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Influence of enzymes with different activity on dynamics of buffalo milk coagulation parameters

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Abstract: *The aim of the present study was to evaluate the effect of 5 species of chymosin with different concentrations on the dynamics of the traits measured by mechanical lactodynamograph, characterizing the coagulation ability of buffalo milk. The coagulation properties of buffalo milk were evaluated on 420 milk samples taken during morning milking from whole milk. Five enzymes (MAXIREN 600, FROMASE 750, MAHIREN XDS, MAXIREN 180 and MAXIREN PREM P) were tested with 4 concentrations each and one control group with a standard, recommended by the manufacturer of the biosensor - mechanical lactodynamometer chymosin (Polo Trade - Computerized Renneting Meter, Italy). The effect of the concentration of each enzyme was determined by examining 20 milk samples. The use of enzymes at various concentrations resulted in substantial variation in the magnitude and direction of changes. Different chymosins with different concentrations actively affect the curd firmness. The shorter coagulation time will provide sufficient time for the curd firming time and its curd firmness. Over 50% of milk samples coagulate by 6 minutes. The effects of enzymes and their different concentrations on measurement were in different groups, which in our opinion was a good prerequisite for using the obtained data for modelling of the measurement process.*

Keywords: buffalo milk, coagulation properties, enzymes, mechanical lactodynamograph

Introduction

The significance of milk coagulation properties in cheese production has become increasingly important in a number of countries: Italy (De Marchi et al., 2008), Estonia (Troch et al., 2017) and Scandinavian countries (Ikonen et al., 2004; Walstra et al., 2006). Milk coagulation properties are confirmed to be associated to the quality and yield of cheese. The cheesemaking conditions, e.g. the type and concentration of the enzyme, incubation temperature, and the different elements of milk composition influence milk coagulation ability. Thus, each individual factor with effect on milk proximate composition has also an effect on milk coagulation properties.

Enzyme-induced coagulation of milk represents modification of casein micelles via casein hydrolysis by rennin (chymosin), followed by calcium-micelle association (Fox and McSweeney, 1998). Usually, chymosin is extracted from calf stomachs and is a mixture of proteases chymosin and pepsin (Andrén, 2002).

Active research is carried out on the use of mechanical lactodynamography for generation of reference data on mid-infrared reflectance spectroscopy (MIRS) calibration (Pretto et al., 2011; De Marchi et al., 2013, 2014). Corrections between milk samples traditionally evaluated with the lactodynamograph and MIRS are moderate to high (Dal Zotto et al., 2008; De Marchi et al., 2009). Researchers believe that MIRS analysis could not be replaced by lactodynamography, yet it could be used at the population level for genetic purposes (Cecchinato et al., 2009).

Several authors (Caroli et al., 1990; Dal Zotto et al., 2008; Bittante, 2011) have discussed the problems associated to this method as negative effects related to accuracy. Obviously, the longer coagulation times would be related to shorter curd firming time and lower curd firmness. This restricts the possibility for measuring all parameters of milk coagulation ability, especially for slowly coagulating cow and buffalo milks – main raw materials in dairy industry.

Clearly, the measurement of milk coagulation properties by lactodynamography would remain a reference method in the future and background for modernisation of instruments using mid infrared rays. In this connection, overcoming of some methodical flaws of the method via modelling of main components influencing the measurement to achieve an improved measurement of curd firmness – a key trait for dairy industry, becomes increasingly important. There are only a few investigations of buffalo milk coagulation properties (Bartocci et al., 2002; Ariota et al., 2007; Potena et al., 2007b).

The aim of the present study was to evaluate the effect of 5 species of chymosin with different concentrations on the dynamics of the traits measured by mechanical lactodynamograph, characterizing the coagulation ability of buffalo milk.

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Material and methods

The coagulation properties of buffalo milk were evaluated on 420 milk samples taken during morning milking from whole milk. A total of 5 enzymes (MAXIREN 600, FROMASE 750, MAHIREN XDS, MAXIREN 180 and MAXIREN PREM P) were tested with 4 concentrations each and one control group with a standard, recommended by the manufacturer of the biosensor - mechanical lactodynamometer chymosin (Table 1). The effect of the concentration of each enzyme was determined by examining 20 milk samples.

