Freezing Point of Heat-treated Drinking Milk in the Czech Republic

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Abstract

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In the Czech Republic, the freezing point of milk is presently used as a quality indicator of cows' raw milk as well as of heat-treated drinking milk, and its limit value is ≤ –0.520°C. Of the total of 295 drinking milk samples examined over a period of one year, 145 were samples of pasteurised milk and 150 were samples of UHT milk. In compliance with the Czech State Standard 57 05 38, the freezing point was determined by a thermistor cryoscope. The measured mean value of the freezing point of the heat-treated drinking milk was –0.515°C ± 0.0078. A total of 207 (70.2%) samples of the heat-treated drinking milk, i.e. 93 (64.1%) samples of pasteurised and 114 (76%) samples of UHT milk, were found above the maximum limit value. The unsatisfactory results of the monitoring of the freezing point of drinking milk emphasise the need for a reassessment of the current system of the milk quality evaluation with respect to this quality index.

Keywords: cow milk; pasteurised milk; UHT milk; physicochemical properties; milk quality

The quality assurance and product safety are among the priorities of advanced market economies and the producers' obligations. For that reason, the quality of raw materials and food products of animal origin must meet the requirements set forth by legal regulations. The freezing point of milk is an important indicator of the milk quality. The freezing point of milk is determined primarily to prove milk adulteration with water and/or to determine the amount of water added (Bhandari & Singh 2003). Freezing point variations have until recently been exclusively attributed to the additions of extraneous water to milk. However, studies in recent years have shown that undesirable dilution of milk with water is by far not the only reason for a rise of the freezing point.

Regular checks of raw cows' milk freezing point, which is one of the quality indices of purchased milk, have been performed in the Czech Republic in central laboratories since 1993, when the maximum limit for the freezing point of cows' raw milk of –0.510°C was set forth in the Czech State Standard (CSS) (ČSN 57 0529 1993, 1998). The CSS was amended in 1995 when the freezing point discrimination limit for cows' raw milk was set at –0.515°C. In 1998, new conditions for the assessment of the freezing point of cows' raw milk were laid down. If subsequent milk sampling

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performed during officially supervised milking proved that the rise of the freezing point had been caused by physiological reasons rather than by technological negligence, no financial penalties for the milk adulteration were imposed. The same limit value for the freezing point (–0.515°C) was also laid down in subsequent legislative regulations from 1999–2003. Decree 203/2003 Sb. newly stipulated the determination of the freezing point of heat-treated drinking milk, and set the limit value at ≤ –0.515°C. In 2004, the maximum limits of the freezing point of both raw and heat-treated drinking milk were changed to ≤ –0.520°C in compliance with EU regulations (Decrees 638/2004 Sb. and 639/2004 Sb.). A freezing point above that value is admissible only under the condition that heat-treated drinking milk is regularly checked for water additions. The freezing point remains a quality index of raw cows' milk in the Czech Republic.

The freezing and the boiling points (–0.522°C and 100.15°C, respectively) and the osmotic pressure (700 kPa at 20°C) are important colligative properties of milk. Of the major milk constituents, lactose and also chlorides jointly make up 75 to 80% of the freezing point value and play the most important role in the so-called freezing point depression of milk. Lactose contributes about 0.296°C to the milk freezing point depression, and chlorides, together with Na^+ and K^+ cations, about 0.119°C. The remaining 20–25% of the freezing point value are affected by other milk constituents, i.e. calcium, magnesium, lactates, phosphates, citrates, urea, etc. (Fox & McSweeney 1998). Fatty globules, casein micelles, and whey proteins have a negligible effect on the milk freezing point depression (Bhandari & Singh 2003). In his study, Kessler (1984) noted that milk fat has no effect on the freezing point of milk and that milk proteins affect it only very little: 2.66% of casein may decrease the freezing point by 0.000001°C, and 0.67% of whey proteins by 0.000407°C.

