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Artificial Neural Networking Model an Approach for the Coagulation Properties of Milk

Vesna K. Hristova[1], M. Ayaz Ahmad[2], Biljana Trajkovska[3], Stefce Presilski[4], Georgi Bonev[5]

Abstract — The analysis and sequence of some technological parameters and milk coagulation properties (MCP) of Holstein Friesian dairy cows have been studied in the present research article. The milk samples have been collected from a local farm's at Pelagonia region, Republic of Macedonia and the experimental work was conducted in the laboratories of the Faculty of biotechnical sciences, R. Macedonia and Tabuk University, KSA. The study illustrates the MCP of cow's milk as well as the effect of milk urea nitrogen level and pH on the coagulum development. The scanning electron microscopy (SEM) of raw milk samples and after rennet addition also has been studied. All the said results/parameters have been compared with the soft computing approach so called artificial neural networking model, and the predictions of soft computing (ANN) model's outcomes were found in a good agreement with the experimental data.

Index Terms —milk coagulation properties (MCP), milk urea nitrogen (MUN), artificial neural networking (ANN) model.

1 INTRODUCTION

Milk is the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing (Codex Alimentarius, 2011) [1]. The composition of milk varies by species, but it always contains significant amounts of proteins (approx. 3.3%), carbohydrate (approx. 4.6%) and fat (approx. 4.3%), as well as a great source of calcium and other components, organic acids, peptides, and vitamins (Heck et al., 2009, Hettinga, 2009) [2]. The milk delivered to dairies is converted into a number of fresh products and manufactured dairy products. Some 68.2 million tonnes of raw milk were used to produce 9.3 million tonnes of cheese in the EU-28 in 2013, while 31.5 million tonnes of raw milk were turned into a similar amount of drinking milk. (EUROSTAT, 2015) [3].

The process of milk coagulation and development of rennet-induced gel (coagulum) is the most significant and the most sensitive process in the production of the rennet curd cheese varieties(Tofanin et al., 2012) [4]. In terms to rennet coagulation the main active component is an enzyme (chymosin) that hydrolyzes the k-caseins which largely contribute to the colloidal stability of casein micelles [5].

According to Lucey J. A., 2002 in reference [5], the percentage of k-caseins proteolysis is about 90% of the unheated milk. Briefly, the particular hydrolysis of κ-casein by chymosin (rennet) leads to a dynamic decrease in the extent of the repulsive forces among the casein micelles and formation of strands composed of aggregated casein micelles that further associate to build up a three-dimensional continuous network (Tuinier and de Kruif 2002; Lucey et al. 2003). [6, 7]

Due to the advancement of technology, so called modern age of technology, the theoretical facts and experiment results were found completely closer to each other. Predictive soft computing methodologies have been used for prediction and controlling many dairy production processes "from farm to dairy industrial scale" providing significant advantages in increased manufacturing efficiency and quality control. Therefore the new technique named artificial neural network (ANN) model would be a very powerful tool to determine some experimental observables and/or facts perfectly in the field of dairy science. Recently, the artificial neural network (ANN) model / technique are frequently applied for identification and in checking of the complex interactions of raw materials, manufacturing settings and attributes of dairy products such as chemical composition, sensory traits, functionality and shelf life [8 – 10].

By using some predictions of such mathematical models, i.e. ANN model, one can decrease the cost of dairy products as well as can be increase the production rate for mankind [11, 12].

From a research point of view the utilization of ANN systems to relate target responses back to input variables settings is providing exceptionally efficient approaches to study the complexities of interactions in dairy products [11, 12].

The present research work has been carried out to obtain a better insight into the problem of rennet coagulation of Holstein-Friesian cow's milk in a local farm's in Pelagonia region, Republic of Macedonia. The study illustrates the MCP of cow's milk as well as the effect of milk urea nitrogen level and pH on the total coagulation time. The scanning electron microscopy (SEM) of raw milk samples and after rennet addition also has been studied. All the said results/ factors have been compared with the soft computing approach so called artificial neural networking model, and the predictions of soft computing (ANN) model's outcomes were found in a good agreement with the experimental data [13, 14].

