU regulation 2073/2005).

Previous studies (Rysha et al.) has shown that all Sharri cheese samples that were analyzed for S. aureus and the results were (>10 CFU/g) don't complain with EU regulation 2073/2005 [12].

Another research made for different type of cheese named Arzua Ulloa produced from raw milk showed result 61.4% comply with EU regulation, 22.8% were acceptable while 15.8% were not acceptable due to high number of S. aureus, E. coli and/or L. monocytogenes [12].

Food contamination by pathogen microorganisms like S. aureus and E. coli is a serious public health problem [10] [14]. The main sources of contamination with S. aureus are the presence of S. aureus in the raw milk, direct contamination during the cheese processing, and cross contamination at the final product [1].

As a conclusion, low or more value S. aureus and E. coli were found in Sharri cheeses. This result shows that Sharri cheese may pose a risk for public health.

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Ribosomal Protein RpL35/uL29 Function and Role in Different Diseases

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Abstract. RpL35/uL29 is member of large subunits. It is shown that RpL35/uL29 participate in different processes in diseases and development. It is shown that RpL35/uL29 is important for ribosome mature. Many authors show that RpL35/uL29 is good indicator for diagnosis. Here we will describe role and function of ribosomal protein RpL35/uL29 in different cancer diseases such as colorectal adenocarcinoma and atherosclerosis.

Keywords: RpL35/uL29, Cancer diseases, RpL35/uL29 Processing

Introduction

RpL35/uL29 play a pivotal role during the assembly of the ribosome. RpL35/uL29 is located close to RpL19/eL19 and RpL25/uL23 in the exit tunnel. Some authors suggest that RpL35/uL29 causes slow growth defects by generating low 60S subunit level. Arx1 participates in ribosome biogenesis. Arx1 binds to pre-60S subunits exactly in the vicinity of the RpL35/uL29 and RpL25/uL23 at the exit tunnel. For release of Arx1 in the cytoplasm from subunit 60S (RpL35/uL29) the protein Rei1 must interact with Arx1 (Greber, B.J. et al., 2016) (Figure 1).

L35/uL29 assembles onto the ribosome in the nucle(ol)us and stably interact with 27S pre-rRNAs, therefore suggesting that it is added early in the assembly pathway. It has been suggested that zebrafish RPL35/uL29, as many other r-protein genes, acts as a haploinsufficient tumor suppressor by an as yet unknown mechanism (Babiano R., and de la Cruz J., 2010).

Yeast L35 is an essential small r-protein of 120 amino acids with a predict mass of 13.9 kDa and a predicts basic pI of 11.36 (Saccharomyces Genome Database, www.yeastgenome.org). As two thirds of the eighty years r-proteins, RpL35/uL29 is encoded by two paralogous genes, RPL35A (SOS1, YDL191W) and RPL35B (SOS2, YDL136W), which both localize to the left arm of chromosome IV.

The coding regions of these two genes are nearly identical, differing in only 3nt out of 362, but resulting in no change in the amino acid sequences of the predicted L35A and L35B proteins (Babiano R., and de la Cruz J., 2010). Human RpL35/uL29 is small protein of 123 amino acids and is encoded by gene which is placed at chromosome 9 q arm (Uechi T., et al., 2001) (Figure 2)

R-protein L35/uL29 together with RpL20/eL20 is expressed from a dicistronic operon in archaea and eukaryotes which follows the general regulatory scheme of r-protein synthesis: when in excess, RpL20/eL20 binds to its own mRNA and directly inhibits the translation of the first cistron of the operon, rpmI, encoding L35/uL29. This inhibition is then transmitted by translational coupling to the second cistron, rpIT, encoding L20/eL20 itself. Haentjens-Sitri J., et al., (2008) show that this is not the case for rpmI-rpITmRNA, the regulation of the synthesis of L35/uL29 and L20/eL20 obeys a competition mechanism between the repressor and the ribosome for binding that mRNA (Haentjens-Sitri et al., 2008).

The expression profile of RpL35/uL29 together with three other ribosomal protein genes (RPL18/eL18, RPL31/eL31, and RPS3/uS3) were validated by RNA blots using additional, independent crosses from the same families. Expression of RPL35/uL29 was monitored throughout early larval development, revealing that these expression patterns were established early in the development (in 2-day-old larvae) (Meyer E., and Manahan D.T., 2010).

Role and function of RpL35/uL29 in Ribosomal Processing

RpL35/uL29 assembles in the nucle(ol)us and stably interacts with 27S pre-rRNAs, therefore suggesting that it is added early in the assembly pathway. RpL35/uL29 participates in processing of 27SA rRNAs. Based on the crystal structure of the yeast 60S ribosomal subunit (Ben-Shem A., et al., 2010), rpL17/uL22, rpL26/uL24, rpL35/uL29, and rpL34/eL37, lie close to each other, adjacent to 5.8S rRNA, whose 5'-end is generated by 27SA3 pre-rRNA processing. Four ribosomal proteins- rpL17/uL22, rpL26/uL24, rpL35/uL29, and rpL37/eL37 specifically cannot assocaye with pre ribosomes when A3 factors are depleted. These four r-proteins bind adjacent to each other on 5.8S rRNA in mature 60S ribosomes in S. cerevisiae (Ben-Shem A., et al., 2010) this results indicates that the presence of A3 factors, which are required for proper formation of the 5-end of 5.8S rRNA, stabilizes this neighborhood of r-proteins within assembling ribosomes. We also show here that in the absence of A3 factors and rpL17/uL22, rpL26/uL24, rpL35/uL29, and rpL37/eL37, Rat1 cannot stop at the B1S site and proceeds beyond this site to turn over 27S pre-rRNA.

A third important function of A3 factors is to ensure stable association of r-proteins rpL17/uL22, rpL26/uL24, rpL35/uL29, and rpL37/eL37 with pre ribosomes. Interestingly, the binding sites in mature ribosomes of rpL17/uL22, rpL26/uL24, rpL35/uL29, and rpl37/eL37 are near each other in domains I and III of 5.8S/25S rRNAs (Ben-Shem A., et al., 2010). Three of the four r-proteins most affected in the A3 mutants are not required for 27SA3 pre-rRNA processing, but are required for 27SB pre-rRNA processing. In the absence of rpL17/uL22, rpL35/uL29 or rpL37/eL37, 27SB pre-rRNA, but not 27SA3 pre-rRNA.

Once A3 factors dissociate from pre ribosomes, it is imperative that this base pairing between 5.8S and 25S rRNAs be maintained. Stable association of rpL17/uL22, rpL26/uL24, rpL35/uL29, and rpL37/eL37 may play a role in maintaining base pairing between these two rRNAs after release of A3 factors, and in mature functioning ribosomes (Sahasranaman A., et al., 2011). In the hypothalamus, numerous ribosomal genes changed their expression under CSDS (14 Rps and 22 Rpl genes). Rps14/uS11, Rps8/eS8, Rps6/eS6, Rps9/uS4, Rps5/uS7, Rps19/eS19, Rps16/uS19, Rps/uS3, Rpsa/uS2, Rps26/eS26, Rps10/eS10, Rpl37a/eL43, Rpl41/eL41, Rpl19/eL19, Rpl23a/uL23, Rpl37/eL37, Rpl8/uL2, Rpl10a/uL1, Rpl36/eL36, Rpl7a/eL8, Rpl2/uL11. Rpl35/uL29, Rpl34/eL34, Rpl0/uL10, Rpl6/eL6, Rpl28/eL28, Rpl18/eL18, Rplp2/P2, Rpl13/eL13, Rpl18a/eL20, Rpl29/eL29 and Rplp1/P1 were upregulated, and Rpl22/uL22, Rps6/eS6, were downregulated. Smagin D.A., et al., suggest that enhanced expression of the Rpl18/eL18 and Rpl35/uL29 genes was overlapped in the hippocampus and hypothalamus (Smagin D.. eta., 2016).

Role and function of RpL35/uL29 in Cancer Diseases

Many RP genes might also be cancer genes in human, where their role in tumorigenesis could easily have escaped detection up to now.

In humans, several ribosomal proteins regulate p53 activity by abrogating Mdm2-induced p53 degradation or by increasing translation of p53 mRNAs. RpL35/uL29 also is associated with the highest tumor incidence. The finding that mutations in so many different RP genes, including S7/eS7, S8/eS8, S15a/uS8, S18/uS13, S29/uS14, L7a/eL8, L13/eL13, L23a/uL23, L35/uL29, L36/eL36, and L36a/eL42, predispose to cancer suggests that a function shared by RPs underlines their role in this phenotype. However, not all RP genes were cancer genes: S12/eS12, S15/uS19, L3/uL3, L24/eL24 and LP1/P1 heterozygotes appeared normal (Amsterdam A., et al., 204b).

A study from Huang L., et al., reports a 11-gene signature for predicting PLNM (predicting lymph node metastasis) in cervical carcinoma which are identified 7 genes (RPL35/uL29, TMSB10, YWHAZ, BTD, LDHA, GUSB and SOD2) were up-regulated in patients without PLNM and down-regulated in patient with PLNM (Huang L., et al., 2011).

Luzp4 is an RNA binding protein that associates with TREX subunits. With mass spectrometry following IP is identified an interaction of Luzp4 with RpL35/uL29 I test cancer (Viphakone N., et al., 2015). The RPL35/uL29, RPS23 and TIMP1 genes were found to be overexpressed in both early and advanced stage colorectal adenocarcinomas (p<0.05) (Lau T.P., et al 2014).

RpL35/uL29 is a good indicator and for other diseases such as development of atherosclerosis (Wang H.X., and Zhao Y.X., 2016). Signal recognition particle (SRP), together with its receptor (SR), mediates the targeting of ribosome-nascent chain complexes to the endoplasmic reticulum. Using protein crosslinking, it was detected that there are distinct modes of binding of SRP to the ribosome. During signal peptide recognition, SRP54 is positioned at the exit site close to ribosomal proteins L23a/uL23 and RpL35/uL29. When SRP54 contacts SR, SRP54 is rearranged such that it is no longer close to L23a/uL23. This repositioning may allow the translocation channel (Pool M.R., et al., 2002).

F-box proteins are best known for their role as substrate receptors of SCF ubiquitin ligases. FbxL16 is member of F-box protein which bind and regulate the function of protein phosphatase 2A (PP2A), a heterotrimeric serine phosphatase that has diverse functions including modulation of TGF beta signaling and cell cycle control. With mass spectrometry is observed that RpL35/uL29 co-immunoprecipitates have low affinity to bind FBXL16 (Honarpour et al., 2014). RpL35/uL29 is regulatory factor involving in the Met-mediated regulation of CSN2 (casein) translation elongation and secretion also (Jiang N., et al., 2015).

Discussion

Here in this manuscript we show that ribosomal protein RpL35/uL29 interact with different ribosomal protein such as Rps14/uS11, Rps8/eS8, Rps6/eS6, Rps9/uS4, Rps5/uS7, Rps19/eS19, Rps16/uS9, Rps/uS3, Rpsa/uS2, Rps2/uS5, Rps26/eS26,Rps10/eS10, Rpl37a/eL43, Rpl41/eL41, Rpl19/eL19, Rpl23a/uL23, Rpl37/eL37, Rpl8/uL2, Rpl10a/uL1, Rpl36/eL36, Rpl7a/eL8, Rpl12/uL11, Rpl35/uL29, Rpl34/eL34, Rplp0/uL10, Rpi6/eL6, Rpl28/eL28, Rpl18/eL18, Rplp2/P2, Rpl13/eL13, Rpl18a/eL20, Rpl29/eL29 and Rplp1/P1 were is upregulated, but in interaction with ribosomal proteins such as Rpl22/uL22, Rps6/eS6, is downregulated, but in interaction with ribosomal proteins such as S7/eS7, S8/eS8, S15a/uS8, S18/uS13, S29/uS14, L7a/eL8, L13/eL13, L23a/uL23, L35/uL29, L36/eL36, and L36a/eL42 shown higher cancer incidence. We can conclude that RpL35/uL29 is good indicator for diagnosis.
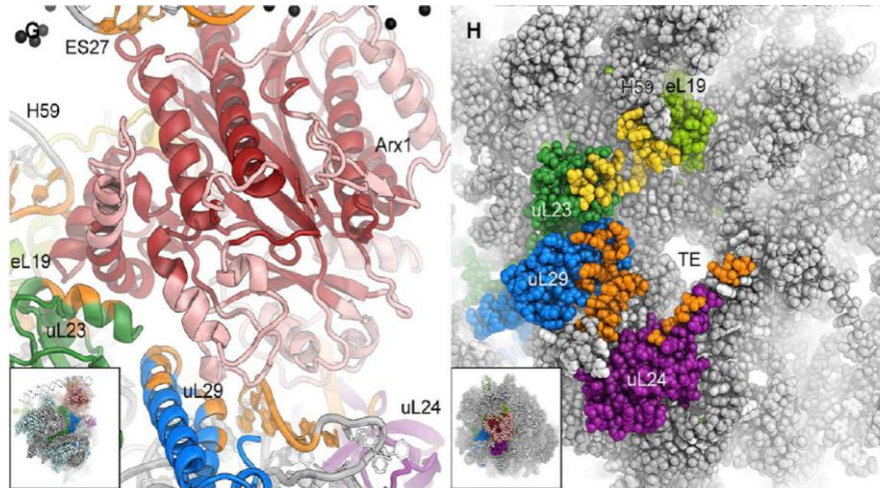
Fig. 1. (G) Arx1 interacts with RpL23a/uL23, RpL26/uL24, RpL35/uL29, and RpL19/eL19 as well as the rRNA in vicinity of the tunnel exit. 60S residues involved in contacts are shown in orange. (H) Contact sites of Arx1 on the 60S subunit with ribosomal proteins. Greber, B.J. et al., Insertion of the Biogenesis Factor Rei1 probes the Ribosomal Tunnel during 60S Maturation. Cell 2016; 164, 1-12
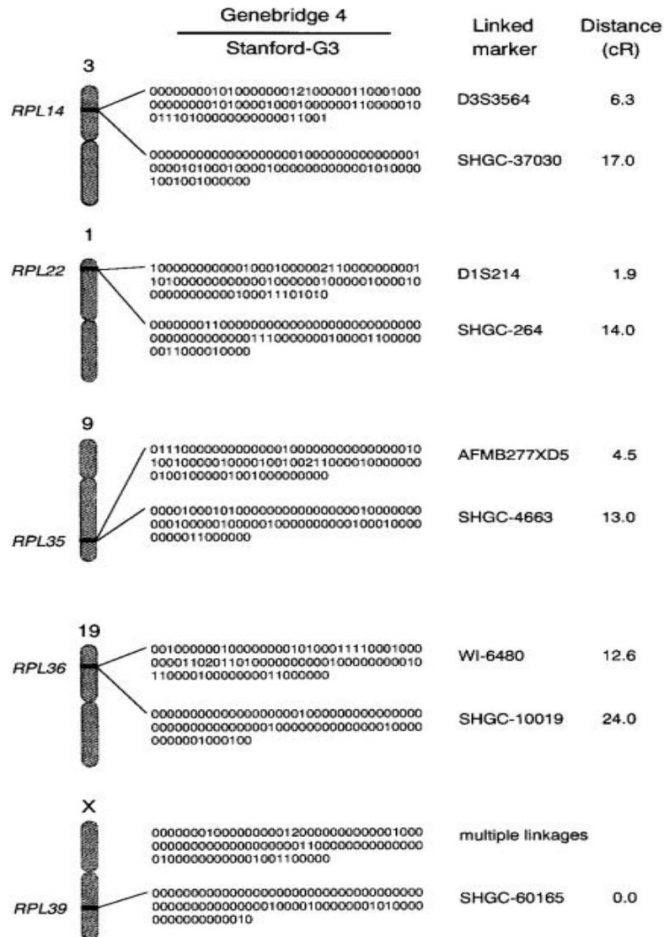
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Cholesterol Analysis by Enzymatic Method in Conventional, Pastured and Organic Eggs

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Abstract. The concentration of cholesterol in three types of eggs has been analyzed through the enzymatic method. The types of analyzed eggs were: conventional, pastured and organic. Thirty five samples were analyzed, the sampling has been random, and the cholesterol analysis was done by GENESYS 10S UV-Vis Spectrophotometer. Analyzes have been conducted in research laboratories of the University for Business and Technology (UBT) - Kosovo. The average of the obtained results indicates a slightly lower cholesterol concentration in the type of pastured eggs compared to other types.

Key words: Egg, Cholesterol, Chicken Feed, Atherosclerosis.

Introduction

Laying hens are members of an order known as Aves. Indigenous chickens are descended from red jungle birds (Gallus gallus).

Chicken meat provides essential vitamins and minerals and is the most affordable source of meat for humans. The chicken egg is like a sealed box with an external structure suitable to assure its internal quality. The peel is essentially composed of $CaCO_3$ (98%), in a polycrystalline structure in parallel layers forming the mammalian node, creating over 8000 pores that serve for gas exchange inside and outside the egg. The internal nutrient core has two compartments which are albumin (59%) and egg yolk (31%), where the main nutrients for human consumption are found. Most of the essential nutrients are in the egg yolk and especially the fat-soluble vitamins present specifically in this part. Essential fatty acids and omega-3s are also present in the egg yolk. (Bertechini A. G, 2013). Specifically, eggs consist of 3 main elements: eggshell (9–12%), egg white (60%) and egg yolk (30–33%). The whole egg is composed of water (75%), proteins (12%), lipids (12%), including carbohydrates and minerals (1%). customers. At the same time, well-being and many other factors can affect egg quality. These factors include breed and strain of layers, dietary composition, bird health, state of the environment and storage, processing and treatment of eggs (Attia, 2016).