In the past, it was believed that milk dilution with water was the principal reason for the rise of the freezing point. It has, however, been demonstrated that 1% of added water will cause an elevation of the freezing point of milk by 0.006°C (Singhal *et al.* 1997). The following internationally accepted interpretations of the freezing point depression have been adopted: where the freezing point is lower than –0.535°C, milk is assumed to be free from added water; the freezing point values between –0.530 and –0.534°C indicate that milk production checks are needed; the freezing point values from –0.525 to –0.529°C indicate a strong probability of the presence of extraneous water; and where the freezing point is –0.525°C or higher, the farmer is asked to prove that no water has been added to the milk (Singhal *et al.* 1997). Milk with the freezing point of ≤ –0.525°C is considered as free from added water (Fox & McSweeney 1998).

Dilution of milk with water may either be intentional, or it may be caused by technological imperfections at the milk primary production level. The principal causes of milk dilutions with water include defects in the construction of milking machines or in sanitation. In such cases, residual and condensing water may find its way to milk (Zee *et al.* 1982; Buchberger 1996). The freezing point of milk may also be affected by the sampling method on the farm. Harding (1995) and Coveney (1993) defined the method of sampling (authentic sampling) that minimises the risk of water being added to milk.

The freezing point of milk is affected not only by the presence of extraneous water but mainly also by the milk constituents that are present in genuine milk. Their concentrations may be influenced by a number of factors, including dairy cow breed, stage of lactation, subclinical mastitis, dairy cows' nutrition, water intake, climatic conditions (heat stress), regional and seasonal influences, CO_2 in milk (Shipe 1959; Demott & Burch 1966; Demott *et al.* 1967, 1968; Eley *et al.* 1978; Bartsch & Wickers 1979; Rohm *et al.* 1991; Wiedemann *et al.* 1993; Šustová *et al.* 2000; Slaghuis 2001 and others). In the Czech Republic, insufficient (protein, energy and mineral) nutrition of dairy cows is considered an important factor affecting the freezing point of raw milk (Hanuš & Jílek 1998).

The freezing point of bovine milk is usually in the range between –0.512°C and –0.550°C, and only very rarely falls outside that range. The average freezing point of raw cows' milk is close to –0.522°C or –0.540°C (Fox & McSweeney 1998). The average freezing point values of samples of bulk tank cows' milk are different in different countries. In bulk tank milk studies, the freezing point of –0.528°C was found in Poland (Kuczaj 2001) and Italy (Coni *et al.* (1997), –0.5209°C in the Netherlands (Slaghuis 2001), –0.523°C in the Czech Republic (Kadlec *et al.* 2004), –0.526°C in Switzerland (Bosset *et al.* 1983) and –0.53856°C in the UK (Coveney 1993). Some authors have pointed out that the freezing point of raw cows' milk has been rising (Foissy *et al.* 1990; Schukken *et al.* 1992; Slaghuis 2001). This fact might be explained by changes in the breeding of dairy cows (genetic factors), their nutrition and management, milking technologies, and the increase in the yield of dairy cows in recent years.

The freezing point of heat-treated drinking milk will depend on the freezing point of the raw milk and the changes of the freezing point during the milk treatment and processing in dairies. The following factors may play a role in the drinking milk production technologies: watering of milk with, e.g., residual water, loss of salts through, e.g. the formation of milk stone, variation of milk acidity caused by, e.g., production of lactic acid during lactose fermentation and gassing (and de-gassing) of milk by portions of dissolved carbon dioxide, oxygen or nitrogen (Kessler 1984). The cooling or heating of milk causes aggregation of soluble salts and their transfer to casein micelles or fatty globules. This reaction is, however, reversible and the freezing point may therefore vary depending on the time lapse between the processing and testing of samples (Sherbon 1999). Rohm *et al.* (1991) ascribed the elevation of the freezing point to changes in the calcium phosphate complex and in the carbon dioxide pressure. The shift was about 0.002°C depending on the temperature and the length of the heating period. Literature data on the effects of the heat treatment on the freezing point of milk vary. Kessler (1984) found that the freezing point of milk after pasteurisation at 74°C for 30 sec was unchanged, but pasteurisation at 85°C for 2.8 s increased the freezing point by 0.002°C, and prolonged pasteurisation at 95°C for 303 s increased the freezing point by 0.001°C. The effect the UHT treatment has depended on the technology used (direct or indirect UHT heating). The direct UHT heating caused a rise of the freezing point by 0.009°C, while the indirect heating by 0.003°C compared with raw milk. Other authors (Singhal *et al.* 1997) reported even greater elevations of the milk freezing point after pasteurisation and the UHT treatment (0.006–0.009°C and 0.023°C, respectively).

