JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of High-Pressure Processing at Elevated Temperatures on Thiamin and Riboflavin in Pork and Model Systems

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High-pressure/high-temperature properties of vitamins in food are important with respect to the new pressure-assisted thermal sterilization method utilizing pressure-induced adiabatic temperature changes. Riboflavin, thiamin, and thiamin monophosphate (TMP) stabilities were assayed in the temperature range from 25 to 100 °C under normal pressure (0.1 MPa) and high pressure (600 MPa) in acetate-buffered (pH 5.5) model solutions, some with added fructose, hemoglobin, or ascorbic acid. Thiamin and riboflavin stabilities were also assayed in minced fresh pork fillet and in rehydrated pork reference material with and without pressure treatment at 600 MPa in the temperature range from 20 to 100 °C. In pork, the vitamins proved to be sufficiently stabile for high-pressure/high-temperature processing. Under similar conditions, vitamin decay in model solutions was up to 30 times faster, especially that of TMP. Thus, it appears that it may not be possible to draw conclusions for the pressure behavior of real food matrices from the results of investigations in food models. A further consequence is that caution is necessary when supplementing foods with synthetic B vitamins preceding high-pressure/high-temperature processing.

KEYWORDS: High pressure; high temperature; thiamin; riboflavin; matrix effects; adiabatic

INTRODUCTION

Thiamin (vitamin B_1) is a water-soluble vitamin found in most whole grains that helps maintain a normal metabolism. In food, the vitamin is found as free thiamin or bound to proteins or to pyrophosphate as cocarboxylase, all exhibiting different stabilities. Generally, thiamin is sensitive to neutral and alkaline pH, air or oxygen, or sulfites and to heat; cooking loss including leaching may be as high as 80% (*1*−*3*). B vitamins generally show high stability in acid solution. Thus, it is possible to autoclave pork samples at 120 °C before analyzing the vitamins. The loss of vitamin B_1 during thermal processing of pork varied between 15 and 50% and was commonly higher in water than during cooking in fat. Conventional canning of meat dishes typically led to vitamin B_1 losses from 20 to 40% (*4*). In model systems, the thermal decay of thiamin could be characterized as a first-order reaction (*5*). This mechanism was also found for thermal treatments of meat and vegetables at various conditions (35−138 °C; pH 4−7; water activity, 0.9−0.99). Reaction rate constants at temperatures from 80 to 140 °C were between 0.0001 and 0.1020 min^{-1} while activation energies (E_a) were from 70 to 130 kJ/mol (*1*−*3*). Only little data are available for the influence of high pressure, a new minimal processing method, on B vitamins. Thiamin and thiamin monophosphate (TMP) in model solutions (pH 5.5) and also in rehydrated lyophilized pork were reported to remain unchanged even after 18 h of high-pressure treatment at 600 MPa and 25 °C (*6*). Also, treatments at lower pressures (200 and 400 MPa) at room temperature for 30 min had no significant effect on the retention of thiamin in a model system with pH 6.7 (*7*). High-pressure treatment of milk at 20 °C and 400 MPa for 30 min resulted in no significant loss of vitamin B_1 (*8*). At present, no systematic investigations on the influence of high pressure at elevated temperatures on thiamin are published.

Riboflavin (vitamin B_2) is a water-soluble vitamin present in most living organisms. It is an active part of different coenzymes that catalyze many oxidation−reduction reactions. These coenzymes have essential roles in several dehydrogenases and oxidases where riboflavin can accept or donate a pair of hydrogen atoms. Meats, poultry, fish, and dairy products are all ample sources, but food processing can destroy up to 80% of this vitamin. The toxicity of riboflavin has never been reported. In meat, riboflavin often occurs as flavin mononucleotide and flavin adenine dinucleotide bound to phosphate and is quite stable against heat (*3*). Under alkaline conditions (pH 8), especially under the influence of light (UV and visible), decay is accelerated (*16*), but the pH of meat during cooking is normally acidic. The more important cause of cooking losses of the water-soluble vitamin seems to be due to leaching. On cooking of pork, thermal losses of riboflavin were found to be below 10% (*3*); also, during sterilization, no essential reduction was found. Assessment of the changes of nutritional and sensory quality of sprouted alfalfa seed treated by high-pressure revealed that the values of riboflavin content did not change very much

