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Coagulation capacity of milk of local Polish and Holstein-Friesian cattle breeds*

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The study evaluated coagulation properties of milk of four cow breeds used in Poland and texture parameters of rennet curds obtained from it. The research material consisted of 733 milk samples collected from two native breeds – Polish Red (n=215) and White-backed (n=229), as well as Simmentals (n=189) and Polish Holstein-Friesians (n=100) (both of the latter are breeds used internationally). The following were determined in the samples: proximate chemical composition, casein content, active acidity (pH) and somatic cell count. Milk coagulation properties (MCP) were analysed using the Lactodynamograph v2 (Foss) in terms of rennet coagulation time (RCT, min), curd-firming time (k_{20}, min) and curd firmness 30 min after rennet addition (a_{30}, mm). Curd texture parameters (fracturability, cohesiveness, adhesiveness, springiness, gumminess, and chewiness) were determined using a Zwick/Roell. Milk from native breeds, especially Polish Red, had the most favourable parameters, i.e. the shortest clotting time (16:31 min, p≤0.01) and curd-firming time (4:51 min) as well as the firmest curd after 30 min (28.04 mm; p≤0.01). Curds from milk of Polish Red cows had the lowest fracturability at the highest cohesiveness and adhesiveness (p≤0.01). Curds from milk of White-backed and Simmental cows had similar parameters, but the gels from White-backed milk were significantly (p≤0.05) the springiest. Milk of Holstein-Friesians had the worst clotting and texture parameters. Prolonged clotting time and curd-firming time negatively affect

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curd texture. Clotting time correlated negatively with curd fracturability (r = -0.457) and chewiness (r = -0.141). In turn, curd firmness after 30 min correlated positively with fracturability (r = 0.684), cohesiveness (r = 0.347), adhesiveness (r = 0.263) and gumminess (r = 0.183). Milk obtained from the native breeds and Simmental cows was shown to have more favourable coagulation parameters than that obtained from Holstein-Friesian cows. Short clotting time, high curd firmness and short curd-firming time determine favourable texture parameters in rennet curds. This milk is therefore a valuable raw material for the production of cheeses.

KEY WORDS: local breeds / coagulation / rennet curd / texture

A significant portion of milk produced around the world is used for cheese production. Global production of cheese from whole cow milk amounted to 18.7 million tonnes in 2014, of which Poland produced 0.74 million tons, ranking sixth. The leading cheese producers include the USA (5.2 million tonnes), Germany (1.9 million tonnes), France (1.8 million tonnes), Italy (1.2 million tonnes) and the Netherlands (0.78 million tonnes) [FAOSTAT, 2018].

Important indicators of suitability of milk for cheese making are its coagulation properties (MCP). The ability to coagulate under the influence of rennet, formation of a firm curd as well as good syneresis capacity and whey drainage are the key features of milk used to make cheese [Cipolat-Gotet *et al.* 2012]. The coagulation parameters of milk are determined in part by its physicochemical properties, i.e. acidity and protein content, including casein [Penasa *et al.* 2016], its somatic cell count [Barłowska *et al.* 2009] and the concentration of rennet added to milk [Pretto *et al.* 2011]. Another important source of MCP variation is the breed of cows [Barłowska *et al.* 2006, De Marchi *et al.* 2007, Penasa *et al.* 2014]. In Poland there are 12 breeds of cows used for dairy purposes, with Black-and-White Holstein-Friesians accounting for about 90% of the active population. In addition to high-production breeds, native cattle breeds are used in Poland as well, including Polish Red and White-backed. Moreover, the Simmental breed is raised locally in mountainous areas. Cows of these breeds are usually raised in traditional, low-input production systems. The food quality policy pursued by the European Union countries is contributing to an increase in consumer interest in foods with specific characteristics resulting from the origin of the raw material or from the their traditional processing. In Europe milk of local breeds is often used for the production of cheeses with the Protected Designation of Origin (e.g. Italian Parmigiano-Reggiano, Grana Padano, Provolone and Asiago), while milk coagulation parameters are taken into account in determining the price of the raw material [Penasa *et al.* 2016]. Also in Poland measures are taken to promote dairy products (including rennet cheeses) produced from milk of local breeds. Farmstead cheeses are produced using traditional methods; the raw material is not standardized in terms of its content of the main milk constituents and no additives are used to improve the technological process. Therefore, the physicochemical properties and palatability of cheese depend primarily on the quality of raw milk.