The freezing point has been newly introduced in the Czech Republic as an index of quality of heat-treated drinking milk. In European countries, a discussion on the limit set forth for the freezing point of heat-treated milk is underway. A number of studies have demonstrated that 29% to 80% of samples of heat-treated drinking milk fail to meet the freezing point limit of ≤ –0.520°C set forth by the EU legislation (Coni *et al.* 1997).

The objectives of the study were to determine the freezing point of samples of heat treated drinking milk from retail shops in the Czech Republic, and compare the stipulated freezing point limits with the requirements of the effective legislation.

MATERIAL AND METHODS

Material

The samples of drinking heat-treated milk were purchased in retail shops (randomly from different producers) 1× monthly over a period of one year. Pasteurised milk (PM) – 145 samples of high-temperature pasteurised homogenised milk, i.e. heated to at least 85°C, with negative peroxidase and phosphatase tests (Decree 124/2004 Sb.), ultra heat treated homogenised milk (UHT, milk for long keeping) – 150 samples of milk whose uninterrupted flow was briefly heated to a high temperature corresponding to the effects of heating it to 135°C for at least 1 sec, and the milk was then aseptically packaged in opaque packaging in order to minimise any chemical, physical, and sensorial changes (Decree 124/2004 Sb.).

The samples were transported to laboratories in cool boxes at temperatures not exceeding 6°C. Before the tests, the samples were stored according to the producers' specifications (UHT milk and pasteurised milk at temperatures not exceeding 24°C and 6°C, respectively). In compliance with CSS (ČSN 57 0538 1998).

Methods

Freezing point determination. In the milk samples, the freezing point determination was performed in compliance with CSS ČSN 57 0538 with a thermistor cryoscope (Milk Cryoscope Model 4D2, Advanced Instruments, Inc., Massachusetts, USA). The thermistor cryoscope was regularly calibrated with standard solutions with the freezing points of –0.408°C and –0.600°C, and the calibration was verified with the reference solution Lactrol (–0.512°C). Freezing points are expressed in degrees centigrade. The uncertainty of the freezing point measurements was set at ± 0.61%. The measurement uncertainty is a combined uncertainty at the probability level of U = 95%, for the expansion coefficient $k = 2$.

Statistical processing of results. The results of examinations were statistically analysed. The results were processed by STAT Plus statistical software (Matoušková *et al*. 1992), with reference to the existing limit value of ≤ –0.520°C for the freezing point of drinking milks. Student *t*-test was used to compare the mean freezing points of UHT and pasteurised milk samples, and to determine the differences between them. In these sets, the frequency of samples whose freezing points were outside the limits was tested using the Chi-square test for differences between frequencies. One sample *t*-test was used to compare the arithmetic means of the freezing points of the sample sets (heat-treated milk, UHT and pasteurised milk; the samples of heat-treated milk in individual months) with a determined freezing point limit (Zar 1999).

RESULTS AND DISCUSSION

Of the total of 295 samples of heat-treated drinking milk examined over a period of one year, 145 of them were those of pasteurised milk and 150 those of UHT milk. The average freezing point, standard deviation, and the minimum and maximum values of the freezing point of pasteurised and UHT drinking milks are shown in Table 1. The drinking milk freezing point averaged at –0.515°C, i.e. 0.005°C above the freezing point laid down as the maximum limit for drinking milk (≤ –0.520°C). The average freezing points of pasteurised and UHT milks were also above the maximum freezing point limit. It follows from Table 1 that no statistical agreement between the freezing point limit values and 99% reliability intervals was found in UHT and pasteurised milk samples. The statistical evaluation of the arithmetic means of the freezing points of the heat treated, UHT, and pasteurised milk samples with relation to the limit value (one sample *t*-test) showed that the differences between the means and the value of –0.520°C was statistically highly significant ($P < 0.01$). The arithmetic mean of the freezing point of drinking milk samples higher than the maximum limit (–0.5169°C) was also found in Austria (Rohm *et al*. 1991). Coni *et al*. (1997) on the other hand, reported a lower average freezing point of UHT milk (–0.522°C) than the maximum limit from Italy.