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10.1021/jf0626821 CCC: $37.00
Published on Web 01/25/2007

Table 1. Preparation and Composition of Vitamins B_1 and B_2 Model Solutions

model solution	preparation and composition
thiamin or TMP in 0.2 mol/L sodium acetate buffer (pH 5.5)	10 mL of thiamin or TMP standard solution was adjusted with 2.5 mol/L sodium acetate to pH 5.5 and diluted with distilled water to 100 mL (=10 μg thiamin or TMP/mL)
thiamin or TMP in 0.2 mol/L sodium acetate buffer (pH 5.5) containing fructose	1.8 g of fructose (Merck) was dissolved in 10 mL of thiamin or thiamin monophosphate standard solution, adjusted with 2.5 mol/L sodium acetate to pH 5.5, and diluted with distilled water to 100 mL (=10 μg thiamin or TMP and 18 mg fructose/mL)
thiamin or TMP in 0.2 mol/L sodium acetate buffer (pH 5.5) containing hemoglobin	1.5 g of hemoglobin (Sigma) was dissolved in 10 mL of thiamin or thiamin monophosphate standard solution, adjusted with 2.5 mol/L sodium acetate to pH 5.5, and diluted with distilled water to 100 mL (=10 μg thiamin or TMP and 15 mg hemoglobin/mL)
thiamin or TMP in 0.2 mol/L sodium acetate buffer (pH 5.5) containing ascorbic acid	100 mg of L-ascorbic acid (Aldrich) was dissolved in 10 mL of thiamin chloride hydrochloride or TMP standard solution, adjusted with 2.5 mol/L sodium acetate to pH 5.5, and diluted with distilled water to 100 mL (=10 μg thiamin or TMP and 1 mg L-ascorbic acid/mL)
riboflavin in 0.2 mol/L sodium acetate buffer (pH 5.5)	10 mL of riboflavin standard solution was adjusted with 2.5 mol/L sodium acetate to pH 5.5 and diluted with distilled water to 100 mL (=10 μg riboflavin/mL)

as a consequence of pressurization with 500 MPa for 10 min or the storage period of 21 days (*9*). At present, data on the pressure stability of riboflavin, especially in meat and under elevated temperatures, are very scarce. Additionally, the knowledge of high-pressure/high-temperature properties of vitamins in food is becoming very important with respect to the new short-time pressure-assisted thermal sterilization method utilizing adiabatic temperature effects due to pressure changes (*10*).

MATERIALS AND METHODS

Preparation of Vitamins B_1 and B_2 Standard Solutions. Thiamin chloride hydrochloride (Sigma), TMP chloride dihydrate (Sigma), and riboflavin (Merck) were dissolved in 0.1 mol of sulfuric acid to give a final concentration of 100 μg/mL. The preparation and composition of individual model solutions for heat and high-pressure experiments are described in **Table 1**.

Preparation of Pork Meat: Lyophilized Pork for Vitamin B_1 Experiments. Samples of about 0.5 g of lyophilized pork purchased from Community Bureau of Reference (BCR, Brussels) were precisely weighed and filled into polyethylene bags (~2 mL); for rehydration, 1.5 mL of water was added, and the bags were then heat sealed.

Preparation of Pork Meat: Pork for Vitamins B_1 and B_2 Experiments. Fresh pork fillet (~500 g) purchased from a local market was minced and homogenized in a mixer. Samples of about 2.5 g of homogenized pork were precisely weighed into Teflon tubes (inner diameter, 0.6 cm) and closed with silicon rubber stoppers. All samples were chilled to 4 °C after preparation if not used immediately and stored until heat and high-pressure treatment and analysis, respectively.