There are studies available on the coagulation properties of milk obtained from cows of Polish native breeds, but they concern only the rennet clotting time

(determined by the Shern method) [Barłowska *et al.* 2009] and evaluation of rennet curd texture [Wolanciuk *et al.* 2016].

The aim of the study was to evaluate coagulation properties of milk obtained from cows of four breeds (including two native breeds) used in Poland and texture parameters of rennet curds obtained from it.

Material and methods

Farm characteristics and milk sampling

The material for the study comprised 733 milk samples taken from four breeds of cows: Polish Red – RP (n = 215, five herds), White-backed – WB (n = 229, five herds), Simmental – SM (189, five herds), and Polish Black-and-White Holstein-Friesian – PHF HO (n=100, one herd). The research was carried out over two years (2016-2018). Milk samples obtained from a complete milking procedure were collected individually from each cow, twice a year (the spring/summer season – June-September and the autumn/winter season – January-March) during test day milking (the AT4 method), and then cooled and transported to the laboratory. Samples were taken from cows in their second to fifth lactation, between days 90 and 210 of lactation. Data regarding their daily yield in the months of milk sampling were obtained from milking performance records (RW-2 reports) kept by the Polish Federation of Cattle Breeders and Dairy Farmers.

The WB, RP and SM cows were kept on small family farms in tie-stall barns. Milking was performed twice a day (every 12 hours) in a direct-to-can or pipeline system. The cows' diet was based mainly on on-farm feed. In the autumn/winter season the diet was based on haylage and hay, while in the spring/summer the cows mainly grazed in the pasture. In both seasons the feed ration was supplemented with on-farm cereal meal (Tab. 1). The average herd size was 11 for RP, 14 for WB and 18 for SM. The PHF HO cows were kept in one herd (450 cows) in a free-stall barn and

Table 1. Feed rations fed to cows on the farms

Family farms			Large-scale farm
Fodder given manually (2 x a day)			Fodder from a feed wagon (1 x a day)
Approximate daily feed ration			daily ration for a cow with yield of 35 kg of milk
Polish Red cows	White-backed cows	Simmental cows	TMR:
Autumn/winter season (November-April)			• maize silage – 17 kg • alfalfa haylage – 2 kg • wet sugar beet pulp - 6 kg • spent grain - 10 kg • CCM (Corn Cob Mix) – 7.5 kg • rapeseed extraction meal – 1.8 kg • cereal meal (36% protein) – 1.5 kg • hay – 1 kg • water – 6 kg dry matter content in feed ration: 41.4%
• fodder beets – 10 kg • hay – 6 kg • barley-triticale (1:1) meal – 1- 2 kg	• haylage – 30 kg • barley-triticale (1:1) meal – 1- 2 kg	• haylage – 35 kg • barley-triticale (1:1) meal – 2- 3 kg	
Spring/summer season (May-October)			
• green pasture forage • barley-triticale (1:1) meal – 1-2 kg	• green pasture forage • barley-triticale (1:1) meal – 1-2 kg	• green pasture forage • barley-triticale (1:1) meal – 2- 3 kg	

fed in the TMR system (total mixed ration). Milking took place three times a day in a carousel milking parlour. The PHF HO cows were maintained in intensive conditions, while the cows of the other breeds were kept under extensive animal management conditions.

Analysis of milk samples

The following were determined in each milk sample: contents of fat, protein and lactose using a Bentley Infrared Milk Analyzer (the dry matter content and protein-to-fat ratio were calculated from these data), casein content according to the AOAC method [2000] and active acidity (pH) using an Elmetron CP-401 pH meter. The somatic cell count (SCC) was determined using the Bentley Somacount 150 in order to exclude milk samples from cows with diseased udders. All milk samples contained below 400,000 SCC per ml.

