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Thiamine in Lipid Systems vs. the Antioxidant Activity of Epigallocatechin Gallate and Caffeine

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Abstract: The aim of this study was to determine correlations between the concentration of thiamine hydrochloride or thiamine pyrophosphate and the antioxidant activity of epigallocatechin gallate (EGCG) and caffeine, as well as thiamine stability. The study was conducted in model systems. Oxidation degree indices of soybean oil (peroxide value and anisidine value LAN) and concentrations of total thiamine were determined. To compare the dynamics of the changes in thiamine content during storage, half-life $T_{1/2}$ was determined. There was a strong correlation between the stability of thiamine and the stability of the oil. Thiamine was particularly sensitive to secondary oxidation products. Higher losses of thiamine introduced in the form of thiamine pyrophosphate were found (4–6%). The addition of tea components increased fat stability and thus reduced thiamine losses. The dynamics of thiamine loss were found to be lower with EGCG than caffeine. The antioxidant activity of these components was significantly reduced when the content of thiamine (1.0–20.0 mg/100 g) was higher than the natural level in foods. In order to maintain thiamine stability and the high activity of the active tea ingredients, it is necessary to consider their simultaneous addition to the systems in concentrations that limit their interactions.

Keywords: thiamine; soybean oil; oxidative stability; epigallocatechin gallate; caffeine

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1. Introduction

Thiamine (vitamin B1) is a water soluble vitamin, occurring both in free form and in bound form as phosphate esters: thiamine monophosphate (TMP), thiamine pyrophosphate (TPP), and thiamine triphosphate (TDP) [1,2]. After absorption, it undergoes phosphorylation to TPP and participates in cellular enzymatic processes associated with carbohydrate, lipid, and branched amino acid metabolisms. Thiamine, in addition to its crucial role in energy metabolism, is also known for its catalytic activity in hexose-monophosphate pathways [3]. Studies further confirm the influence of thiamine on the immune system [3,4]. Thiamine depletion decreases naive B cells without affecting IgA+ plasma cells via the impairing of tricarboxylic acid cycle activity and finally impairs the initiation of antigen-specific antibody responses [5,6].

Studies show that thiamine therapy should be a crucial part of treatment plans and health policies to prevent the development or progression of dementia and Alzheimer's disease [7,8].

Thiamine is widely distributed in plant and animal products. This vitamin is found in high concentrations in yeast, cereal products (especially whole wheat), legume seeds (especially fermented), and meat (especially pork). Small amounts of thiamine can also be synthesized by intestinal microorganisms [9]. Unfortunately, thiamine is one of the most labile vitamins [4,10–14]. Analyses of cooked meals has shown a 50% loss of thiamine on average. This instability of thiamine was confirmed by previous studies related to both high temperature processing and storage [15,16]. Therefore, globally, many populations may be exposed to clinical or subclinical deficiencies of this vitamin. Clinical symptoms develop within the following three weeks of a deficient thiamine intake [17,18].

Frequent deficiencies of this vitamin can be caused by its inadequate dietary intake. This may result from the intake of small portions of food, as well as the consumption of highly processed products [4,12,19]. For a group of consumers who exclude or avoid certain categories of food, supplementation may be an alternative source of many nutrients.

Very high doses of thiamine, i.e., up to 3 g/day, are used in the treatment of numerous diseases [20–22]. Food fortification may constitute an attractive source of many nutrients, including thiamine. However, it may also be possible that the introduction of increased levels of thiamine could have detrimental consequences to the stability of other components [23,24].

Other studies also indicate that functional foods with tea extracts are taken into consideration as elements that may potentially aid the treatment of dementia and Alzheimer's disease [25–27]. Moreover, EGCG therapy may be helpful in treating the parenchymal and vascular symptoms of amyloidosis [28]. Antioxidants are interesting compounds for scientists because of their anti-ageing and anti-inflammatory properties [22,29]. Researchers also indicate the importance of antioxidants for the physiological functions of the liver, the kidneys, the digestive system, and the prevention of cardiovascular diseases and cancer [7,25–27].

Results of earlier studies indicate that phenolic compounds from natural antioxidants, especially rosemary and tea, show strong antioxidant activities both in lipid systems and food [30–35]. It was found that mixing oils, e.g., soybean oil, with different concentrations of green tea extract led to an increase in its stability against oxidation [36,37].

Tea leaves at various degree of fermentation (oxidation) have been proved to have health-promoting properties, as they significantly reduce the risk of various diseases, including cardiovascular diseases [37,38]. Tea extracts have significant anti-inflammatory, antimicrobial, anticarcinogenic, antihypertensive, neuroprotective, cholesterol-lowering, and thermogenic properties [39]. Some studies suggest that tea and its bioactive polyphenolic components have numerous health-beneficial effects, including the prevention of various diseases such as diabetes, arthritis, stroke, and obesity [40–42].

Our preliminary studies [43,44] have shown that the addition of tea extracts has increased the oxidative stability of fat and thus has reduced the loss of thiamine. This analysis has shown the greatest protective effect of white and green tea extracts, which has strongly correlated with the highest antioxidant efficiency. Moreover, the earlier data suggest that the presence of thiamine may influence the antioxidant activity of tea extracts with varying degrees of fermentation [5,6]. However, there is no data in scientific publications that would prove which types of bioactive components of tea are more active towards thiamine. The high antioxidant efficiency of EGCG was found, but there is no data on the stability of thiamine in the presence of this component. Moreover, there is no data on the activity of thiamine toward these active tea components.

Therefore, the assumed null hypothesis (H_0) was that the concentration of thiamine and antioxidant activity of epigallocatechin gallate (EGCG) and caffeine are related to each other, at $p > 0.05$. In case the null hypothesis was rejected, an alternative hypothesis (H_1) was assumed, according to which there are no correlations between the aforementioned variables.

2. Materials and Methods

2.1. Sample Preparation

Thiamine hydrochloride and thiamine pyrophosphate (Merck) were assumed as thiamine models. The study was conducted in model systems, where soybean oil (Flota Logistic Sp. z o.o. Tychy, Poland) systems with active tea components (PhytoLab GmbH & Co. KG; Vestenbergsgreuth, Germany) and thiamine hydrochloride or thiamine pyrophosphate were adopted in weight. Active tea components—(-)-epigallocatechin gallate (EGCG) (99.54%) and caffeine (99.94%)—were added at the following amounts: 0.04, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mg/100 g of oil. Thiamine at different concentrations was added to soybean oil with EGCG or caffeine. Thiamine was added at the following amounts: 0.01,

0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, and 0.8 (0.01–0.8 mg/100 g—the natural thiamine level in food products) as well as 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 9.0, 13.5, 16.0, 18.0, and 20.0 mg/100 g (1.0–20.0 mg/100 g—enriched products).

The samples were stored at 30 ± 1 °C, without access to light. In order to obtain a uniform weight, 20 g samples with a 1.3–1.5 cm thick layer were mixed continuously. They were stored, and the degree of soybean oil oxidation was measured on Days 0, 5, 7, 11, 15, 17, 21, 25, 28, and 31.

2.2. Methods

On the established days, soybean oil oxidation degree indices were determined: peroxide value and anisidine value LAN.

2.2.1. Peroxide Value

Peroxide value [45,46] was based on the quantitative determination of the iodine that was released from potassium iodide under the influence of active oxygen from peroxides in fat. The peroxide value was expressed in milliequivalents (meq) of active oxygen per kilogram of oil.

2.2.2. Anisidine Value

The determination of the anisidine value (mEq/kg) [47] was based on the spectrophotometric determination of the yellow complex concentration formed as a result of the reaction of secondary fat oxidation products with the p-anisidine solution. The absorbance was measured at 350 nm, both before and after reaction.

Based on the indicators of lipid oxidation, the protection factor (Wo) was calculated. This was the ratio between the time necessary for a particular sample to reach the peroxide value 50 ($meq0_2/kg$) and the corresponding time for the control sample. Wo > 1 indicates the antioxidant properties of the additive, whereas Wo > 1 indicates the pro-oxidative properties of the additive [48].

In order to compare the influence of elevated amounts of thiamine on the antioxidative properties of EGCG and caffeine, the differences between the protection coefficient of the component and that of a sample without thiamine, as well as that of a sample with thiamine added at an amount of 2.0 mg/100 g of oil, were calculated. A greater difference indicated the higher 'vulnerability' of the extract to the high doses of thiamine hydrochloride.

2.2.3. Thiamine Stability

On the set days of storage, the samples with oil, thiamine, and active tea components (EGCG or caffeine) were determined using the thiochromium method [49,50], which included an analysis of quantitative changes in the free (thiamine hydrochloride) and bound (thiamine pyrophosphate) forms.

