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OBTAINING AN ASSORTMENT OF FRESH CHEESE BY COAGULATION WITH LETTUCE (*LACTUCA SATIVA)* EXTRACT

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Abstract

The aim of this work was to evaluate the milk-clotting potential of lettuce (Lactuca sativa). Two extracts from this plant were obtained, by dissolving in a cold water, first, by a simple aqueous extraction and the second, by a concentration of simple extract. These two extracts were compared in their milk-clotting activity with a commercial animal rennet, chymosin. The analysis consisted in the characterization of raw cow's milk used by physico-chemical and microbiological analyses and characterization of the fresh cheese curd obtained by physico-chemical, microbiological and sensory analyses. The yield of the process was calculated in each of the three cases and a comparison between them was performed. The obtained results revealed a good milk-clotting activity for both tested plant rennets.

Key words*: cheesemaking, Lactuca sativa, milk-clotting activity, plant rennet.*

INTRODUCTION

Milk-clotting is the main stage of cheese production. It is made with milk-clotting enzymes that are prepared by proteolytic enzymes, this being the oldest application of them known for thousands of years.

The researches targeting the milk-clotting with the aid of plant coagulants has shown a growing interest in the industry of milk and dairy products, due to the easy availability of raw materials and simple extraction processes (Shah et. al., 2014). Another argument in the use of rennets obtained from plant sources is that the use of vegetable proteases in the process of obtaining cheeses promotes greater acceptability of this range of products from people with a vegetarian diet, to which are added certain benefits represented by the fact that they can improve their nutritional intake with various bioactive compounds from plant sources used in the process of milk coagulation.

The aim of this study was to evaluate the milk-clotting potential of lettuce (*Lactuca sativa*) and the possibility of using extracts obtained from this plant in dairy products such as cow's milk fresh cheeses. Leaves of lettuce have been indicated as a source of milk-clotting enzymes, which can substitute animal rennet (Derso & Dagnew, 2019).

Lettuce belongs to *Asteraceae (Compositae)* botanical family. The origin of the lettuce is quite controversial, most hypotheses claim that the appearance of the current form involved four species of European origin: *Lactuca sativa, L. saligna, L. serriola* and *L. virosa* (Kesseli et al., 1991). It has been cultivated since ancient times by the Egyptians, Greeks and Romans and is also popularly called lettuce. Lettuce is an annual plant with a short growing season. It is cultivated for its leaves and heads, which are eaten mostly fresh. The heads contain large amounts of vitamins (C, A, K, B complex), mineral salts (720 mg per 100 g, of which 234 mg potassium, 37 mg calcium, 24 mg phosphorus, 11 mg magnesium, the rest being iron and zinc) (Burzo et al., 2005), as well as significant amounts of sugars, polyphenols and cellulose. It is a low-calorie vegetable, being recommended in all diets.

The consumption of lettuce reduces the risk of heart disease, cancer and cataracts. Lettuce is very rich in vegetable fibers, which can significantly reduce cholesterol and prevent constipation. Also, it can induce a feeling of satiety much faster and thus help to lose weight or maintain weight within optimal limits. Lettuce is a remineralizing, purifying, emollient vegetable (Pârvu, 2006). The leaves can be eaten fresh, as salad, in early spring and autumn. The

storage temperature is around 1°C and the shelf life is usually two weeks (Lagunovschi-Luchian, 2014).
According with Shah et al. (2014), a serine protease named lettucine from *Lactuca sativa*, were identified with milk-clotting activity.

MATERIALS AND METHODS

In order to perform the experiments, a series of materials were used to make the curd obtained from a plant source, such as cow's milk, salad extracts, as well as laboratory reagents, which were used to perform physico-chemical analyses of the curd samples, whey, but also of the raw milk, in two repetitions for each.

Obtaining the plant extract

A comparative study was performed, in the simulation of the process of obtaining fresh cheeses, two extracts from *Lactuca sativa*, obtained as a crude extracts by dissolving the shredded and grinding plant in distilled water at cold: the first extract was obtained, as a supernatant, by filtration and centrifugation of the plant-water mix and, in the case of the second extract, the supernatant-base was concentrated by precipitation with ammonium sulphate, followed by centrifugal separation and dissolution of the precipitate in pH 5.5 citrate buffer solution. Ammonium sulphate precipitation of proteins is a widely used technique in enzyme purification, which takes advantages of the desolvation effect caused by high concentrations of salts (Duarte et al., 2009)
The preparation of cold plant extracts that were used as sources of vegetable curd in the coagulation of cow's milk in the process of fresh cheese production is shown schematically in figure 1 and figure 2, which represent the working protocol to obtain the simple crude extract of *Lactuca sativa,* at cold, respectively the working protocol to obtain the concentrated crude extract of *Lactuca sativa,* at cold.
In relation to a standardized product, the coagulation of milk with chymosin, the animal milk-clotting enzyme frequently used in industrial processes, was used as control sample. The commercial product used was CHY-MAX, manufactured by CHR.HANSEN.

