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"LUCIAN BLAGA" UNIVERSITY OF SIBIU
FACULTY OF AGRICULTURAL SCIENCES, FOOD INDUSTRY AND ENVIRONMENTAL PROTECTION

Summary
PhD Thesis

RESEARCH ON THE ENRICHMENT OF QUALITY AND NUTRITIONAL PROPERTIES OF DAIRY PRODUCTS WITH ADDED NATURAL BIOACTIVE PRODUCTS

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PREFACE

Doctoral studies took me on a path to knowledge to a new era of nutrition and having perspective concepts obtain as many functional natural products with bioactive major impact on health. Father of medicine Hippocrates said: "We are what we eat" and today's society brings results and evidence.

Completion of the thesis is a balance of activities over many years, during which I had the opportunity to meet and work with great people he faces now my thoughts of gratitude, respect and friendship.

Respectful address thank ***Mr. Dean Prof. Dr. Tita Ovidu****, scientific leader of the work, the professionalism that guided me on the path to the title of Doctor of Science, for all times comply competence and scientific guidance for real support given throughout the period of the PhD thesis and preparing .*

During university research skills began to take shape and come to life along with distinguished people who work encouraged me, believed in me and helped me and hence the formation of this thank Mrs. conf.univ.dr.Tita Mihaela.

I thank in particular ***Mr. Otto Ketney, PhD lecturer*** *continued support given to me during the study and thesis development, materialized through a fruitful collaboration, particularly useful suggestions, in a word, by a constant in my life support. His presence was a support that made it possible and support research and development of the whole material.*

European projects have been my chance to improve scientific training as part of a PhD study by implementing competitive doctoral programs.

During the preparation of this thesis, I benefited from scholarships awarded by the research project No. 88/1.5/S/60370 projects under the Operational Programe Human Resources Development (HRD), financed from EU Structural Funds.

Special thanks to ***Mr.prof.dr.Endre Mathe*** *and the entire collective of the laboratory wich he runs, for careful guidance during the internship at the research center "Agricultural and Molecular Research Institute, College of Nyíregyháza" in Hungary during the period January 2012 - April 2012.*

Thank you family supported me, encouraged me and understood all these years, throughout the course of doctoral internship, my parents being dedicated to this success.

"I do not think there is any emotion that may cross the human heart like that felt by the inventor sees a creation of the mind moving towards success, such emotions make a person forget food, sleep, friends, love, everything."

N.Tesla

CONTENT

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ABBREVIATIONS

α-TE	α-tocopherol
DNA	Deoxyribonucleic acid
ACP	Acyl-acyl carrier protein
ACN	Acetonitrile
AOAC	Association of Official Analytical Chemists
aw	Water activity
CoA	Coenzyme A
Brix	1 gram of sucrose in 100 grams of solution, is the concentration of the solution in percent by weight (% w / w)
CAFA	caffeic acid
DPPH	2, 2-diphenyl-1-picrilhidrazil
EDTA	ethylene diamine tetra acetate
FA	Acid ferulic
FAD	Flavinadenin dinucleotide
FoSHU	Food with special specification
FMN	flavinmononucleotide
FRAP	2, 2 '-Diphenyl-1-picrilhidrazil
FT-IR	Fourier transform infrared spectrometry
GC-ECD	Gas chromatograph detection in electron capture detector
GC-MS	Gas chromatograph with mass spectrometer detection in
GPT	glutamate-piruvattransaminaze
HACCP	Hazard analysis and critical control points
HBV	hepatitis B
HDL	high-density lipoprotein
HPLC	High Performance Liquid Chromatography
HPLC-MS	high performance liquid chromatograph with detection in mass spectrometer
IC	Ion Chromatograph
IR	infrared radiation / Spectrometry in
IUPAC	International Union of Pure and Applied Chemistry
LAB	Lactic acid bacteria
LC-MS	detection in liquid chromatography mass spectrometer
LDH-L	L-lactate dehydrogenase
MSF	Ministry of Health and Family

NAD	Nicotinamidadenin dinucleotide
Nm	Newton meter
N	Newton
UN	United Nations
WHO	World Health Organization
PROC	Protocatechic Acid
PR	proteins.
PLP	Pyridoxal 5-phosphate
RDS	relative standard deviation
UFLC	ultra-fast liquid chromatography
UV	Ultra Violet UV radiation / UV Spectrometry
UV / VIS	spectroscopy in the field ultraviolet spectrophotometry and visible
TPP	coenzyme thiamine pyrophosphate
SA	salicylic acid
SNF	solids non-fat substance
Sr	Standard deviation
VA	Vanillic acid

KEYWORDS:

Sea buckthorn, probiotics, bioactive lactic culture, yogurt, dairy products, antioxidants, nutrition.

STRUCTURE

The doctoral thesis with the theme " Research on the enrichment of quality and nutritional properties of dairy products with added natural bioactive products" includes 291 pages , 138 figures and graphs, 61 tables, 249 references many recent time and is structured in two parts: bibliographic study and personal research.

- Documentary study that describes:
    - the need to improve the nutritional value of food
    - theoretical aspects of nutritional components of milk and sea buckthorn
    - national and international legislation regarding the addition of nutritional supplements / bioactives in food;
- Personal research which presents:
    - the organization of the class researches describing the theme of motivation, research objectives, indicators included in the study and organizing researches
    - materials and methods with a detailed description of material to researches and analysis of the main methods used
    - Experimental results including - results and discussion, partial conclusions
    - Final conclusions and development trend of research in describing the contributions and perspectives of further research, the materialization of the research, bibliography, appendices.

CHAPTER 1. NUTRITIONAL VALUE OF FOODS

The diet leaves its impact on the entire human life starting from embryonic development, causing health and work capacity. It is a permanently acting factor influencing the development of metabolic processes and regulator exchange processes while food is the potential source. But the needs of the body and food intake are not always in an optimal balance, it is disrupted by numerous factors like social, economic, technological and cultural, etc. *(scribd.ro)*.

Functional foods are products that are consumed preferably as the current diets due to their biologically active compounds that contribute for maintaining human health *(Serban, 2009)*.Collection and study of new functional foods is essential for contemporary stage, but the human body is subject to a variety of attacks, which require maximum protection, adaptation and maintenance of balance. By their action specific bioactive compounds such foods can help maintain body balance on normal parameters. *(Serban, 2009)*

Developing a new dairy-free probiotic food is an expensive process. Food companies have traditionally funded research for new food product formulations, but the stakes are higher for lactose-free products, for both food companies and consumers *(Walzem, 2004)*.

1.1. BIOACTIVE COMPONENTS FROM SEA BUCKTHORN

Sea buckthorn (*Hippophae rhamnoides L.*), fam. Elaeagnaceae. is bushy shrub, rustic, found in small groups or large shrubs on sand and gravel, the gravel along the river, green land, rocky rib fractures, rocks, especially on salinizes geological formations, coastal regions by mountain floor on large surfaces Sub Carpathians Wallachia and Moldavia, is isolated in the valley of river Olt, Arges ,River Fair etc.

New research at the Emil Grigorescu pharmacologist, biochemist John Brad, foresters Haralamb Athanasius, S. Corlăţeanu, E. Beldeanu, N. Bogdan, E. Untaru, C. Traci etc. have demonstrated the importance of this species in different areas. I. *(SA, 2007)* who introduced sea buckthorn in fruit tree culture.

1.2. SOME PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION OF SEA BUCKTHORN

Sea buckthorn berries have a very high content of vitamins, minerals, trace elements, antioxidants, phytohormones etc. Sea buckthorn berries contain vitamin C in a ratio of two times more than currants and ten times more than citrus. Vitamins A, B1, B2, B6, B9, E, K are also present in fruits of sea buckthorn in significant concentrations. In addition to these vitamins many other bioactive substances are also present in fruits of sea buckthorn (about 200 in number after some evaluations), these fruits contain β-carotene in greater proportion than the carrot and other carotenoids, microelements such as P, Ca, Mg , K, Fe, Mo, cellulose, proteins with a high content of essential amino acids (especially lysine) complex oils (saturated and unsaturated fatty acids, sterols), organic acids such as malic acid, succinic acid, ursolic acid and so on, and flavonoids identical to those of Gingkobiloba. Remarkable contents of hormonal substances are found in fruits and leaves of sea buckthorn, especially serotonin, substance recognized as having special physiological effects related to the central nervous system, protein synthesis, simulation of imunoinductor etc. *(ECOTECH, 2010; ULIAN, 2009)*

1.2.1. Nutritional value, and therapeutic importance of the fortified dairy products with sea buckthorn

Nutritional values of sea buckthorn fruit are based on its known composition *(Beveridge, Li, Oomah, & Smith, 1999)* and the relationship of this composition to human nutritional requirements *(Magherini, 1986)* The fruit including seeds contains large amounts of essential oils and vitamin C *(Centenaro, Capietti, Pizzocaro, & Marchesini, 1977; Novruzov & Aslanov, 1983)*). Generally, sea buckthorn fruits are very high in health promoting compounds *(Jeppsson & Gao, 2000)*.

The medicinal benefits of sea buckthorn oil has propelled research in the efficient extraction of oil and components thereof, their characterization and the understanding of the health-related effects of oil and it sassociated components.

CHAPTER 2. THEORETICAL ASPECTS OF NUTRITIONAL COMPONENTS OF MILK AND SEA BUCKTHORN

2.1. CARBOHYDRATES

The main types of carbohydrates found in milk as lactose *(Agency, 2002; Holland, Welch, Unwin, Buss, Paul, & Southgate, 1993)*. It is a disaccharide formed from galactose and glucose. Lactose forms about 54% of the total non-fat milk solids. *(Saxelin, Korpela, Mäyrä-Mäkinen, Mattila-Sandholm, & Saarela, 2003)*.

2.2. PROTEIN

Milk protein content did not differ significantly although commercial yogurts may have higher levels of protein due to the addition of skimmed milk during processing, which increases the protein content of the final product *(Adolfson, 2004)*.

Sea Buckthorn contains high amounts of protein, especially albumin and globulin *(Li, Beveridge, & Canada, 2003)*.

Some lactic acid bacteria used as starter cultures in milk and fermentation, and probiotic bacteria such as L. acidophilus and B. bifidum produce b -d -galactosidase *(Lourens-Hattingh & Viljoen, 2001)*.

2.3. LIPIDS

Milk fat is a natural fat, with physical, chemical, and biological properties unique to contribute to the appearance, texture, flavor, and stability of dairy products. Milk fat is an energy source, essential fatty acids, fat soluble vitamins, and other components of health promotion.

2.4. LACTIC ACID BACTERIA AND PROBIOTICS

The role of probiotics, mainly of the generally *Lactobacillus and Bifidobacterium* are used in the treatment of gastrointestinal infections is increasingly documented as an alternative or complement to antibiotics, with the potential to decrease the use of antibiotics or reduce their adverse effects*(Myllyluoma, 2007)*. Probiotic bacteria are live microbial strains that, when applied in adequate doses, beneficially affect the host animal by improving its intestinal microbial balance. *(Smit, 2003)*.

2.5. ANTIOXIDANT CAPACITY OF SEA BUCKTHORN

Antioxidants are bioactive substances that prevent or inhibit oxidation reactions promoted by oxygen or peroxides and thus protects cells from damage caused by oxidative stress.

Sea buckthorn is a natural reserve of anti-oxidants and therefore, it's efficacy to protect against oxidative stress has been evaluated by some investigators.The antioxidant and immunomodulatory properties of sea buck-thorn in vitro have also been determined *(Geetha, Sai Ram, Singh, Ilavazhagan, & Sawhney, 2002)*.

2.6. CONTENT OF SEA BUCKTHORN POLYPHENOLS

Phenolic acids are generally not abundant in most plants. There are a few exceptions: gallic acid (1.5) and salicylic acid (SA; 1.8)*(Vermerris & Nicholson, 2007)*

Sea buckthorn does not contain anthocyanins typical for red and blue berries, and only very minor amounts of ellagitannins (hydrolysable tannins) have been detected *(Koponen, Happonen, Mattila, & Törrönen, 2007)*. Like most biological compounds, the type and amount of phenolics in sea buckthorn berry vary depending on the origin, year of harvest, ripeness, processing and storage *(Chen, Zhang, Xiao, Yong, & Bai, 2007; Gao, Ohlander, Jeppsson, Bjork, & Trajkovski, 2000; Yang, 2009)*.

2.7. WATER SOLUBLE VITAMINS OF SEA BUCKTHORN, MILK AND FERMENTED MILK PRODUCTS

2.7.1. Vitamin B1

Thiamin (vitamin B1) is included among the water-soluble vitamins. In alkaline solutions, as well as in the presence of oxidants and radiation, the vitamin is unstable and loses its biological activity. Whole cows' milk contains about 37 mg thiamin 100 g^{-1} , mostly in the free form.*(Biesalski & Back, 2002d)*.

2.7.2. Vitamin B2

Riboflavin is a B-group vitamin is soluble in water and ethanol. It actively participates in metabolic processes, antibody formation, skin cells and red blood cells. Riboflavin plays a very important and multiple role in body. It enters into the constitution of the FMN and FAD dehydrogenase, contributing to oxidation-reduction reactions. In cows' milk, the free form prevails (61% riboflavin, 26% FAD, 11% hydroxyethyl form, traces of three other derivatives), whereas in other foodstuffs, the protein-bound forms predominate. In human milk, about one- to two-thirds of riboflavin is furnished as FAD.*(Biesalski & Back, 2002c)*.

2.7.3. Vitamin B3

Niacin (nicotinic acid and nicotinamide) is part of the nucleotide-nicotinamide coenzymes (NAD) and phosphorylated form (FDAN). Both coenzymes involved in many redox reactions of metabolism. Amino acid tryptophan can be converted to niacin and this conversion requires vitamin B6 (pyridoxine). Niacin defciency or pellagra is most prevalent in countries where maize is the staple food and appropriate processing techniques, i.e. treatment with alkaline solutions, to release niacin from its protein-bound form, niacytin, are unknown (e.g. Africa and the Far East)*(Biesalksi & Back, 2002)*.

2.7.4. Vitamin B5

Pantothenic acid (from Greek pantothenate - everywhere), or vitamin B5, is the most common vitamin, which is present in almost all dishes. Defciency does not easily occur because the vitamin is widely distributed in foods. Defciency symptoms include the burning feet syndrome, muscle cramps, weakness, vomiting, disturbances of the digestive tract and impaired motor function *(Biesalski & Back, 2002b)*

2.7.5. Vitamin B6

Pyridoxine occurs in three different forms: pyridoxine, pyridoxamin and pyridoxal. The vitamin is involved in the metabolism of amino acids as coenzyme of transaminases. Vitamin B6 is essential for humans, most animals and numerous microorganisms. Most researchers agree that vitamin B6 intake should be related to protein intake, yet the ratio varies depending on the study design used to obtain the values. *(Biesalski & Back, 2002e)*.

2.7.6. Vitamin B7

Biotin, or vitamin B7 or vitamin H, is a vitamin of group B. The average biotin concentration in cows' milk is 3.5 mg biotin 100 g^{-1} and is affected little by exo-genous or endogenous factors. Human milk contains less biotin (on average 0.58 mg 100 g^{-1}) and the daily and interindividual variations are higher. Biotin losses due to different processing conditions are small and usually do not exceed 20%. *(Biesalski & Back, 2002a)*.