In our study, a large number of samples failed the freezing point standards laid down for drinking heat-treated milk (Table 2). A total of 207 (70.2%) samples, of which 93 (64.1%) were samples of pasteurised and 114 (76%) of UHT milk, failed the test. Studies in other European countries confirmed that a high percentage of samples fail the EU maximum limits set forth for heat-treated drinking milks. In Italy, 29% of UHT samples failed (Coni *et al*. 1997). Coni *et al*. (1997) also compared the number of unsatisfactory samples in Italy with the situation in the Netherlands (where 61% of the heat-treated milk samples failed) and in France (80% samples of UHT milk failed).

Table 1. Statistical evaluation of the freezing point of heat-treated drinking, pasteurised and UHT milks

Freezing point (°C)	Heat-treated drinking milk*	UHT milk	Pasteurised milk
n	295	150	145
Min	–0.531	–0.528	–0.531
Max	–0.463	–0.463	–0.490
Mean	–0.515[a]	–0.514[a,b]	–0.516[a,b]
SD	0.0078	0.0082	0.0066
CV(%)	1.51	1.59	1.27
CI (99%)	–0.516 to –0.514	–0.516 to –0.512	–0.518 to –0.515

n = number of samples, min = minimum value, max = maximum value, SD = standard deviation of the mean, *CV*(%) = coefficient of variation of the mean, CI = confidence interval

*heat treated drinking milk = pasteurised and UHT milks

[a]the difference between the arithmetic mean of the set and the limit value of the freezing point of –0.520°C in the one sample *t*-test was statistically highly significant ($P = 0.01$)

[b]the differences between arithmetic means for UHT and pasteurised milks as calculated by the Student *t*-test are statistically highly significant ($P = 0.01$)

Table 2. Numbers of tested samples of heat treated drinking, pasteurised and UHT milks, and their evaluation with respect to the maximum limit value

	Heat-treated drinking milk	UHT milk	Pasteurised milk
Number of samples (n)	295	150	145
Number of nonconforming samples (n_1)	207	114*	93*
% nonconforming samples (% n_1)	70.2	76	64.1

*the number of nonconforming samples was higher among UHT milks, and the difference was statistically highly significant ($P < 0.05$, χ^2 test)

It is not easy to determine the reason for the elevation of the freezing point in drinking heat-treated milks. The freezing point value depends primarily on the quality of raw milk. If some negative factors come into play at the primary production level and the milk freezing point is close to –0.520°C, the maximum limit of the freezing point is likely to be exceeded during the dairy treatment and milk processing. The average freezing point value of bulk tank samples of raw cows' milk reported in quality assessments of milk supplied in the Czech Republic in 2004 was –0.524°C (Kopáček 2005). The average freezing point of the heat-treated drinking milk in our study (Table 1) is by 0.009°C higher than that of raw milk. The difference is lower in pasteurised milks (0.008°C) than in UHT milks (0.01°C). The Student t-test showed a statistically highly significant difference between the mean freezing points of UHT milk samples and pasteurised milk samples ($P = 0.01$). The non-compliancy frequency among UHT milk samples was higher than among pasteurised milk samples, and a frequency comparison (χ^2 test) showed that the difference was statistically highly significant ($P = 0.026$). The fact that the number of unsatisfactory samples of UHT milk is higher than that of pasteurised milk (Table 2) corroborates the assumption that the changes in the freezing point taking place during UHT treatment of milk are greater (Singhal *et al.* 1997; Drbohlav *et al.* 2004). Investigating the effects of the technological processes on the milk characteristics in the Czech Republic, Drbohlav *et al.* (2004) collected samples from the production processes of pasteurised and UHT milks. They concluded that the freezing point rises with the growing intensity of the heat treatment. The freezing point rise in pasteurised and UHT milks was 0.0053°C and 0.008°C, respectively. The freezing point rose without any significant change in the composition of milk which might suggest a higher water content. Of the milk constituents, the best indicator in this respect is solids-not-fat. In Italy, the average freezing point of UHT milk of –0.522°C was by 0.006°C higher than that of raw milk (Coni *et al.* 1997).

