

Influence of high-pressure treatment on ribonucleoside contents and enzyme activities in milk

Einfluss einer Hochdruckbehandlung auf Ribonucleosid-Gehalte und Enzym-Aktivitäten in Milch

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Summary

Ribonucleosides are naturally occurring bioactive substances. In milk, they belong to the group of minor constituents and show a species-specific composition pattern. As technological treatments influence the ribonucleoside content, they are suitable chemical parameters to characterize the effect, e.g. of heat treatment, on milk and milk products. The aim of the present study was to examine the influence of a high-pressure treatment on the ribonucleoside contents in milk, and on the activity of the milk enzymes alkaline phosphatase and adenosine deaminase in different milk samples. In raw milk samples, a high-pressure dependent release of ribonucleosides was determined, whereas in pre-heated milk samples the content changes were considerably smaller. According to the current knowledge, this release is enzyme-controlled. The studies suggest that the high-pressure treatment induces a correlation between adenosine deaminase activity and the enzyme-induced release of inosine. For adenosine deaminase activity, high pressure seems to be protective at a processing temperature of 80 °C.

Keywords:

ribonucleosides, high pressure, milk, adenosine deaminase, alkaline phosphatase

Zusammenfassung

Ribonucleoside sind natürlich vorkommende bioaktive Substanzen. In Milch gehören sie zur Gruppe der minderen Inhaltsstoffe und zeigen ein speziesspezifisches Gehaltsmuster. Da eine technologische Behandlung den Ribonucleosid-Gehalt beeinflusst, sind Ribonucleoside geeignete chemische Parameter zur Beschreibung der technologischen Behandlung von Milch und Milchprodukten, z. B. der Milchwärmebehandlung. Das Ziel der vorliegenden Untersuchung war die Überprüfung des Einflusses einer Hochdruckbehandlung auf Milch-Ribonucleosid-Gehalte und auf die Aktivitäten der Milchenzyme alkalische Phosphatase und Adenosindesaminase in unterschiedlichen Milchproben. In Rohmilchproben wurden hochdruckabhängige Ribonucleosid-Freisetzungen bestimmt, wohingegen in thermisch vorbehandelten Milchproben die Gehaltsveränderungen wesentlich geringer waren. Nach derzeitigem Kenntnisstand sind diese Freisetzungen enzymgesteuert. Die Hochdruckbehandlung bewirkt einen Zusammenhang zwischen der Adenosindesaminase-Aktivität und den Gehalten des enzymatischen Produkts Inosin. Für Adenosindesaminase scheint Hochdruck bei 80 °C Prozesstemperatur protektiv zu sein.

Kennwörter:

Ribonucleoside, Hochdruck, Milch, Adenosindesaminase, alkalische Phosphatase

Introduction

Ribonucleosides belong to the group of naturally occurring bioactive compounds. They are secreted as products of cellular RNA and ribonucleotide metabolism into physiological fluids such as blood, milk, and urine. Considered under structural aspects, naturally occurring ribonucleosides are N-glycosides of pyrimidines and purines [1]. Ribonucleotides are orthophosphoric acid esters of ribonucleosides.

Different bioactive effects of dietary ribonucleosides have been described, including the ability to enhance gut growth and maturation [2] and to influence the ac-

tivity of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) in the small intestine of rats [2–5]. Ribonucleosides have an effect on human cells, therefore cytochemical investigations showed that several ribonucleosides can induce apoptosis in human cells, and therefore may be potentially anticarcinogenic compounds [6, 7]. In addition, chemically modified ribonucleosides are applied as pharmaceutically active compounds in the treatment of different illnesses, and naturally modified ribonucleosides serve as valuable pathobiochemical marker molecules for cancer [for review: 7].

Dietary ribonucleosides are ingested mainly as nu-

cleoproteins and are converted enzymatically in the course of intestinal digestion to monomeric compounds [8, 9]. It has been shown that ribonucleotides and related compounds are semi-essential [10]. Due to the biochemical and trophochemical properties of dietary ribonucleotides and ribonucleosides [11–13], the supplementation of infant formulae and follow-on formulae with ribonucleotides has been allowed by a regulation of the European Commission [14]. Some reviews report on the effects of ribonucleotides and ribonucleosides for nutritional applications, in particular in infant nutrition [15–20].