A Jenway model 6200 fluorometer (Jenway, Stone, UK) (an input filter with a maximum of 365 nm and an output filter with a maximum of 435 nm) was used for the measurement of thiochromium fluorescence. All determinations were made in duplicate.

2.2.4. Statistical Analysis

The obtained results were subject to statistical analysis using STATISTICATM PL 13 (StatSoft) software. In order to determine the strength of the correlation between the variables, Pearson's linear correlation coefficients (r) were calculated (r) [51]. The data analyzed were from two independent samples, and seven measurements for each sample were taken; n = 14. The comparison of the dynamics of changes in thiamine content during the storage was conducted by linear regression analysis of the values obtained experimentally. Half-life $T_{1/2}$ was determined, i.e., the time within which the initial thiamine content decreased by half [9]. The accuracy of the models was estimated using the coefficient of determination (R^2) and the root mean square error (RMSE). The significance level for all analyses was set at 5%.

3. Results

3.1. Antioxidative Effect of EGCG and Caffeine in the Presence of Thiamine Hydrochloride and Thiamine Pyrophosphate

The analysis showed that the higher the concentration of EGCG and caffeine was, the higher the antioxidant activity. The lowest oil oxidation indices (Tables S1–S12) were found at concentrations of 4.0 and 6.0 mg/100 g of EGCG and caffeine. Moreover, for these concentrations, indexes of the protection factor Wo (Figure 1) were the highest. It was found that the samples with EGCG exhibited a higher antioxidant activity, whereas a lower activity was observed in the samples with caffeine. There was also a significant correlation between the amount of thiamine hydrochloride or thiamine pyrophosphate in the system and the oxidative stability of soybean oil in the presence of the tea components under analysis, especially EGCG (Table 1 and Tables S1–S12, Figure 1). However, the size and the direction of these relationships depended on the concentration level of both the hydrochloride thiamine and pyrophosphate thiamine. The lipid oxidation indicators did not increase in the systems whose thiamine content corresponded to a low level in food products (0.01–0.1 mg/100 g). Meanwhile, in the samples containing thiamine hydrochloride or thiamine pyrophosphate at amounts from 0.1 to 1.0 mg/100 g, the oil oxidation indices even decreased. The protection factors (Wo) for these samples were the highest.