Heat and High-Pressure Treatment. A high-pressure multivessel system was used with five autoclaves of 20 mL each for pressures up to 800 MPa and temperatures up to 100 °C manufactured by aad GmbH (Frankfurt, Germany). The system was fully programmable, allowing reproducible pressure build-up times (SPS, Siemens, Germany). Pressure was generated by a multistage pneumatic pressure intensifier. The pressure transmitting fluid was a mixture of one-third water and two-thirds of a glycol-based fluid (aad GmbH, Germany). The experiments were done at 0.1 (normal pressure) and 600 MPa (high pressure). Vitamin B_1 (thiamin and TMP) model solutions and rehydrated lyophilized pork were treated at 25, 40, and 60 °C for 1, 3, and 18 h while thiamin model solutions in acetate buffer were treated at 80 and 100 °C for 15 min only. Vitamin B_2 (riboflavin) model solutions were treated at 25, 40, 60, 80, and 100 °C for 15 min only. Pressure build-up was programmed from 80 s of pressure release to 40 s. Every assay using model systems and pork samples was independently performed in at least duplicate. All samples were chilled rapidly (within approximately 3 min) to 20 °C after heat and high-pressure treatment. Thiamin and TMP model solution samples and rehydrated lyophilized pork meat were stored at −18 °C up to analysis (5−6 weeks). All other samples were stored maximally for 1 day at 4 °C until analysis.

Analysis of Vitamins. The determinations were carried out by high-performance liquid chromatography (HPLC) according to refs *11*−*13*. While the model solutions of thiamin, TMP, and riboflavin except dilution required no further preparation, the B vitamins of pork had to be extracted for HPLC analysis by acid and enzymatic treatment.

Acid Treatment. Amounts of the pork sample (2.5 g each) were weighed and quantitatively convicted in a conical flask with 50 mL of 0.1 M hydrochloric acid and autoclaved at ~120 °C for 30 min. After it was cooled to 20 °C, the solution was diluted with water to a volume of 100 mL and filtered (filter paper). For vitamin B_1, B_2 determination of 12.5 mL of filtrate was adjusted to pH 4.0 with 2.5 mol/L sodium acetate, 40 mg of taka-diastase (Pfaltz&Bauer, CT) was added, and it was incubated at 37 °C for 18 h. After it was cooled to 20 °C, the solution was adjusted to pH 3.0 with 0.1 mol of hydrochloric acid and then diluted with water to 20 mL and filtered (filter paper). Aliquots of filtrate were ultrafiltered and diluted with methanol (2:1). Because impurities of vitamin B1 were found in the used taka-diastase, analytical results given were systematically enzyme blank corrected.

HPLC Determination. The HPLC system consisted of an Agilent 1100 Series binary pump, column oven, autosampler, fluorescence detector, and an Agilent ChemStation. Vitamin B_1 (postcolumn derivatization method): stationary phase: Purosphere^R RP C_{18}, end capped, 5 μm, 250 mm × 4.0 mm (Merck, Darmstadt, Germany); mobile phase: methanol:0.01 mol of ammonium phosphate buffer (pH 3.5), containing 1 g of tetraethyl ammonium chloride (Sigma) and 1 g of sodium heptanesulfonate (Sigma)/L (30:70); flow rate, 1.5 mL; injection, 2 μL (1−10 ng of thiamin); postcolumn reagent, 0.4 g/L potassium hexacyanoferrate(III) (Sigma) solved in 15% sodium hydroxide solution; flow rate (pump 2), 0.3 mL/min; reactor, tefzel capillary (2.5 m, 0.33 mm i.d.); fluorometric detection, 365 nm (excitation), 435 nm (emission); and retention time, ~1.6 min of TMP and 7.2 min of thiamin. Vitamin B_2: The stationary phase was the same as for vitamin B_1; mobile phase: methanol:0.01 mol of ammonium phosphate buffer (pH 3.5), containing 1 g of tetraethyl ammonium chloride (Sigma) and 1 g of sodium heptanesulfonate (Sigma)/L (25:75); flow rate, 1.5 mL; injection, 20 μL (1−5 ng of riboflavin); fluorometric detection, 450 nm (excitation), 525 nm (emission); and retention time of riboflavin, ~9.2 min.