Cholesterol is an extremely important biological molecule that plays a role in membrane structure and is a precursor for the synthesis of steroid hormones, bile acids and vitamin D. High serum cholesterol is a major risk factor for human cardiovascular disease such as is coronary heart disease and stroke are the number one most common diseases in America (Tabas, 2002). Excess cholesterol in the bloodstream can form plaque (a thick, hard deposit) on the walls of the body arteries. The creation of cholesterol or plaque causes the arteries to become thicker, harder and less flexible, slowing and sometimes blocking blood flow to the heart. When blood circulation is restricted, angina (chest pain) may occur (Hongbao Ma, 2004). Cholesterol deposition on egg yolk may also be affected by food. The inclusion of specific foods in commercially grown fatty chicken diets, such as unsaturated fatty acid rich vegetable

oils are used to alter the lipid profile of the egg and reduce the cholesterol content in the egg (Faitarone, 2013). A medium-sized boiled egg (50 g) contains 78 kcal of energy, 6.29 g of protein, 0.56 g of carbohydrates, and 5.3 g of total fat, of which 1.6 g is saturated, 2.0 g is unsaturated fat and 186 mg is cholesterol. In 1968, the American Heart Association (AHA) recommended that dietary cholesterol consumption be no more than 300 mg daily and emphasized that no more than three yolks should be consumed per week.

Recent epidemiological studies usually indicate a lack of association between dietary cholesterol and / or egg intake and the risk of CVD in the general population. However, there seems to be a more consistent relationship between egg intake and CVD in diabetics, however, this has not always been observed. (N.Blesso, 2018)

Materials and Methods

Sampling and sample preparation

For quantitative determination of the cholesterol, we have obtained eggs from three types of farms:
• The farm which produces organic eggs;
• The farm which produces conventional eggs and;
• The farm which produces eggs known as pastured eggs.
A total of 35 samples were analyzed and the sampling was random, of these 15 were conventional eggs, 10 organic eggs, and 10 pastured eggs.

Laboratory work

Extraction procedure

In the beginning, we have gently opened the egg. After that, using the peel, we have divided the egg yolk by white, and discarded the white. The egg yolk we added to a glass and recorded its mass and volume. After, we have divided the egg yolk into 2 equal portions and have followed the steps below;

We have added an equal volume of acetone to each tube. This should be done in a glass tube for centrifugation. We have mixed the material vigorously for 2 minutes and then we have centrifuged it (100rpm, 15 min). After that, we have decanted the acetone and repeated the same three times. Finally, we assembled the acetone fractions and finally allowed the acetone to evaporate. Acetone extract should contain cholesterol and egg yolk pigments.

Principle of enzymatic action of cholesterol reagent

The cholesterol reagent contains cholesterol-esterase, cholesterol-oxidase and turnip peroxidase.

$$cholesterol \; + \; O_2 \; \xrightarrow{\;cholesterol\; oxidase\;} \; cholestenone \; + \; H_2O_2$$

$$H_2O_2 \; + \; phenol \; + \; 4-amino-antipyrine \; \xrightarrow{\;HRP\;} \; colored \; product \; + H_2O$$
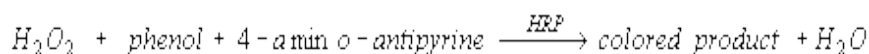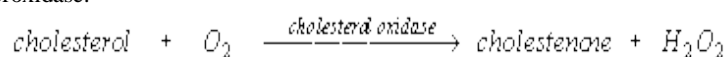
Since only free cholesterol is a substrate for cholesterol oxidation, any cholesterol esters present in the sample must first be hydrolyzed with the aid of the cholesterol - esterase enzyme. Free cholesterol then reacts with another enzyme known as cholesterol - oxidase, whereby cholestenone is formed and hydrogen peroxide is released which reacts with a substance known

as 4-amino-antipyrin and at the end of the reaction the colored product is formed, whose intensity is measured by spectrophotometer and is in direct proportion to the concentration of cholesterol in the sample.

Working procedure

• Reset the acetone fraction to a few ml of isopropanol (you should know the amount);
• Mix the isopropanol sample;
• Take 1 ml of sample and place it in another tube;
• Add 5 ml of isopropanol and mix the sample;
Use the table below to build your calibration curve and cholesterol analysis. Put the reagents directly in the analytical kit.
Note: count the kiwis on top and then add reagents to the kiwis according to the instructions given in the table below. You should add the last cholesterol reagent and relatively at the same time to each kiwi.
After adding the reagent to all cells, start (calculate) the time. Do not mix the kiwis, but shake them gently with your finger every 5 minutes. Incubate the kiwis for 10 minutes.

Table 1. Laboratory working procedure

Component	Blank	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Water	100μL	90 μL	90 μL	90 μL	90 μL	90 μL	0 μL
1 mg/ml standard	-------	10 μL	-------	-------	-------	-------	-------
2 mg/ml standard	-------	-------	10 μL	-------	-------	-------	-------
4 mg/ml standard	-------	-------	-------	10 μL	-------	-------	-------
8 mg/ml standard	-------	-------	-------	-------	10 μL	-------	-------
Lipid sample	-------	-------	-------	-------	-------	5 μL	50 μL
Cholesterol reagent	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

Finally we read the absorbance at 500 nm in Genesys10S UV-Vis Spectrophotometer and used the graph (calibration curve) to determine the cholesterol concentration (mg/ml) in the sample that we have analyzed.

Results and Discussion

Table 2. Cholesterol in conventional eggs

The Number of The Sample	Egg Yolk Weight (g)	Cholesterol Concentration in Diluted Egg Yolk (mg / ml)	Total Cholesterol Concentration in Egg Yolk (mg)
K1	18.46	0.690	152.84
K2	15.83	1.015	192.80
K3	18.36	0.820	180.66

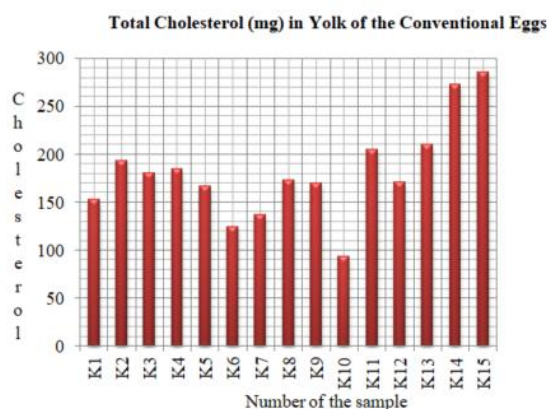| K4	16.83	0.911	183.98
K5	17.29	0.800	165.98
K6	17.82	0.580	124.02
K7	15.79	0.720	136.42
K8	14.56	0.987	172.44
K9	15.55	0.910	169.80
K10	16.70	0.465	93.180
K11	13.41	1.270	204.36
K12	17.10	0.830	170.31
K13	14.95	1.080	209.89
K14	18.06	1.160	272.34
K15	16.26	1.351	285.57

Figure 1. Total cholesterol concentration in commercial egg yolk

According to the US Department of Agriculture, a large egg contains about 186 mg of cholesterol, and the whole is found in the egg yolk. Although other data related to the cholesterol concentration in the egg are found in the literature, one of them dominates and that is that in an egg of average size, the concentration of cholesterol in the egg yolk is about 200 mg.

A total of 15 conventional eggs were analyzed. Egg yolk was first measured in gram, then cholesterol was calculated in diluted egg yolk, and finally the total cholesterol concentration is calculated in conventional egg yolk.

The obtained results after spectroscopic analysis indicate an average cholesterol concentration in conventional egg yolk of 180.97 mg, which is in accordance with the data from the US Department of Agriculture regarding to the mean concentration of cholesterol in the egg yolk. From the obtained results we can see also that that the mass of the egg yolk is not directly proportional to the cholesterol concentration For example, if we analyze sample K11 we can see that despite the fact that the mass of the egg yolk is relatively small (13.41 g) the concentration of cholesterol in the diluted sample and consequently the total concentration in the sample is higher (204.36mg) compared to some other samples which contain a greater initial egg yolk mass. Finally, from the results we can see that commercial egg have higher average cholesterol concentration in egg yolk compared to other analyzed egg (pastured and organic).

Table 3. Cholesterol in pastured eggs

The Number of The Sample	Egg Yolk Weight (g)	Cholesterol Concentration in Diluted Egg Yolk (mg / ml)	Total Cholesterol Concentration in Egg Yolk (mg)
E1	16.73	0.290	64.590
E2	18.41	0.504	120.62
E3	17.34	0.529	119.24
E4	18.83	0.627	153.48
E5	14.79	0.715	137.47
E6	15.63	0.422	85.740
E7	18.23	0.850	201.44
E8	15.40	0.979	195.99
E9	18.24	0.807	191.35
E10	19.14	0.997	248.07
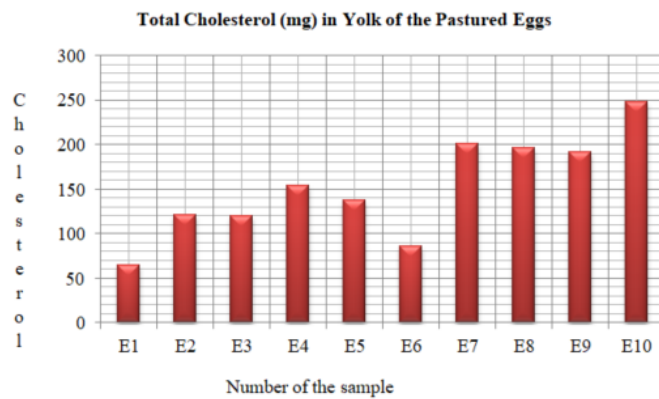
Figure 2. Total cholesterol concentration in pastured egg yolk

The obtained results from the analysis of the pastured eggs also show an average concentration of cholesterol in the yolk within the recommendations given by the US Department of Agriculture. From the ten analyzed samples we found that the average concentration of cholesterol in the egg yolk is 151.799 mg. If we compare these results with the average of the results obtained from conventional and organic egg we can see that the average concentration of cholesterol in this type of egg is lower.

As with conventional eggs, mass of the egg yolk does not necessarily determine the total cholesterol concentration in the egg yolk. For example, if we compare sample E1 and E5 we can see that despite the fact that in the sample E1 egg yolk weighs more (16.73 g) than sample E5 (14.79 g), the total cholesterol concentration in the sample E1 (64,590 mg) is significantly lower compared to the sample E5 (137.47mg).

Table 3. Cholesterol in organic eggs

The Number of The Sample	Egg Yolk Weight (g)	Cholesterol Concentration in Diluted Egg Yolk (mg / ml)	Total Cholesterol Concentration in Egg Yolk (mg)
O1	18.26	1.140	270.61
O2	19.58	0.297	73.810
O3	15.33	0.720	143.48
O4	16.21	0.490	103.25
O5	19.67	0.724	185.13
O6	14.55	0.819	154.91
O7	16.63	0.877	189.59
O8	17.32	1.065	239.79
O9	15.93	0.589	121.89
O10	19.38	1.076	271.08
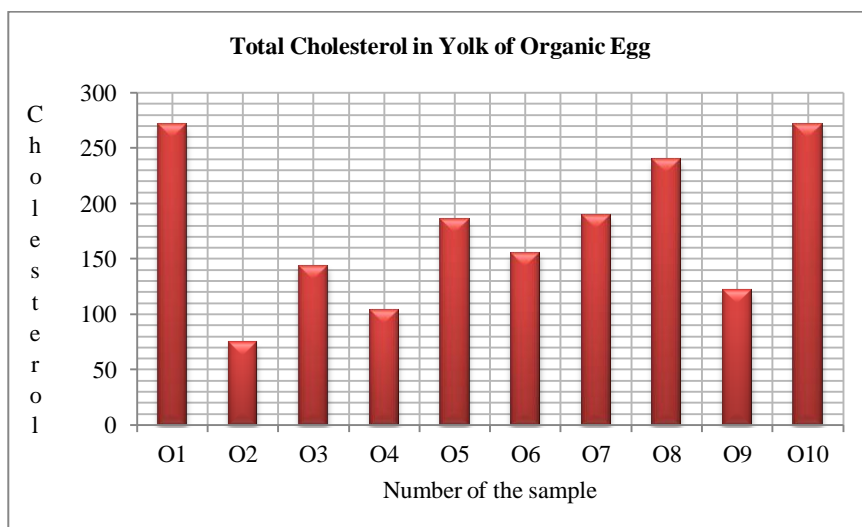
Figure 3. Total cholesterol concentration in commercial egg yolk

Even in the case of organic eggs, as in the previous two cases, the obtained results show an average cholesterol concentration within the recommendations given by the US Department of Agriculture. From the ten analyzed samples we found that the average cholesterol concentration in the egg yolk is 175.354 mg. If we compare these results with the average of the results obtained from conventional and pastured egg, we can see that the average concentration of cholesterol in this type of egg is lower compared to conventional eggs and slightly higher compared to pastured eggs. Even in this type of eggs the measure of the egg yolk does not determines the final concentration of cholesterol.

Conclusions

After analyzing the cholesterol concentration in 35 egg samples we can conclude that the average cholesterol concentration in three analyzed groups does not exceed the limits or recommendations given by the US Department of Agriculture in relation to the average cholesterol concentration in the egg yolk (186 mg). The average concentration of cholesterol in conventional egg yolk has been slightly higher compared to pastured and organic eggs. The average cholesterol concentration in the yolk of pastured eggs has been slightly lower compared to conventional and organic egg. The egg yolk weight is not always directly proportional to the cholesterol concentration. Apart from the way of nutrition, the concentration of cholesterol in the egg is probably determined by a number of other factors that make this issue quite complex. Upon completion of this study work we recommend the consumers to avoid excessive consumption of eggs (the daily requirement for cholesterol is 300 mg), namely not to consume more than 2-3 whole eggs per day even though "The American Heart Association" suggests one egg (or two white eggs) a day for people who eat it as part of a healthy diet, while for those with heart disease, type 2 diabetes or people with high levels of bad cholesterol (LDL), the American Heart Association (AHA), and the National Cholesterol Education Program (NCEP) suggest limiting cholesterol intake to 200 milligrams a day.

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Parasite Fauna in Common Carp (Cyprinus carpio L., 1758) from Fish Cage Culture System on Tikvesh Reservoir (N. Macedonia)

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Abstract. Over the years, fish cage culture system has become one of the economically viable methods of large-scale production of high-value food fishes. Although, cage farming has many economic advantages, diseases are one of the major limiting factors to the successful production. The aim of this study was to determine the presence of parasite fauna in common carp (Cyprinus carpio L. 1758) from fish cage farms on Tikvesh reservoir (N. Macedonia). A total of 206 specimens of common carp from this fish cage farms were examined for parasitological investigations. Infestation with parasite was determined in 121 specimens (58.74 %). In common carp from this reservoir, the presence of 5 parasite species was established: Trichodina sp., Dactylogyrus extensus, Eudiplozoon nipponicum, Bothriocephalus opsariichthydis and Ergasilus sieboldi. In confined conditions such as cages where the stocking density is very high and the resultant stress might act as conductive factor for pathogens to cause diseases. High stocking densities coupled with fluctuations in environmental conditions and/or stress can favor parasite proliferation leading to significant mortalities in net-cage-reared fishes.

Keywords: parasites, fish, common carp, cage culture system

Introduction

Growing global population, reduction of natural fish stocks, and the increasing demand are the major drivers for increasing fish production. Aquaculture remains the only option to meet these demands and globally, the share of aquaculture is projected to rise to 62% of the total fish production by 2030 [1]. However, considering the limitations of the traditional aquaculture systems due to environmental issues, carrying capacities etc., it has been recognized that cage culture has many advantages. Over the years, cage culture has become one of the economically viable methods of large-scale production of high-value food fishes.

Although, cage fish farming has many economic advantages, like any other animal production system, diseases are one of the major limiting factors to the successful production. Increasing intensification and lack of adequate health management measures result in frequent occurrence of diseases. Since the basic cage culture practices are similar in all the regions, disease problems encountered will largely depend on the species being cultured, environmental conditions and management practices [2].

The aim of this study was to determine the presence of parasite fauna in common carp (Cyprinus carpio L. 1758) from fish cage culture system on Tikvesh reservoir (N. Macedonia). The Tikvesh reservoir is situated on the River Crna Reka, 12 kilometers on the southwest of

Kavadarci town. It occupies territory of 14 km2 and is 29 km long. The total gross area of the reservoir is 475 million m3 of water. The water from the Tikvesh reservoir is used for irrigation, production of electricity, as well as for fish production in cage systems. On average, the production of fish cage farms on Tikvesh reservoir ranges from 5-35 kg per 1 m3 volume of water, depending on the fish species and the technology of cultivation. On this reservoir, in total volume of 70.000 m3 of water, 2.400 tons of fish can be raised.

There are approximately 30 cage farms for carp breeding, with about 500 cages in the waters of Tikvesh reservoir. Cage farms are characterized by different number of cages, often with dimensions 5 m x 5 m x 5 m (with a total volume of 125 m3).

Fig.1. Tikvesh reservoir

Fig.2. Fish cage farms on Tikvesh reservoir (original)

Materials and methods

The fish were caught using net or by local anglers. The specimens were placed in plastic containers and transferred alive to the laboratory. During the dissection, the gill filaments, the eyes, the fins, the intestines and the skin were examined under the stereomicroscope. All parasites found in each individual fish were identified and enumerated. The parasite specimens were fixed in 70% alcohol to be observed under light microscope. During the study period, data on parasite species were categorized according to season. The environmental factors were not measured in this study. Total numbers of parasites were determined directly by numerical count. The number of fish examined, fish infected, prevalence and mean intensity (total and by seasons) are given in table 1.

Classical epidemiological variables (prevalence and mean intensity) were calculated according to [3].

The parasite specimens were identified using reference keys of [4] and [5].

During the examinations at Laboratory for fish diseases in Hydrobiological Institute in Ohrid (R. N. Macedonia), stereomicroscopes „Zeiss"- Stemi DV4 and „MBS 10", as well as light microscope „Reichart" were used.

Results and discussion

 A total of 206 specimens of common carp from this fish cage culture system were examined for parasitological investigations. Infestation with parasite was determined in 121 specimens (58.74 %).
In common carp from this reservoir, the presence of 5 parasite species was established:
      1. *Trichodina* sp. on fins in common carp in autumn;
      2. *Dactylogyrus extensus* on gills in common carp in spring, summer, autumn and winter;
      3. *Eudiplozoon nipponicum* on gills in common carp in spring;
      4. *Bothriocephalus opsariichthydis* in intestines in common carp in autumn;
      5. *Ergasilus sieboldi* on gills in common carp in spring.
Data on fish examined, fish infected, as well as the percent of infestation with parasites (total and by seasons) in common carp from Tikvesh reservoir are given in Table 1.

