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A PRELIMINARY STUDY ON THE POTENTIAL OF ROMANIAN NATIVE FLORA TO OBTAIN MILK-CLOTTING PLANT PROTEASES

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Abstract

The increase in cheese consumption worldwide, in symbiosis with the promotion and consolidation of lacto-vegetarian diets, as well as with the support of foods with religious restrictions (Halal, Kosher), has imposed a sustained trend of identifying plant proteases with milk-clotting activity. In the spirit of this trend, appeared the necessity to scan the native flora of Romania, to find the local resources of vegetal milk-clotting enzymes. This article presents a series of tests performed on indigenous plants like Taraxacum officinale, Rumex acetosa, Lactuca sativa, Urtica dioica, to identify and evaluate the potential for milk coagulation, knowing that they are sources of plant protease. The research involved the use of plant tissues as such, but also in order to obtain extracts that were later used in milk coagulation. The analysis consisted in establishing the milk-clotting activity (according with Soxhlet method), but also physic-chemical and sensory analyses. The obtained results revealed milk-clotting activity on almost all plants researched. A more accurate characterization of the purified plant milk-clotting enzyme would be interesting to be performed in the future.

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

INTRODUCTION

Milk is a natural product of mammals with the role of feeding and providing immune protection for newborns. It has also been a valuable source of food for humans since prehistoric times. The domestication of animals took place more than 6,000 years ago. Sanskrit writings attest to the fact that milk was already an important food at that time. It was so valuable that the ancient Hindus valued welfare according to the number of cattle. Over time, cows have become sacred animals, and are still considered a part of India's population (Ciocârlie, 2002).

Among animal foods, milk is the most complete and most easily assimilated food by the body, being one of the staple foods in human nutrition, especially during growth, because it contains all the substances necessary for normal growth and development of the body. Milk is the food strictly necessary to feed the sick, the elderly and all those who work in a toxic environment or in difficult working conditions. Being an excellent food product, a very varied assortment of food products can be prepared from it. Historical documents show that the pieces of cheese were brought as a gift to the kings of that time and also served as objects of exchange for nomadic peoples (Guzun, 2001). Enzymatic coagulation of milk is an important step in the cheese-making process. In general, coagulation of milk with calf rennet is the most used procedure. Research over time has shown that several plant proteases have the ability to coagulate milk (Liburdi et al., 2019). They are generally extracted from the leaves and stems of plants.

According Abebe et al. (2020), the high prices of calf rennet, refusal to accept cheese made from animal rennet in general and porcine rennet by vegetarians and Muslims, respectively, requested the need to substitute animal rennet with easily available, relatively cheap, and acceptable source of rennet for cheese preparation.

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 enzymes, known for thousands of years. Historically, clot extracted from ruminants' stomachs is the most widely used source of enzymes in the manufacture of cheese (Bornaz et al., 2010), although there is evidence of early use of coagulation enzymes in microorganisms and plants. Genetically engineered clot substitutes produced by microorganisms have been shown to be suitable substitutes for animal rennet, but growing interest has been directed at plants rennet, it means, enzymes for coagulating milk extracted from plants (Manzoor et al., 2014).

Animal rennet is a complex of enzymes produced in the stomach of any mammal, including proteases that coagulate milk, producing its separation into a solid part (curd) and a liquid part (whey). The presence of these enzymes is very important in the stomachs of young mammals for digesting the breast milk with which they are breastfed. The main active enzyme in the clot is called chymosin or renin (EC 3.4.23.4), along with pepsin and lipase. The natural calf rennet is extracted from the inner mucosa of the fourth chamber of the stomach of young, unweaned calves. The rennet extracted from older calves, fed on grass or cereals, contains less or no chymosin, but more pepsin. This rennet can only be used for some special types of milk and cheese. Each ruminant species produces a special type of rennet to digest the milk of its own species, so there is, for example, a goat's rennet for coagulating goat's milk or a lamb's rennet for coagulating sheep's milk.

The researches targeting the clotting of milk with the aid of plant coagulants has shown a growing interest in the industry of milk and dairy products, due to both the easy availability of raw materials and simple extraction processes (Shah et al., 2014). Another argument in the use of clots 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 the fact that they can improve their nutritional intake with various bioactive compounds from plant sources used in the process of milk coagulation.

In view of the aspects mentioned above, in our study it has been established as objective the evaluation of the coagulation potential of some vegetal extracts coming from plants harvested from the spontaneous flora, from our country, in the processing of cow's milk. The targeted plants were *Taraxacum officinale* (dandelion), *Rumex acetosa* (sorrel), *Lactuca sativa* (lettuce) and *Urtica dioica* (nettle).

