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Histamine concentration in selected types of cow's milk taking into account storage conditions

Zawartość histaminy w wybranych rodzajach mleka krowiego z uwzględnieniem warunków jego przechowywania

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Abstract

Introduction and Objective. Milk, besides nutrients, can also contain undesirable substances, including biogenic amines, e.g. histamine, which can cause severe poisoning. Considering consumer safety, it is important to know the concentrations of histamine not only in milk placed on the market, but also in stored milk. The aim of this study was to analyze histamine concentrations during the storage of cow›s milk.
Materials and Method. UHT (n=21) and pasteurized (n=20) milks were investigated in the study. Histamine concentration was determined by ELISA. Concentrations were measured on the day the milks were opened and after 24h, 48h, and 7 days of refrigerated storage. The determined histamine concentrations were compared with the MLP values for this monoamine. The EDI for milk-derived histamine and the percentage of EDI in the NOAEL and LOAEL values were calculated.
Results. Histamine concentrations varied, but did not exceed MLP values. Higher concentrations of this biogenic amine were associated with the type of heat treatment (UHT), fat content (≤1.5%), and storage time (7 days after opening). The protein content of milk significantly affected histamine concentrations only after 7 days of storage – milks with ≥3.3 g protein/100 ml had the highest histamine concentrations. The percentage ratio of EDI/NOAEL and LOAEL did not exceed 100% throughout the storage period, regardless of the exposure scenario. The highest EDI/NOAEL values were recorded for sensitive individuals: 1.8% (day 0) – 2.2% (day 7).
Conclusions. Histamine concentration was significantly higher in UHT milks than in pasteurized milks and in those with a ≤1.5% fat content compared to the 2% and ≥3.0% milks. The concentration of histamine in milk increased as a function of time. The examined milk can be considered safe in terms of histamine content at any stage of storage.

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Key words
histamine, milk, exposure assessment, storage time, UHT milk, pasteurized milk

Streszczenie

Wprowadzenie i cel pracy. Mleko, oprócz składników odżywczych, może zawierać substancje niepożądane, w tym aminy biogenne. Jedną z nich jest histamina, która może być przyczyną poważnych zatruć. Z uwagi na bezpieczeństwo konsumentów ważna jest znajomość stężeń histaminy nie tylko w mlekach wprowadzanych na rynek, ale również w tych przechowywanych. Celem pracy była analiza stężenia histaminy w mlekach krowich podczas ich przechowywania.
Materiał i metody. Badaniu poddano mleka UHT (N = 21) i pasteryzowane (N = 20). Stężenie histaminy oznaczono metodą ELISA. Pomiary stężeń wykonano w dniu otwarcia pojemników z mlekiem oraz po 24 h, 48 h i 7 dniach przechowywania ich w warunkach chłodniczych. Oznaczone stężenie histaminy porównano z wartościami MLP dla tej monoaminy. Obliczono EDI dla histaminy pochodzącej z mleka oraz procentowy udział EDI w wartościach NOAEL i LOAEL.
Wyniki. Stężenie histaminy było zróżnicowane, ale nie przekraczało wartości MLP. Wyższe stężenie tej aminy biogennej powiązano z rodzajem obróbki termicznej (UHT), zawartością tłuszczu (≤ 1,5%) oraz czasem przechowywania (7 dni od otwarcia). Koncentracja białka w mleku wpływała istotnie na stężenie histaminy jedynie po 7 dniach przechowywania – mleka zawierające ≥ 3,3 g białka/100 ml wykazywały najwyższe stężenie histaminy. Procentowy stosunek EDI/NOAEL i LOAEL nie przekraczał 100% w całym okresie przechowywania, niezależnie od scenariusza narażenia. Najwyższe wartości EDI/NOAEL odnotowano dla osób wrażliwych: od 1,8% (dzień 0.) do 2,2% (dzień 7.).
Wnioski. Zawartość histaminy była istotnie wyższa w mlekach UHT niż w pasteryzowanych oraz w tych o zawartości tłuszczu ≤ 1,5% w porównaniu do mlek 2% i ≥ 3,0%. Stężenie histaminy w mleku rosło w funkcji czasu, osiągając najwyższą wartość

po 7 dniach. Badane mleko na każdym etapie przechowywania można uznać za bezpieczne pod względem zawartości histaminy.

Słowa kluczowe
histamina, mleko, ocena narażenia, czas przechowywania, mleko pasteryzowane, mleko UHT

INTRODUCTION

Histamine (2-[4-imidazolyl] ethylamine) is a heterocyclic monoamine that can be produced in the human body (endogenous histamine) and supplied with food (exogenous histamine) [1, 2]. This compound, as a biogenic amine, is present in foods rich in protein, where it is formed as a result of bacterial decarboxylation of L-histidine. The microorganisms that produce histamine in food include primarily Gram-positive bacteria, especially the *Lactobacillus* species (e.g. *L. curvatus*), less frequently Gram-negative bacteria (e.g. *Enterobacter cloacae*), yeasts (e.g. *Debaryomyces hansenii*) and moulds (e.g. *Geotrichum candidum*) [3, 4]. The highest concentrations of this compound can be observed in fresh fish and fish products. It is found in products such as beer, wine, fermented vegetables, and meat. Due to the presence of high-quality protein, it can also be found in dairy products such as milk, cottage cheese, cheese or yogurt [3].