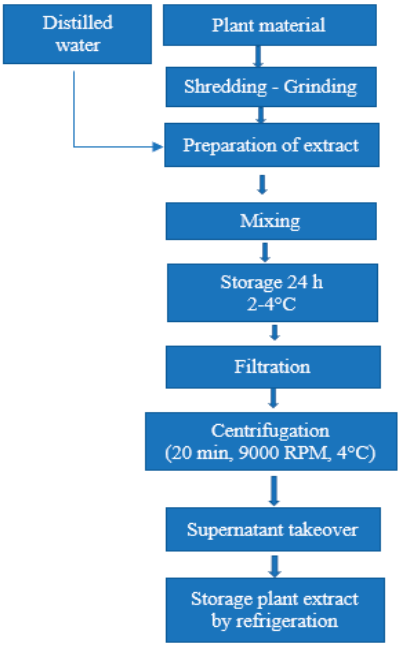

Figure 1. Working protocol used to obtain *Lactuca sativa* simple extract

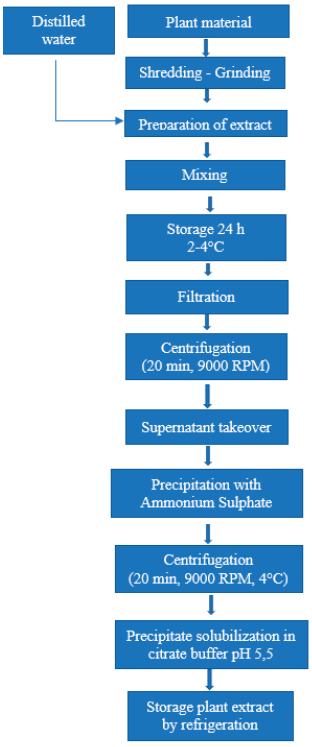

Figure 2. Working protocol used to obtain *Lactuca sativa* concentrated extract

Physico-chemical and microbiological analyses of raw milk

The raw milk was purchased from a free-market Farm Dispenser.

Physico-chemical analysis

Determination of the physico-chemical properties of raw milk using the Ekomilk device

The Ekomilk device is an automatic, fast and specially designed laboratory equipment used for the analysis of the main physico-chemical quality parameters of cow's, sheep's, goat's or buffalo's milk.

The Ekomilk device provides a large number of measurements, using ultrasound technology, and a 50 ml of milk is required to analyse all parameters. The milk samples must have a temperature between 5-35°C, the measurement time is 90 seconds, and the device analyses the fat, dry matter, density, protein and water added to the milk.

Determination of pH

Before performing the pH meter measurements, the instrument must be calibrated using standard buffer solutions. To determine the pH of the milk sample, the beaker was filled 2/3 full, with milk and the electrode was completely immersed. The value is read 15-20 seconds after immersion.

Determination of ionic calcium in milk by complexometric method

Calcium ion dosing is done by complexing with the disodium salt of ethylenediaminetetraacetic acid (Na_2H_2EDTA) or complexon III, in the presence of murexid as an indicator, (Campeanu et al., 1993).

Dosing of milk casein

The determination of casein is based on the principle of its precipitation at isoelectric pH (4.6 for cow's milk).

It is very important not to exceed the isoelectric pH value, as casein is resolubilized and the casein content of the sample is calculated from the amount of sodium hydroxide which participated in the complete solubilization of the casein (Campeanu & al., 1993, Căpriţă & Căpriţă, 2008).

Dosing of reducing carbohydrates by the Schoorl method

The Schoorl method can directly dose soluble reducing carbohydrates based on the redox reaction with Fehling's reagent. The amount of directly reducing carbohydrates in the sample is deduced from the tables which establish a correlation between the volume of sodium thiosulphate used in the titration and the directly reducing carbohydrates (Vasu et al., 1985).

Microbiological analysis

For the raw milk samples, microbiological analyses were performed on specific culture media.

Total aerobic count

This parameter refers to the quantification of the number of microorganisms that grow in aerobiosis, at 30°C, after incubation 72h ± 3h.

According to REGULATION (EC) NO. 853/2004 of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs, the maximum Plate Count at 30 °C allowed by law is 100.000 CFU / ml for raw milk from dispenser.