Statistical Analysis. All reactions were performed in at least triplicate, and duplicate samplings were made to every reacted sample for HPLC elution. Data were presented as means with standard deviations and significance levels. Statistical significance was tested using advanced statistical package of Microsoft Office software, Student's *t* test.

Table 2. Effect of High-Pressure Treatment at Elevated Temperatures on Vitamin B_1 (Thiamin and TMP) Content in Model Solutions of pH 5.5

	treatment			relative content (%)[a]				
				thiamin		thiamin monophosphate		
model solutions	temp (°C)	pressure (MPa)	time (min)	*x*	*s*	*x*	*s*	*n*
thiamin or TMP	~20	0.1	untreated	100.0	2.9	100[b]	3.0	9
in sodium acetate buffer (pH 5.5) containing fructose, hemoglobin, and ascorbic acid	25	0.1	1080	100.0	3.0	99.0	3.0	9/9
	25	600	1080	100.0	3.0	99.0	3.0	9/9
thiamin or TMP	40	0.1	60	100.0	2.4	98.7	2.8	9/8
in sodium acetate buffer (pH 5.5) containing fructose and hemoglobin	40	0.1	180	99.4	2.8	95.2*	2.5	9/9
	40	0.1	1080	97.0*	2.3	83.2**	2.5	8/8
thiamin or TMP	40	600	60	99.4	3.0	98.3	2.9	8/6
in sodium acetate buffer (pH 5.5) containing fructose and hemoglobin	40	600	180	98.5	2.8	94.1**	3.0	8/7
	40	600	1080	92.5***	2.5	77.0***	2.5	8/8
thiamin or TMP	40	0.1	60	98.5	2.0	98.0	2.0	2/3
in sodium acetate buffer (pH 5.5) containing ascorbic acid	40	0.1	180	96.3	2.5	94.1	2.4	3/2
	40	0.1	1080	94.0*	2.0	81.0*	2.0	3/3
thiamin or TMP	40	600	60	96.8	3.0	88.4*	2.8	2/3
in sodium acetate buffer (pH 5.5) containing ascorbic acid	40	600	180	90.2*	2.0	81.3**	2.7	3/2
	40	600	1080	87.0**	2.5	73.5**	2.2	3/2
thiamin or TMP	60	0.1	60	99.7	2.7	85.2	2.3	10/8
in sodium acetate buffer (pH 5.5) containing fructose and hemoglobin	60	0.1	180	98.5	2.5	82.3	2.4	8/9
	60	0.1	1080	91.0***	2.0	75.6	2.6	9/6
thiamin or TMP	60	600	60	98.5	2.5	82.3	2.8	12/8
in sodium acetate buffer (pH 5.5) containing fructose and hemoglobin	60	600	180	96.0*	2.2	76.3	2.2	9/8
	60	600	1080	84.0***	2.0	65.8	2.3	7/6
thiamin or TMP	60	0.1	60	95.6	2.5	81.0	2.4	3/3
in sodium acetate buffer (pH 5.5) containing L-ascorbic acid	60	0.1	180	84.0**	2.0	78.0	3.0	3/2
	60	0.1	1080	80.0**	2.0	71.0	2.5	3/3
thiamin or TMP	60	600	60	94.0*	2.7	74.0	2.2	3/3
in sodium acetate buffer (pH 5.5) containing L-ascorbic acid	60	600	180	80.4**	2.5	69.0	2.0	3/2
	60	600	1080	72.0**	2.2	63.0	2.2	2/2
thiamin or TMP	80	0.1	15	99.6	3.1			5
in sodium acetate buffer	80	600	15	98.5	2.8			5
(pH 5.5) containing fructose	100	0.1	15	98.0	3.0			5
and hemoglobin	100	600	15	94.5*	2.7			5

[a] Related to content in untreated samples [relative content in % = $(C_i/C_0) \times 100$], where C_0 = content in untreated samples; C_i = content in samples after treatment; and n = number of analyses. [b] Composition of untreated samples; see **Table 1**; x = mean value; s = standard deviation or range of variation. *, **, and ***, significantly different from untreated sample at $p < 0.5$, $p < 0.1$, and $p < 0.01$.