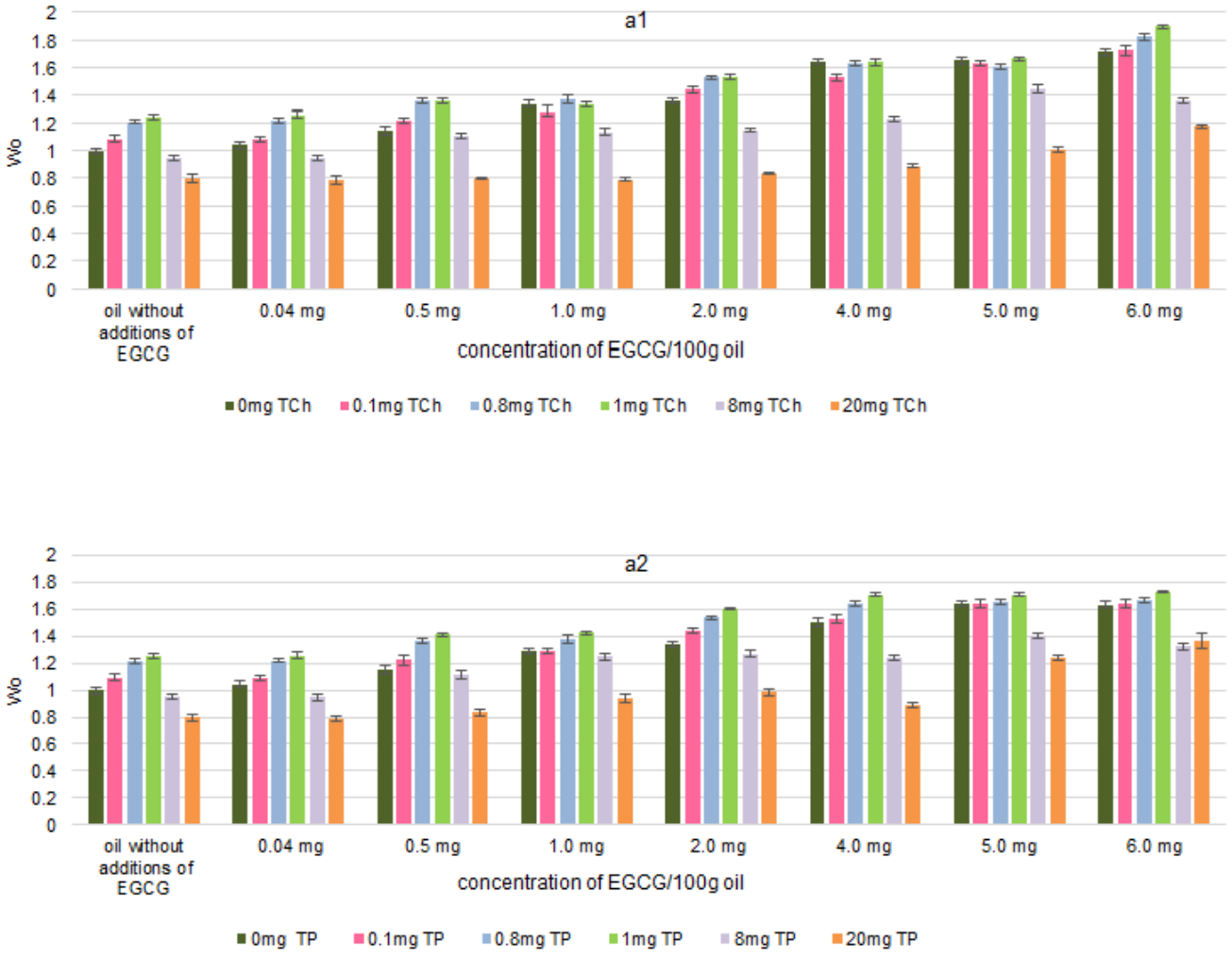

Figure 1. *Cont.*

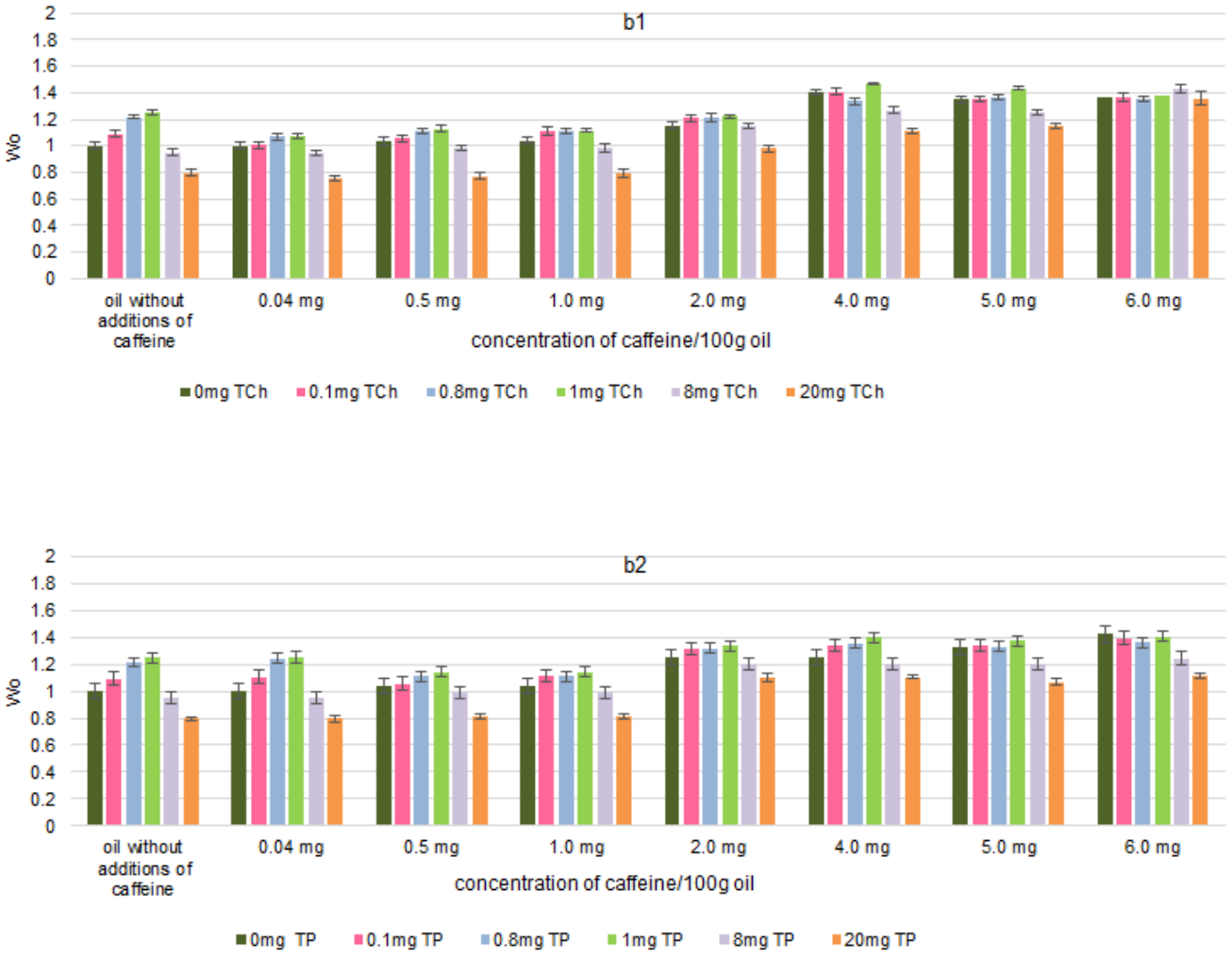

Figure 1. The protection factor (Wo) of EGCG (**a**) and caffeine (**b**) in the presence of thiamine hydrochloride (1) and thiamine pyrophosphate (2).

In the samples containing thiamine at concentration ranges of enriched products (above 1.0–20.0 mg/100 g), there was a statistically significant positive correlation between the indicators of primary (peroxide value) and secondary (anisidine value) oxidation products of soybean oil systems with the addition of EGCG or caffeine. A high correlation coefficient indicates a stronger correlation between the increased content of thiamine hydrochloride and thiamine pyrophosphate (over 1.0 mg/100 g) in the system and the reduced antioxidant activity of both EGCG and caffeine (Table 1). The addition of thiamine, at amounts above 1.0 and 20.0 mg/100 g, diminished the protection factor Wo (Figure 1, Tables S1–S12), defined as the time necessary for a particular sample to reach the peroxide value 50 (meq0_2/kg). For example, the analysis, for samples with thiamine concentrations of 0.1 mg/100 g, showed a protection factor on the highest level, while solutions containing the most thiamine (20 mg/100 g) showed a decrease in the protection factor on the lowest level (16–34%). A table containing all the remaining thiamine concentration data is included in the Supplementary Material Tables S1–S12. There was a decrease in the protection factor (Wo) observed for both EGCG and caffeine at these concentrations. The highest decrease in the protection factor was noted at a thiamine concentration of 20 mg/100 g and an EGCG or caffeine concentration of 4 mg/100 g. However, a significant decrease in the protection factor was noted at a thiamine concentration above 6 mg/100 g. It was found that the antioxidant activity of both EGCG and caffeine was also more vulnerable to thiamine pyrophosphate than thiamine hydrochloride. For example, the analysis, for samples with EGCG at 4 mg/100 g and a thiamine concentration of 20 mg/100 g, showed a decrease in

the protection factor on the level of 46% in the presence of thiamine hydrochloride, and on the level of 49% in the presence of thiamine pyrophosphate.

A lower correlation was found for samples with the addition of both EGCG and caffeine, especially at higher concentrations. The analysis showed that the higher the concentration of EGCG and caffeine was (above 5.0 mg/100 g), the lower the decrease was in the protection factor of both EGCG and caffeine in the presence of a high concentration of thiamine.

Table 1. Correlation coefficients between the Protection Factor (Wo) of EGCG and caffeine, and the content of thiamine hydrochloride and thiamine pyrophosphate.

Concentration [mg/100 g] EGCG/		Correlation Coefficient of Wo and Content of Thiamine Hydrochloride			
EGCG/ Caffeine	Thiamine Hydrochloride	Thiamine Hydrochloride		Thiamine Pyrophosphate	
		EGCG	Caffeine	EGCG	Caffeine
0.5 mg	0–0.06	0.178 ^{NS}	0.185 ^{NS}	0.111 ^{NS}	0.121 ^{NS}
	0.08–1.0	0.793 ***	0.889 ****	0.721 ***	0.865 ***
	1.0–20	−0.984 ****	−0.974 ****	−0.955 ****	−0.974 ****
1.0 mg	0–0.06	−0.162 ^{NS}	0.178 ^{NS}	−0.155 ^{NS}	0.098 ^{NS}
	0.08–1.0	0.771 ***	0.540 **	0.724 ***	0.540 **
	1.0–20	−0.901 ****	−0.887 ***	−0.905 ****	−0.965 ****
2.0 mg	0–0.06	−0.155 ^{NS}	0.171 ^{NS}	−0.121 ^{NS}	0.112 ^{NS}
	0.08–1.0	0.775 ***	0.560 **	0.722 ***	0.560 **
	1.0–20	−0.943 ****	−0.849 ***	−0.901 ****	−0.849 ***
3.0 mg	0–0.06	0.126 ^{NS}	−0.013 ^{NS}	0.115 ^{NS}	−0.018 ^{NS}
	0.08–1.0	0.677 **	−0.517 ***	0.621 ***	−0.514 ***
	1.0–20	−0.917 ****	−0.718 ***	−0.900 ****	−0.721 ***
4.0 mg	0–0.06	−0.170 ^{NS}	−0.013 ^{NS}	−0.056 ^{NS}	−0.021 ^{NS}
	0.08–1.0	0.647 **	−0.106 ^{NS}	0.633 **	−0.111 ^{NS}
	1.0–20	−0.851 ***	−0.612 **	−0.832 ***	−0.550 **
5.0 mg	0–0.06	−0.200 *	−0.032 ^{NS}	−0.130 ^{NS}	−0.032 ^{NS}
	0.08–1.0	−0.319 *	0.141 ^{NS}	−0.220 *	0.141 ^{NS}
	1.0–20	−0.726 ***	−0.443 **	−0.426 **	−0.463 **
6.0 mg	0–0.06	0.121 ^{NS}	0.108 ^{NS}	0.061 ^{NS}	0.055 ^{NS}
	0.08–1.0	0.348 *	−0.111 ^{NS}	0.167 ^{NS}	−0.111 ^{NS}
	1.0–20	−0.638 **	−0.349 *	−0.408 **	−0.249 *
Without additions	0–0.06	0.158 *		0.063 ^{NS}	
	0.08–1.0	0.724 ***		0.695 **	
	1.0–20	−0.881 ***		−0.851 ***	

**** linear relationship very strong; *** linear relationship significant; ** linear relationship moderate; * linear dependence weak; NS no linear relationship at: $p \leq 0.05$; $n = 14$.

3.2. Thiamine Stability

The total thiamine content in the samples was also measured. A considerable loss of thiamine was observed after 31 days of storage (Tables 2 and 3; Figure 2). It was found that the thiamine losses, among 31 days storage, followed a simple linear regression model (R^2 = 0.99–0.96) (Tables 2 and 3). There was a considerable loss of thiamine—from 10 to more than 21% for thiamine hydrochloride, and from 12 to more than 28% for thiamine pyrophosphate (Figure 2). It was found that the stability of thiamine depended on the forms of thiamine. A lower stability of thiamine was confirmed in the samples with thiamine pyrophosphate, regardless of the changing storage conditions. The analysis of all predictors' influence on thiamine changes (Tables 2 and 3) revealed that the type of active component and the initial thiamine and active component content significantly affected the loss of thiamine.

Table 2. Dynamic of changes in thiamine hydrochloride content (mg kg^{-1}) over 31 days in the presence of tea components. (EGCG and caffeine) and soybean oil shown as values of the half time of thiamine losses ($T_{1/2}$) and coefficients in regression equations.

