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Modelling of Buffalo Milk Coagulation Kinetics after Addition of Enzymes at Different Concentrations by Means of Mechanical Lactodynamography

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Abstract

The aim of the present study was to evaluate the possibility for modelling milk coagulation kinetics using enzymes with different concentrations. Buffalo milk coagulation properties were evaluated in 420 milk samples. Five enzymes were tested/MAXIREN 600, FROMASE 750, MAHIREN XDS, MAXIREN 180 and MAXIREN PREM P/, each with 4 concentrations along with one control group with standard chymosin recommended from the manufacturer. The analyses were performed by means of a biosensor – mechanical lactodynamograph (Polo Trade – Computerized Renneting Meter). The statistical analysis was done by Principal Component Analysis/PCA. Curd firmness of buffalo milk could be predicted with very high reliability through the other tested milk coagulation parameters. This allows for increasing the accuracy of measuring a_{30} regardless of rennet coagulation time and curd firming time values. The established values in our opinion support the thesis about the specific effect of enzymes on buffalo milk despite their concentration. The enzyme MAXIREN PREMP that satisfied at the highest extent our preliminary expectations for curd firmness exhibited the lowest negative values with respect to factor 1 and high positive ones with respect to factor 2. This enzyme also showed the shortest rennet coagulation time, the earliest curd firming time and high curd firmness.

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Buffalo Milk, Coagulation Kinetics, Enzymes, Concentrations, Mechanical Lactodynamography

Introduction

The interest to milk coagulation properties in research community and industry is continuously increasing, mainly because the relative share of milk which is processed into cheese also increases.

A number of studies confirm the significance of milk coagulation properties for cheese yield and quality /Bynum and Olson, 1982; Aleandri *et al.*, 1989; Martin *et al.*, 1997; Wedholm *et al.*, 2006; De Marchi *et al.*, 2008, Yordanova *et al.*, 2012, Beneduci *et al.*, 2010).

Coagulation properties of milk are associated with cheese yield and quality/Clark *et al.*, 2000; Storry and Ford, 1982a; Okigbo *et al.*, 1985b; Oster-sen *et al.*, 1997/. The conditions for cheese production including the type of concentration of rennin, incubation temperature, and different elements of milk composition influence milk coagulation ability /Storry and Ford, 1982b; Okigbo *et al.*, 1985b/. Thus, every factor with effect on milk quality is also important for milk coagulation properties /Storry *et al.*, 1983; Politis and Ng-Kwai-Hang, 1988; Aleandri *et al.*, 1989/. When kappa casein is hydrolysed, casein micelles become

unstable and thus, precipitated by calcium. The aggregation or shrink age of casein micelles occurs during the non-enzyme stage/Garnot and Olson, 1982; Storry and Ford, 1982a; Walstra *et al.*, 1984/. Rennet coagulation time (RCT) is the point when the curd formed by casein micelles is sufficient to be seen. The time to curd firmness of 20 mm (k_{20}) measures the rate of curd firming after the coagulation has begun. The ideal variant for producers is to decrease RCT and to increase CR as both parameters have an influence on technological time for milk processing into cheese.

Traditionally, milk coagulation properties are expressed through rennet coagulation time (RCT), time to curd firmness of 20 mm (k_{20}) and curd firmness 30 min after enzyme addition (a_{30}), measured by Formagraph. Various instruments are used for assessment of milk coagulation properties, e.g. Formagraph (Foss Electric, Hillerød, Denmark) or computerized meters which perform measurement of rennet coagulation time (RCT, min) and curd firmness 30 min after chymosin addition (a_{30} , mm), although there are also alternative systems based on optical, thermal and vibrational methods. (O'Callaghan *et al.*, 2002; Kübarsepp *et al.*, 2005; Cecchinato *et al.*, 2009; DeMarchi *et al.*, 2009). Researchers which have studied coagulation properties of buffalo milk are only few (Bartocci *et al.*, 2002; Ariota *et al.*, 2007; Potena *et al.*, 2007b, Karabashev *et al.*, 2012).