1. Faculty of Biotechnical Sciences, University of St. Clement Ohridski, Partizanska, bb 7000 Bitola, Macedonia. E-mail: v.primavera79@gmail.com
2. Physics Department, Faculty of Science, P.O. Box 741, University of Tabuk - 71491, Saudi Arabia. E-mail: mayaz.alig@gmail.com
3. Faculty of Biotechnical Sciences, University of St. Clement Ohridski, Partizanska, bb 7000 Bitola, Macedonia. E-mail: trajkovskab55@gmail.com
4. Faculty of Biotechnical Sciences, University of St. Clement Ohridski, Partizanska, bb 7000 Bitola, Macedonia. E-mail: presilskistevo@yahoo.com
5. Faculty of Agriculture, Student Campus, Trakia University, Stara Zagora, Bulgaria. E-mail: gbonev@uni-sz.bg

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2 MATERIALS AND METHOTDS USED

2.1 Milk Samples Collection and Laboratory Analyses

Holstein-Friesian breed milk samples were taken at one dairy farm in the Bitola district in the Republic of Macedonia which is situated at an altitude of 1,233 metres above the Adriatic sea level. The dairy cows on the farm were fed ad libitum throughout the year as a total mixed ration, supplemented with concentrate according to standard practice and the cows were never turned out to graze. Rations before and after calving were formulated to exceed National Research Council recommendations (NRC, 2001) [15], and the residues of the dietary feed were generally observed in the herd. All cows received the same lactation diet for ad libitum intake throughout the experimental period post calving. The milk samples were collected from the morning milking of the dairy cows (6.00 - 7.00 hours). In accordance with the rules for milk sampling, the milk samples were manually taken from the individual collector of the milking De Laval system in with a special sterile plastic cups (50ml) [16]. Samples were transported to the laboratory by movable refrigerator and kept in at the same temperature < 10 °C during the determination of milk quality parameters. The analysis of MCP was carried out following M. Mele, R. Dal Zotto et al. (2009); [17] briefly, milk samples (10 mL) were heated to 35 °C and 200 μL of rennet was added.

2.2 Coagulation of Milk

Examination and coagulation of milk were performed within 3 hours after milking.Milk from the selected experimental cows was transported in containers from inox-steel, prechilled at a temperature up to 8^0 C at the "Laboratory of dairy chemistry and technology", Faculty of biotechnical sciences, Bitola. In the double bottom stabilizer, 5 kg of milk from each selected cow was heated to the renneting temperature of 35-37 ^{0}C and was added rennet powder, according to the rules of the company-manufacturer. The mixture was manually mixed well and the initial clotting time T (min), the time period starting from the addition of rennet to the first appearance of clots of milk solution, was recorded. Total clotting time T (min) was also measured and for normal coagulation was reported for extended testing period of 45 -60 minutes [18].

2.3 Determination of Milk Coagulation Activity

The rennet was commercial powder (CHY–MAX POWDER EXTRA NB, CHR. HANSEN, the strength of the enzyme being 2235 IMCU/g) Rennet solution of 0.4% was prepared and an appropriate amount of this solution was taken to give a visually observed coagulation time of approximately 10 minutes in milk. For measurement of the milk coagulation time the following two steps were used.

(i) Effect of Temperature

The milk samples were adjusted to pH 6.6 -6.8 by slow addition of 1 M HCI, placed in a water bath, and the coagulation time was measured at temperatures over the range at 30 °C to 40 °C.

(ii) Effect of pH

All samples were equilibrated at 35 °C and the coagulation time determined at pH between 6.20 and 7.00. And the Calcium sensitivity was evaluated by measuring the coagulation time after addition of an appropriate amount of calcium chloride.

2.4. Visual Method for Coagulation Time Measurement

Coagulation time identified as time from rennet addition to the formation of the first visible floccules was measured visually. Actually the coagulation time was defined as the time required for the first appearance of graininess in the moving film of milk samples on the surface of the glass walls of the beaker [16 -18]. A quantity of 25 ml by volume of each sample was measured into a beaker of 125ml and placed it in a water bath at 35 °C up to 45 minutes. After that we added an appropriate volume of rennet solution to it and then we measured the coagulation time with the help of visual method.

2.5 Scanning Electron Microscopic (SEM) Analysis

Scanning electron microscopy (SEM) was used successfully by many investigators to reveal the microstructures of curd as well as to observe the modification in microstructure of the coagulated milk.