Concentration [mg/100 g]		Dynamic of Change in Thiamine Content Over 31 Days									
		EGCG					Caffeine				
EGCG/ Caffeine	Thiamine	$T_{1/2}$ [Days]	R^2	RMSE	$Y = ax + b^{-1}$		$T_{1/2}$ [days]	R^2	RMSE	$Y = ax + b^{-1}$	
					Coeff. a 24 h^{-1}	b				Coeff. a 24 h^{-1}	b
0.5	0.01	79.65	0.99	0.00005	$-(4.1 \pm 0.01) \times 10^{-5}$	1.01 ± 0.001	71.18	0.99	0.00007	$-(8.2 \pm 0.00) \times 10^{-5}$	1.01 ± 0.000
	0.06	79.82	0.97	0.00026	$-(2.4 \pm 0.04) \times 10^{-4}$	1.06 ± 0.000	70.75	0.99	0.06255	$-(3.7 \pm 0.02) \times 10^{-4}$	1.06 ± 0.000
	0.1	80.92	0.97	0.02738	$-(1.3 \pm 0.03) \times 10^{-4}$	1.12 ± 0.001	69.53	0.98	0.00081	$-(6.2 \pm 0.02) \times 10^{-4}$	1.11 ± 0.000
	0.2	80.14	0.97	0.00088	$-(8.1 \pm 0.00) \times 10^{-4}$	1.22 ± 0.002	71.74	0.98	0.00140	$-(1.1 \pm 0.02) \times 10^{-3}$	1.23 ± 0.000
	1.0	82.86	0.97	0.07186	$-(3.6 \pm 0.01) \times 10^{-3}$	2.84 ± 0.001	75.23	0.98	0.10058	$-(5.0 \pm 0.02) \times 10^{-3}$	2.75 ± 0.000
	8.0	77.83	0.97	0.02467	$-(3.5 \pm 0.05) \times 10^{-2}$	2.99 ± 0.003	67.48	0.98	0.06913	$-(5.3 \pm 0.01) \times 10^{-2}$	3.12 ± 0.003
	16.0	74.88	0.96	0.20155	$-(8.4 \pm 0.04) \times 10^{-2}$	1.14 ± 0.007	63.34	0.98	0.14957	$-(1.2 \pm 0.03) \times 10^{-1}$	9.95 ± 0.006
	20.0	67.78	0.99	0.15864	$-(1.3 \pm 0.01) \times 10^{-1}$	4.77 ± 0.008	63.81	0.99	0.15143	$-(1.4 \pm 0.01) \times 10^{-1}$	4.47 ± 0.008
1.00	0.01	83.52	0.99	0.00006	$-(4.9 \pm 0.00) \times 10^{-5}$	1.01 ± 0.000	70.17	0.99	0.00008	$-(7.8 \pm 0.00) \times 10^{-5}$	1.01 ± 0.000
	0.06	80.38	0.97	0.00035	$-(2.3 \pm 0.07) \times 10^{-4}$	1.06 ± 0.000	72.42	0.98	0.00046	$-(3.4 \pm 0.01) \times 10^{-4}$	1.06 ± 0.000
	0.1	80.89	0.97	0.00032	$-(4.2 \pm 0.01) \times 10^{-4}$	1.10 ± 0.000	70.56	0.99	0.00383	$-(5.8 \pm 0.06) \times 10^{-4}$	1.10 ± 0.000
	0.2	82.37	0.97	0.00055	$-(7.5 \pm 0.05) \times 10^{-4}$	1.25 ± 0.001	71.90	0.98	0.00149	$-(1.2 \pm 0.02) \times 10^{-3}$	1.25 ± 0.000
	1.0	82.98	0.97	0.08374	$-(4.2 \pm 0.07) \times 10^{-3}$	2.82 ± 0.000	75.03	0.98	0.10009	$-(5.0 \pm 0.03) \times 10^{-3}$	2.74 ± 0.000
	8.0	78.85	0.97	0.02443	$-(3.5 \pm 0.00) \times 10^{-2}$	2.98 ± 0.003	70.64	0.98	0.06017	$-(4.8 \pm 0.04) \times 10^{-2}$	3.30 ± 0.003
	16.0	69.75	0.96	0.17299	$-(9.1 \pm 0.07) \times 10^{-2}$	8.98 ± 0.006	63.68	0.97	0.18432	$-(1.1 \pm 0.03) \times 10^{-1}$	7.48 ± 0.006
	20.0	74.89	0.99	0.15937	$-(1.3 \pm 0.07) \times 10^{-1}$	5.94 ± 0.008	63.16	0.96	0.29129	$-(1.4 \pm 0.04) \times 10^{-1}$	4.23 ± 0.008
3.00	0.01	83.52	0.98	0.00003	$-(4.3 \pm 0.00) \times 10^{-5}$	1.01 ± 0.001	69.78	0.97	0.00011	$-(6.5 \pm 0.00) \times 10^{-5}$	1.01 ± 0.000
	0.06	80.38	0.99	0.00046	$-(2.4 \pm 0.01) \times 10^{-4}$	1.06 ± 0.000	69.86	0.98	0.00056	$-(3.7 \pm 0.02) \times 10^{-4}$	1.06 ± 0.000
	0.1	80.89	0.98	0.08285	$-(3.8 \pm 0.05) \times 10^{-4}$	1.10 ± 0.000	69.80	0.98	0.00085	$-(6.6 \pm 0.04) \times 10^{-4}$	1.12 ± 0.000
	0.2	82.37	0.98	0.00074	$-(7.8 \pm 0.07) \times 10^{-4}$	1.25 ± 0.001	70.59	0.97	0.00234	$-(1.3 \pm 0.04) \times 10^{-4}$	1.25 ± 0.000
	1.0	82.98	0.99	0.06827	$-(3.5 \pm 0.06) \times 10^{-3}$	2.82 ± 0.000	72.51	0.97	0.11669	$-(5.9 \pm 0.03) \times 10^{-3}$	2.99 ± 0.000
	8.0	78.85	0.96	0.03103	$-(3.3 \pm 0.02) \times 10^{-2}$	2.98 ± 0.003	72.35	0.97	0.07762	$-(4.3 \pm 0.03) \times 10^{-2}$	2.81 ± 0.003
	16.0	69.75	0.96	0.18356	$-(9.6 \pm 0.01) \times 10^{-2}$	8.98 ± 0.006	70.40	0.99	0.08552	$-(9.5 \pm 0.03) \times 10^{-2}$	8.87 ± 0.006
	20.0	74.89	0.98	0.16432	$-(1.0 \pm 0.04) \times 10^{-1}$	5.94 ± 0.008	63.93	0.98	0.19509	$-(1.4 \pm 0.01) \times 10^{-1}$	4.79 ± 0.008
5.00	0.01	72.49	0.98	0.00011	$-(8.2 \pm 0.02) \times 10^{-5}$	1.02 ± 0.000	71.55	0.99	0.00013	$-(1.2 \pm 0.00) \times 10^{-4}$	1.02 ± 0.000
	0.06	72.79	0.99	0.00040	$-(3.3 \pm 0.03) \times 10^{-4}$	1.06 ± 0.000	71.87	0.98	0.00064	$-(3.8 \pm 0.02) \times 10^{-4}$	1.06 ± 0.000
	0.1	72.87	0.98	0.00076	$(5.9 \pm 0.01) \times 10^{-4}$	1.12 ± 0.000	71.83	0.98	0.00105	$-(8.0 \pm 0.02) \times 10^{-4}$	1.13 ± 0.000
	0.2	73.60	0.98	0.00149	$-(1.1 \pm 0.00) \times 10^{-4}$	1.25 ± 0.001	73.58	0.99	0.00202	$-(1.4 \pm 0.02) \times 10^{-3}$	1.24 ± 0.000
	1.0	74.94	0.99	0.10646	$-(5.4 \pm 0.04) \times 10^{-3}$	2.99 ± 0.000	72.18	0.98	0.13223	$-(6.7 \pm 0.02) \times 10^{-3}$	3.00 ± 0.000