Ribonucleosides are minor constituents of the non-protein nitrogen (NPN) fraction of milk and they show a species-specific composition pattern [16]. Some studies report on the influence of technological treatment on the contents of ribonucleosides, and on the activity of the milk enzyme adenosine deaminase (EC 3.5.4.4), e.g. during milk heat treatment. Furthermore, the determination of ribonucleosides is suitable in the differentiation of butter types. In addition, the milk enzyme adenosine deaminase is an appropriate heat indicator in milk. Therefore, characterizing ribonucleoside pattern has potential application to act as an indicator for the description of technological treatments of milk and milk products [for review: 21].

High-pressure treatment is an interesting technological process for preservation of food products. Numerous studies report on the effect of high-pressure treatment on milk and milk products and its influence on constituents and properties, e.g. about the effects of high-pressure treatment on indigenous milk enzyme activity, mineral balance in milk, and about milk fat globules as well as particle size distribution of casein micelles in milk [for review: 22]. Main focus of many studies is the investigation of interactions and influence of high-pressure treatment on milk proteins [for review: 23], e.g. about pressure induced changes in the rennet coagulation properties [24] and cheese-making properties of heated milk [25]. Recent studies report of heat impact on pressure induced denaturation of β -lactoglobulin [26]

and on the antigenic response of whey proteins influenced by high pressure and heat treatment [27]. In the first study on the high-pressure induced changes of ribonucleoside contents [28], a release of unmodified ribonucleosides was observed in high-pressure treated raw milk samples. These changes in ribonucleoside contents were also registered in case of addition of ribonucleoside monophosphates. In the present study, the influence of high-pressure treatment at different experimental conditions on the ribonucleoside contents and activities of ALP and ADA in drinking milk samples, different skimmed milk samples and raw milk samples are represented and discussed.

Material and methods

1. Configuration of the high-pressure device

The high-pressure treatment of the milk samples was performed in a high-pressure device (Fig. 1), where a pressure of approx. 5 bars of compressed air is transmitted on hydraulic oil (hydraulic oil EP Econa 46, DEA, Hamburg, Germany) in the high-pressure pump (Bolenz & Schäfer, Biedenkopf/Lahn, Germany) according to the principle "big plunger – small plunger" in the ratio 1024:1. The respective temperature of the hydraulic oil is obtained with a thermostat (Julabo FP40, Julabo Labortechnik, Seelbach, Germany).

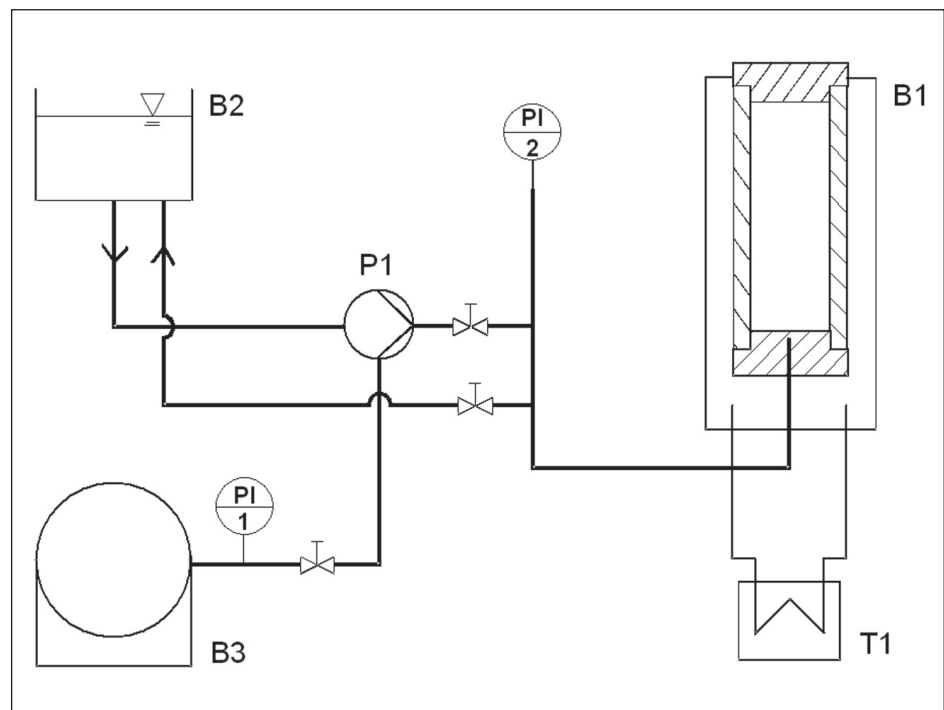


Fig. 1: Flow diagram of the high-pressure device;
B1: high-pressure vessel (volume: 20 mL); B2: oil reservoir; B3: air reservoir;
P1: high-pressure pump; T1: heating/cooling unit; PI: pressure indicator.