Table 1. Description of enzymes and concentration of enzymes in the experiment, μL

Description of enzymes	Concentration of enzymes in the experiment, ml			
FROMASE® 750 TL liquid microbial coagulant obtained from a selected strain of *Rhizomucor miehei*. Milk coagulation activity ≥ 750 IMCU/ml	0.08	0.13	0.18	0.23
MAXIREN PREMIUM P liquid chymosin yeast extracted from *Kluyveromyces lactis* and *Aspergillus niger* Milk coagulation activity 1:27000≥ 300 IMCU/ ml	0.22	0.27	0.30	0.35
MAXIREN® XDS liquid chymosin yeast extracted from *Kluyveromyces lactis* Milk coagulation activity ≥ 620 IMCU/ml	0.10	0.15	0.20	0.25
MAXIREN 180 100% liquid chymosin yeast extracted from *Kluyveromyces lactis* Milk coagulation activity 1:17000≥ 180 IMCU/ ml	0.37	0.40	0.45	0.50
MAXIREN® 600 liquid chymosin yeast extracted from *Kluyveromyces lactis* Milk coagulation activity ≥ 600 IMCU/ml	0.10	0.15	0.20	0.25
NATUREN Plus 215/0.8l animal rennet manufactured from the extract of the fourth stomach (vells) of either calves or lambs Milk coagulation activity - 215 IMCU/ml	0.20			

The analyses were performed by means of a biosensor – mechanical lactodynamometer (Polo Trade - Computerized Renneting Meter, Italy). This technique monitors viscosity behaviour of milk samples placed at a constant temperature, induced by addition of standard enzyme. Viscosity changes are measured by means of loop pendulums immersed in coagulating milk. The device used a computer system to transform the resistance experienced by moving pendulums consequently to coagulum formation into a graph (Figure 1).

Milk coagulation ability is determined by three traits:

- Rennet coagulation time (RCT);
- Curd firming time (k_{20});
- Curd firmness (a30 / A_{30}).

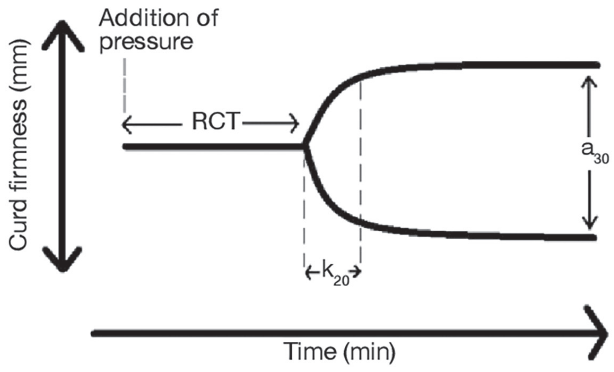

Figure 1. Typical fork-shaped diagram obtained by Formagraph and CRM (inspired from Ikonen et al., 2004; Dal Zotto et al., 2008; Bittante, 2011) by Troch et al., 2017

Principle Component Analysis (PCA) was used for statistical processing of data. The distribution of parameters by groups was presented through Scatterplot-PCA by two Factors via Multivariate Exploratory techniques-Principal component & Classification analysis.

Multivariate Exploratory techniques - Cluster Analysis - Euclidean distances was used to evaluate the effect of different enzymes and concentrations.

Results and discussion

The results from the analysis of distribution, frequency and dominance of buffalo milk curd firmness are presented in Table 2 and Figure 2. The highest distribution percentage was found out for milk with curd firmness 45 mm – 22.38%, followed by curd firmness of 50 mm – 21.19%. A low percentage of milk sample had a less firm coagulum – 25 mm (2.38%). In general, it could be summarised that the different chymosins and their various concentrations had an active influence on curd firmness. It should be noted that this parameter was dependent on the other two milk coagulation properties: rennet coagulation time (RCT) and curd firming time (k_{20}). The obviously shorter coagulation time would provide sufficient time for the other two stages. In a study with 476 Bulgarian Murrah buffaloes reared in three farms in Bulgaria, it was found out that the highest proportion of animals (40.8%) produced milk coagulating within 11.3-18 min (Karabashev et al., 2018). More than 80% of milk samples included in the present study coagulate within 18 min. In this study (Table 3, Figure 3), milk coagulated mainly up to the 6th minute – in over 50% of samples. A similar distribution percentage was exhibited by samples coagulating within 7, 8 and 9 minutes – 15.95, 12.85 and 12.85%, respectively. It could be affirmed that the enzymes used at tested concentrations offered possibilities for modelling the coagulation rate which could contribute successfully for improvement of curd firmness measurement A_{30}.