The technical development of Formagraph and the availability of computerized renneting meter allowed automated measurements and continuous registering of data in device's memory. A number of research studies carried out modelling of milk coagulation dynamics through rheo meters (Douillard, 1973, 1986; McMahon *et al.*, 1984; O'Callaghan and Guinee, 1996). Nowadays, NIRS and other optic devices are used for coagulation monitoring (O'Callaghan *et al.*, 2002; Kübarsepp *et al.*, 2005a; Fagan *et al.*, 2007); and optical records are transformed into traditional parameters. Furthermore, such parameters could be directly predicted by MIRS from fresh milk without provoking coagulation, using adequate calibration algorithms (Dal Zotto *et al.*, 2008; De Marchi *et al.*, 2009).

Investigations on the use of mechanical lactodynamograph for generation of reference data for MIRS calibration are currently performed/DeMarchi *et al.*, 2009/, продължават.

Numerous authors discuss the problems associated to mechanical lactodynamograph /Ikonen *et al.*, 1999; De

Marchi *et al.*, 2007, Cecchinato and Carnier, 2011; Cecchinato *et al.*, 2011/ as well as some effects with negative impact on its precision/Ikonen *et al.*, 1999, 2004; Cassandro *et al.*, 2008; Cecchinato *et al.*, 2011/.

Obviously, the longer coagulation time is related to shorter curd firming time and lower curd firmness.

In Bulgaria, the issues related to evaluation of buffalo milk coagulation properties by means of mechanical lactodynamography were not addressed so far.

The aim of the present study was to evaluate the possibility for modelling milk coagulation rate using enzymes with different concentrations.

Materials and Methods

Buffalo milk coagulation properties were evaluated in 420 milk samples. Five enzymes were tested/MAXIREN 600, FROMASE 750, MAHIREN XDS, MAXIREN 180 and MAXIREN PREM P/, each with 4 concentrations along with one control group with standard chymosin recommended from the manufacturer. The analyses were performed by means of a biosensor – mechanical lactodynamograph (Polo Trade – Computerized Renneting Meter). This technique implies monitoring of the viscosity behaviour of milk samples under constant temperature whose coagulation is induced by addition of standard enzyme. Viscosity changes are registered by oscillating pendulums immersed in coagulating milk. The device converts the changes in pendulum movements resulting from curd formation into a diagram via the computerized system.

Milk coagulation properties are defined by three parameters:

Rennet coagulation time /RCT/
Curd firming time / k_{20} /
Curd firmness / a_{30} /

Results and Discussion

Descriptive statistics of buffalo milk data

The RCT mean values and variances are presented in *Table 1*. The obtained RCT values with the different enzymes and their concentrations were within a broad range. The highest coagulation rate was obtained after milk treatment with different concentrations of FROMASE 750 (from 1.20 to 2.58min) and MAXIREN

PREM P (from 1.19 to 1.42min). The values with these two enzymes differed statistically significantly from all other groups and controls. A statistically significant lower RCT was obtained with high concentrations of the enzyme MAHIREN 600 (from 0.15 to 0.25), with respective RCT values from 3.23 to 2.91min. The values obtained with the different concentrations of the enzyme MAHIREN XDS were similar to those of the standard (from 5.9 to 7.04min), whereas the RCT values with the enzyme MAXIREN 180 exceeded those of the standard (7.23–8.77min) for the different concentrations while RCT for the concentration of 0.50 ml was similar to that with the standard.

Curd firming times are presented in *Table 2*. The most statistically significant shortening of this parameter was achieved with the highest concentrations of FROMASE 750 / 0.23 ML (1.17min), as well as with the highest concentration of MAXIREN PREMP / 0.35 ML (0.19min). In all other cases, curd firming time did not differ substantially from that of the standard except for middle and highest concentrations of MAXIREN 180 – 0.400 and 0.50 ml, which yielded values higher than those of the standard.