Milk is characterized by its high nutritional value, and its chemical composition varies depending on the species and breed of animal. The composition of milk is also influenced by non-genetic factors, i.e. season, climate, breeding conditions, health status, previous pregnancies/lactations, composition of feeds consumed, and age of the animal [5, 6]. In 2013, a total of 781 million tonnes of milk was produced worldwide, of which cow's milk accounted for 85.9% of the total production. In 2016 in the countries of the European Union, the production of cow's milk amounted to 163 million tonnes, which accounted for 96.8% of the total milk production in these areas. It is estimated that over 95% of all dairy products produced in developed countries come from cow's milk [5, 7, 8].

Raw milk is characterized by a low microbiological quality; therefore, in order to produce a safe final product, the milk is subjected to technological processes. Heat treatment of milk extends its shelf life and minimizes the risk of poisoning by reducing or eliminating microorganisms, including pathogenic microorganisms. The most common methods of heat treatment used in milk production include pasteurization and ultra-high temperature (UHT) treatment, with the removal of both vegetative and persistent forms possible only during the UHT process [9]. Unfortunately, incorrect packaging and storage conditions of milk may favour microbiological contamination which, in turn, may result in an increase in the content of biogenic amines, including histamine [10]. The longer the time and the higher the storage temperature, the higher the concentration of histamine in the product. Due to the thermostability of histamine, heat treatment processes do not allow its decomposition. However, thermal methods are crucial for the elimination of microorganisms involved in the synthesis of biogenic amines [2, 11]. It is estimated that in the case of pasteurized milk, the histamine content is usually between 0.3–0.7 ppm, while in UHT milk it can be as high as 0.8 ppm. In the case of fermented dairy products, these concentrations can be up to 10 times higher [3, 4, 12].

The final toxic effect of histamine is influenced primarily by the amount of its consumption with food. Its toxicity will also depend on individual factors, such as the activity of enzymes that break down histamine or the functioning of the kidneys. In addition, the presence of other biogenic amines in food (e.g. cadaverine) may intensify its toxic effects [4, 13]. Excessive amounts of histamine consumed with food products can cause poisoning. Most cases of histamine poisoning concern poisoning with fish from the suborder Scombridae (e.g. tuna, mackerel); however, symptoms of poisoning may also occur after eating other products containing large amounts of this compound. Symptoms of poisoning may appear very quickly, up to a few minutes after consumption of a contaminated product, but within 24 hours from consumption [3, 11]. The symptoms of histamine poisoning include numerous skin reactions (urticaria, rash, oedema, erythema), symptoms from the digestive system (nausea, vomiting, diarrhea) and the nervous system (headaches, loss of vision, tingling, cramps). High accumulation of this compound in the body may also result in bronchospasm, respiratory failure, palpitations and tachycardia [11, 13].

Determining the limits of histamine toxicity is problematic because the tolerance of this compound is very individual and depend on, among others, allergies, food intolerances, or from taking certain medications that inhibit the activity of enzymes involved in the decomposition of exogenous biogenic amines [14]. In 2011, the European Food Safety Authority (EFSA) carried out a qualitative risk assessment of the presence and toxicity of biogenic amines in food. According to the Agency observations, it is assumed that the consumption of histamine in the amount of up to 50 mg/person/meal by healthy people should not cause negative health effects [14, 15]. Unfortunately, food supervision restricts histamine checks to fishery products only. This may pose a risk to consumers in relation to other products containing biogenic amines, especially for people in risk groups [3, 16].

The presence of histamine in food products is not the only scientific issue of interest. An interesting issue is the changes in its concentrations that occur during storage of the products. For this reason, the purpose of this study is to determine the content of histamine and to analyze changes in its concentrations in milk, depending on the time and conditions of storage. In addition, the study attempts to estimate the health risk to consumers in relation to the determined levels of this biogenic amine.

MATERIALS AND METHOD

Experimental material. The study comprised 41 samples of cow›s milk (21 UHT milk and 20 pasteurized milk), purchased from randomly selected retail stores in the Silesian Province of south-west Poland. Characteristics of the studied milks are presented in Table 1.

The purchased milk came from Polish producers, and the entire study was conducted within their shelf life. The milks were stored in accordance with the manufacturer›s recommendations until the analysis was performed (fresh milks T=1–6°C; UHT milks T=2–25°C). Milk samples were

Table 1. Characteristics of the cow›s milk tested

Variable	Subgroup	n	%
Type of heat treatment of milk	UHT	21	51,2
	Pasteurization	20	48,8
Fat content in milk [%]	≤1,5	7	17,1
	2,0	16	39,0
	≥3,0	18	43,9
Protein content [g] in 100 ml milk	3,0	10	24,4
	3,1-3,2	17	41,5
	≥3,3	14	34,1

n – number; % – percentage

collected and analyzed on the day the milks were opened (day 0) and 24hr (day 1), 48hr (day 2) and 7 days (day 7) after opening. Sampling times were established based on producers' recommendations and scientific reports [17]. During the study period, all milks were stored at refrigeration temperature (T=0–5°C).

Samples of cow's milk were prepared for the determination of histamine concentrations according to the recommendations of the producer of the test used in the analysis. From each milk sample, 4 mL were taken under sterile conditions, and then centrifuged (10 min / 3000 × g / 22°C). The upper lipid layer was removed from each of the centrifuged milk samples, and 20 µL of skimmed milk vortexed (IKA VORTEX Genius 3) with 4 mL of 10 mM PBS, with the addition of 0.05% Tween 20 (Sigma-Aldrich). In further analyses, 100 µL of the above mixture was used.

