" LUCIAN BLAGA" UNIVERSITY OF SIBIU

FACULTY OF AGRICULTURAL SCIENCES, FOOD INDUSTRY AND ENVIRONMENTAL PROTECTION

DOCTORAL DISSERTATION

SUMMARY

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PH.D. CANDIDATE
Eng. Alina Florentina DAN

Sibiu, 2014

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RESEARCH REGARDING THE PRODUCTION AND THE IMPROVEMENT OF QUALITY AND NUTRITIONAL VALUE OF ALMOND MILK AS A POSSIBLE SUBSTITUTE FOR COW'S MILK

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FORWARD

The sweet taste of the new wine that my grandfather used to give me drink directly from the wine press and the fragrance of salty almonds roasted by my grandmother, are memories which are part of my childhood spent in an area with a multiple significance: historical, agricultural and viticultural. I spent my childhood among the Tohan hills, the vineyard and the beautiful, sweet smell of the almond flowers. People say that this is the place where prince Nicolae of Romania met his love, Ioana Dolette, giving up his royal rights in order to marry her.

Sitting on my grandparents' porch, in spring, I used to admire those hills which looked as if they were snowed with white almond flowers. I'm sure that the root of my curiosity about these fruit comes from here. And my answers lie in my university studies, but mostly in my doctoral studies under the mentorship of ***Professor Doctor Eng. Ovidiu Tita.*** *His engineering experience, his professionalism, his scientific and didactic competence and above all, his patience during my research activity, helped me finish my thesis. I would like to express my gratitude to my teacher, Professor Doctor Eng. Ovidiu Tita for all his support and permanent guidance.*

My deepest thanks goes also to ***Professor Doctor Josep Antoni Tur Mari****, head of the Comunitary Nutrition and Oxidative Stress Research Group from the Department of Food Biology and Health Sciences, University of Balearic Island , Palma de Mallorca, Spain which facilitated my internship performance outgoing the department he leads.*

In the same time, I would like to thank Professor Doctor Antoni Pons Biescas, member of Comunitary Nutrition and Oxidative Stress Research Group from the Department of Food Biology and Health Sciences, University of Balearic Island , Palma de Mallorca, Spain. for the permanent guidance during the whole internship.

I would also like to thank the entire group of PhDs from the department of Department of Food Biology and Health Sciences, University of Balearic Island , Palma de Mallorca, Spain. for their support.

Many thanks also to the teachers' staff from the Faculty of Agricultural Sciences, Food Industry and Environment Protection from Sibiu and also to the assessment committees for their references concerning the growth of the quality research activity.

Last but not least, I would like to thank my family and my closest friends for their moral support and understanding on the entire research period. I dedicate my success to them. Thank you.

Alina Florentina Dan

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Table of contents

THE FIRST PART
Documentary study

The documentary study includes three chapters.

CHAPTER I. ORGANIZATION OF RESEARCH

In section 1.1 , *Institutional and organizational frame where research is conducted* shows the institutional frame where research was conducted : The University of Balearic Island, Department of Biology and Phenomenal Sciences, Spain, Lucian Blaga University Sibiu , Faculty of Agricultural Sciences , Food Industry and Environmental Protection and Bioef Laboratory.
Subchapter 1.2 - *Thesis Motivation and research objectives*

Starting from the idea of functional food , I decided to investigate a food , a drink made from almonds, generically named almond milk ,drink that meet the requirements and functions in order to fall within this category of products and to replace as much as possible cow milk. Also , the need for this research comes from the fact that it is very important to know the current situation on the acquisition, production and marketing of the product and uses of almond milk .

The objectives of this research:

- Establish the manufacturing stages technology of almond milk
- Analyze almond milk in terms of physicochemical characteristics to optimize the addition of different nutrients and flavors in order to improve the quality and nutritional value
- HACCP analysis throughout the process of almond milk production Choosing from the diversity of nutrients those that improve the quality and nutritional value of almond milk
- Developing and optimizing a functional product with experimental studies that have followed the addition of carob powder , rosehip powder or lactoferrin in laboratory
- Highlighting nutritional potential of almond milk with added carob powder , rosehip powder , or lactoferrin
- Preparation and characterization of a functional product in terms of textural and sensory characteristics
- Undertake a study on a sample of 10 children aged 3 to 5 years who received almond milk lactoferrin
- Obtaining a product meeting certain consumer requirements
- Making a comparative analysis between almond milk and cow milk

CHAPTER II. DOCUMENTARY STUDY ON OBTAINING PROCESS AND CHARACTERISTICS OF ALMOND MILK

Subchapter 2.1 - *Characterization of raw material – almonds*
Subsection 2.1.1 - *Historical area of distribution , cultural history* includes information regarding area of almond distribution , almond crop production at worldwide, European and national level .
Subsection 2.1.2 - *Maximum limits of pesticide residue contented in almonds assigned to consumption* covers the limits imposed by the Codex Alimentarius for pesticides residue in almonds
Subsection 2.1.3 - *Technical characteristics and composition of almond* regards shape, size, yield of almond kernels .
In **section 2.1.3.2** - *Composition of macro - and micronutrients* , the main ingredients of almonds are shown: fat, protein, fiber , sugars, minerals and vitamin content , nutritional values .
Subsection 2.1.4 - *Effects of almond consumption* - presents some results of research on the effects that almond consumption had on various categories of consumers.
In the **subchapter 2.2** - *Methods for obtaining almonds milk and its characteristics* are given a series of already known processes for the preparation of a drink , generically named almond milk. This subchapter includes also the characteristics of the product.
Subchapter 2.3 - *Application of almond milk* – is detailing the main areas where almond milk has been used successfully by date , its beneficial effect being demonstrated (in case of animal milk lactose intolerance, in order to decrease LDL) .

CHAPTER III. POSSIBILITIES OF IMPROVING THE QUALITY AND NUTRITIONAL VALUES OF ALMOND MILK

Section 3.1 , *The current study on the possibilities of improving the quality and nutritional value of almond milk*
In **section 3.1** - *The current study on the possibilities of improving the quality and nutritional value of milk almond* – shows some studies regarding the possibilities of enriching almond milk: by the addition of fruit juices or teas and correcting osmolality- isotonic drink (Antonio Pons Biescas , 2011), adding different flavors .
Subchapter 3.2 - *Raw materials used to improve the quality and nutritional value of almond milk* - is divided into three chapters, each of them presenting a raw material chosen in order to improve the quality and nutritional value of almonds milk.
In **section 3.2.1** , *Carob powder* is described carob powder (the family to which it belongs , chemical composition, nutritional values) .
Subchapter3.2.2 - *Rosehip powder* - includes aspects of the family of which belongs , the purpose that fruits are used for, nutritional values , effects of consumption) .
Subchapter 3.2.3 - *Lactoferrin* - is divided into three chapters which present :
Subchapter 3.2.3.1 - *Overview* , includes data on the glycoprotein lactoferrin spread, concentrations of various fluids, structure.

Subchapter 3.2.3.2 - *Physical and chemical properties*- includes data on amino acid composition of bovine and human lactoferrin , physical and chemical properties of bovine lactoferrin , recommended doses
Subchapter 3.2.3.3 – *Biological Functions* describes the inhibitory effect of lactoferrin on bacteria , antibacterial mechanism, mechanism of antiviral activity , current applications of lactoferrin .

Part Two
EXPERIMENTAL

The experimental part includes the following four chapters.

