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CHANGES OF QUALITY OF RENNETS DURING STORING

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Abstract

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This work deals about evaluation of qualitative parameters of rennets. During the six months storing of rennets were determined following qualitative parameters: pH, rennet coagulation time, activity (strange) of rennet and dose of rennet, and finally were used instrumental methods: Near infrared spectroscopy (NIR) for recognizing age of rennets and spectrophotometric methods of determining the color of rennets. The theory found in the references suggests, that the activity of rennet should decrease by an average of 1–2 % per month, but the results are showing, that are changing quality of rennets namely mostly activity (strange) of rennet, which was decreased by 33% per half year. In analysis of color of rennets weren't observed major changes by the whole group. Some statistical differences were detected in the partial values L*a*b*, most advantageous were evaluation by rennet total color change during storage, which were higher than noticeable change in four samples. The least color changes ($P > 0.05$) were observed in microbial rennets, with no values observed ($\Delta Eab < 2$) that can be recognized even by the human eye when subjected to parallel comparison. It was found that the NIR analysis can be used to recognizing of rennets, which are different ages.

rennet, qualitative parameters, NIR spectroscopy, color

Milk coagulation properties are of great importance as they significantly influence cheese yield and quality (Kubarsepp *et al*.; 2005, Tabayehnejad *et al*., 2012). The use of rennet in cheese making dates back to approximately 6000 BC (Fox and Mc Sweeney, 1997; Moschopoulu, 2011).

The nomenclature of enzymes is marked by the long history, during which the nature of enzymes was realized, and knowledge about their identity and diversity gradually increased. The first name for the milk-clotting enzyme was chymosin, derived from the Greek word for gastric liquid 'chyme', given by Jean-Babtis Deschamps to the main enzyme from the fourth stomach of the calf (Law and Tamine, 2010).

Rennet contains two main acid proteolytic enzymes (chymosin and pepsin) secreted in the fourth stomach (abomasum) of unweaned ruminants (calves, lambs or kids) (Crabbe, 1993) and these fractions depend on the age of the animals when slaughtered, and their previous diet; in commercial products, chymosin varies between approximately 50 % and 95 %. Because world cheese production increased by a factor of approximately 3.5 since 1961, but rennet supply decreased, because of the limited availability of calf stomachs, it was necessary to search for substitutes (Jacob *et al*., 2011).

Many proteinases can induce the coagulation of milk but most are too proteolytic or have the incorrect specificity and hence cause reduced cheese yield or defective cheeses. Only six proteinases – bovine, porcine and chicken pepsins and the acid proteinases of *Mucor miehei*, *M. pusillus* and *Endothia parasitica* – have been found to be more or less satisfactory for some or all cheese varieties (Fox and Stepaniak, 1993). Research on rennet substitutes continues, but the significance of this work is overshadowed by the production of chymosin from genetically engineered microorganisms. The gene for calf chymosin (or prochymosin) has been cloned in selected microorganisms (e.g. *Kluyveromyces lactis*, *Escherichia*

coli and *Aspergillus niger*) (Foltman, 1989; Teuber, 1990; Fox and Stepaniak, 1993). These rennet's substitutes are termed "coagulants". Amongst coagulants, we can also classify some rennet enzymes derived from plants, such as bromelain, papain etc. (Jacob *et al.*, 2011).

Most commercial suppliers offer standardized rennet, which are available in liquid, powder, or paste form, and are stable for a period of months at given temperatures. The rennet is produced from either fresh, dried, or frozen stomachs, which are cut and minced. The rennet is extracted by water, buffer, or salts (Pometto *et al.*, 2006). Rennet solutions of rennet are less stable and the substance decomposes in various speeds. Quality of rennet has an influence on the quality of rennet curd as well as the final quality of cheese (Teplý *et al.*, 1976). As considered by many authors, Teplý *et al.* (1976), Wong *et al.* (1999), Nájera *et al.* (2003), Arvanitoyannis (2009) and Fuquay *et al.* (2010), essential quality properties of commercial rennet comprise: pH, high enzymatic activity, stability, strength and microbiological purity as well as durability.

The optimum value of rennet pH is 4 to 6. If the value is increased above 6.5, there is a rapid loss of enzymatic activity of rennet (Teplý *et al.*, 1976). Milk clotting activity, i.e. the capability for specific κ-casein hydrolysis, is the most important property of enzymes used in cheese production and only a few analytical methods have been established to measure it (Jacob *et al.*, 2011).