Table 2. Infestation with parasites in common carp (Cyprinus carpio) from fish cage culture system on Tikvesh reservoir (N. Macedonia)

Seasons	Number of examined fish	Number of infected fish	Percent of infestation (%)
Spring	55	35	63.64
Summer	51	21	41.17
Autumn	54	26	48.15
Winter	46	39	84.78
TOTAL	206	121	58.74
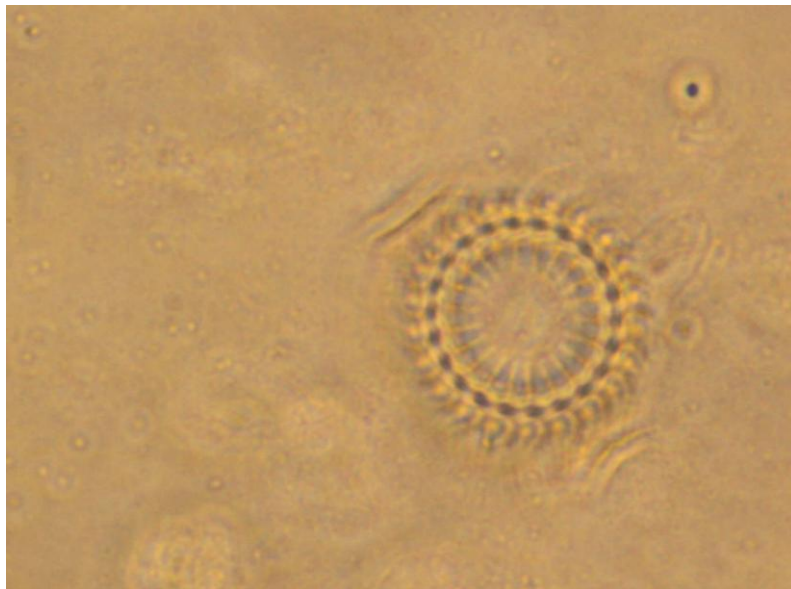
Fig.3. Trichodina sp. on fins in common carp (Cyprinus carpio) from fish cage culture system on Tikvesh reservoir (original)

Fig.4. Eudiplozoon nipponicum (clamps) on gills in common carp (Cyprinus carpio) from fish cage culture system on Tikvesh reservoir (original)

Fig.5. Eudiplozoon nipponicum (posterior part) on gills in common carp (Cyprinus carpio) from fish cage culture system on Tikvesh reservoir (original)

Fig.6. Bothriocephalus opsariichthydis in intestines in common carp (Cyprinus carpio) from fish cage culture system on Tikvesh reservoir (original)

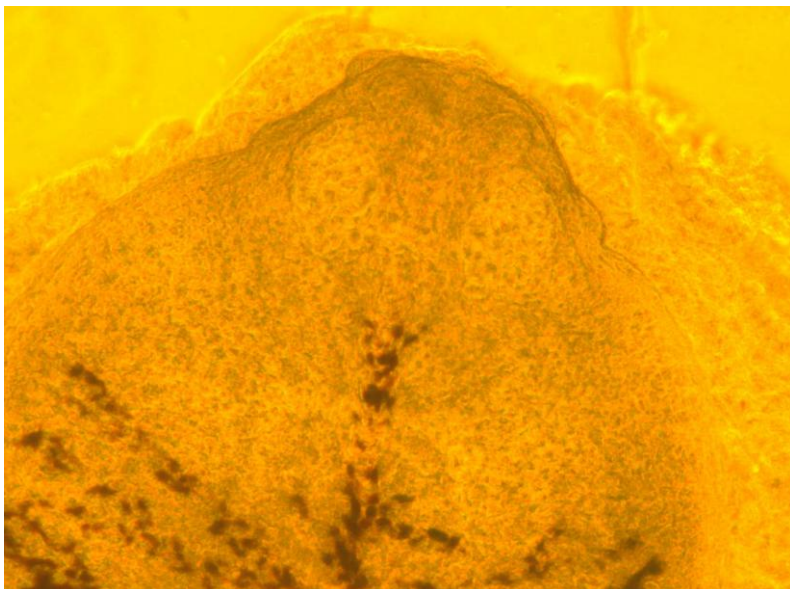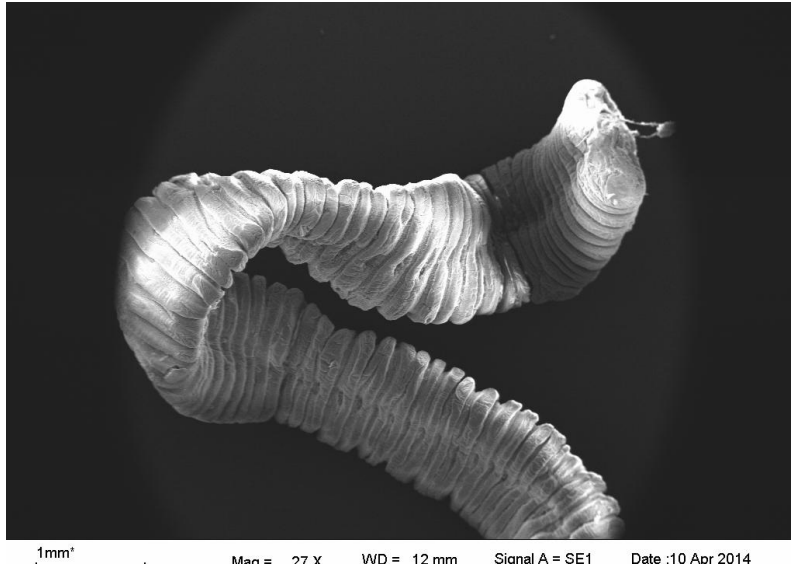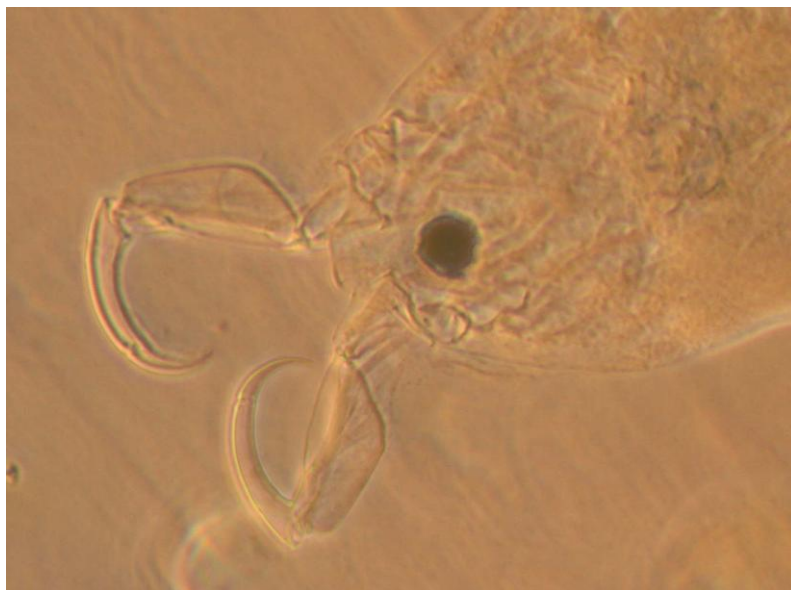Fig.7. Ergasilus sieboldi (anterior part) on gills in common carp (Cyprinus carpio) from fish cage culture system on Tikvesh reservoir (original)
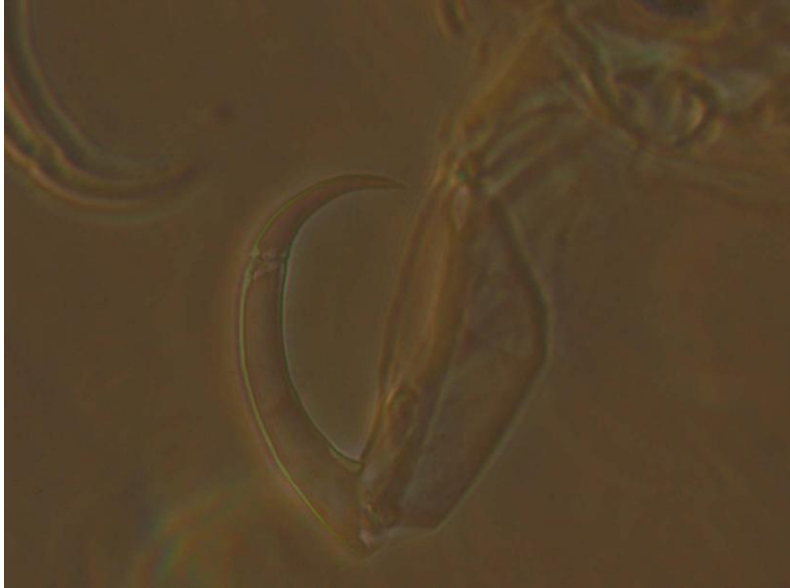
Fig.8. Ergasilus sieboldi (hooks) on gills in common carp (Cyprinus carpio) from fish cage culture system on Tikvesh reservoir (original)

Except some protozoans, most of the economically important parasites infecting farmed fishes are ectoparasitic in nature, of which copepods are considered serious parasites causing mortalities. Ectoparasites feed on mucous, tissues, and blood/body fluids and the damage caused by their attachment and feeding activities may pave way for secondary infections. Major pathology associated with some ectoparasitic infestation includes damage to the epithelial layer (skin and gills) resulting in hemorrhagic lesions on the skin and osmoregulatory dysfunction. They are also reported to act as vectors of some of the pathogenic viruses and bacteria besides making the fishes susceptible to secondary infection. Economic losses can be quantified in terms of direct mortalities, secondary infections, poor/reduced growth and expenses for treatment. Open cage farms facilitate easy transmission of parasites from wild to farmed fish and vice versa thereby causing unforeseen consequences in sympatric wild fishes.

In confined conditions such as cages where the stocking density is very high, the resultant stress might act as conductive factor for pathogens to cause diseases. High stocking densities coupled with fluctuations in environmental conditions and/or stress can favor parasite proliferation leading to significant mortalities in net-cage-reared fishes.

As in other aquaculture systems, environmental factors such the temperature, salinity, dissolved oxygen, suspended particulate matters etc., are critical and any adverse changes in these parameters would make the fish susceptible to diseases. Similarly, crowding and handling stress and feed management also play a crucial role. However, as any other farming system, health management practices involving early detection of infection and prophylactic and therapeutic treatment are of paramount importance.

Conclusions

A total of 206 specimens of common carp from fish cage culture system on Tikvesh reservoir (N. Macedonia) were examined for parasitological investigations and infestation with parasite was determined in 121 specimens (58.74 %).

In common carp from this reservoir, the presence of 5 parasite species was established: Trichodina sp., Dactylogyrus extensus, Eudiplozoon nipponicum, Bothriocephalus opsariichthydis and Ergasilus sieboldi.

Many of the biosecurity measures which are employed in land based aquaculture systems will not have much relevance to cage farming, as the system is highly dynamic. However, a thorough understanding of pathogens, disease process, diagnosis, epidemiology and control measures are essential for better health management of farmed fishes in cages.

The treatment or control of parasitic diseases in fish can be achieved if the following parameters are addressed:

(1) Identification of the parasite;

(2) Obtaining knowledge about their life cycle;

(3) Awareness of the environmental needs of the parasite, such as host specificity, optimum temperature, pH, nutrition and other metabolic needs;

(4) Determining their geographical range;

(5) Determining the effect of host immunological mechanisms on the parasite;

(6) Learning the control and methods of diseases treatment caused by the relevant parasite.

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Chemical composition and nutritional value of Raspberry fruit (Rubus idaeus L.)

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Abstract. Raspberries fruits (Rubus idaeus L.) as important part of our diet are known for their sensory and nutritional characteristics, because of high content of essential nutrients that are beneficial for biological activity in human health. A high consumption of plant foods, such as raspberries, appears to decrease the risk of obesity, diabetes, heart disease, and overall mortality. It can also promote a healthy complexion, increased energy, and overall lower weight. Nutritional quality was analyzed through by chemical parameter of fresh raspberry samples from plantation in Kosovo area at the ripe stage. The following quality parameters were determined in the raspberry's fruits: pH, dry matter, total soluble solids (TSS/°Brix), total acidity (TA), TSS/TA ratio, total sugars, reducing sugars, sucrose, vitamin C, protein, and lipids. Results can be used to inform potential raspberry farmers about the nutritional qualities and consumer preferences for raspberries. The rich nutritional composition of the studied fruits makes them a very special diet. All statistical analysis was performed using the MS Excel program and SPSS 22.0 statistics software.

Keywords: Raspberry, nutrient quality, fruits

Introduction

Berry fruits, consumed fresh or processed, are nutritionally valuable food, as they are rich sources of minerals, vitamins, sugars, dietary fibers, organic acids, macro and micronutrients of pharmacological interest, and polyphenolic compounds. They have gained attention because of their potential for improving human health.

Raspberry fruits (*Rubus idaeus L.*) is an important berry crop for both the fresh and the processing market. Raspberry, is an important commercial product in fresh or processed form due to its nutritional, medicinal, and cosmetics uses [1],[2]. The term "nutraceutical" indicates a fortified food or a dietary supplement that is held to provide health or medical benefits in addition to its basic nutritional value.

Nutraceuticals are also called functional food. Raspberries fruits contain high concentrations of important nutrients, bioactive compounds and phytochemicals.Raspberry fruit is considered as a low energy fruit that is comprised primarily of natural carbohydrates, with the main sugar form being fructose, a feature that makes berry fruit very popular among consumers. Raspberry is also an excellent source of vitamin C. It is well known that vitamin C has health and wellness attributes that make berry fruit very popular among consumers. [2].

Raspberries fruits contribute to the nutritional value of a diet. Raspberries also contain phytochemical components with documented biological activity, many of which were initially investigated based on their in vitro antioxidant properties. Some of these compounds are now recognized for their ability to influence cell signaling pathways that affect receptors,

transporters, gene expression, and other cellular events. The package of nutrients and bioactive components that the raspberries deliver suggest their important protective role in human health [3].

Fruits and vegetables are a primary food source providing essential nutrients for sustaining life. They contain a variety of phytochemicals, such as phenolic acids and flavonoids, which have been associated with many health benefits. Detailed information about the health-promoting components of wild raspberries is needed to give a better insight into their use as functional foods and as ingredients in pharmaceuticals, nutraceuticals, and medicines [1].

Raspberry fruit is very interesting for consumers because of its pleasant aroma and color, low calories, and high nutrient value, with health benefits that manifest in the high amounts of antioxidants [4].

Raspberry (*Rubus idaeus L.*) are very popular fruit in Kosovo, cultivar of them is developed in Kosovo, where is commercially cultivated under several climatic conditions and cultivation modes. Raspberry production in Kosovo is experiencing a period of rapid growth. Currently, over 1,000 ha of raspberries are planted throughout Kosovo. The average raspberry yield in Kosovo is 9-12 tons per hectare. The main raspberry varieties are Polka with 75% of total production, Willamette with 10%, Meeker 10% and others 5%.

However, quality parameters and nutritional value have not been determined in raspberries fruits during ripening, especially in fruit grown under organic cultivation. Literature regarding to this raspberry cultivar is scarce. This is the first research of this type in Kosovo and it should give us a novel result. This study has been designed to investigate the nutritional quality of raspberry fruits with ripening time by using some parameters (dry matter, total soluble solids (TSS), pH, total acidity (TA), TSS/TA ratio, total sugars, reducing sugars, sucrose, vitamin C, protein, lipids.

Materials and methods

Materials

Fruits of raspberry (*Rubus idaeus L.*) from five raspberry cultivars, were grown at the different plantation located in the Kosovo and harvested at commercial maturity stage in the season 2019.Sampling was carried out on 3 trees growing in garden. Approximately 1 kg of fruits of uniform ripeness (red color) were harvested from each tree and transferred to laboratory at the day of harvest and stored overnight at

+4∘C.Analyses were carried out next day. Fruits were washed with demineralized water, and after removing surface water, air dried and then mashed using a kitchen blender to prepared for further analysis. Three replicates were used for analysis.

Chemical analysis

Nutritional properties

Determination of nutritional properties of raspberry fruits were performed according the standard methods of the AOAC [7]. Total soluble solids content (TSS) measured using Abbe refractometer calibrate against sucrose and expressed in ∘Brix. Titratable acidity (TA) was measured according to AOAC Method and expressed as milligrams of citric acid. The sugar-acid ratio of samples was determined by calculation as described below in equation: $Sugar-acid\ ratio = (TSS\ (°Brix))/(\%\ TA)$. pH was measured using pH/mv meter, and dry matter (DM) was measured in triplicate by drying 5 g of the fresh fruits at 105∘C until constant weight (4-6 hours). Determination of lipids was done by Soxhlet extraction after digestion of

the samples by hydrochloric acid hydrolysis, followed by extraction of the fats with petroleum ether. After the extraction, lipid content was determined by weighing. Protein was determined by the Bradford method with some modifications. Gelatin is commonly used to create the standard curve, and the absorption is measured at 545 nm in a spectrophotometer. Reducing sugar was determined using the method of Lane and Eynon and Fehling's solution as described by AOAC [7]. Total sugars were determined by the phenol-sulfuric acid method by Nielsen [8]. Glucose is commonly used to create the standard curve, and the absorption is measured at 490 nm. The sucrose mass fraction was determined by calculation from the difference between total and reduced sugars. Vitamin C content was estimated using spectrophotometric method with 2,4-dintrophenylhydrazine as an indicator [1],[2]. Freshly processed fruit (1 g) was homogenized in a mortar with a pestle with metaphosphoric acid (5% metaphosphoric acid in 10% acetic acid solution in water), filtered and treated with 85% sulphuric acid solution and 2,4-dintrophenylhydrazine, and then incubated at 60 ∘C for 60 min in a water bath. Absorbance was measured at 520 nm in a spectrophotometer (Genesys 10S UV-Visible,) for estimation of vitamin C in the fruits.

Statistical analysis

All data were expressed as the mean ± standard deviation of triplicate experiments. All statistical analysis performed using the MS Excel program and SPSS 22.0 statistics software Differences were tested for significance using the ANOVA procedure, with a significance level of $p < 0.05$.

Results and Discussion

The results of the chemical composition and nutritional components of the fresh raspberry fruits analyzed in period August 2019, in the five locations and cultivars are given in Tables 1-2 and figure 1.