Milk coagulation properties (MCP) were analysed using a Lactodynamograph v2 (Foss Italia, Italy). A 10 mL volume of milk, heated to 35°C, was mixed with 200 μL of rennet solution (Hansen Naturen Plus 215, diluted in distilled water to obtain a 1.2% solution (wt/vol), with a final value of 0.0513 IMCU/milk ml). The properties recorded were rennet coagulation time (RCT, min) as the time interval between the addition of rennet and gelation, curd-firming time (k_{20}, min) as the time between gelation and the attainment of curd firmness of 20 mm and curd firmness at 30 min after the addition of rennet (a_{30}, mm).

Rennet curds were prepared according to the method described by Ziarno [2006] with our own modifications. A 50 ml volume of milk was heated in a water bath at 35°C and then treated with rennet (0.0513 IMCU/ml milk). The parameters defining the texture of the curds obtained (fracturability, cohesiveness, adhesiveness, springiness, gumminess and chewiness) were determined using a Zwick/Roell BDO-FB0.5TS Proline testing machine (Zwick GmbH and Co, Ulm, Germany). A cylindrical die with a diameter of 45 mm and a height of 5 mm penetrated the curd twice in succession to a depth of 25 mm at a speed of 1 mm/s. The compression cycles were separated by a 2 s relaxation phase. The measurements and results were recorded in TestXpert II software.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's test (for unequal sample sizes) was used to compare means of the milk daily yield, chemical composition, pH and coagulation properties as well as texture parameters of rennet clots for different cattle breeds (RP, WB, SM and PHF HO). Differences between means at $p \leq 0.05$ and $p \leq 0.01$ were considered statistically significant. The Tables present mean values and standard deviations. The relationships between curd texture parameters and milk coagulation properties were determined using Pearson's correlation coefficients (r). The computations were performed using STATISTICA ver. 13 software Dell Inc. [2016].

Results and discussion

Cows of local breeds (RP and WB) and Simmentals, raised in traditional breeding systems, produced significantly ($p \leq 0.01$) less milk (about three-fold) than PHF HO cows (43.41 kg), but with higher contents of dry matter (Tab. 2). Milk obtained from Polish Red cows had a particularly favourable composition, with significantly ($p \leq 0.01$) more protein (including casein) and fat. In contrast, milk from PHF HO cows had the most favourable protein-to-fat ratio (0.94). Cecchinato *et al.* [2011], who assessed the milk parameters of Italian Holstein-Friesians, reported slightly higher fat and protein contents (3.88 g/100 g and 3.45 g/100 g, respectively) at a lower daily yield (32.4 kg) compared to our study. Lower milk yields in local breeds have also been demonstrated by other authors [Chiofalo *et al.* 2000, Gandini *et al.* 2007, Litwińczuk *et al.* 2012]. In a study by Chiofalo *et al.* [2000], cows of the Sicilian Modicana breed produced 20.4 kg/day less milk than Holstein-Friesians, with a 0.58 p.p. higher fat content. Varotto *et al.* [2015], when comparing yields of cows of the local breed Rendena and Holstein-Friesians and the quality of their milk, showed that the local cows produced significantly ($p \leq 0.05$) less milk (by 9.7 kg) with a 0.22 p.p. (percentage point) lower fat content ($p \leq 0.05$), but with higher contents of protein (by 0.05 p.p.) and lactose (by 0.06 p.p.) − $p \leq 0.05$. In a study by Litwińczuk *et al.* [2012], the daily yields of local breeds ranged from 12.6 kg in Polish Red to 16.9 kg in White-backed cows, as compared to 19.1 kg in Simmentals and 25.9 kg in Holstein-Friesians.