Taraxacum officinale is an herbaceous, perennial plant, with a height of 20-50 cm, with truncate leaves, arranged in a basal rosette and with yellow flowers grouped in calatids. The parts used for medicinal use are: roots that contain bitter principles, young leaves and juice. The plant is rich in triterpenoids that have anti-inflammatory properties (Oroian, 2018). More recent research has shown that in addition to salicylic acid, the leaves also contain vitamins A, B, C and D, and a substance called choline, which is also found in the gallbladder (Hoffmann, 2016).

Rumex acetosa it is a perennial plant found in spontaneous flora in almost all regions of the globe. It is a very popular crop plant in some regions. It is cultivated for its leaves rich in iron, vitamins and oxalic acid, having the advantage that it appears very early (Gescher et al., 2011). It has the highest content of oxalic acid (approximately 5.27%) in dry matter. From a medical point of view, it is considered a vegetable with emollient action due to the high content of oxalic acid, which gives it its specific sour taste (Drăghici, 2009).

Lactuca sativa has been cultivated since ancient times by the Egyptians, Greeks and Romans and is also popularly called lettuce. Lettuce was brought to the new world by Christopher Columbus. It is an annual plant with a short vegetation period. It is cultivated for its leaves and heads, which are eaten mainly 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 sugar, polyphenols and cellulose. It is a low-calorie vegetable, being recommended in all diets. Eating this plant reduces the risk of heart disease, cancer and cataracts. Are very rich in vegetable fiber, which can significantly reduce cholesterol and prevent constipation, can induce a feeling of satiety much faster and thus help to lose weight or keep weight within optimal limits. *Lactuca sativa* is a remineralizing, purifying, emollient vegetable (Pârvu, 2006). The leaves can be eaten fresh, in the form of a salad, in early spring and autumn.

Urtica dioica, considered in ancient times the queen of plants, we must not miss our diet. It is consumed only in spring, from March to May, when they are young. It ensures a constant supply of biologically active substances, which restore our tone in the spring. Nettle is rich in chlorophyll, mineral salts (calcium, magnesium, potassium, iron, silicon), protein, pantothenic acid, folic acid, vitamins (B1, B2, C, K, beta-carotene). Nettle vitaminizes and remineralizes the body, eliminates anemia, balances the body's defense system with detoxifying effect, has a beneficial effect in bronchitis and asthma, fights cough, promotes the elimination of uric acid by the kidneys, is a hair tonic and fights dandruff. It is found in spontaneous flora all over the globe except in very cold or excessively hot areas. It is an herbaceous, dioecious, perennial plant that grows up to 150 cm in height. In the soil it forms a thin, cylindrical, whitish, long and branched rhizome. The stems are straight, with 4 edges the leaves are opposite, toothed at the edges. Both the stem and the leaves are provided with stinging hairs. At the base, the leaves are cordate. It blooms in June (Drăghici, 2009)

MATERIALS AND METHODS

The experiments were carried out in the “Engineering Processing” and “Food Biotechnology” laboratories, from the Faculty of Biotechnologies, USAMV Bucharest.

Raw milk. As coagulation substrate was used non-pasteurized raw cow milk delivered in the automatic equipment from UASMV Bucharest campus, originated from the didactical farm Belciugatele.

Plants sources of vegetal proteases. All the plants tested in this study (*Taraxacum officinale*, *Rumex acetosa*, *Lactuca sativa* and *Urtica dioica*) were harvested during spring season from natural habitats in Bucharest-Ilfov geographical. For the test the plants were conditioned as aqueous extracts, as follows: sort the leaves, flowers, roots and stems; crushing and grinding of the selected plant material takes place; the preparation of the extract consists in the addition of distilled water over the ground vegetable material; stir the mixture; followed by filtration of the mixture and centrifugation for 15 minutes at 40°C, then separation of the supernatant to obtain the extract; the last step is to store the extract in refrigerated conditions at 4°C.