Coliform bacteria

A sample is seeding using the technique of incorporating the inoculum into a Mac Conkey agar culture medium to count coliform bacteria. *Staphylococcus* can multiply in milk at high temperatures and produces enterotoxins retained by casein and found in cheeses made from contaminated milk. The culture media used for *Staphilococcus* determination was Manitol.

Total combined yeasts and molds count (TYMC)

Samples are performed according to the preparation standards specific to each matrix which provide for the preparation of samples for analysis, initial suspension and decimal dilutions for the microbiological examination, also complying with the provisions of SR EN ISO 7218: 2007 / A1: 2014.

Yeasts and molds can contaminate machinery and storage facilities in dairy plants.

Testing of milk-clotting activity

The research aim was to simulate the technological process of obtaining fresh curd cheeses (see Figure 3) and to establish the coagulation yield using two extracts of *Lactuca*

sativa, by comparison with the coagulation yield obtained with a commercial rennet based on chymosin.
The following simulation steps were as follows: (a) to pasteurize raw cow's milk, at 72°C, for 20 seconds and, immediately after, to cool it down to 35°C; (b) the milk-clotting enzyme is added to the pasteurized and cooled milk and it follows an incubation of 16 h at 30°C, stage in which the milk is coagulated; (c) after processing of the curd by cutting and stirring, light heating to 35°C, to favor the separation of the whey; (d) the last step is to separate the whey curd and evaluate the samples obtained (Figure 3).
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The notations used for the curd samples thus obtained were:
P1 - sample of milk + 0.025% chymosin CHY-MAX;
P2 - sample of milk + 10% simple aqueous extract of *Lactuca sativa*;
P3 - sample of milk + 0.81 % concentrated aqueous extract of *Lactuca sativa*;

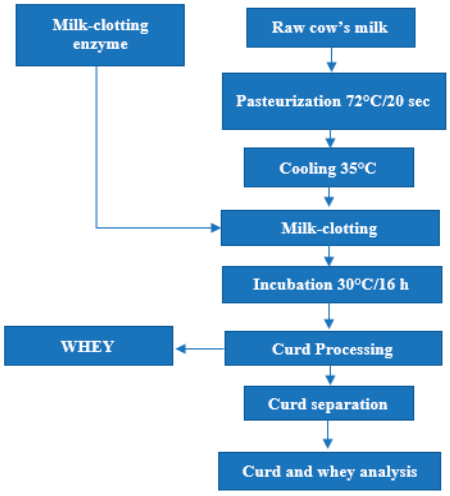

Figure 3. Working protocol used to obtain milk curd with salad extracts

Determination of yield
The calculation of the yield when the quantities of milk, raw material and cheese are known is obtained by the following formula:

$$R\ (\%) = (100 \times CB) / CL$$

In which:
CB - the amount of cheese (curd) obtained, in grams;
CL - the amount of coagulated milk, in liters.

Physico-chemical analysis of the curd

Determination of the water activity index (aw)
The curd sample is inserted into the thermostatic boxes of the determination device aw.
After 30 minutes of incubation, the sample is placed in the reading chamber of the device. When the aw value stabilizes, the values are read on the device screen.

Determination of total dry matter
Determination of dry matter by thermobalance is a fast and reliable method of determining the moisture content using the thermogravimetric principle. Thermogravimetry consists of weighing the sample before and after heating to determine the moisture content from the mass difference.
The sample is prepared at the time of the measurement. This prevents the exchange of moisture with the environment. The sample is evenly distributed in a thin layer on the weighing pan to obtain reproducible results. If the sample is applied unevenly, it will cause unhomogeneities in the heat distribution in the heated sample, resulting in incomplete drying or extending the measurement time.

Microbiological analysis of the curd
For the fresh cheese curd obtained, microbiological analyzes were performed on specific culture media.

Total aerobic count
This parameter refers to the quantification of the number of microorganisms that grow in aerobiosis, at 30 °C, after incubation 72 h ± 3 h.
According to REGULATION (EC) NO. 853/2004 of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs, the maximum Plate Count at 30 °C allowed by law is minim 10^4 CFU/g – maxim 10^5 CFU/g for cheeses from raw milk.
Microbiological analyzes for the fresh cheese curd obtained, were performed on specific culture media, as described in 2.2.2 section.