CHAPTER IV. STUDIES AND EXPERIMENTAL RESULTS OBTAINED DURING ALMOND MILK PRODUCTION

Subchapter 4.1 – *Establishing steps for almond milk production process*
4.1.1 *Boiling almonds* - is done in order to deactivation of lipoxygenase in almond and in order to remove the skin. This step involves immersing of almonds in water at 100 º C for 10 minutes. For almond milk preparation was only used the white cotyledon .
4.1.2 *Preparation of the syrup* , the step of preparing a syrup of sucrose.
4.1.3 *Grinding almonds* , contains information about how should be done the grinding of almonds
4.1.4 *Addition of carob powder , rosehip powder and lactoferrin powder and mixing them with syrup and almonds* – This step is performed using a Thermomix robot by mixing almond powder with those powders , together with syrup for 1 minute .
4.1.5 *The addition of preservatives* presents data about preservatives used .
4.1.6 *Maceration* - sets the main parameters for insoluble material extraction .
4.1.7 *Filtering data* - shows information about the removal of inappropriate particles.
4.1.8 *Pasteurization* - present the conditions of the pasteurization of almond milk (90 º C for 5 minutes , followed by a sudden cooling to 4 ° C)
4.1.9 *HACCP Study on manufacturing process of almond - milk* contains information about a HACCP system implementation in the manufacturing process of almond milk (HACCP scheme on the technological process , determining risks, establishing the limits of control critical points for the process to be carried out safely , establishing methods to monitor the parameters of the critical control points) .
The technological scheme and the identification of critical control points are shown in Figure 4.1

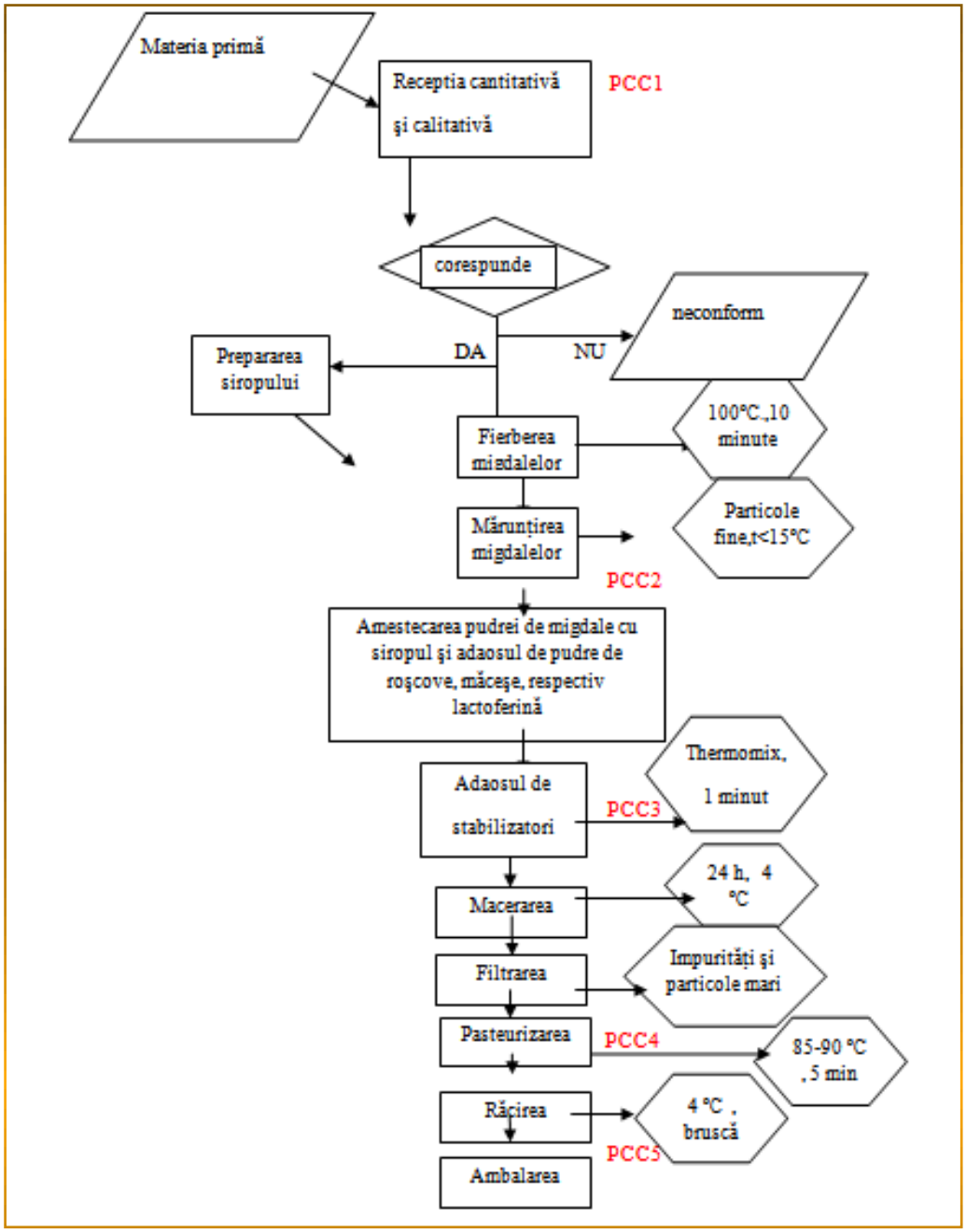


Fig.4.1 Flow diagram for the manufacture of almond milk

Subchapter 4.2 *Methods of analysis and control*

Subchapter 4.2.1 - *Sensory analysis* - refers to the principle of methods for assessing the appearance, texture , taste , color, smell of almond milk , the principle of sample preparation , and the expression of results.

Sensory characteristics of almond milk are shown in the table below.

Table nr.4.2 Sensory characteristics of almond milk

Caracteristici	**Rezultatul aprecierii**
Aspect	Lichid omogen, palescent, fără corpuri straine în suspensie şi fără sediment
Consistenţă	Fluidă
Culoare	albă
Miros	Plăcut
Gust	plăcut, dulceag

Subchapter 4.2.2 - *Determination of pH* – presents the principle of the method , equipments and utensils used , calibration mode , sample preparation , data and calculations .

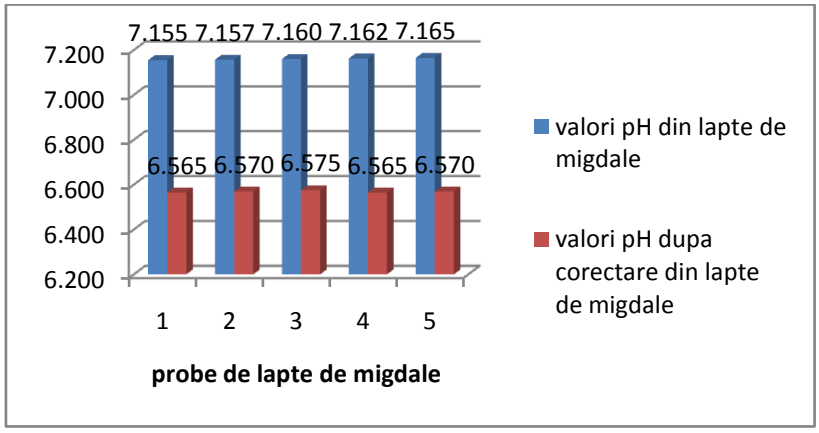


Figure 4.3 Statistical diagram for pH values of almond milk

Subchapter 4.2.3 - *Determination of ash* - includes the principle of the method , equipments, tools and reagents used , calculations and data obtained.

Table 4.3 Values of ash content from almond milk

Proba	**ID**	**m creuzet (g)**	**m proba (g)**	**m proba creuzet(g)**	**Rezultat (%)**
Lapte migdale	I	43,6201	10,0818	43,6285	0,083

	II	43,6201	10,0818	43,6285	0,083318
	III	43,6305	10,0707	43,6386	0,080431
	IV	43,6507	10,0786	43,6588	0,080368
	V	43,6604	10,0806	43,6684	0,07936

The ash content of almond milk presented lower values compared to the ash content from animal milk, which means a low mineral content .