Mean values and variances of a_{30} are presented in *Table 3*. A statistically significant increase in curd firmness was achieved only with different concentrations of the enzyme MAXIREN PREM except for 0.30 (from 45.25 to 47.95mm). It should be noted that with FROMASE 750, RCT and k_{20} values were close to those with MAXIREN PREM, although curd firmness was insignificantly different from that obtained with the standard

With the enzyme MAXIREN 180, curd firmness values were statistically significantly lower than those with the standard, in association with the first two milk coagulation parameters (35.00 to 38.55 mm).

PCA results for enzymes with respect to the three parameters are presented on *Fig 1 and Table 4*. The results evidenced rather large variations of effects of different enzymes on milk coagulation. It could be affirmed that three groups were formed, namely enzymes with negative values for the two factors-MAHIREN 600, enzymes with negative values for the factor 1 and various values for factor 2 - FROMASE 750, enzymes with predominantly positive values for the two factors - MAXIREN 180, MAHIRENXDS and control group. It should be noted that MAXIREN PREMP exhibited the lowest negative values for factor 1 and high positive

values for factor 2. These enzymes showed the highest coagulation rate and earliest time to curd firming time and curd firmness. These values in our opinion support the hypothesis about the specific effects of enzymes regardless of their concentration. Undoubtedly, the highest negative values were found out with almost all concentrations of MAXIREN PREMP, whereas the highest positive values – with the different concentrations of MAXIREN 180.

PCA of parameters RCT, a_{30} , k_{20} for 21 enzymes are presented on *Fig. 2 and Table 5*. Factor 1 describes 82.02% of the variation while factor 2 - 11.55%. All parameters except for a_{30} were with positive values for factor 1 and factor 2, where enzymes were negative but rather close to zero (0.013).

Table 6, Fig. 3 and 4 present statistically significant regression relationships between a_{30} and RCT, as well as between k_{20} and a_{30} . It could be seen that curd firmness could be predicted with the other measured parameters. This allowed for increasing the accuracy of a_{30} measurement regardless of RCT and k_2 values.

The obtained results definitely support the hypothesis for possible modelling of milk coagulation parameters. One of the problems with the used method is that not all milk samples coagulated within 30 minutes/Ikonen *et al.*, 1999; De Marchi *et al.*, 2007/. This is an important problem when slowly coagulation milks (like buffalo milk) are evaluated from the point of view of statistical analysis of studied samples/Cecchinato and Carnier, 2011; Cecchinato *et al.*, 2011/.

The results indicated that despite the achieved considerable acceleration of the curd firming time in buffalo milk k_{20} , the desired effect for increasing curd firmness was not present in many of experimental groups. This is in line with the conclusions of several researchers that the k_{20} parameter could not be evaluated in milk samples with long RCT, as curd firming did not allow a 20-mm oscillation interval within 30 minutes. This parameter is important for the utility of milk coagulation properties evaluation. Also, in such cases, the repeat ability and reproducibility of k_{20} is lower than those of RCT, which is a reason for excluding k_{20} estimates regardless of the practical significance of this index of the optimum time for curd cutting. Lastbutnotleast, the parameter a_{30} is strongly dependent on rennet coagulation time both phenotypically and genetically /Ikonen *et al.*, 1999, 2004; Cassandro *et al.*, 2008; Cecchinato *et al.*, 2011/.