Animal rennet used in experiments. Rennet is an enzyme used to coagulate milk in the process of forming cheeses. Commercially purchased rennet, called IDEAL, is a source of chymosin, obtained by fermentation (Figure 1) and was employed as control. The use instructions refer to the use of 2 ml of solution for 10 l of warm milk (35°C) and shaking the solution before use. The milk-clotting time mentioned in the instructions is 45 minutes. The net mass of the package is 100 ml, and the coagulation power of the curd is 180 IMCU*/ml, (*international milk coagulation units). Indications: Heat the milk to 35ºC and add 2 ml of rennet to 10 L of milk**.**

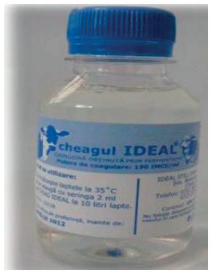

Figure 1. Commercial Rennet IDEAL

Methods used in experiments. In order to perform the experiments to highlight the ability of coagulation with plant extracts, different steps were performed, as follows. In an initial step the physical and chemical analysis of the raw cow's milk, was analyzed by the use of an EKOMILK analyser which provides information regarding the fat percentage, dry matter, density, added water, protein content; also, by classical tools, were determined the milk pH (pH-meter) and milk acidity (titration)). Further, the vegetal extracts from *Taraxacum officinale, Rumex acetosa, Lactuca sativa, Urtica dioica*, were prepared; the cleaned plants were prepared as aqueous extracts and these vegetal extracts were added in milk (20 ml aqueous extract in 100 ml milk); samples were incubated for coagulation at 35°C; the resulting curd was analyzed for the water activity, dry matter and organoleptic properties.

Physico-chemical analysis. In order to perform the experiments, the following physico-chemical analyzes were determined: determination of pH, water activity, dry matter, determination of acidity, milk-clotting activity and determinations using the Ekomilk device.

Determination of pH. was performed with the aid of a pH meter of the INOLAB 720 WTW series type with automatic temperature compensator.

Water activity was measured by the use of a Novasina system.

Dry matter. The principle of the method is based on removing water from the product by heating at a temperature of 100-110°C and calculating the dry mass in the sample according to the below formula

$$\% \text{ dry matter} = [(m_2 - m_0)/(m_1 - m_0)] * 100$$

m_1-mass of the ampoule and the product to be analyzed, in g; m_2-mass of the ampoule and the residue after drying, in g; m_0-mass of the ampoule, in g**.**

Determination of acidity. The acidity was determined by titration with an alkaline NaOH solution until the milk sample is neutralized in the presence of phenolphthalein as an indicator by the aid of an automatic titrator Schott Titronic basic type

Milk Clotting Activity. One of the important properties of coagulating enzymes is their coagulation capacity quantified by Milk Clotting Activity (MCA). MCA is expressed by the amount of milk, taken in volumes, at 35°C, for 40 minutes (2400 seconds). MCA is calculated with the formula:

$MCA = (2400*V)/(T*E)$ in which MCA = Milk Clotting Activity; E = volume of coagulated milk (in liters); T = coagulation time (in seconds).

The coagulation time represents the necessary period from the moment of the introduction of the coagulating enzyme in the milk until the appearance of the first curd flakes.

The coagulated milk sample is left until the curd is hardened and its appearance is appreciated as hard, firm curd, soft curd, dusty curd.

Soxhlet units are defined as the volume of fresh milk that can be coagulated by a unit volume of curd in 40 minutes (2400s) at 35°C (Costin G. M., 2003).

Yield calculation. The calculation relation is as follows:

$R\ (\%) = 100 * CB\ /\ CL$, where: R - yield (%); CB - the amount of cheese / curd obtained (kg) and CL - the amount of milk used (l). (Palicica et al., 2007)

Organoleptic analysis. In order to perform the sensory analysis of curd samples, certain quality indicators are monitored, such as: appearance, appearance in section, color, consistency, smell and aroma of the samples (Banu C., 2007), and the scoring system is performed using the method by comparison with a unit scoring scale (Table 1), from 1 to 5.

The notations used for the curd samples thus obtained were: PMI - sample of 100 ml of milk + 0.02 µl of commercially purchased animal rennet; P1 - test of 100 ml milk + 20 ml aqueous extract of *Rumex acetosa*; P2 - sample of 100 ml milk + 20 ml aqueous extract of *Taraxacum officinale*; P3- 100 ml milk + 20 ml extract of *Urtica dioica*; P4 - 100 ml milk + 20 ml *Lactuca sativa* extract.