Analytical methods. Histamine concentrations were determined by enzyme-linked immunosorbent assay (ELISA), using commercial RIDASCREEN® Histamine assay (Art. No: R1601) from R-Biopharm (Germany). The tests were performed in accordance with the instructions. Each milk sample was analyzed in duplicate. While performing the tests, an ELMI DTS-2 microplate thermal shaker, MW-12A scrubber and Mindray MW-12A microplate reader (λ=450 nm) were used.

The concentration of histamine in milk was determined based on the standard curve, the relationship of the absorbance of the standard/sample (B) to the absorbance of the blank (B0) standard (B/B0×100%) (OY) to the concentration of µg/L standards (OX). The RIDA SOFT Win. Net (version 1.99) programme was used to obtain the standard curve (Fig. 1) and to calculate the concentration of histamine in the samples. When determining the actual histamine concentration in cow's milk (µg/L), a dilution factor of 200 was taken into account. The limit of detection (LOD) for histamine in milk was 100 µg/L, and specificity – 100% for histamine and about 0.01% for 3-methylhistamine.

The calculated histamine concentrations were compared with the Maximum Permissible Limits (MPLs) established for the content of this monoamine in foodstuffs. The recommendations of international organizations were taken into account during the comparison (Tab. 2).

Evaluation of daily histamine intake from milk. Based on the average consumption of milk in the daily diet, the body weight of the average consumer and the concentration of histamine in the analyzed products, the estimated daily

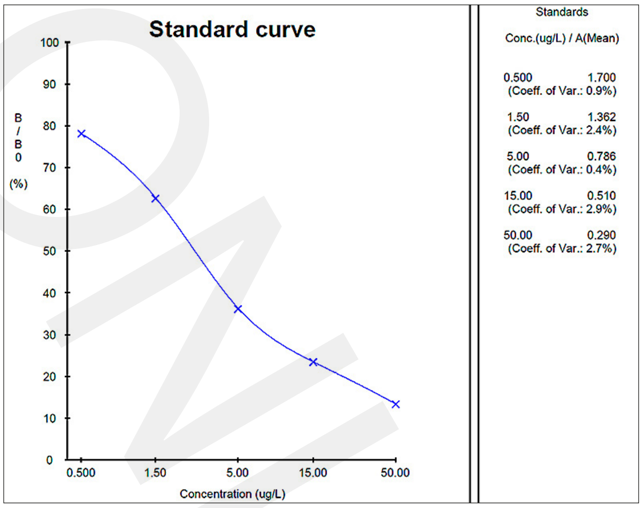

Figure 1. The standard curve for histamine

Table 2. Maximum Permissible Limits (MPLs) for histamine content determined by selected international organizations [15, 18, 19]

International organization	MPLs
European Food Safety Authority (EFSA); Food and Drug Administration (FDA)	5 mg/100 g =50 mg/kg*
Food and Agriculture Organization of the United Nations (**FAO**) World Health Organization (WHO)	10 mg/100 g =100 mg/kg*

* due to the state of aggregation of the studied material (liquid), the values expressed in grams (g) were related to milliliters (mL) (product-milk), and those in kilograms (kg) to liters (L)

intake (EDI) of histamine with milk was calculated according to the following equation:

$$\mathrm{EDI} = \frac{C_h \times M_m}{bw}\ [\mathrm{mg/kg\ b.w./day}]\ [20]$$

where:
C_h – concentration of histamine in the analyzed milk [mg/L]. Calculations were performed for 3 scenarios: 1) minimum histamine concentration recorded; 2) average histamine concentration recorded; 3) maximum histamine concentration recorded (the so-called worst-case scenario). Each of the scenario was related to both the concentration of histamine determined on the day of opening the milk (day 0) and after 7 days of its storage in refrigeration conditions (day 7).
M_m – average daily milk consumption in Poland [mL/day], based on the 2021 Polish Statistics Report, the value of 2.90 L of milk/month/person, i.e. 0.0967 L/day, was assumed [21]. Due to the lack of precise data for the consumption of cow's milk only, the term 'milk', according to the Polish Statistics Report, refers to '*milk from cows and other animals, fresh, pasteurized, UHT, homogenized*.' The effect of culinary processing of milk on histamine concentrations was not included since biogenic amines show high thermostability during traditional methods of thermal treatments [3, 4].
bw – average body weight of the consumer [kg]. According to the EFSA recommendations, when the body weight of an adult European citizen is unknown, 70 kg should be taken as the average body weight [22].

The EDI values for the average histamine concentration in milk on the day of opening and during storage were compared with the histamine concentration established for

Table 3. Accepted NOAEL and LOAEL for histamine within the context of adult consumers, including susceptible individuals [15, 19, 23]

NOAEL / LOAEL		Value
NOAEL	Susceptible individuals (individuals with histamine intolerance)	5 mg/person*/meal**= 0.0714 mg/kg b.w./meal
	Healthy individuals	50 mg/ person*/meal**= 0.714 mg/kg b.w./meal
LOAEL	Healthy individuals	75 mg/person*/meal**= 1.0714 mg/kg b.w./meal

* - according to EFSA estimates, an average „person" is a consumer with a body weight of 70 kg; ** - values are given per meal, since histamine is usually excreted from the body within a few hours (the risk of cumulative effect is insignificant); due to the lack of data on the average amount of milk consumed per meal, the worst-case variant was assumed - the average consumption of milk in Poland during the day, as a whole, was treated as per 1 meal

which no-observed-adverse-effect level (NOAEL) and the lowest-observed-adverse-effect level (LOAEL) (Tab. 3).

