Additionally, amino derivatives of TA were synthesized using green chemistry technologies to modify TA [30]. The reaction process was controlled, and product formation was detected using a selective method of light absorption with ultraviolet rays (UV-254). Thin-layer chromatography (TLC) analyses were conducted on Silufol UV-254 plates [31]. Visualization was achieved by spraying with a 1% solution of ninhydrin to detect amines. Melting points were measured using a Fisher-Johns device.

Subsequently, the molecular characteristics of the target compound, 2-hydroxyethan-1-aminium(2R,3R)-3-carboxy-2,3-dihydroxypropanoate and 1-benzyl-3,4-dihydroxy-pyrrolidine-2,5-dione, were elucidated by Nuclear Magnetic Resonance (NMR) analysis. The NMR spectra were registered on a spectrometer Varian Mercury-300 at operating frequencies 300.077 MHz (^{1}H), 75.46 MHz (^{13}C), and chemical shifts were reported with respect to TMS (Tetramethylsilane) in $CDCl_3$ (Deuterated chloroform). EACS and benzylimide were synthesized and investigated as new nitrogen-containing derivatives of natural tartaric acid. The synthesis modifications proposed by us were based on methods described in the literature [32].

The process of TA benzylimide (1-benzyl-3,4-dihydroxy-pyrrolidine-2,5-dione, also known as Benzylimide of Tartaric Acid) synthesis was carried out by following procedure: benzylamine (0.1 mol) and L-(+)-tartaric acid (0.1 mol) was refluxed for 8 – 10 h using a Dean-Stark apparatus. As the solvent *o*-xylene was used, at the temperature 183 – 185 °C. The reaction mixture was then cooled to ambient temperature, and the resulting crystalline product was separated by vacuum filtration. After washing portions of hexane, the filtrate was recrystallized from an acetonitrile-toluene mixture (v:v = 1:1). The final product was obtained as a pale solid powder with a 92% yield, m_p=200 – 202°C. ^{1}H NMR spectrum in $CDCl_3$, δ, ppm (J, Hz): 4.38 (d, J = 4.6 Hz, 2H, -C$\underline{H}_2$Ph), 4.5 (d, J = 7.2 Hz, 2H, -C$\underline{H}$-C$\underline{H}$-), 6.3 (d, J = 5.3 Hz, 2H, -O$\underline{H}$), 7.3-7.2 (m, 5H, ArH).

The process of 2-hydroxyethan-1-aminium-(2R,3R)-3-carboxy-2,3-dihydroxypropanoate (TA Colamine Complex) synthesis was carried out by the following procedure: TA (0.1 mol) was dissolved in H_2O (30 mL) and ethanolamine (0.1 mmol) was dissolved in EtOH (20 mL) before two solutions were mixed and incubated in a cold-water bath (at a temperature of -3 °C – 0 °C, created by the mixture of ice and NaCl) for 1 h. The precipitated salt was filtered off over suction and washed with diethyl ether affording TA colamine complex in the form of white crystals with a 95% yield, m_p=181-182 °C. ^{1}H NMR spectrum in DMSO-d_6 (Deuterated dimethyl sulfoxide) with addition of CF_3COOH, δ, ppm (J, Hz),: 2.8 (br.s., 3H, $N^+\underline{H}_3$-CH_2-), 2.92 (m, 2H, N^+H_3-C$\underline{H}_2$-), 4.3 (s, 2H, HO-C$\underline{H}$-COO^-), 7.8 (br.s., 3H, -OH).

Antioxidant activity assessment: Several methods assess antioxidant capacity, including ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), FRAP (ferric reducing antioxidant power), CURPAC (cupric reducing antioxidant capacity), and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays [33]. For this purpose, the spectroscopic method was applied, using DPPH 0.2 mM absolute methanol solution [34-36]. All the measurements we performed by the application of a spectrophotometer in the visible region at 517 nm, which corresponds to the absorption maximum of DPPH. The DPPH radical scavenging activity was calculated using the following equation (1):

$$DPPH\ scavenging\ effect\ (\%) = \{1-(A_{sample\ 517\ nm}/A_{control\ 517\ nm})\}\times 100 \quad (1)$$

Where: $A_{control\ 517\ nm}$ represents the absorbance value of the controlled reaction measured at 517 nm, using distilled water in place of test sample. $A_{sample\ 517\ nm}$

represents the absorbance value of the testsample, measured at 517 nm, in presence of TA N-containing derivatives.

All the measurements were repeated five times and were performed at room temperature (23 °C). Photometric studies were conducted using a Thermo Scientific Multiskan GO spectrophotometer (517 nm). For the assessment of its value, the aqueous solutions were prepared by the consequent multiple dilutions of the initial 1 mg/mL ascorbic acid solution up to concentrations of 10, 20, 40, 80, and 500 mcL, which were also diluted to a total volume of 2000 mcL. The mentioned solutions were mixed with 0.2 mM methanol solutions of DPPH. After the incubation of the mixture in the darkness for 30 min, the optical densities (D) of all the solutions were measured [37- 38].