Abb. 1: Fließschema des Hochdruckversuchsstands;
B1: Hochdruckbehälter (20 mL Volumen); B2: Ölbehälter; B3: Pressluftflasche;
P1: Hochdruckpumpe; T1: Thermostat; PI: Druckmesser.

2. Preparation of high-pressure treated milk samples

The milk samples were filled into polyethylen bags (Eydam, Kiel, Germany; volume: two times 4 mL per high-pressure treatment) and heat-sealed. Afterwards they were inserted in the isolated high-pressure vessel (volume: 20 mL; Bolenz & Schäfer, Biedenkopf/Lahn, Germany) and treated at 500 MPa high pressure over 20 min pressure holding time at the specified temperature. Parallely, milk samples were heated at normal pressure at the respective temperature in a thermostat (Julabo FP40, Julabo Labortechnik, Seelbach, Germany).

3. Determination of ribonucleoside contents

High-pressure treated and normal-pressure treated samples were adjusted to pH 3.50 using concentrated formic acid, centrifuged (13.000 x g, 30 min, 18 °C) and the resulting sera were prepared for liquid chromatographic analyses by applying membrane filtration (0.2 µm pore size). These acid preserved sera (injection solutions) were stored at -24 °C until analysis.

The ribonucleosides in the sera were quantitatively determined using an automated dual-column HPLC analyzer. The function of this analyzer was already described in detail in former publications [29–31]. The main principles of the method are: The apparatus consists of a modular HPLC system (Merck-Hitachi, Darmstadt, Germany) combining chemoselective affinity and size exclusion properties in the pre-column (40 x 3 mm I.D.), filled with bonded phase material – modified phenylboronic acid substituted vinyl polymer – according to [32] with a reversed-phase analytical column (250 x 4,6 mm I.D., Supelcosil LC18-S, Supelco, Taufkirchen, Germany) connected with an automated six-port switching valve. A Diode Array Detector (Merck-Hitachi, Darmstadt, Germany) was

used for the detection of the ribonucleosides (measuring wavelength: 260 nm). The quantification of the ribonucleosides was carried out with an external standard, in which the following ribonucleosides were contained: adenosine (Ado), cytidine (Cyd), guanosine (Guo), inosine (Ino), uridine (Urd), 1-methyladenosine (m1Ado), N6-methyladenosine (m6Ado) and N6-dimethyladenosine (m6,2Ado) purchased from Sigma Chemie, Taufkirchen, Germany. Hypermodified ribonucleoside N6-carbamoyl-L-threonyl-adenosine (t6Ado) was synthesized according to [33]. The analysis of HPLC chromatograms was performed with HSM-Chromatography Data Station Software version 4.1 (Merck-Hitachi, Darmstadt, Germany).

4. Determination of enzyme activity

The determination of alkaline phosphatase (ALP) activity was performed with the Fluorophos™ method according to DIN EN ISO 11816-1 (2006). The limit of detection of this method is 10 mU/L. The determination of adenosine deaminase (ADA) activity was carried out by specific addition of the substrate adenosine (Sigma, Taufkirchen, Germany) to the milk sample, which was heated at a defined temperature of 37 °C for 10 min. The enzymatic product inosine was quantified by use of the dual-column HPLC [30, 34]. The limit of detection of the described direct method is 13 mU/L.

Results and discussion

1. High-pressure treatment of raw milk and preheated raw milk

In exploratory investigations, the ribonucleoside contents as well as the ALP activity were examined in non-heated high-pressure treated raw milk as well as in raw milk samples that were preheated for 5 min

ribonucleoside	Raw milk*				Preheated raw milk (80 °C/5 min)*			
	normal pressure/ 50 °C/20 min		500 MPa/ 50 °C/20 min		normal pressure/ 50 °C/20 min		500 MPa/ 50 °C/20 min	
	[µmol/L]	± s. d.	[µmol/L]	± s. d.	[µmol/L]	± s. d.	[µmol/L]	± s. d.
Cyd	3.77	0.073	12.82	0.003	2.76	0.016	2.58	0.002
Urd	11.50	0.091	15.12	0.007	13.79	0.006	13.18	0.011
m1Ado	0.25	0.010	0.24	0.006	0.19	0.000	0.17	0.015
Ino	0.32	0.013	0.20	0.010	1.40	0.019	1.30	0.017
Guo	0.24	0.008	0.24	0.008	0.35	0.001	0.29	0.011
Ado	0.84	0.006	1.23	0.004	0.28	0.002	0.34	0.001
m6Ado	n. d.**	-	n. d.	-	n. d.	-	n. d.	-
t6Ado	0.38	0.037	0.36	0.004	0.31	0.001	0.36	0.037
m6,2Ado	n. d.	-	n. d.	-	n. d.	-	n. d.	-

* fat content: 3.92%; protein content: 3.45%

** n. d.: not detectable

Tab. 1: Ribonucleoside contents [µmol/L] in normal-pressure treated and in high-pressure treated raw milk and preheated (80 °C/5 min) raw milk.