Based on the calculated EDI values, NOAEL and LOAEL values, % EDI/NOAEL and % EDI/LOAEL were determined according to the equation:

$$\% \frac{\text{EDI}}{\text{NOAEL/LOAEL}} = \frac{\text{EDI}}{\text{NOAEL/LOAEL}} \times 100 \text{ [24]}$$

A ratio of % EDI/NOAEL or % EDI/LOAEL <100 indicates that the EDI for histamine is lower than the NOAEL or LOAEL for this compound, which consequently poses no risk to the consumer [24].

Statistical analysis. The results were statistically prepared using Statistica 13.3 software StatSoft Poland (Kraków, Poland). Quantitative data were presented as median (Me) with the values of lower and upper quartile (Q1-Q3). The results were also described as numbers (n) with percentages (%). Normality of the distribution was tested using the Shapiro-Wilk W test, and based on histograms and curve parameters for skewness and kurtosis. The Mann-Whitney U (p^U) test was used to assess the dependence of histamine concentration on nominal variables, i.e. type of thermal treatment of milk. In the analysis of the relationship between histamine concentration and ordinal variables (categorized fat and protein content), the Kruskal-Wallis test (p^{KW}) with *post-hoc* analysis was used. The significance level of $\alpha = 0.05$ was assumed.

Friedman's test (p^F), for dependent samples with pair-wise comparative analysis was used to compare changes in histamine concentration during milk storage.

Linear regression was applied to describe the relationship between histamine concentration and selected characteristics of the analyzed milks. The normality of the distribution of the residuals was assessed by graphical interpretation. Strength of the linear relationship was checked based on the coefficient of determination (R^2) and standard error (SE). The assumption of no autocorrelation of the variables was assumed to be fulfilled for the Durbin-Watson (d^{D-B}) statistic of 1.5–2.5. Collinearity of the residuals was ruled-out based on a variance inflation factor (VIF) above 0.5.

RESULTS

Concentration of histamine in milk with regard to selected characteristics of the studied material and storage time. The concentration of histamine, determined in 41 samples of cow›s milk during storage was variable, but in none of the products did it exceed MPLs. Higher concentrations of this biogenic amine were associated with the type of heat treatment (UHT), fat content (1.5%), and storage time (7 days after opening).

On the day of opening (day 0), the average histamine concentration in pasteurized milk was 0.56 mg/L and after 24 h and 48 h of storage it increased by 0.01 mg/L (Me=0.57 mg/L) and 0.07 mg/L (Me=0.63 mg/L), respectively. The highest average concentration of histamine in pasteurized milk was recorded 1 week after opening (day 7) (Me=0.77 mg/L). For UHT milk, histamine concentrations determined immediately after opening were 0.74 mg/L, 0.77 mg/L after 24 h, 0.80 mg/L after 48 h and 0.85 mg/L after 1 week of refrigerated storage (Tab. 4).

The fat content of the milk significantly differentiated the content of histamine in the products on each day of analysis. Milks with a ≤1.5% fat content had significantly higher histamine concentrations compared to products with a fat content more than 3%. The minimum concentration of histamine, at the level of 0.29 mg/L, was recorded for milk with a fat content of ≥3.0%, on the first day of the study (0h), while the maximum level of this biogenic amine was observed after 7 days in milks containing 2.0% and ≤1.5% fat – 1.10 mg/L and 1.03 mg/L, respectively (Tab. 4).

The histamine concentration in milk was also analyzed depending on the protein content declared by the producer. No significant relationship was found between these variables on the day of opening and after 24 h and 48 h. However, measurements made after 7 days of milk storage in a refrigerator indicated that the milks containing more than 3.3 g of protein, on average, had the highest concentrations of histamine (Tab. 4).

The storage time of milk had a significant effect on the histamine content in the products. The average concentration of this monoamine was lowest on the day the milk was opened (Me=0.62 mg/L), and was significantly different from the concentration of this chemical compound recorded after 24 h, 48 h and 7 days of refrigerated storage ($p^F<0.01$) (Fig. 2).

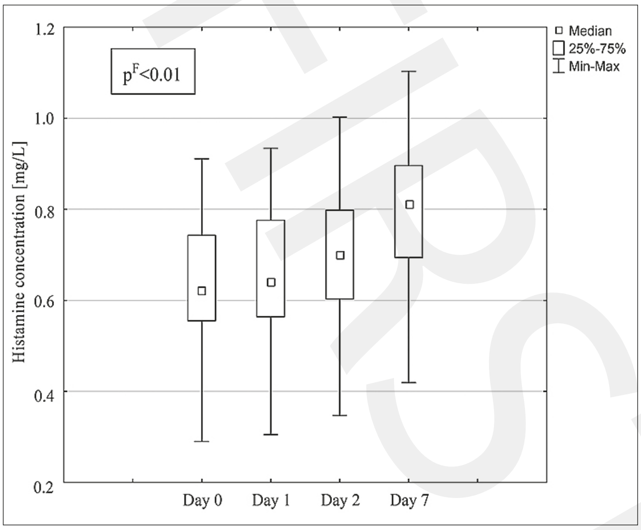

Figure 2. The average histamine concentration during storage

Evaluation of the relationship between histamine concentration and selected milk characteristics (linear regression). Evaluation of the relationship between histamine concentrations and selected milk characteristics, showed that