Table 1. The chemical composition of the raspberry fruits

Sample	pH	W(DM)/%	TSS/∘Brix	TA/ %	TSS/TA ratio
A_1	3.74 0.2	9.03 0.1	9.00±0.1	0.91 0.1	9.89 0.1
A_2	3.80 0.2	8.47 0.1	11.00±0.1	0.86 0.1	12.79 0.2
A_3	3.81 0.1	8.96 0.1	11.00±0.1	0.89 0.2	12.35 0.2
A_4	3.70 0.1	9.04 0.1	9.00 0.1	0.95 0.2	9.47 0.2
A_5	3.65 0.1	8.44 0.1	10.00 0.1	0.80 0.2	12.50 0.2

Data are expressed as average value   standard deviation of three replicates

Table 2. The nutritional composition of the raspberry fruits

Sample	Vitamin C mg/100g	Total sugars g/100g	Reducing sugars g/100g	Sucrose g/100g	Lipids g/100g	Proteins g/100g
A_1	46.70 0.1	4.27 0.1	3.67 0.2	0.60 0.2	0.55 0.1	0.80 0.1
A_2	48.18±0.3	4.45 0.3	3.79 0.1	0.66 0.3	0.52 0.2	0.75 0.1
A_3	46.44 0.3	4.64 0.1	3.95 0.1	0.69 0.1	0.59 0.1	0.81 0.2
A_4	49.57 0.1	4.82 0.1	4.04 0.1	0.75 0.1	0.65 0.2	0.69 0.3

A5	50.57 0.1	4.32 0.1	3.54 0.1	0.78 0.1	0.58 0.2	0.72 0.3

Data are expressed as average value   standard deviation of three replicates

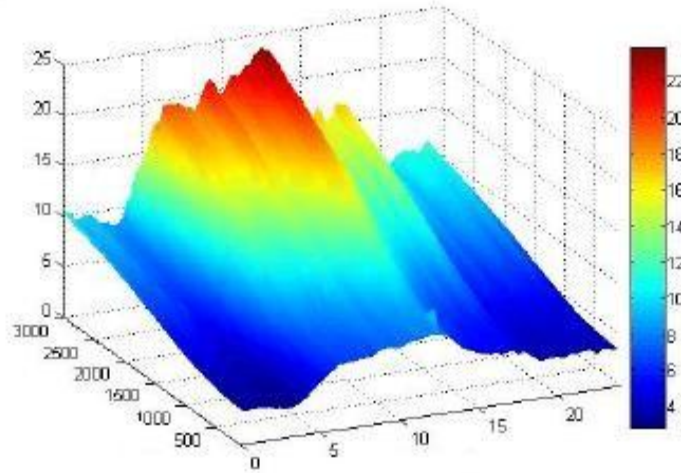Fig.1. The 3D visualization data of chemical parameters of strawberries fruits

pH values tend to be lower, ranging from 3.65±0.2 to 3.81±0.1, where the higher pH has A3 samples with 3.81 ± 0.1, these values are similar to many other authors studies, author reference [1]. The values for raspberry fruit of the total dry matter ranged from 8.44% ± 0.1 to 9.04% ± 0.1.

Soluble solids TSS/∘Brix content in fresh raspberries fruits ranged from 9.00 $\pm$ 0.1

°Brix to 11.00 $\pm$ 0.1°Brix, the values show very good amounts of soluble solids compared to the study of author reference [1]. Samples of raspberries contained about 0.80%± 0.2 to 0.95% ± 0.1 of citric acid.

Total acidity values for raspberries fruits were somewhat lower, which caused it's also lower TSS/TA ratio. The relationship between total soluble solids and total acidity is very important in determining fruit quality. In numerous researches conducted on different raspberry cultivars, the total soluble TSS/TA ratio was found to be very important, because it provides information on the balance of sugars and acids in the fruit. Raspberry fruits are a good source of vitamin C. Content of vitamin C in raspberries fresh fruit essentially different among the researched cultivars.

Samples of raspberries contained more vitamin C 46.44±0.1mg to the 50.57 ±
0.1mg/100g, where the highest amounts are samples A4 and A5. If we compare these results with other authors in most cases, we will see that the raspberry we have explored have average values compared to author reference [3]. From the above mentioned, the four raspberry varieties in our study might be considered as rich in vitamin C. The total number of sugars present in the raspberry fruit varied from 4.27
± 0.1g to 4.82±0.1g/100g. The comparing these fruits, we can infer that the raspberry fruit do not constitute itself as a good source of sugars, which are at a lower level than that reported by other authors on different cultivars from different parts of the world [1],[3].Protein values were estimated between 0.69 ± 0.1 g to 0.81 ± 0.2 g per 100 g raspberries fruits, which is lower with the report of authors reference [3]. Lipid contents is 0.52 ± 0.2g to 0.65 ± 0.2 g/100 g which is consistent with the report of authors reference [3]. The obtained results can be useful in

clarifyng the quality of fruits and traditional products in order to their promotion and application as food additive and nutraceutical.

Conclusion

The present study reveals that the Raspberries fruits (Rubus idaeus L.) grown and developed at the Kosovo region were rich in vitamin C, sugars and minerals. Their nutritional value and quality attributes make them suitable for consumption. Tested fruit presented a considerably high content of vitamin C and moderate to high content of sugars and low content of lipids and proteins, giving nutritional and health relevance to this fruit, and these raspberries could be a promising dietary supplement and cosmetic supplement to address the needs of vitamin C and nutrients.

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The gross margin of beef farms – the case of Kosovo and Albania

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Abstract. The aim of this paper was to analyze the economic efficiency of beef farms in Kosovo and Albania. It's a descriptive and quantitative survey and the random sampling technique was used to select the respondents, in Kosovo 35 farms, managing mainly Simmental breed; and in Albania 19 farms, mainly Holstein and crossbred beef breeds. Two methods of data analysis were used, namely: descriptive statistics, and gross margin analysis. Data on: meat production, farm expenses and returns, fodder production, and feed bought in the market for each farm were recorded during the first half of 2016. In Kosovo, the Gross Margin per Calf is 230.13 Euro, the price of meat sold 2.32 Euro/kg and the slaughtered weight 517,88 Kg; while in Albania these figures are 173.10 Euro, 2.6 Euro/kg and 277.89, respectively. It is a must that extension service to train the farmers for better: management of their farm, feeding system, fodder production, animal health etc.

Key words: gross margin per farm, income per farm, meat cost, fattening calves.

Introduction

Agriculture and rural development continue to play an essential role in the economy of Kosovo and Albania, being assessed as a motor of economic development. Both countries continue to be predominantly rural economies with 9.1 percent of the GDP generated by agriculture in Kosovo [10], and about 18% in Albania [23]. Agriculture is also the largest employing sector, accounting for it employs about 35% of the active force in Kosovo [18] and 40% in Albania [7].

The growth of livestock production is very important for the economic development of the two countries. The cattle sector is one of the most important sub-sectors in agriculture of both countries as it provides about 98% of milk and 60,4% of meat in Kosovo [6], and 85% of milk and 44.7% of meat in Albania [8].

Small-scale farming system is dominant for beef production, in both countries, and such farms continue to produce in the traditional way and market their animal origin products through informal channels. The number of cattle in Kosovo is approximately 260 000 of which 115 000 heads are slaughtered every year. While in Albania these numbers are 470 000 and 120 000 respectively. In Kosovo one farm family as average is managing 3.9 cattle and it is estimated that today there are about 91,200 livestock farms [1]1, while in Albania are managing 2.29 cattle [8].

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Beef production in both countries is a secondary activity and is focused on calf fattening that comes from dairy farms mainly oriented to milk production (mainly Holstein crossbred in Albania [4] and Simmental in Kosovo2.

Livestock production in both countries suffers from a low level of competitiveness, due to low production efficiency and high production costs, and producers are forced to accept low incomes by not complying with imported products [24], [4].

In Kosovo, most of the imported beef is coming from the imported live animals, mainly from Serbia, Croatia, Bulgaria, and Czech Republic [11], while in Albania most of it is imported as frozen meat from Latin America [12].

One way to compare enterprises that make use of the same resources on the property is by using the gross margins. For a farm enterprise the gross margin is one measure of profitability that is a useful aid to enterprise planning. The starting point for construction of cash flow budget and assessment of whole farm profitability can be the calculation of gross margin. Also it can be used to assist in assessing the opportunity to develop new farm enterprises. Gross margin profit is the difference between the annual gross income for that enterprise and the variable costs directly associated with the enterprise. The requirement into the future will be to maintain profitable farming systems in the face of ever increasing cost structures and production challenges. Improving our skill and knowledge of all aspects of our farm business will be the key to meeting the challenge. In farm business management, the focus is on getting the most from existing land and assets [19].

Standard gross margin is the barometer of efficiency at crop level allowing the comparison between various production targets in vegetal and animal sectors of the EU agriculture. Gross margin is proportionally influenced by gross product and reverse proportionally by variable cost [14].

"The advantage of gross margin is the fact that it allows the comparison, in terms of profitability, between various activities running in a farm" [16]. The gross margin is recognized as an important benchmark for success in determining competitive production capability, and is used in comparing enterprise across the EU within the Farm Accountancy Data Network [5]. While PwC (2011) concludes that margin analysis is a neglected measure in the company. Valuable knowledge can be gained by understanding what exactly is affecting your margin. Gross margin analysis for revenue management factors can be very helpful because analysis can determine your key issues. Once issues that negatively affect the gross margin are understood, measures can be taken to improve the situation.

The gross margin method began to be used in the early 1960's, in the United Kingdom, to analyze and plan the revenues from agricultural holdings [15]. The gross margin method is easy to use and that was the reason for its widespread use. This method comprises three phases for a given product: (i) gross income calculation, (ii) variable cost and its structure, and (iii) gross margin calculation per unit of surface or animal.

One of the major prerequisites for increasing beef production in the country and the number of calves for fattening is the farm profit [20]. Meat production is an important component of agricultural production as well as of the gross domestic product in general and furthermore it contributes to employment. Most studies [22], [21] emphasize that weight gain is the main factor for the farms fattening calves.

Profitability variability and economic efficiency of fattening operations are also heavily influenced by the price of calves slaughtered [20]. But on the other hand, the input costs, especially feeding costs, are the main factors affecting the cost of production [13]. To achieve success in competitive markets fattening calf prices should reflect changes in feed prices [25].

This paper aimed to make a comparison between the profitability of beef farms in Kosovo and Albania. In addition, the paper presents an analysis of beef production in various farms and also

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[2] From the visit and interviews of the 35 beef farms of the study conducted by the first author of this paper.

the main aspects of economic efficiency for increasing profitability and competitiveness in beef sector. For this purpose, the data were collected from farms of seven regions of Kosovo and six regions of Albania. They were processed according to the specific methodology for calculating the gross margin and profit.

The results provide some information that can help farmers who manage beef farms, as well as all stakeholders in the meat industry to improve economic performance.

Materials and Methods

This study, in both countries, was conducted to collect farm data pertaining to revenue and expenses on beef farms to make an economic analysis based on gross margin. The gross margin is calculated as the difference between total income and the variable cost. Variable cost includes the cost of:

- feed (from farm fodder production and feed bought in the market),
- labor (from family member and hired labor),
- veterinary service (including and insemination),
- water,
- electricity,
- transportation, and miscellaneous.

The random sampling techniques were used to select the respondents. In Kosovo were monitored and interviewed 35 beef farms, while in Albania were monitored 19 beef farms. In both countries the interviewed took place during the first half of 2016.

Data collection: In both countries, a structured questionnaire was used for collection of all information related to beef farming. In each country the questionnaires were discussed with a panel of three specialists, to verify its content and validity, as well was tested with three farmers, to avoid confounding questions and for clarity. Face-to-face interviews were conducted. According to the questionnaire the following data were recorded:

Daily body gain of calves in fattening; (ii) Production of farm meat; (iii) Quantity of meat sold; (iv) The price of meat sold; (v) Expenses on fodder products; (vi) Expenses for animal feed purchased on the market; (vii) Expenses for veterinary service; (viii) Expenses for electricity, water, travel, land rent, and fuel; (ix) Annual wage of workers; (x) Farm Income from meat sales (IpFmeat).

In addition to the incomes and expenditures (cited above), technical data has been collected, such as:
- type of animal feed used (including premix),
- the percentage of feed consumed compare with total expenses,
- the percentage of compound feed compare with total expenses.

Data analysis: For data analysis was developed a model in Microsoft Excel program, while the statistical data processing was done with Statgraphics Centurion XVI.

Results and Discussions

Kosovo

The Gross Margin per fattening calves is an important measure to determine how successful it has historically been to operate meat production from calves into fattening as an indicator of financial success and for the future.

Data on the number of fattening calves per farm, slaughter weight, calf weight at the begingn of fattening period, daily weight gain, fattening days, meat price sold, Income per Farrm (IpF) meat, variable cost per farm, Gross Margin per calf (GMpC), the cost of one kg of meat, the market sales ratio vs total beef production, the price of meat sold, the cost of feed to the variable cost and the cost of the concentrate to the cost of feed are summarized in Table 1 below:

Table 1: Technical data of beef farms in Kosovo

Country	No,of farms	No, of fattening calves per farm	Slaughtered weight (kg/head)	Weight at the beginning of fattening (kg/head)	Daily body gain (kg)
Kosovo	35	17,38	517,88	135,29	1,301

Country	Days in fattening	Meat sold vs meat produced (%)	Price of meat sold (€/kg)	IpF meat (€)	Variable cost per farm (€)
Kosovo	294	100	2,32	24792	19 920

Country	Gross margin per calf (€)	Meat Cost (€/kg)	Feed expenses vs variable cost (%)	Concentrate feed vs feed feed (%)
Kosovo	230.13	1,99	43.42	55.65

From the processing of the data obtained by the questionnaire, it appears that on farms with 1-10 fattening calves there are significant differences between farms that breed 1-10 fattening calve and those with over 11 heads:

- Farms are managing 5-120 heads of fattening calves with an average of 17,38 heads.
- Small farms (30% of them) have an average loss of € 152.3 for calves (from € 64 to € 289 for calves), while in medium farms only 20% of them come with an average loss of € 155.7 per calf (€ 37.1- € 374.9 per calf);
- The initial weight of calves ranges from 50 kg up to 320 kg of
- Specialized fattening farms which mainly buys the calves from the import. The live weight at the end of fattening period ranges from 200 to 800 kg. Most small farms sell the calves when reach the weight of 200-650 kg, while large farms sell them when are 500-750 kg.
- The calves in this study have reached the slaughtering weight in 180-540 days, with a daily gain variation of 638 to 2080 gr/day/calf. While the sales price of meat is € 2.2- € 3.0 Euro/kg of live weight.
- In the large farms the daily gain is 11.7% higher than in small farms or 1454 g/calf/ day versus 1240 g / calf / day.
- The sales price is 6.2% higher in large farms (2.42 €/kg compared to 2.27 €/kg in small farms).
- IpCalf has a variation from € 515 Euro to € 1913 Euro, while GM for a calf varies from -374.9 Euro to 970.5 Euro.
- The cost per 1 kg of living weight varies from € 1.66 to € 2.82, but the cost of small farms is 12.45% higher than large farms or (€ 2.058 vs € 1.83).
- The cost of feed to variable costs varies from 25.0 to 77.6%. Large farms have an indicator of 43.13% while small farms 43.55%.
- The cost of concentrate feed on the cost of feed varies from 21.7 percent to 78.1 percent, where the cost of small farms is 57.2 % and the large farms 52.3%.

- Small farms (13%) have benefited on average per farm € 404 (for animal feed) from MAFRD subsidies, while 47% of medium-sized farms have benefited on average € 7868 (for investment).
- Small farms (35%) receive advice from the public extension service, while for the medium farms this figure is 47%, which also affects the best breeding of fattening calves.
- Small farms (70%) buy calves from bazaar without knowing their origins, while medium farms buy from the farms known to them, and this is one of the reasons they have better calf daily body gain.
- Small farms buy calves in a weight much less (80%) than large farms, which in some cases have not completed the breeding season.

Albania

The technical data for the beef farms in Albania are summarized in Table 2 below:

Table 2: Technical data of beef farms in Albania

Country	No,of farms	No,of fattening calves per farm	Slaughtered weight (kg/head)	Weight at the beginning of fattening (kg/head)	Daily body gain (kg)
Albania	19	47,63	277,89	111,84	0,870

Country	Days in fattening	Meat sold vs meat produced (%)	Price of meat sold (€/kg)	IpF meat (€)	Variable cost per farm (€)
Albania	188,16	100	2,60	35 961	27 716

Country	Gross margin per calf (€)	Meat Cost (€/kg)	Feed expenses vs variable cost (%)	Concentrate feed vs feed feed (%)
Albania	173,10	2,19	63,4	53,02

Variation in the number of heads for fattening calf farms is very large, ranging from 6 heads to 400 heads, however, farms with 15 to 25 calves dominate.

- Large farms manage 87.1 heads of fattening calves, while small farms only 12.1 heads.
- The initial weight of calves ranges from 35 kg (farms to fattening their calves) up to 230 kg of specialized fattening farms which mainly buys the calves from the import. The live weight at the end of fattening period ranges from 200 to 450 kg. Most small farms sell the calves when reach the weight of 200-220 kg, while large farms sell them when are 350-450 kg.
- The calves in this study have reached the slaughtering weight in 70-300 days, with a daily gain variation of 571.4 to 1 200 gr/day/calf. While the sales price of meat is 2.17-3.07 Euro/kg of live weight.
- In the large farms the daily gain is 6.7% higher than in small farms or 903.4g/calf/ day versus 847.0g / calf / day.

- The sales price is 2.3 % higher in small farms (2.65 €/kg compared to € 2.59 in large farms) but this is because part of them sell their own meat not in the regular and approved markets. The variable farm cost varies from € 1 087.6 to € 239 103.7.
- IpCalf has a variation from € 178.7 to € 1448.9, while GM for a calf varies from € -5.65 Euro to € 751.5.
- The cost per 1 kg of living weight varies from € 1.66 to € 2.82, but the cost of small farms is 1.41% higher than large farms or (€ 2.16 vs € 2.13).
- The cost of feed to variable costs varies from 55.4 to 71.3%. Large farms have an indicator of 64.64% while small farms 63.19%.
- The cost of concentrate feed on the cost of feed varies from 42.8% to 65.7%, where the farms with most calves having the highest percentage of the report because they use more concentrate feed that is purchased (and has a price high), compared to farms with up to 20 calves that use less concentrates and a portion of maize is own production.