Tab. 1: Ribonucleosid-Gehalte [µmol/L] in normaldruck- und in hochdruckbehandelter Rohmilch sowie in vorerhitzter (80 °C/5 min) Rohmilch.

at 80 °C before high-pressure treatment (Tab. 1). The data of the pressured samples were compared with the data of samples tempered at 50 °C/20 min under normal pressure (Tab. 1), in order to obtain an approximate description of the influence of the high pressure under deduction of the influence of the respective temperature. The results reveal that increases in contents of cytidine, uridine and adenosine were observed in the pressured raw milk sample, i.e. these ribonucleosides were released during pressure processing. It was striking that the inosine contents showed an opposite trend.

By contrast, in preheated raw milk (80 °C/5 min), only minor high-pressure caused changes of ribonucleoside contents were determined (Tab. 1). In pressured raw milk, the ALP activity was 459 ± 6 U/L, in raw milk treated at normal pressure it amounted to 463 ± 43 U/L. In preheated raw milk, a small activity increase was generated by high-pressure processing (pressured sample: 83 ± 0 mU/L; normal pressure sample: 27 ± 6 mU/L).

The observed release and changes of ribonucleoside contents in high-pressure treated raw milk samples compared to the minor changes in preheated samples confirm the results of a former study [28]. In addition, these investigations displayed that the high-pressure treatment of aqueous ribonucleoside monophosphate solutions did not yield a noticeable release of ribonucleosides, i.e. under the experimental conditions applied there was no pressure-dependent dephosphorylation of ribonucleotides detectable [28].

2. High-pressure treatment of HTST and UHT drinking milk samples

In further investigations, high-temperature short-time (HTST) and ultra-high-temperature (UHT) heated drinking milk samples were high-pressure treated at 500 MPa over 20 min pressure holding time. In addition to the ribonucleoside contents, activities of ALP and ADA were detected.

In the HTST-heated milk sample no striking changes in the ribonucleoside contents were determined by direct comparison of a tempered sample under normal pressure and a tempered sample under high pressure (Tab. 2). A minimal activity reduction of 37 ± 2 mU/L to 30 ± 0 mU/L caused by high-pressure was observed for ALP. The ADA activity was 811 ± 41 mU/L in milk tempered at normal pressure at 50 °C and 905 ± 3 mU/L in the high-pressure treated sample, that means, high-pressure treatment caused a slight increase in the ADA activity.

Also in UHT drinking milk no striking changes in the ribonucleoside content were found by direct comparison of normal pressure and high-pressure treatment (Tab. 2). The results of the ALP determination revealed 321 ± 16 mU/L in the normal-pressure treated sample, while 305 ± 10 mU/L were found in high-pressure treated milk sample. While UHT milk is ALP-negative shortly after heating, the small enzyme activities detected are ascribed to the reactivation of ALP during storage of the UHT milk [35].

As expected, ADA activities could not be determined in UHT milk. Once heat-denatured, ADA was not reactivated by the high-pressure treatment applied. In ad-

ribonucleoside	High-temperature short-time heated drinking milk*				Ultra-high-temperature drinking milk*			
	normal pressure/ 50 °C/20 min		500 MPa/ 50 °C/20 min		normal pressure/ 50 °C/20 min		500 MPa/ 50 °C/20 min	
	[µmol/L]	± s. d.	[µmol/L]	± s. d.	[µmol/L]	± s. d.	[µmol/L]	± s. d.
Cyd	7.54	0.087	7.71	0.037	15.26	0.017	15.48	0.365
Urd	23.16	0.282	21.62	0.090	22.87	0.023	23.06	0.408
m1Ado	0.22	0.002	0.25	0.002	0.43	0.010	0.41	0.004
Ino	1.57	0.003	1.63	0.053	n. d.**	-	n. d.	-
Guo	1.13	0.004	1.14	0.021	0.28	0.002	0.29	0.004
Ado	0.06	0.003	0.11	0.008	0.90	0.003	0.90	0.019
m6Ado	n. d.	-	n. d.	-	0.05	0.007	0.05	0.001
t6Ado	0.41	0.003	0.38	0.006	0.53	0.038	0.67	0.079
m6,2Ado	n. d.	-	n. d.	-	n. d.	-	n. d.	-
ALP [mU/L]	37	2	30	0	321	16	305	10
ADA [mU/L]	811	41	905	3	< LOD***	-	< LOD	-