The first commercial rennet with a standardised enzymatic activity was that developed and sold by Franz Soxhlet, the Austrian pharmacist. Since then, the Soxhlet unit has been widely used for the characterization of rennet strength (milk-clotting activity), although it has been somewhat modified over time and today a new kind of unit is used (Fuquay *et al.*, 2010). The Soxhlet method is very easy and therefore is still used by traditional cheese making in small farm or small dairy also today (Jacob *et al.*, 2011). Calculated rennet strength in Soxhlet units is refers to the volume of raw milk, which can be clotted by one volume unit (1 ml or 1 g) of enzyme in 40 min (2 400 s) at 35 °C. This result is expressed as the ratio (e.g. 1: 5 000, i.e. 1 ml of rennet is coagulated 5 000 ml of milk) (Teplý *et al.*, 1976; Pometto *et al.*, 2006; Fuquay *et al.*, 2010; Law and Tamine, 2010; Tabayehnejad *et al.*, 2012).

It should be emphasized that this method should be regarded as a mere indicative determination of rennet strength, because the resulting strength depends on pH and quality of the milk and is not related to any reference standard of rennet. Therefore, the 1997 ISO/IDF (International Dairy Federation) introduced methods 199 (ISO/IDF 2006 – sheep and goats), 157 (ISO/IDF 2007 – cattle) and 176 (ISO/IDF 2012 – microbial rennet), which use standardized substrates (skimmed milk with 0.05% $CaCl_2$, pH 6.5, at 32 °C) to determine the overall activity of coagulation enzymes (Czech technical standard ISO No. 11815 (57 0525), 2008; Jacob *et al.*, 2011; Tabayehnejad *et al.*, 2012). The resulting rennet strength is compared with the reference standard of rennet, which has the same composition as the rennet sample. The strength of the clotting enzymes is expressed in International Milk Clotting Units (IMCU) (Jacob *et al.*, 2011). Table I is showing orientation conversion between Soxhlet unit and International Milk Clotting Units.

The rennet strength decreases when the substance is handled and stored improperly, with light, heat and shaking exerting clear detrimental effects on the rennet. Therefore, the substance should be stored in a closed container at a dark place and temperature below 10 °C, this considered to ensure rennet durability and stability (Teplý *et al.*, 1978; Arvanitoyannis, 2009). While rennets can resist to even very high temperatures when dry, the efficiency of liquid rennet is reduced already at room temperature, the intensity of which relating to the strength of the solution (Teplý *et al.*, 1978). If stored properly, rennet will not reduce its activity (strength) by more than 1–2 % per month (Arvanitoyannis, 2009; Fuquay *et al.*, 2010).

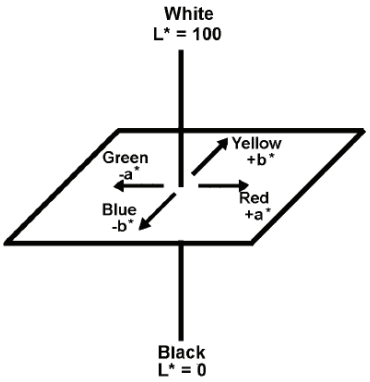
Our work also discusses the use of spectroscopy in the visible (UV VIS) and near infrared (NIR) regions in determining changes in quality parameters of rennet.

The initial judgment about the quality of food or beverages is influenced by appearance and notably by its color (Lawless and Heymann, 1998). Thus, color is one of the main parameters of the quality of wines, especially of red ones. The color provils information about defects, the type, and the conservation of wines during storing, and it has also an important influence on the overall acceptability by consumers (De Simon *et al.*, 2008).