Subchapter 4.2.4 - *Determination of fat content* - includes the principle of the method , description of equipment, tools , reagents, way of determination and expression of results . To determinate the fat content of almond milk I used the Gerber method.

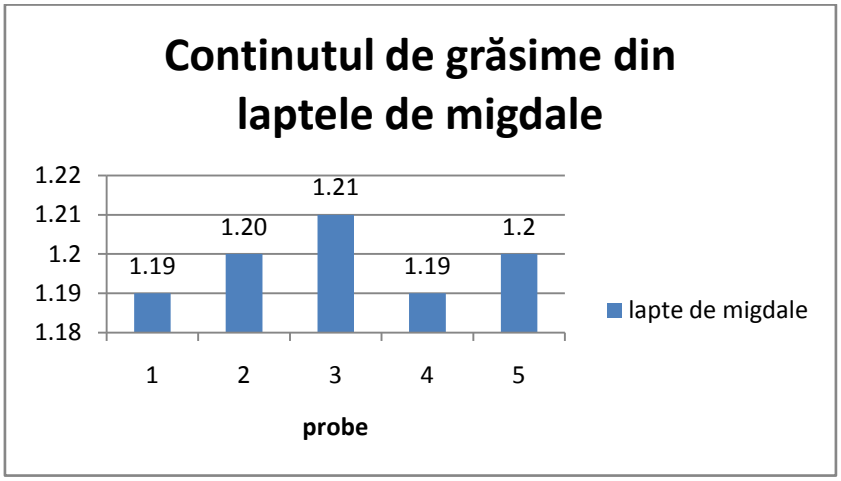


Fig . 4.6 Statistical diagram of fat content results of almond milk.

Subchapter 4.2.5 - *Determination of protein* - presents the principle of the method , equipment, reagents , tools , results . Protein content was done by spectrophotometry (Spectrophotometer Shimadzu, model UV -2401) using the Lowry reagent and the Folin Ciocalteau reagent.

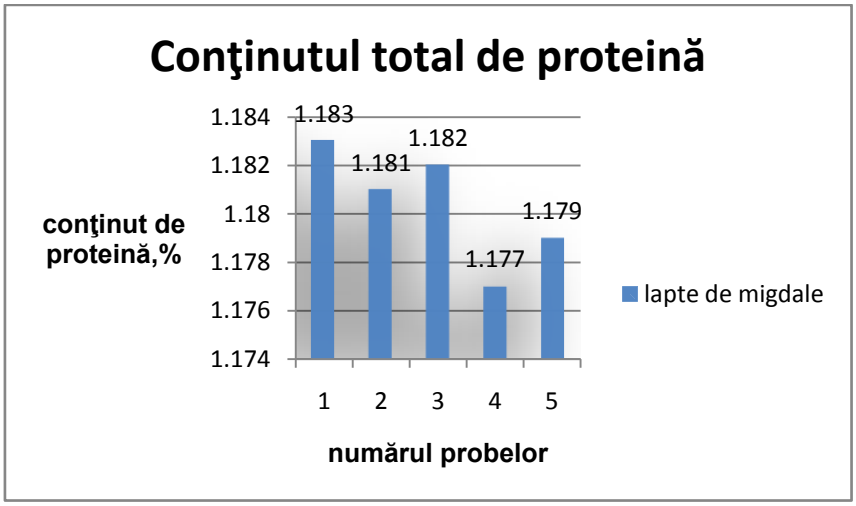


Fig.4.9 Statistical diagram of total protein content values of almond milk

It is known that the higher value of protein content is found in animal milk . However, it is also known the fact that some people have allergies to animal milk proteins , manifested by eczema, cramps , diarrhea .Taking this into consideration the amount of vegetable protein in almond milk is not negligible.

Subchapter 4.2.6 - *Determination of total phosphorus* – shows the principle of the method , equipment, reagents , the calibration curve , results, and statistical processing . The principle of the method chosen for the determination of phosphate in almond milk consists of ashing to remove the organic part , the ashes will contain acid-soluble phosphates which reacts with molybdate to form a compound that is reduced by a reducing agent such as ascorbic acid at blue molybdenum, that can be read at 823 + 1 nm. (AOAC 995.11) . Reading samples was made with a spectrophotometer Shimadzu UV -2401 model .

Fig.4.10 Sample preparation for the determination of phosphorus in almond milk (personal archive)

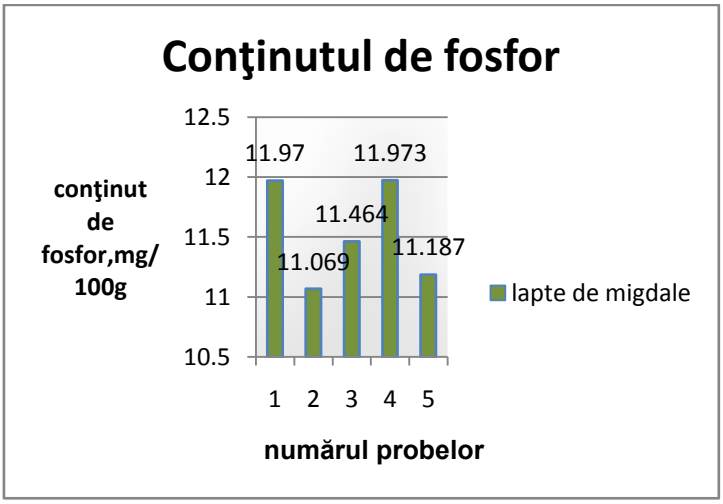


Fig . Figure 4.13 Statistical values of phosphorus content of almond milk

Phosphorus almond milk is responsible for regulating heart beats and normally functioning of the kidneys , for the strengthens of teeth and bones. It also helps in cases of fatigue , irritability, difficulty in breathing , stress, numbness or sudden changes in weight. Phosphorus , like other minerals contained in almond milk helps in reviving the body and restore its energy. **In section 4.2.7** - *Determination of total polyphenols* are shown the principle of the method , the equipment , utensils, results.

The antioxidant activity of polyphenols is largely due to its redox property, enabling them to act as reducing agents (Torabian S , 2009) . These natural antioxidants can sweep away free radicals and can also break these chains (Amarowicz and others 2004). Determination of polyphenols in almond milk was made by UV / VIS spectrophotometry , using Folin Ciocalteau reagent.

Table 4.7 The values of total polyphenol content of almond milk

absorbanţa	concentraţia în cuva	umol/L lapte de migdale
0.112	74.000	170.200
0.112	74.000	170.200
0.109	72.000	165.600
0.128	84.667	194.733
0.099	65.333	150.267
0.102	67.333	154.867

In section 4.2.8 - *Determination of sterols and tocopherols* are shown the principle of the method, equipment, tools , reagents , separation and identification chromatograms and results .

The healthy effect of vitamin E in the body is due to the eight types of tocopherol isomers , named after the Greek alphabet . α - TE is the most active form of vitamin E in biological terms . Almonds are considered one of the greatest sources of α - tocopherol (Chen and others , 2006). Sterols are a class of alcohols with complex structures, which play an important role in the metabolism of the organism. The category includes sterols and phytosterols . Separation , identification and quantification was done by high performance liquid chromatography (HPLC). The method uses a column packed with different materials (stationary phase) , a pump that pushes mobile phases through the column and a detector that shows the retention times of the molecules . The equipment used was a Shimadzu system

Fig.4.14 Shimadzu system controller (personal archive)

The retention time of the sterols is between 50 minutes and 75 minutes , as shown in Figure 4.27 and the for fucosterol is 51 min .