Organoleptic analysis of the curd
The methods of sensory analysis used consist of the method of organoleptic analysis performed with a group of panelists and the method of color determination with a spectrocolorimeter.
The method principle. In order to perform the sensory analysis of cheese samples, certain

quality indicators are monitored, such as: appearance, color, texture, smell and aroma of the samples. The scoring system has been performed using the method by comparison with a unit scoring scale, from 1 to 5. The number of points awarded to each quality indicator is awarded on the scale described in Table 1.

Table 1. 5-point scale for assessing the quality of the curd (source: Banu, 2007)

Quality appreciation step	Points number	General description of the degree of appreciation
Excellent	5	Excellent quality
Very good	4	Quality in full compliance with the specifics of the product
Good	3	Good quality, appropriate
Satisfactory	2	The product has slight defects that can be accepted
Unsatisfactory	1	The product has obvious, multiple and systematic defects
Altered	0	The product has significant defects and can no longer be consumed

Next, the average score (Pm), which represents the arithmetic mean of the results of the evaluation by points of a sensory characteristic and the weighted average score (Pmp) assigned to each sensory characteristic, have been calculated using the relation below:

Pmp = Pm x fp or Pmp = Pm x fi x ft

In which:

fp - the weighting factor that represents the product between fi and ft;

fi - the factor of importance, which indicates the extent to which each sensory characteristic participates in the quality of the product (the sum of the factors of importance is equal to 1, and the values of the factors of importance are established for each product in the standards of sensory analysis);

ft - the transformation factor with the help of which one passes from the 5-point scale to the 20-point scale, in order to establish the quality of the product. The transformation factor is equal to 4.

Finally, the total average score (Pmt) has been calculated by summing the values of the weighted average scores from all sensory characteristics. The total average score is expressed to one decimal place (Banu, 2007).

Each quality indicator has its own weight in relation to the other indicators. The individual scores given to each sensory characteristic are recorded in the summary sheets, which are presented in Table 1.

2.7. Determination of color using a spectrocolorimeter

A HunterLab MiniScanTM XE Plus spectrocolorimeter was used to measure the color of the samples, for which the working conditions were: Geometry of the device: 45º / 0º; Viewing area: LAV; Illuminant: D65; Observer: 10º; Color system: CIELAB'76.

RESULTS AND DISCUSSIONS

Coagulation of milk with vegetable milk-clotting enzymes is essential in the cheesemaking process. Previously, several plant sources (*Taraxacum officinale, Rumex acetosa, Lactuca sativa, Urtica dioica*) were used to study the coagulation capacity of milk (Nitu et al., 2021). These plants are found in the spontaneous and cultivated flora of Romania, so they were harvested and used in the form of aqueous extracts. When testing a potential replacement for animal rennet, it is particularly important to perform a milk coagulation test.

From the plant sources studied in the previous article, lettuce (*Lactuca sativa*) presented the best premises to be used as a substitute for commercial animal rennet.

Results of physico-chemical determinations of cow's milk used as raw material

Table 2 shows the results of the physico-chemical analyses obtained for the cow's milk sample, using Ekomilk device and pH-meter, and also analyses obtained for the raw material milk sample, in terms of casein content, ionic calcium content and lactose content.

Table 2. Results of physico-chemical analysis of cow's milk used as raw material

Sample	Analyzed parameters								
	pH	Fat (%)	Non-fat dry mater (%)	Density (g/cm³)	Water added (%)	Protein (%)	Casein (g/100 ml)	Ionic calcium (mg/100 ml)	Lactose (g/100 ml)
Raw cow milk	6.7±0.1	3.66±0.1	8.2±0.1	1.028±0.001	0±0.1	3.2±0.1	2.87±0.01	326.6±0.1	4.56±0.1
Reference values*	6.4-6.7	3.5±0.1	8-8.5	1.027-1.033	0	3-3.2	-	-	-

*State Standard STAS 143-84 on the quality of raw cow's milk

The results show that the raw milk used in the experiments is of a good quality, which is in line with current standards.
The determined casein content is optimal, as it must be at least 80% of the total protein content. At a protein content of 3.2 g / 100 ml milk, as shown in Table 2, the casein content should be at least 2.56 g / 100 ml milk. The result obtained, 2.87 g casein / 100 ml milk gives the auspices of a good coagulation yield, casein representing the substrate of the curd.
The ionic calcium content represents the ability of milk to form a three-dimensional structure during coagulation, through calcium bridges.
According to Walstra et al., 2006, the minimum ionic calcium content is 120 mg / 100 ml. The determined calcium ion content, 326.6 mg / 100 ml, indicates a high potential for the three-dimensional structure of the raw milk used.
The determined lactose content is within the normal content of cow's milk 4.2-4.6 g / 100 ml (Walstra et al., 2006) and will influence the use of whey resulted from the cheesemaking process, as a substrate. for obtaining probiotic biomass.