Table.1 Mean values and variations of RCT

Enzymes	RCT - Means	RCT - N	RCT - Std.Dev.	RCT - Variance	RCT - Std.Err.
Standard	7.389a	20,00	0.93	0.86	0.21
MAHIREN 600/0.1ML	6.848c	20.00	0.65	0.42	0.15
MAHIREN 600/ 0.15 ML	3.225d	20.00	0.60	0.36	0.13
MAHIREN 600/ 0.20 ML	3.529d	20.00	0.22	0.05	0.05
MAHIREN 600/ 0.25 ML	2.912d	20.00	0.30	0.09	0.07
FROMASE 750 / 0.08 ML	2.576d	20.00	0.32	0.11	0.07
FROMASE 750 / 0.13 ML	1.483e	20.00	0.19	0.04	0.04
FROMASE 750 / 0.18 ML	1.299e	20.00	0.10	0.01	0.02
FROMASE 750 / 0.23 ML	1.205e	20.00	0.05	0.00	0.01
MAHIREN XDS/ 0.1ML	7.039a	20.00	1.64	2.70	0.37
MAHIREN XDS/ 0.15ML	6.766c	20.00	0.40	0.16	0.09
MAHIREN XDS/ 0.2 ML	6.899c	20.00	0.59	0.35	0.13
MAHIREN XDS/ 0.25 ML	5.903c	20.00	0.39	0.15	0.09
MAXIREN PREM P/0.22 ML	1.419e	20.00	0.21	0.05	0.05
MAXIREN PREM P/0.27 ML	1.316e	20.00	0.09	0.01	0.02
MAXIREN PREM P/0.30 ML	1.236e	20.00	0.05	0.00	0.01
MAXIREN PREM P/0.35 ML	1.190e	20.00	0.06	0.00	0.01
MAXIREN 180 /0.37 ML	8.774b	20.00	0.66	0.44	0.15
MAXIREN 180 /0.40 ML	8.084b	20.00	0.98	0.97	0.22
MAXIREN 180 /0.45 ML	8.011b	20.00	0.52	0.28	0.12
MAXIREN 180 /0.50 ML	7.323a	20.00	0.58	0.33	0.13
All groups	4.496	420.00	2.86	8.20	0.14

Table.2 Mean values and variations of k_{20}

Enzymes	k_{20} - Means	k_{20} - N	k_{20} - Std.Dev.	k_{20} - Variance	k_{20} - Std.Err.
Standard	0.408a	20.000	0.270	0.073	0.060
MAHIREN 600/ 0.15 ML	0.293a	20.000	0.061	0.004	0.014
MAHIREN 600/ 0.20 ML	0.314a	20.000	0.214	0.046	0.048
MAHIREN 600/ 0.25 ML	0.279a	20.000	0.090	0.008	0.020
FROMASE 750 / 0.08 ML	0.332a	20.000	0.066	0.004	0.015
FROMASE 750 / 0.13 ML	0.233a	20.000	0.077	0.006	0.017
FROMASE 750 / 0.18 ML	0.249a	20.000	0.075	0.006	0.017
FROMASE 750 / 0.23 ML	0.173b	20.000	0.055	0.003	0.012
MAHIREN XDS/ 0.1ML	0.718c	20.000	1.547	2.395	0.346
MAHIREN XDS/ 0.15ML	0.401a	20.000	0.279	0.078	0.062
MAHIREN XDS/ 0.2 ML	0.373a	20.000	0.235	0.055	0.053
MAHIREN XDS/ 0.25 ML	0.324a	20.000	0.059	0.003	0.013
MAXIREN PREM P/0.22 ML	0.263a	20.000	0.084	0.007	0.019
MAXIREN PREM P/0.27 ML	0.225a	20.000	0.077	0.006	0.017
MAXIREN PREM P/0.30 ML	0.233a	20.000	0.092	0.008	0.021
MAXIREN PREM P/0.35 ML	0.188b	20.000	0.067	0.004	0.015
MAXIREN 180 /0.37 ML	0.571a	20.000	0.405	0.164	0.091
MAXIREN 180 /0.40 ML	0.918d	20.000	1.571	2.469	0.351
MAXIREN 180 /0.45 ML	0.462a	20.000	0.274	0.075	0.061
MAXIREN 180 /0.50 ML	0.739c	20.000	0.532	0.283	0.119
MAHIREN 600/0.1ML	0.474a	20.000	0.305	0.093	0.068
All groups	0.389	420.000	0.548	0.300	0.027