Table 2. Daily yield, chemical composition and acidity of milk from the analysed cow breeds

Item	RP (n=215)		WB (n= 229)		SM (n=189)		PHF HO (n=100)	
	mean	SD	mean	SD	mean	SD	mean	SD
Daily yield (kg)	12.43^{A}	4.58	14.86^{A}	4.09	17.61^{A}	8.52	43.41^{B}	8.27
pH	6.79	0.11	6.77	0.10	6.80	0.07	6.75	0.05
Fat (g/100 g)	4.61^{B}	0.70	4.26^{AB}	0.82	3.99^{AB}	0.65	3.44^{A}	0.76
Protein (g/100 g)	3.48^{B}	0.37	3.40^{B}	0.41	3.38^{B}	0.44	3.20^{A}	0.31
Casein (g/100 g)	2.72^{B}	0.33	2.62^{B}	0.37	2.63^{B}	0.37	2.47^{A}	0.26
Lactose (g/100 g)	4.86	0.23	4.85	0.24	4.88	0.22	4.86	0.17
Dry matter (g/100 g)	13.61^{B}	0.89	13.17^{AB}	1.04	12.90^{A}	1.01	12.16^{A}	0.87
P/F[#]	0.76	0.10	0.81	0.15	0.86	0.10	0.94	0.23

[#]P/F protein-to-fat ratio.
[AB]Within rows means bearing different superscript differ significantly at $p \leq 0.01$.

Analysis of milk coagulation properties is crucial when assessing suitability of raw milk for cheese production. Milk obtained from the native breeds and Simmental cows had more favourable parameters (Tab. 3). Milk of the Polish Red cows had the most favourable parameters in this respect, with the shortest clotting time (16:31 min, $p \leq 0.01$), the shortest curd-firming time (4:51 min) and the highest ($p \leq 0.01$) curd firmness after 30 minutes (28.04 mm). A short rennet clotting time and a high firmness value translate into higher cheese yield and its better quality [Pretto *et al.* 2013]. In turn, milk of Simmental and White-backed cows coagulated within a similar time

Table 3. Milk coagulation properties of the analysed cow breeds

Item	RP (n=215)		WB (n=229)		SM (n=189)		PHF HO (n=100)	
	mean	SD	mean	SD	mean	SD	mean	SD
RCT (min)	16:31^{A}	6:52	19:29^{B}	5:28	19:25^{B}	5:57	20:16^{B}	4:57
k_{20} (min)	4:51^{A}	2:23	5:19^{A}	2:33	6:51^{B}	4:47	5:55^{B}	1:45
a_{30} (mm)	28.04^{B}	10.67	25.44^{A}	11.25	23.06^{A}	9.87	21.94^{A}	10.46

RCT – rennet coagulation time; a_{30} – curd firmness 30 min after rennet addition; k_{20} – curdfirming time;
^{AB}Within rows means bearing different superscript differ significantly at $p \leq 0.01$.

(19:25 and 19:29 min, respectively), whereas curds obtained from milk of Simmental cows required significantly ($p \leq 0.01$) more time to become firm (by 1:32 min). Milk obtained from the Holstein-Friesian cows had the least favourable coagulability, i.e. the longest clotting time (20:16 min) and lowest curd firmness after 30 minutes (21.94 mm). Similarly, Varotto *et al.* [2015] when comparing coagulation properties of milk obtained from cows of the native Italian breed Rendena and Holstein-Friesians showed that milk of the local breed had more favourable coagulation parameters, i.e. clotting time was 2:10 min faster ($p \leq 0.05$) and curd was 4.8 mm firmer after 30 min ($p \leq 0.05$). The coagulation times for milk of Polish and Italian Holstein-Friesians were similar (20:16 and 21 min, respectively), as was curd firmness after 30 min (21.94 and 21 mm, respectively). When analysing coagulability of milk obtained from six breeds (the typical dairy breeds Holstein-Friesian, Brown Swiss and Jersey, and dual-purpose breeds, i.e. Simmental and the native breeds Rendena and Alpine Grey), Stocco *et al.* [2017] also found the best parameters for milk of the native breeds, especially Rendena. Milk of these cows clotted more than 20% faster than that of Holstein-Friesians, while curd was as much as 36% firmer after 30 minutes.