Based on the collected data a grading curve was constructed using the least squares to a linear correlation in the concentration range 0 - 0.002 mg/ml ascorbic acid (see Fig. 1, y = 35509 x + 0,5706, where y is the light absorption number, correlation coefficient R^2 = 0.9482). The mean relative deviation was 2.23% (n = 5). The value of EC_{50} for ascorbic acid was defined as 0.0014 mg/mL due to the constructed calibration curve.

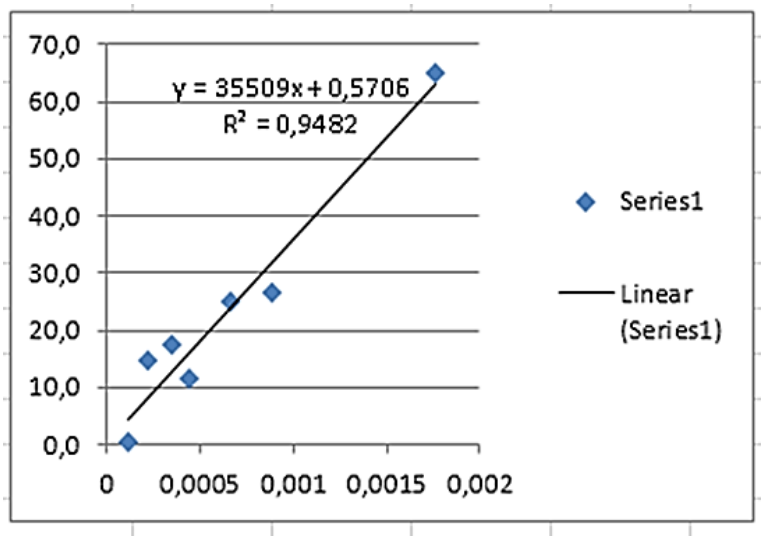

Figure 2. The graphical presentation of DPPH neutralization dependance from the concentration of ascorbic acid in the titration solution.

Anti-spoilage activity test

The anti-spoilage efficacy of TA benzylimide and colamine complex was assessed in two distinct feed formulations: fish feed and chicken feed. MODUS GRANUM Company (https://modusgranum.am/) supplied the feeds and their compositions. Fish feed preservation utilized "Vitasil", containing 40% formic acid, 30% acetic acid/peracetic acid, 15% sodium formate, 2% sodium benzoate, and 13% water (0.83% feed mass) (https://agrovitex.ru/catalog/vitasil). Chicken feed contained 0.5% sodium hydrosulfate. Tartaric acid derivatives were added to the feed at 0.1% by weight. During batch preparation at the plant, individual samples were taken without antimicrobial additives, and then the final form of feed was applied.

The testing derivatives of TA were added to feeds without commercial antimicrobial additives using the same methodology, which is used for the feed production industry. To ensure uniform distribution, TA benzylimide was dissolved in DMSO, and TA colamine complex in water, as crystalline materials cannot be evenly dispersed in feed. 10 grams of feed were taken, and pre-prepared

solutions were added by spraying. Control samples included a negative control, comprising 10 mL water, TA, and DMSO, to assess the residual antimicrobial activity and moisture effects. The study incorporated a negative control sample, consisting of 10 mL water, TA, and DMSO, to evaluate baseline antimicrobial activity and moisture impact. For the positive control samples the commercial preservative "Vitasil" was applied [39].

The resulting masses were transferred into small air-permeable bags and stored in room conditions for 4 months. After 4 months, all samples were inoculated onto the surface of solid sterile nutrient agarised cultural media (30 ml of meat peptone agar) in 90 mm Petri Dishes then were incubated for 72 h at 30-37 °C in aerobic conditions. After cultivation, the presence of bacteria was recorded by growth detection, using the method of counting of CFU (colony-forming unit) [40].

RESULTS:

The results of the studies of pure TA, its colamine derivative and TA benzylimide synergist antioxidant activity as feed additives for chicken and fish feeds are presented on Table 1.

Table 1. The comparison of the synergist antioxidant activity of tartaric acid (TA) derivatives.

N	DPPH	AA	TA	TA CC	TA B	W	D_{517}	S, %
1	2000	-	-	-	-	2000	1.667	0
2	2000	-	40	-	-	1960	1.664	0.2
3	2000	-	-	40	-	1960	1.665	0.2
4	2000	-	-	-	40	1960	1.666	0.1
5	2000	40	40	-	-	1920	0.725	56.6
6	2000	40	400	-	-	1560	0.674	59.6
7	2000	40	-	40	-	1920	0.640	61.7
8	2000	40	-	400	-	1560	0.573	65.7
9	2000	40	-	-	40	1920	0.708	57.6
10	2000	40	-	-	400	1560	0.621	62.8

N – sample number (1-6); S – scavenging; TA – tartaric acid water solution (1 mg/mL) in mcL; TA CC – tartaric acid colamine complex water solution (1 mg/mL) in mcL; TA B – tartaric acid Benzylimide MeOH solution (1 mg/mL) in mcL; AA – Ascorbic acid water solution (1 mg/mL) in mcL; DPPH – DPPH, MeOH solution (1 mg/mL) in mcL; W – water in mcL.