2.7.7. Vitamin C.

Vitamin C has two biologically active vitamins, L-ascorbic acid and the two-electron reduction producing the dehydro-L-ascorbic acid. Both have antiscorbutic activity. Ascorbate is oxidized reversibly with the loss of electrons to form one ascorbate free radicals semi-dehidroascorbic acid, which is further oxidized to dehydro-L-ascorbic acid. The contribution from supplements was 5.8% for men and 8.6% for women *(Morrissey, 2002a)*.

2.8. FAT SOLUBLE VITAMINS OF SEA BUCKTHORN, MILK AND FERMENTED MILK PRODUCTS

2.8.1. Vitamin A

Vitamin A has three primary forms: retinol, retinal and retinoic acid. The main chemical form in food is retinol. It is found only in foods of animal origin. Some carotenoids, and especially b-carotene from fruits, vegetables and some oils, can be converted in the body to some extent into retinol. Beta-carotene is therefore called provitamin A. This conversion is far from complete and only a small part of the ingested β-carotene (probably less than one-sixth) is converted into retinol.*(Schaafma, 2002)*.

2.8.2. Vitamin D

Vitamin D is a group of lipo-soluble vitamins. Vitamin D is the generic descriptor for all steroids having similar biologica l activ ity as cho lecalciferol or vitamin D3 . *(van Staveren & de Groot, 2002)* .

2.8.3. Vitamin E

In 1922, *Evans and Bishop (1922)* they found a soluble dietary component that was essential to prevent fetal death and sterility in rats fed a diet containing rancid lard. This was originally called 'factor X' or 'antisterility factor',but was later named vitamin E. *(Morrissey & Kiely, 2002)*.

2.8.4. Vitamin K

Vitamin K (the coagulation vitamin) was discovered in the 1930 by *Dam (1935)* as a result of investigations into the cause of an excessive bleeding disorder in chickens fed on a fat-free diet. Its isolation and structural determination were accomplished in 1939 and its metabolic function was defined only after a new amino acid, ┐ -carboxylglutamic acid, was dis-covered in bovine prothrombin in 1974. Vitamin K is essential for the blood clotting process, where it serves as an essential cofactor for the specific carboxylation of a number of vitamin K-dependent coagulation proteins. *(Morrissey, 2002b)*.

2.9. MINERALS FROM SEA BUCKTHORN, MILK AND FERMENTED MILK PRODUCTS

In milk, 14% of iron occurs in milk fat, which is associated with fat cell membrane. About 24% of iron is related to casein probably phosfoserine residues of caseins, while 29% is bound to proteins whey and 32% is associated with a low molecular weight fraction. Distribution of copper in milk was reported as 2% in the proportion of fat, 8% protein bound whey, 44% casein and 47% in a low molecular weight fraction.

2.10. ORGANIC ACIDS FROM SEA BUCKTHORN, MILK AND FERMENTED MILK PRODUCTS

2.10.1. Citric acid

Food is the most versatile and widely used in acidic foods. Citric acid is found in many natural products and is one of the most important acids involved in plant respiration. More fresh fruits such as lime and acid fruits have a pronounced taste due to the presence of citrate ions.

2.10.2. Malic acid

Malic acid is similar to citric acid, having acidifying effects due to its own taste, but the "burst" effect is not given by citric acid. It is found widely in fruits and is strongly associated with apples, although prevalent in many fruits and vegetables. Sea Buckthorn is a great source of malic acid.

2.10.3. Tartric acid

Tartaric acid has a strong taste; natural fruits enhance aroma and synthetic flavors, especially grapes and blueberries. Tartaric acid is a natural component fruit, including many currants, raspberries, blueberries, grapes and dietary acidifiers.

2.10.4. Lactic acid

Lactic acid is one of the most widespread acids in nature and one of the oldest used in food. Due to its taste, it is easily differentiated from other food acids, is also used to increase fruit flavor in drinks and pastries.

CHAPTER 3. NATIONAL AND INTERNATIONAL LEGISLATION ON THE ADDITION OF BIOACTIVE SUPPLEMENT IN FOOD

Food laws have existed since ancient times; there is always a need for quality control and food safety. Today such control is designed to protect consumer's health to prevent misrepresentation on food composition, thus leading to the countries of the European union space to enact strict control and food safety (Fuller, 2001)

Bioavailability, therefore, is not only an important concept in the field of nutritional science but also an important food value.

Addition of some nutrients has also formed the basis of marketing strategies in product development. In general, the main criteria for selecting nutrients to be added to foods are that they are shown to be necessary, safe and effective *(Technology, 1975)*

The addition of nutrients also requires careful attention to food regulations, labelling, nutritional rationale, cost, the acceptability of the product to consumers and a careful assessment of technical and analytical limitations for compliance with label declarations. *(Richardson, 1990)*.

3.1. CODEX ALIMENTARIUS

According to the General Principles for adding food essential nutrient CAC / GL 09-1987 (amended 1989, 1991) they are intended:

- To provide guidance to those responsible for developing guidelines and legal texts relating to the addition of essential nutrients to foods.
- To establish a uniform set of principles for the rational addition of essential nutrients to foods
- To maintain or improve the overall nutritional quality of food by adding one or more essential nutrients to a food whether or not it is normally contained in the food in order to prevent or correct a deficiency of one or more nutrients in the population or specific population group*(Alimentarius, 2010; Commission, 2009)*

CHAPTER 4. RESEARCH ORGANIZATION

4.1. INSTITUTIONS AND ORGANIZATIONS THAT UNDERTAKE RESEARCH

Locations where this research was conducted were research center "Agricultural and Molecular Research Institute, College of Nyíregyháza" in Hungary and doctoral research laboratories inside the Lucian Blaga University of Sibiu, Faculty of Agricultural, Food and Environmental Protection Sibiu, where the laboratories of these institutions have emerged and completed results to be found in the sentence.

4.2. MOTIVATION AND RESEARCH OBJECTIVES, INDICATORS AND ORGANIZATION RESEARCH STUDIED

4.2.1. Research objectives

The main objectives of the study are:

- Analysis of raw materials in terms of chemical composition and nutritional product in order to optimize the addition of bioactive substances rich in vitamins and minerals.
- Determine the main macronutrients (vitamins, minerals, organic acids, polyphenols) from raw milk and Sea Buckthorn.
- Preparation and characterization of functional dairy product in terms of sensory characteristics, texture and microscopy.
- Improvement and optimization of technology to obtain milk with added biologically active natural compounds.
- Determination of vitamins, minerals and other nutritional values of the product functional compounds, yogurt and health impacts and product characterization in terms of nutrition.

4.2.2. Stages of research

Phases of research were conducted as planned organization of the research presented in (Figure 4.1)

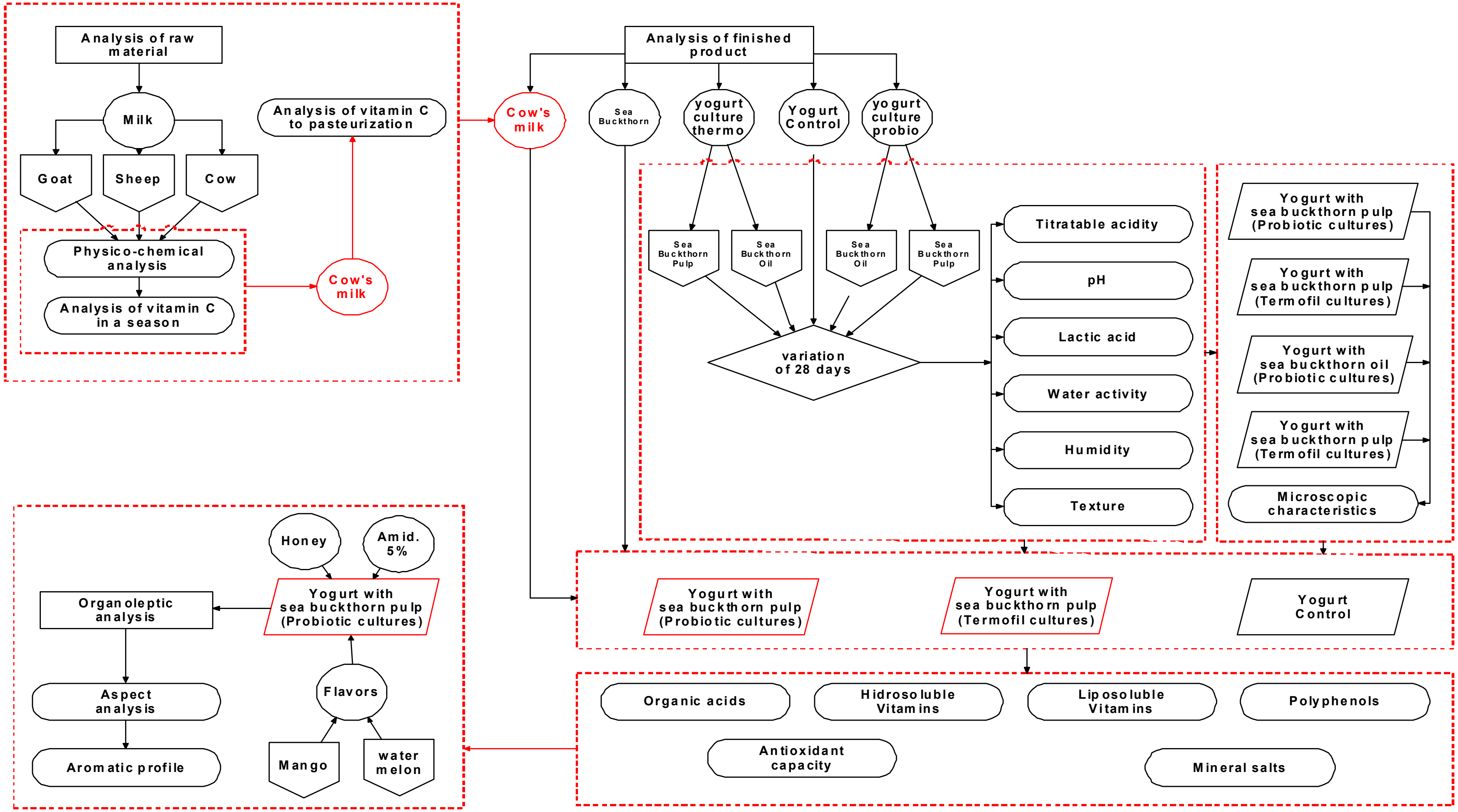


Figure 4.1 *Organization scheme of research*

CHAPTER 5. MATERIALS AND METHODS

5.1. SUBJECT OF MATERIAL RESEARCH

5.1.1. The milk

Sampling was achieved from Alțâna farm , which were then labeled and placed in a cold box at 4 ° C, and were taken to the laboratory where they were analyzed. Sampling procedure was accompanied by a card which included individual identification number of the animal, lactation number, date of harvest, the quantity harvested and physico-chemical indicators analyzed: fat, protein, SNF, lactose, pH, density, conductivity and freezing point. These determinations were made according to season and stage of lactation.

Besides the nutritional aspects, it has been well recogni zed that bioactive components in human milk influence the immune status of the neonate; the bioactivities of milk not only pro vide protection but also ‘‘educate’’ the infant immune system in the early postnat al period *(Baldi, 2005)* , that is why milk is analyzed in terms of raw material quality and nutritional point of view.

5.1.2. Sea Buckthorn

Sea buckthorn form that has been added to yogurt is fresh pulp by removing seeds and oil to maintain viable shaped bioactive components.

We have chosen several forms of Sea buckthorn, and Sea buckthorn in optimal form was added.

Sea buckthorn used was purchased from SC NP Prod SRL, based in Marpod com, Sibiu County and work point in com Rosia, Sibiu County.

Sea buckthorn oil has been used already prepared, supplied by SC Hofigal Export-importance, as one of the leading suppliers of natural products in Romania

5.1.3. Lactic cultures

The main cultures are used:

- Culture Hansen Yo ® Flex.Advance 2.0 consists of the following cultures:
  - *LactobacillusLactis*subsp.Cremoris
  - *Lactobacillusdelbrueueckii*subsp.bulgaricus
  - *StreptococcusThermophilus*
  - *LactobacillusMesenteroidessubsp.*
- Culture ABT-10, Hansen probiotic
  - *LactobacillusAcidophilus*
  - *Bifidobacterium*
  - *StreptococcusThermophilus*
  - *Bifidobacterium*

5.1.4. Yogurt

Yogurt was prepared in the laboratory according to the proposed technological scheme for obtaining yogurt and in accordance with the choice of raw material, by milk of sheep, and cow.

Bioactive yoghurt was obtained with added sea buckthorn with the proportion of 3%, 5%, 7% as fresh fruit pulp and 1%, 2%, 3% sea buckthorn oil, followed by cooling thermostat as planned and storage at 4 ° C.

5.1.5. Honey

Because honey is a complete food for essential amino acids and vitamins necessary for human body. It is therefore desirable future research to obtain honey based products to cover these nutritional needs.

Honey with pollen and honey with Sea Buckthorn meet nutritional requirements to meet the needs of carbohydrates, amino acids, vitamins and a lesser need for fat.

5.1.6. Starch

The second polysaccharide after cellulose is widespread universal vegetable starch. As cellulose, starch is composed only of D-glucose. The plants are in fruit, seeds and tuberculosis, reserve starch, insoluble in water, but can be easily converted into glucose or its derivatives by enzymatic reactions.

5.1.7. Flavors

Because some consumers do not like the taste of sea buckthorn, slightly astringent have used natural essence of melon and mango flavors for being very pleasant and acceptable. Proportion was tested and recommended by the supplier of natural flavors.

Mango (Mangifera indica L.) is one of the most popular tropical fruits due to its different sensorial attributes. Extensive studies of the volatile constituents in mango have identified more than 300 free volatiles *(Shibamoto & Tang, 1990*) and about 70 glycosidically bound compounds *(Pino, Mesa, Muñoz, Martí, & Marbot, 2005)*

5.2. SAMPLE PREPARATION

Samples were encoded by lactic cultures used and the addition of bioactive components of sea buckthorn oil and sea buckthorn pulp.

Samples were in thermophilic culture component Hansen Yo ® Flex.Advance 2.0 were noted with C2, and those that were inoculated with probiotic cultures ABT-10Hansen, probiotic C1. Samples with added oil were marked with U U1 1%, 2%, U2, U3-3% sea buckthorn oil. Samples were dried Sea Buckthorn added / extract were denoted by K, or adding 3% for K1, K2, K3, adding 5% to 7% in milk cultures. Inoculation was made with 20 g culture to 1liter milk. The milk was standardized to a fat content of 2.8% using Pearson's square method.

CHAPTER 6. RESULTS AND DISCUSSION

6.1. RESEARCH ON THE COMPOSITION OF GOAT MILK, COW, SHEEP IN TERMS OF PHYSICAL AND CHEMICAL CHARACTERISTICS

We analyzed raw milk from different animal species or cow, sheep and goat in terms of physicochemical characteristics. The chemical composition of milk varies according to season; feed, age, lactation period as mentioned by *Jenness and Wong (1988)*

Goat milk was the main variation in physical and chemical indicators such as fat ranged between 3.95% and 5.21% in summer/ winter, dry matter ranged between 8.95% and 9.64% summer/ winter, protein between 3, 42% 3.55% winter and autumn, lactose between 4.01% and 4.13% in summer and winter, pH between 6.41% and 6.52% winter/ spring.