Table.3 Mean values and variations of a_{30}

Enzymes	a_{30} - Means	a_{30} - N	a_{30} - Std. Dev.	a_{30} - Variance	a_{30} - Std. Err.
Standard	43.050a	20.000	10.013	100.261	2.239
MAHIREN 600/ 0.15 ML	43.150a	20.000	9.549	91.187	2.135
MAHIREN 600/ 0.20 ML	39.900a	20.000	9.442	89.147	2.111
MAHIREN 600/ 0.25 ML	41.900a	20.000	8.783	77.147	1.964
FROMASE 750 / 0.08 ML	43.200a	20.000	9.157	83.853	2.048
FROMASE 750 / 0.13 ML	45.100a	20.000	9.679	93.674	2.164
FROMASE 750 / 0.18 ML	42.350a	20.000	9.853	97.082	2.203
FROMASE 750 / 0.23 ML	42.750a	20.000	9.602	92.197	2.147
MAHIREN XDS/ 0.1ML	43.050a	20.000	10.013	100.261	2.239
MAHIREN XDS/ 0.15ML	41.050a	20.000	9.355	87.524	2.092
MAHIREN XDS/ 0.2 ML	41.850a	20.000	9.377	87.924	2.097
MAHIREN XDS/ 0.25 ML	42.300a	20.000	9.274	86.011	2.074
MAXIREN PREM P/0.22 ML	46.200b	20.000	10.056	101.116	2.249
MAXIREN PREM P/0.27 ML	45.250b	20.000	10.417	108.513	2.329
MAXIREN PREM P/0.30 ML	43.100a	20.000	9.245	85.463	2.067
MAXIREN PREM P/0.35 ML	47.950b	20.000	9.528	90.787	2.131
MAXIREN 180 /0.37 ML	35.000d	20.000	8.079	65.263	1.806
MAXIREN 180 /0.40 ML	37.900d	20.000	7.636	58.305	1.707
MAXIREN 180 /0.45 ML	40.900a	20.000	7.840	61.463	1.753
MAXIREN 180 /0.50 ML	38.450d	20.000	8.049	64.787	1.800
MAHIREN 600/0.1ML	38.550d	20.000	9.361	87.629	2.093
All groups	42.045	420.000	9.513	90.506	0.464

Table.4 PCA table of enzymes with respect to RCT, a_{30} , k_{20}

Factor scores, based on correlations (MILK BUFFALO AVERAGE PCA)		
	Factor 1	Factor 2
Standard	0.29645	0.91501
MAHIREN 600/0.1ML	0.88648	-0.90199
MAHIREN 600/ 0.15 ML	-0.47836	-0.11086
MAHIREN 600/ 0.20 ML	-0.0101	-1.46266
MAHIREN 600/ 0.25 ML	-0.39596	-0.79002
FROMASE 750 / 0.08 ML	-0.49754	0.01606
FROMASE 750 / 0.13 ML	-1.05307	0.27821
FROMASE 750 / 0.18 ML	-0.71833	-0.92959
FROMASE 750 / 0.23 ML	-0.91975	-1.11989
MAHIREN XDS/ 0.1ML	0.82589	2.34697
MAHIREN XDS/ 0.15ML	0.44075	-0.1137
MAHIREN XDS/ 0.2 ML	0.31051	0.13651
MAHIREN XDS/ 0.25 ML	0.03426	-0.01586
MAXIREN PREM P/0.22 ML	-1.13752	0.91732
MAXIREN PREM P/0.27 ML	-1.10791	0.28787
MAXIREN PREM P/0.30 ML	-0.84616	-0.66984
MAXIREN PREM P/0.35 ML	-1.51655	1.33372
MAXIREN 180 /0.37 ML	1.74575	-1.82529
MAXIREN 180 /0.40 ML	1.95178	1.06963
MAXIREN 180 /0.45 ML	0.73622	0.26469
MAXIREN 180 /0.50 ML	1.45317	0.37371

Table.5 PCA table of milk coagulation parameters RCT, a_{30} , k_{20} for 21 enzymes

Factor-variable correlations (factor loadings). based on correlations (MILK BUFFALO AVERAGE PCA) Active and Supplementary variables *Supplementary variable		
	Factor 1	Factor 2
RCT	0.931799	0.125219
A30	-0.876528	0.472631
K20	0.907788	0.327825
*Enzymes	0.403891	-0.012955

Table.6 a_{30} -RCT and a_{30} - k_{20} regression relationships with mean values