Several researchers [13], [20] report large variation in relation to the cost of feed at variable cost (43.24-85.9%). Of all the cost items, the highest standard deviation was for the feed cost, which indicates a high variability and the opportunity for optimizing and reducing these costs.

The challenge for beef farmers is to select the feeding system which provides adequate nutrition for the beef production system, while minimizing both fixed and variable costs. Producing and utilizing home-grown feed crops at low cost requires very good levels of management to ensure a high yield of highly digestible herbage is achieved [2], [24], [4].

The higher the price you get for your livestock, the greater the income - but not necessarily the profit. Successful producers aim to improve product quality (red meat) and evaluate selling options to maximize the price received, or to minimize price fluctuations. However, producers have a much greater chance of improving profitability by managing the quantity of product produced and controlling cost structures [3].

The Statgraphics Centurion XVI program was used for statistical data processing for the indicators listed below:
- IpF meat vs the number of calf in fattening;
- Meat cost (€) vs weight gain;
- The cost slaughtered weight (carcass).
The results are as follows:
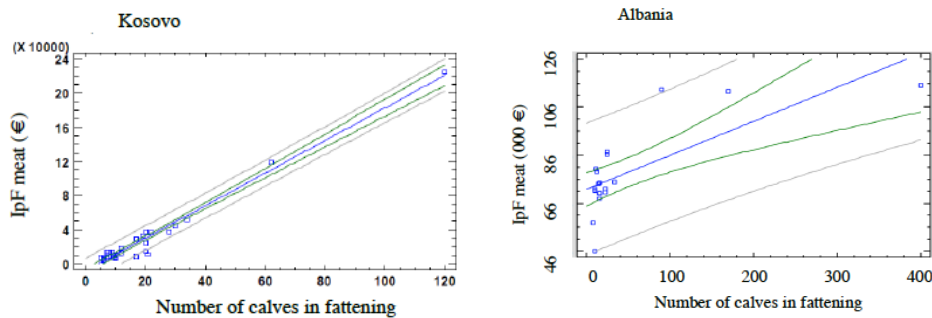
Fig. 1. IpF meat vs Number of fattening calves. There are differences between the IpF meat and the correlation coefficient in the farms of both countries.

Kosovo: IpF meat = -8382.64+ 1909.78 * Heads of calves in fattening. The correlation coeficient is equal with 0.985676, showing a strong relation between variables.

Albania: IpF meat= 11556.953+576.622*fattening calves. The correlation coeficient is equal with 0.6738 showing a relatively strong relation between variables. Since the P-value in the

ANOVA table, for both cases is less than 0.05, there is a statistically significant relationship between IpF meat and number of calves in fattening at the 95.0% confidence level.

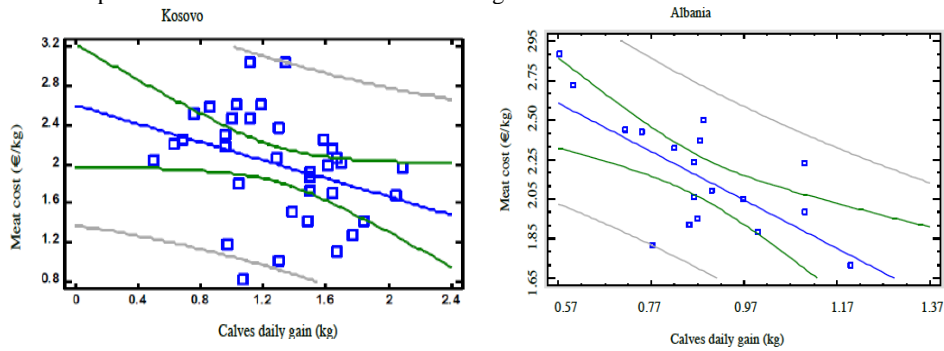Fig. 2. Meat cost (€) vs Calves daily gain. There are differences between the Meat cost (€) in relation with the calves daily gain and the correlation coefficient in the farms of both countries.
Kosovo: Meat cost (€) = 2.596 - 0.466*calf daily gain (Kg). The correlation coefficient equals -0.3406, indicating a relatively weak relationship between the variables.
Albania: Meat cost (€)= 3.656-1.117*calves daily gain (Kg). The correlation coefficient equals -0.1512, indicating a negative relatively weak relationship between the variables. Since the P-value in the ANOVA table, for both cases is less than 0.05, there is a statistically significant relationship between meat cost and calves daily gain at the 95.0% confidence level.
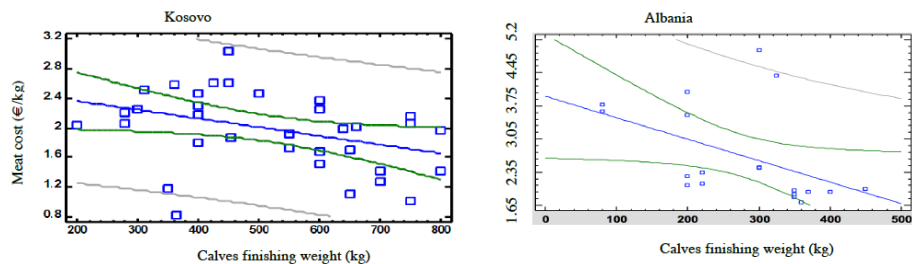
Fig. 3. Meat cost (€) vs Calves finnishing weight (kg). There are differences between the Meat cost (€) in relation with the calves finishing weight in both countries, while the correlation coefficient is in the same level.
Kosovo: Meat cost (€)= 2.598 - 0.001*calf finishing weight. The correlation coefficient equals -0.3626, indicating a relatively negative weak relationship between the variables.
Albania: Meat cost (€)= 2.596 - 0.466*calf daily gain (Kg). The correlation coefficient equals -0.3943, indicating a relatively weak relationship between the variables. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between meat cost and calves finishing weight at the 95.0% confidence level.

Conclusions

Kosovo and Albania are not sufficient in beef production and will take time to increase the production near the needs of consumers.

The public and private extension agents should find ways to make the farmers aware of the relative importance of all their financial inputs, in terms of their contribution to the cost of production per kilogram of meat produced on the farm. In addition, the extension task is to train farmers for improving the management of production as it is still the decisive factor in profitability

In Kosovo, the Gross Margin per Calf is € 230.13, the price of meat sold € 2.32 per kg and the slaughtered weight 517,88 Kg; while in Albania these figures are € 173.10, € 2.6 per kg and 277.89, respectively.

Several farms in both countries have negative Gross Margin and is a must for extension service to train farmers to keep the financial record per each crop and production. The extension service needs to train the farmers for better: management of their farm, feeding system, fodder production, animal health etc.

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Determination of Total Bacteria Count, Somatic Cells and Physico - Chemical Parameters of Raw Milk in Peja, Gjakova and Prizreni Region

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Abstract. The aim of our research was to determine the quality of raw cow's milk in three regions of Kosova through total bacteria count (TBC), somatic cell count (SCC) and physico-chemical parameters. A total of 120 samples were collected from farms during the period January – June 2019. Microbiological evaluation was conducted based on the definition of total bacterial count and somatic cell count. Also, physico-chemical parameters tested include fat, protein, lactose, Solid Non Fat (SNF), total solids content (TS), freezing point depression (FPD), density and acidity oSH. The obtained results showed that TBC count was: in Peja region 21/120, in Gjakova region 61/120 and in Prizreni region 41/120 samples belonged to the III class. The SCC count was: in Peja region 54/120, in Gjakova region 45/120 and in Prizreni region 62/120 samples belonged to the III class. In positive samples were also found changes of the physical-chemical parameters.
Keywords: raw cow's milk, TBC, SCC, physic-chemical parameters

Introduction

Dukagjini Plain is a flat part of the western region of Kosovo, characterized by a milder climate and different agricultural crops, while pastures and forests are found in the lake plains. Therefore it is important to investigate milk composition and microbiological characteristics in order to know the raw milk quality. Milk composition varies according to factors such as breed, age, mammary gland health, lactation stage, nutritional management and season [7]. Mastitis, particularly the subclinical type, is one of the most persistent and widely spread disease conditions of importance to milk hygiene and quality among dairy cattle worldwide [4]. Milk is a complex biological fluid and by its nature and a very favorable product for microbial growth, especially bacterial pathogens [2]. Depending on which manipulation milk undergoes, biological and physico-chemical properties of milk can slightly vary from the activity of microorganisms. Thus, the number of bacteria in milk directly influences the quality and safety of dairy products [3]. The control of total quality of milk is an essential component of the dairy sector and should be performed for a few reasons. Firstly, it helps farmers, collectors, processors and others involved in the milk productive chain to determine the condition of the quality as well as the weak spots in their ongoing activities. Secondly, it helps agencies involved in monitoring the quality of milk to meet consumer expectations for a final product of high quality, with high security and biological values [1]. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass [5]. This study was undertaken to examine quality of raw cow's milk in three regions of Kosova through total bacteria count (TBC), somatic cell count (SCC) and physico-chemical parameters.

Materials and methods

Sampling

In this study 120 raw cow's milk samples were collected from the three regions of Kosova - Peja, Gjakova and Prizren. Samples were collected from the farms during the period January – June 2019. All the laboratory tests of this work were carried out in the laboratory of Food and Veterinary Agency (FVA) during the research period. 40 ml of raw milk was collected using clean sterile glass bottles with azidol. The samples were put in an ice box in $2 – 4^oC$ and delivered to the FVA in Prishtina.

Total bacterial count (TBC)

Total bacterial count was determined as described by ISO 21187:2004 IDF 100B-1991:200. This method was used for total bacterial counts and individual bacteria count in raw milk, using BactoScan TM- Foss's apparatus, based on FC+ (flow cytometry) as the key for accurate bacterial counts, and categorization of bacterial cells. Every passing cell is registered by photo electronics attached to the microscope. BactoScan™ FC+ measures Individual Bacteria Count and displays results in IBC, but their results are presented also in Colony Forming Units (CFU), which can be obtained by a plate count method. 40 ml of fresh milk is placed in plastic bottles and stored at $2-4^oC$. Manually are mixed for 1 min. Samples are placed in Bactoscan strips and the apparatus receives 4.5 ml of sample for analysis. The results are automatically output to the software for 9 min. (6). The evaluation of the quality of laboratory tests is based on these standards:
$\leq$ 80,000 EXTRA class; $\leq$ 100.000 class I; $\leq$ 300.000 class II; $\geq$ 300.000 class III

Somatic cell count (SCC)

For the counting of somatic cells was used Fluorescence Optical Electronic Method (Fossomatic Minor). 40 ml of fresh milk is placed in plastic bottles at $38-40^oC$ for 7 min. It is manually stirred for 1 min and placed manually in the Fossomatic Minor pipette. The apparatus automatically receives 1 ml of sample for analysis. The results are automatically output to the software.

Physico-chemical analysis

MilkoScan FT 120 is a device that measures physico-chemical parameters. This device works on the principle of infrared spectrophotometry according to ISO 9622 standard and analyses were performed in Food and Veterinary Agency of Kosova. The parameters tested with this MilkoScan FT 120 are: milk fat, milk protein, lactose, non-fat solids, and total solids, freezing point, density and acidity.

Results and Discussion

In this study we aimed to investigate the microbiological quality of 120 raw milk samples, collecting during the period January – June 2019. We determined that 61/120 milk samples had values above 300,000 cfu/ml - III class in Gjakova region, 41/120 samples in Prizreni region and 21/120 samples in Peja region. These results are presented in figure 1.

Figure 1. TBC evaluation in milk samples for certain region, during the period January – June 2019

The average number of TBC count over the period January - June 2019 for the III class with over 300,000 cfu / ml was higher in Gjakova region with average value 10.2 and with lower values in Prizren region with average values 6.8. The average value results are presented in figure 2.
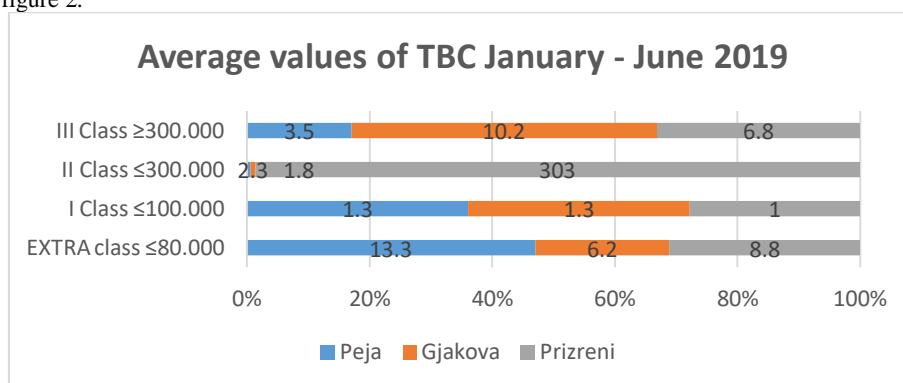
Figure 2. Average values of TBC evaluation in certain region, during the period January – June 2019

In figure 3 are presented the results of somatic cell count during the period January – June 2019. The results indicate that the higher number of SCC (III class ≥500.000 SC/ml) were in Prizreni region with 62/120samples, 54/120 samples in Peja region and the smallest number 45/120 samples in Gjakova region. This results indicate lower scale of mastitis in Gjakova region.
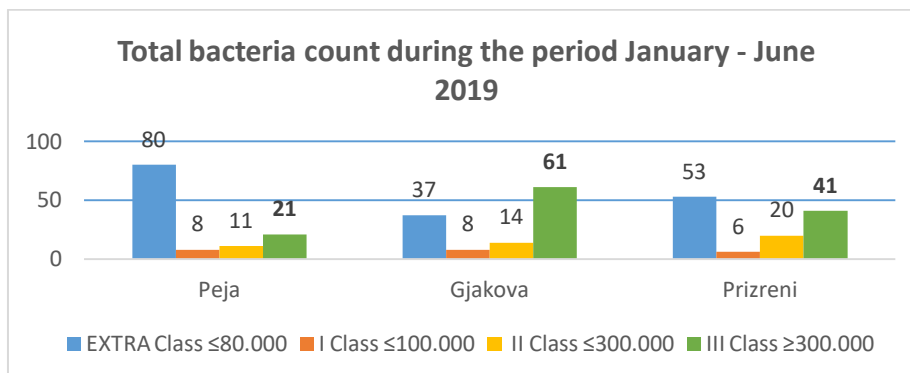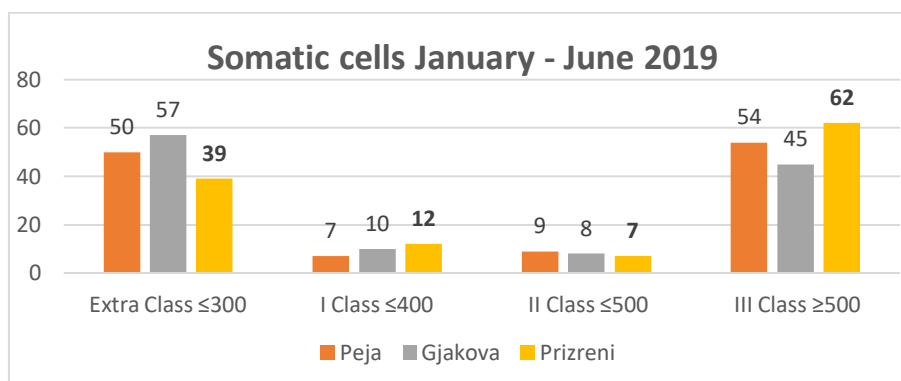
Figure 3. SCC evaluation in milk samples for certain region, during the period January – June 2019.

The average number of SCC over the period January - June 2019 for the III class ≥ 500.000 SC/ml was higher in Prizreni region with average value 10.3, with average value 9.2 in Peja region and with lower average value 7.5 in Gjakova region. These results are presented in figure 4.
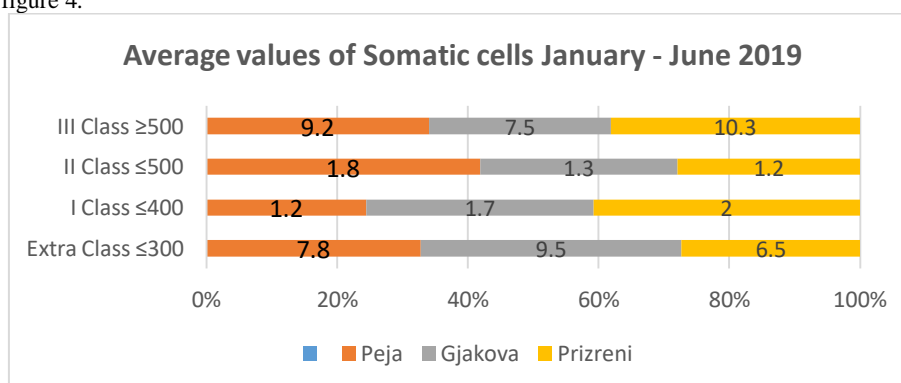
Figure 4. Average values of SCC for certain region, during the period January – June 2019.

The following tables will present the physico-chemical data and the correlations between these parameters in the three regions included in this study. Results were expressed as average values of physico-chemical parameters. Generally the changes in physico-chemical parameters and the correlations between them are in accordance with changes in microbiological parameters. Table 1 shows correlation coefficient between physico-chemical parameters in Peja region. High correlation were between Fat (3.2%) and TS (8.5), Protein with acidity. There is also a high correlation between Lactose and SNF, and SNF and density. High correlation was and between TS and acitidty.