Goat milk density in the four seasons remained around 1.029 g/cm3 or 1.033 g/cm^3.

Lactose production is important, as long as it regulates the release of water by breast tissue. At the same time, the quantity of milk is strongly linked to the lactose. Milk lactose concentration is rather constant, generally reaching values of 4.4%.

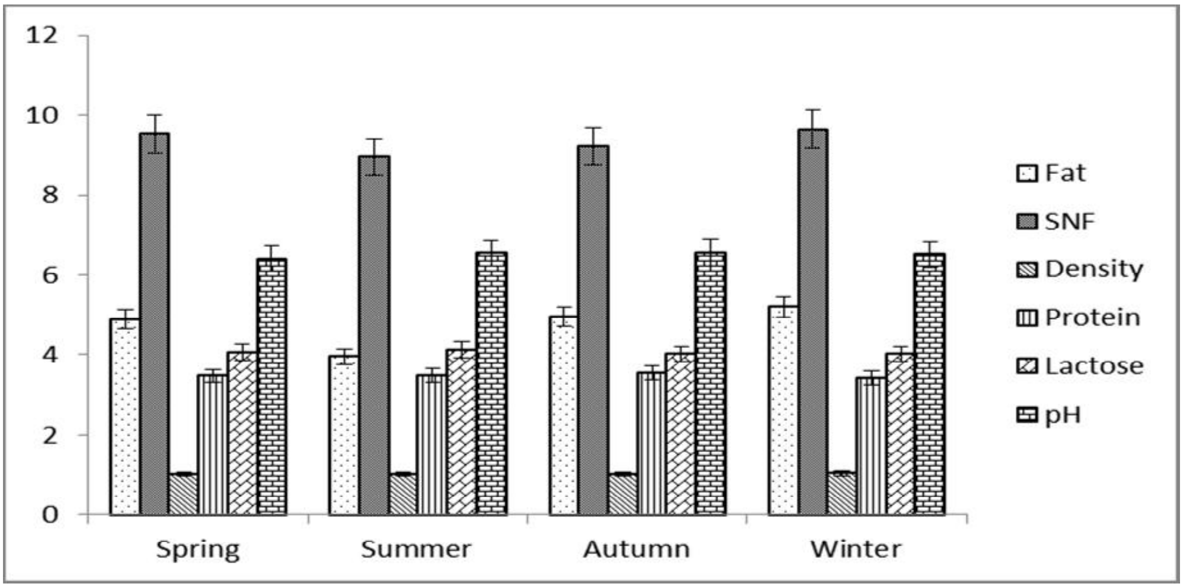


Figure 6.1 Variability of physico-chemical and average values of goat milk according to season from Alțâna farm, Sibiu County

Variability of physico-chemical parameters analyzed for goat milk collected from the farm Alțâna, Sibiu, is shown in Figure 6.1. The results indicate a slight increase for protein, fat and solid not fat in the summer season due to animal feed. Similar data on the variation of chemical and physical parameters were obtained and *Jandal (1996)* to compare these parameters with milk obtained from other species (cow and goat).

Mean maximum solid non-fat obtained from analyzes exceeds the value determined by *Chintescu and Toma (2001)* 8,9%, 8.9%, which was 9.64% in the winter. The content of protein and fat that have the greatest average value of 3.42% throughout the winter because of different feeding during winter. The biological value and digestibility coefficient of goat milk casein were found to be 89.29 and 92.42, respectively *(Kumar, Chandra, & Zachdeva, 1986)*.

Cow's milk had especially important variations in fat content, so it stood between 4.7% and 5.78% autumn/ winter, dry density, protein and lactose were not important variables.

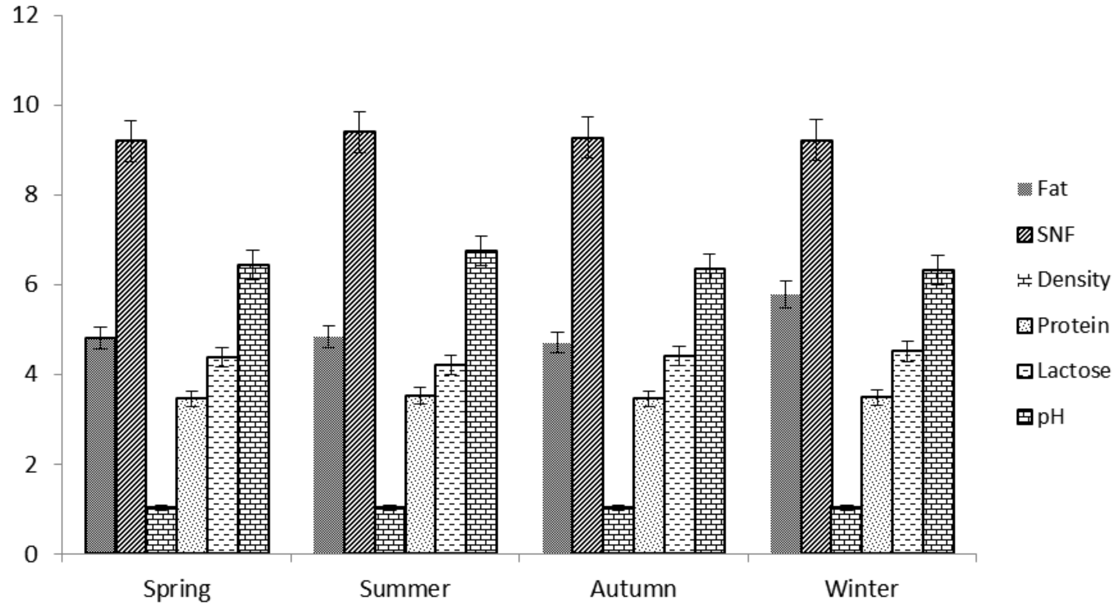


Figure 6.2 Variability of physico-chemical and average values of cow milk according to season from Alțâna farm, Sibiu County

Variability of physico-chemical parameters analyzed for cow's milk collected from the farm Alțâna, Sibiu, is shown in Figure 6.2. The results obtained indicate slight increase in fat in autumn season due animal feed.

If sheep milk experimental results show important variations fat content ranged from 6.99% in winter and summer respectively while 7.17% variation in the protein content of 5.82% and 6.11% in summer and autumn.

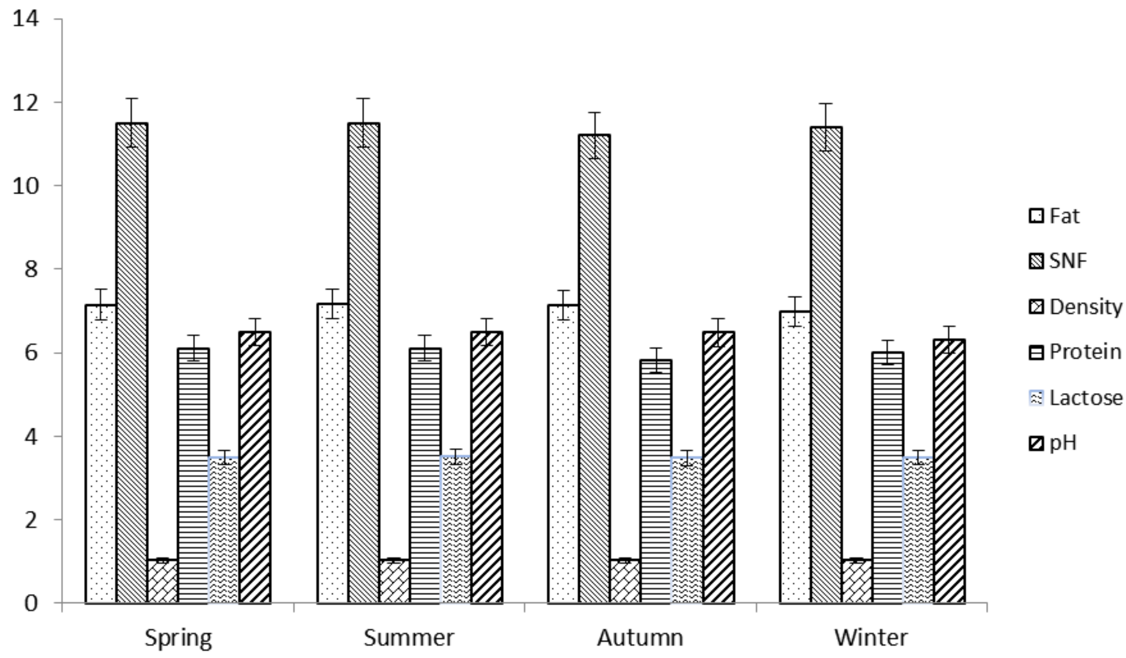


Figure 6.3 Physico-chemical variability and average values of goat milk according to season from Alțâna farm, Sibiu County

Significant variations of physico-chemical parameters analyzed for sheep milk are shown in Figure 6.3, pH, density and lactose remain approximately constant with small deviations compared to fat and protein vary depending on the season.

6.2. SEASONAL VARIATION OF VITAMIN C CONTENT IN SHEEP, GOAT AND COW MILK

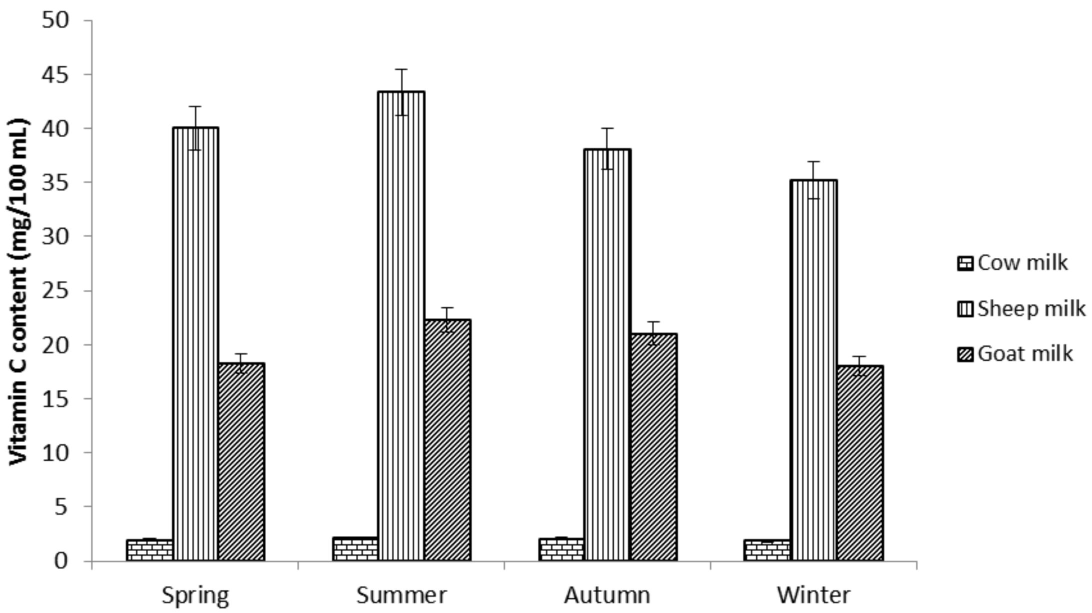


Figure 6.4 Variability ascorbic acid content of cow's milk, sheep and goat milk depending on the season from Alțâna farm, Sibiu County

The content of vitamin C in raw milk collected from 165 cows on the farm of Sibiu Altan was determined by 2, 6-dichloro-fenolindofenol method described by *(Tita Mihaela-Adriana, 2002).*

Monitoring nutritional quality of milk during the period of validity highlighted important factor due to the high sensitivity of vitamins from oxidation, and the ongoing development of Maillard reaction during storage as described made by *Gliguem and Birlouez-Aragon (2005).*

Correlated with experimental data obtained above, *Gliguem and Birlouez-Aragon (2005)* have shown that vitamin C degradation was especially influenced by the type of package, using a 3-layer opaque bottles was associated with complete oxidation of vitamin C by 1 day of storage, while 6-layered opaque bottle, which was an oxygen barrier, in this case vitamin C decreased slowly to reach 25% of the initial concentration after 4 days of storage.

The studies analyzed the vitamin C in quantitative terms for three types of milk, milk generally is a poor source of vitamin C. Vitamin C content was analyzed by seasons, and the results indicate that there are significant differences between values.

Milk composition, cow, goat, sheep regarding major components depend on the season, but also feeding. Levels of vitamin C in raw sheep milk was about five times higher than pasteurized cow's milk, and demonstrated similar studies by *Scott and BISHOP (1986).*

6.3. PASTEURIZATION EFFECT ON ASCORBIC ACID, DEHYDROASCORBIC ACID FROM COW'S MILK

Ascorbic and dehydroascorbic acid (vitamin C), are sensitive to heat and therefore their concentrations in milk are affected by pasteurization.

At this stage of the thesis were determined concentrations of ascorbic acid plus dehydroascorbic acid in milk samples after pasteurization (62.5 0C, 30 min) and (100 0 C, 5 min). Both methods resulted in a significant decrease in the levels of ascorbic acid plus dehydroascorbic acid (93.34% and 66.7%) and ascorbic acid (91.66% and 58.34%). Based on these observations, it is recommended that milk can be heat treated using slow pasteurization. In addition, ascorbic acid is proposed as an indicator of the degree of heat treatment.

In the literature there are several methods of pasteurization developed for this purpose *(Henderson, Fay, & Hamosh, 1998; Israel-Ballard, Chantry, Dewey, Lönnerdal, Sheppard, Donovan, et al., 2005; Lepri, Del Bubba, Maggini, Donzelli, & Galvan, 1997)* which lead to an effective pasteurization with as little loss of vitamins.

Jensen (1995) showed that ascorbic acid and tocopherols are crucial for antioxidant activity and also immunomodulation *Miquel, Alegría, Barberá, Farré, and Clemente (2004)* showed that ascorbic acid and tocopherols are sensitive to light, oxygen and temperature; therefore, their concentrations could be affected by pasteurization.

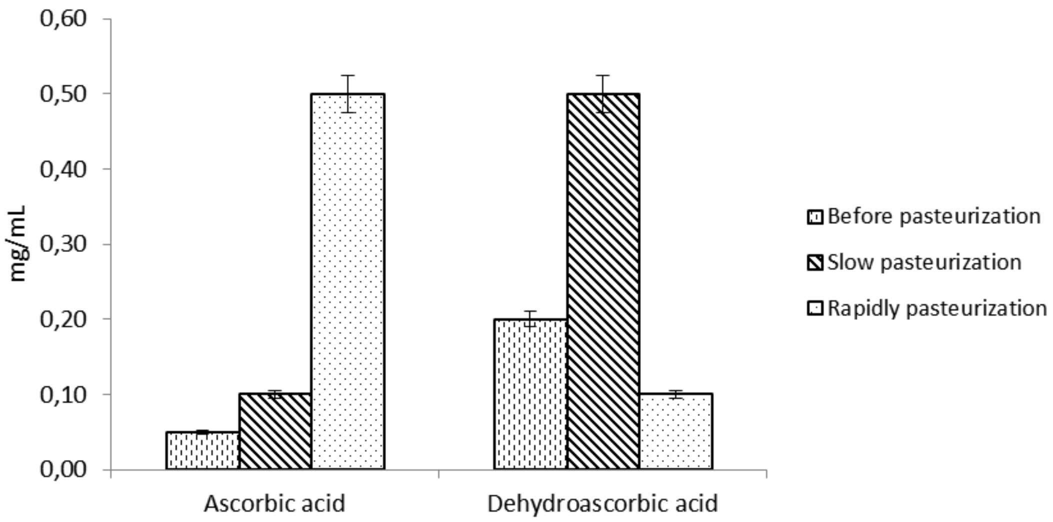


Figure 6.1 Variation of ascorbic acid by applying / not applying heat treatment

To examine the effect of ascorbic acid and dehydroascorbic pasteurization, these compounds were analyzed before and after heat treatments. In Figure 6.5 is shown the effect of pasteurization by slow or fast heating methods which led to a significant decrease ($P <0.05$), in the concentration of ascorbic acid and dehydroascorbic acid by about 93.34% ascorbic acid for slow pasteurization, 66.7% acid ascorbic for rapid pasteurization and 91.66% respectively dehydroascorbic acid for rapid pasteurization, slow pasteurization 58.34% dehydroascorbic acid. Similar studies conducted in the thesis were performed către *Moltó-Puigmartí, Permanyer, Castellote, and López-Sabater (2011)* just for them after pasteurization total amount of vitamin C and ascorbic acid were approximately 20% and 16% lower than untreated samples.