Microbiological analysis

The results of the quantification of microbiological parameters are summarized in Table 3.

Table 3 Results of microbiological analyzes in the milk sample (CFU / mL)

Microbiological indicator	Total number of aerobic germs (NTG)	TYMC (total combined yeasts and molds count)	***Staphylococcus***	Coliforms bacteria
Value	4.3×10^7	4.9×10^5	4.3×10^1	2×10^5

Visual aspects of the results from the microbiological analyzes can be observed in Figure 4.

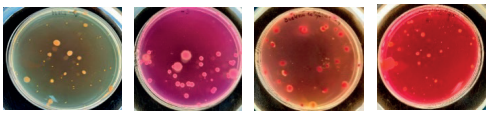

Figure 4. On-plate aspects of the results of microbiological analyses in raw milk (from left to right: NTG, TYMC fungi, coliform bacteria, staphylococci)

The results of the microbiological analyses, presented in Figure 4 and centralized in Table 6, give an overview of the quality of the raw milk.
Thus, regarding the Plate Count, the result obtained, 4.3 x 107 CFU / mL is higher than the one provided in REGULATION (EC) NO. 853/2004 of 29 April 2004 which establish specific hygiene rules for on the hygiene of foodstuffs, of 100.000 CFU / mL for raw milk, possible causes being faulty handling in the vending machine supply, quality of packaging provided; by using pasteurization in the treatment of milk intended to obtain the fresh cheese curd, the pathogenic germs will be eliminated.
The result obtained for the total number of yeasts and filamentous fungi (TYMC), 4.9×10^5 CFU / mL, indicates a contamination of raw milk with yeasts and molds, potentially obtained from the contact of milk with the atmosphere; these microorganisms are destroyed by a properly applied pasteurization regime.
It is observed that staphylococci were also detected, 4.3×10^1 CFU / mL, but no confirmatory tests were performed; their presence indicates, in principle, that the sample is not compliant, but by pasteurization these microorganisms will be destroyed.
The total number of coliform bacteria determined, 2×10^5 CFU / mL is an indicator of the degree of hygiene in which the milk was obtained and handled, the raw milk being non-compliant; these bacteria are also destroyed by a pasteurization regime.

Testing of milk-clotting activity

According to Lo Piero et al., (2002), lettucine from *Lactuca sativa* has the highest milk-clotting activity at 50°C. For these experimental tests, the decision to have the incubation for coagulation at 30°C was taken considering the intended comparison with the commercial animal rennet.
After coagulation and processing of the curd, they led to the following quantitative results, in Table 5. It is observed that in the case of the two salad extracts, the amount of curd obtained is higher by 45%, which represents a significant increase, which is also reflected in the calculation of yield. A first expression of the yield is expressed also in Table 5.
It is observed that the most efficient coagulation yield is obtained when using the concentrated lettuce extract, being approximately 23% higher than the yield obtained when using the animal

rennet and 45% higher than the yield obtained when using the gross lettuce extract.

Physico-chemical characterization of the curd

Determining water activity

The results of the determinations are presented in Table 4.

It is known that a lower value of the water activity index leads to an increase in the shelf life of the finished product. The results obtained from the determination of the water activity index of the cow's milk curd with the addition of animal rennet and the vegetable coagulants obtained from *Lactuca sativa* are presented in the Table 4.

Table 4. Total dry matter and water activity of the curd samples obtained

	P1	P2	P3
Total dry matter, %	41.925	44.248	35.838
Water activity, aw	0.990	0.985	0.975

The sample of milk with animal rennet, registered the highest value of the water activity index, respectively 0.990. Also, sample 2 of milk with simple extract of *Lactuca sativa* registered a close value of 0.985, and sample 3 of milk with concentrated extract of *Lactuca sativa* had the lowest value of the water activity index, 0.975. Therefore, the data obtained for the three samples are comparable.

Determination of total dry matter

The fresh cheese curd resulting from the three cases of the experimental determinations was recorded with the total dry matter contents shown in Table 4.

The resulting curd quantities, with the dry matter content described above, were recalculated to a standard 40% fresh cheese dry matter content. The physical yield of the cheeses will be recalculated in the Table 5, resulting in a recalculated standard yield.