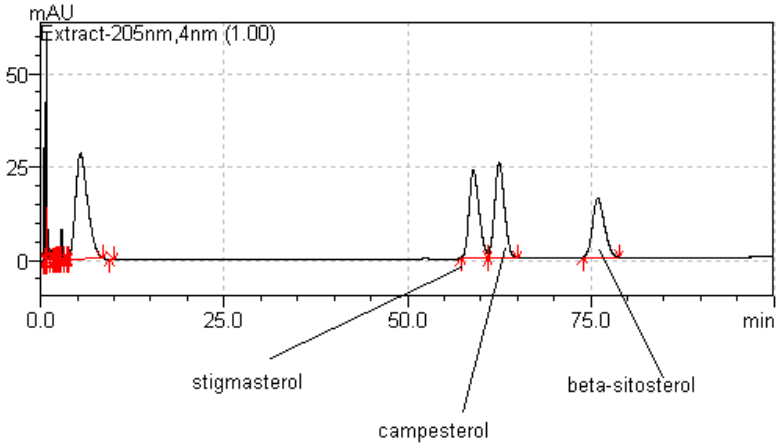


Fig . 4.27 The chromatogram of separation and identification of stigmasterol , campesterol and beta-sterol (personal archive)

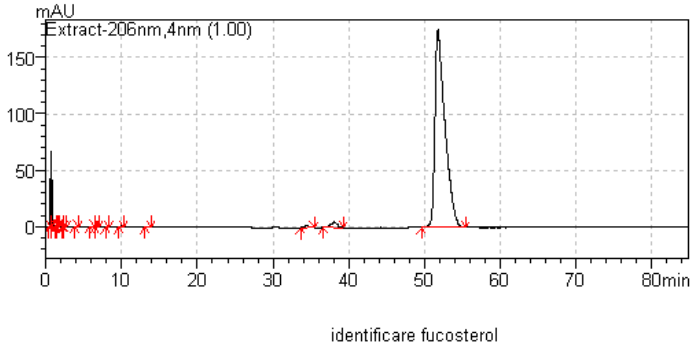
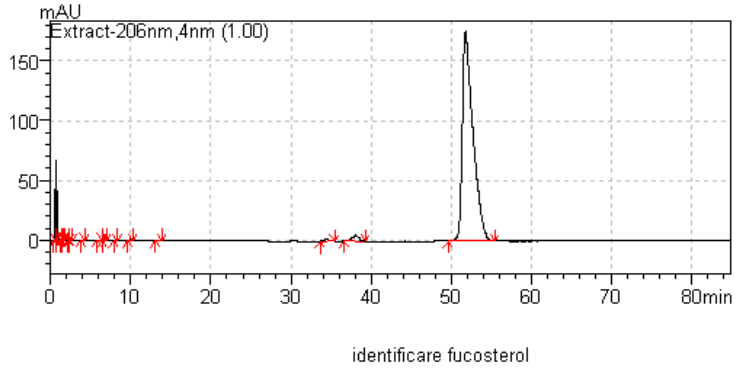


Fig . 4.30 The chromatogram of separation and identification of fucosterolului (personal archive)



The chromatogram for alpha tocopherol indicated the retention time at 79 min (Figure 4.32)

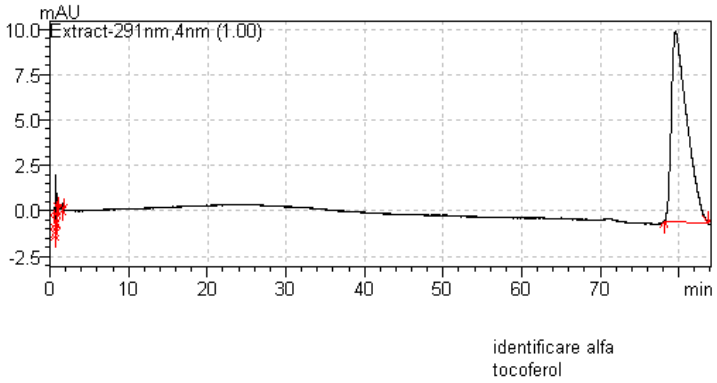


Fig.4.32 The chromatogram separation and identification of alpha tocopherol (personal archive)

Table 4.9 The values for the content of sterols and tocopherols from almond milk

	Fucosterol mg/ L lapt migdale	**Stigmasterol mg/ L lapte migdale**	**Campesterol mg/ L lapte migdale**	**Betasitosterol mg/ L lapte migdale**	**Alfatocoferol mg/ L lapte migdale**
Lapte de migdale 19.07.2011	49.2	8.65	4.2	46.145	4140.59
Lapte de migdale 21.07.2011	44.075	8.2	4.1	45.1	4340.875

Lapte de migdale 25.07.2011	46.85	9.225	4.1	46.125	4402.375
Lapte de migdale 29.07.2011	48.5	9.2	4.1	45.25	4405.45

Subchapter 4.2.9 - *Determination of calcium , magnesium , potassium , sodium , zinc, iron* refers to the importance of minerals to the body , the principle of the method used (atomic absorption spectrophotometry) spectrophotometer structure, tools and reagents used , sample preparation , calibration curves , results and statistical interpretation .
Sample preparation is done according to the scheme 4.48 .

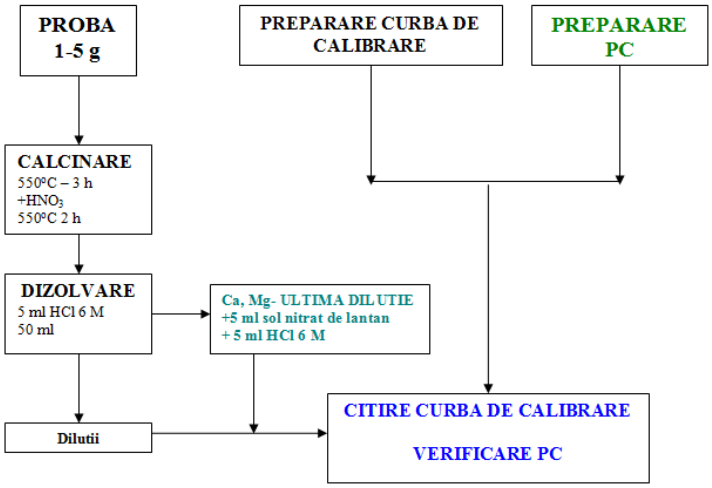


Fig . 4.48 Scheme of work for the determination of Ca, Mg, K , Na , Fe , Zn by atomic absorption spectrophotometry

Spectrophotometer used to read samples was Sens AA .

Fig . 4.47 The absorption spectrophotometer Sens AA (personal archive)

The values obtained for the mineral content of almond milk are shown in Fig . 4.54 A and B.

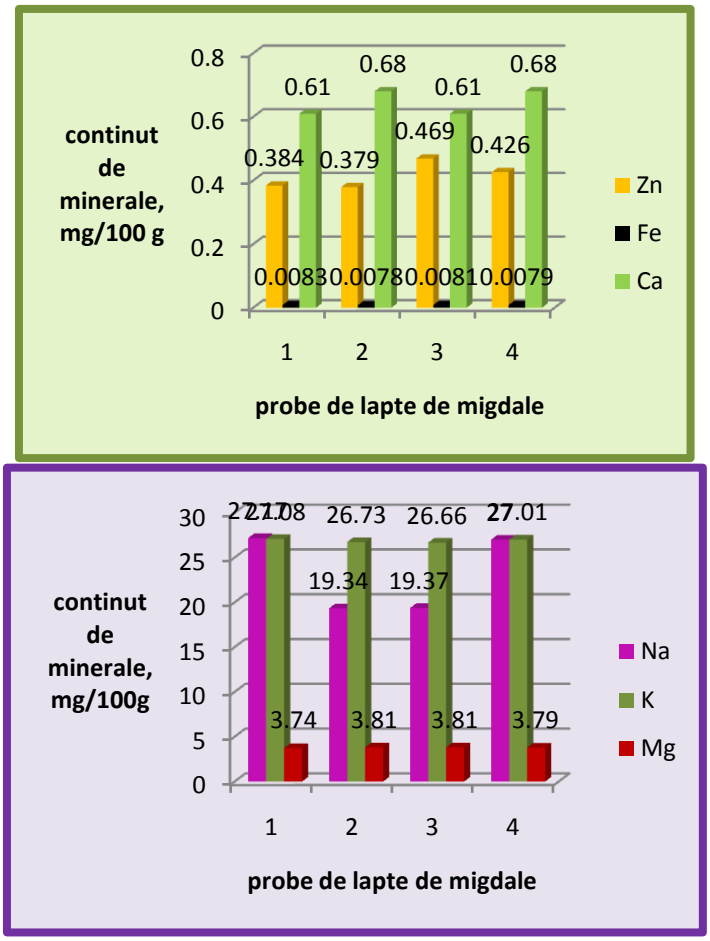


Fig . 4.54 a and b: Graph of the values obtained for Ca, Mg , K , Na , Zn , Fe in almond milk samples