Equation	R	SEE	F	P
Y=45.430-0.753 RCT	0.726	0.86	21.24	0.0002
Y=45.817-9.951 K 20	0.665	1.11	15.03	0.001

The statistical analysis was done by Principal Component Analysis/PCA

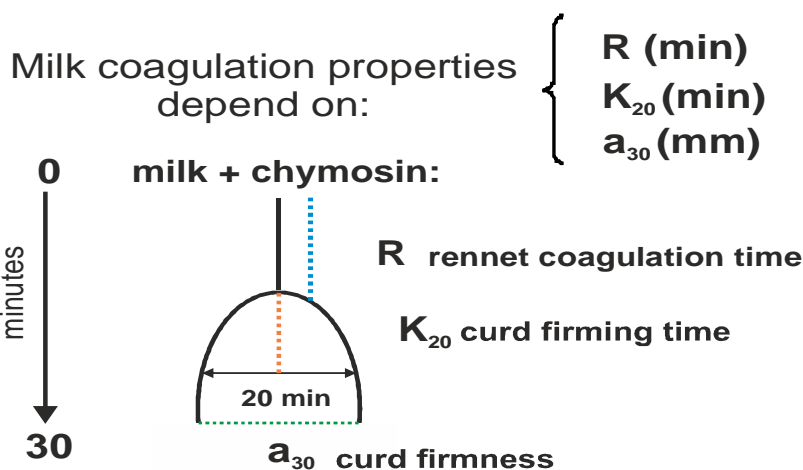


Fig.1 PCA of enzymes for 3 milk coagulation parameters (RCT, a_{30} , k_{20})

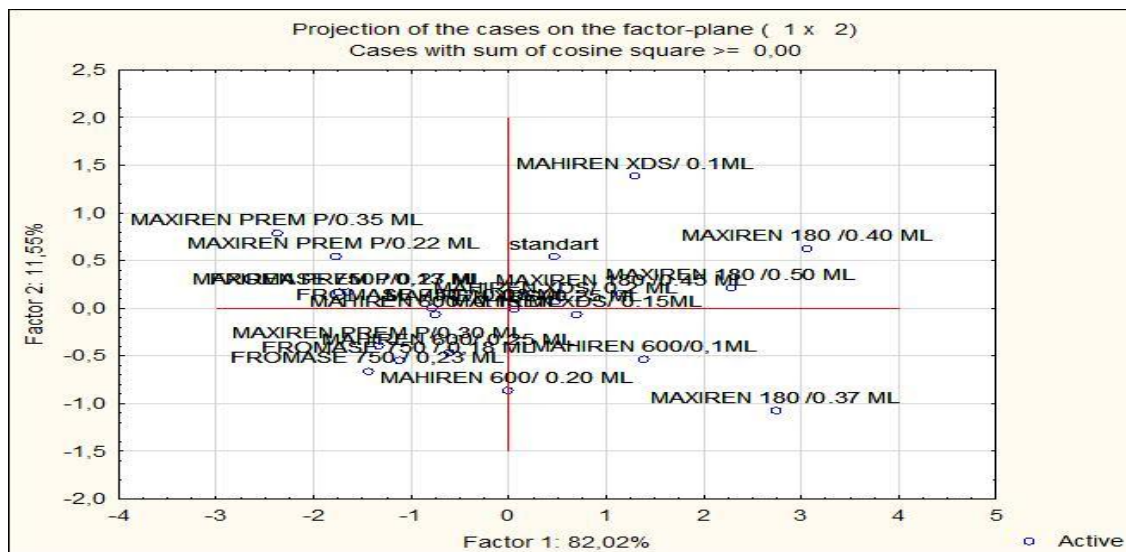


Fig.2 PCA plots of milk coagulation parameters RCT, a_{30} , k_{20} for 21 enzymes