Table 5. Quantities of cheeses obtained in the tests – Physical and Recalculated standard yield

Data/Sample	P1	P2	P3
CB, kg	0.295	0.500	0.725
CL, liters	2	4	4
Physical yield, Rphy, %	14.75	12.5	18.125
Total Dry Matter, %	41.925	44.248	35.838
CB Standard Recalculated at 40% dry matter kg	0.309	0.553	0.650
Recalculated yield, Rrec, %	15.460	13.828	16.239

The results obtained show that fresh cheese obtained by coagulation with concentrated extract of *Lactuca sativa* had the highest yield, being 5% higher than the yield of fresh cheese by coagulation with commercial rennet CHY-MAX and 17.5% higher than the yield obtaining cheese by coagulation with simple extract of *Lactuca sativa.*

The addition of the source of concentrated vegetable coagulant enzyme from *Lactuca sativa* in cow's milk leads to the lowest value of the water activity index, which implies an increase in the shelf life of the finished product. The curd sample obtained with the addition of simple extract from *Lactuca sativa* also reached a lower value of the aw index than the curd obtained from the milk sample in which a quantity of chymosin was added, which recorded the highest value. of the water activity index.

Following the determination of the dry matter for the curd samples obtained, it was found that the milk sample in which the concentrated extract of *Lactuca sativa* was added had the lowest dry matter content and therefore the lowest energy value.

Microbiological characterization of the curd

The curd samples were analysed 5 days after obtaining, being kept in refrigeration conditions. The values of the microbiological parameters of the three samples are detailed in Table 6.

Table 6. Values analysed in curd samples

Sample/ Microbiological indicator	Total number of aerobic germs (NTG)	***Staphylococcus***	Coliforms bacteria
P1 Milk + chymosin	6.75×10^7	0	2.15×10^6
P2 Milk + simple extract of lettuce	2.8×10^{10}	0	2.92×10^9
P3 Milk + Concentrated extract of lettuce	7.60×10^9	0	6.7×10^8

Visual aspects of the microbiological analysis can be seen in Figure 5 for Aerobic colony count, respectively Figure 6 for coliforms.

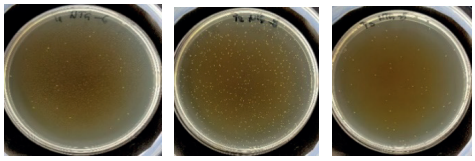

Figure 5. Visual aspects of Aerobic colony count analysis in different curd samples (P1, P2 P3)

The results of the microbiological analyzes performed on the curd samples obtained give an overview of the fresh cheese manufacturing process and storage conditions.
In the case of aerobic colony count it is observed that by the addition of the curd of vegetal origin the total load is higher by 2-3 logarithmic units in relation to the milk coagulated with enzyme of animal origin.

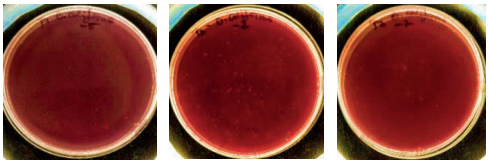

Figure 6. Visual aspects of coliform analysis in different curd samples (P1, P2 P3)

Staphylococci were not present in any of the s analysed sample, thus the samples being compliant. In the case of total coliform bacteria it is observed that the addition of plant extract increases the contamination by 2-3 logarithmic units.
The source of the contamination may be due to the defective microbiological quality of the plant extract and the inadequate handling of the material. It is recommended that this extract also undergo sterilizing filtration so that microbiological indicators are kept within the limits of admissibility.
The results obtained following the microbiological analyses indicates a contamination of the three curd samples after at least 5 days of storage in refrigeration conditions, which indicated the highest values in the case of sample P2, coagulated with simple *Lactuca sativa* extract, followed by those of sample P3, coagulated with concentrated extract of *Lactuca sativa*.
The explanation of this phenomenon lies reside even in the process of concentration, by precipitation and centrifugation, by removing the supernatant can be removed some of the microbiological contaminants.
The next concern in the process of obtaining plant coagulant extracts should be the treatments applied in order to remove contaminants.

Organoleptic characterization of the curd

Cheesemaking is a more complex process that involves concentrating protein along with a variable fraction of fat and minerals, eliminating a significant amount of water and lactose (Costin, 2003).
Sensory determination of the samples of milk coagulated with milk-clotting enzyme from the same plant source, *Lactuca sativa*, obtained simply or concentrated, by comparison with the sample of milk coagulated with commercial chymosin, consisted in the use of two methods, described in the materials and methods section, respectively of scoring quality indicators according to sensory analysis and determination of the colour of the curd samples using the Hunterlab spectrocolorimeter.
The sensory analysis was performed by a sensory panel of 20 people (staff and students from the Faculty of Biotechnology). The characterization of the tasting team is described in Table 7. It could be noticed that the panel consisted of people of different ages (between 10 and 55 years old). and the females were predominant (70%); meanwhile, in the group 35% were smokers.