RESULTS AND DISCUSSION

Thiamin and TMP in Model Solutions. Table 2 shows the thiamin and TMP retention vs treatment time at 25, 40, and 60 °C under normal pressure (0.1 MPa) and high pressure (600 MPa) in 0.2 mol/L acetate buffer (pH 5.5) containing fructose, hemoglobin, and ascorbic acid. In correspondence to earlier results (*6*), no changes in the concentrations of thiamin and TMP were found at 25 °C neither at 0.1 MPa nor under high pressure (600 MPa) and not even after 18 h of treatment. However, at temperatures of 40 °C and above, the high-pressure treatment markedly accelerated the degradation rate of thiamine and especially that of TMP in these model systems. The reactions followed first-order kinetics in the temperature range from 25 to 60 °C with and without pressure where the goodness of fit of exponential regression was generally $R^2 > 0.98$. Reaction rate constants (k) and activation energy (E_a) were evaluated by regression and by using the Arrhenius model (see **Table 4**). Under pressure, the rate constants were always higher (from 30% to about 4-fold). All used model solutions of TMP were much more pressure sensitive at 40 and 60 °C than the corresponding thiamin model solutions. Independent of the further additives, TMP decay at those temperatures was substantially higher than that of thiamin. Ascorbic acid as an additive seemed to catalyze the thermal decay of thiamine as well as of TMP with or without pressure. The increased losses in presence of ascorbic acid may be explained by oxidation of ascorbic acid to dehydroascorbic acid through residual oxygen and subsequent oxidative decay of thiamine and TMP by action of dehydroascorbic acid (*14*).

Thiamin and TMP in Pork. Vitamin B_1 content in untreated pork fillet used for the experiments ranged at about 0.87 mg/100 g (variation coefficient ~2%) and corresponded to literature values for pure pork. In **Table 3**, retention data for vitamin B_1 in rehydrated pork lyophilizate and in fresh minced pork fillet

Table 3. Effect of High-Pressure Treatment at Elevated Temperature on Vitamin B_1 Content of Pork Meat

	treatment			vitamin B_1[a] content (mg/100 g)		vitamin B_1[a] retention in %[b]		
sample	temp (°C)	pressure (MPa)	time (min)	*x*	*s*	*x*	*s*	*n*
	∼20	0.1	untreated	0.697[c]	0.017	100.0	2.4	4
pork lyophyllisate,	20	0.1/600	1080	0.699	0.018	100.2	2.5	8
rehydrated	40	0.1/600	1080	0.695	0.015	99.7	2.1	8
(BCR reference)	60	0.1	1080	0.630	0.019	88.5***	2.2	4
	60	600	1080	0.596	0.018	82.3***	2.6	4
	∼20	0.1	untreated	0.870[c]	0.016	100.0	0.9	5
	25	0.1/600	45	0.870	0.009	100.0	0.5	2/2
	60	0.1	15	0.870	0.006	100.0	0.3	2
	60	0.1	30	0.867	0.005	99.7	0.3	2
	60	0.1	45	0.865	0.004	99.4	0.2	2
	60	600	15	0.865	0.003	99.4	0.2	2
	60	600	30	0.862	0.004	99.1	0.2	2
	60	600	45	0.860	0.004	98.8	0.2	2
	80	0.1	15	0.864	0.004	99.3	0.2	2
pork filet, fresh,	80	0.1	30	0.858	0.004	98.6	0.2	2
minced	80	0.1	45	0.844	0.003	97.0*	0.2	2
	80	600	15	0.860	0.005	98.8	0.3	2
	80	600	30	0.847	0.003	97.4*	0.2	2
	80	600	45	0.830	0.003	95.4**	0.2	2
	100	0.1	15	0.786	0.003	93.2***	0.2	2
	100	0.1	30	0.762	0.003	87.6***	0.2	2
	100	0.1	45	0.728	0.004	83.7***	0.2	2
	100	600	30	0.782	0.005	89.9***	0.3	2
	100	600	30	0.727	0.003	83.6***	0.2	2
	100	600	45	0.679	0.003	78.0***	0.2	2

[a] Calculated as thiamin chloride hydrochloride. [b] Related to vitamin B_1 content in untreated samples; retention in % = $(C_i/C_0) \times 100$. [c] C_0 = content of vitamin B_1 in untreated samples; x = mean value; ± = standard deviation ($n < 3$, range of variation); and n = number of analyses. *, **, and ***, significantly different from untreated sample at $p < 0.5$, $p < 0.1$, and $p < 0.01$.