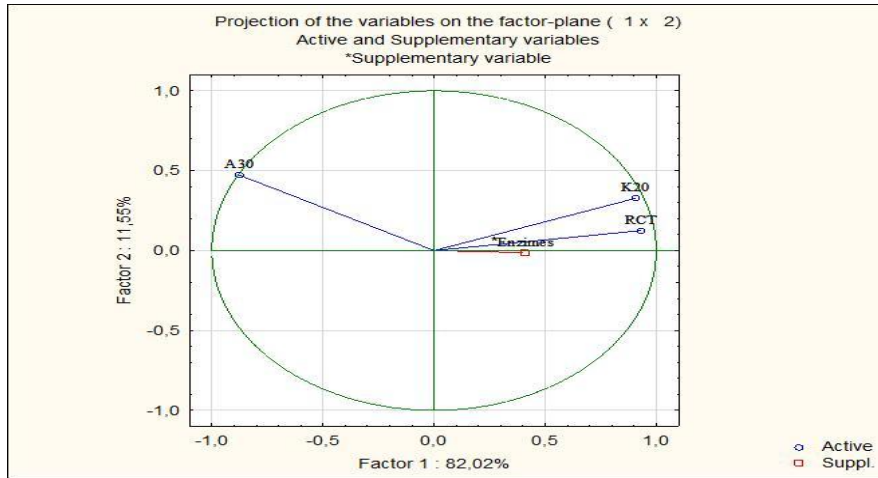


Fig.3 a_{30} - k_{20} regression scatter plot using mean values

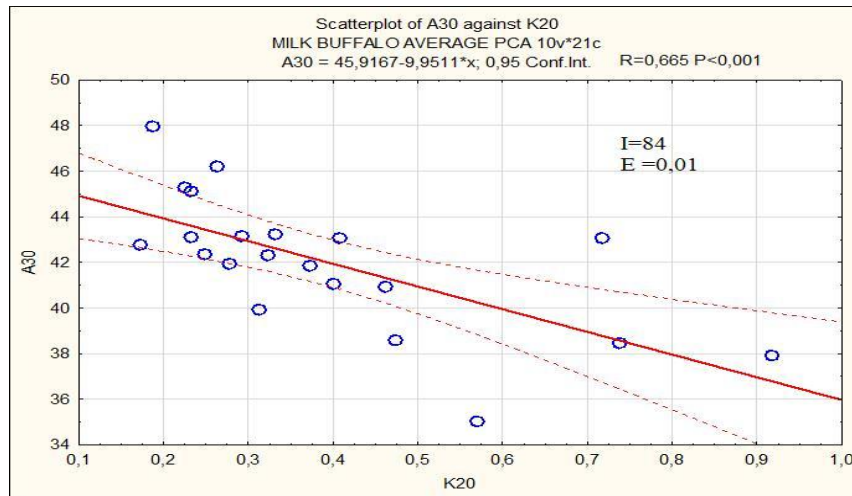
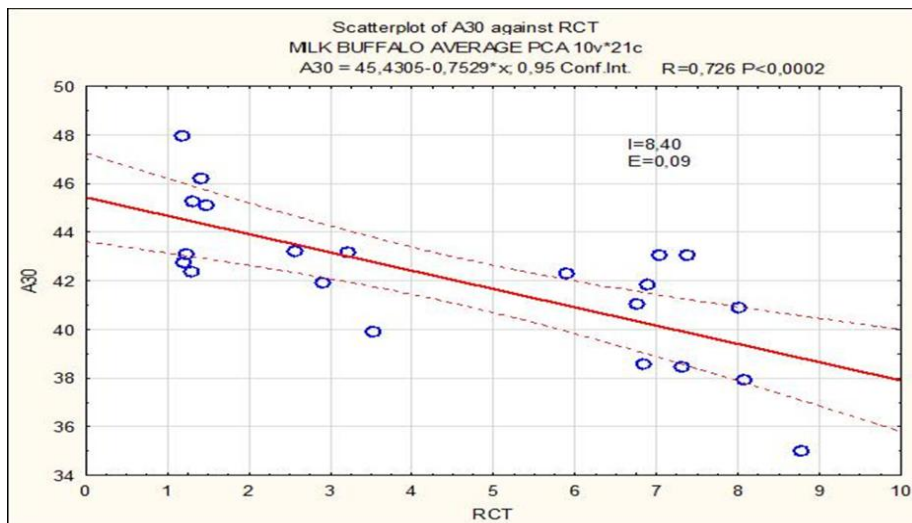