Table 4. Histamine concentration [mg/L] in cow›s milk during storage, including selected characteristics of the tested material

Characteristics of milk	Histamine concentration [mg/L]			Statistical analysis
	Me (Q1-Q3)	Min	Max	
Day 0				
Type of heat treatment of milk				
UHT	0.74 (0.69-0.81)	0.42	0.91	pU<0.00001
Pasteurization	0.56 (0.41-0.59)	0.29	0.68	
Fat content of milk [%]				
≤1.5	0.77 (0.70-0.81)	0.65	0.87	pKW<0.05 (*≤1.5% vs. ≥3.0%)
2.0	0.59 (0.58-0.77)	0.51	0.91	
≥3.0	0.58 (0.41-0.71)	0.29	0.79	
Protein content [g] in 100 mL of milk				
3.0	0.68 (0.62-0.73)	0.29	0.81	pKW=0.16
3.1-3.2	0.59 (0.41-0.71)	0.36	0.88	
≥3.3	0.67 (0.57-0.85)	0.51	0.91	
Day 1 (24h after opening)				
Type of heat treatment of milk				
UHT	0.77 (0.70-0.82)	0.44	0.93	pU<0.00001
Pasteurization	0.57 (0.42-0.60)	0.31	0.60	
Fat content of milk [%]				
≤1.5	0.80 (0.70-0.82)	0.66	0.89	pKW<0.05 (*≤1.5% vs. ≥3.0%)
2.0	0.60 (0.59-0.78)	0.52	0.93	
≥3.0	0.59 (0.41-0.71)	0.31	0.82	
Protein content [g] in 100 mL of milk				
3.0	0.68 (0.62-0.74)	0.31	0.81	pKW=0.16
3.1-3.2	0.60 (0.43-0.72)	0.37	0.88	
≥3.3	0.68 (0.59-0.86)	0.52	0.93	
Day 2 (48h after opening)				
Type of heat treatment of milk				
UHT	0.80 (0.73-0.89)	0.48	1.00	pU<0.00001
Pasteurization	0.63 (0.50-0.68)	0.35	0.77	
Fat content of milk [%]				
≤1.5	0.84 (0.78-0.87)	0.69	0.91	pKW<0.05 (*≤1.5% vs. ≥3.0%)
2.0	0.69 (0.66-0.83)	0.57	1.00	
≥3.0	0.61 (0.49-0.73)	0.35	0.89	
Protein content [g] in 100 mL of milk				
3.0	0.72 (0.68-0.79)	0.35	0.87	pKW=0.28
3.1-3.2	0.67 (0.50-0.79)	0.41	0.90	
≥3.3	0.72 (0.66-0.89)	0.57	1.00	
Day 7 (7 days after opening)				
Type of heat treatment of milk				
UHT	0.85 (0.77-0.94)	0.50	1.10	pU<0.01
Pasteurization	0.77 (0.61-0.82)	0.42	0.91	
Fat content of milk [%]				
≤1.5	0.90 (0.83-0.94)	0.74	1.03	pKW<0.005 (for all comparisons)
2.0	0.84 (0.80-0.93)	0.69	1.10	
≥3.0	0.69 (0.57-0.77)	0.42	0.92	
Protein content [g] in 100 mL of milk				
3.0	0.76 (0.70-0.85)	0.42	0.91	pKW=0.28
3.1-3.2	0.79 (0.62-0.84)	0.50	0.99	
≥3.3	0.87 (0.80-0.95)	0.69	1.10	

Me - median; Q1 - first quartile (lower quartile); Q3 - third quartile (upper quartile); p^{U} - Mann-Whitney U-test; p^{KW} - Kruskal-Wallis test (with post-hoc analysis for p<0.05)

throughout the study, histamine content was significantly lower in pasteurized milk compared to UHT milk. The greatest difference in histamine concentrations was observed on the day of opening and after 24 h of milk storage – on those days, pasteurized milk exhibited 0.25 mg/L less histamine content compared to UHT milk (Tab. 5).

The fat content of milk also had a significant effect on the measured concentration of histamine. On day 0 (opening day) and day 1 (24 hours after opening), milks with a fat content of 2.0% and ≥3.0% represented a lower concentration of this compound by 0.06 mg/L, compared to milks with a maximum fat content of 1.5%. However, after a week of storage, the concentrations of histamine in milks in the 2.0% and ≥3.0% fat categories were already lower by 0.14 mg/L, compared to the milks in ≤1.5% category (Tab. 5).