CHAPTER V. IMPROVING THE QUALITY AND NUTRITIONAL VALUE OF ALMOND MILK

Subchapter 5.1 ,*The necessity and justification of research* motivates the research that was conducted in order to improve the quality and nutritional value of almonds milk. **Subchapter 5.2** –The *national and international legislation on adding additional foods* shows the General Principles from Codex Alimentarius for the addition of essential nutrients to foods . **Subchapter 5.3** - *Addition of carob powder* , discuss the possibility of improving the almond milk with carob powder . Three kind of samples were taken in work and after the sensory analysis was done, it was established the optimal dose of carob powder to add .

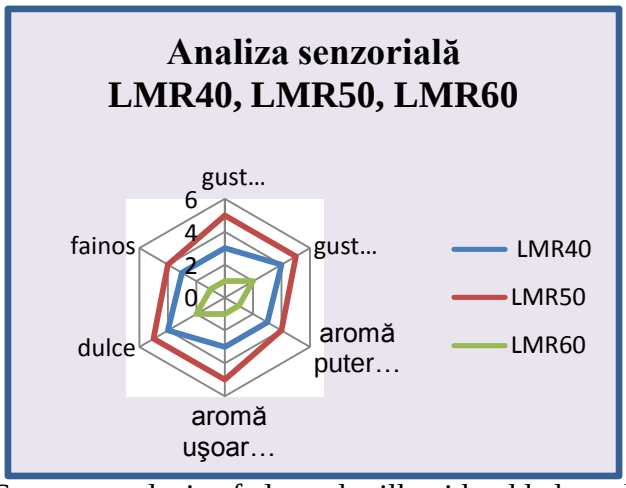


Fig . 5.1 Sensory analysis of almond milk with added carob powder

Subchapter 5.3.1 - *Determination of calcium in carob powder and in samples of almond milk with added carob powder* , makes reference to the results obtained in enriched almond milk . The calcium content from carob powder (Figure 5.3) and from almond milk enriched with carob powder(Fig. 5.4). was determinate by atomic absorption spectrometry

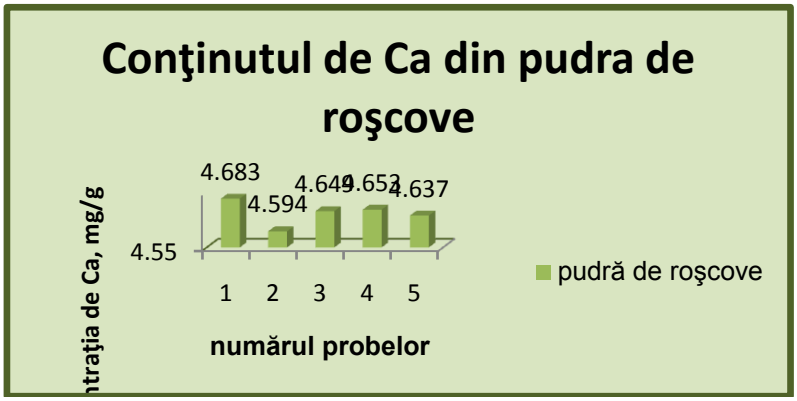


Fig.5.3 The content of calcium from carob powder

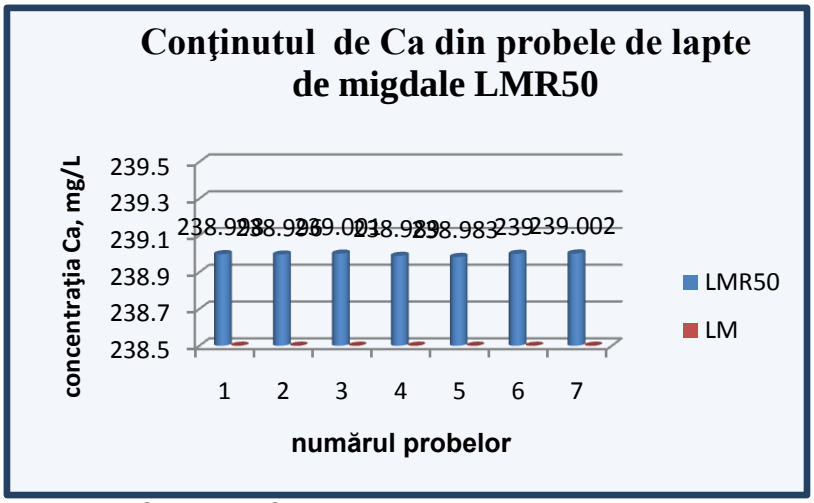


Fig . 5.4 The content of calcium from almond milk samples LM , respectively LMR50

It is known that for the absorption of Ca in the body, it is necessary that Ca / P ratio be bigger than 1.4 , so that it was necessary to analyze also the phosphorus content of almond milk samples . The analysis of phosphorus in almond milk samples with carob powder added was made by spectrophotometry, based on the reaction of molybdenum with phosphates , which form a complex that is reduced to molybdenum blue , as was explained in a previous chapter . The values obtained for LMR50 phosphorus content are given in Figure 5.5 .

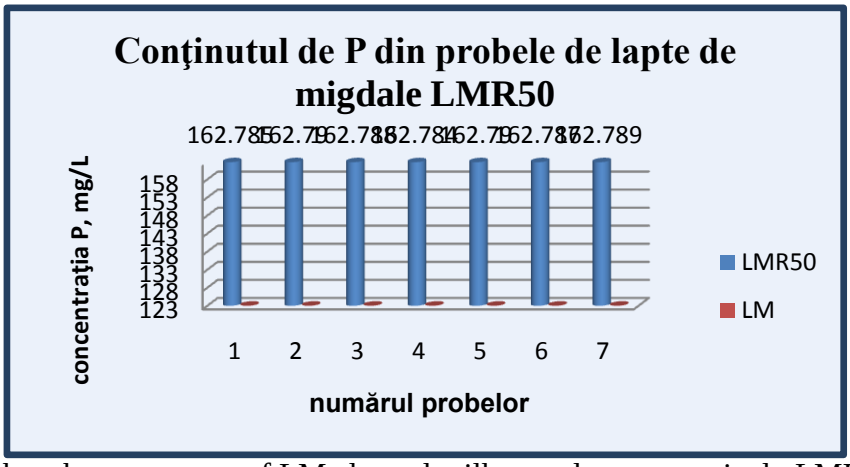


Fig.5.5 The phosphorus content of LM almond milk samples , respectively LMR50 samples.

The results showed a mean of 238.99 mg / L,for LMR50 samples and the phosphorus value was 162.78 mg / L. The ratio Ca / P is about 1.425 .

Subchapter 5.4 - *Addition of rosehip powder* , discuss the possibility of enriching almond milk with rosehip powder . Three kind of samples were taken in work and after the sensory analysis was done, it was established the optimal dose of rosehip powder to add .