Paľo *et al.* (1992) reported that the rise of the freezing point attributable to the heat treatment is insignificant compared to dilution with water due to technological reasons. Another factor contributing to a rise of the milk freezing point during the dairy treatment and processing is the milk dilution with residual water and water condensate from the production machinery surfaces. Each square meter of the equipment surfaces that milk comes into contact with during processing contributes 40 ml water (Rohm *et al.* 1991; Paľo *et al.* 1992). Milk adulteration levels range from 0.3% (Paľo *et al.* 1992) to 0.5% (Rohm *et al.* 1991). The greatest milk watering occurs in milk pasteuriser and filling machine at their entering end (Paľo *et al.* 1992).

Seasonal variability of the freezing point of raw cows' milk also affects the freezing point of heat-treated drinking milk (Drbohlav *et al.* 2004). The highest values of the freezing point are found in the summer months (May and June, sometimes July and August) and in the early autumn (September, October). The high freezing points in spring months are probably linked with the start of the grazing period for dairy cows and the use of green fodder. As concerns the summer months, the reason probably lies in higher milk yields and the ensuing decrease in the milk component and fat-free dry matter contents (Šustová *et al.* 2000). Variability may be induced by an exposure of dairy cows to heat stress, stage of lactation and, most importantly, dairy cows' nutrition (Buchberger 1996; Hanuš & Jílek 1998; Hanuš *et al.* 2003). Seasonal influences can be observed even now, although total mixed rations (TMR) based on year-round stored forages are used on almost all

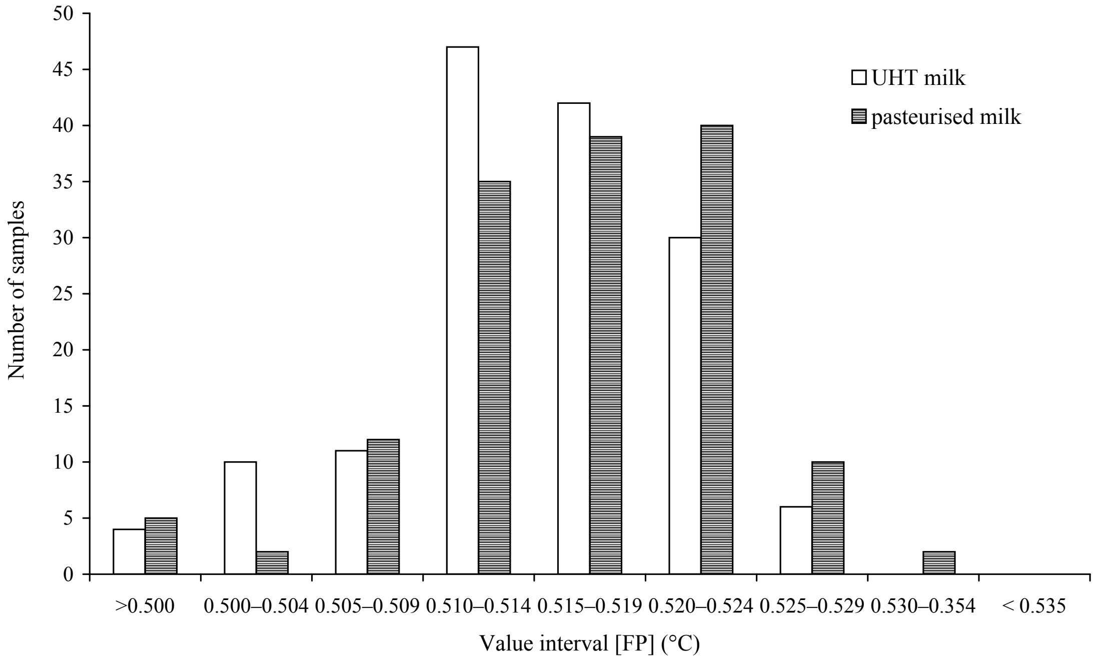

Figure 1. Frequency of freezing point values of pasteurised and UHT milks at value interval

farms in the Czech Republic. In 2003, the freezing points of raw cows' milk were the lowest from October to November, and the highest from May to June (Kadlec *et al.* 2004). Also Šustová *et al.* (2000) recorded the highest average freezing point values in May and the lowest ones in November and December. The data partly corresponding to these results are also shown in Figure 2. In May, the highest average freezing point was found, and the number of unsatisfactory samples was also the highest. The lowest freezing point value was found in June, which, however, may have been caused by the small number of the samples tested. Using the one sample t-test, a statistically highly significant difference ($P < 0.01$) in the arithmetic means of freezing points between the heat-treated milk samples and the freezing point limit value was found in January, February, March, April, May, November