Table 7. Characterization of the sensory panel

Gender		Female	Male
Ratio, %		70,00	30,00
Smokers, %		42.86	16,67
Age, %	0-20 years	57,14	16,67
	20-30 years	14,29	16,67
	40-50 years	14,29	33,33
	>50 years	14,29	33,33

The sensory analysis performed on the appearance of the fresh cheese curd, which should have a well-shaped shape. The analysis of the consistency or texture of the curd is intended to be elastic, compact, and the colour is characteristic, white and glossy, almost uniform in the same container. The smell of the analysed samples must be pleasant, specific to fermentation, without foreign odour. The appearance of the analysed whey should be clear or slightly opalescent.
The sensory description of the curd and whey samples resulting from the experiments is presented in Table 8.

Table 8. Sensory description of curd and whey samples

Sample P1			
Characteristics	Curd	Appearance	Firm consistency, with the appearance of porcelain in the section, easy expulsion of whey, without gas bubbles
	Whey	Colour	White
		Taste	Pleasant, specific
		Smell	Pleasant, specific
		Appearance	Clear, slightly opalescent
		Colour	Yellow-greenish
		Taste	Sour, pleasant
		Smell	Pleasant, specific
Sample P2			
Characteristics	Curd	Appearance	Friable consistency, sandy appearance, compact, without whey expulsion, gas bubbles in section
	Whey	Colour	greenish white
		Taste	Pleasant, specific, slightly sour
		Smell	Pleasant, specific
		Appearance	Opalescent, fat separation on the surface
		Colour	Yellowish
		Taste	Sour, pleasant
		Smell	Pleasant, specific
Sample P3			
Characteristics	Curd	Appearance	Suitable consistency, slightly soft, slightly sandy appearance, with whey expulsion, rare gas bubbles in section
	Whey	Colour	Greenish white
		Taste	Pleasant, specific, slightly sour
		Smell	Pleasant, specific
		Appearance	Slightly opalescent, slightly greasy on the surface
		Colour	Yellowish
		Taste	Sour, pleasant
		Smell	Pleasant, specific

The centralized results of Total average score (Pmt), following the processing of the data obtained from the tasting team are presented in Figure 7.

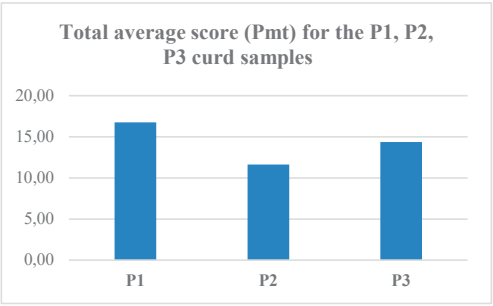

Figure7. Graphical representation of Total average score (Pmt) for the P1, P2, P3 CURD SAMPLES

The results obtained from the sensory analysis of the tasted curd samples suggest that P1 sample of milk with chymosin was preferred, being the sample that obtained the highest average total score, respectively 16.74.

This was followed by P3 milk sample with concentrated *Lactuca sativa* extract which obtained a total average score of 14.36 and P2 milk sample with simple *Lactuca sativa* extract, which obtained the average total score of 11.62.

The degree of organoleptic appreciation of the fresh cheese curd obtained with concentrated extract of *Lactuca sativa* is quite close to that obtained with commercial rennet.

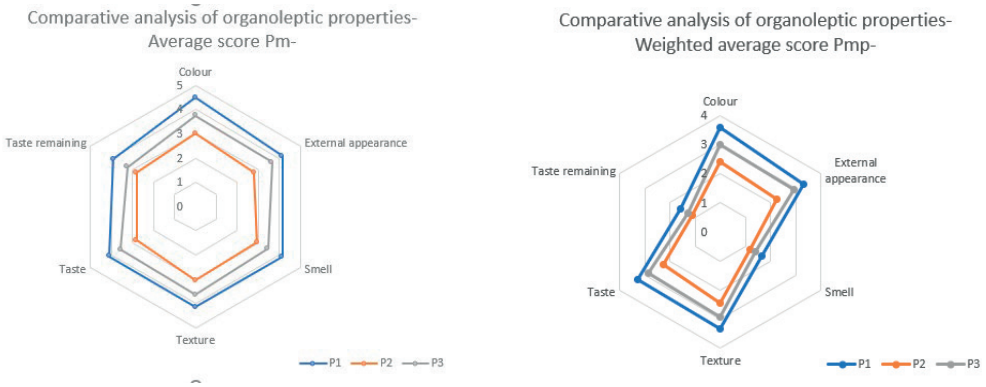

Figure 8. Graphical representation of the sensory characteristics of the curd (Pm-Average score and Pmp-weighted average score)