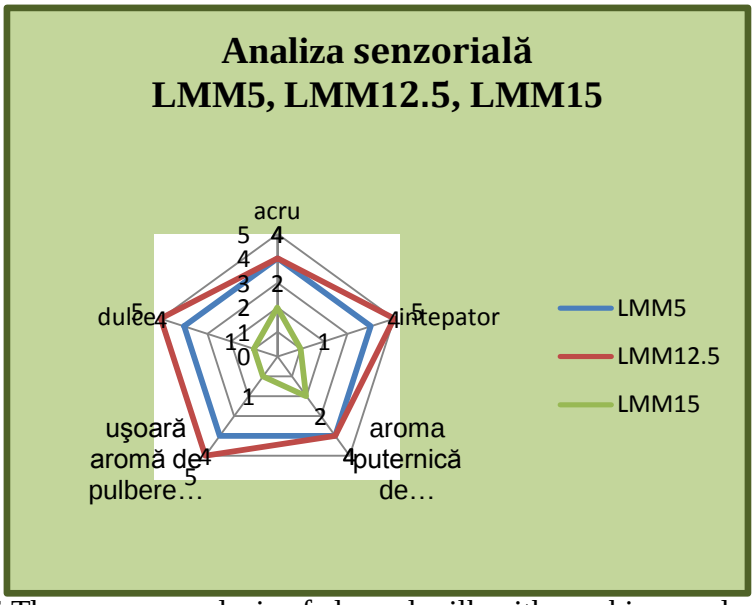


Fig.5.6 The sensory analysis of almond milk with rosehip powder added

The analysis of pH samples with added rosehip powder showed that this addition has a considerable influence on physical and -chemical properties of the manufactured product . More specifically, if the addition of rosehip powder was 1.5 %, the pH of the product decreased by 0.5 units, which influenced product quality. Administration of 0.5-1.25 % Rosehip powder leads to a unessential development of pH value (decreased only 0.05-0.09 units). Sensorial quality of the product with the addition of 1.25 % Rosehip powder is considerably high . Adding increased amounts of rosehip reduces the quality of the product , which is probably due to high acidity , but also to the dark color of the product .

Subchapter 5.4.1 - *Ascorbic acid dosage* refers to the principle of the method , experimental protocol, dosing results, the effect of pasteurization on ascorbic acid content from almond milk, stability studies conducted on almond milk with rosehip powder added .

Section 5.4.1.1- *The experimental protocol of ascorbic acid dosage*

The determination of ascorbic acid content from rosehip powder , respectively from almond milk , was based on the reaction between ascorbic acid and 2,6- dichlorophenol .

Subchapter 5.4.1.2 *The results of ascorbic acid dosage from almond milk* indicated values between 38.8 and 40.3 mg / L (fig.5.10)

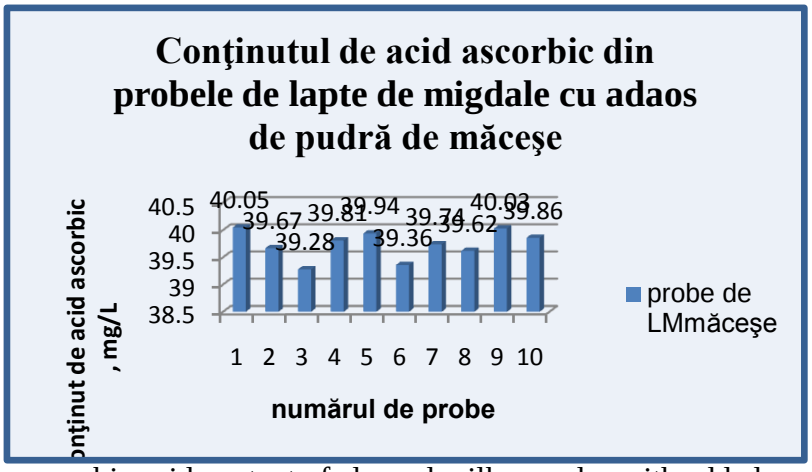


Fig . 5.10 The ascorbic acid content of almond milk samples with added rosehip powder

Subchapter 5.4.1.3 – *The effect of pasteurization on ascorbic acid content from almonds milk.*
The ascorbic acid is sensitive to heat, which makes pasteurization modifying this content. The concentrations of ascorbic acid were determined in samples of almond milk with added rosehip powder before and after pasteurization (Figure 5.11)
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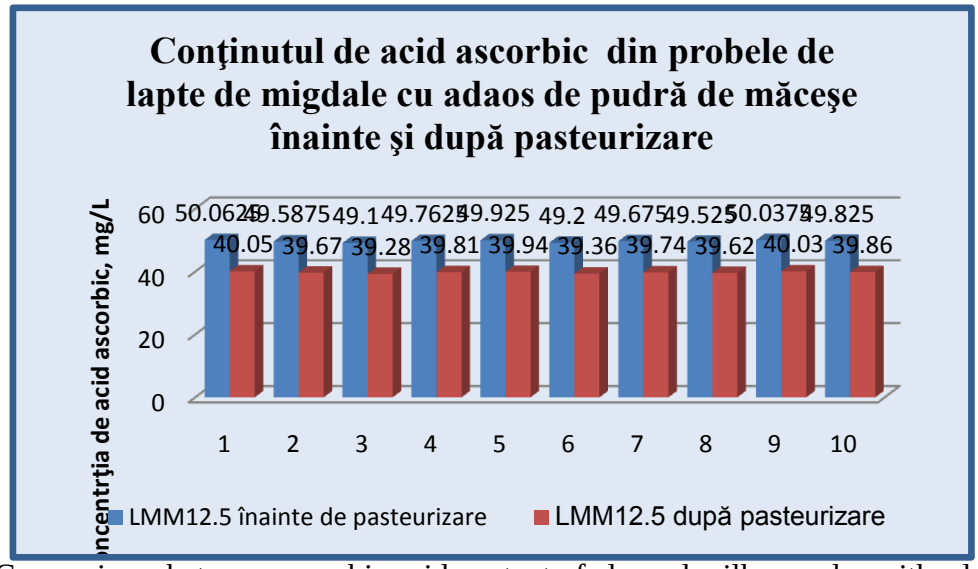

Fig . 5.11 Comparison between ascorbic acid content of almond milk samples with added rosehip powder before and after pasteurization

The results revealed a reduction of around 20 % in the ascorbic acid content of the almond milk samples with rose hip powder added after pasteurization compared with the results for ascorbic acid content of the almond milk samples before pasteurization This is due to the thermal sensitivity of ascorbic acid .

Subchapter 5.4.1.4 *Stability studies conducted on almond milk with rosehip powder added*
Vitamin C is a vitamin sensitive to light , oxygen and temperature. Therefore a research on the variation of the concentration of this vitamin in almond milk samples with rosehip powder added

was necessary. We have considered the five samples of almond milk with rosehip powder added LMM12.5 . Ascorbic acid content was determined after a month, after two months, respectively after 3 months.
The ascorbic acid from almond milk samples with rosehip powder added degrades in time by a linear function.

Table 5.3 The evolution of ascorbic acid content from almond milk samples with rosehip powder added

Luni	Rezultat (mg)	Regăsire (%) A	Ln(A/100)
0	**39,736**	**100**	**0**
1	**39.6167**	**99.7**	**- 0.003004**
2	**39.3903**	**99.13**	**- 0.008738**
3	**39.3346**	**98.99**	**- 0.010151**

If the concentrations are calculated after 24 months of storage under natural conditions, in samples will be found 95, 38 % of the initial ascorbic acid according to the equation in the chart below (Figure 5.17) .