* high-temperature short-time heated (HTST) drinking milk and ultra-high-temperature (UHT) drinking milk: fat content: 3.5%

** n. d.: not detectable

*** LOD (limit of detection) of the direct ADA determination in milk: 13 mU/L

Tab. 2: Ribonucleoside contents [µmol/L] and activities of ALP and ADA in normal-pressure treated and in high-pressure treated drinking milk samples.

Tab. 2: Ribonucleosid-Gehalte [µmol/L] und Aktivitäten von ALP und ADA in normaldruck- und in hochdruck-behandelten Trinkmilch-Proben.

dition, it is interesting to note that also in heat treated drinking milk no striking high-pressure induced changes were identified in the ribonucleoside contents.

3. High-pressure treatment of fat-reduced raw milk samples

In a former study, it was shown that the fat content of raw milk has an impact on the ADA activity [36]. In further investigations it was examined whether the fat content of milk samples exerts an influence on ribonucleoside contents. In raw bulk milk sample (mean=4.68% fat) an increase of cytidine and uridine caused by high-pressure treatment was recorded (Tab. 3). For a "cautious" rising of cream, raw milk sample was stored for 20 h at 6 °C in a creaming cylinder. Subsequently, the fat-reduced milk (2.40% fat) was drained of and subjected to high-pressure treatment under the experimental conditions described above. Compared to high-pressure treated raw milk, the cytidine and in particular the uridine release was

lower (Tab. 3). The milk prepared by rising of cream was further centrifuged gently at 100 x g for 10 min, thus reducing the fat content from 2.40% to 2.20%. During high-pressure treatment of this milk sample, less cytidine was released than in the fat-reduced milk and in untreated raw milk samples. For uridine, the situation was vice versa.

In the raw milk samples, high-pressure treatment led to a minor change of the ALP activity (Tab. 3). In the fat-reduced milk samples, a lower ALP activity was determined and only marginal differences in enzyme activity could be detected. In raw milk, an ADA activity of 673 ± 17 mU/L was determined in milk sample tempered at normal pressure, whereas 318 ± 8 mU/L were found in the pressure treated samples. Thus, ADA was partially inactivated by high pressure at 50 °C (Tab. 3). In fat-reduced or fat-reduced and centrifuged milk samples, the high-pressure induced inactivation was also observed. It is noticeable that the activity reduction of approx. 53% caused by high pressure in all

ribonucleoside	Raw milk*		Raw milk fat-reduced***		Raw milk fat-reduced and centrifuged****	
	normal pressure/ 50 °C/ 20 min	500 MPa/ 50 °C/ 20 min	normal pressure/ 50 °C/ 20 min	500 MPa/ 50 °C/ 20 min	normal pressure/ 50 °C/ 20 min	500 MPa/ 50 °C/ 20 min
	[μ mol/L] \pm s. d.	[μ mol/L] \pm s. d.	[μ mol/L] \pm s. d.	[μ mol/L] \pm s. d.	[μ mol/L] \pm s. d.	[μ mol/L] \pm s. d.
Cyd	5.45 0.093	11.32 0.041	9.34 0.083	14.56 0.102	6.85 0.070	9.45 0.185
Urd	9.01 0.230	11.33 0.028	13.74 0.134	13.86 0.111	10.92 0.114	10.16 0.128
m1Ado	0.17 0.003	0.20 0.014	0.22 0.018	0.18 0.007	0.13 0.003	0.16 0.021
Ino	0.08 0.008	n. d.** -	0.19 0.008	n. d. -	0.30 0.008	n. d. -
Guo	0.07 0.009	n. d. -	0.08 0.008	n. d. -	0.14 0.018	0.08 0.006
Ado	0.12 0.004	0.07 0.001	0.11 0.005	0.10 0.001	0.12 0.007	0.06 0.012
m6Ado	n. d. -	n. d. -	n. d. -	n. d. -	n. d. -	n. d. -
t6Ado	0.35 0.001	0.35 0.011	0.47 0.004	0.44 0.007	0.39 0.011	0.38 0.009
m6,2Ado	n. d. -	n. d. -	n. d. -	n. d. -	n. d. -	n. d. -
ALP [U/L]	790 13	799 33	647 34	650 2	615 13	612 13
ADA [mU/L]	673 17	318 8	562 42	265 8	567 24	260 9

* raw milk: fat content: 4.68%; protein content: 3.55%

** n. d.: not detectable

*** raw milk was stored for 20 h at 6 °C in a creaming cylinder; fat content: 2.40%; protein content: 3.59%

**** raw milk fat-reduced by rising of cream (see ***) was centrifuged with 100 x g for 10 min; fat content: 2.20%; protein content: 3.59%

Tab. 3: Ribonucleoside contents [μ mol/L] in high-pressure treated raw milk and in fat-reduced, high-pressure treated raw milk.