The more favourable coagulation parameters of milk from native breeds were reflected in texture parameters of rennet curds (Tab. 4). The best curds were obtained from milk of Polish Red cows. They had significantly ($p \leq 0.01$) the lowest fracturability as well as the highest cohesiveness and adhesiveness. Curds from milk of the White-backed and Simmental cows had similar parameters, but gels obtained

Table 4. Texture parameters of rennet clots obtained from milk of the analysed cow breeds

Item	RP (n=215)		WB (n= 229)		SM (n=189)		PHF HO (n=100)	
	mean	SD	mean	SD	mean	SD	mean	SD
Fracturability (N)	4.01^{C}	1.24	3.41^{B}	1.15	3.49^{B}	1.22	2.95^{A}	0.81
Cohesiveness (mJ)	0.62^{B}	0.26	0.53^{A}	0.21	0.52^{A}	0.22	0.47^{A}	0.18
Adhesiveness (N)	2.21^{b}	0.90	2.01^{ab}	0.73	2.06^{ab}	1.10	1.85^{a}	0.18
Springiness	1.79^{ab}	0.88	2.04^{b}	1.19	1.96^{ab}	1.11	1.63^{a}	1.09
Gumminess (N)	0.45	0.15	0.40	0.09	0.42	0.11	0.40	0.10
Chewiness (N)	0.80	0.46	0.83	0.56	0.85	0.56	0.67	0.50

$^{aA...}$Within rows means bearing different superscript differ significantly at: small letters – $p \leq 0.05$; capitals – $p \leq 0.01$.

from milk of White-backed cows were significantly ($p \leq 0.05$) the springiest. There were no significant differences in gumminess or chewiness of these curds. In an earlier study by Wolanciuk *et al.* [2016] comparing texture parameters of rennet curds obtained from milk of cows raised on low-input farms and cows kept in an intensive rearing system, the former had significantly higher springiness (by 0.34; $p \leq 0.05$) and cohesiveness (by 0.03 N, $p \leq 0.01$), but lower hardness (by 1.35 N, $p \leq 0.01$). The best curds were obtained from milk of Polish Red cows, which had the highest values for springiness, chewiness and cohesiveness and the lowest for hardness, although the differences in comparison with the other local breed, i.e. White-backed, amounted to only about 10%.

In cheese technology one of the most important steps in the production process is the coagulation of milk proteins. The course of this process depends in part on the content of dry matter components in milk. Table 5 presents correlation coefficients between coagulation parameters and both the daily yield of cows and physicochemical properties of their milk. The content of dry matter was shown to be positively and moderately correlated with curd firmness after 30 min (r = 0.200, p=0.000). Higher dry matter contents also reduced curd-firming time (r = -0.308, p=0.000). There was no relationship between dry matter content and milk coagulation time. Contents of protein, casein and fat were positively and moderately correlated with curd firmness after 30 min (r = 0.273, p=0.000, r = 0.287, p=0.000, r = 0.136, p=0.04, respectively) and negatively with curd-firming time (r = -3,339, p=0.000, r = -0.323, p=0.000, r = -0.236, p=0.000). There were no correlations between milk coagulation parameters and lactose content or the protein-to-fat ratio. Significant correlations were found between milk pH and clotting time (r = 0.249, p=0.000) and curd firmness after 30 minutes (r = -0.199, p=0.000). The daily yield of cows was negatively correlated with curd firmness after 30 minutes (r = -0.165, p=0.031). Similarly, Manuelian *et al.* [2018] showed a negative correlation between daily yield and curd firmness (r = -0.06, $p \leq 0.05$) and a positive correlation with curd-firming time (r = 0.32; $p \leq 0.05$). Manuelian

Table 5. Pearson's correlation coefficients between coagulation parameters and daily yield, chemical composition and acidity of milk