Table 1. Scoring scale for organoleptic analysis

Quality appreciation stage	**Number of points**	**General description of appreciation stage**
Excelent	5	Excelent quality
Very good	4	Quality fully according with the specific of the product
Good	3	Good quality, proper
Satisfying	2	The product has slight defects that can be accepted
Unsatisfying	1	The product has obvious, multiple and systematic defects
Adulterated	0	The product has severe defects and can no longer be consumed

RESULTS AND DISCUSSIONS

The study of the effect of using plant coagulants that have as a source plants from spontaneous and cultivated flora, respectively *Taraxacum officinale, Rumex acetosa, Urtica dioica* and *Lactuca sativa*, in the process of obtaining cheeses, on the sensory and physico-chemical qualities of the finished product was performed by using as raw material unpasteurised cow's milk according to the working methods described above.

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

Obtaining cheese can be described as the process of removing water, lactose and some mineral salts from milk in order to produce a concentrate of fat and protein (Ciocîrlie, 2002).

Transforming milk into cheese 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).
The Table 2 shows the results of the physico-chemical analyzes obtained for the cow's milk sample.

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

Sample	Analyzed parameters						
	pH	Acidity (°T)	Fat (%)	Non-fat dry mater (%)	Density (g/cm³)	Water added (%)	Protein (%)
Raw cow milk	6.6	19	3.53	7.97	1.027	0	3.01
Reference values*	6.4-6.7	15-21	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

As can be seen in the table above, the analyzed cow's milk had a pH of 6.6 and an acidity of 19 degrees Thorner, elements that reflect its quality and freshness, according to the reference values in STAS 143-84. Following the determinations performed, a fat percentage of 3.53% and a dry matter value of 7.97% were recorded. Also, cow's milk had a density of 1,027g/cm^3 and a total protein content of 3.01%, values that fall within the reference ranges of STAS 143-84 for fresh milk.

Results of physico-chemical determinations

The curd samples obtained by adding vegetable rennet, from different plant sources, were analyzed physico-chemically, and the results obtained are presented as followed.
The curd samples obtained from the addition of plant rennet were analyzed physico-chemically in order to determine the resulted volume of whey, the amount of curd obtained, the determination of the pH of the whey, the water activity index of the curd samples, the dry matter content of the curd samples and the calculation of the yield, and the results obtained are presented in Table 3.
As shown in Table 3 the control sample of milk with rennet Ideal which is comparable to sample 2 of milk with *Taraxacum officinale* extract, both resulting in the same value of 83 ml. The other vegetal extracts registered relatively higher whey volume (an increase of about 18.5% in volume) which is a satisfactorily result.

Table 3. Curd samples - Results of physico-chemical determinations

Sample code number	Whey volume (ml)	pH whey	aw curd	Dry matter curd, %	Yield, %
PMI	83	4.73	0.892	57.74	11.52
P1	99	4.56	0.888	34.51	14.76
P2	83	4.16	0.892	23.38	25.54
P3	98,5	5.05	0.894	66.67	6.59
P4	98	5.27	0.889	46.01	6.34

In terms of water activity, the data obtained for all samples are comparable and vary between 0.88 and 0.89, which is an index value that support an increase in the shelf life of the finished product.
The pH of the analyzed whey had close values, between 4.16 and 5.27, which indicates a slightly acidic environment that favored the coagulation of cow's milk, while the initial milk pH was around 6.6 (Table 3).
The dry matter determined for the curd samples obtained reveals both the degree of separation of the curd from the whey following the filtration / separation operation, and the ability of the three-dimensional structure of the curd to retain water. This varies in a fairly large range for the analyzed samples, respectively 23.38-66.67.
Regarding the efficiency of the coagulation process (yield, %), which reflects the amount of curd obtained from the volume of milk tested, respectively 100 ml of milk, it is observed in Table 3 that the lowest value was presented by the P4 sample with *Lactuca sativa* (6.34%), closely followed by the P3 sample with *Urtica doica* (6.59%). Almost double yield was registered in the case of sample P1 with *Rumex acetosa* (14.76%) while for the sample P2 with *Taraxacum oficinale* the volume of separated whey was significantly higher 25.54%). Actually, *Taracaxum officinale* has been reported before as a potential milk coagulant (Mahajan & Chaudhari, 2014). Also, the possibility of using *Rumex* juice in the amount of 9 ± 0.5% as a coagulant of milk proteins has been confirmed by Grek et al. 2017.
The MCA (Milk Clotting Activity) was determined under small scale laboratory conditions, in 20 ml glasses tubes (Figure 2).
The results obtained from the determination of the milk clotting activity of cow's milk curd

with the addition of different vegetable coagulants are presented in the Table 4.