Table 2. *Cont.*

Concentration [mg/100 g]		Dynamic of Change in Thiamine Content Over 31 Days									
		EGCG					Caffeine				
EGCG/ Caffeine	Thiamine	$T_{1/2}$ [Days]	R^2	RMSE	$Y = ax + b^{-1}$		$T_{1/2}$ [days]	R^2	RMSE	$Y = ax + b^{-1}$	
					Coeff. a 24 h^{-1}	b				Coeff. a 24 h^{-1}	b
5.00	8.0	75.96	0.96	0.07356	$-(3.8 \pm 0.09) \times 10^{-2}$	2.86 ± 0.003	69.13	0.98	0.04546	$-(5.5 \pm 0.01) \times 10^{-2}$	3.01 ± 0.003
	16.0	69.70	0.98	0.56298	$-(3.6 \pm 0.05) \times 10^{-2}$	3.17 ± 0.007	68.15	0.98	0.16410	$-(1.1 \pm 0.03) \times 10^{-1}$	8.67 ± 0.006
	20.0	69.59	0.98	0.15284	$-(1.2 \pm 0.01) \times 10^{-1}$	4.48 ± 0.008	64.05	0.98	0.19239	$-(1.5 \pm 0.01) \times 10^{-1}$	4.55 ± 0.008
6.00	0.01	71.49	0.97	0.00015	$-(9.5 \pm 0.05) \times 10^{-5}$	1.02 ± 0.001	63.52	0.99	0.00013	$-(1.2 \pm 0.03) \times 10^{-4}$	1.02 ± 0.000
	0.06	72.00	0.99	0.00030	$-(3.3 \pm 0.03) \times 10^{-4}$	1.06 ± 0.001	68.94	0.97	0.00064	$-(3.8 \pm 0.01) \times 10^{-4}$	1.06 ± 0.000
	0.1	72.62	0.98	0.00082	$(6.4 \pm 0.05) \times 10^{-4}$	1.13 ± 0.001	66.42	0.98	0.00105	$-(8.0 \pm 0.01) \times 10^{-4}$	1.13 ± 0.000
	0.2	72.01	0.98	0.00155	$-(1.2 \pm 0.03) \times 10^{-4}$	1.25 ± 0.001	67.44	0.98	0.00202	$-(1.4 \pm 0.01) \times 10^{-3}$	1.24 ± 0.000
	1.0	73.75	0.99	0.10723	$-(5.4 \pm 0.04) \times 10^{-3}$	2.83 ± 0.001	69.37	0.98	0.13223	$-(6.7 \pm 0.05) \times 10^{-3}$	3.00 ± 0.000
	8.0	78.63	0.99	0.03904	$-(3.4 \pm 0.03) \times 10^{-2}$	2.97 ± 0.003	65.57	0.99	0.04546	$-(5.5 \pm 0.01) \times 10^{-2}$	3.01 ± 0.003
	16.0	73.21	0.98	0.12157	$-(8.4 \pm 0.05) \times 10^{-2}$	7.91 ± 0.006	62.94	0.98	0.16410	$-(1.2 \pm 0.06) \times 10^{-1}$	8.67 ± 0.006
	20.0	70.83	0.97	0.18008	$-(1.1 \pm 0.04) \times 10^{-1}$	4.43 ± 0.008	61.17	0.98	0.19239	$-(1.5 \pm 0.04) \times 10^{-1}$	4.55 ± 0.008
Without additions	0.01	68.16	0.97	0.00011	$-(6.0 \pm 0.00) \times 10^{-5}$	1.01 ± 0.000					
	0.06	68.53	0.97	0.00063	$-(3.4 \pm 0.01) \times 10^{-4}$	1.06 ± 0.000					
	0.1	69.66	0.97	0.00092	$(5.2 \pm 0.03) \times 10^{-4}$	1.09 ± 0.001					
	0.2	70.62	0.97	0.00201	$-(8.1 \pm 0.03) \times 10^{-4}$	1.22 ± 0.000					
	1.0	67.78	0.97	0.11460	$-(1.1 \pm 0.02) \times 10^{-3}$	2.77 ± 0.000					
	8.0	65.00	0.97	0.08939	$-(6.3 \pm 0.03) \times 10^{-2}$	2.70 ± 0.003					
	16.0	61.82	0.96	0.23382	$-(1.1 \pm 0.03) \times 10^{-2}$	5.45 ± 0.006					
	20.0	57.64	0.99	0.19417	$-(1.6 \pm 0.02) \times 10^{-1}$	3.40 ± 0.008					

Simple linear regression equation y = ax + b (the linear least-squares method; y—dependent variable; x—independent variable; a—independent variable coeff./slope of the line; b—intercept). Coeff. a/24 h—change of coeff. a in 24 h storage time. R^2—coeff. of determination (the square of correlation coefficient *r*). The coeff. A/24 h shows the dynamics of changes in thiamine content. Half time $T_{1/2}$ (in days) is the time in which the initial thiamine content decreases by half.

Table 3. Dynamic of changes in thiamine pyrophosphate content (mg kg^{-1}) over 31 days in the presence of tea components. (EGCG and caffeine) and soybean oil shown as values of the half time of thiamine losses ($T_{1/2}$) and coefficients in regression equations.

Concentration [mg/100 g]		Dynamic of Change in Thiamine Content Over 31 Days									
		EGCG					Caffeine				
EGCG/ Caffeine	Thiamine	$T_{1/2}$ [Days]	R^2	RMSE	$Y = ax + b^{-1}$		$T_{1/2}$ [days]	R^2	RMSE	$Y = ax + b^{-1}$	
					Coeff. a 24 h^{-1}	b				Coeff. a 24 h^{-1}	b
0.5	0.01	78.91	0.99	0.00004	$-(4.1 \pm 0.04) \times 10^{-5}$	1.01 ± 0.000	71.43	0.99	0.00006	$-(8.0 \pm 0.03) \times 10^{-5}$	1.01 ± 0.000
	0.06	78.83	0.97	0.00014	$-(2.4 \pm 0.03) \times 10^{-4}$	1.06 ± 0.000	70.32	0.98	0.06249	$-(3.7 \pm 0.00) \times 10^{-4}$	1.06 ± 0.000
	0.1	80.27	0.97	0.00003	$-(4.6 \pm 0.02) \times 10^{-5}$	1.01 ± 0.000	68.28	0.99	0.00055	$-(6.5 \pm 0.05) \times 10^{-4}$	1.11 ± 0.000
	0.2	79.62	0.97	0.00055	$-(7.8 \pm 0.07) \times 10^{-4}$	1.22 ± 0.000	69.66	0.99	0.00095	$-(1.3 \pm 0.05) \times 10^{-3}$	1.23 ± 0.000
	1.0	82.14	0.97	0.07093	$-(3.6 \pm 0.01) \times 10^{-3}$	2.81 ± 0.000	73.85	0.98	0.10240	$-(5.2 \pm 0.04) \times 10^{-3}$	2.74 ± 0.000
	8.0	76.39	0.97	0.03813	$-(3.7 \pm 0.01) \times 10^{-2}$	2.90 ± 0.003	66.39	0.99	0.04389	$-(5.5 \pm 0.04) \times 10^{-2}$	3.16 ± 0.003
	16.0	75.46	0.96	0.05601	$-(7.7 \pm 0.08) \times 10^{-2}$	8.46 ± 0.006	61.76	0.99	0.09857	$-(1.2 \pm 0.03) \times 10^{-1}$	9.27 ± 0.006
	20.0	65.38	0.99	0.15683	$-(1.4 \pm 0.00) \times 10^{-1}$	5.42 ± 0.008	59.36	1.00	0.10918	$-(1.6 \pm 0.01) \times 10^{-1}$	4.70 ± 0.008
1.00	0.01	79.94	0.99	0.00006	$-(5.1 \pm 0.01) \times 10^{-5}$	1.01 ± 0.000	69.34	0.99	0.00007	$-(8.2 \pm 0.05) \times 10^{-5}$	1.01 ± 0.000
	0.06	80.18	0.97	0.00035	$-(2.3 \pm 0.01) \times 10^{-4}$	1.06 ± 0.000	72.11	0.98	0.00047	$-(3.5 \pm 0.03) \times 10^{-4}$	1.06 ± 0.000
	0.1	79.85	0.97	0.00043	$-(4.3 \pm 0.04) \times 10^{-4}$	1.12 ± 0.000	69.76	0.99	0.00281	$-(6.0 \pm 0.02) \times 10^{-4}$	1.10 ± 0.000
	0.2	80.14	0.97	0.00104	$-(7.1 \pm 0.01) \times 10^{-4}$	1.22 ± 0.000	70.47	0.99	0.00140	$-(1.3 \pm 0.01) \times 10^{-3}$	1.25 ± 0.000
	1.0	78.57	0.97	0.08723	$-(4.5 \pm 0.02) \times 10^{-3}$	2.82 ± 0.000	73.73	0.99	0.10627	$-(5.3 \pm 0.02) \times 10^{-3}$	2.75 ± 0.000
	8.0	76.43	0.97	0.03620	$-(3.7 \pm 0.00) \times 10^{-2}$	2.90 ± 0.003	70.34	0.98	0.05975	$-(4.8 \pm 0.01) \times 10^{-2}$	3.20 ± 0.003
	16.0	73.21	0.96	0.12450	$-(8.6 \pm 0.04) \times 10^{-2}$	9.07 ± 0.006	60.98	0.99	0.14877	$-(1.2 \pm 0.02) \times 10^{-1}$	8.23 ± 0.006
	20.0	65.85	0.99	0.17048	$-(1.3 \pm 0.04) \times 10^{-1}$	4.45 ± 0.008	59.90	0.98	0.19947	$-(1.6 \pm 0.00) \times 10^{-1}$	4.94 ± 0.008
3.00	0.01	81.08	0.98	0.00002	$-(4.6 \pm 0.05) \times 10^{-5}$	1.01 ± 0.000	66.96	0.99	0.00008	$-(7.2 \pm 0.05) \times 10^{-5}$	1.01 ± 0.000
	0.06	77.23	0.97	0.00017	$-(2.8 \pm 0.01) \times 10^{-4}$	1.06 ± 0.000	67.09	0.99	0.00041	$-(4.1 \pm 0.01) \times 10^{-4}$	1.06 ± 0.000
	0.1	78.00	0.97	0.00043	$-(4.3 \pm 0.00) \times 10^{-4}$	1.10 ± 0.000	67.52	1.00	0.00044	$-(7.2 \pm 0.04) \times 10^{-4}$	1.12 ± 0.000
	0.2	79.71	0.97	0.00070	$-(8.8 \pm 0.04) \times 10^{-4}$	1.25 ± 0.000	67.90	0.99	0.00154	$-(1.4 \pm 0.00) \times 10^{-3}$	1.25 ± 0.000
	1.0	79.80	0.97	0.08040	$-(4.1 \pm 0.04) \times 10^{-3}$	2.81 ± 0.000	69.48	0.99	0.13142	$-(6.7 \pm 0.05) \times 10^{-3}$	2.99 ± 0.000
	8.0	74.90	0.97	0.06003	$-(3.9 \pm 0.01) \times 10^{-2}$	2.91 ± 0.003	69.69	0.99	0.05405	$-(4.8 \pm 0.01) \times 10^{-2}$	2.90 ± 0.003
	16.0	72.40	0.96	0.13497	$-(8.6 \pm 0.04) \times 10^{-2}$	8.47 ± 0.006	68.19	1.00	0.06643	$-(1.0 \pm 0.01) \times 10^{-1}$	8.57 ± 0.006
	20.0	72.22	0.99	0.15062	$-(1.1 \pm 0.04) \times 10^{-1}$	4.13 ± 0.008	61.38	0.99	0.11964	$-(1.5 \pm 0.02) \times 10^{-1}$	4.99 ± 0.008
5.00	0.01	69.98	0.99	0.00008	$-(8.9 \pm 0.00) \times 10^{-5}$	1.02 ± 0.000	70.17	0.99	0.00011	$-(1.0 \pm 0.00) \times 10^{-4}$	1.02 ± 0.000
	0.06	70.35	0.97	0.00031	$-(3.6 \pm 0.03) \times 10^{-4}$	1.06 ± 0.000	70.18	0.99	0.00044	$-(3.7 \pm 0.00) \times 10^{-4}$	1.06 ± 0.000
	0.1	70.38	0.97	0.00057	$-(6.4 \pm 0.04) \times 10^{-4}$	1.12 ± 0.000	69.71	0.99	0.00068	$-(7.3 \pm 0.04) \times 10^{-4}$	1.13 ± 0.000
	0.2	71.17	0.97	0.00114	$-(1.2 \pm 0.03) \times 10^{-3}$	1.25 ± 0.000	71.30	0.99	0.00127	$-(1.2 \pm 0.03) \times 10^{-3}$	1.25 ± 0.000
	1.0	72.59	0.97	0.11652	$-(5.9 \pm 0.02) \times 10^{-3}$	2.99 ± 0.000	69.15	1.00	0.13277	$-(6.7 \pm 0.03) \times 10^{-3}$	3.00 ± 0.000