Table 1. Pearson correlation coefficient between Physico – chemical parameters in Peja region

Peja region January - June 2019	Fat 3.2%	Protein 3.0 %	Lactose	SNF	TS 8.5	FPD -0,517oC	Density 1.028-1.0328	Acidity 6.5 - 7.8 *SH

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	Fat 3.2%	Protein 3.0 %	Lactose	SNF	TS 8.5	FPD -0,517oC	Density 1.028-1.0328	Acidity 6.5 - 7.8 *SH
Fat 3.2%	1							
Protein 3.0 %	0.307	1						
Lactose	-0.429	-0.547	1					
SNF	-0.190	0.310	0.626	1				
TS 8.5	0.928	0.485	-0.246	0.183	1			
FPD -0,517oC	-0.737	-0.736	0.279	-0.378	-0.903	1		
Density 1.028-1.0328	-0.547	0.246	0.587	0.892	-0.203	-0.087	1	
Acidity 6.5 - 7.8 *SH	-0.551	0.831	-0.508	0.203	0.781	-0.894	0.049	1

Table 2. Average values of physico-chemical parameters during period January - June 2019 in Peja region

Physico-chemical parameters January - June 2019 in Peja region	Fat 3.2%	Protein 3.0 %	Lactose	SNF	TS 8.5	FPD -0,517oC	Density 1.028-1.0328	Acidity 6.5 - 7.8 *SH
January	4.1	3.4	4.4	8.8	12.9	-0.7	1031.1	9.1
February	3.6	3.3	4.6	9.0	12.6	-0.6	1031.8	7.8
March	3.5	3.2	4.5	8.7	12.3	-0.6	1031.2	7.4
April	3.8	3.2	4.5	8.8	12.6	-0.6	1031.0	7.4
May	3.8	3.3	4.6	8.9	12.7	-0.6	1031.6	8.1
June	3.9	3.2	4.6	8.8	12.7	-0.6	1030.9	7.6
Minimale value	3.548	3.171	4.397	8.7295	12.311	-0.6523	1030.9	7.37
Maximale value	4.074	3.3905	4.6275	8.9525	12.922	-0.57765	1031.8	9.1005
Average	3.8	3.27	4.5	8.8	12.6	-0.6	1031.2	7.9
Standard Deviation	0.22	0.09	0.10	0.09	0.25	0.03	0.37	0.72

In table 3 is presented correlation coefficient between physico-chemical parameters in Gjakova region. High correlation were between Fat (3.2%) and TS (8.5) and Fat and Acidity. There is also a high correlation between Protein with TS and acidity. High correlation was and between lactose and SNF and SNF with density and TS with Density and Acidity.

Table 3. Pearson correlation coefficient between Physico – chemical parameters in Gjakova region

Gjakova region January - June 2019	Fat 3.2%	Protein 3.0 %	Lactose	SNF	TS 8.5	FPD -0,517oC	Density 1.028-1.0328	Acidity 6.5 - 7.8 *SH
Fat 3.2%	1							
Protein 3.0 %	0.592	1						
Lactose	-0.452	0.088	1					
SNF	0.208	0.828	0.631	1	1			

	Fat 3.2%	Protein 3.0 %	Lactose	SNF	TS 8.5	FPD -0,517oC	Density 1.028-1.0328	Acidity 6.5 - 7.8 *SH
TS 8.5	0.807	0.914	0.058	0.745	-0.842			
FPD -0,517oC	-0.366	-0.895	-0.498	-0.978	0.597	1		
Density 1.028-1.0328	0.018	0.755	0.665	0.962	0.967	-0.933	1	
Acidity 6.5 - 7.8 *SH	0.859	0.870	0.006	0.682	0.967	-0.810	0.562	1

Table 4. Average values of physico-chemical parameters during the period January - June 2019 in Gjakova region.

Physico-chemical parameters January - June 2019 in Gjakova region	Fat 3.2%	Protein 3.0 %	Lactose	SNF	TS 8.5	FPD -0,517oC	Density 1.028-1.0328	Acidity 6.5 - 7.8 *SH
January	4.1	3.5	4.3	8.8	12.9	-0.6	1030.9	8.3
February	3.6	3.4	4.6	9.1	12.7	-0.6	1032.3	7.9
March	3.6	3.2	4.4	8.5	12.2	-0.6	1030.0	7.3
April	3.7	3.1	4.5	8.6	12.3	-0.6	1030.3	7.3
May	3.9	3.3	4.5	8.8	12.8	-0.6	1030.8	8.1
June	3.6	3.1	4.5	8.6	12.3	-0.6	1030.6	7.6
Minimale value	3.6	3.134	4.3095	8.5285	12.168	-0.62185	1029.95	7.2535
Maximale value	4.104	3.469	4.6295	9.1025	12.9495	-0.5761	1032.3	8.3375
Average	3.8	3.27	4.5	8.7	12.5	-0.6	1030.8	7.8
Standard Deviation	0.21	0.14	0.13	0.24	0.32	0.02	0.97	0.44

In Prizreni region correlation coefficient were with high values between Fat with Lactose, TS and acidity, Lactose with SNF, and SNF with TS. These results are presented in table 5.

Table 5. Pearson correlation coefficient between Physico – chemical parameters in Prizreni region

Prizreni region January - June 2019	Fat 3.2%	Protein 3.0 %	Lactose	SNF	TS 8.5	FPD -0,517oC	Density 1.028-1.0328	Acidity 6.5 - 7.8 *SH
Fat 3.2%	1							
Protein 3.0 %	0.519	1						
Lactose	0.343	-0.281	1					
SNF	0.664	0.360	0.794	1				
TS 8.5	0.971	0.553	0.481	0.819	1			
FPD -0,517oC	-0.318	-0.605	0.162	-0.221	-0.338	1		
Density 1.028-1.0328	-0.055	0.299	-0.184	0.015	-0.018	0.513	1	
Acidity 6.5 - 7.8 *SH	0.891	0.217	-0.840	-0.680	-0.266	-0.449	-0.332	1

Table 6. Average values of physico-chemical parameters during the period January - June 2019 in Prizreni region

Physico-chemical parameters January - June 2019 in Prizreni region	Fat 3.2%	Protein 3.0 %	Lactose	SNF	TS 8.5	FPD -0,517oC	Density 1.028-1.0328	Acidity 6.5 - 7.8 *SH
January	3.8	3.4	4.2	8.6	12.5	-0.6	1031.1	8.7
February	4.0	3.4	4.5	8.9	12.9	-0.6	1030.8	8.1
March	4.1	3.4	4.6	9.0	13.1	-0.6	1031.3	7.4
April	3.7	3.2	4.5	8.8	12.5	-0.6	1031.0	7.6
May	4.3	3.3	4.6	8.9	13.2	-0.6	1030.8	8.0
June	3.8	3.2	4.5	8.8	12.6	-0.6	1030.8	7.9
Minimale value	3.6565	3.2195	4.242	8.6125	12.46	-0.6172	1030.8	7.3585
Maximale value	4.3005	3.408	4.565	8.976	13.2025	-0.597	1031.25	8.701
Average	3.9	3.32	4.5	8.8	12.8	-0.6	1031.0	7.9
Standard Deviation	0.26	0.08	0.14	0.15	0.30	0.01	0.19	0.55

Conclusions

- The study showed that the total bacteria count belonging to the third class of classification with ≥300.000 cfu/ml was higher in the Gjakova region with 61 samples.
- The number of SCC was higher in Prizreni region with 62 samples which belongs to third class of classification with ≥500.000 SC/ml, which may be an indicator of mastitical diseases of milking cows.
- The studied samples show a correlation between physico-chemical parameters which is in line with the increase in total bacterial count and somatic cell count.
- More attention should be focused in the health care of milking cows, appropriate handling with milk, transportation at low temperatures from farms and collecting points to the dairies, in order to avoid contamination of milk with causative agents of infection as a risk to population health.

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STATISTICAL PROCESS CONTROL OF THE WORT FOR BEER PRODUCTION OF "PEJA BEER"

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Abstract. This study evaluates the process of production wort for beer production of "Peja Beer". Samples of wort were taken for the period of ten days for measurable characteristics: original extract, pH and color. The process was conducted under real production conditions, where every four hours 315 hl of wort was produced. The Shewhart Control Graph for Individual Measurements was taken to determine if the process was with respect to each quality characteristic statistically under control. Based on the obtained results, we came to the conclusions that the process was under control. The upper and lower control limit for the original extract and pH were within the specifications while the color must be corrected.

Keywords: Wort, beer, pH, original extract, color, control graphs

Introduction

Wort Production

Beer is a fermented aqueous drink based on starch and flavoured by hops. The basic ingredients of beer are water; starch source such as malted barley able to be fermented (converted into alcohol): a brewer's yeast to produce the fermentation; and a flavouring, such as hops, to offset the sweetness of the malt.[9]

The fundamental processes of brewing are malting, mashing, lautering, boiling, fermentation, maturation, filtration and packaging.

Malting is the process where barley grain is made ready for brewing, converting barley to malt. Malting is broken down into three steps in order to help to release the starches in the barley. First, during steeping, the grain is added to a vat with water and allowed to soak for approximately 40 hours. During germination, the grain is spread out on the floor of the germination room for around 5 days.[5]

The final part of malting is kilning when the malt goes through a very high temperature drying in a kiln; with gradual temperature increase over several hours. When kilning is complete, the grains are now termed malt, and they will be milled or crushed to break apart the kernels and expose the cotyledon, which contains the majority of the carbohydrates and sugars; this makes it easier to extract the sugars during mashing. [7]

Mashing is the process in which malt grist, solid adjuncts, and water are mixed together at a suitable temperature for the malt enzymes to convert the various cereal components into fermentable sugars and other nutrients. Mashingconverts the starches released during the malting stage into sugars that can be fermented. The milled grain is mixed with hot water in a

large vessel known as a mash tun. In this vessel, the grain and water are mixed together to create a cereal mash. During the mash, naturally occurring enzymes present in the malt convert the starches (long chain carbohydrates) in the grain into smaller molecules or simple sugars (mono-, di-, and tri-saccharides). The principal enzymes responsible for starch conversion are β and α-amylases but also proteases, leading to a mixture of sugars and peptides or amino acids, producing wort of the desired composition.[3],[6]

This conversion is called saccharification which occurs between the temperatures 60-70oC. The result of the mashing process is a sugar-rich liquid or "wort", which is then strained through the bottom of the mash tun in a process known as lautering. Prior to lautering, the mash temperature may be raised to about 75–78°C (known as a mashout) to free up more starch and reduce mash viscosity. Additional water may be sprinkled on the grains to extract additional sugars (a process known as sparging).

After mashing, when all the starch has been broken down, it is necessary to separate the liquid extract (the wort) from the solids. Wort separation is important because the solids contain large amounts of protein, fatty material, silicates and polyphenols (tannins). This insoluble, undegraded part of the malted barley grain is allowed to settle to form a bed in the mash tun and the sweet wort is filtered through it (lautering). The filtered wort is used as the fermentation medium to produce beer. The residual solid fraction obtained after this stage is known as brewer's spent grains.[3]

Following extraction of the carbohydrates, proteins, and yeast nutrients from the mash, the clear wort must be conditioned by boiling in the kettle. After filtration, the wort is transferred to the brewing kettle, where it is boiled during at least one hour with the addition of hops. Boiling is needed to isomerize the hop alpha acids, to strip out unwanted malt and hop volatiles, to denature proteins and coagulate proteins/polyphenols as hot break, and to fix the wort composition by terminating all enzymic and microbiological activity surviving the mashing process. The purpose of wort boiling is to stabilize the wort and extract the desirable components from the hops, which will confer typical beer qualities, such as bitter taste, flavor, and foam stability. At the end of the boiling period, the hopped wort is transferred to a vessel known as a whirlpool, where further separation of hop residues (spent hops) and the trubaceous matter (hot break) takes place.[3],[6],[8] The hop residues, which are then useless, are dumped directly as being of no further value.

After boiling and clarification, the wort leaving the whirlpool has to be cooled in preparation for the addition of yeast and subsequent fermentation. Wort is usually cooled through plate heat exchangers. The principal changes that occur during wort cooling are as follow: cooling the wort to yeast pitching temperature, formation and separation of cold break and oxygenation of the wort to support yeast growth.

Shewhart Individuals Control Chart

Individuals control charts are statistical tools used to evaluate the central tendency of process over time. Individuals control charts are used when it is not feasible to use averages for process control. Control charts for individuals are often used to monitor batch processes, such as chemical processes, where the within-batch variation is so small relative to between-batch variation that the control limits on standard X chart would be too close together. Range charts are used in conjunction with individuals charts to help monitor dispersion.[10]

Points outside of these control limits are signals indicating that the process is not operating as consistently as possible; that some assignable cause has resulted in a change in the process.Similarly, runs of points on one side of the average line should also be interpreted as a signal of some change in the process. When such signals exist, action should be taken to identify and eliminate them.[11]

Calculation of moving range
The difference between data point, x_i, and its predecessor, x_{i-1}, is calculated as $MR_i = |x_i - x_{i-1}|$.

For "m" individual values, there are m-1 ranges.

Next, the arithmetic mean of these values is calculated as $\overline{MR} = \frac{MR_1 + MR_2 + \cdots + MR_{m-1} + MR_m}{m-1}$

If the data are normally distributed with standard deviation σ then the expected value of $\overline{MR}$ is d2σ = 2σ/√π.[11]

Calculation of moving range control limit

The upper control limit for the range (or upper range limit) is calculated by multiplying the average of the moving range by 3.267:

UCLr = 3.267 $\overline{MR}$ (**1.1**)

The value 3.267 is taken from the sample size-specific D4 anti-biasing constant for n=2, as given in most textbooks on statistical process control.[4]

Calculation of individuals control limits

First, the average of the individual values is calculated:

$$\bar{x} = \frac{x_1 + x_2 + \cdots + x_{n-1} + x_m}{m} \qquad (\mathbf{1.2})$$

Next, the upper control limit (UCL) and lower control limit (LCL) for the individual values (or upper and lower natural process limits) are calculated by adding or subtracting 2.66 times the average moving range to the process average:

UCL=$\bar{x}$ + 2.66$\overline{MR}$ (**1.3**)

UCL=$\bar{x}$– 2.66$\overline{MR}$ (**1.4**)

The value 2.66 is obtained by dividing 3 by the sample size-specific d_2 anti-biasing constant for n=2, as given in most textbooks on statistical process control.[4]

Materials and Methods

The statistical process control of the wort for beer production was performed at Beer Factory "Peja Beer". The chemical analyses were done at the Factory Laboratory.

Raw Materials

Water for the mush production is used from the spring of White Drini.

For mush production was used malted barley produced from Scarlet type two row barley in Croatia Nova Gradishka.

Lupulo (Humulus Lupolus): Germany – CO2– Hop Extract Hallertauer magnum, Hop Styrian Aurora and Hallertau Perle PELET TYPE 90, Slovenia.

Mash Preparation

For mash production was used 100% malted barley. Malt is milled with conditioning milling in ratio of: malt with water, 1: 3.

Grinding of malt was done at 45ºC. The process of mashing production is done by infusion, figure 2.1. The breaks and temperatures used for Phytase was 45ºC, Protein rest 52ºC, Maltose rest 63ºC and Saccharification rest 72ºC. The whole mass was then heated to 76 °C and held for 10 minutes. At the end of the heat treatment, all the mass is transferred to the drain to remove the filtrate. After withdrawing the whole mush and removing the trub, the obtained mush was

boiled for 60 minutes at 95 °C, with addition of hops in three doses. The first dose of hops was of 50%, the second dose of 30% and the third dose of 20%.
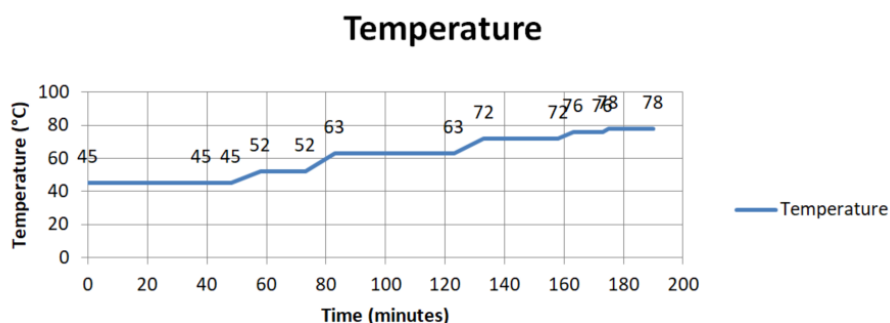

Fig. 2.1. Temperature vs Time in infusion method

Methods of Analysis

Original Extract

The determination of original extract was made according to the method (EBC Metod: 8.5).
By Beer-analyzer was determined the wort original extract expressed in percentage (%).
The following devices were used: Alkoolizerbeer, Anton Paar DMA 4500, densitymeter, Sp-1m Sample changer (Anton Paar GmbH, Germany) and cuvette.
In the beer-analyzer, the cuvette were placed in the following order: the first cuvette with distilled water, the second cuvette with alcohol, the third cuvette with mush, the fourth cuvette with NaOH and the other four cuvette are filled with distilled hot water at 60 °C, while the number of cuvette were taken according to the number of samples we analyzed.
Wort samples were filtered through filter paper. The filtered wort was poured into the cuvette, placed in a beer analyzer where measurements were made and the results read.
The Original Extract (EO) was calculated by Balling formula:

$$OE(\%) = \frac{(2.0665 \cdot A) + E_R}{100 + (1.0665 \cdot A)} \cdot 100 \qquad \textbf{(3.1)}$$

where are: EO = original extract of wort in percentage, Plato, A = content of alcohol by mass (% m/m), E_R = real extract of beer in percentage, Plato.

Wort Color

The wort color is determined by spectrophotometer (EBC Method: 8.5)
The absorbance of wort was measured at a wavelength of 430 nm in a 10 mm cuvette. The color in EBC (European Brewing Convention) units was obtained by multiplying the absorbance by a given factor. The color measurement was performed by Agilent 8453 spectrophotometer (Agilent Technologies, Santa Clara, California), cuvette dimension of 10 mm, membrane filter holder, membrane filters porosity of 0.45 micron. The spectrophotometer was adjusted to 430 nm wavelength, having a precision of ± 0.5 nm. The sample was not diluted so that we have absorbance at 430 nm, within the linearity of the spectrophotometer. Measurements were made in the room of 10 mm. The sample was filtered through a membrane

filter to which 1 g/liter of kiselgur was added for clarification of the sample. The calibration of the spectrophotometer was done by setting the wavelength at 430 nm.

The cuvette was filled with distilled water and the apparatus was calibrated at absorbance of 0.00. The cuvette was filled with wort from the sample, and then read the absorbance.