I: *Approximate conversion between different units of activity and milligrams for the main enzymes in calf bovine rennet and adult bovine rennet (Law and Tamine, 2010)*

Enzym	Soxhlet's unit	IMCU*
1 mg chymosin A	1:24,400	291
1 mg chymosin	1:18,750	223
1 mg pepsin	1:5,500	81
1 IMCU chymosin A	1:85	
1 IMCU chymosin B	1:85	
1 IMCU pepsin	1:70	

* International Milk Clotting Units (IMCU)



1: *The Diagram, which is representing the CIEL* a* b* color space (Takiwaki et al., 2007)*

The CIE (Commission Internationale de 1'Eclairage) – L*a*b is' the most popular standardized color system. RGB (red, green, and blue) color coordinates of the images were converted into quasi – L'a*b' values using formulae similar to those defined by CIE. These values were compared with CIEL*a*b* values simultaneously measured with a colorimeter. A ***Lab*** color space is a color-opponent space with dimension ***L*** for lightness and ***a*** and ***b*** for the color-opponent dimensions, based on nonlinearly compressed CIE XYZ color space coordinates (**Fig. 1**) (Takiwaki *et al*., 2007).

FT NIR (Near-infrared spectroscopy with use Fourier transformation) is a physical non-destructive method, which use the interaction between incident radiation and a thin layer of sample of the material (Burns and Ciurtzak, 2007). Physically, it is based on the absorption or reflection of electromagnetic radiation (usually 800 to 2,500 nm, i.e. from 12,500 to 4,000 cm^{-1}) (Osborne, 2000). This method enables to give complete information about composition of sample (Mlček, 2008).

The objective of this study was to evaluation of qualitative parameters of rennet (pH, rennet clotting time, strange of rennet and dose of rennet) during the six months storing in the fridge (4 ± 2 °C) whit using laboratory and instrumental methods.

MATERIAL AND METHODS

Rennets

For analyzes were used six different liquid of rennets:

- Pepsin natural rennet with the strength of 1:10 000 (1),
- chymosin natural rennets with the strength of: 1:5 000 (2), The largest total colour change ΔE^*_{ab} from standard was achieved in rennet (6) that was also one of the most distinctive colour, and rennet (3), which developed a slight turbidity. The least colour changes ($P > 0.05$) were observed in rennets (4) and (5), with no values observed ($\Delta E_{ab} < 2$) that can be recognised even by the human eye when subjected to parallel comparison/ml (3) and 145 IMCU/ml (4),
- chymosin microbial rennets with the strength of: 950 IMCU / ml (5) and 1,000 IMCU/ml (6).

The rennets were storaged in a refrigerator by temperature 5 ± 1 °C during the six months. The samples were analyzed immediately after first opening rennet and after three and six months storing in the refrigerator (4–6 °C). This time of the procedure was chosen in relation to quality parameters of liquid rennet, which should be the same for at least 3 months of storing under the required conditions.

Preparation of standard milk substrate

For assays of qualitative properties of rennets were used restored whole, semi-skimmed and skimmed milk powder of the same batch to ensure the same quality substrate during half year. Restore of milk was carried out according to Czech technical standard No. 57 0105-3 (2003). Because we used the heat-treated milk, it was necessary to add $CaCl_2$ before renneting. For achieved of the same milk clotting time of the dried milk as the raw milk, it was tested the addition of solution 36% $CaCl_2$ at intervals of 20 to 120 ml. Optimum milk clotting time was reached by the addition of 100 ml of $CaCl_2$. All measures milk clotting time were three times repeated and for the following calculations were used arithmetic mean values.

Laboratory analysis

In a chemical laboratory were determined the following qualitative properties of rennets: pH with using WTW (Ger) pH meter according to method Czech technical standard No. 57 0107-12 (570107) (2008), strength of rennet – formula **1** and dose of rennet – formula **2** according to Soxhlet (Gajdůšek, 1997).

$$S = \frac{volume\ (ml) \times 2400}{rennet\ clotting\ time\ (s)}, \quad (1)$$

$$D = \frac{M}{S} \times \frac{35}{\mathrm{t}} \times \frac{40}{T}, \quad (2)$$

where **S** is the strength of rennet according to Soxhlet, **D** is the dose of rennet in ml (1 000 ml), **t** is the temperature by renneting in °C (35 °C), **T** is the milk clotting time in minutes (40 minutes).

Decrease activity of rennets from opening was calculated according to the formula **3**.

$$Decreased\ activity\ of\ rennets = \left[\left(\frac{a}{b} \right) - 100 \right] \cdot 100\ .\ (\%) \quad (3)$$

Decrease activity of rennet in the third month: **a** is the strange of rennet after opening, **b** is the strange of rennet in the third month.

Decrease activity of rennet after six months opening: **a** is the strength of rennet in the third month and **b** is the strength of rennet in the sixth month.