Table 4. Milk Clotting Activity of different plant extract proteases

Sample code number	Milk Clotting Activity (%)
PMI – Commercially rennet	0.0089
P1 – *Rumex acetosa*	0.0038
P2 – *Taraxacum officinale*	0.0017
P3 – *Urtica dioica*	0.0048
P4 - *Lactuca sativa*	0.0029

Table 4 shows the results obtained from the coagulation power test, according to the method proposed by Soxhlet. Thus, it is observed that the highest coagulation power is found in sample 3 with *Urtica dioica*, followed by sample 1 with *Rumex acetosa*, sample 4 with *Lactuca sativa* and sample 2 with *Taraxacum officinale*. However, the MCA's recorded in the plant extract samples have lower values than the commercially purchased rennet reference sample.

Different authors have used many different methods and units, so this makes it difficult to compare the coagulation capacity of different coagulants of plant origin. An important role is also played by a prior selection of the type of milk on which the coagulation capacity of these coagulants from plant sources is tested, in order to obtain specific or desired varieties of cheese. Despite the successes achieved for certain types of preparations, the production of cheeses obtained from coagulation with clot of vegetable origin on an industrial scale is quite limited and marginal.

Figure 2. Milk Clotting Activity Determination under small scale laboratory conditions

Results of sensorial analysis

The sensory analysis of the milk samples coagulated with vegetal rennet from different vegetal sources consisted in the use of the method, described in the previous chapter, respectively the awarding of points to the quality indicators according to the sensory analysis. The results are described in Table 5.

Table 5. Senzory Analyses - Average scores

Sample code number	**Number of points awarded to quality indicators**					**Average score**
	Appearance	Texture	Firmness	Smell	Color	
PMI	4	4	3	4.5	5	4.1
P1	4	4	3	4	4.5	3.9
P2	4	4	2	4	5	3.8
P3	1	1	0.5	4	4.5	2.2
P4	2	2	1.5	4	4.5	2.8

The highest score was obtained by the PMI sample with Ideal rennet, followed at a very short interval by sample 1 with *Rumex acetosa* and sample 2 with *Taraxacum officinale*.

Lower scores were obtained in sample 4 with *Lactuca sativa* and sample 3 with *Urtica dioica*. The appearance of the curd is shown in Figure 3.

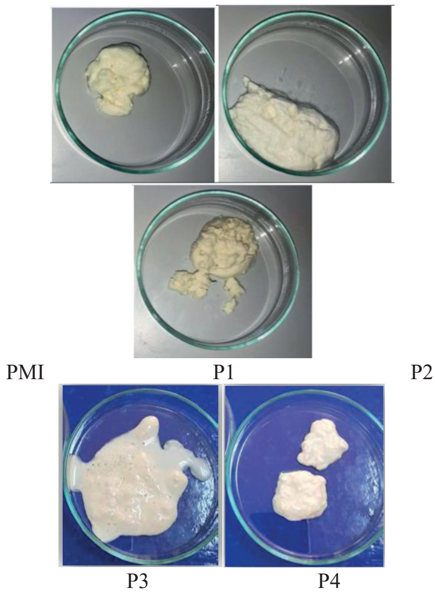

Figure 3. Curd obtained with different types of vegetal rennet (PMI - Commercially rennet; P1 - *Rumex acetosa;* P2 - *Taraxacum officinale;* P3 - *Urtica dioica* P4 - *Lactuca sativa*)

CONCLUSIONS

Following the study carried out and based on data from the literature, a series of conclusions can be issued, which are presented below.

Coagulation of milk with coagulating enzymes of vegetable origin is a feasible solution in the cheese-making process.

Several plant sources have been used to study the coagulation capacity of milk, including *Taraxacum officinale, Rumex acetosa, Urtica dioica, Lactuca sativa*. 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 studying a potential replacement for animal clot, it is particularly important to perform a test of milk coagulation activity.

From the experimental results presented in this article, a series of conclusions can be drawn, which are briefly presented below.

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.

Following the determination of the dry matter for the curd samples obtained, it was observed that the milk sample to which the *Taraxacum officinale* extract was added had the lowest dry matter content and therefore the lowest energy value.

In the milk sample in which *Rumex acetosa* extract was added, a curd formed very well separated from the whey, obtaining the largest volume of whey among the analyzed samples.

The addition of a source of fresh and ground plant rennet, respectively of *Rumex acetosa*, in cow's milk leads to obtaining a low value of the water activity index, which implies an increase in the shelf life of the finished product. In this respect, the results obtained underlined the fact that the sample with the addition of *Rumex acetosa* reached the lowest value of the aw index. At the opposite pole was the test milk sample clotted with commercial animal rennet, which recorded the highest value of the water activity index.