Table 4. Rate Constants (k), Activation Energies (E_a), and 10% Loss Time Intervals (H_{10}) for Vitamin B_1 Degradation in Model Systems and in Pork Meat Treated by Heat and High Pressure[a]

	treatment		0.1 MPa			600 MPa		
sample	temp (°C)	range (min)	$k \times 10^{-3}$ (min^{-1})	E_a (kJ/mol)	H_{10} (min)	$k \times 10^{-3}$ (min^{-1})	E_a (kJ/mol)	H_{10} (min)
thiamin model solutions	25	0–1080	0			0		
(pH 5.5) containing	40	0–1080	0.028		∼3570	0.073		∼1370
fructose and	60	0–1080	0.087	49	∼1150	0.157	33	640
hemoglobin	80	0–15	0.267	57	∼375	1.483	91	∼99
	100	0–15	1.008	88	∼74	3.420	72	∼27
	25	0–1080						
thiamin model solution	40	0–180	0.203	67	∼560	0.570	32	175
(pH 5.5) containing		180–1080	0.173			0.245		
ascorbic acid	60	0–180	0.947		175	1.211		84
		180–1080	0.041			0.123		
TMP	25	0–1080	0.009		∼185[a]	0.009		∼185[a]
model solutions	40	0–180	0.268	108	∼600)	0.342	100	∼420
(pH 5.5) containing		180–1080	0.166			0.238		
fructose and	60	0–60	2.669		37	3.247		31
hemoglobin		60–1080	0.109			0.199		
	25	0–1080	0.009		∼185[a]	0.009		∼185[a]
TMP	40	0–60	0.337	187	∼297	2.055	281	∼175[a]
model solution		60–180	0.338			0.698		
		180–1080	0.167			0.011		
(pH 5.5) containing	60	0–60	3.510	102	28	5.018	39	20
ascorbic acid		60–1080	0.315			0.583		
		60–1080	0.101			0.104		
pork meat lyophyllisate,	25	0–1080	0			0		
rehydrated (BCR	40	0–1080	0.020	71	∼83[a]	0.020	91	∼83[a]
reference)	60	0–1080	0.110		909	0.180	91	555
	25	0–45	0			0		
pork meat filet, fresh,	60	0–45	0.115	81	∼870	0.287	81	∼348
minced	80	0–45	0.602	92	∼166	0.981	81	∼102
	100	0–45	4.138	105	24	5.762	98	17
pork puree[b]	120	0–45	20.4	108	5			

[a] Hours. [b] Ref *2*; ∼, estimated value; k_1, constant of relative reaction velocity calculated by regression ($y = 100e^{kt}$); H_{10} value, time for 10% reduction of vitamin B_1 content = $(\ln 10/9)/k = \sim 0.1/k$; E_a, activation energy = $(R \times T_1 \times T_2/T_2–T_1) \times \ln k_2/k_1$); R = 8.309 J/°C; T = temperature (Kelvin).

Table 5. Influence of High-Pressure Treatment at Elevated Temperatures on Vitamin B_2 (Riboflavin) Content in Buffer Solutions and in Pork Meat