UV VIS spectroscopy

The transmittance spectra were measured with spectrophotometer CM-3500d (Konica – Minolta, Japan), using 1-cm plastic cuvettes. Measurements were taken every 10 nm between 380 and 780 nm with 8° light reflection angle. From the spectra, the color coordinates were calculated using the CIE method with L*, a* and b* values and ΔE^*_{ab} according to formula **3** (Sojčat, 2003). All measures were three times repeated.

$$\Delta E^*_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}, \quad (3)$$

where ΔE^*_{ab} is total color change, ΔL*, Δa* and Δb* are the differences in the values of each color coordinate from the standard.

FT NIR spectroscopy

FT NIR reflectance spectra of rennets were collected by the spectrophotometer Thermo Nicolet ANTARIS (Thermo Nicolet, USA). Spectra of samples were measured in the transmittance mode in a transflectance cuvette with optical part 1 mm, with number 80 scans in wave number 10,000–4,000 cm^{-1} and spectral resolution 4 cm^{-1}. Each sample was measured twice and average spectrum was used to create of calibration models. The some spectra were adjusted before using into calibration. The calibrations were created by program TQ Analyst with using algorithm Discriminate Analysis (DA). DA is a classification technique, which make use of identity of spectrum of sample with the spectrum of standard and separate the spectrum of sample into characterize same of the pre-defined classes. If the spectrophotometer is able to distinguish the spectrum belonging to different groups, thus is creating a cluster, which is containing identical spectrums, which have identical characteristics (Nicolet CZ, 2010).

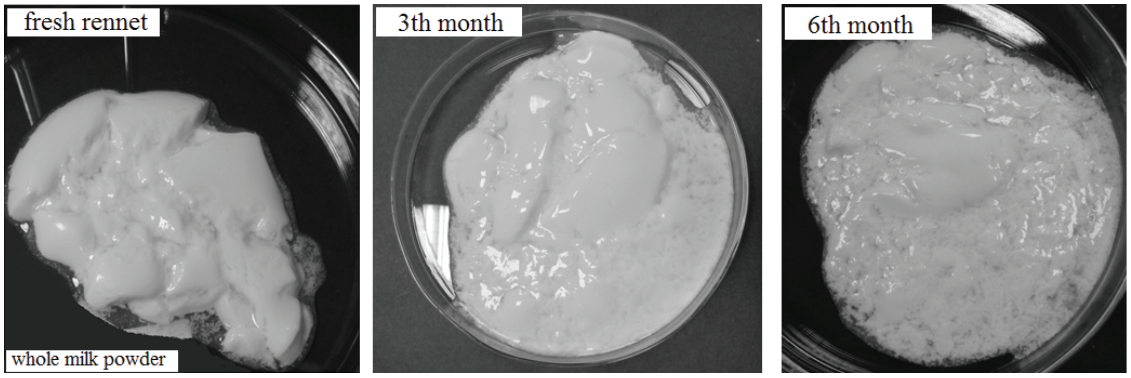
For statistical evaluation was used the program EXCEL 2003 and UNISTAT 5.1.

RESULTS AND DISCUSSION

Rennet samples were analysed for basic quality properties immediately after initial opening and subsequently after 3 and 6 months of storage in the refrigerator. The main requirement on the quality of rennet is the rennet stability, which is characterised by the standard power of the substance (Fox *et al.*, 1993). Figure 2 clearly shows an illustrative example of the impact of changes in quality parameters of rennet during storage over the six-month period, which was strikingly manifest in the quality of the resulting curd obtained.

Results of rennet chemical analyse are summarised in Table II.

In each rennet pH ranged over a half of year from 4.00 ± 0.02 to 5.98 ± 0.06, with the values not increasing above pH 6.5 even after six months. The greatest changes occurred in rennet (2), where the pH increased within 6 months from 5.47 ± 0.02 to 5.98 ± 0.06. However, the rennet strength, which includes the time of coagulation of milk, was changing rather significantly during storage. The theory found in the references suggests, that the activity of rennet should decrease by an average of 1–2 % per month (Arvanitoyannis, 2009; Fuquay *et al.*, 2010), the results achieved however not confirming this. The rennet activity under the conditions of the study decreased only after 3 months by 16 %, which after 6 months was up to 26 %. This level of activity reduction is two times higher than it should be in theory. It should be however noted that the determination of strength of rennet by Soxhlet method is only approximate, and the results can be affected by both the quality of milk used and the method of handling the rennet when analysed any further. Such a decline may however occur in the field as well, at a small farm/cheese manufacturing plant, if the correct methods of handling of rennet is not followed or rennets are exposed to room temperature over longer periods of time. It is therefore necessary to establish at least indicative strength of the rennet before applying it to milk and calculate the appropriate dosage that