Fig.4 a_{30} -RCT regression scatter plot using mean values



Bittante, 2011, indicates that curd firmness was strongly negatively correlated to rennet coagulation time and that k_{20} could not be measured. It attempted to create a model in which curd firmness was a function of RCT over a 30-min interval. The tested model was: $CF_t = CF_{px} (1 - e^{-kCFx(t-RCT)})$. The obtained regression equations were logical and in line with author's conclusions. The fact that we were not able to draw an equation reliable enough to reflect the relationship of both RCT and k_{20} vs a_{30} supports the abovementioned thesis.

The mutual relationship between milk coagulation parameters shown both by us and the results of other authors/Ikonen *et al.*, 1999, 2004; Cassandro *et al.*, 2008; Cecchinato *et al.*, 2011/ provide arguments to support the experimental approach chosen by us, The results made clear that the time course of effects of various enzymes at different concentrations was not always consistent and did not always follow a definite trend.

In all types of milk, curd firmness (a_{30}) reached a maximum and then gradually decreased. This is due to syneresis (Calvo and Balcones, 2000), which induces expulsion of whey from the gel even when curd was not firmed. This occurs because the containers used for evaluation of milk coagulation properties are small (10 mL).

The most obvious difference between animal species consists in the circumstance that in the milk of small ruminants (goats) the syneresis usually occurs rather earlier than in cow or buffalo milk. Maximum curd firmness (a_{30}) values for cow milk are attained within 30 min from rennet addition. The default setting of lactodynamographs for analysis of cattle milk is mainly intended to evaluate coagulation and curd firming, and not syneresis. Also, the milk of cows and goats reacts differently to acidification, temperature changes, addition of calcium and changes in rennet concentration (Bencini, 2002).

The results from our experiments with buffalo milk supported entirely the hypothesis that if milk is analysed with the same instrument and under the same experimental conditions (Cecchinato *et al.*, 2012a), buffalo milk coagulated earlier and attained a higher a_{30} values than cow milk. Traditional instruments for analysis of milk coagulation properties were more important for the production of buffalo cheese (Bartocci *et al.*, 2002; Zicarelli, 2004; Ariota *et al.*, 2007; Potena *et al.*, 2007 a, b; Bartocci and Terramocchia, 2010), as compared to goat cheese. The comparison of data obtain

with milk of different animal species confirmed that modelling of curd firmness (a_{30}) should be re-evaluated if the aim is to obtain a reliable information. The obtained experimental data and their analysis add further to conclusions of discussed research works. They also discovered opportunities for modelling and using of established statistically significant relationships for prediction of curd firmness (a_{30}) from values of RCT and k_{20} .

Conclusions and Recommendations

Curd firmness of buffalo milk could be very accurately predicted from the other two parameters subject to measurement, this allows for increased accuracy of a_{30} measurement regardless of rennet coagulation time and curd firming time values.

The highest buffalo milk coagulation rate was achieved with the different concentrations of FROMASE 750 (from 1.20 to 2.58min) and MAXIREN PREM P (from 1.19 to 1.42min). Values obtained with both enzymes were statistically significantly different from those of the standard and other experimental group.

Statistically significant shortened curd firming time of buffalo milk was demonstrated for the highest concentrations of FROMASE 750 / 0.23 ML (1.17min), as well as with the highest concentration of MAXIREN PREMP/0.35 ML (0.19min).

A statistically significant increase in curd firmness of buffalo milk (from 45.25 to 47.95mm). Was achieved only with the different concentrations of MAXIREN PREM except for 0.30

In our view, established values support the thesis that despite their concentration, enzymes had a specific effect on buffalo milk. The enzyme MAXIREN PREMP which satisfied at the highest extent our preliminary expectations for curd firmness, had the lowest negative values for factor 1 and high positive one for factor 2. This enzyme showed also the shortest rennet coagulation time, the shortest curd firming time and high curd firmness.

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