In the literature there are few studies that have addressed the effects of pasteurization on milk ascorbic acid, however research conducted in this thesis emphasize that, as in the case of loss of ascorbic acid in milk caused by the heat treatment may result from increasing the conversion rate of ascorbic acid to dehydroascorbic confirmed by similar studies in this regard by *Naidu (2003)* , while the total average concentration of ascorbic acid detected in fresh samples was similar to that reported so *Buss, McGill, Darlow, and Winterbourn (2001)*. However, the decreases observed in the experimental data of the thesis were higher than the values determined by *Van Zoeren-Grobben, Schrijver, Van den Berg, and Berger (1987)*a nd *Randoin and Perroteau (1950)*, which used a shorter pasteurization cycle (65 0C, 20 min).

6.4. VARIATION OF TITRATABLE ACIDITY

6.4.1. Variation of titratable acidity

Changes in titratable acidity for yogurt with added sea buckthorn oil, inoculated with probiotic cultures had values between 85 to 140 ^{0}T from the first day of inoculation until day 28 when C1U1 sample with 1 ml of sea buckthorn oil, 90^{0}T up at 153 ^{0}T from day 0 to day 28 when C1U2 sample with 2 ml of sea buckthorn oil and if C1U3 sample with values between 97 to 160 ^{0}T from final day to day 0. Compared with the values obtained for the blank (no added sea buckthorn oil) sea buckthorn oil sample values were lower and therefore the addition of sea buckthorn oil influences the fermentation process and the development of lactic cultures.

If adding yogurt samples obtained from culture of thermophilic acid Sea Buckthorn where C2U1 sample with addition of 1 ml of sea buckthorn oil was from 102 to 150 ^{0}T from the first day until day 28, the acidity of the sample C2U2 ranged from 134^{0}T up to 166 ^{0}T from day zero to day 28 and if C2U3 sample with 3 ml of sea buckthorn oil acidity ranged from 130^{0}T to 170^{0}T from day zero to day 28. Compared with the control sample that had ranging from 130 up to 175 ^{0}T yogurt samples with added sea buckthorn thermophilic cultures obtained were close so the addition of sea buckthorn oil has influenced the development of lactic cultures.

Variation acid in yogurt samples obtained with the addition of sea buckthorn pulp was 100 to 150 ^{0}T C1K1 sample from the first day until day 28, C1K2 sample acidity ranged from 113 to 114 ^{0}T from day 0 to day 28 and if C1K3 sample acidity values were 125 to 168 ^{0}T compared with values obtained for blank samples added Sea Buckthorn directly influenced the acidity because Sea Buckthorn contains many acids.

Acid variation yogurt with added sea buckthorn and thermophilic culture was inoculated with high levels of acidity than the above options, as follows: C2K1 sample had values of 137 to 160 ^{0}T from the first day until day 28, the sample C2K2 acidity values were from 145 to 172 ^{0}T and acidity C2K3 sample ranged from 158 to 180 ^{0}T, compared to the values obtained for blank samples can be seen that the values for yogurt with added sea buckthorn were much higher therefore the addition of sea buckthorn positively influence digestion and also there was an acid intake contributed to some extent to increased acidity.

The measurement is a composite one including the natural acidity of the milk and the developed acidity arising from bacterial activity but, as the natural acidity should not vary a great deal (assuming that the milk is standardised for total solids), titratable acidity is a reasonable indication of the performance of the starter culture. (Tamime & Robinson, 1999).

6.5. VARIATION OF pH

The most important buffering components of milk are caseins, phosphates and citrates, although quantitative assignment of the buffering capacity to these constituents is rather dificult. *(Bhandari & Singh, 2003)*

pH variation for the test sample was determined over a period of 28 days, measuring the pH of 7 in 7 days from the first day.

In samples of yogurt with probiotic cultures obtained and added sea buckthorn oil were obtained variations characterized by a slight increase and then decrease as follows: C1U1 sample originally had a pH of 4.3 and then increased at day 14 remained constant at 4.6 weeks and again at day 28 decreased the value of 4.1, C1U2 initial sample pH was 4.2 with a slight increase in the next 14 days to 4.5 and then a sudden drop in on 28 for 4. C1U3, if the initial sample had a pH of 4.1 to 4.3 increased after 7 days and remained constant throughout the period, compared with the control sample that initially had pH of 4.6 decreased to 4.3 throughout the period. Increases in pH during storage period of 28 days due to Retrieved buckthorn oil, compared with the control fermentation processes where pH decrease was consistent.

Yoghurt samples produced by thermophilic culture with added oil had similar variations of the above as follows: C2U1 sample had initial pH 4.6 and decreased to 4.1 C2U2 initial sample had pH 4.3, on day 7 increased to 4.4 remained constant until day 21, then it began to fall, and in C2U3 sample pH was initially 4.2, decreased slightly on day 7, maintaining constant until day 28. Comparing the data with the control sample that was initially approached pH 4.7 C2U1 this sample decreased uniformly until the day 28-4 pH can say that the addition of sea buckthorn oil significantly influenced by pH variations in samples that have sea buckthorn oil had more C2U2 and that C2U3.

PH variation for samples of yoghurt and probiotic cultures added sea buckthorn pulp had lower values in samples with higher added Sea Buckthorn as follows: C1K1 sample originally had reached 4.6 pH that dropped to 4.3 on day 28, the sample C1K2 originally had remained constant 4.3, 14 days after the day 28 decreased to 4 and C1K3 sample initially had a pH of 3.9 reached after a period of 28 days to 3.8 i.e. the lowest value of all samples. Compared with the control sample which was initially 4.6 and finally 4.2 it can be seen that the addition of sea buckthorn pulp pH influenced by a greater decrease in samples containing higher Sea Buckthorn similarly as acidity.

Samples with the addition of Sea Buckthorn and inoculated with thermophilic culture variations were observed similar to those obtained from probiotic cultures, pH values were 4.3 for sample C2K1 first day to 4.1 on day 28, for C2K2 initial sample pH was 4.4 and decreased on day 14 to 4, remained constant throughout the period of analysis and sample C2K3 initial pH was 4.1 and at day 28 reached 3.8, this value was the lowest of all samples analyzed, compared with the control which was initially 4.7 and 4.3.

Finally it can be seen that the addition of sea buckthorn influenced similarly as for pH determination of acidity, this mainly due to the acid content of sea buckthorn.

6.6. VARIATION OF LACTIC ACID

Variation of lactic acid was determined over a period of 28 days; measurements were made at an interval of 7 days.

If C1U1 lactic acid sample has values between -9.7- 7.63 g/100 g of product from the first day until day 28 compared with lactic acid sample C1U3 where minimum values are between 0.33-2.9 g /100 g sample and the control sample without addition of sea buckthorn oil, in which sample values are approximately similar C1U1. As can be seen the addition of seabuckthorn oil increased lactic acid content negatively.

If lactic acid C2U1 sample values between -17.96- 11.6 g/100 g of product from the first day until day 28 compared with lactic acid sample C2U3 where minimum values are between 3.15-5.9 g/100 g sample and the control sample without added Sea buckthorn oil where sample values are approximately similar C2U1. As we see the addition of sea buckthorn oil negatively affect growth of lactic acid content similar to yogurt from probiotic culture.

If thermophilic yogurt with added sea buckthorn oil, so if C1K1 lactic acid sample has values between -17.3-11.46 g/100 g of product from the first day until day 28 compared with the sample C1K3 where lactic acid peaks recorded between 18.45- 25.8 g/100 g sample and the control sample without pulp Sea Buckthorn where sample values are approximately similar C1K2. As we see the addition of pulp sea buckthorn positively increase lactic acid content. Increased lactic acid content may be due to a small extent of sea buckthorn pulp addition of organic acids the contents Sea Buckthorn.

If C2K1 lactic acid sample has values between -24.1- 19.26 g/100 g of product from the first day until day 28 compared with the sample C2K3 where lactic acid peaks recorded between 26.57-28.0 g/100 g sample and the control sample without pulp the Sea Buckthorn where the values are approximately similar C2K2 sample. As we see the addition of pulp Sea Buckthorn influence Sea Buckthorn enrichment lactic acid positively. Increases in lactic acid Content may be due to a small extent and sea buckthorn pulp due to its content addition of organic acids in it. As shown Sea Buckthorn influence addition of pulp positively, increased lactic acid content similar to yogurt from probiotic culture.

6.7. VARIATION OF MOISTURE AND WATER ACTIVITY

6.7.1. Change in water activity

Water activity varies from day 0 to day 28 to test C1U1 from 0.901 – 0.995 and in C1U3 sample water activity was from 0.912-0.975 compared with the control where water activity ranged from 0.89-0.97.

Water activity varies from day 0 to day 28 to test C2U1 from 0.961 – 0.972 and in C2U3 sample water activity was from 98.2-99.8 compared with the control where water activity ranged from 0.978-0.992, values recorded were far superior and without significant variations when compared with yogurt (probiotic cultures) samples obtained.

Water activity varies from day 0 to day 28 to test C1K1 from 0.90 – 0.987 and in C1K3 sample water activity was from 0.902-0.945 compared with the control where water activity ranged from 0.912-0.978.

Water activity varies from day 0 to day 28 to test C2K1 from 0.97 -0.998 and in C2K3 sample water activity was from 0.979-0.996 compared with the control where water activity ranged from 0.98-0.999 , values recorded were far superior and without significant variations when compared with yogurt (probiotic strain) samples obtained.

6.7.2. Humidity variation

Humidity varies from day 0 to day 28 to test C1U1 from 86.74-86.94% and C1U3 sample humidity was from 87.32-87.52% compared with the control where humidity ranged from 88.23-88.43%, variations in the analyzed period of 28 days were very small tending to constant values.

Humidity varies from day 0 to day 28 to test C2U1 from 86.84-87.12% and C2U3 sample humidity was from 85.78-86.06% compared with the control where humidity ranged from 84.76-85.04%, variations in the analyzed period of 28 days were extremely small almost constant.

Humidity varies from day 0 to day 28 to test C1K1 from 86.74-86.94% and C1K3 sample humidity was from 88.9-89.22% compared with the control where humidity ranged from 89.1-89.42% recorded varaţion in the analyzed period of 28 days were extremely small almost constant.

Humidity vary from day 0 to day 28 to test C2K1 from 85.2-85.56% and if C2K3 sample humidity was from 86.98-87.34% compared with the control where humidity ranged from 84.76-85.12%.

6.8. VARIATION OF TEXTURE PARAMETER

Texture means diferent things to diferent people, and the perceived textural characteristics expected from diferent dairy foods vary widely. In general, the food texture is evaluated using instrumental and sensory methods *(Rosenthal, 1999; Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004)*.

The perceived textural attributes of a given dairy food are influenced by a variety of factors such as how we chew the food and how much chewing force is applied. Given this complexity, it is very diifcult, if not impossible, to objectively measure and characterize texture. Therefore, human sensory evaluation has been the cornerstone of food texture characterization. Humans are particularly sensitive in identifying textural diferences between two samples, whereas instruments can quickly provide a quantitative measurement on an absolute scale. Owing to limitations of cost and variety of new dairy food, eforts are continually made in designing instrumental methods for texture evaluation. *(Nollet & Toldra, 2009)*

In this chapter, the aim is primarily to measure the textural attributes from thermophilic and probiotic yogurt.

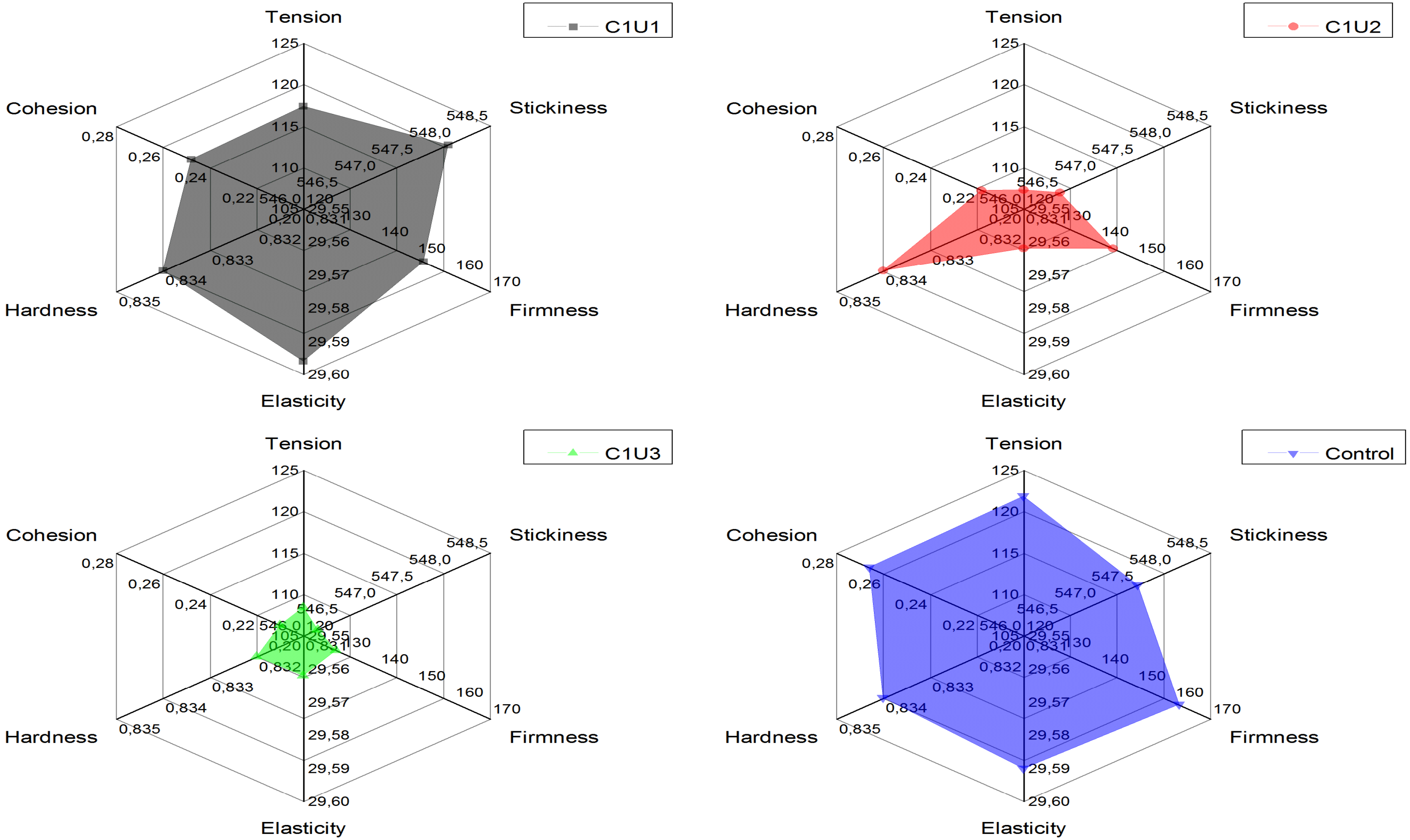


Figure 6.5 Textural characteristics of yoghurt with added of sea buckthorn oil (probiotic cultures)