The protein content of milk also significantly affected the levels of histamine – a higher protein content was

Table 5. Evaluation of the relationship between histamine concentration in milk during storage and its selected characteristics (linear regression - progressive stepwise method)

Dependent variable - histamine concentration	Factor - predictor	b	SE for b	p value
Day 0				
$R^2_{(adjusted)}$ = 0.775; SE = 0.075; d^{D-B} = 2.00; VIF=0.225; p<0.0001				
Histamine concentration [mg/L] (day 0)	Type of milk heat treatment (UHT**/ pasteurized)	-0.25	0.025	<0.0001
	Fat content of milk (≤1,5%** / 2,0% / ≥3,0%)	-0.06	0.016	<0.0001
	Protein content [g] in 100 mL of milk (3,0** / 3,1-3,2 / ≥3,3)	0.08	0.017	<0.0001
Day 1 (24h after opening)				
$R^2_{(adjusted)}$ = 0.780; SE = 0.075; d^{D-B} = 1.97; VIF=0,220; p<0.0001				
Histamine concentration [mg/L] (day 1)	Type of milk heat treatment (UHT**/ pasteurized)	-0.25	0.025	<0.0001
	Fat content of milk (≤1,5%** / 2,0% / ≥3,0%)	-0.06	0.017	<0.0001
	Protein content [g] in 100 mL of milk (3,0** / 3,1-3,2 / ≥3,3)	0.08	0.017	<0.0001
Day 2 (48h after opening)				
$R^2_{(adjusted)}$ = 0.730; SE = 0.08; d^{D-B} = 1.99; VIF=0,270; p<0.0001				
Histamine concentration [mgL] (day 2)	Type of milk heat treatment (UHT**/ pasteurized)	-0.22	0.027	<0.0001
	Fat content of milk (≤1,5%** / 2,0% / ≥3,0%)	-0.07	0.018	<0.0001
	Protein content [g] in 100 mL of milk (3,0** / 3,1-3,2 / ≥3,3)	0.08	0.018	<0.0001
Day 7 (7 days after opening)				
$R^2_{(adjusted)}$ = 0.610; SE = 0.09; d^{D-B} = 1.99; VIF=0,390; p<0.0001				
Histamine concentration [mg/L] (day 7)	Type of milk heat treatment (UHT**/ pasteurized)	-0.09	0.021	<0.0001
	Fat content of milk (≤1,5%** / 2,0% / ≥3,0%)	-0.14	0.032	<0.0001
	Protein content [g] in 100 mL of milk (3,0** / 3,1-3,2 / ≥3,3)	0.09	0.021	<0.0001

SE – standard error, R^2(adjusted) – adjusted coefficient of determination; d^{D-B} – Durbin-Watson d-statistic; VIF – variance inflation factor; ** – reference group associated with a higher histamine content in the milk. Milks with a categorized protein content of 3.1–3.2 and ≥3.3g, represented 0.08–0.09 mg/L higher histamine concentrations than those containing 3.0 g of protein per 100 mL (Tab. 5). The estimation errors and significance of the obtained parameters (<0.0001) confirmed the good fit of the model which, depending on the day of analysis, explains 61–78% of the variability of histamine concentration in cow›s milk (Tab. 5).

Estimation of histamine exposure. EDI of histamine with milk was calculated depending on the recorded histamine concentration (according the scenarios described in the methodology). On the day of milk opening, EDI ranged from 0.4 µg/kg b.w./day (0.0004 mg/kg b.w./day) to 1.3 µg/kg b.w./day (0.0013 µg/kg b.w./day), depending on the recorded histamine concentration. The percentage ratio of EDI to NOAEL and LOAEL, regardless of histamine concentration, did not exceed 100%. The highest EDI/NOAEL value of approximately 1.8% was recorded for potential milk consumption (with the maximum histamine concentration determined) by people with intolerance to this monoamine (Tab. 6).

A similar estimation of histamine intake was made for milk stored in refrigerated conditions. The highest EDI values were obtained for milk stored for 7 days. Depending on the adopted scenario, the EDI for histamine after a week of milk storage, ranged from 0.58 µg/kg b.w./day (0.00058 mg/kg b.w./day) to 1.5 µg/kg b.w./day (0.0015 µg/kg b.w./day). Estimated daily intake of histamine did not exceed the limit values, and had a maximum of about 2.2% of the NOAEL value established for people intolerant to this biogenic amine (Tab. 6).

Table 6. EDI and percentage ratio of EDI to NOAEL (for sensitive adult), NOAEL and LOAEL (for healthy adult), for three recorded levels of histamine concentrations in milk

Scenario for minimum (1), average (2), maximum (3) determined histamine concentrations	Histamine concent. (C_h) [mg/L]	EDI [mg/kg b.w./day]	EDI/ NOAEL (sensitive adult) [%]	EDI/ NOAEL (healthy adult) [%]	EDI/ LOAEL [%]
Day 0					
(1)	0.29	0.000401	0.56	0.056	0.037
(2)	0.62	0.000856	1.20	0.120	0.080
(3)	0.91	0.001260	1.76	0.176	0.117
Day 1 (24h after opening)					
(1)	0.31	0.000428	0.60	0.060	0.040
(2)	0.64	0.000884	1.24	0.124	0.083
(3)	0.93	0.001285	1.80	0.180	0.120
Day 2 (48h after opening)					
(1)	0.35	0.000484	0.68	0.068	0.045
(2)	0.70	0.000966	1.35	0.135	0.090
(3)	1.00	0.001381	1.93	0.193	0.129
Day 7 (7 days after opening)					
(1)	0.42	0.00058	0.812	0.0812	0.0542
(2)	0.81	0.00112	1.567	0.1567	0.1044
(3)	1.10	0.00152	2.127	0.2127	0.1418

EDI – estimated daily intake; LOAEL – lowest observable adverse effects level; NOAEL – no-observed-adverse-effect level

DISCUSSION

Histamine is found in a variety of foods intended for human consumption. Low concentrations of this compound usually do not pose a threat to the life and health of consumers [25]. Unfortunately, there may be a situation in which a product with a high histamine concentration is consumed which may pose a potential risk to the consumer. In addition, people with intolerance to this compound should also be taken into consideration as even small amounts can cause health problems (depending on individual tolerance) [11]. The greatest risk of histamine poisoning is posed by fish, followed by cheese; this, however, does not change the need to conduct researches on other food products in this respect [15].