Table 3. *Cont.*

Concentration [mg/100 g]		Dynamic of Change in Thiamine Content Over 31 Days									
		EGCG					Caffeine				
EGCG/ Caffeine	Thiamine	$T_{1/2}$ [Days]	R^2	RMSE	$Y = ax + b^{-1}$		$T_{1/2}$ [days]	R^2	RMSE	$Y = ax + b^{-1}$	
					Coeff. a 24 h^{-1}	b				Coeff. a 24 h^{-1}	b
5.00	8.0	72.98	0.97	0.04316	$-(4.3 \pm 0.01) \times 10^{-2}$	2.89 ± 0.003	66.62	1.00	0.03185	$-(5.4 \pm 0.02) \times 10^{-2}$	3.10 ± 0.003
	16.0	67.19	0.96	0.12365	$-(1.1 \pm 0.04) \times 10^{-1}$	8.65 ± 0.006	65.18	1.00	0.07008	$-(1.1 \pm 0.06) \times 10^{-1}$	8.76 ± 0.006
	20.0	66.68	0.99	0.10806	$-(1.3 \pm 0.02) \times 10^{-1}$	4.44 ± 0.008	60.98	1.00	0.08624	$-(1.6 \pm 0.05) \times 10^{-1}$	5.00 ± 0.008
6.00	0.01	68.60	0.99	0.00012	$-(1.0 \pm 0.02) \times 10^{-4}$	1.02 ± 0.000	61.69	0.99	0.00011	$-(1.3 \pm 0.00) \times 10^{-4}$	1.02 ± 0.000
	0.06	69.52	0.97	0.00030	$-(3.6 \pm 0.00) \times 10^{-4}$	1.06 ± 0.000	67.69	0.97	0.00064	$-(3.9 \pm 0.04) \times 10^{-4}$	1.06 ± 0.000
	0.1	70.16	0.97	0.00072	$-(7.0 \pm 0.01) \times 10^{-4}$	1.13 ± 0.000	64.27	1.00	0.00050	$-(8.7 \pm 0.04) \times 10^{-4}$	1.13 ± 0.000
	0.2	69.66	0.97	0.00111	$-(1.3 \pm 0.03) \times 10^{-3}$	1.25 ± 0.000	65.31	0.99	0.00125	$-(1.5 \pm 0.03) \times 10^{-3}$	1.25 ± 0.000
	1.0	71.61	0.97	0.11489	$-(5.8 \pm 0.02) \times 10^{-3}$	2.82 ± 0.000	67.38	0.99	0.14344	$-(7.2 \pm 0.03) \times 10^{-3}$	3.01 ± 0.000
	8.0	76.49	0.97	0.04143	$-(3.7 \pm 0.02) \times 10^{-2}$	2.91 ± 0.003	63.96	1.00	0.03639	$-(5.8 \pm 0.00) \times 10^{-2}$	3.08 ± 0.003
	16.0	70.98	0.96	0.14023	$-(9.0 \pm 0.01) \times 10^{-2}$	7.68 ± 0.006	60.69	0.99	0.10329	$-(1.2 \pm 0.00) \times 10^{-1}$	9.54 ± 0.006
	20.0	67.68	0.99	0.14243	$-(1.3 \pm 0.02) \times 10^{-1}$	4.52 ± 0.008	58.86	1.00	0.11489	$-(1.7 \pm 0.04) \times 10^{-1}$	5.13 ± 0.008
Without additions	0.01	63.89	0.97	0.00028	$-(6.5 \pm 0.01) \times 10^{-5}$	1.01 ± 0.000					
	0.06	63.98	0.96	0.00077	$-(3.7 \pm 0.04) \times 10^{-4}$	1.06 ± 0.000					
	0.1	65.75	0.98	0.00082	$-(6.4 \pm 0.03) \times 10^{-4}$	1.09 ± 0.000					
	0.2	66.77	0.97	0.00190	$-(1.3 \pm 0.04) \times 10^{-3}$	1.22 ± 0.000					
	1.0	63.56	0.96	0.13237	$-(6.5 \pm 0.04) \times 10^{-3}$	2.79 ± 0.000					
	8.0	60.88	0.96	0.11588	$-(6.3 \pm 0.02) \times 10^{-2}$	2.83 ± 0.003					
	16.0	52.14	0.96	0.28795	$-(1.3 \pm 0.02) \times 10^{-1}$	6.53 ± 0.006					
	20.0	53.76	0.97	0.27690	$-(1.8 \pm 0.04) \times 10^{-1}$	3.57 ± 0.008					

Abbreviation as in Table 1.