Item	Daily yield (kg)	pH	Fat (%)	Protein (%)	Casein (%)	Lactose (%)	P/F#	Dry matter (%)
RCT(min)	0.118	0.249	0.012	0.071	0.058	-0.060	0.065	-0.003
	p=0.074	p=0.000	p=0.802	p=0.140	p=0.215	p=0.208	p=0.178	p=0.960
a_{30} (mm)	-0.165*	-0.199	0.136	0.273	0.287	-0.080	0.030	0.200
	p=0.031	p=0.000	p=0.004	p=0.000	p=0.000	p=0.092	p=0.529	p=0.000
k_{20} (min)	0.131	0.097	-0.236	-0.339	-0.323	-0.042	0.042	-0.308
	p=0.096	p=0.093	p=0.000	p=0.000	p=0.000	p=0.476	p=0.475	p=0.000

#P/F protein-to-fat ratio; RCT – rennet coagulation time; a_{30} – curd firmness 30 min after rennet addition; k_{20} – curd-firming time; p – p-value.

et al. [2018] also reported that the casein content in milk positively influences curd firmness (r = 0.15, p≤0.05), while it has a negative effect on curd-firming time (r = -0.69, p≤0.05). These relationships were also reported by Penasa *et al.* [2016]. In turn, Maciel *et al.* [2016] in an analysis of milk quality in Danish Holstein-Friesians showed a significant relationship between its clotting time and the cows' daily yield, protein and casein content in milk, as well as its pH.

Analyses also showed relationships between milk coagulation parameters and texture of rennet curds (Tab. 6). Clotting time was negatively correlated with curd fracturability (r = -0.457, p=0.000) and chewiness (r = -0.141, p=0.017). Curd firmness after 30 min (a_{30}) was positively correlated with its fracturability (r = 0.684, p=0.000), cohesiveness (r = 0.347, p=0.000), adhesiveness (r = 0.263, p=0.000) and gumminess (r = 0.183, p=0.002). Curd-firming time (k_{20}) was negatively correlated with all the analysed parameters. The results indicate that prolonged clotting time and curd-firming time negatively affect texture parameters. High curd firmness after 30 minutes is reflected in lower fracturability, as well as higher cohesiveness and adhesiveness, which is important in curd processing during cheese production. The parameters analysed are important when the curd is being cut. If the process is performed improperly, cheese yield is reduced due to loss of fat and curd fines into whey, which may negatively affect\ quality of the final product [Hansen *et al.* 2010].

Table 6. Pearson's correlation coefficients between milk coagulation parameters and texture of rennet curds

Item	RCT	a_{30}	K_{20}
Fracturability (N)	-0.457***	0.684***	-0.472***
Cohesiveness (N)	-0.100	0.347***	-0.214***
Adhesiveness (mJ)	-0.013	0.263***	-0.201**
Springiness	-0.112	-0.004	-0.047
Gumminess (N)	-0.081	0.183**	-0.256***
Chewiness (N)	-0.141*	0.083	-0.149*

RCT – rennet coagulation time; a_{30} – curd firmness 30 min after rennet addition; K_{20} – curd-firming time.
*p≤0.05 **p≤0.01 ***p≤0.001.

The present study is the first to analyse the results of instrumental assessment of coagulation properties for milk of cow breeds used in dairy production in Poland, including native ones. The information obtained from the instrumental test (determination of RCT – rennet clotting time; a_{30} – curd firmness at 30 minutes after the addition of the coagulation enzyme; and k_{20} – the time needed for the gel to attain a specified firmness – curd-firming time) provides a more comprehensive characteristic of milk coagulation properties and therefore a more objective assessment of raw milk in terms of its suitability for the production of rennet cheeses.

In conclusion, the cattle breed significantly influences coagulation properties of milk. Milk obtained from native breeds and Simmental cows was shown to have more favourable coagulation parameters than that obtained from Holstein-Friesian cows.

Milk of Polish Red cows was distinguished by particularly favourable properties. Short clotting time, high curd firmness and short curd-firming time determine favourable texture parameters in rennet curds, i.e. their low fracturability and high cohesiveness. The low yield of native breeds is compensated for by the high quality of the milk produced. This milk is therefore a valuable raw material for the production of cheeses, which should be distinguished with a special quality designation. This could improve profitability of farms raising native cattle breeds and at the same time provide greater potential for the development of local rural communities.

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