Tab. 3: Ribonucleosid-Gehalte [μ mol/L] in hochdruckbehandelter Rohmilch und in fettreduzierter hochdruck-behandelter Rohmilch.

three milk samples was obviously independent of the fat content. Due to the fact that ADA converts adenosine to inosine, the lower inosine contents detected in samples treated by high pressure may be explained by a process-related reduction of ADA activity.

4. High-pressure treatment of raw milk at different temperatures

For the results previously presented, the parameters hydrostatic pressure, pressure holding time and temperature were not modified. In order to investigate the influence of a varying heat impact during high-pressure treatment, the parameter temperature was modified, whereas the hydrostatic pressure and pressure holding time remained unchanged.

When raw milk (fat content: 4.68%; protein content: 3.55%) was treated at 50 °C and 60 °C at 500 MPa increasing cytidine contents of approx. 6 µmol/L were observed in relation to samples treated at normal pressure. The release was lower when milk was treated at 70 °C and 80 °C (Tab. 4). Regarding uridine (Tab. 4), increasing contents at 50 °C and 60 °C were detected as a result of high-pressure treatment, whereas the uridine contents were higher in samples treated at 70 °C and, in particular, in those prepared at 80 °C under normal pressure.

At different temperatures, the changes in the inosine content were of particular interest (Tab. 4): In the temperature range 50–70 °C, inosine was not detected in high-pressure treated samples. In normal-pressure treated samples, the inosine concentrations increased with increasing temperatures. At 80 °C, however, the inosine concentration was considerably higher in the pressed sample than in the sample heated at normal pressure (Tab. 4).

In former investigations it was shown that ADA in milk has an interesting activity behaviour. An increasing of ADA activity, and therefore of inosine formation was determined in the temperature range 45–75 °C. In HTST-heated drinking milk, ADA is available in an activated form, whereas in high-temperature heated (e.g. 85 °C/4 s) and in UHT-heated drinking milk the enzyme is completely inactivated [34, 35].

In the present investigations, the ADA activities detected in high-pressure and normal-pressure treated milk samples at different temperatures (Fig. 2) could indicate a relation to the detected inosine contents (Tab. 4). The described thermal activation of ADA in milk was observed in the normal pressure samples up to the range of 70–75 °C, and complete inactivation occurred at 80 °C. In comparison to the normal-pressure treated samples, high-pressure treatment in the temperature range of 50–70 °C caused inactivation of ADA, whereas at 80 °C it caused activation. It was shown for the first time that high-pressure treatment presumably caused a conformational change of the enzyme molecule at 80 °C so that the thermal denaturation was stopped by high-pressure treatment or partly compensated, i.e. high pressure essentially maintained ADA in the activated form.

In samples treated under normal pressure, thermal inactivation of the ALP was detected (Fig. 3). If, however, 500 MPa high pressure are given at the respective temperature "to the system", either reactivation occurred or the thermal inactivation was slowed down, i.e. at temperatures >50 °C the high pressure (500 MPa) has a protective effect opposite to the heat-induced denaturation of the ALP. For ALP, the protective effects of high-pressure impact as well as the antagonistic effects of pressure and temperature treatments on ALP activity were also described by Ludikhuyze et al. [37].

raw milk*	Cyd		Urd		Ino	
	[µmol/L]	± s. d.	[µmol/L]	± s. d.	[µmol/L]	± s. d.
50 °C:						
normal pressure/50 °C/20 min	5.45	0.093	9.01	0.230	0.08	0.008
500 MPa/50 °C/20 min	11.32	0.041	11.33	0.028	n. d.**	-
60 °C:						
normal pressure/60 °C/20 min	6.29	0.116	8.83	0.012	0.85	0.017
500 MPa/60 °C/20 min	12.58	0.203	11.47	0.156	n. d.	-
70 °C:						
normal pressure/70 °C/20 min	8.64	0.157	11.01	0.276	0.96	0.008
500 MPa/70 °C/20 min	11.34	0.035	10.73	0.034	n. d.	-
80 °C:						
normal pressure/80 °C/20 min	5.53	0.032	10.89	0.075	0.10	0.009
500 MPa/80 °C/20 min	8.40	0.280	9.88	0.140	1.18	0.049

* raw milk: fat and protein content and data at 50 °C see also Tab. 3.