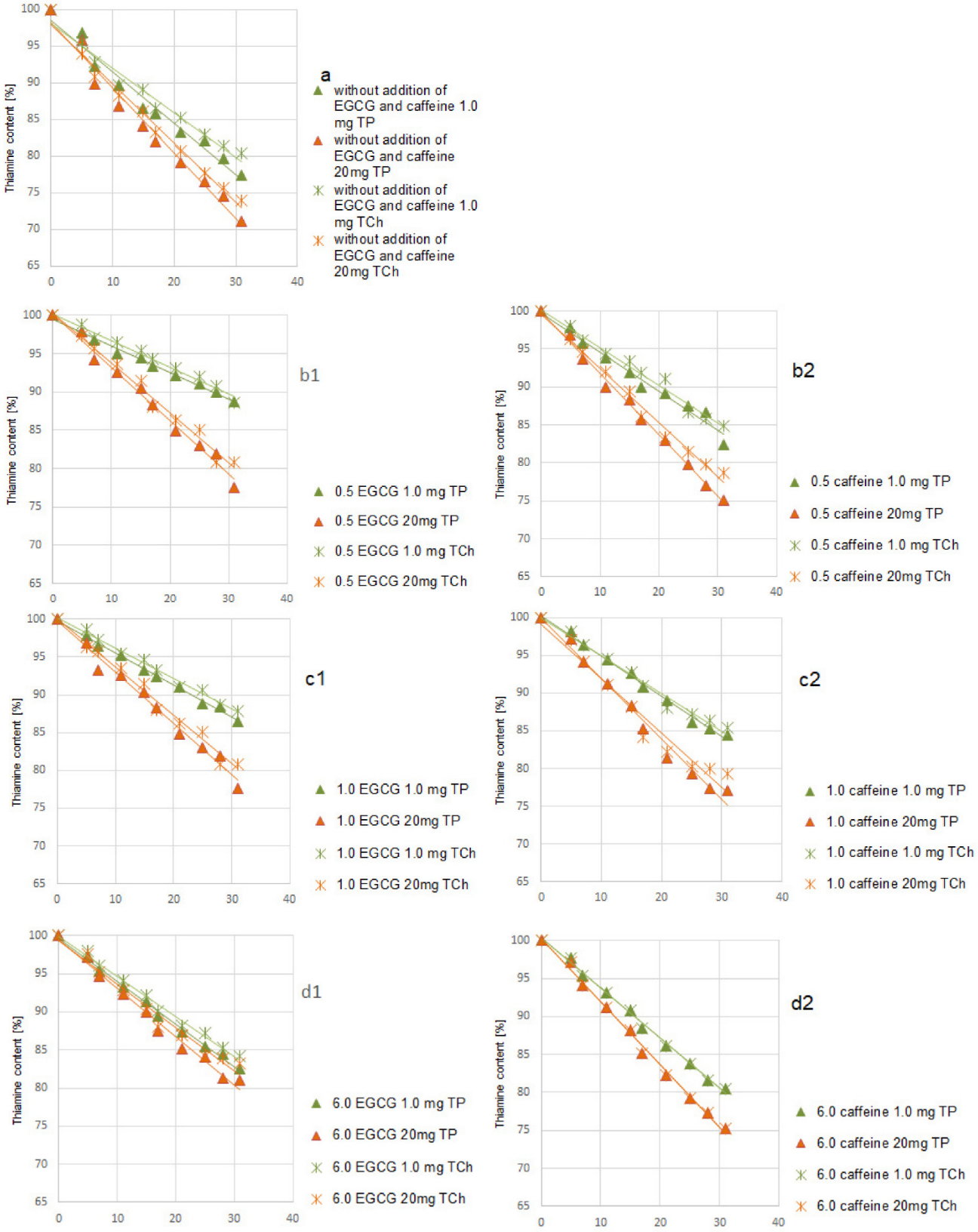

Figure 2. Contents of thiamine pyrophosphate (TP) and thiamine hydrochloride (TCh) in soybean oil among storage at 30°C in the presence of EGCG (1) and caffeine (2) at concentrations of 0.5 mg/100 g (**b**), 1.0 mg/100 g (**c**), and 6.0 mg/100 g (**d**) and without the addition of caffeine and EGCG (**a**).

The analysis of the dynamics of changes in values of thiamine hydrochloride and thiamine pyrophosphate content (Tables 2 and 3) showed the highest dynamics of thiamine loss with oil and without additions of active components (EGCG or caffeine). The highest losses of thiamine, both thiamine hydrochloride and thiamine pyrophosphate, with higher thiamine concentrations—exceeding 8 mg/100 g—were found.

The analysis of thiamine stability in model oil systems containing tea ingredients showed the protective effect of both EGCG and caffeine. However, the protective effect of EGCG toward thiamine was found to be higher than in the case of caffeine. It was found that the half-life $T_{1/2}$ of thiamine values showed lower dynamics of thiamine loss with EGCG than caffeine. The thiamine half-lives $T_{(1/2)}$ in the presence of caffeine were up to 20% times shorter than they were in the presence of EGCG.

The losses of thiamine with EGCG were situated in the range of 10–20% and with caffeine of 12–23%.

There was a strong negative correlation between the stability of thiamine and the instability of oil (Table 4). The analysis showed that the higher the value of fat oxidation was, the higher the losses of thiamine were. Thiamine was particularly sensitive to secondary oxidation products (Table 4).

Table 4. Correlation coefficients between thiamine hydrochloride or thiamine pyrophosphate stability and peroxide value. The Anisidine Value and Protection Factor of AGCG and caffeine.

Concentration of EGCG or Caffeine [mg/100 g]	EGCG or Caffeine	Correlation Coefficients between Thiamine Hydrochloride Stability and Value of Antioxidant Activity		
		Peroxide Value	Anisidine Value	Protection Factor
Thiamine Hydrochloride				
Without Additions		−0.930 ****	−0.970 ****	0.929 ****
0.5 mg	EGCG	−0.901 ****	−0.947 ****	0.945 ****
	caffeine	−0.922 ****	−0.939 ****	0.886 ***
1.0 mg	EGCG	−0.908 ****	−0.949 ****	0.931 ****
	caffeine	−0.884 ***	−0.886 ***	0.904 ****
2.0 mg	EGCG	−0.949 ****	−0.984 ****	0.989 ****
	caffeine	−0.761 ***	−0.750 ***	0.778 ***
3.0 mg	EGCG	−0.947 ****	−0.950 ****	0.978 ****
	caffeine	−0.431 **	−0.397 *	0.574 **
4.0 mg	EGCG	−0.908 ****	−0.927 ****	0.930 ****
	caffeine	−0.278 *	−0.245 *	0.355 *
5.0 mg	EGCG	−0.880 ***	−0.890 ***	0,892 ***
	caffeine	−0.261 *	−0.231 *	0.360 **
6.0 mg	EGCG	−0.780 ***	−0.801 ***	0,804 ***
	caffeine	−0.440 **	−0.423 **	0.274 *
Thiamine Pyrophosphate				
Without Additions		−0.940 ****	−0.992 ****	0.991 ****
0.5 mg	EGCG	−0.981 ****	−0.947 ****	1.000 ****
	caffeine	−0.922 ****	−0.939 ****	0.936 ****
1.0 mg	EGCG	−0.998 ****	−0.990 ****	1.000 ****
	caffeine	−0.884 ***	−0.886 ***	0.904 ****
2.0 mg	EGCG	−0.999 ****	−0.984 ****	0.999 ****
	caffeine	−0.761 ***	−0.750 ***	0.778 ***

Table 4. *Cont.*

Concentration of EGCG or Caffeine [mg/100 g]	EGCG or Caffeine	Correlation Coefficients between Thiamine Hydrochloride Stability and Value of Antioxidant Activity		
		Peroxide Value	Anisidine Value	Protection Factor
3.0 mg	EGCG	−0.998 ****	−0.990 ****	0.998 ****
	caffeine	−0.431 **	−0.397 *	0.574 **
4.0 mg	EGCG	−0.998 ****	−0.987 ****	0.998 ****
	caffeine	−0.278 *	−0.245 *	0.355 *
5.0 mg	EGCG	−0.858 ***	−0.860 ***	0.861 ***
	caffeine	−0.261 *	−0.231 *	0.360 **
6.0 mg	EGCG	−0.812 ****	−0.803 ***	0.808 ****
	caffeine	−0.400 **	−0.403 **	0.278 *

**** linear relationship very strong; *** linear relationship significant; ** linear relationship moderate; * linear dependence weak; NS no linear relationship at: $p \leq 0.05$; $n = 14$.

The addition of EGCG or caffeine increased fat stability and thus reduced thiamine losses. However, this depended on the type of the tea component and its concentration. It was found that the greatest protective effect was achieved with EGCG.

The best results for thiamine hydrochloride stability (between 80% and 90%) in the presence of EGCG were observed in the concentration regions of 0.04–3 mg thiamine/100 g and 3.0–4.0 mg EGCG/100g. Moreover, the half-life $T_{1/2}$ of thiamine values showed lower dynamics of thiamine loss for these concentrations of EGCG. The thiamine content in these systems containing thiamine hydrochloride and EGCG below 4 mg/100 g was strongly correlated with the highest antioxidant activity of EGCG. However, this correlation was already lower in systems with EGCG concentration exceeding 4 mg/100 g. In these samples, the oxidative activity of EGCG was the highest, but the losses of thiamine were also higher.