	treatment			vitamin B_2				
				μg/100 g		retention (%)[a]		
sample	temp (°C)	pressure (MPa)	time (min)	x	s	x	s	n
riboflavin in buffer solution pH 5.5	20	0.1	untreated	480[b]	30	100	6.3	5
	25	600	15	480	30	100	6.3	5
	60	0.1/600	15	480	30	100	6.3	10
	80	0.1/600	15	480	29	100	6.0	10
	100	0.1/600	15	466	25	97*	5.2	10
	20	0.1	untreated	170[b]	10	100	5.9	4
	25	0.1	45	242	2	142*	1.2	2
	25	600	45	220	3	129*	1.8	2
	20	0.1	untreated	164	4	100	2.5	2
	60	0.1	15	178	2	109*	1.2	2
	60	0.1	30	208	2	128**	1.2	2
	60	0.1	45	214	1	131**	0.6	2
	60	600	15	162	2	98	1.2	2
	60	600	30	172	2	105	1.2	2
	60	600	45	186	2	113**	1.2	2
pork filet, fresh, minced	20	0.1	untreated	136	2	100	1.5	2
	80	0.1	15	150	2	110	1.5	2
	80	0.1	30	156	2	115**	1.5	2
	80	0.1	45	160	1	118**	1.0	2
	80	600	15	168	2	124**	1.5	2
	80	600	30	170	3	125**	2.2	2
	80	600	45	174	2	128**	1.5	2
	20	0.1	untreated	152	3	100	2.0	2
	100	0.1	15	184	3	121**	2.0	2
	100	0.1	15	198	2	130**	1.3	2
	100	0.1	30	210	2	138**	1.3	2
	100	600	15	192	2	127**	1.3	2
	100	600	30	202	2	133**	1.3	2
	100	600	45	198	2	130**	1.3	2

[a] Retention in % = $(C_i/C_0) \times 100$. [b] C_0 = riboflavin content in untreated samples; x = mean value; s = range of variation; and n = number of analyses. *, **, significantly different from untreated sample at $p < 0.5$ and $p < 0.1$.

are given. At 20−25 °C, vitamin B_1 was stable under pressure as well as without pressure. Treatments of lyophilizate at 40 °C and 0.1 or 600 MPa showed only minor decay even after 18 h of treatment. According to the calculated rate constants given in **Table 4**, treatment time of about 83 h would be afforded for 10% decay (H_{10}). Retention (%) and treatment time at all used temperatures and pressures showed a good exponential correlation indicating first-order reactions. At 60 °C with and without pressure, when comparing H_{10} times, losses in both kinds of meat samples were similar and clearly higher than those in thiamin model solutions containing fructose and hemoglobin. However, they were substantially lower than in model solutions containing ascorbic acid and all containing TMP. After 15 min of pressure treatment of minced pork at 600 MPa and 100 °C, a vitamin B_1 loss of about 10% is to be expected. In contrast, conventional heat sterilization (20 min at 121 °C) may cause losses up to 45%.

Vitamin B_2 (Riboflavin). Table 5 summarizes the effects of high-pressure treatment at elevated temperatures on vitamin B_2 content in buffer solutions and in minced fresh pork meat. Riboflavin in buffer solutions of pH 5.5 overcomes 15 min treatments at temperatures from 20 to 100 °C and 0.1 and 600 MPa without any loss. In fresh minced pork, the initial riboflavin content was between 130 and 170 mg/100 g according also to literature values. Interestingly, the content increased by 40% within 45 min after preparation without any treatment (25 °C). Under pressure, the increase was a little smaller (30%). This behavior was also found at the higher temperatures of 60, 89, and 100 °C with the main increase within the first 15 min of treatment. The increase of riboflavin is obviously caused by liberation of chemically or physically bound vitamin and may be interpreted as a matrix effect, the mechanism of which was not investigated in this work. It is concluded that in fresh minced pork fillet as well as in rehydrated lyophilized pork, thiamin was found to be protected from destruction due to heat and/or pressure through matrix effects. However, while at 600 MPa and 60 °C, rehydrated pork needed 550 min for 10% vitamin loss, and the model solution containing TMP and ascorbic acid showed the same decay within 20 min, which is nearly 30 times faster! Regarding, for example, a recent fatal incidence with thiamin-deficient soy-based infant formula (*15*), this has to be taken into account when infant formula is supplemented with vitamin B_1. A further consequence is that it may be not possible to draw conclusions for the pressure behavior of real food matrices from the results of investigations in food models. Riboflavin is known to be heat-resistant, and in this work, it is demonstrated to be also pressure-resistant under elevated temperatures. Pressure and heat in this case were found to cause the liberation of bound vitamin from the food matrix.