** n. d.: not detectable

Tab. 4: Ribonucleoside contents [µmol/L] in high-pressure treated and in normal-pressure treated raw milk samples at different temperatures.

Tab. 4: Ribonucleosid-Gehalte [µmol/L] in hochdruckbehandelten und in normaldruckbehandelten Rohmilchproben bei unterschiedlichen Temperaturen.

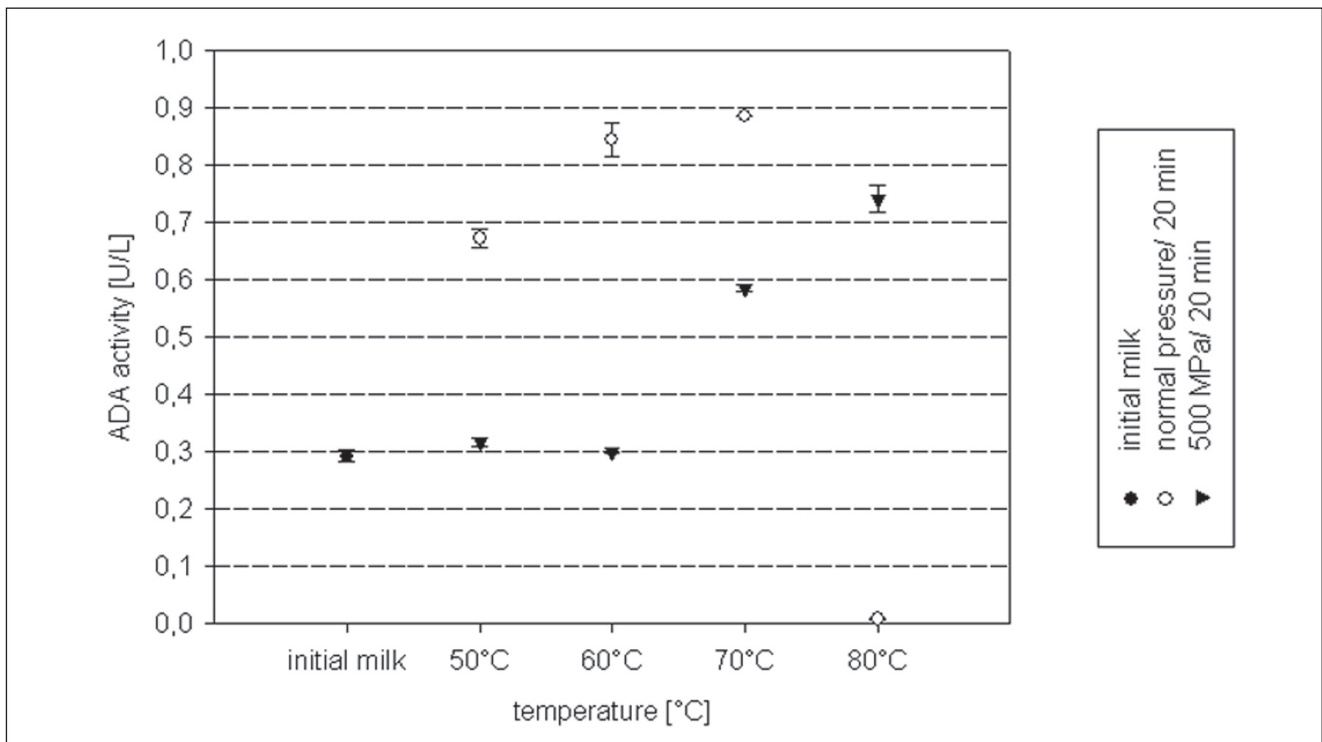


Fig. 2: ADA activity [U/L] in high-pressure treated and in normal-pressure treated raw milk samples at different temperatures.

Abb. 2: ADA-Aktivität [U/L] in hochdruckbehandelten und in normaldruckbehandelten Rohmilchproben bei unterschiedlichen Temperaturen.