For SEM analysis, freeze dried, reconstituted samples of cow milk have been used. After adjusting the pH values 6.6 - 6.8 the samples were equilibrated at 35 °C and coagulation monitored by visual observation. The samples were taken before and after adding the rennet at time intervals up to the visually observed coagulation time. The freeze-fracturing technique has been applied to the milk samples for SEM analysis without adding any cryoprotectives. The freeze-fracturing was carried out in a modified Batzers BAF 300 unit, in an object's temperature up to -150 °C. One can much more details related to such experimental in reference (Muller et. al., 1982) [13 - 14]. The scanning electron microscope (SEM) images have been taken out of Japan made, JSM 6390A (JEOL Japan) at a dissimilar magnification of the above prepared samples at Physics Department, Faculty of Science, University of Tabuk, Saudi Arabia. Before SEM examine, the prepared samples were layered with gold in a vacuum coating unit. The cross section areas ranging of samples were approximately 1 cm to 5 microns in width and the magnification ranging of SEM was the order of 20X to approximately 30,000X, with spatial resolution of 50 to 100 nm [14].

3 RESULTS AND DISCUSSIONS

Amongst all 148 cows, only 25 selected cows were followed and re-sampled on several occasions to evaluate possible changes in coagulation properties according to the milk urea nitrogen level. On the basis of milk urea nitrogen (MUN) level, the cows/samples were divided in two groups, group 1st (milk urea nitrogen level < 6.5 mmol/L) (10 cows) i.e. normal milk urea nitrogen level (MUN) and group 2nd (milk urea nitrogen level > 6.5 mmol/L) i.e. high milk urea nitrogen level (15 cows). The observed MUN levels, pH values and total coagulation times of cow's milk have been depicted in Table 1. In the same table the predicted values of the total coagulation times by soft computing / simulations named artificial neural networking model also have been shown.

The lowest MUN level was measured 3.60, the pH value was 6.58 ± 0.01 and the coagulation time was 45 minutes for

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the (10) cows with normal MUN levels, whereas the highest values for MUN levels was 5.40, the pH was 6.84 ± 0.15 and the coagulation time was 55 minutes for the same group of cows (1st group).

TABLE 1
DIRECT MEASURED COALGULATION TIME ALONG WITH SIMULATED RESULTS OF ANN MODEL

Milk Sample/ Cow I.D. No.	Milk Urea Nitrogen (MUN) level	pH values of raw milk	Total Coagulation Time in (min.) measured	Total Coagulation Time in (min.) by ANN model
Samples with normal (MUN) level < 6.50mmol/L (1st group)				
6708	4.85	6.58 ± 0.01	53	27 ± 5
8875	5.40	6.64 ± 0.02	45	23 ± 4
4618	4.80	6.65 ± 0.05	54	27 ± 5
7536	4.30	6.67 ± 0.06	55	28 ± 6
1717	4.80	6.70 ± 0.07	50	25 ± 3
1621	5.25	6.73 ± 0.09	45	23 ± 4
1629	3.60	6.76 ± 0.10	51	26 ± 5
1413	4.40	6.78 ± 0.12	45	23 ± 3
1182	5.10	6.81 ± 0.12	55	28 ± 5
798	4.60	6.84 ± 0.15	50	25 ± 4
Samples with high (MUN) level > 6.50mmol/L (2nd group)				
3489	7.15	6.64 ± 0.05	52	29 ± 5
8847	7.90	6.57 ± 0.02	57	25 ± 4
9304	10.85	6.75 ± 0.05	45	28 ± 5
4972	9.70	6.67 ± 0.06	60	29 ± 6
1288	9.20	6.61 ± 0.07	59	28 ± 3
8365	11.10	6.56 ± 0.09	48	27 ± 4
7375	12.40	6.61 ± 0.10	60	25 ± 5
1829	12.50	6.69 ± 0.12	55	28 ± 3
3085	12.75	6.60 ± 0.12	51	25 ± 5
5918	10.20	6.81 ± 0.13	51	29 ± 4
8327	8.50	6.63 ± 0.09	53	30 ± 5
922	11.10	6.68 ± 0.10	42	25 ± 4
5802	7.40	6.62 ± 0.12	55	28 ± 5
5840	9.35	6.68 ± 0.12	56	29 ± 6
1746	9.20	6.70 ± 0.15	59	28 ± 7

Further, for the 2nd group of (15) cows with high MUN levels, the lowest MUN level was measured 7.15, the pH value was 6.56 ± 0.09 and the coagulation time was 48 minutes, whereas the highest values for MUN levels was 12.75, the pH value was 6.81 ± 0.13 and the coagulation time was 60 minutes for the same group of cows (2nd group).