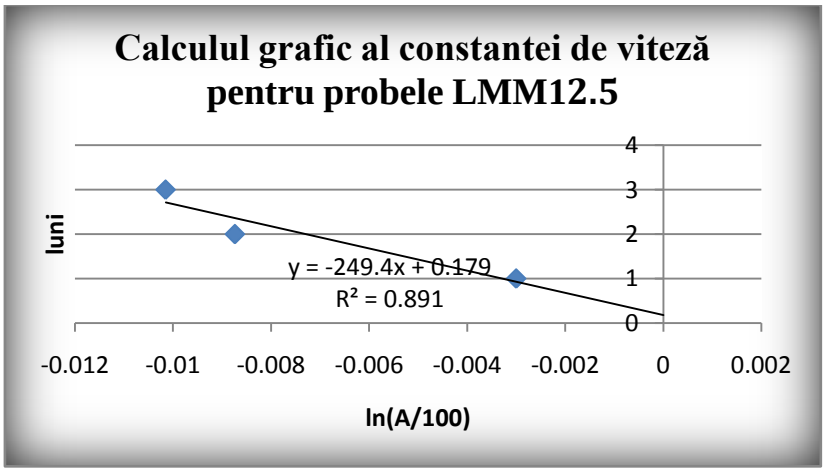

Fig . 5.17 The graph of constant speed for LMM12.5

Subchapter 5.5 - *Addition of lactoferrin* – deals with information regarding the principle of the method, information on the principle used in the determination of lactoferrin from almond milk with lactoferrin added, equipment used , stability studies conducted on almond milk with lactoferrin,in time variation of lactoferrin content from almond milk samples with lactoferrin added, the influence of light on the content of lactoferrin, the results of an experiment on children fed with almond milk with rosehip powder, carob powder or lactoferrin.

In section 5.5.1 - L*actoferrin dosage* is shown the method for determining the content of lactoferrin in samples of almond milk with lactoferrin added. The method used was ELISA immunoassay method . The principle of this method is the competitive antigen -antibody reaction in the free micro plates sites. To determine Lf a kit purchased from laboratories Taradon Elisa and CER Groupe Belgium was used.

The samples taken were noted LMLf1 , LMLf2, and LMLf3 for the addition of 1, 2.5 , and 3 g of lactoferrin / L almond milk .

According to EFSA the addition of lactoferrin may be about 120 mg/100 g for a soft drink, 200 mg/100 g for milk destinated to children and 300 mg/100 g for milk formulas for athletes .

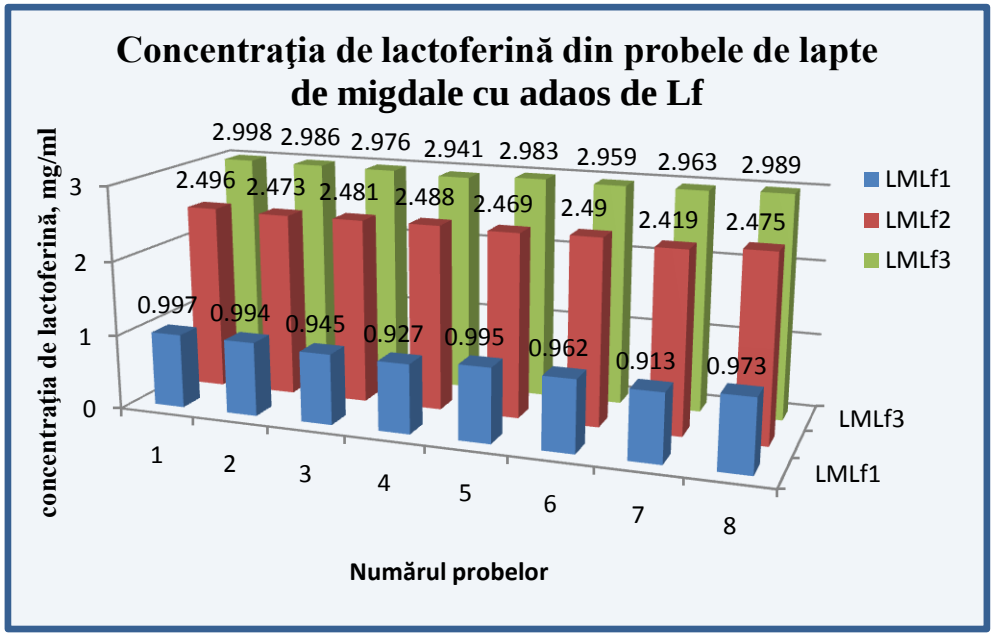

Fig . 5.21 Lactoferrin content of almonds milk samples LMLF

Subchapter 5.5.2.1 *Effect of pasteurization on the lactoferrin from almond milk samples*

Lactoferrin stability is a key issue when it comes to using it as additives in various products for nutrition. The stability of lactoferrin from almond milk was investigated at different temperatures and pasteurization times . The temperature varied from 80 ° to 100 ° C, the heating time was 5 minutes. The results showed that the stability of lactoferrin depended very much on the pH of the solution. For an almond milk with an alkaline pH value turbidity or even gels appeare (Fig.5.22) fact demonstrated also by Abe , 1991.

Fig . 5.23 Almond milk with added lactoferrin (turbidity at the pasteurization temperature due to alkaline pH , personal archive)

Fig 5.24 Almond milk with lactoferrin added (formation of gels due to the high temperature of pasteurization 100 ° C. , personal archive)

In order to adjust the pH value to 6, acid citric was added in almond milk with lactoferrin. The final conclusion was that the pasteurization of almond milk with lactoferrin added to be done at 90 º C for 5 minutes.

Subchapter 5.5.2 .2 *In time variation of lactoferrin content from almond milk samples with lactoferrin added* .

The samples of almond milk with lactoferrin added was stored at 4 ° C for 60 days , in two types of package: some of them dark colored and other crystalline. The purpose of this experiment was to observe the influence of light of the amount of lactoferrin from the sample . As shown in the results , lactoferrin content of almond milk samples in dark packages decreases with about 2 % after 30 days and 2.2 % after 60 days (Fig. 5.26) .

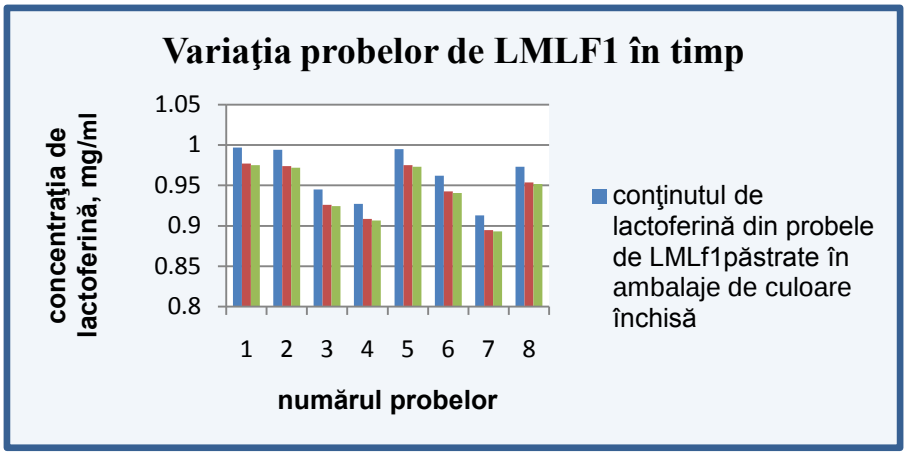

Fig . 5.26 The variation of LMLf1 samples kept in dark packages

In the other case, almond milk samples stored in crystalline packages lactoferrin content was reduced by about 5 percent after 30 days, which indicates the sensitivity of lactoferrin to the light (Figure 5.29)