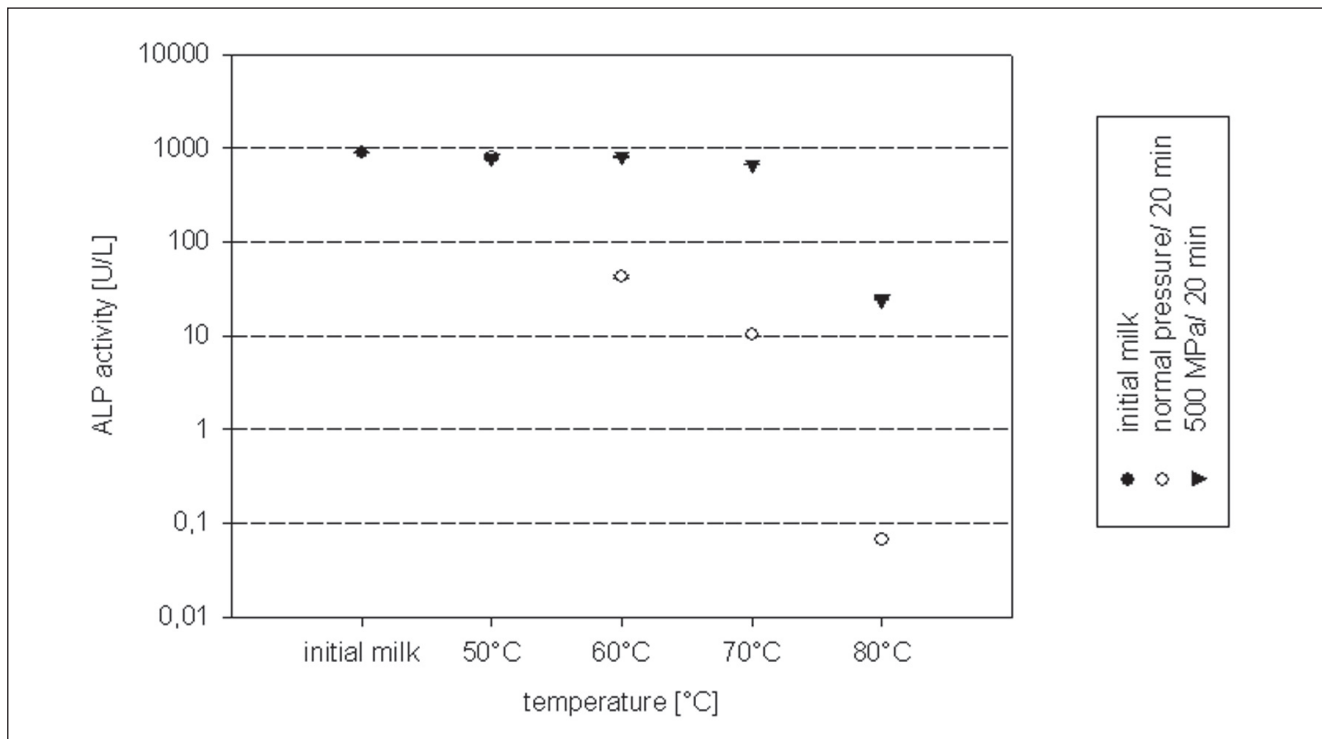


Fig. 3: ALP activity [U/L] in high-pressure treated and in normal-pressure treated raw milk samples at different temperatures.

Abb. 3: ALP-Aktivität [U/L] in hochdruckbehandelten und in normaldruckbehandelten Rohmilchproben bei unterschiedlichen Temperaturen.

Conclusion

Studies on ribonucleoside contents and on the activity of ADA and ALP in high-pressure treated milk samples were carried out.

The release of ribonucleosides observed in high-pressure treated raw milk samples and, presented for the first time also in fat-reduced raw milk samples, confirm the results from a former study [28]: The release is enzyme-controlled, because the content changes are considerably smaller in preheated milk samples. The impact of high-pressure treatment under defined temperature conditions indicates a relation between ADA inactivation/ADA activation and inosine contents in the respective milk samples.

During high-pressure treatment at 80 °C ADA largely preserves its thermally active form, i.e. at 80 °C, a protective high-pressure influence is observed compared to thermal inactivation. In further investigations, it should be checked to which extent this protective effect influences the thermal stability of the milk enzyme.

ALP converts ribonucleotides to ribonucleosides by dephosphorylation. However, according to the current knowledge, the ribonucleoside release in pressured samples can be only partly explained by the activity of the indigenous ALP present. In order to obtain further information on the contents and content changes of valuable ribonucleosides in high-pressure treated milk, future investigations should also deal with the influence of high-pressure treatment on the activity of further milk enzymes, which are relevant for ribonucleoside content pattern, e.g. nucleases.

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