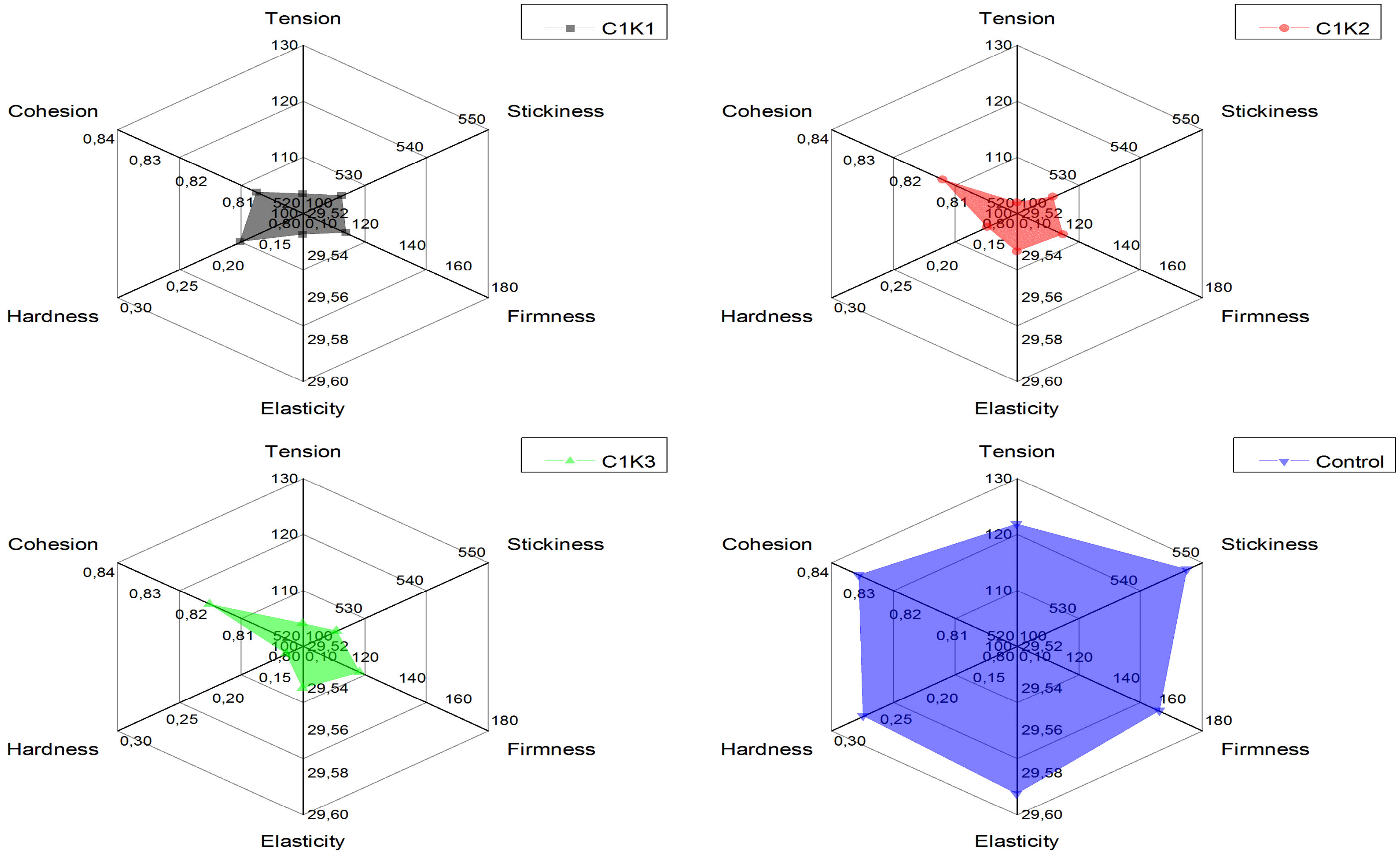


Figure 6.6 Textural characteristics of yoghurt with added of sea buckthorn pulp (probiotic cultures)

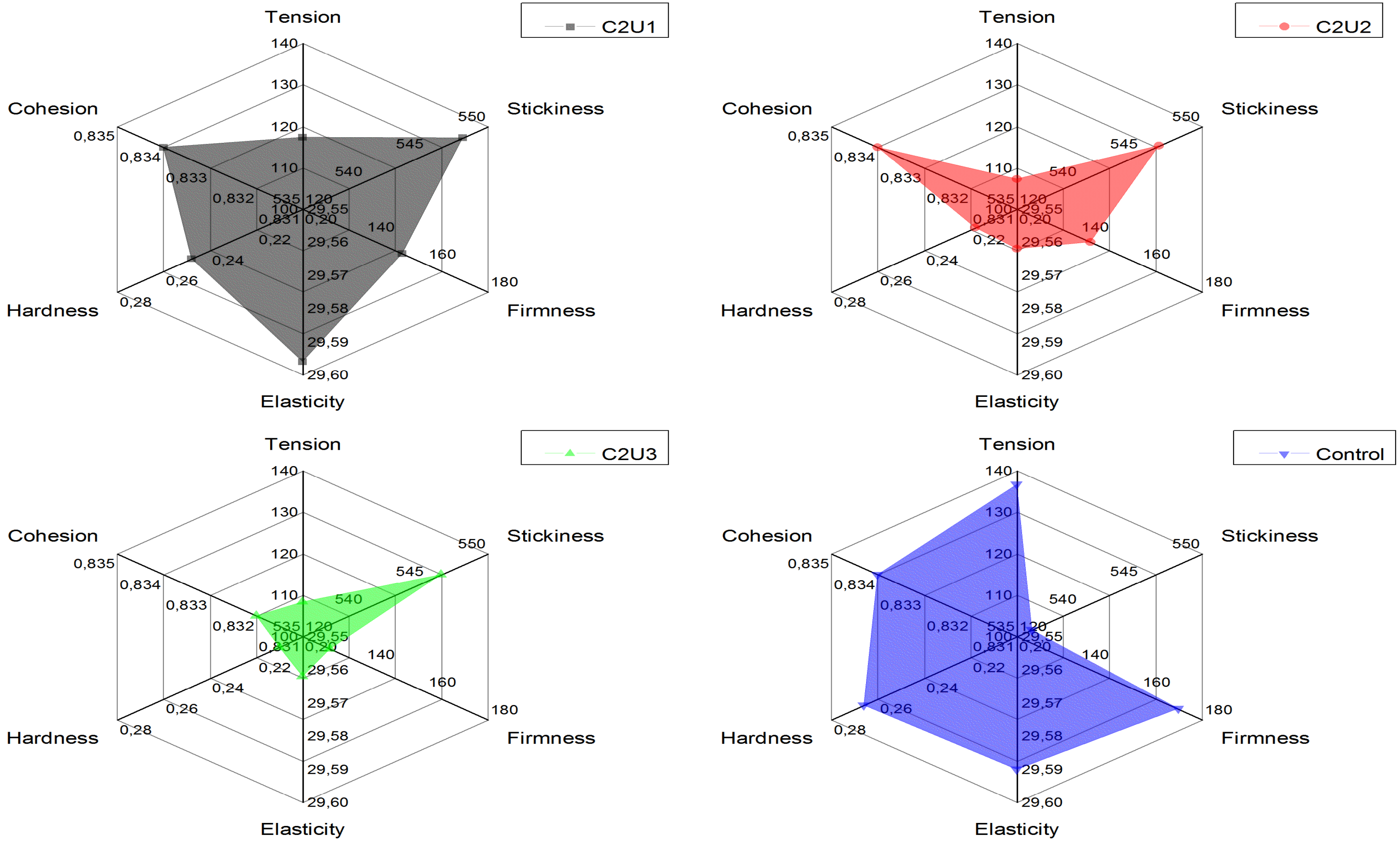


Figure 6.7 Textural characteristics of yoghurt with added of sea buckthorn oil (thermophilic culture)

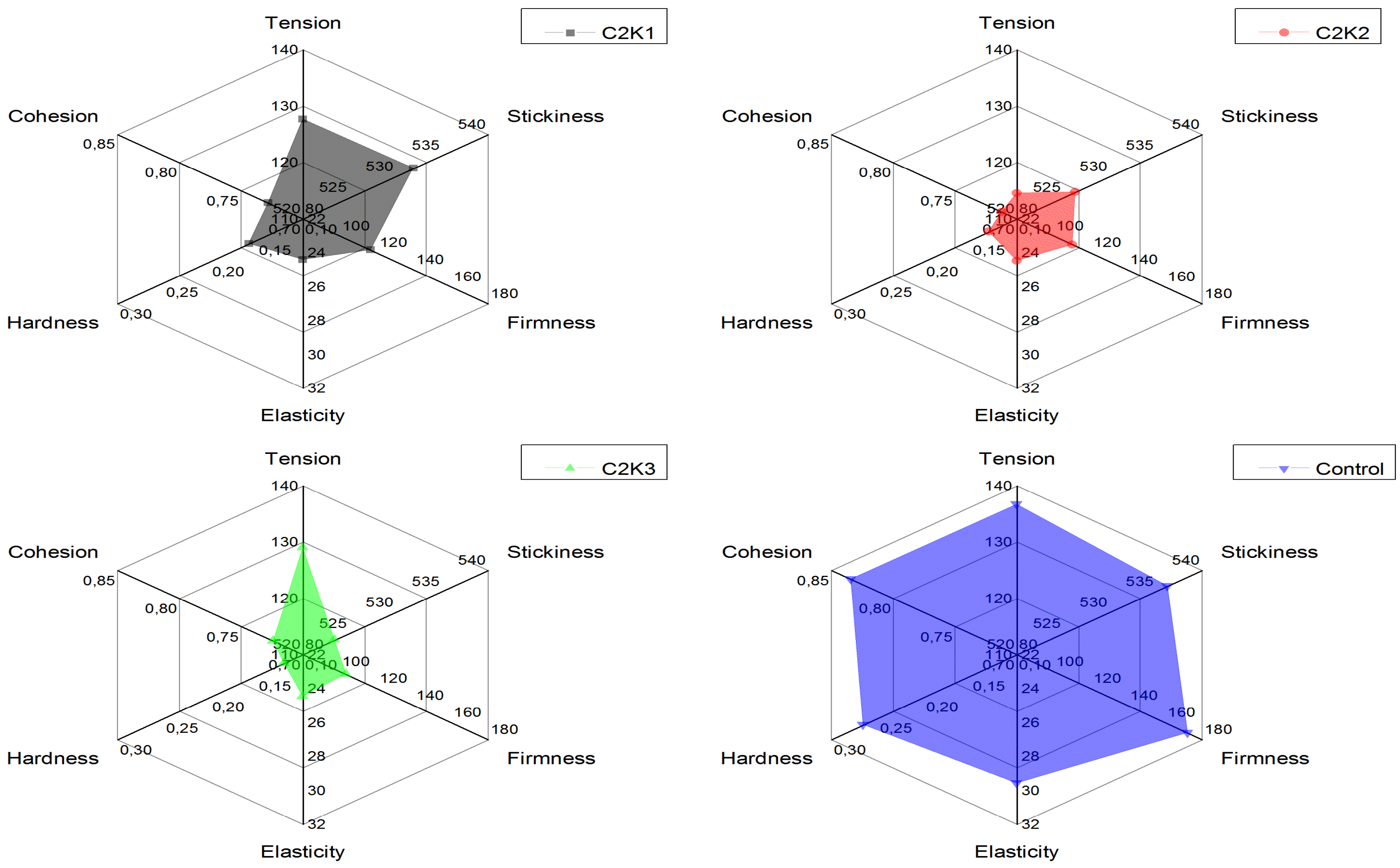


Figure 6.8 Textural characteristics of yoghurt with added sea buckthorn pulp (thermophilic culture)

6.9. MICROSCOPIC CHARACTERISTICS OF YOGURT STRUCTURES

6.9.1. Yogurt with Sea buckthorn pulp (probiotic cultures)

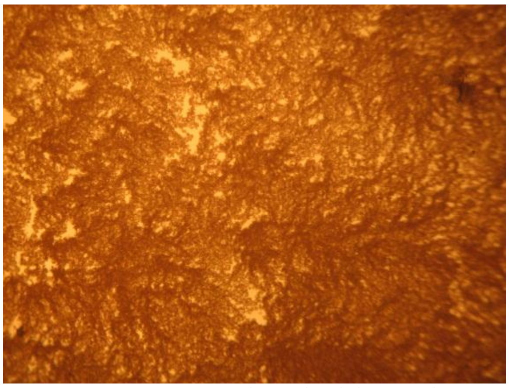


Figure 6.9 Yogurt with sea buckthorn pulp 5x

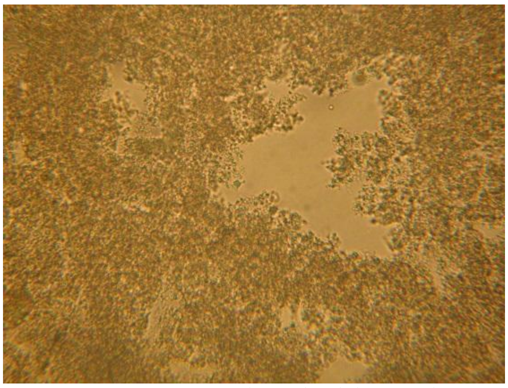


Figure 6.10 Yogurt with sea buckthorn pulp 10x

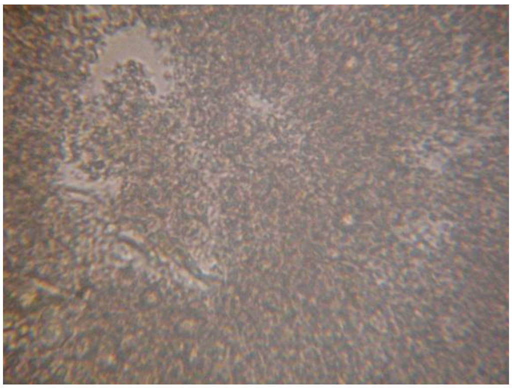


Figure 6.11 Yogurt with sea buckthorn pulp 45x

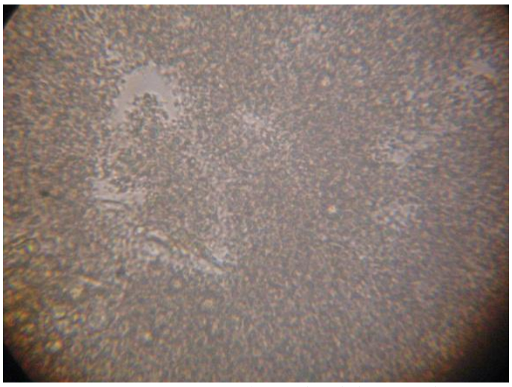


Figure 6.12 Yogurt with sea buckthorn pulp 90x

In figure 6.9 we can see the yogurt with Sea Buckthorn observed through a microscope lens 5x, it presents the structure homogeneous in the picture Figure 6.10 can be seen in more detail sea buckthorn pulp yogurt with a 10x objective in this case is homogeneous structure yogurt. In Figure 6.11 we can see the yogurt with sea buckthorn pulp observed under a microscope with a 45x objective, it presents the structure homogeneous in the picture Figure 6.12 can be seen in more detail yogurt with Sea Buckthorn with a 90x objective in this case yogurt has homogeneous structure