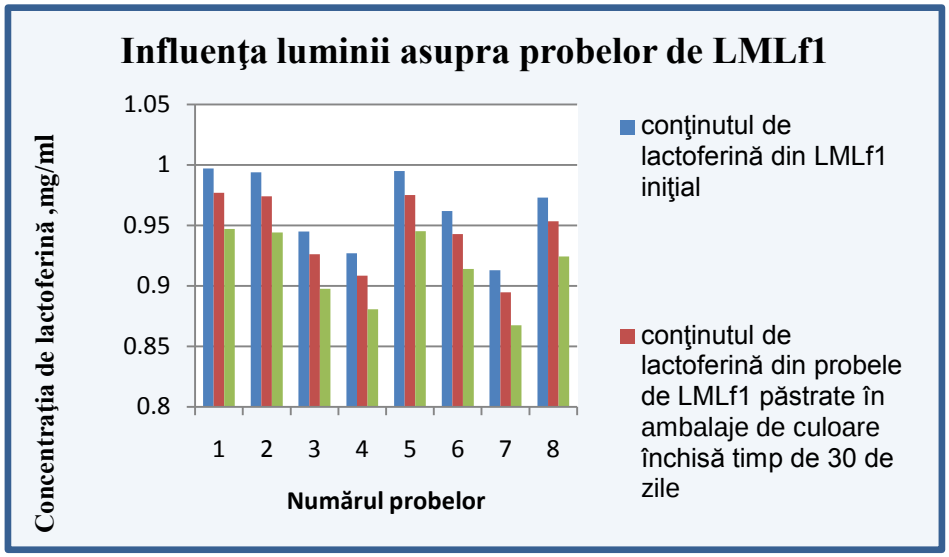

Fig.5.29 The influence of light on LMLf1

As a result of these determinations it is indicated to preserve almond milk with lactoferrin added in dark packages.

Subchapter 5.5.3 – *The results of an experiment on 20 children aged between 3 and 5 years. fed with almond milk with lactoferrin added, carob powder added, rosehip powder added*

In order to determine the effects of the product and to choose a product to please consumers we made an experimental study . The study was conducted on 20 children , aged between 3 and 5 years. The children were given the three types of almond milk and had to choose the one that they find the best in terms of taste . The majority agreed by the children was the one with carob powder added, followed by the one with lactoferrin added and on the last place was the one with rosehip powder added . The explanation can be derived from the fact that almond milk with carob powder added resembles in terms of appearance with animal milk with chocolate .

In the next phase the children were fed with almond milk with lactoferrin added for two months . The Almond milk formula with lactoferrin administered to them contained 250 mg/100 ml . Administration of milk was made to each child independently by the family and with its consent. During this study were regularly determined height and weight of children by nurse assigned kindergarten.

Data was collected on the frequency of diarrhea , respectively about its duration or the frequency and also about the appearance of viral respiratory infections . Also, two children were monitored closely because they were diagnosed with cow milk intolerance. The results showed that the number of virus diseases found significantly decreased. Children showed no changes of stagnation or improper physical development at that age during administration .

Children who are lactose intolerant milk appreciated this milk as tasty , and the symptoms manifested with animal milk administration (vomiting , abdominal cramps, bloating , diarrhea) have disappeared.

CHAPTER VI. COMPARATIVE ANALYSIS BETWEEN COW'S MILK AND ALMOND MILK

The scientific documentary and experimental research in order to develop this work started from the premise that almond milk is a functional food . New trends in functional foods nutrition delineated from the rest , just to recognize nutritional values and to be consumed by everyone. Approaching the problem from this point of view , it is known that dairy products are considered functional foods . Cow's milk is the main supplier of substances necessary for growth . The values for protein substances , minerals , calcium , phosphorus, are considerably higher than those of almond milk , protein content , calcium and phosphorus are responsible for absorption and deposition of these minerals in the matrix protein .

Milk fats consist of triglyceride - rich in saturated fatty acids. Almond milk consist of unsaturated fatty acids.

Cow 's milk minerals are composed of macro (Ca, K , Na , Mg), and micro elements (Fe , Cu, Zn , Al) . Cow's milk contains 135 mg/100 g Ca, almond milk contains 0.68 mg / 100 g Phosphorus is contained in cow's milk in amount of 75 mg/100 g , and the proportion from almond milk is 11.97 mg / 100g .

Sodium found in cow's milk in an amount of 75 mg/100g and in almond milk- 26.89 mg/100g. Regarding potassium content ,the gap between cow's milk and almond milk is higher (1300 mg/100g compared to 26.66 mg/100g) , and magnesium case the differences is from 90 mg/100 g to 3 79 mg/100g .

Iron is present in almond milk in small amounts (0.1 mg/100 g) and must be compensated by eating other foods rich in iron. Almond milk contains 0.0079 mg/100g . Regarding the content of vitamin E, α - tocopherol acetate is present in fresh cow's milk in a proportion of 45.5 μg/100g , content that decreases with its degreasing to 4.5 μg/100g (Suprya Kaushik , 2001) .

Almond milk contains about 440.5 mg/100 ml .

The taste of cow's milk is sweet , pleasant, because lactose . The lactose is responsible for the taste of the milk but it is also responsible for a number of gastrointestinal problems , especially at people with lactose intolerance due to the inability of the organism to digest it . Symptoms they have are bloating , flatulence , diarrhea , abdominal pain .

Besides this, cow's milk can cause allergies , especially at children aged up to 12 months. Symptoms consist of eczema type rash , swelling of the face , lips , eyes, eyelids, runny kind respiratory symptoms , dry cough , digestive symptoms (diarrhea with mucus and bloody streaks , vomiting food) , anemic syndrome , lack of appetite food, leading to edema that may require hospitalization.

The approach of almond milk as functional food is made also on the presumption that it does not contains lactose so it does not produce all the symptoms found in some cow milk consumers , bringing nutrient intake yet the body . The almond milk is a food handling in order to obtain extra benefits by reducing the risk of disease occurrence , is a classic food , but incorporated as a dietary supplement may be beneficial and nutritional .His presentation was that of a food normally with no modified features . From the point of view of prevention of disease, almond milk with the addition of rose hip powder can prevent deficiencies of vitamin C , almond milk with the addition of carob may be used by people with diabetes due to reduced caloric content compared to the cocoa .Almond milk with lactoferrin has been shown to reduce symptoms of lactose allergy in animal milk . Obviously , further research and clinical trials are needed in order to support these claims .

Experimental research suggests that , although it can not replace cow's milk , requiring daily ingestion of large quantities to ensure daily intake of nutrients, animal milk is not suitable to the needs of a particular sector of consumers. Moreover , the possibilities of enriching the quality and nutritional value of almond milk (with added rosehips ,carob or lactoferrin) come to demonstrate that its importance as a functional food is not negligible.

CHAPTER VII. CONTRIBUTIONS AND FURTHER TRENDS IN RESEARCH DEVELOPMENT

The objectives of this thesis was made possible both by the accumulation of data and

information from the literature, and especially by their application in experimental measurements . The personal contributions to this thesis can be defined as:

- Establish manufacturing process of almond milk ;
- HACCP study for the manufacture of almond milk ;
- Identify the main characteristics of almond milk ;
- Determination of the main components of almond milk ;
- Choosing some natural components (lactoferrin , carob powder , rosehip powder) which added in almond milk to enrich both the quality and nutritional value;
- Undertake a study on a certain sector of consumers of the three types of milk to get a feedback about their assessment and the effects of using them.

Regarding , the future directions of development of this research, the following can be considered

-the possibility of putting almond milk on the Romanian market, in conjunction with certain manufacturers , or in micro factories ;

- the opportunities for enrichment almond milk with other ingredients , so to fold strictly to a consumer sector , like diabetics , people with lactose intolerance , people with cardiovascular problems ;
- Research the effect of almond milk with lactoferrin in clinical trials ;

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