2: *The appearance of curds using rennet of different ages (fresh, stored in the refrigerator over three and six months)*

II: *Quality parameters of rennets analysed*

Rennet	Month	pH	Rennet clotting time [s]	*Strength of rennet [1:x]	*Decreased activity of rennet from opening [%]	*Dose of rennet [ml]
1	0	4.01 ± 0.01 [a]	46 ± 4.99 [a]	5,255	-	0.19
	3	4.01 ± 0.02 [a]	51 ± 4.92 [a]	4,675	11	0.21
	6	4.00 ± 0.02 [a]	61 ± 1.70 [b]	3,913	26	0.26
2	0	5.47 ± 0.02 [a]	56 ± 4.55 [a]	4,260	-	0.23
	3	5.58 ± 0.02 [b]	74 ± 8.38 [b]	3,258	24	0.31
	6	5.98 ± 0.06 [c]	92 ± 2.83 [c]	2,599	39	0.38
3	0	5.55 ± 0.01 [a]	45 ± 3.68 [a]	5,294	-	0.19
	3	5.55 ± 0.01 [a]	45 ± 7.36 [a]	5,294	0	0.19
	6	5.51 ± 0.01 [a]	55 ± 1.25 [b]	4,390	17	0.23
4	0	5.68 ± 0.02 [a]	33 ± 4.32 [a]	7,273	-	0.14
	3	5.73 ± 0.02 [b]	50 ± 0.47 [b]	4,832	34	0.21
	6	5.67 ± 0.02 [a]	56 ± 3.30 [c]	4,260	41	0.23
5	0	5.61 ± 0.01 [a]	15 ± 0.47 [a]	16,364	-	0.06
	3	5.70 ± 0.01 [b]	17 ± 0.47 [b]	13,846	15	0.07
	6	5.63 ± 0.02 [a]	16 ± 1.25 [b]	14,694	10	0.07
6	0	5.42 ± 0.02 [a]	10 ± 0.47 [a]	24,828	-	0.04
	3	5.45 ± 0.01 [a]	11 ± 0.82 [a]	21,818	12	0.05
	6	5.37 ± 0.01 [b]	12 ± 0.82 [a]	20,000	19	0.05

1 – Pepsin natural rennet with the strength of 1:10,000; 2; 3; 4 – chymosin natural rennets with the strength of: 1:5,000; 150 IMCU/ml and 145 IMCU/ml; 5 and 6 are chymosin microbial rennets with the strength of: 950 IMCU / ml and 1,000 IMCU/ ml., the pH and coagulation time values are arithmetic means with standard error of the mean, *For formulas calculating was used only means of values

is needed for optimal curdling and the quality of the resulting cheese. As can be see from the results, even the calculated dose of the rennet used was significantly changing during storage. In most cases, the quantity of the rennet increased during storage by an average of 2 ml, since it should be borne in mind that for the process of curdling the respective quantity of milk to be optimal, the calculated dose of rennet should be doubled when applied. Only for the rennet (5) and (6), almost the same dose of rennet would be used after a half of year as that applied after opening. Determining the dose of rennet is very important because it significantly affects the resulting quality of the curd/cheese. If the quantity of rennet to curdle milk is low, the resulting curd is soft and fragile, whilst if applying more rennet in curdling, the curd is stiff and poorly releasing whey (Gajdůšek, 1997).

The appearance of the rennets during storage changed significantly ($P < 0.05$) in four of the six types of rennet, (1), (2), (3) and (6). For colour analysis results, please refer to Table III.

The largest total colour change ΔE^*_{ab} from standard was achieved in rennet (6) that was also one of the most distinctive colour, and rennet (3), which developed a slight turbidity. The least colour changes ($P > 0.05$) were observed in rennets (4) and (5), with no values observed ($\Delta E_{ab} < 2$) that can be recognised even by the human eye when subjected to parallel comparison.