From the analysis of the graphical representtations of the average scores and the weighted average scores obtained after the tasting session of the three samples of fresh cheese curd (Figure 8), it is observed the quite clear delimitation of the preferences for sample P1 (milk coagulated

with chymosin), closely followed by sample P3 (coagulated milk with concentrated *Lactuca sativa* extract), sample P2 (coagulated milk with simple *Lactuca sativa* extract) being on the last place in the tasters' preferences.

Determination of colour using a spectrocolorimeter

The samples of fresh cheese curd obtained in the experimental determinations were analysed, both immediately after obtaining and at 5 days.

The results obtained from the colour determinations, with the help of the HunterLab spectrocolorimeter, of the curd samples, respectively the numerical values are presented in graphical form in Figure 9.

As can be seen, the colours of the analysed curd samples are very close, located grouped in the yellow spectrum of the analysis.

Following the physico-chemical determinations carried out on the raw cow's milk used as raw material for the preparation of the curd, a very good quality and freshness was found, and the values recorded were within the limits provided by the standards in force.

The curd obtained from milk treated with concentrated extract of *Lactuca sativa* had a higher production yield than the curd obtained from milk treated with commercial rennet chymosin. The lowest yield was obtained in the case of the curd obtained from milk treated with simple extract of *Lactuca sativa*.

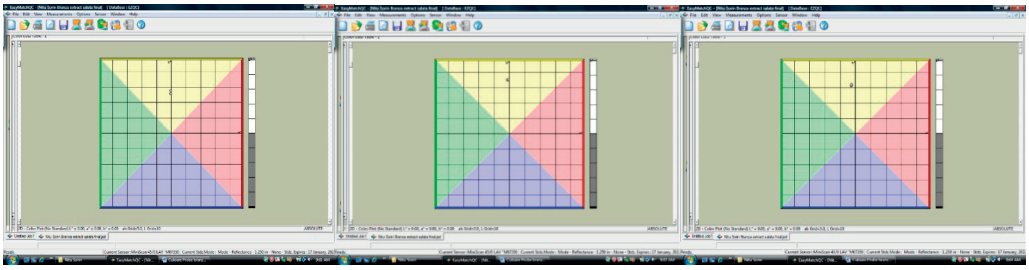

Figure 9. Graphical representation of colorimetric analyses for curd samples analysed at baseline and after 5 days (a-P1; b-P2; c-P3); From left to right: a. Sample P1 - initial (top point), after 5 days (bottom point); b. Sample P2 - initial (top point), after 5 days (bottom point); c. Sample P3 - initial (top point), after 5 days (bottom point)

CONCLUSIONS

The data obtained from the physico-chemical analyses of the raw milk and the final product aswaell as the results from organoleptic analyzes of the curd samples with the addition of *Lactuca sativa* plant extracts, obtained differently, led to promising results in terms of quality and preference of the finished products. The same cannot be said for microbiological indicators, as milk was not compliant. Pasteurization can solve these microbiological issues.

In the milk sample in which a simple extract of *Lactuca sativa* was added, a very well separated curd was formed from the whey, with the highest dry matter content of the three curd samples obtained.

Regarding the yields obtained from the technological simulations performed, in the first phase the physical yield was expressed, by reporting the quantities of physical curd obtained to the volumes of milk raw material used.

The recalculated standard manufacturing yield, performed at 40% dry matter, is an important indicator of the milk-clotting capacity of the plant enzymatic extracts, providing a more accurate picture of the efficiency. For instance, the curd obtained from milk treated with concentrated *Lactuca sativa* extract had a 5% higher manufacturing yield than the curd obtained from milk treated with commercial rennet-chymosin. The lowest yield was obtained in the case of the curd obtained from milk treated with simple extract of *Lactuca sativa,* less than 17.5% than that obtained with concentrated extract of *Lactuca sativa.*

The comparative analysis of the organoleptic properties of the three curd samples clearly indicates the preferences of the tasting team, the P1 sample being preferred to the P3 sample in terms of all sensory characteristics. The P2 test was on the last place in the preferences of the tasting team in terms of all the sensory characteristics.

The studied *Lactuca sativa* plant extracts have shown that they have the potential to coagulate cow's milk and can be used successfully as a milk-clotting enzyme from the vegetable source in the process of obtaining fresh cheeses.

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