The colour of the undiluted sample was calculated using the formula:

Color (EBC unit) = A x f x 25   (**3.2**)

where A= absorbance at 430 nm in a 10 mm cuvette and f= dilution factor and the result was expressed in units of EBC in two significant digits.

Wort pH

The wort pH was determined by the Method (EBC Method: 8.17.)

Working tools:
- pH – meter, HACH HQ430d flexi,
- standard solution for calibration of the apparatus,
- test tubes and
- distilled water.

Sample preparation:
AErlenmeyer flask is obtained in which the wort is heated till 20°C the sample is filtered then we have determined the properties of wort.

Working procedure:
After calibration of the pH-meter with puffer solution, the electrode is rinsed with distilled water and dried thoroughly then immersed in the sample, mixing the solution with the electrode and allowing it to stand slightly (until the pH value does not changes in pH –meter), then we read the pH value. Mush standard pH values ranged from 5.2-5.6.

Results and Discussion

Calculations of the different control cutoff points are exhibited as Follows.

For original extract:

$$\bar{x} = \frac{13.29 + 13.18 + 13.05 + \cdots + 13.01 + 13.1 + 13.22}{60} = 13.10483$$

$\bar{x} = 13.10483$ (CL:Control Limit or Central Line)

$$MR_1 = |x_2 - x_1| = |13.18 - 13.29| = 0.11$$
$$MR_{59} = |x_{60} - x_{59}| = |13.22 - 13.10| = 0.12$$
$$\overline{MR} = \frac{0.11 + 0.13 + 0.03 + \cdots + 0.04 + 0.09 + 0.012}{59} = 0.07949$$

$\overline{MR} = 0.07949$

UCL = Upper Control Limit

LCL = Lower Control Limit

$$UCL = \bar{x} + 3\frac{\overline{MR}}{d_2} = 13.10483 + 3\frac{0.07949}{1.128} = 13.3162$$

$$LCL = \bar{x} - 3\frac{\overline{MR}}{d_2} = 13.10483 - 3\frac{0.07949}{1.128} = 12.8934$$
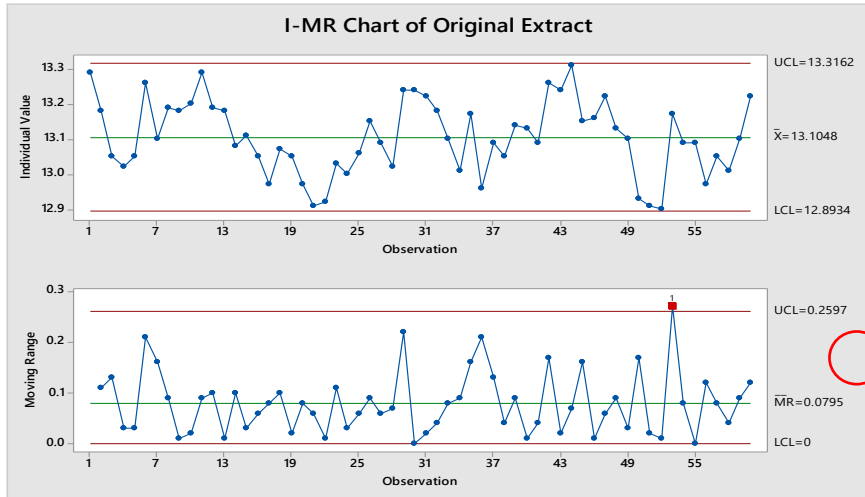
Figure 4.1. Control charts for individual observations of original extract and for the moving range

For pH:

For the average of the individual values

$$\bar{x} = \frac{5.27 + 5.30 + 5.35 + \cdots + 5.32 + 5.22 + 5.27}{60} = 5.2555$$

$\bar{x} = 5.2555$ (CL:Control Limit or Central Line)

$$MR_1 = |x_2 - x_1| = |5.30 - 5.27| = 0.03$$
$$MR_{59} = |x_{60} - x_{59}| = |5.27 - 5.22| = 0.005$$

$$\overline{MR} = \frac{0.03 + 0.05 + 0.02 + \cdots + 0.12 + 0.10 + 0.05}{59} = 0.050169$$

$\overline{MR} = 0.050169$

UCL = Upper Control Limit

LCL = Lower Control Limit

$$UCL = \bar{x} + 3\frac{\overline{MR}}{d_2} = 5.2555 + 3\frac{0.050169}{1.128} = 5.3889$$
$$LCL = \bar{x} - 3\frac{\overline{MR}}{d_2} = 5.2555 - 3\frac{0.050169}{1.128} = 5.1221$$
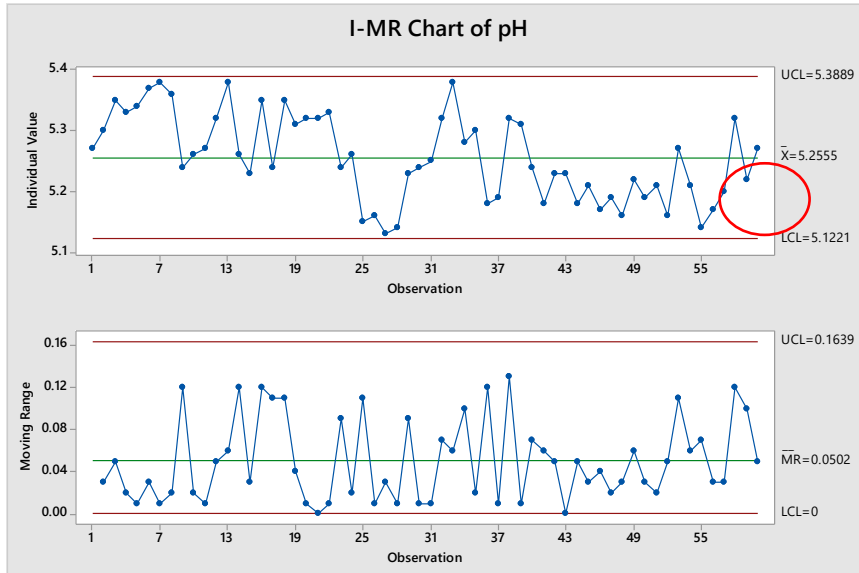
Figure 4.2. Control charts for individual observations of pH and for the moving range

For Color:

For the average of the individual values

$$\bar{x} = \frac{6.4 + 8.1 + 6.7 + \cdots + 8.1 + 7.1 + 7.7}{60} = 7.832$$

$\bar{x} = 7.832$(CL:Control Limit or Central Line)

$$MR_1 = |x_2 - x_1| = |6.4 - 8.1| = 1.70$$
$$MR_{59} = |x_{60} - x_{59}| = |7.7 - 7.1| = 0.06$$

$$\overline{MR} = \frac{1.70 + 1.40 + 0.80 + \cdots + 0.00 + 1.00 + 0.6}{59} = 0.9949$$

$\overline{MR}$=0.9949

UCL = Upper Control Limit

LCL = Lower Control Limit

$$UCL = \bar{x} + 3\frac{\overline{MR}}{d_2} = 7.832 + 3\frac{0.9949}{1.128} = 10.478$$

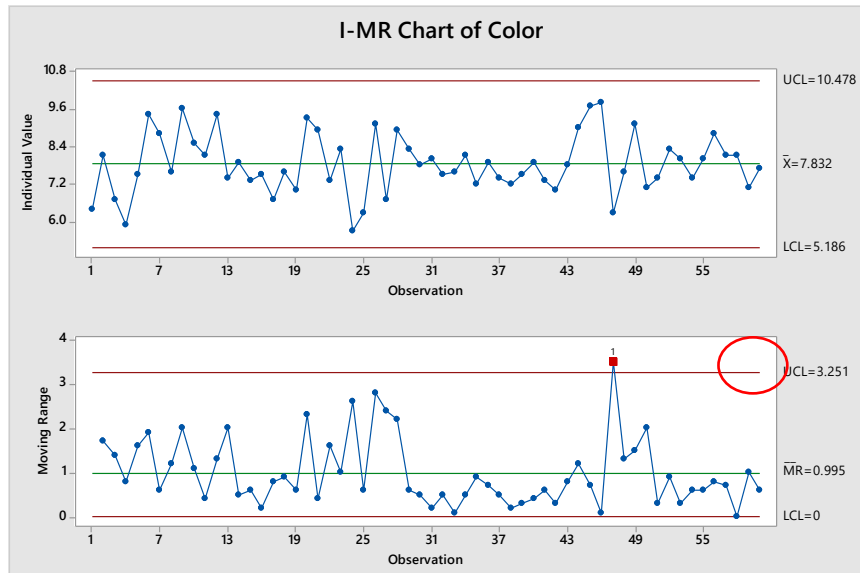$$LCL = \bar{x} + 3\frac{\overline{MR}}{d_2} = 7.832 - 3\frac{0.9949}{1.128} = 5.186$$

Figure 4.3. Control charts for individual observations of color and for the moving range

Based on obtained results of measurable characteristics of wort such as original extract, pH, and color, we came to the conclusion that the process of wort production is statistically under control.

Range charts are used together with individual charts to observe the distribution of variables.

From the control charts for the original extract, we came to the conclusion that the process is under control and within specification given that we have a average value (center line) of 13.105%, the upper control limits, UCL is 13.32% and lower control limits, LCL is 12.89%, while the values set by the company for the specifications we have, the average value of 13%, while the lower specification limits, LSL is 12.7% and upper specification limits, USL is 13.3%..Observation 53 points these are outside the moving range control limit.

From the control charts for pH we conclude that the process is under control and within specification given that we have a average value (center line) of 5.25, the upper control limits, UCL is 5.38 and the lower control limits, LCL is 5.12 while the values set by the company for the specifications we have for the lower specification limits, LSL the value of 5.2 and the upper specification limits, USL the value of 5.4.Observation 41 to 51 points are outside the individual control limits.

From the control charts for color we conclude that the process is under control and out of specification given that we have a average value (center line) of 7.83 EBC, upper control limits, UCL is 10.48 EBC and the lower control limits, LCL is 5.19 EBC while the values set by the company for the upper specification limits, USL the value of 9.5 EBC and for the lower specification limits, LSL the value of 6.5 EBC.Observation 47 points these are outside the moving range control limit.

Conclusions

1. According to control charts for individual observations of pH and for the moving range from 41 to 51 points are outside the individual control limits thus should be identified and eliminated in the process.

2. Specifications for wort color must be corrected.
3. The brewing company should apply the statistical process quality control, SPC method to improve and maintain the quality of its product by controlling the raw material and all stages of the brewing process such as the process of wort production, the process of fermentation and maturation, the process of filtration, filling and packaging.
4. The company should establish a Statistical Quality Control unit and employ trained personnel to monitor the progress of the processes and to ensure durable and reliable quality characteristics of their final products.
5. The company should maintain accurate statistical data on their production in order to help the statistical control unit to have access to improve the quality of subsequent products.

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Production of Non-Alcoholic Beer

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Abstract. This study is conducted on the basis of market demand and J.S.C. "Birra Peja", Peja, Kosovo, beer factory management demand for a quality non-alcoholic beer. The study shows the entire technological production process of producing alcohol-free beer of 0% alcohol and 6.0 - 6.2% basic extract. The work done in the production was monitored by the laboratory of the factory "Birra Peja". The work was also monitored in the laboratory of the brewery "Union" in Ljubljana, Slovenia, the National Institute of Health in Pristina, and the Peja Agricultural Institute. Chemical and microbiological analyzes were carried out based on methods according to the European Convention on Breweries (ECB) and Mitteleuropäische Brautechnische Analysenkommission e. V. (Central European Commission for Brewing Analysis) or MEBAK. From laboratory analyzes and sensory evaluations of beer quality, we have come to the conclusion that non-alcoholic or zero-alcohol beer is of good quality, and, considering the high quality, it should be produced in "Birra Peja".

Keywords: Basic extract, Non-alcoholic beer, ECB, MEBAK

Introduction

There are several efficient and safe ways to produce non-alcoholic beer. Based on Kunze's methodology on beer production, we decided that the beer must be natural and with no added ingredients of modified origin (1961, 2004). We produced beer that has all the characteristics of the beer with alcohol but without alcohol or a level of zero-alcohol. The study was concerned with alcoholic fermentation which does not produce alcohol. We have worked with four different yeast yield rates 2.0×107, viable cells (wort ml), which yeast at the beginning of fermentation did not contain alcohol or had 0% alcohol (Schuster et al., 1976).

The used raw material for the production of this beer was the same as when producing beer with alcohol. As raw material for the production of non-alcoholic beer was used Croatian barley malt, quality analysis results of which will be presented below.

The yeast that is used is the second generation in our fermentation process. The results obtained showed that the yield rate turned a higher maximum number of yeast culture cells, higher amount of fermentable sugar obtained and ethanol production rate, lower diacetyl and pentandione in green beer, as well as higher amounts of high alcohols and esters. The results of the process of obtaining the wort show that this method itself was even more efficient as a controlled method for obtaining wort with limited amounts of fermentable sugars resulting in the composition of the production product; ethanol, and concentrations of diacetyl, high alcohols and ethers in green beer.

The used method of obtaining higher concentration of wort and high gravity beer, which beer after the fermentation process was interfered with dehydrated water containing maximum 0.002% oxygen. The beer dilution process was done after the fermentation was completed to a certain extent and during the filtration of the finished product until the initial extract of 6.2%.

Chemical and microbiological analyzes were carried out based on methods according to the European Convention on Breweries (ECB) and Mitteleuropäische Brautechnische Analysenkommission e. V. (Central European Commission for Brewing Analysis) or MEBAK (Anger et al., 2005).

The beer obtained after fermentation and maturation contains a raw material odor (milled mater with water. We had the option to remove this fragrance through chemical aromatic compounds that would have given the flavor of our choice (vermicelli or beer flavor). However, we chose a different method that was based on purifying the aroma through gases, which do not alter the taste and quality of the beer but only affect the elimination of the unpleasant odor.

The purpose of this paper was to study the production of non-alcoholic beer in the technological process.

Material and Methods

The 6.2oP wort is prepared from boiling 100% malt with a one decoction procedure. Water from the "Drini i Bardhë" spring with strength of 9.5°dGH. For this study, was used Aurora (bitter) and Golding (aromatic) humulus lupulus with Slovenian origin with the ratio of 70:30%. During the process, we have added 6.3 gr α-acide/hl to the wort. The yeast used for the production of these beers is Saccharomyces carlsbergensis with the previously stated concentration of yeast cells per ml. We have cooled the wort by withdrawing oxygen. We used a tool for stabilizing polyphenols. The main fermentation was carried out and completed at 4oC, in which case the total amount of the extract was not consumed. This method was based on Narziss's recommendations (1976).

Yeast, temperature, amount of carbon dioxide, amount of ethanol, and gravity were continuously monitored during the fermentation process.

Results and Discussion

The natural production of beer was conducted in order to complete this study successfully. So primary in this study are the advantages and not the dependencies on the raw material or the technological process of brewing.

By doing the cost-benefit analysis of the final product, savings were noticed from the initial stages of production of this beer. The cost of filtering beer has fallen in proportion to the index beforehand. We should mention about 15% energy savings in wort preparation, about 15% energy savings during the main and supplementary fermentation, capacity utilization compared to beer produced by wort at 6.2°P for about 50%. Also due to changes in production recipes based on the methodology by Pajaziti (2014), we have been able to reduce the substances used for stabilization (protein and polyphenols) by 50%. As we see in the tables and histograms, we have changes in the amount of secondary fermentation products such as the increase of the amount of esters and the decrease of the amount of high alcohols, in which case there is a change of the taste of the beer produced with these modifications. Therefore, the beer produced by this method due to the halved amount of polyphenols has taste stability over-time because the oxidation of polyphenols does not happen. Also, below are presented the results of two tasting groups, one from "Birra Peja" and the other from "Union" brewery, who conducted tasting separately. In this case, the beer produced by this method compared to the beer produced without alcohol in the region has been scored higher.

Table 3. Results of beer tasting at "Union" brewery, Ljubljana.

Beer tasting at "Union" brewery, Ljubljana						
Taster	Sample					
	S	T	G	A	K	M
Taster #1	3.30	3.20	3.30	3.20	3.20	3.20
Taster #2	3.20	3.30	3.20	3.20	3.20	3.20
Taster #3	3.20	3.30	3.20	3.20	3.20	3.20
Taster #4	3.20	3.30	3.20	3.20	3.20	3.20
Taster #5	3.10	3.20	3.30	3.20	3.20	3.30
Taster #6	3.10	3.30	3.20	3.20	3.20	3.20
Average scoring	3.18	3.27	3.23	3.20	3.20	3.21
Evaluation	Very good	Excellent	Excellent	Very good	Very good	Very good

Table 2. Results of beer tasting at "Birrfa Peja" brewery, Peja.

Beer tasting at "Birra Peja" brewery, Peja					
Organoleptic characteristics	Sample				Maximum points
	A	B	C	D	
Taste	1.00	1.00	1.00	2.50	3
Smell	1.20	1.00	1.00	1.50	2
Colour	4.20	4.00	4.00	4.50	5
Clarity	3.40	3.00	2.00	4.00	4
Foam	4.00	3.00	3.00	5.50	6
Full points	13.80	12.00	11.00	18.00	20
Evaluation	Very good	Very good	Excellent	Excellent	

Table 3. Beer analysis results.

Date of work	26.03.2019	23.05.2019	min/max
Basic extract %	6.04	6.06	6.0-6.2
Real extract %	5.74	5.75	
Apparent extract %	5.67	5.68	
Real rate of fermentation %	5.03	5.24	
Appar. rate of ferment. %	6.09	6.34	
Alcohol %v/v	0.19	0.20	0.0-0.30
Density 20/20	1.0223	1.0224	
CO_2 g/l	5.2	5.3	4.7-5.7
pH	5.10	5.17	5.0-5.3
Color EBC	6.80	7.0	6.5-8.0

Bitter EBC	19.6	19.0	18-20
O_2 total mg/l	0.15	0.14	0-0.5
Polyphenols mg/l	68.19	72.14	

Table 4. Results of analysis of secondary fermentation products.