The content of histamine in milk compared to other foods, especially fish and cheese, is at a relatively low level. The average concentrations of histamine are assumed to be 0.3–0.7 ppm (0.3–0.7 mg/L) for pasteurized milk, and up to 0.8 ppm (0.8 mg/L) for UHT milk [4]. Histamine contents recorded in the current study on the day of opening the milks are in accordance with the above – an average of 0.56 mg/L for pasteurized milk and 0.74 mg/L for UHT milk. In comparison, Yilmaz et al. [26] observed lower concentrations of histamine in milk – 0.16 mg/L for pasteurized milk and 0.20 mg/L for UHT milk. However, it is important to remember that concentrations of biogenic amines in foods can vary even within a single food group. Milewski et al. [27] studied the effect of season of production on the health properties and composition of goat milk and rennet cheeses. The total BA content was found to be 1,056.4 mg/kg in the cheeses made from the milk of goats fed during the winter, which was nearly twice as high as that to cheeses made from the milk of goats fed during the summer feeding period – 567.6 mg/kg.

In the current study, a correlation was found between the type of heat treatment used in the production process, and the content of histamine in cow's milk on each day of analysis (0h, 24h, 48h and 7 days after opening). It was shown that pasteurized milks are characterized by significantly lower levels of this biogenic amine, compared to UHT-treated milks. On the other hand, research conducted in Turkey (ELISA method) by Yılmaz et al. [26] showed no statistically significant differences between the levels of histamine for pasteurized and UHT milks (p>0.05) on the day of opening. A similar dependence in relation to the own study was also observed by Czerniejewska-Surma and Żochowska [28] (colorimetric method according to the Polish Standard), where the UHT cow's milk analyzed by the authors, on the day of opening contained an average of 2.51 mg/100g of histamine (arithmetic mean of 4 parallel markings), and characterized by its higher concentration compared to pasteurized cow's milk (1.55 mg/100g).). Unfortunately, there are no scientific reports that would explain the reason for the differences in BA concentration, between UHT and pasteurized products. It is possible that the longer shelf life of UHT products, and thus the possible longer protein degradation which encourages the production of biogenic amines, plays a key role. Studies have shown that extra-cellular enzymes produced by natural microflora, mainly those with protease activity, have thermal stability, and are thus a major factor in changing the stability of UHT milk during storage [29].

Analysis showed that the concentration of histamine in milk is also influenced by the percentage of fat. It was observed that products with a fat content of less than 1.5% were characterized by a statistically significantly higher concentration of the biogenic histamine amine on each day of the analysis, compared to milks with a fat content exceeding 3%. Similar observations were made in the case of the study of powdered milk – skimmed products had the highest content of histamine (3.18 mg/100ml) in relation to the other milks tested. However, it should be emphasized that skimmed milk powder differs significantly in terms of production methods (concentration and dehydration of the raw material), compared to milk in its traditional form, which also has a significant impact on the content of biogenic amines in food [28]. El-Desoki [30] used cheeses made from raw cow's milk with a standardized fat content of 1.5 and 3% as controls to determine the effect of heat treatment of milk on the formation of free amino acids and biogenic amines in low-fat Egyptian hard Ras cheese. Control samples with lower fat content were characterized by higher concentrations of biogenic amines in all analyzes conducted by the author. It seems that water activity (a_w) may play a key role in this relationship. A_w decreases with increasing fat content, which has an inhibitory effect on proteolytic bacteria, thus limiting the availability of precursor amino acids in BA synthesis [31].

The current study showed a significant influence of storage conditions on the values of histamine concentration levels in milk. The obtained concentrations were the lowest on the day of milks opening (0 h; Me=0.62 mg/l), and systematically increased with the passage of time during storage under refrigerated conditions, reaching the highest value on the last day of the study (day 7; Me=0.81 mg /L) (p^F<0.01). An increase in histamine concentration over time was also observed by Marijan et al. [32] in the case of ripening of Livno cheese from Bosnia. The authors compared the values of this monoamine for 2 different parts of the cheese – centre and rind – during ripening, i.e. 0–105 days. On the first day of the study (day 0), histamine levels were determined to be 0.13 mg/kg for the rind and 4.33 mg/kg for the middle part of the cheese. On the last day (day 105), the concentration of this compound increased to 24.10 mg/kg in the rind and to 80.97 mg/kg in the middle part of the cheese.