LITERATURE CITED

(1) Mauri, L. M.; Alzamora, S. M.; Chirife, J.; Tomio, M. J. Kinetic parameters for thiamin degradation in food and model solutions of high water activity. *Int. J. Food Sci. Technol.* **1989**, *24*, 1−9.

(2) Bognar, A.; Püchner, H. J. Influence of cooking methods on the nutritional value of pork. *Fleischwirtschaft* **1983**, *63*, 943−951.

(3) Bognar, A. Vitamin losses during storage and cooking of food. *Nutrition* **1995**, *19*, 411−416, 478−483, 551−554.

(4) Zacharias, R.; Bognar, A. Quality of sterilized meals. In *Schulverpflegung mit Industriell Hergestellten Sterilisierten Speisen*; Bundesministerium für Ernährung, Ed.; Landwirtschaft und Forsten and Federal Research Centre for Nutrition: Stuttgart/Karlruhe, 1977; pp 40−93.

(5) Felicotti, E.; Esselen, W. B. Thermal destruction rates of thiamin in pureed meat and vegetables. *Food Technol.* **1957**, *10* (12), 13.

(6) Bognar, A.; Butz, P.; Kowalski, E.; Ludwig, H.; Tauscher, B. Stability of thiamin in pressurized model solutions and pork. In *Nutritional, Chemical and Food Processing Implications of Nutrient Availability*; Schlemmer, U., Ed.; Bioavailability '93, Proceedings, Part 2; Berichte BFE-R-93-O1; Fed. Res. Ctr. Nutr. Food: Karlsruhe, Germany, 1993; pp 352−356.

(7) Sancho, F.; Lambert, Y.; Demazeau, G.; Largeteau, A.; Bouvier, M.; Narbonne, J.-F. Effect of ultra-high pressure on hydrosoluble vitamins. *J. Food Eng.* **1999**, *39*, 247−253.

(8) Sierra, I.; Vidal-Valverde, C.; Lopez-Fandino, R. Effect of high pressure on the vitamin B_1, and B_6 content of milk. *Milk Sci. Int.* **2000**, *55*, 365−367.

(9) Gabrovska, D.; Paulickova, I.; Maskova, E.; Fiedlerova, V.; Kocurova, K.; Pruchova, J.; Strohalm, J.; Houska, M. Changes in selected vitamins, micro-organism counts, and sensory quality during storage of pressurised sprouted seed of alfalfa (Medicago sativa L.). *Czech J. Food Sci.* **2005**, *23* (6), 246−250.

(10) Meyer, R. S.; Cooper, K. L.; Knorr, D.; Lelieveld, H. L. M. High-pressure sterilization of foods. *Food Technol.* **2000**, *11*, 67−72.

(11) Bognar, A. Determination of vitamin B1 in food by high-performance liquid chromatography and post-column derivatization. *Fresenius J. Anal. Chem.* **1992**, *343*, 155−156.

(12) EN 14122. Food−Determination of vitamin B_1 by HPLC, 2003.

(13) EN 14152. Food—Determination of vitamin B_2 by HPLC, 2003.

(14) Fernández, A.; Butz, P.; Bognar, A.; Tauscher, B. Antioxidative capacity, nutrient content and sensory quality of orange juice and an orange-lemon-carrot juice product after high pressure treatment and storage in different packaging. *Eur. Food Res. Technol.* **2001**, *213*, 290−296.

(15) Fattal-Valevski, A.; Kesler, A.; Sela, B. A.; Nitzan-Kaluski, D.; Rotstein, M.; Mesterman, R.; Toledano-Alhadef, H.; Stolovitch, C.; Hoffmann, C.; Globus, O.; Eshel, G. Outbreak of life-threatening thiamine deficiency in infants in Israel caused by a defective soy-based formula. *Pediatrics* **2005**, *115* (2), E233−E238.

(16) Toyosaki, T.; Hayashi, A. Structural analysis the products of milk riboflavin photolysis. *Milchwissenschaft—Milk Sci. Int.* **1993**, *48* (11), 607−609.

Received for review September 19, 2006. Revised manuscript received December 6, 2006. Accepted December 14, 2006.

JF0626821