Peja Beer	26.03.2019	23.05.2019
Diacetyl (mg/l) 0.1	0.0060	0.0084
Pentanedione (mg/l) 0.6	0.00	0.00
DMS (mg/l) 0.03-0.12	0.015	0.019
Acetaldehyde (mg/l) 2-20	0	0
Etyl Acetate (mg/l) 5-30	0.50	0.57
Iso-Amyl Acetat (mg/l) 1-5	0.00	0.00
Propanol (mg/l) 5-30	0.00	0.00
Iso-Butanol (mg/l) 5-20	1.20	1.24
Iso-Amyl Alcohol (mg/) 50-60	2.4	2.5

Conclusion

This study was conducted in the period between October 2017 – May 2019 and by comparing the results it was concluded that non-alcoholic beer produced at "Birra Peja" meets the most the quality requirements as compared to beers produced at other factories that have been studied for comparison. The main drawback of the beer produced by this method is that it is not 0.00% v/v alcohol. It is worth mentioning that this non-alcoholic beer produced in "Birra Peja" has a colloidal and microbiological viability of 12 months.

In consultation with the factory's management, we have come to the conclusion that the continuous production of these beers should begin as soon as possible. The beer is of high quality, it is drinkable and has satisfactory colloidal stability.

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Bacteriocin production by lactic acid bacteria (LAB) isolated from traditional cheese

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Abstract. Lactic Acid Bacteria (LAB) are a group of bacteria that are found as natural microbiota in various ecosystems. They can produce a number of antimicrobial metabolites, including organic acids and other organic components, hydrogen peroxide and bacteriocins. The aim of this study was the evaluation of antibacterial activity of LAB isolated during production and maturation of traditional Rugova cheese. Samples for analysis were collected from different points of Rugova region and were transported to the laboratory under constant cooling conditions. The bacterial isolation was performed using standard methods and the isolates of LAB were identified down to the species level using a Biotyper Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Out of 140 tested isolates 105 had the ability to produce bacteriocins. The ability of bacteriocin production by LAB isolated from Rugova cheese can be taken as a measure of quality and safety of this traditional product.

Keywords: Lactic acid bacteria, bacteriocin, cheese, MALDI-TOF MS

Introduction

Lactic Acid Bacteria (LAB) are a group of bacteria that are found as natural microbiota in various ecosystems. They are used to produce a variety of fermented foods, they are important constituents in pharmaceutical formulations and as probiotics in functional foods. The LAB can produce a number of antimicrobial metabolites, including organic acids and other organic components, hydrogen peroxide and bacteriocins [1]. Bacteriocins are ribosomally synthesized peptides with antimicrobial activity. They are secreted to the extracellular medium [2] and can be classified in three classes [3]. Class I are short peptides (19–50 amino acids) that are often post-translationally modified resulting in the non-standard amino acids, such as lanthionine and others. A prominent member of this class is Nisin A. Class II are termostabile peptides like Pediocin PA-1, lactococcin G, enterocine As-48, ect. Class III comprises large and heat labile proteins. Best known bacteriocins of class III are Colicin, produced by Escherichia coli or various helveticins produced by Lactobacillus helveticus [4-5]. LA bacteria are used to produce a large variety of fermented foods, they occur in pharmaceutical formulations and as probiotics in functional foods [6]. Traditional cheeses are known for a high diversity of microbial communities, dominated by LAB, which are important for developing organoleptic

characteristics of cheese [7-8]. Kosovo produces a variety of traditional cheeses by traditional methods, however little information is available about microbial activities in this products [9]. The aim of this study was the evaluation of antibacterial activity of LAB isolated during production and maturation of traditional Rugova cheese.

Materials and methods

All samples analyzed in this study were collected from different points of Rugova region (Fig. 1), and were transported to the laboratory under constant cooling conditions. Traditional cheese produced by farmers in Rugova region, were collected in different stage of production and maturation.
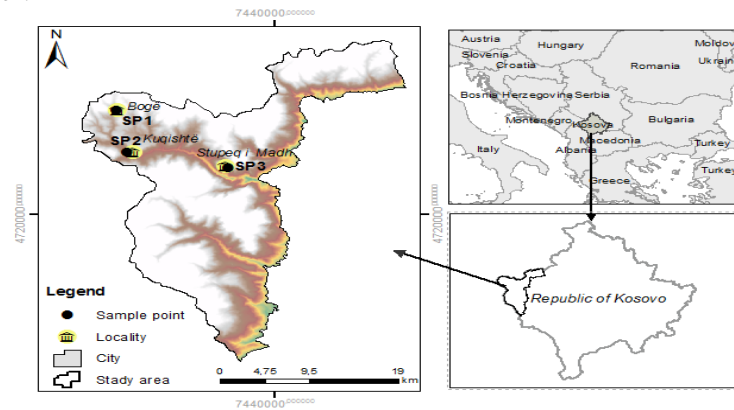

Fig. 1. National Park "Bjeshkët e Nemuna" and sampling points.

For bacterial isolation, 1 ml of appropriate dilutions was plated on M17 and MRS agar medium respectively. The plates were incubated at 30°C and 37°C for 48h under aerobic and anaerobic conditions. Gram positive and catalase negative bacteria were selected randomly from M17 and MRS agar. Pour plating technique was used for bacterial purification, by repeated plating in the same agar until pure cultures were obtained [9-11]. The isolates of LAB were identified down to the species level using a Biotyper Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Agar overlay techniques were used to demonstrate the antibacterial activity of natural LAB isolated from traditional Rugova cheese, against 5 indicator strains [12].

Results and Discussion

After identification of our isolates by MALDI TOF-MS, 140 were selected to investigate their bacteriocine production ability in traditional Rugova cheese. Out of 140 tested isolates 105 had the ability to produce bacteriocins against 5 indicator bacteria (Fig. 2).
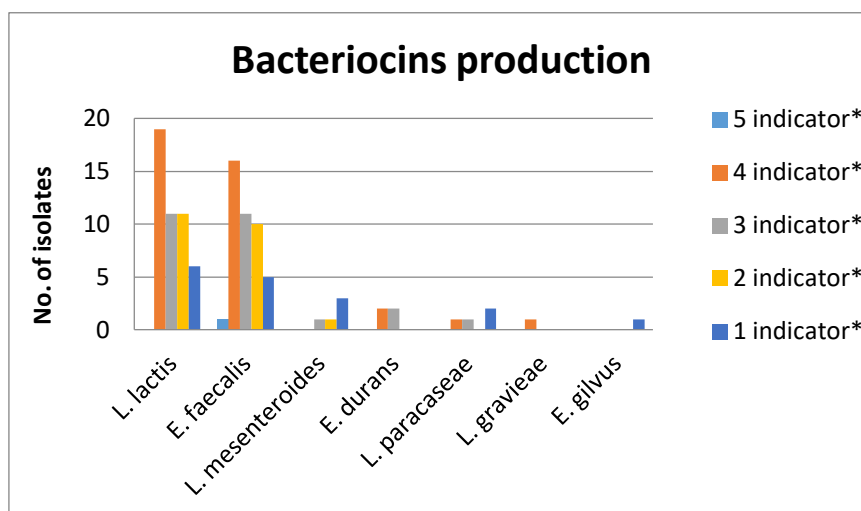
*Number of indicator bacteria.

Fig. 2. The number of isolates that have the ability to produce bacteriocins against indicator bacteria

Forty-seven isolates of Lactococcus lactis showed antibacterial activity against at least one-indicator bacteria. Other authors have found little or lack of activity of bacteriocin production of lactococci against Listeria spp. [13]. Forty-three isolates of Enterococcus faecalis showed bacteriocin production against 1 to 5 indicator bacteria. Only one isolate (FA25) had the ability to inhibit the growth of Listeria moncytogenes with a clearly visible inhibition zone. The ability of huge number of isolates of genus Enterococcus for bacteriocin production is in harmony with the findings of other authors [14-16]. Leuconostoc mesenteroides is present with 5 isolates in Rugova cheese that had ability to produce bacteriocin against 3 indicator bacteria. Leuconostoc mesenteroides are used in Swiss type cheese because of metabolite secreted by this bacteria, which also are acceptable for other foods [17-18]. Others bacterial species (Enterococcus durans, Latobacillus paracaseae, Lactococcus grrvieae and Enterococcus gilvus), had low antibacterial activity against indicator bacteria.

Conclusions

The large number of bacteriocin producers demonstrates the great assertiveness of the natural LAB microbiota over potentially existing pathogens. Thus, the ability of bacteriocin production by LAB isolated from Rugova cheese can be taken as a measure of quality and safety of this traditional product. Further investigation is needed to characterize these bacteriocins and elucidate their antibacterial mechanisms.

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A Research of Some Pathogen Microorganisms in Traditional Sharri Cheese

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Abstract. Sharri cheese is a traditional hard, fatty and salty type of cheese produced in Sharri, Gora, Opoja and Shtirpca towns in the Sharr Mountains region. Sharri cheese is produced by processing sheep milk, cow milk or their mixture. The aim of this study was to determine the microbiological quality of traditionally and industrially produced Sharri cheese. In total 58 samples of cheese were examined. Staphylococcus aureus was found in 57 samples. Escherichia coli were determined in 34 samples and the number of E. coli were found between 1×10^1 CFU/g to 4.5×10^6 CFU/g. According to our results, Sharri cheese can be risky in terms of public health as it contains several foodborne pathogens.

Keywords: Sharri cheese, microbiology, Staphylococcus aureus, Escherichia coli

Introduction

In order for a person to live a healthy and long life, he must have adequate and balanced nutrition throughout his life. Adequate and balanced nutrition increases people's productivity and success rate in society. It is sufficient for a person to consume various nutrients (proteins, fat, carbohydrates, vitamins, minerals and water) in foods continuously and regularly throughout his life [4].
With unbalanced and malnutrition high blood pressure, weight gain or deficiency, growth-developmental retardation, decreased human productivity and diseases such as cardiovascular disorders, muscle / bone loss and so on are emerging. Diseases such as physical and mental disorders have various problems during a person's life [6].
The milk group is a food group containing most nutrients and provides a large portion of the daily need. Dairy and cheese products contain many important vitamins and minerals such as calcium, phosphorus, magnesium, proteins, vitamins A, D, E, K, B vitamins [3]. There are different types of cheeses in the world where they can be classified according to: type of milk used during cheese production (cow, sheep, buffalo, coat etc.), fat percentage (full fat, half fat, fat cheese, double cream, cream, low fat), consistency of cheese (very strong, strong, half strong, half soft, soft, fresh cheese), fermentation type, surface (hard, soft, spicy, molds), interior (holes, molds) [5].
Milk and milk products create an excellent environment for pathogenic microorganisms. Milk and dairy products can be important sources of foodborne diseases. One of the most important foodborne intoxication in world is caused by Staphylococcus aureus. The illness comes from taking the enterotoxins formed in food. The disease can appear within 2-4 hours by taking 1-10μg of enterotoxin in the body through food. S. aureus can be found in the human body, in the animal body, at food processing sites. Toxins formation can occur when the milk is taken from

the diseased animal with mastitis, pasteurized milk is contaminated and stored under inappropriate temperature conditions [2].

Another type of foodborne intoxication is caused by Escherichia coli. This type of bacteria forms hemorrhagic colitis which causes diarrhea, hemolytic, uremic syndrome, and death. Minimal infection dose is known to be 10-100 cfu. The source of this bacterium may be unpasteurized products but also pasteurized dairy products that have been contaminated by various causes such as unhygienic conditions of production, personnel hygienic conditions [2].

Sharri cheese is a type of cheese produced traditionally Strpce, Opoje, Dragas villages in Sharri mountain region. Production places usually are placed above 2000m and the cheese is produced on summer months. After the milking from sheeps is finished they add the rennet and coagulate the milk without pasteurizing it. Besides traditionally produced cheese, there are some industrial producers in region that produce Sharri cheese with pasteurized cow milk instead of sheep milk [7] [13]. The old processing methods are still used in traditional cheese production. The microbiological quality of the cheese depends on several factors such as: unpasteurized raw material, lack of hygienic conditions in the production sites, lack of standardization of the cheese process where it is influenced by the individual processor. In previous years (Rysha et al) research has shown that 75% of raw milk samples do not comply with EU legislation, while cheese samples produced from raw milk do not comply with EU legislation [12]. The aim of this study was to determine the microbiological quality of traditionally and industrially produced Sharri cheese.

Materials and Methods

58 cheese samples from traditional and industrial producers were taken in sterile conditions from different regions and were transported in cold chain. 30 samples were from traditionally producers and 28 samples were from industrially producers. Analyzes were conducted at Istanbul University- Cerrahpasa, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology. Sharri cheese samples were taken in spring time from May to June 2019 and analyzes were performed in June 2019.

Manufacture of Sharri Cheese

Traditionally produced Sharri cheese is made from fresh milk taken after the milking of sheep's is finished. Rennet is added and the coagulation period lasts about 60 minutes. After the curd is cut, whey is drained and is filtered with a piece of cloth about 10-12 hours. The oval cheese up to 10kg matures on wooden boards for 2 weeks. Cheese is broken by hands and put on brine containing approximately 10% salt. Cheese is ready to eat after 45 days [7].

Industrially produced cheese is made after the milk is filtered and pasteurized. Culture and rennet is added and the coagulation period lasts about 45 minutes. After the curd is cut, whey is drained by pumps and oval cheese is formed with a piece of cloth. Oval cheese maturates in cold rooms maximum 8°C for 5-7 days. After that cheese is broken by hands and put in brine containing approximately 7-10% salt. Cheese is ready to eat because of pasteurization process on raw milk.

Microbiological analyses

Determination of E. coli

The method used for the identification of E. coli was ISO 16649-2-- Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of β-glucuronidase- positive

Escherichia coli—Part 2: Colony-count technique 4°C using 5-bromo4-chloro-3-indolyl β-D-glucuronide.

Determination of S. aureus

The method used for the identification of S. aureus was ISO 6888-1—Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of coagulase- positive staphylococci (Staphylococcus aureus and other species- Part 1: Technique using Baird- Parker agar medium.

Results and Discussion

In total 58 samples of traditionally and industrially produced Sharri cheese were analyzed. All of the cheese samples have undergone heat treatment. Shown in Table 1 at the end of the microbiological analysis of E. coli, according to EU legislation limits for E. coli that can be found on the cheeses that are made from milk that are undergone heat treatment are 100 CFU/g to the maximum of 1000 CFU/g. The results of our analyses show that 16 samples of traditionally produced cheese are under 100 CFU/g, 3 samples are within limits 100 CFU/g to 1000 CFU/g while 11 samples containing more than 1000 CFU/g do not complain with the EU regulation 2073/2005. From industrial producers according to EU legislation limits for E. coli that can be found on the cheeses that are made from milk that are undergone heat treatment are 100 CFU/g to the maximum 1000 CFU/g. The results shown on Table 1 of our analyses show that 15 samples of traditionally produced cheese are under 100 CFU/g, 6 samples are within limits 100 CFU/g to 1000 CFU/g while 7 samples containing more than 1000 CFU/g do not complain with the EU regulation 2073/2005.

Table 1: The result of the analysis of E. coli in Sharri cheese

	<100 cfu/g	100 cfu/g to 1000 cfu/g	>1000 cfu/g
Traditional	16 samples	3 samples	11 samples
Industrial	15 samples	6 samples	7 samples

Shown in Table 2 at the end of the microbiological analysis of S. aureus, according to EU legislation limits for S. aureus that can be found on the cheeses that are made from milk that are undergone heat treatment are 100 cfu/g to the maximum 1000 cfu/g. The results of our analyses show that all of the 58 samples of traditionally and industrially produced cheese are above the limit 1000 cfu/g so these cheese samples do not complain with the EU regulation 2073/2005.

Table 2: The result of the analysis of S. aureus in Sharri cheese

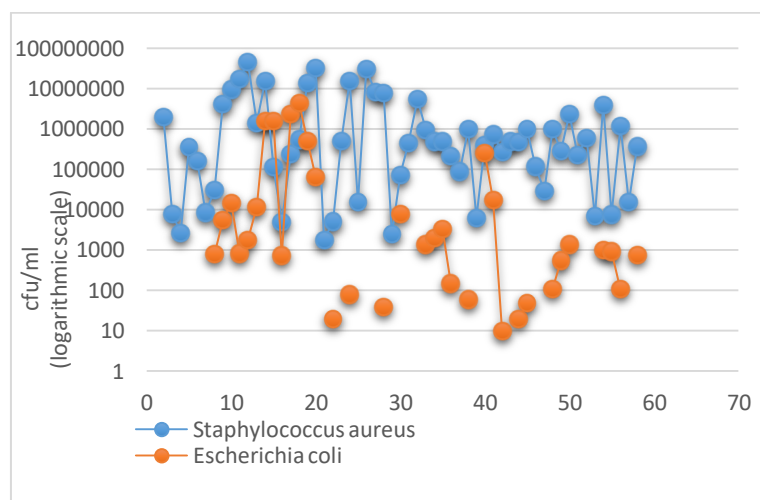|	<100 cfu/g	100 cfu/g to 1000 cfu/g	>1000 cfu/g
Traditional	-	-	30 samples
Industrial	-	-	28 samples

Figure 1: Presence of E. coli and S. aureus count in Sharri cheese.

This study has shown that at the end of the microbiological analysis of E. coli, according to EU legislation, from traditional producers only 10% of the samples are within the limit while 36% of the samples do not comply with (EU regulation 2073/2005). From industrial producers 21% of the samples are within the limit while 25% of the samples do not comply with (EU regulation 2073/2005).

At the end of the microbiological analysis of S. aureus, in the traditional and industrial method all cheese samples are out of the permissible limit (EU regulation 2073/2005).

Previous studies (Rysha et al.) has shown that all Sharri cheese samples that were analyzed for S. aureus and the results were (>10 CFU/g) don't complain with EU regulation 2073/2005 [12]. Another research made for different type of cheese named Arzua Ulloa produced from raw milk showed result 61.4% comply with EU regulation, 22.8% were acceptable while 15.8% were not acceptable due to high number of S. aureus, E. coli and/or L. monocytogenes [12].

Food contamination by pathogen microorganisms like S. aureus and E. coli is a serious public health problem [10] [14]. The main sources of contamination with S. aureus are the presence of S. aureus in the raw milk, direct contamination during the cheese processing, and cross contamination at the final product [1].

As a conclusion, low or more value S. aureus and E. coli were found in Sharri cheeses. This result shows that Sharri cheese may pose a risk for public health.

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