An increase in the content of histamine biogenic amine as a function of cheese ripening time (vat milk cheese) was also observed by Wechsler et al. [33]. The authors noted a doubled histamine content in the cheese after 90 days of ripening (82–121 mg/kg), compared to day 45. One of the few studies relating to the analysis of the dependence between the level of histamine in milk and storage conditions – time and temperature, is the study by Czerniejewska-Surma and Żochowska from 2003 [28]. The authors showed a significant linear relationship between storage time and histamine content for pasteurized milk stored at refrigerated temperature (3±1°C). In the case of pasteurized milk stored in refrigerated conditions, the content of this monoamine increased up to 48 h after opening; however, after 72 and 96 h, a slight decrease in its content was observed. The content of histamine in UHT milk at refrigerated temperature slowly increased until 72 h after opening, and then decreased significantly after 96 h.

The differences observed in the concentrations of this monoamine during milk storage are primarily due to the bacterial decarboxylation of histidine into histamine. It is noteworthy, that in the current study, the average concentration of histamine in the samples increased systematically until day 7 of storage, which is contrary to the study of Czerniejewska-Surma and Żochowska, and could be explained not only by differences in sampling time (0h, 24h, 48h without 72h and 96h), but also by the analytical method used [28, 31].

Histamine can be found in a variety of foods intended for human consumption. Low concentrations of biogenic amines in food (<100 mg/kg) generally do not pose a serious health risk to consumers as they are degraded by detoxification enzymes in the gastrointestinal tract. However, it has been reported that people sensitive to this monoamine have experienced adverse effects when consuming lower concentrations of histamine with food, compared to healthy individuals. It is assumed that histamine intolerance is the result of reduced activity of the intestinal diamine oxidase, also known as diaminoxidase (DAO) [11]. Nowadays, foods with high concentrations of biogenic amines still pose a health risk to consumers [1, 34]. It is estimated that about 40% of all food poisoning outbreaks in the United States and Europe are the result of poisoning with fish rich in histamine, known as scombrotoxism [35]. According to data published by EFSA and ECDC (European Centre for Disease Prevention and Control), between 2010 – 2017, there were 599 outbreaks of histamine food poisoning reported in the European Union, while in 2021, there were 47 such outbreaks – 209 cases of poisoning, 16 of which required hospitalization [36, 37].

The current study shows that taking into account the histamine content in milk consumption does not pose a risk to human health, including sensitive individuals,. Independently of the adopted scenario (minimum, average and maximum marked levels of histamine), the percentage ratio of EDI to NOAEL did not exceed 100% for both the day of opening the cow's milk (0h) and on the last day of the study (day 7). Turkish researchers concluded similarly, finding that histamine concentrations measured in commercially available milk and cheese did not pose a risk to public health [26]. However, Liu et al. [38] studied exposure of the Chinese population to histamine contained in cheese. Based on the analysis, the researchers found that high concentrations of histamine in cheese were likely to pose a risk to children aged 3–10 years.

There were similar conclusions from a study by Reinholds et al. [39] that assessed dietary exposure to mycotoxins, heavy metals and biogenic amines in ripened mould cheeses for different European populations. There was no risk to consumers for heavy metals and mycotoxins present in mould cheeses, but for biogenic amines the risk rates were at high levels (worst-case scenario). For histamine, the highest risk was determined for French adolescents for high levels of cheese consumption – 26.6% for acute oral reference doses (aRfD). On the other hand, Lo Magro et al. [40] observed that the percentage contribution of histamine dose originating from seafood consumption (total: fresh, frozen, preserved) to the NOAEL value, with the average exposure of an adult person (18–64 years old), as a mean, was 7.7%. For comparison, in the most likely exposure scenario for the same group of consumers, the value was only 0.72%. Based on the results, there was no significant health risk from the consumption of histamine with seafood. In contrast, Petrović et al. [20] reported that 0.04% of the Serbian population is exposed to the unfavourable effects of histamine, with an average estimated daily fish consumption of 0.0274 mg/kg b.w./day for the adult population.

It is quite difficult to compare levels of exposure to histamine due to the wide variety of analyzed products, especially considering the varying levels of consumption of specific food categories in different regions of Europe and worldwide. However, it seems that compared to other food groups, milk, especially pasteurized milk, is among the safest, even for people with histamine intolerance.

Due to the small number of reports on the histamine content in milk, research should be continued, and the results of the current study should be extended to other factors that may affect the final concentration of histamine in these products. However, it is also important to develop new legislation on the acceptable limits of potentially toxic compounds present in food products, not only for histamine, but also for other biogenic amines that could present a risk to consumer health. It is also worth considering the permissible values of histamine not only for fish and fishery products, but also for other groups of food products.

CONCLUSIONS

The results of laboratory tests and analysis of the obtained results allow formulation of the following conclusions:

1) histamine content was significantly higher in UHT-treated milks than in pasteurized milks, and in those with a fat content of less than 1.5%, compared to those with higher fat levels;
2) the storage time of the milk significantly affected the histamine content of the products. The average concentration of histamine was lowest on the day of opening the milk and significantly differed from the concentration recorded after 24h, 48h and 7 days of storage at refrigerated temperature;
3) in terms of histamine content, the tested cow›s milk can be considered safe for the consumer at each stage of its weekly storage. MLP values set by selected international organizations for histamine, were not exceeded, and the health risk to consumers was assessed as low.

The research conducted and the conclusions formulated relate only to cow's milk, which is not the only dairy product consumed by consumers. Therefore, continuation of the study is recommended of changes in histamine concentrations in other products belonging to the 'dairy' category (such as yogurts, cheeses), and in milk from other animal species (e.g. goat milk), taking into account the conditions of storage, since high concentrations of histamine may pose a threat to potential consumers.

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