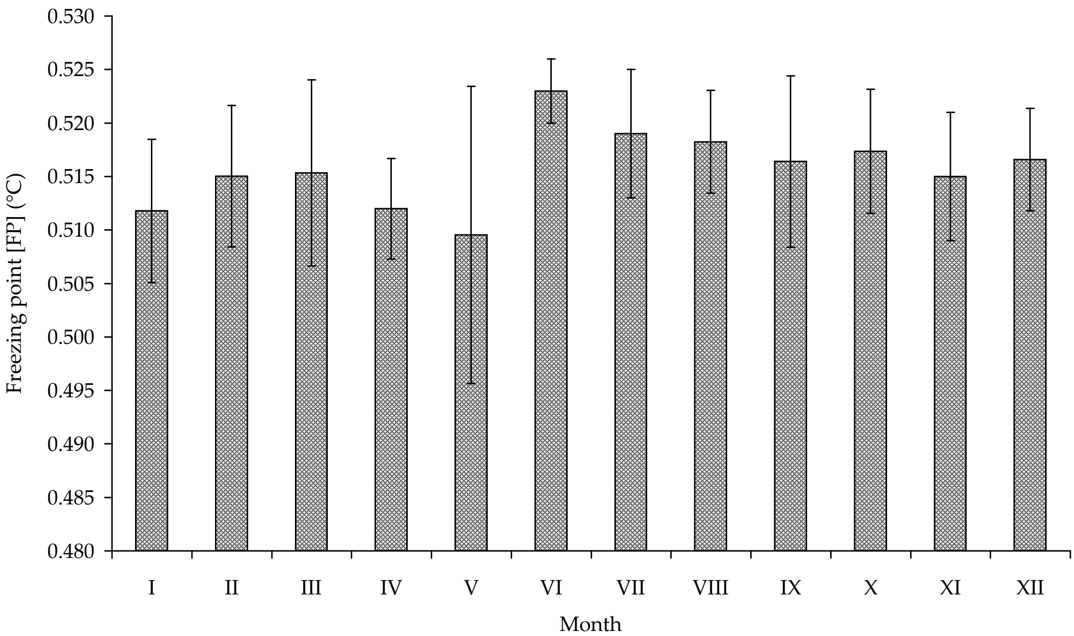

Figure 2. Variability of the freezing point of heat-treated drinking milk throughout the year

and December; and in September and October, the same difference was statistically significant ($P < 0.05$). No statistically significant differences between the mean freezing point values and the limit values (Figure 2) were found only in three months of the year (June, July and August).

The freezing points of bulk tank samples of raw cows' milk in the Czech Republic (Kadlec *et al.* 2004) have been for a number of years characterised by the fact that most of the values ascertained (83.1% in 2003) were in the –0.515 to –0.529°C range. In 2003, only 2.6% of samples were < –0.535°C, and 1.1% of samples were > –0.505°C. It follows from Figure 1 that the freezing points of the heat-treated drinking milk samples rise during technological processes and their values shift closer to 0°C (by 0.005°C on average). The freezing points of most of the samples are in the –0.510 to –0.524°C range, and the freezing point of none of the samples was < –0.535°C (Figure 1).

The issues relating to the freezing point of raw milk have been extensively dealt with by a large number of authors. The issues relating to the freezing points of drinking milks, on the other hand, have remained very topical because the literary data on them are rather scanty and the issues have also been largely neglected in real practice.

CONCLUSION

Decree 638/2004 Sb., which came into effect on January 1, 2005, changed the maximum limit of the freezing point of heat-treated milk to –0.520°C. The existence of an identical maximum freezing point limit for both raw and heat-treated drinking milks in the Czech Republic contributes to the perpetuation of an inadequate system for the determination of the freezing point of heat-treated drinking milk. Unsatisfactory results of the monitoring of the freezing point of heat-treated drinking milk (70.2% of unsatisfactory samples) point in this respect to the need for a reassessment of the current system of the milk quality evaluation.

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