Therefore, one can conclude from Table 1, that the studied parameters, i.e. MUN levels and the total coagulation time were found little bit in higher order for the 2nd group with (MUN) level > 6.50 m-mol/L rather than the 1st group with (MUN) level < 6.50 m-mol/L. Such obtained results were also found in better agreement with the other worker in the field of dairy science [8 – 17 and references therein].

The total coagulation time was also predicted by soft computing of ANN model and it is depicted in the same Table 1 for each individual cow's in both the cases of MUN levels (normal and high). In all the samples the visually measured total coagulation time for both the milk samples were found approximate twice in the comparison of prediction of ANN model. In both the cases / groups of cow's milk, the predicted total coagulation times, found arbitrary in order of time value with standard errors and also its values found to be approximated within the order of 28.5 ± 6 minutes. These results suggested that the milk composition was influenced by the dietary treatment and some other genetic and paragenetic factors.

To acquire a better understanding on the extent of the aggregation of the casein micelles during renneting, the structural state of the raw milk proteins and casein curds after adding rennet were investigated using SEM technique (Figure 1. a-d). Practically, the prime initiative was to find out the structural changes and protein degradation after coagulation process, at both milk samples with normal and with high milk urea nitrogen level.

In the present SEM analyses, the main goal was to find a visual illustration of the coagulation processes and to demonstrate the structural changes in cow's milk.

(a)

Fig. 1 (a). A Scanning Electron Microscopic picture of normal MUN levels milk sample before the addition of rennet.

(b)

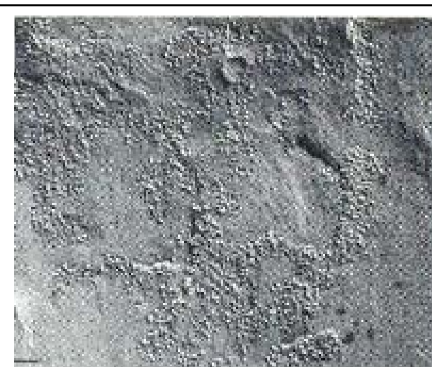

Fig. 1(b). A Scanning Electron Microscopic picture of normal MUN levels milk sample after the addition of rennet.

(c)

(d)

Fig. 1(d). A Scanning Electron Microscopic picture of high MUN level milk sample after the addition of rennet.

Scanning electron micrographs obtained have been depicted in Fig.1 (a) and Fig. 1(b), for the milks sample of group 1st cows (10 cows), before and after the addition of rennet respectively. And the same Fig.1 (c) and Fig. 1 (d) were scanned for the milk of high urea nitrogen level > 6.5 mmol/L) i.e. 2nd group of cows (15 cows).

From all the figures it is clear that the cow's milk appeared as almost special shape particles, and composed of numbers of submicelles. The visually obtained coagulation times of cow's milk were 5 minutes. In the samples the onsets of aggregation were also pragmatic in SEM approximate of 70% of the coagulation time.

4 Conclusions

Based on our present experimental work entitled "artificial neural networking model an approach for the coagulation properties of milk" of the Holstein-Friesian Cows' Milk in Republic of Macedonia", we can draw the following valuable conclusions:

The influence of the technological parameters of the total coagulation time in milk coagulation process as milk urea nitrogen (MUN) level and pH values has been concluded and found it in a good agreement with the other worker in the same research field.

The total coagulation time measured in present milk samples were found twice to the simulated results, which suggested that the milk composition was influenced by the dietary treatment and some other factors which would be our future field of interest.

In the present research work, we did not find a significant effect of temperature and the pH values over the total coagulation time and also coagulum properties, but it is exited by some other authors.

Based on the present experimental work, one can say that such types of study are worth mentioning for the advancement of recent dairy technology. The produced results show a lot of good agreement with the results obtained by various authors.

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