The aim of the FT NIR analysis was to determine whether discerning the age of rennet is possible using spectrophotometers. The transmittance mode was selected for the measurement of samples, one that is recommended by many authors to measure liquid samples (Osborne, 2000). To create calibration models, we used 18 samples of rennets measured immediately after opening, and then after three and six months of storage in the refrigerator. The spectra were adjusted for the calibration by means of the first derivative within the region from 7 265.37 to 5 401.21 cm^{-1}. Figure 3 clearly shows the division of the spectra into clusters and the discriminant cross.

With the results achieved, we can conclude that the spectrophotometer can discern with high accuracy various ages of rennet. FT NIR method can serve as a reference method to determine very quickly the fact that the rennet as a result of the storage period no longer meets the same quality parameters as it had at the beginning of its use.

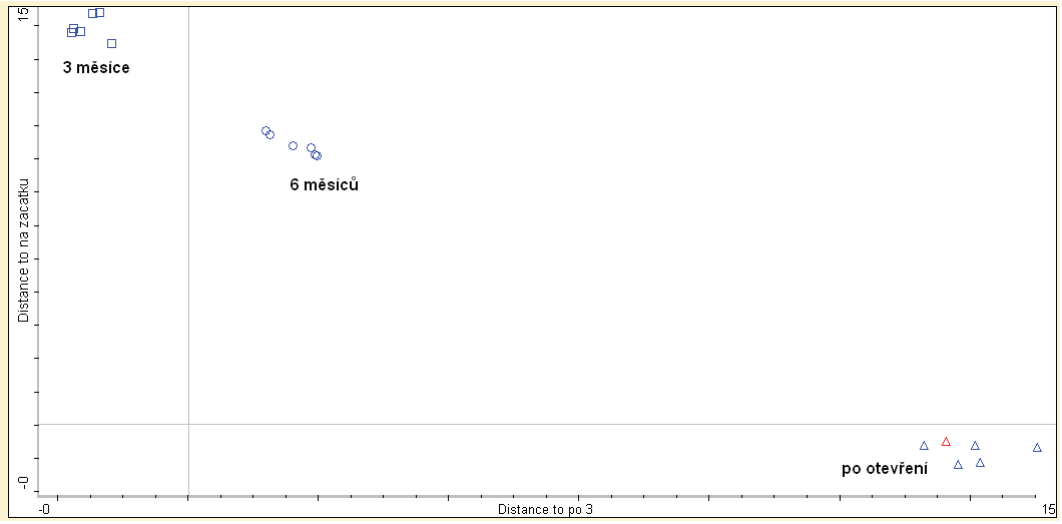
CONCLUSION

The results of the laboratory analysing show that the change in rennet quality is most particularly associated with a decrease in the strength of rennet, which after three months decreased by 16 %, whilst declining after six months by 26 %, which is a 2-fold higher level of decreased activity than it should be in theory. Such a decrease in rennet strength is also associated with the required quantity of rennet