6.9.2. Yogurt with Sea buckthorn oil (probiotic cultures)

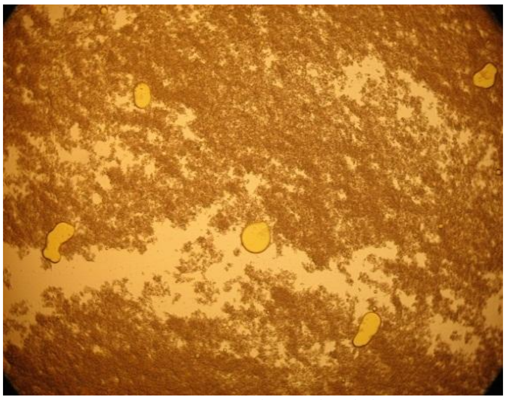


Figure 6.13 Yogurt with Sea buckthorn oil 5x

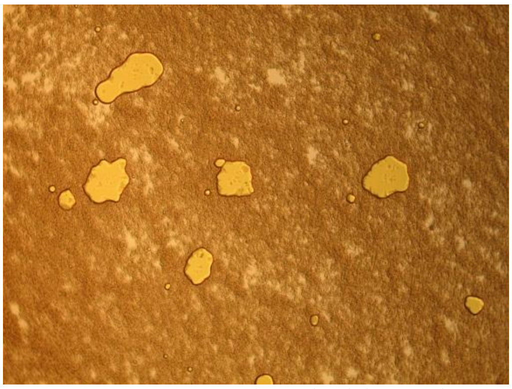


Figure 6.14 Yogurt with Sea buckthorn oil 10x

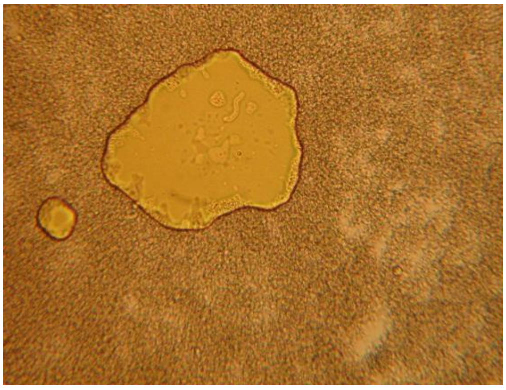


Figure 6.15 Yogurt with Sea buckthorn oil 45x

Figure 6.16 Yogurt with Sea buckthorn oil 90x

In Figure 6.13 we can see the appearance of sea buckthorn oil yogurt observed through a microscope lens 5x, it presents the structure of sea buckthorn oil dispersed uniformly in the image above, in figure 6.14 can be seen more uniformly dispersed oil in yogurt.

In Figure 6.15 you can see sea buckthorn oil globule yogurt separately identified in the table showing the distribution uniformity of yogurt, as in Figure 6.16 oil globules are obvious, also Lactococcus Lactis strain was observed.

6.9.3. Yogurt with Sea buckthorn pulp (thermophilic culture)

If thermophilic yogurt culture is observed in optical microscope, small globular formations and its composition in different quantities and we indicate differences due to cultural homogeneity use.

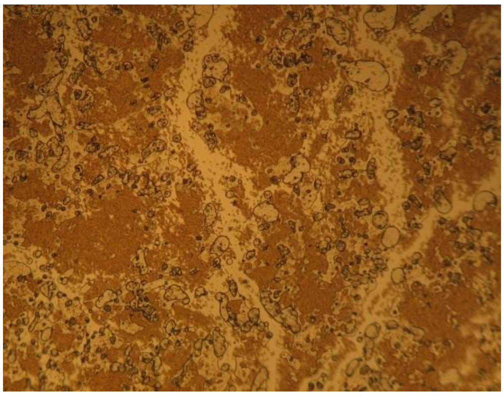


Figure 6.17 Yogurt with Sea buckthorn pulp 5x

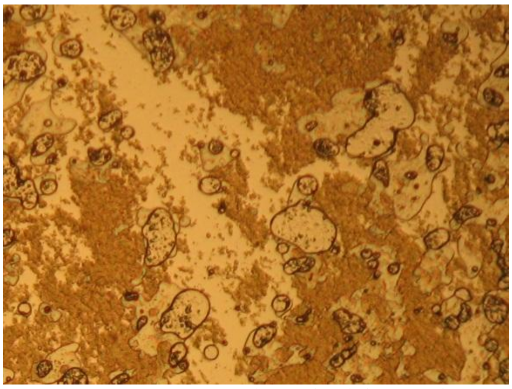


Figure 6.18 Yogurt with Sea buckthorn pulp 5x

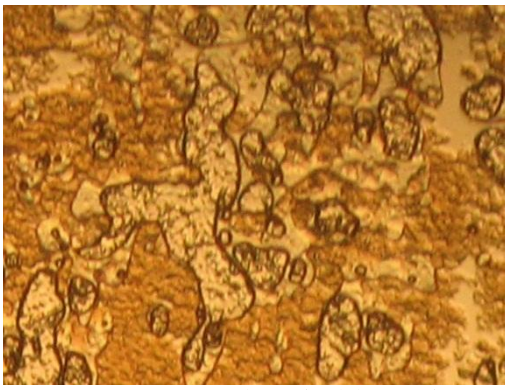


Figure 6.19 Yogurt with Sea buckthorn pulp 45x

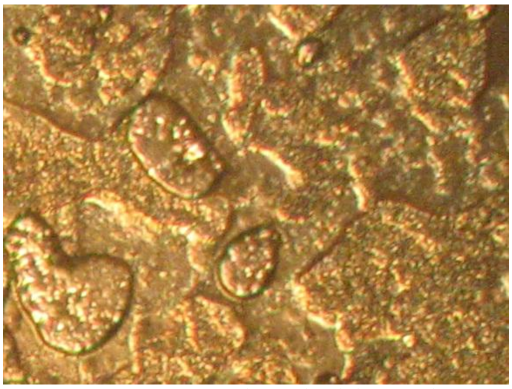


Figure 6.20Yogurt with Sea buckthorn pulp 90x

In Figure 6.17 we can see the look of yogurt with sea buckthorn pulp observed through a microscope lens 5x, it presents uneven and unstable structure in the image above figure 6.18 can be seen more fat globules and air uniformly dispersed in yogurt, increased by an objective 10x.

Figure 6.19 is observed with a 45x objective more air and tend synersis due to thermophilic culture and in Figure 6.20 with an objective 90x more complex structure.

6.9.4. Yogurt with Sea buckthorn oil (thermophilic culture)

Considering the above cases in order to stabilize the sea buckthorn oil in yogurt and to obtain textural characteristics appropriate.

Figure 6.20 Yogurt with Sea buckthorn oil 5x

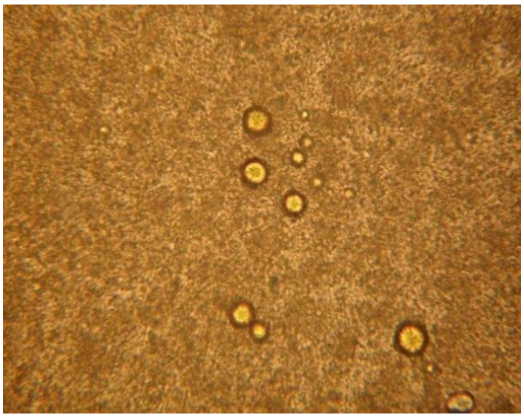


Figure 6.21 Yogurt with Sea buckthorn oil 10x

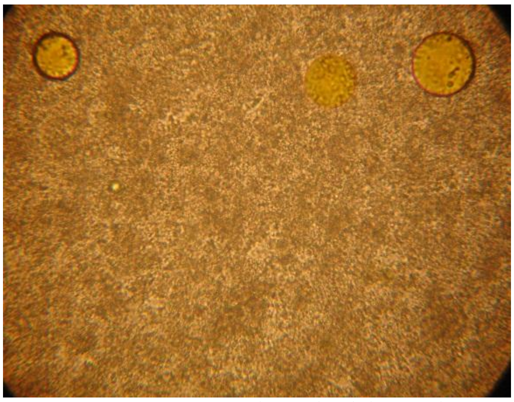


Figure 6.22 Yogurt with Sea buckthorn oil 45x

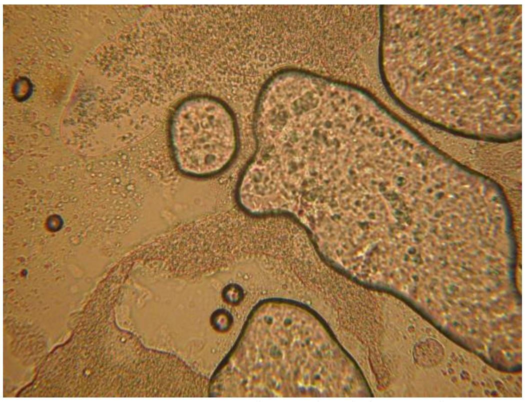


Figure 6.23 Yogurt with Sea buckthorn oil 90x

In Figure 6.20 we can see the appearance of sea buckthorn oil yogurt and thermophilic culture seen through a microscope lens 5x, it presents uniform structure and sea buckthorn oil globules in this case are spread more evenly.

Figure 6.21 in the image can be seen more sea buckthorn oil globules dispersed uniformly in yogurt marry a 10x objective in Figure 6.22 is observed with a 45x objective fewer and sea buckthorn oil globules in Figure 6.23 as an objective 90x.

6.10. CONTENT OF ORGANIC ACIDS

Organic acids in dairy products come from the growth of microorganisms *(Langsrud & Reinbold, 1973; Upreti, McKay, & Metzger, 2006; Zeppa, Conterno, & Gerbi, 2001)* and hydrolysis of milk fat *(Bevilacqua & Califano, 1989; De Jong & Badings, 2005; F., Virto, Martin, Najera, Santisteban, Barron JR, et al., 1997)*.

The roles organic acids play in dairy products include reducing pH, contributing to flavor attributes, inhibiting spoilage and pathogenic microorganisms, and indicating bacterio-logical quality *(Collins, McSweeney, & Wilkinson, 2003; Drake, Carunchia Whetstine, Drake, Courtney, Fligner, Jenkins, et al., 2007; Marshall & Harmon, 1978)*.

The largest amount of acid in sea buckthorn was the (malic acid 1.851 g / L) while the highest proportion of acid found in milk and yogurt (lactic acid 0.254,1.352 g / L), respectively (malic acid 0.088 g / L and 0.074g / L).

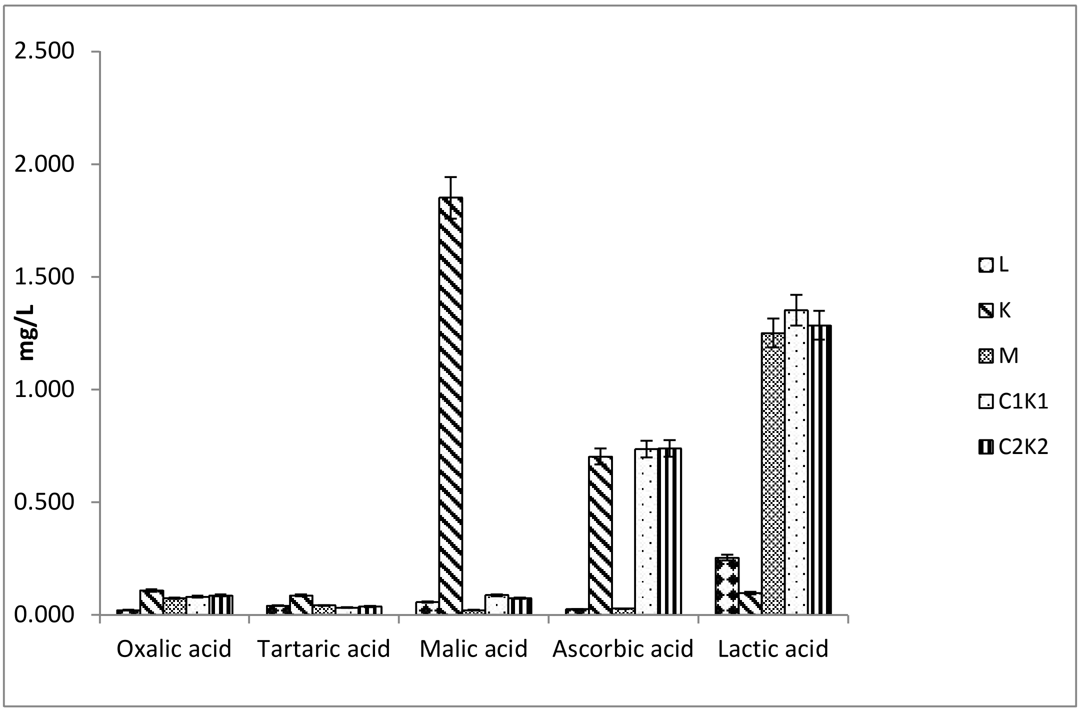


Figure 6.24 Variation of organic acids

6.11. CONTENT OF SOLUBLE VITAMIN

In terms compared to soluble vitamin content of all samples analyzed, the highest amount of vitamin B1 was found in the pulp of sea buckthorn (0.0523 g / L) and vitamin B5 in milk (0.043 g / L) while the proportion more than vitamin B1 was found in milk (0.003 g / L) and vitamin B7 (0.0006 g / L). between probiotic yogurt with sea buckthorn pulp and thermophilic yogurt with sea buckthorn pulp semnificativeîn differences regarding the content of vitamin B2 such probiotic yogurt is 0.00324 g / L while thermophilic yogurt with sea buckthorn pulp is much more that 0.0257 g / l. There are also differences in the case of vitamin B7 is in a much greater 0.00145 g / L at sea buckthorn pulp yogurt with probiotics compared with 0.0025 g / L at sea buckthorn pulp thermophilic yogurt. Differences also exist Siin vitamin B1 which probiotic yogurt with sea buckthorn pulp is 0.00234 g / L and thermophilic yogurt pulp of sea buckthorn 0.00025 g / L, the differences between the samples vitamins yogurt probiotic cultures are due to activity.