Table 2. Analysis of distribution, frequency and dominance of buffalo milk A_{30} values, %

Variable: A_{30}, Distribution: Normal (MILK BUFFALO) Kolmogorov-Smirnov d = 0.06099, p<0.10 Chi-Square=55.92808, df=6 (adjusted), p=0.00000

	OF	CO	PO	C%O	EF	CE	PE	C%E	OE
<= 20.00000	1	1	0.2381	0.2381	4.30277	4.3028	1.02447	1.0245	-3.3028
25	10	11	2.38095	2.619	11.06547	15.3682	2.63464	3.6591	-1.0655
30	54	65	12.85714	15.4762	27.78024	43.1485	6.61434	10.2734	26.2198
35	57	122	13.57143	29.0476	53.23404	96.3825	12.67477	22.9482	3.766
40	41	163	9.7619	38.8095	77.87137	174.2539	18.5408	41.489	-36.8714
45	94	257	22.38095	61.1905	86.96202	261.2159	20.70524	62.1943	7.038
50	89	346	21.19048	82.381	74.1401	335.356	17.6524	79.8467	14.8599
55	38	384	9.04762	91.4286	48.25434	383.6103	11.48913	91.3358	-10.2543
60	31	415	7.38095	98.8095	23.97437	407.5847	5.70818	97.044	7.0256
65	4	419	0.95238	99.7619	9.09149	416.6762	2.16464	99.2086	-5.0915
70	1	420	0.2381	100	2.63106	419.3073	0.62644	99.8351	-1.6311
< Infinity	0	420	0	100	0.69273	420	0.16494	100	-0.6927

*OF- Observed– Frequency; CO- Cumulative–Observed; PO- Percent–Observed; C%O- Cumulative %–Observed; EF- Expected–Frequency; CE- Cumulative–Expected; PE- Percent–Expected; C%E- Cumulative %–Expected; OE- Observed-Expected.

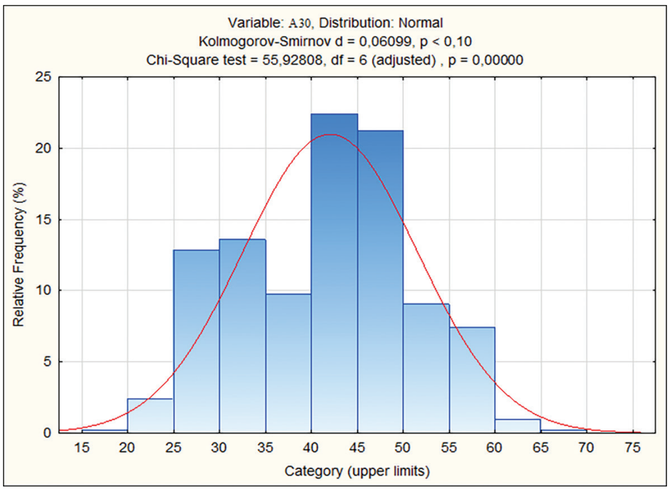

Figure 2. Plot of distribution, frequency and dominance of buffalo milk A_{30}, %

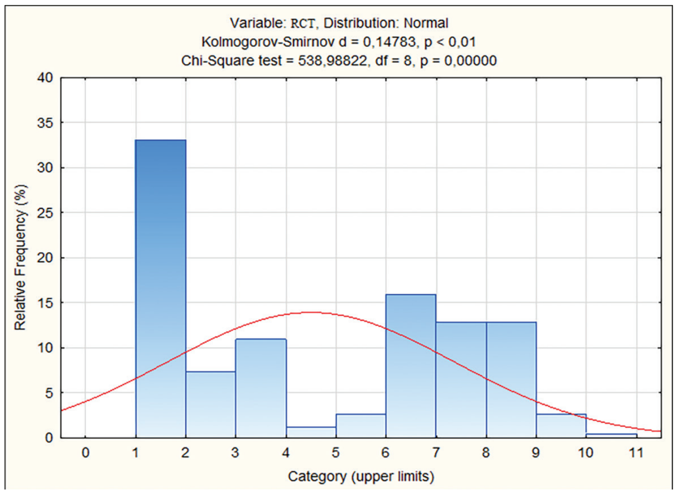

Figure 3. Plot of distribution, frequency and dominance of buffalo milk RCT, %

Table 3. Analysis of distribution, frequency and dominance of buffalo milk RCT, %

Variable: RCT, Distribution: Normal (MILK BUFFALO) Kolmogorov-Smirnov d=0.14783, p<0.01 Chi-Square=538.98822, df=8, p=0.00000