The statistical analysis ($T_{1/2}$) of thiamine hydrochloride in the presence of caffeine showed also a lower thiamine loss than that of the samples without the addition of active components. A protective effect of caffeine toward thiamine was also found, although this effect was lower than that of EGCG. However, the size and the direction of these relationships depended also on the concentration level of caffeine. The thiamine content in the systems containing thiamine hydrochloride and caffeine was less correlated with the antioxidant activity of caffeine than EGCG was (Table 4). Only at low concentrations of caffeine (to 2.0 mg/100 g) was a stronger correlation with thiamine stability and with antioxidant activity found. The lowest correlation for thiamine hydrochloride stability and antioxidant activity of caffeine was observed at concentrations exceeding 4 mg/100 g. In these samples, the losses of thiamine were high, despite the antioxidative activity of caffeine.

4. Discussion

The results of our study are consistent with the findings of earlier research on the antioxidant activity of tea components [35,44,52,53]. The antioxidant activity of green, oolong, and white tea has been shown [44,54–56]. It has also been found that caffeine is an antioxidant capable of preventing lipid peroxidation [57,58]. Other research has confirmed the relationship between the oxidative stability of sunflower oil and the caffeine content and antioxidant activities [59]. Previous studies have also shown that the peroxide anion binding efficiency of tea extracts decreased in the following order: oolong tea > green tea > black tea [60–63]. Our present studies confirmed higher antioxidant activities for EGCG than caffeine. Previous research has also revealed that the antioxidant activity of black tea is slower than that of all the others, and the high antioxidant activity of green teas has been correlated with a high EGCG concentration [64]. It has been confirmed that epigallocatechin gallate is the dominant antioxidant compound in the tea extracts [65–68].

Our research confirmed a reduction in the antioxidant activity of EGCG and caffeine in the samples containing thiamine at high concentrations (above 8.0 mg/100 g). Moreover, earlier studies have shown the highest correlation coefficient between the high concentration of thiamine and the decrease of the antioxidant activity of green and white tea extract [43,44,48,69]. This may be due to the fact that that the antioxidant efficacy of catechins, including EGCG, depends not only on the chemical structure but also on the environmental conditions [70]. Studies show that catechins achieve the highest stability at a pH ranging between 4 and 6 [34]. Reactions such as oxidation and/or polymerization, even for a pH above 6.0, have been observed in other research [71].

Previous research has confirmed that green tea polyphenols accelerate pro-oxidant reactions depending on experimental conditions. The differences between those studies may be associated with the pH of the solutions and the concentrations of thiamine. Other studies found that the pH ranged from 5.36 to 6.96 due to the range of thiamine concentrations studied: 1 and 27 mg/mL, respectively [70,71]. Results of the theoretical studies of bond dissociation energy (BDE) have also shown the possible formation of a complex of thiamine and epigallocatechin gallate. The enthalpy of formation of this complex is 5.8 and 5.4 kcal/mol. The enthalpy of the BDE complex increases and hydrogen-bonded thiamine with EGCG reduces the antioxidant activity of EGCG. The bond dissociation energy increases and OH groups of the complex are less available and less effective as hydrogen donors [43,44,72].

When analyzing the stability of thiamine, in the course of 31 days of storage, it was found that thiamine in the form of pyrophosphate exhibits a lower stability. This is confirmed by previous studies related to both high-temperature processing and storage [9,11]. This could be explained by the fact that a smaller amount of activation energy is required to break down the thiazole ring of thiamine [7,15,73,74].

Our studies found that the thiamine losses followed a simple linear regression model. This was confirmed by the coefficient of determination (R^2 = 0.99–0.96) and the root means square error (RMSE). The degradation patterns were consistent with those reported in previous studies [73,75]. The previous research confirmed that the degradation of thiamine followed a simple linear regression model and ended when the samples had approximately 40% thiamine.

The highest losses of thiamine were found with thiamine concentrations above 16 mg/100 g. Previous studies confirmed a higher pH range in a pediatric formulation containing high amounts of thiamine [70,71]. Thiamine is stable under acidic conditions but is labile in alkaline conditions with the opening of the thiazole ring and yields the thiol form. At low pH, thiamine is present with a positive charge on both the pyrimidine N1 nitrogen (pKa1 $\approx$ 4.8) and thiazole N_3 nitrogen. At a pH above 6.0, thiamine is a cation with a positive charge on the thiazole N_3 nitrogen. With a further increase in pH, its behavior is complex, passing through an uncharged pseudobase intermediate to yield its negatively charged thiol form (pKa2 $\approx$ 9.2) [7,73].

Our research showed a correlation between the stability of thiamine and the instability of oil. Previous research has also confirmed the relationship between fat oxidation and thiamine losses [44]. Our studies also confirmed a higher instability of thiamine in the presence of oxidation products, especially secondary products [43]. The earlier research results have also shown the high instability of thiamine in the presence of fat and that the addition of high-oxidized fat accelerated the total thiamine losses, when compared to low-oxidized fat, both after thermal processing and the storage of meat, e.g., chicken meat [50]. The earlier research results have shown that the oxidation transformation of thiamine proceeds via two independent pathways: with ($2H^+ + 2e^-$) splitting from the NH_2 group of the pyrimidine ring with the formation of tricyclic thiamine, which is then converted into thiochrome, and with the opening of the thiazole ring and the loss of HS [74,76,77].

Our results showed that the addition of tea components, especially EGCG, increased the oxidative stability of fat and thus reduced the loss of thiamine. Earlier studies also

indicate the existence of certain correlations between the activity of antioxidants and the content of thiamine [43,44].

Research results have shown a strong positive correlation between the instability of fat and a lower stability of thiamine [40,78]. It was found that the losses of thiamine hydrochloride during storage are correlated with the production of fat oxidation products, in particular with the content of the secondary products of that process. This correlation may be connected with thiamine sensitivity to redox factors that open its thiazole ring [79,80].

A higher loss of thiamine was observed in the samples with caffeine. These samples were characterized by the lowest correlation between the antioxidant activity of caffeine and thiamine stability. At low concentrations of tea components (0.08 and 0.8 mg/100 g), caffeine is in a stronger correlation with thiamine than EGCG. These results indicate that caffeine may have formed complexes with the thiamine forms. Earlier theoretical studies showed the possible formation of hydrogen-bonded complexes of thiamine with myricetin [79,81]. There were higher losses of thiamine in the samples containing EGCG at amounts exceeding 5 mg/100 g. Moreover, EGCG may have formed complexes with thiamine. Preliminary research has confirmed the possible formation of a complex of epigallocatechin gallate and thiamine [72]. It was found that the amounts of losses depended on the forms of thiamine. Moreover, earlier studies showed higher losses of thiamine introduced in the form of thiamine pyrophosphate [9].

5. Conclusions

It was found that the concentration of thiamine and antioxidant activity of epigallocatechin gallate (EGCG) and caffeine are related to each other. The results of our study are consistent with the findings of earlier research on the antioxidant activity of EGCG and caffeine. It has been confirmed that the antioxidant activity of EGCG is higher than that of caffeine. The addition of tea components, especially EGCG, increased the oxidative stability of fat and thus reduced the loss of thiamine. However, our research also confirmed that this antioxidant activity of EGCG and caffeine in the presence of soybean oil was significantly reduced when the content of thiamine was higher than the natural level in foods, above 8.0 mg/100 g.

Therefore, in order to maintain thiamine stability and the high activity of the active tea ingredients and thiamine stability, it is necessary to consider their simultaneous addition to the systems in concentrations that limit their interactions. Accordingly, these encouraging insights may be interesting for nutritionists as well as food producers offering products for consumers who are at high risk of thiamine deficiency, such as people with Alzheimer's disease as well as vegans and vegetarians. However, further research is necessary to discover ways to block the interaction of thiamine with test compounds.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su13094644/s1. Table S1: Peroxide value to samples with thiamine hydrochloride and EGCG. Table S2: Peroxide value to samples with thiamine pyrophosphate and EGCG. Table S3: Peroxide value to samples with thiamine hydrochloride and caffeine. Table S4: Peroxide value to samples with thiamine pyrophosphate and caffeine. Table S5: Anisidine value LAN to samples with thiamine hydrochloride and EGCG. Table S6: Anisidine value LAN to samples with thiamine pyrophosphate and EGCG. Table S7: Anisidine value LAN to samples with thiamine hydrochloride and caffeine. Table S8: Anisidine value LAN to samples with thiamine pyrophosphate and caffeine. Table S9: The protection factor (Wo) to samples with thiamine hydrochloride and EGCG. Table S10: The protection factor (Wo) to samples with thiamine pyrophosphate and EGCG. Table S11: The protection factor (Wo) to samples with thiamine hydrochloride and caffeine. Table S12: The protection factor (Wo) to samples with thiamine pyrophosphate and caffeine.

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