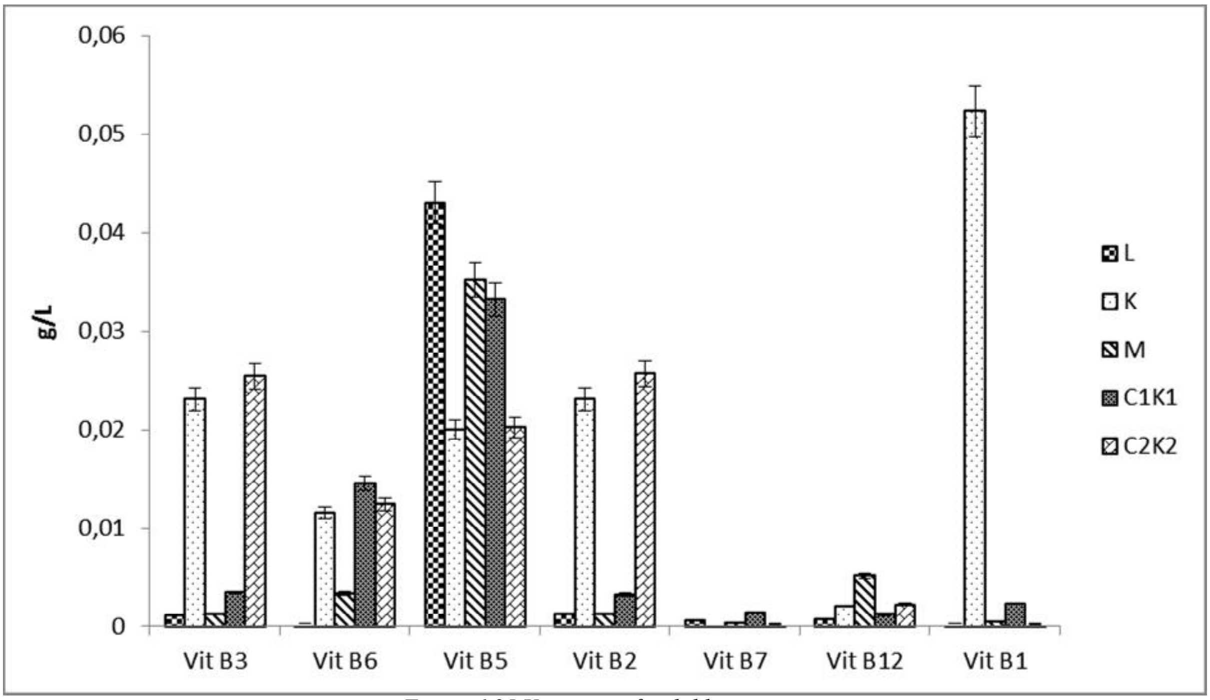


Figure 6.25 Variation of soluble vitamins

6.12. CONTENT OF FAT SOLUBLE VITAMIN

In all samples analyzed, the highest amount of vitamin K1 found in milk and yogurt samples was (0.02557 g / L) and that vitamin A probiotic yogurt (0.024162 g / L) while the smallest proportion of vitamin K2 was found in milk (0.000002 g / L).

From milk, yogurt and Sea Buckthorn it seems no difference in terms of some fat-soluble vitamins such vitamin D3 and E is found in much larger quantities in sea buckthorn pulp.

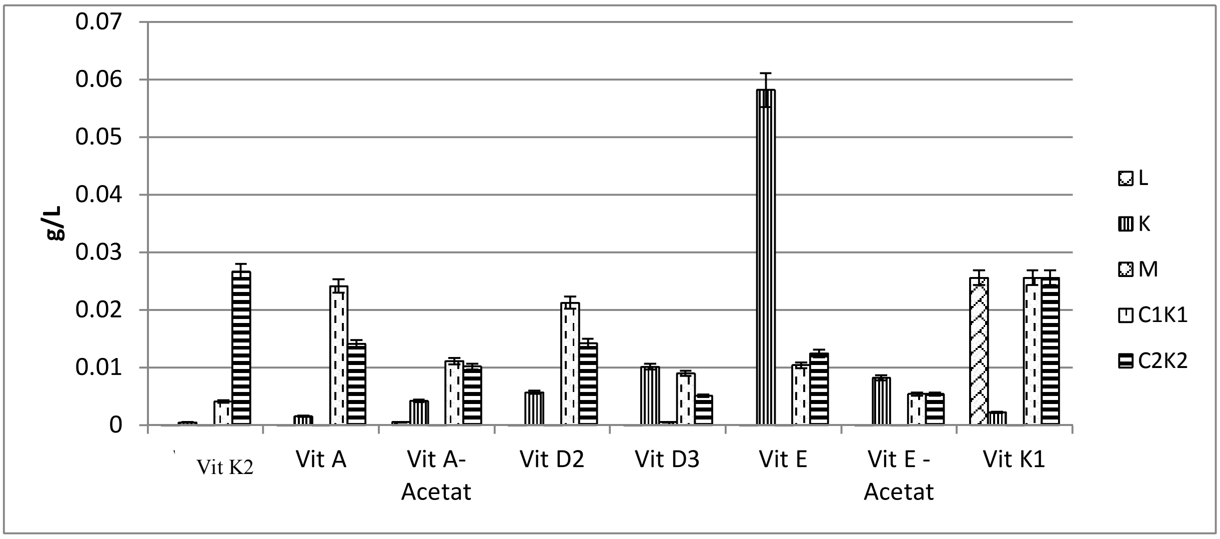


Figure 6.26 Variation of fat soluble vitamin

6.13. CONTENT OF MINERALS

Variation is significant especially in minerals like that found in the highest amount in probiotic yogurt with sea buckthorn pulp 1452.75 mg / kg. A significant contribution was in milk containing as 1120.23mg/Kg and the smallest amount in the Sea Buckthorn 319.24 mg / kg. According to other research, the Ca concentration in bovine milk is relatively constant, about 1 g/L. Contents of Mg and Zn in milk also show only small variations. Mg is ubiquitous in foods, and milk is a good source, containing about 100 mg/L. Zn is an essential part of several enzymes and metalloproteins. Milk is a good Zn source, containing about 4 mg/L *(Haug, Hostmark, & Harstad, 2007)*.

The mineral amount found in probiotic yogurt is largest, as the Sea Buckthorn had an outstanding contribution containing Cu of 7.32 mg / kg, while the lowest amount was found in milk, Fe was found in the amount higher in probiotic yogurt with pulp made from sea buckthorn, sea buckthorn intake containing 3212.24 mg / kg instead the smallest amount found in yogurt (control), Mg was found in the highest amount in the Sea Buckthorn 1035.54 mg probiotic yogurt / kg intake was provided by Sea Buckthorn to 857.58 mg / kg, instead the smallest amount found in yogurt (control), Zn was found in the highest amount in probiotic yogurt with sea buckthorn pulp of 18.24 mg / kg and contribute to 14.25mg/kg and had Sea Buckthorn with a smaller amount found in milk as mentioned by *Haug, Hostmark, and Harstad (2007)* as Zn is an essential part of many enzymes and metalloproteins, Milk is a good source of zinc, containing about 4 mg / L.

As seen mineral salt content in Mg, Zn had Sea Buckthorn pulp. A small proportion of Ca is also bound to α-lactoalbumin, with one atom of Ca per protein molecule *(Hiraoka, Segawa, Kuwajima, Sugai, & Murai, 1980)*. A large proportion of Cu, Zn, and Mn is bound to casein. Fe and Mn are partly bound to lactoferrin *(Renner, 1989)*.

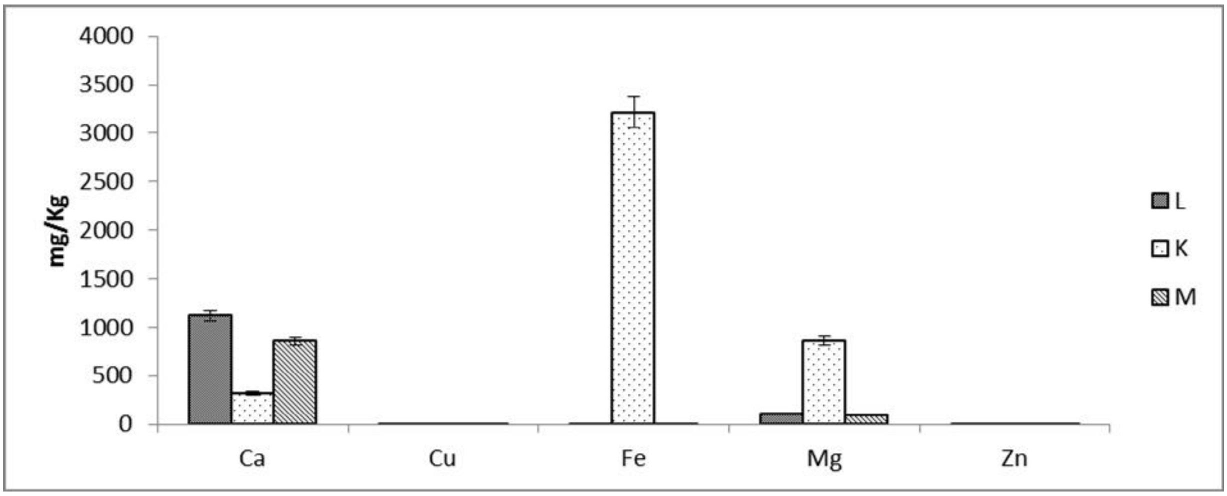


Figure 6.27 Content of minerals

6.14. CONTENT OF TOTAL POLYPHENOLS

As shown in milk and yogurt (blank) the polyphenols are in a large amount found in probiotic yogurt with Sea Buckthorn of 762 mg gallic acid /L, this value is due to the contribution of Sea Buckthorn in composition to that found 754 mg gallic acid /L.

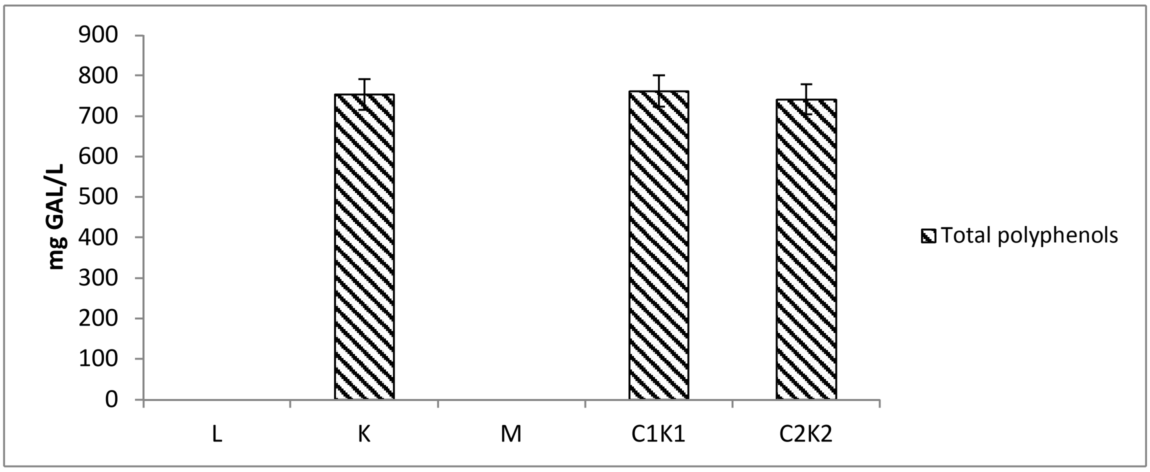


Figure 6.28 Variation of polyphenols

6.15. ANTIOXIDANT CAPACITY

Milk had detectable values for antioxidant capacity instead found quantifiable values representative of probiotic yogurt with sea buckthorn pulp and important contribution had Sea Buckthorn.

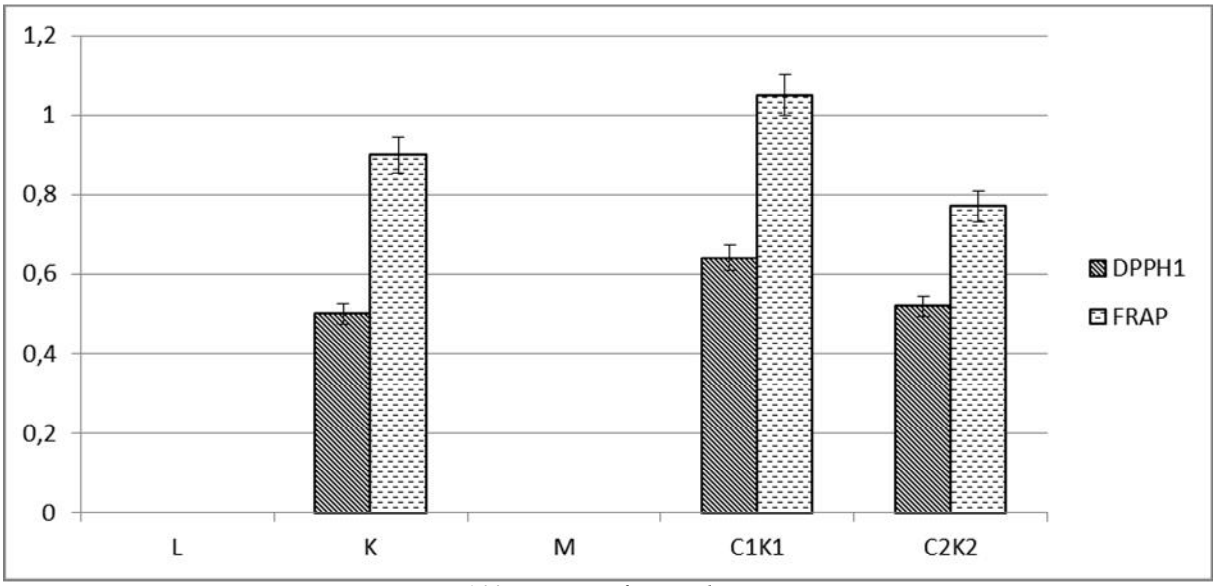


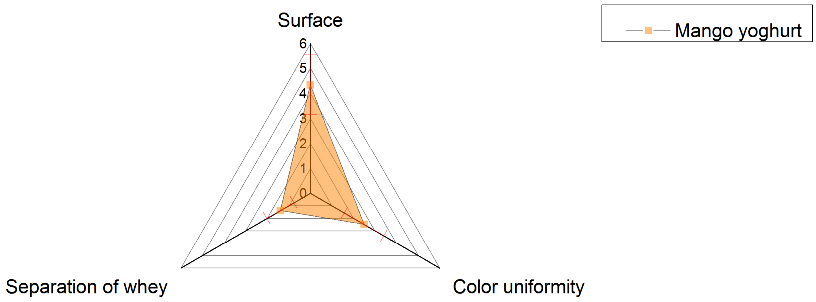
Figure 6.29 Variation of antioxidant capacity

6.16. ORGANOLEPTIC ANALYSIS FINISHED PRODUCT

6.16.1. The features regarding appearance of sea bucktorn pulp yogurt with mango and melon flavors

Given the outstanding results with added probiotic culture buckthorn pulp has gone on improving the nutritional value of yogurt in terms of flavor. Sea buckthorn performed very well in terms of sensory, color, texture but after some consumers to walk the extra option of added flavors (mango and melon) for added flavor and yogurt accepted by all consumers.

Mean for watermelon yogurt was 4.09 points and for the mango flavor 4.34 points from a maximum of 5 points on the scale of values ("very strong") for color consistency scores were 2.37 points yogurt with melon and 2.49 indicating a moderate uniformity of color and if whey separation scores were 1.24 for watermelon flavored yogurt and 1.38 for mango flavored yogurt, values indicating a very low synersis to the poor.