	OF	CO	PO	C%O	EF	CE	PE	C%E	OE
<= 1.00000	0	0	0	0	46.64613	46.6461	11.10622	11.1062	-46.6461
2	139	139	33.09524	33.0952	33.86012	80.5062	8.06193	19.1682	105.1399
3	31	170	7.38095	40.4762	45.7664	126.2727	10.89676	30.0649	-14.7664
4	46	216	10.95238	51.4286	54.826	181.0987	13.05381	43.1187	-8.826
5	5	221	1.19048	52.619	58.21154	239.3102	13.85989	56.9786	-53.2115
6	11	232	2.61905	55.2381	54.77907	294.0893	13.04264	70.0213	-43.7791
7	67	299	15.95238	71.1905	45.68808	339.7773	10.87811	80.8994	21.3119
8	54	353	12.85714	84.0476	33.77324	373.5506	8.04125	88.9406	20.2268
9	54	407	12.85714	96.9048	22.12697	395.6776	5.26833	94.2089	31.873
10	11	418	2.61905	99.5238	12.84837	408.5259	3.05914	97.2681	-1.8484
< Infinity	2	420	0.47619	100	11.47408	420	2.73192	100	-9.4741

*OF- Observed– Frequency; CO- Cumulative-Observed; PO- Percent–Observed; C%O- Cumulative %-Observed; EF- Expected–Frequency; CE- Cumulative-Expected; PE- Percent–Expected; C%E- Cumulative %-Expected; OE- Observed-Expected.

A number of studies associated this trait with the yield in cheese production. The cheese yield could increase by 2 to 10% when milk with good cheesemaking properties is used (Aleandri et al., 1989; Pretto et al., 2013).

The use of chymosins at various concentrations could modulate the curd firming time of buffalo milk in the desired direction. As could be seen from Table 4 and Figure 4, the majority of samples – 90.71% have short curd firming time – 0.5 min, whereas 8.5% of samples have κ_{20} values of 1.5 min. Chymosin is the principal and most active component provoking specific cleavage of the peptide bond Phe105-Met106 of κ-CN. Chymosin-induced coagulation of milk could be described through three phases (Vallas et al., 2010). Off milk is analysed with the same instrument under the same experimental conditions (Cecchinato et al., 2012), buffalo milk coagulates earlier and attains high A_{30} value compared to cow milk.

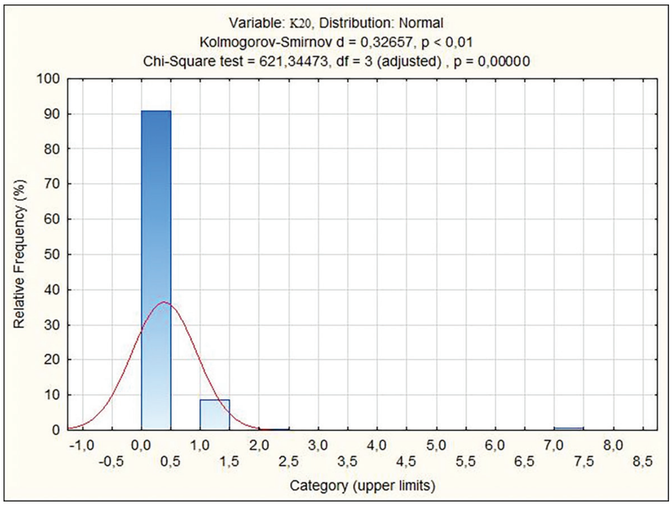

Figure 4. Plot of distribution, frequency and dominance of buffalo milk a K_{20}, %

Table 4. Analysis of distribution, frequency and dominance of buffalo milk K_{20}, %

Variable: K_{20}, Distribution: Normal (MILK BUFFALO) Kolmogorov-Smirnov d=0.32657, p<0.01 Chi-Square=621.34473, df=3 (adjusted), p=0.00000									
	OF	CO	PO	C%O	EF	CE	PE	C%E	OE
<= -0.50000	0	0	0	0	22.0583	22.0583	5.25198	5.252	-22.058
0	0	0	0	0	78.4201	100.4784	18.67144	23.9234	-78.42
0.5	381	381	90.71429	90.7143	143.3626	243.841	34.13395	58.0574	237.637
1	0	381	0	90.7143	120.5846	364.4256	28.71062	86.768	-120.585
1.5	36	417	8.57143	99.2857	46.6286	411.0542	11.10206	97.87	-10.629
2	0	417	0	99.2857	8.256	419.3102	1.96571	99.8358	-8.256
2.5	1	418	0.2381	99.5238	0.6652	419.9754	0.15838	99.9941	0.335
3	0	418	0	99.5238	0.0242	419.9996	0.00577	99.9999	-0.024
3.5	0	418	0	99.5238	0.0004	420	0.00009	100	0
4	0	418	0	99.5238	0	420	0	100	0
4.5	0	418	0	99.5238	0	420	0	100	0
5	0	418	0	99.5238	0	420	0	100	0
5.5	0	418	0	99.5238	0	420	0	100	0
6	0	418	0	99.5238	0	420	0	100	0
6.5	0	418	0	99.5238	0	420	0	100	0
7	0	418	0	99.5238	0	420	0	100	0
7.5	2	420	0.47619	100	0	420	0	100	2
8	0	420	0	100	0	420	0	100	0
< Infinity	0	420	0	100	0	420	0	100	0