III: *The colour of each rennet within the CIELAB system*

Rennet	Month	Color			
		L*	a*	b*	ΔE^*_{ab}
1	0	91.28 ± 0.03 [a]	–0.61 ± 0.01 [a]	27.60 ± 0.02 [c]	-
	3	89.16 ± 0.01 [b]	–0.23 ± 0.01 [b]	32.73 ± 0.01 [a]	5.56*
	6	90.74 ± 0.01 [a]	–0.52 ± 0.01 [a]	29.00 ± 0.01 [b]	1.5
2	0	95.72 ± 0.05 [a]	–0.87 ± 0.03 [a]	12.40 ± 0.02 [b]	-
	3	93.75 ± 0.02 [b]	–0.60 ± 0.02 [b]	15.35 ± 0.04 [c]	3.56*
	6	95.22 ± 0.03 [a]	–0.74 ± 0.03 [a]	13.61 ± 0.04 [a]	1.32
3	0	91.38 ± 0.01 [a]	–1.90 ± 0.03 [a]	27.09 ± 0.07 [b]	-
	3	88.08 ± 0.05 [b]	–1.28 ± 0.02 [a]	33.22 ± 0.03 [a]	6.99*
	6	89.61 ± 0.10 [b]	–1.63 ± 0.06 [a]	28.88 ± 0.06 [b]	2.54*
4	0	98.40 ± 0.01 [a]	–0.95 ± 0.08 [a]	5.81 ± 0.04 [b]	-
	3	98.36 ± 0.02 [a]	–1.07 ± 0.05 [a]	6.34 ± 0.03 [a]	0.54
	6	98.39 ± 0.02 [a]	–0.98 ± 0.06 [a]	5.89 ± 0.04 [b]	0.09
5	0	98.14 ± 0.03 [a]	–0.32 ± 0.04 [a]	4.08 ± 0.03 [a]	-
	3	98.21 ± 0.07 [a]	–0.34 ± 0.02 [a]	4.13 ± 0.03 [a]	0.09
	6	97.95 ± 0.04 [a]	–0.31 ± 0.03 [a]	4.20 ± 0.02 [a]	0.22
6	0	55.04 ± 0.04 [a]	25.35 ± 0.05 [b]	71.88 ± 0.09 [a]	-
	3	47.97 ± 0.05 [c]	29.36 ± 0.03 [a]	71.94 ± 0.08 [a]	8.13*
	6	53.63 ± 0.05 [b]	26.49 ± 0.07 [b]	72.49 ± 0.10 [a]	1.91

All values were reported as the means and standard deviations ± SD
[a, b, c] Means with various superscripts show significant differences between rows (P<0.05)
*Total color changes (ΔE^*_{ab} > 2.0) are with superscript and show just notified difference
1 – Pepsin natural rennet with the strength of 1:10,000; 2; 3; 4 – chymosin natural rennets with the strength of: 1:5,000; 150 IMCU/ml and 145 IMCU/ml; 5 and 6 are chymosin microbial rennets with the strength of: 950 IMCU/ml and 1,000 IMCU/ml, L* – lightness, a* and b* – colour-opponent dimensions, the ΔE_{ab} value of each rennet is related to the first measurement (0)



3: *Discriminant analysis to discern the rennets: rennet after opening (Δ), 3 months old (□) and 6 months old (o)*

needed for curdling milk, which during six months increased by an average of 2 ml to reach 1,000 ml of milk. The changed appearance of rennets over time was also confirmed and can be successfully described through colour measurements using a spectrophotometer, as in other materials and products. The extent of colour difference was however not the same in each of the rennets, with some in fact remaining unchanged as regards colour, while in others a slight or rather significant turbidity was tracked, which may be indicative of contamination caused during handling. It was found that the FT NIR spectroscopy can serve as a reference method for rapidly determining quality parameters, i.e. testing the fact that the rennet no longer possess the same quality parameters during

the storing period as were those at the beginning of use.

The experiment has yielded a recommendation of the necessity of storing the rennet under conditions specified by the manufacturer on the packaging and ongoing checking quality parameters, which certainly depends on the quantitative packaging used by potential consumers as well. This experiment is a sub-part of rennet quality monitoring under various conditions and testing the rennets quality using diverse instrumental methods.

SUMMARY

Milk coagulants are essential for cheese making and one of the most important enzymes in the food industry. This work summarized the results of qualitative properties (pH, rennet clotting time, strange of rennet and dose of rennet) of rennets during the six months storing in the fridge (4 ± 2 °C), which were obtained with using laboratory and instrumental methods (UV VIS and FT NIR). For the analysis were used the six different liquids rennets. It was confirmed that there is a change in quality of rennet, particularly as regards strength, this on average reducing during the storage period twice as much than was expected. Such a decrease in rennet strength is also associated with the required quantity of rennet needed for curdling milk, which during six months increased by an average of 2 ml to reach 1,000 ml of milk. Instrumental methods can be used as quick reference methods to detect variations in the quality of rennet. The change of rennet appearance over time was confirmed, so it can be successfully described using the CIELAB system. The largest total color change ΔE*ab from standard ($P > 0.05$) was achieved in microbial rennet with strange 1000IMCU, that was also one of the most distinctive color, and chymosin natural rennet with strange 150IMCU, which developed a slight turbidity. FT NIR analysis can be used to discern various ages of rennet with high probability. The experiment has yielded a recommendation of the necessity of storing the rennet under conditions specified by the manufacturer on the packaging and ongoing checking quality parameters, which certainly depends on the quantitative packaging used by potential consumers as well.

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