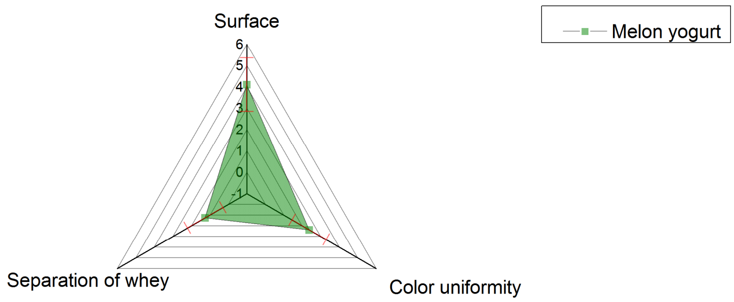


Figure 6.30 Characteristics of yogurt aspect profile of mango and melon flavor

In Figure 6.30 is presented in graphic form the look of probiotic yogurt variability of sea buckthorn flavored melon and mango in both cases it is observed that acceptability scores were at a normal level for the area, uniformity of color and whey separation.

6.16.2. The features regarding aroma profile of yogurt with mango and melon flavors

Aqueous aromatic taste profile features ranged between 1.04 points for yogurt flavored with melon and mango yogurt 1.22 for the intensity scale this score is low i.e. low observable for bitterness were obtained values between 0.31 points melon yoghurt and mango yogurt 0.32 points. Metallic taste had very low average values being 0.23 for yogurt with melon and mango. Oxidized flavor had very low observable values were 0.12 points watermelon flavored yogurt and Mango yogurt 0.13 points, also weakly acidic taste was felt by tasters with values between 0.77 points watermelon flavored yogurt and 0.72 points mango flavored yogurt. Bland taste was very weak with similar values for both types of yogurt. Pungent taste was slightly felt the melon flavored yogurt with 0.42 points compared with 0.21 points for mango flavored yogurt and sour taste was felt slightly weaker, the watermelon flavored yogurt by 0.95 points compared to mango flavored yogurt which was 1.35 points. Rancid flavor was very weak felt similar values for both types of yogurt and astringent flavor had values of 0.89 points watermelon flavored yogurt and that 1.25 points mango flavored yogurt. Sweet taste was of 2.43 points watermelon flavored yogurt and mango yogurt 2.53 points because the mango is also sweet; it was the one who obtained the highest score for all parameters analyzed.

Among the analyzed parameters color, flavor and aroma are important factors that determine acceptance or rejection of a food *Hussain and Atkinson (2009)*.

Figure 6.31 shows the main characteristics of aromatic profile of yogurt with mango and melon flavor, the figure can be seen as mango flavored, yogurt has a higher score than one with the astringent taste of mango; sweet and sour aqueous and watermelon flavored yogurt has higher values than the mango flavor to taste pungent.

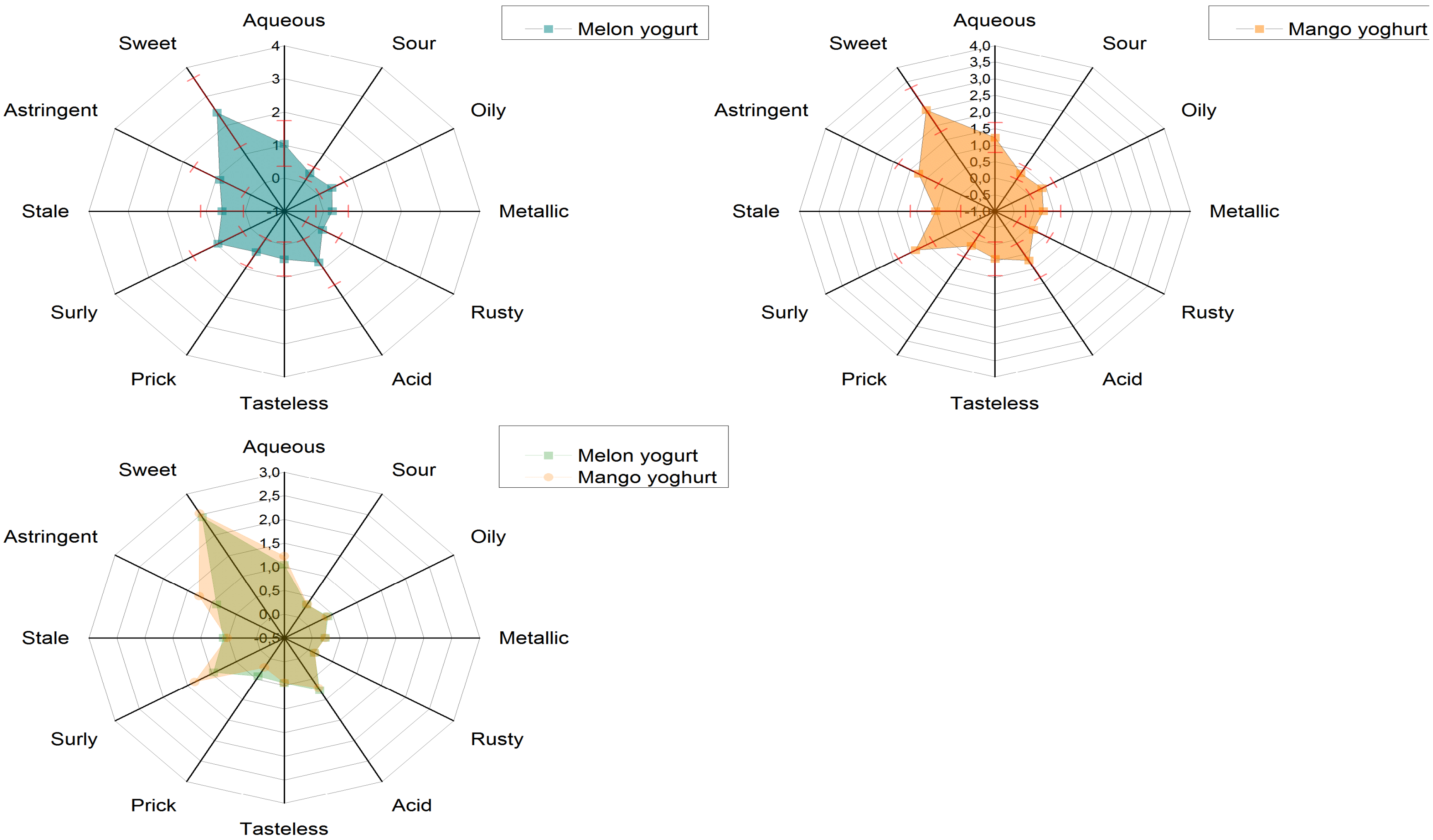


Figure 6.31 Characteristics of the aromatic profile of mango flavored yogurt and melon

CAP.7 CONCLUSIONS AND TRENDS OF DEVELOPMENT RESEARCH

7.1. CONCLUSIONS

In a hierarchy of foods with positive physiological response, cataloged functional or nutraceutical bioactive dairy products with added probiotics will be situated on a very important place.

Nutritional value of dairy products is clearly dependent on acid availability and digestibility of nutrients constituents, phenomena including the link between these constituents changes is caused by lactic bacteria. Thus analyzed the raw material, cow's milk as sour milk energy value is close to that of raw materials, research undertaken high nutritional value is due to retrieved compounds of sea buckthorn's nutritional value and production of new substances (aromatic products, vitamins, organic acids).

It follows from the above results and discussion that the analytical and applied research, and remarkable coefficient of originality and a huge amount of work the following conclusions:

Texture and organoleptic characteristics of the final product:

- dairy flavor is one of the qualities most valued by consumers, sea buckthorn has a key role in enhancing compounds in sea buckthorn and those developed by bacteria present in cultures.
- the texture statistical analysis is not affected by the addition of sea buckthorn, is a very critical parameter as the ability to analyze dairy products is an invaluable tool that can have lasting benefits for the dairy industry.
- in addition to the nutritional value of dairy products is critical wellbeing and satisfaction induced sensory characteristics.

Organic acids:

- dairy products are a source of organic acids, especially ascorbic acid which was supplemented by the addition of sea buckthorn, a great source of ascorbic acid (vitamin C).
- malic acid and ascorbic acid are identified by bringing sea buckthorn containing organic acids, mainly two together constituting about 90% of total acids of fruit.
- the finished product besides organic acids brought by sea buckthorn is representative of lactic acid and oxalic acid in small quantities and tartric acid. Throughout the lactic fermentation, milk lactose content is reduced transformed into lactic acid. Positive digestion and human intestinal tract is accelerated due to lactic acid.
- the results showed that lactic acid in yogurt with added sea buckthorn from thermophilic culture is a lesser amount than the probiotic, because of the action used by starter culture and fermentation factors.

- ascorbic acid is the nutrient most commonly consumed as a supplement, particularly among the elderly population.
- interpretation of results using ANOVA program indicate significant differences on organic acid content of the samples analyzed.

Water soluble vitamins:

- milk is a poor source of water soluble vitamins, vitamin B5 is in the majority, and the amount of vitamin B1 is smaller.
- Sea Buckthorn is a natural multi-vitamin, this super fruit containing all essential vitamins in adequate proportions of category soluble vitamin B3, B5, B1 and B2 in large amounts and minimum proportions of B1.
- thermophilic yogurt with added probiotic and sea buckthorn compared with blank yogurt are higher in vitamin content, being found in optimal proportions vitamin B5, B6 followed by B1, B12, B3, B2, B7.
- vitamin content of fermented yogurt is influenced by raw material content, but increase thermophilic starter culture and probiotic content thiamine, riboflavin as a consequence of their biosynthesis by starter culture.
- statistical analysis shows that there are significant differences between samples of the vitamin content.

Soluble vitamins

- vitamin K in its two forms of vitamin K1 and K2 is predominant in the sea buckthorn and the final product, milk is a poor source of vitamin K.
- phylloquinone (K1) is the main source of vitamin K.
- Sea Buckthorn is a great source of vitamin E and observed results are in according to the latest research on this super fruit polyvitamin.
- vitamin D identified two types D3 and D4 were seen as being in short supply.
- statistical analysis shows that there are significant differences between samples of raw milk, blank yogurt,sea buckthorn yogurt with sea buckthorn .

Minerals:

- there is a very little information on the nutritional aspects of trace elements than others, and a considerable amount of current research is conducted to clarify the role of such minerals in nutrition.
- nutritional information on the addition of minerals and mineral identification from sea buckthorn, milk and yogurt and have considered their level quantitatively..
- highlight content of Fe and Ca, followed by Mg and quantitative nutritional aspect and bioavailability of calcium and phosphorus, as measured by intestinal absorption is higher in fermented dairy products because of the possibility of absorption of these elements.
- the importance of minerals and expected health benefit by adding sea buckthorn, is a great source of minerals, especially Fe and Mg.
- factors affecting the bioavailability of minerals are still difficult to understand, and the possible role of enhancers (eg, lactose, ascorbate, citrate, fosfopeptid, lactoferrin) or inhibitors (eg, protein, calcium, phosphate) of minerals from different types of milk is more important..
- finally, understanding the nutritional importance of trace elements in milk and milk products will also benefit by improving our knowledge of the fundamental aspects of minerals, such as their roles in nutrient

requirements and metabolism, and quantitative relationship between food consumption and health.

Polyphenols

- sea buckthorn berries are rich sources of flavonoids and phenolic acids (salicylic acid and gallic).
- milk and yogurt are in lack of these polyphenols (flavonoids, flavonol glycosides and proanthocyanidins).
- thermophilic yogurt and probiotic polyphenols are a source of positive health effects due to antioxidant effects, anti-inflammatory, antimicrobial, anti-proliferative their flavonoid found in fruits of sea buckthorn.
- flavonoids may have beneficial effects on cardiovascular disease risk and may affect carcinogenesis and Bio indicator's antioxidants in plasma.

Antioxidant capacity

- dairy products do not show antioxidant capacity, as some studies are said to have given only minor significance of certain compounds
- Sea buckthorn is a natural reservoir of antioxidants, particularly due to higher content of vitamin C, E, flavonoids, carotenoids, sterols and polyunsaturated fatty acids (omega 7).
- yogurt with major content of bioactive component of sea buckthorn has a special antioxidant capacity of health benefits.
- anticarcinogenic effects of carotenoids may be at least due to partly antioxidant activity.
- statistical analysis indicated significant differences between samples, both the FRAP and DPPH methods.
- special interest on food supplementation for antioxidant capacity is primarily due to the impact on health.

Probiotic dairy products are part of functional foods, which are made from fermented milk selected from a culture or a concentrate of bacteria that improve the microbial balance in the gut, hence the positive and beneficial influence of these products are on health and integrity of the human body, given that over 80% of our immune system is concentrated on gastro-intestinal tract. Lately phytotherapeutic drug production increased dramatically in our country and we take pride in being a country with most medicinal plants /1000 people with a tradition of thousands of years, which proves the continuity of our people, and their desire to be beneficial, as recorded by ancient writings history of mankind .

Bioactive yoghurt with sea buckthorn will provide an outlet for any milk that is available, and especially the issue of "lactose intolerance" in yogurt will become very low (compared to lactose liquid milk), this topic is under investigation .

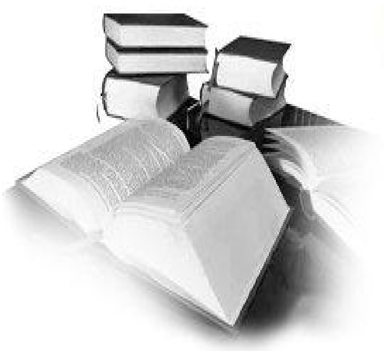
Yogurt in this validity of 28 days is based on the fact that the physical, chemical, microbiological and sensory, characteristics must be acceptable for consumption.

7.2. FUTURE DEVELOPMENTS IN RESEARCH

Current studies confirm the interest of the consumers undoubtedly in fermented dairy products, leaving the most diverse culinary attractions, natural and therapeutic acid products. To natural flavors, probiotic functional food trend has captured the attention of the health and recovery of the most nutritious fruits as sea buckthorn.

This thesis founded and confirmed the idea that food can promote / protect health through biologically active components that it contains; these are of great value to the scientific community by opening future research trends that could not be included in this thesis due to heavy workload such as:

- Promotion of sea buckthorn and inclusion in food as many forms and complex forms due to nutritional and therapeutic unparalleled values; as being declared by superfruit of 2012.
- Analysis shows that polyunsaturated fatty acids, especially omega-7 unique bioactive compound found in increased quantities in sea buckthorn have amazing effects on the body, undertaken recent studies on skin diseases, tissue regeneration and hair.
- Information and call for adequate nutrition and diversified bioactive natural products became important because it reinforced the idea that "eating healthy to treat the disease, it is better than using a particular medication.
- Promoting functional foods and attention for clinical health due to stress, fatigue, additives used, fast food and food chemically synthesized, people are more concerned about body care and continuous information.
- Study metabolites resulting in fermentation processes, and promoting health through biological activity of bacteria used.
- Use of sea buckthorn as an alternative recovery technology, for example, in the production of kefir, probiotic ice cream with the addition of sea buckthorn, sea buckthorn milk with bifidus, even some types of cheese.
- Proven bioavailability of bioactive yogurt fortified with underbrush, through monitoring the consumption in time, with absorption of calcium, other minerals, vitamins and other bioactive compounds.



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