*OF- Observed-Frequency; CO- Cumulative-Observed; PO- Percent-Observed; C%O- Cumulative %-Observed; EF- Expected–Frequency; CE- Cumulative–Expected; PE- Percent–Expected; C%E- Cumulative %-Expected; OE- Observed-Expected.

Figure 5 presents the results from the cluster analysis of data about the effect of different enzymes and their concentrations on parameters of milk coagulation properties. The results show that according to its effect on the 3 parameters, the standard was in the same group with the lowest dose 0.1 ml of MAHIREN XDS. The higher doses of the same enzyme were in the next group with MAXIREN 180 - 0.45 ml, both of them being subgroups of one common effect. Similar effects had the enzymes MAHIREN and MAXIREN 180 - 50 ml, which had similar influence to the lower concentration of MAXIREN 180 - 0.45. All enumerated enzymes formed a large group of enzymes and concentrations with similar effects but different magnitude on the three parameters characterising buffalo milk coagulation ability - RST, A_{30} and K_{20}.

The next large group comprised two major subgroups of enzymes with similar influence. Enzymes included in this group were the MAXIREN PREM and FROMASE 750 with different concentrations. It should be noted that MAHIREN 600 was the only enzyme encountered in the two large groups with similar effects. The first of subgroups with similar effects included MAHIREN 600 0.15 ml and FROMASE 750 - 0.08 ml.

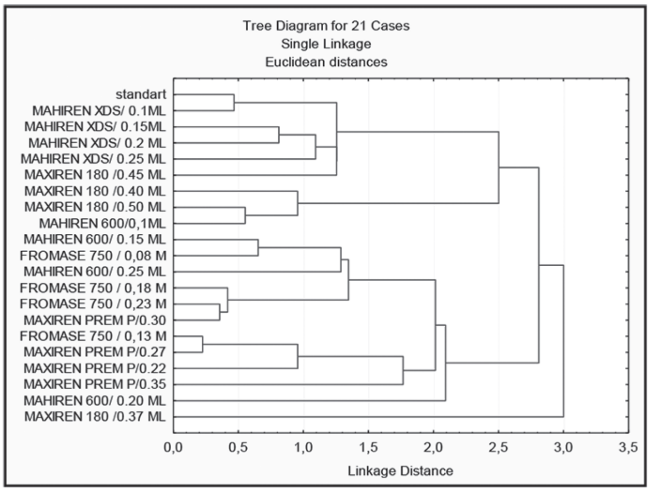

Figure 5. Cluster analysis of enzyme groups according to three parameters: RCT, A_{30} K_{20} in buffalo milk

The obtained results supported the hypothesis for possible modelling of measurements of parameters characterising buffalo milk coagulation. The traditional instruments of milk coagulation properties analysis are more important in the production of buffalo cheese (Wedholm et al., 2006; Ariota et al., 2007; Potena et al., 2007a,b; Bartocci et al., 2010) than in the production of goat and sheep cheese. The comparison of the data obtained with milk from different species confirmed that modelling of A_{30} should be reconsidered in order to obtain some useful information.

Conclusion

The use of enzymes (MAXIREN 600, FROMASE 750, MAHIREN XDS, MAXIREN 180 and MAXIREN PREM P) at various concentrations resulted in substantial variation in the magnitude and direction of changes of buffalo milk coagulation parameters (RST, A_{30} and K_{20}). Different chymosins with different concentrations actively affect the curd firmness. The shorter coagulation time will provide sufficient time for the curd firming time and its curd firmness. Over 50% of milk samples coagulate up to 6 minutes. The effects of enzymes and their different concentrations on measurement were in different groups, which in our opinion was a good prerequisite for using the obtained data for modelling of the measurement process.

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