Regarding the coagulation activity, the best results were obtained with the extract of *Urtica dioica*, the value being, but at half compared to the animal rennet used.

The results obtained after the organoleptic analysis of the curd samples obtained with plant rennet from different plant sources suggest that the sample with the addition of *Rumex acetosa* was preferred, being the sample that obtained the highest score, respectively 4.6 on a 5-point scale.

Data obtained from physico-chemical analyzes of the raw material and the finished product and organoleptic analyzes of curd samples with the addition of different plant coagulants led to interesting results in terms of quality and preference of finished products. From the point of view of preservability, there was a decrease in the values of the aw index in the case of the addition of coagulants compared to the control sample.

The studied plants, respectively *Rumex acetosa*, *Taraxacum officinale*, *Urtica dioica* and *Lactuca sativa* proved that they have the potential to coagulate cow's milk and can be a source of milk-clotting enzymes in cheese-making.

REFERENCES

Abebe, B., & Emire, S. (2020). Manufacture of fresh cheese using east African *Calotropis procera* leaves extract crude enzyme as milk coagulant, *Food Sci. Nutr.,* 8, 4831-4842.

Banu C. (coord.) (2007). *Food quality and sensory analysis*, 103-109. Bucharest, RO: Agir Publishing House.

Bornaz, S., Guizani, N., Fellah, N., Sahli, A., Slama, M.B., & Attia, H. (2010). Effect of Plant Originated Coagulants and Chymosin on Ovine Milk Coagulation, *International Journal of Food Properties,* 13(1), 10-22.

Burzo, I., Dobrescu, A., Badulescu, L., Mihaescu, D., & Balan L. (2005). *Plant Physiology, VIII, Useful Plant Substances,* 355-356. Bucharest, RO: Elisavaros, Publishing House.

Ciocîrlie, N. (2002). *Biotechnology for obtaining and processing raw materials of animal origin. The milk*, 149-157. Bucharest, RO: Printech Publishing House.

Costin, G.M. (2003). *The science and engineering of cheese making*, 30-37. Galați, RO: Academica Publishing House.

Drăghici, E.M. (2009). *Vegetable culture greens*, 67-69. Bucharest, RO: Ceres Publishing House.

Gescher, K., Hensel, A., Hafezi, W., Derksen, A., & Kühn, J. (2011). Oligomeric proanthocyanidins from *Rumex acetosa* L. inhibit the attachment of herpes simplex virus type-1. *Antiviral Research,* 89(1), 9-18.

Grek, O., Tymchuki, A., Chubenko, L., & Ovsiienko, K. (2017). Research on the quality indicators of curd products based on protein-herbal clots. *Food and Environment Safety,* XVI (4), 262-268.

Guzun, V., Musteaţă, Gr., Rubţov, S., Banu, C., & Vizireanu, C. (2001). *Milk industrialization*. Chişinău, MD: Tehnica-Info Publishing House.

Hoffmann, D. (2003). *Medical herbalism*. *Rochester, VT: Healing Arts Press.*

Liburdi, K., Boselli, C., Giangolini, G., Amatiste, S., & Esti, M. (2019). An Evaluation of the Clotting Properties of Three Plant Rennets in the Milks of Different Animal Species. *Foods (Basel, Switzerland)*, *8*(12), 600. https://doi.org/10.3390/foods8120600

Mahajan, R.T., & Chaudhrai, G.M. (2014). Plant latex as vegetable source for milk clotting enzymes and their use in cheese preparation, *Int. J. Adv. Res.,* 2 (5), 1173-1181.

Manzoor, A.S., Shabir, A.M., & Mohd, A.P. (2014). Plant proteases as milk-clotting enzymes in cheesemaking: a review, *Dairy Science and Technology,* 94, 5–16.

Oroian, S., & Samarghitan, M. (2018). Flora from Faragau Area (Mures County) as potential source of medicinal plants. *Acta Biologica Marisiensis*, 1(1), 60-70.

Palicica, R., Mateiu, L., & Pîrvulescu, L. (2007). *Milk processing. Elements of technological and economic calculation*, 93. Timișoara, RO: Orizonturi Universitare Publishing House.

Pârvu, C. (2006). *The Plant Universe*. Bucharest, RO: ASAB Publishing House.

Shah, M., Mir, S., & Paray, M. (2014). Plant proteases as milk-clotting enzymes in cheesemaking: a review, *Dairy Science & Technology, EDP sciences/Springer,* 94(1), 5-16.