The antioxidant activity measurements revealed that, in the absence of ascorbic acid, neither pure TA nor its derivatives (colamine complex and benzylimide) exhibited significant antioxidant properties. Specifically,after 30 minutes of interaction with DPPH, no substantial antioxidant effect was observed. The mean of the difference of the optical density value for the observed solution was only 0.03 units Organic acids are known to enhance ascorbic acid's antioxidant activity through significant synergistic effects [41]. As the next step of research, the anti-spoilage properties assessment was carried out. The results of anti-spoilage tests are presented in Fig. 3.

Figure 3. Anti-spoilage test of TA derivatives, as the feed additive for different feeds.

A: Fish feed, B: chicken feed. Samples: 1 – the industrial feed composition without preservative additives; 2 – the industrial feed with 0.83% "Vitasil" preservative; 3 – the industrial feed composition with the addition of 0.1% TA; 4 – the industrial feed composition with an addition of 0.1% TA colamine complex, 5 – the industrial feed composition with the addition of 0.1% TA benzylimide; 6 – the negative controls with the addition of 10 ml water and DMSO.

According to the obtained data, in all test samples, which contained the new synthetic derivatives of tartaric acid, the growth of bacteria was less pronounced. The maximum antimicrobial effect was expressed by TA benzylimide, in which the minimum intensity of microbial growth was demonstrated. Colamine complex of tartaric acid was characterized by a low antimicrobial effect, but it was more notable in comparison to samples with the industrial compositions with "Vitasil" or sodium hydrogen sulfate (or sodium bisulfate), preservatives application [42].

The study of the anti-spoilage effect of TA N-containing derivatives has demonstrated several antimicrobial effects against spoilage microbes. It was indicated that in the case of samples with N-containing TA derivatives application, the titer of bacteria was 2.2×10^2 – 2.5×10^2 CFU for 1 g feed, stored within four months. For the positive control samples of the industrial feed composition with "Vitasil" and sodium hydrosulfate preservative the mean titer was 3.7×10^2 – 3.8×10^2. Analysis of the negative control samples, which consisted of water and DMSO, yielded remarkably consistent mean titer values, with a narrow range of 5.2×10^2 to 5.4×10^2.

The results indicate that the residual antimicrobial activity of DMSO does not substantially influence the growth of feed spoilage microorganisms. In contrast, humidity is a critical factor in their development and the subsequent deterioration of feed quality during prolonged storage.

DISCUSSION:

The absence of ascorbic acid revealed no antioxidant activity in TA and its derivatives after 30 minutes of DPPH exposure, yet synergistic effects emerged when they were used together. The value of the optical density of the solution was changed by about 0.03 units. Research showed that TA colamine complex, TA benzylimide, and pure TA exhibit marked synergistic antioxidant properties when paired with ascorbic acid [43].

When applied at the same doses, or even at 10 times higher concentrations (400 mcL stock solution), the TA colamine complex showed the maximum synergy effect, exceeding pure TA by 5%. The TA benzylimide effect was 1% higher. Our findings suggest that TA derivatives outperform sodium sulfate, potentially enhancing fish and chicken feed quality by replacing sodium sulfate, which has several drawbacks (adverse effects on animal blood biochemistry, etc.) [44-46]. In accordance with that, TA N-containing derivatives application potentially might increase the level of food safety for humans.

Our comprehensive study demonstrates the promising prospects of researching TA's biological properties and synthetic derivatives, opening avenues for innovative applications. Tartaric acid's dual antioxidant and antimicrobial functionality presents opportunities for improved food and feed preservation methods and the creation of cutting-edge packaging solutions.

Studies on the antimicrobial properties of amino derivatives of tartaric acid demonstrated effectiveness against a variety of microorganisms, including fish pathogens, human opportunistic pathogens, and foodborne spoilage agents. The obtained data have proved the presence of synergic antioxidant activity in TA and the colamine complex of TA. The creation of novel tartaric acid derivatives, especially those with amine functionality, represents a promising frontier in drug design and synthesis. The compounds synthesized demonstrate theoretical potential for varied biological effects, applicable to multiple areas of agriculture and healthcare.

Abbreviations: ABTS, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), allowable daily intake (ADI); BAA, bioactive compounds; CFU, colony-forming unit; CURPAC, cupric reducing antioxidant capacity; FRAP, ferric reducing antioxidant power; TA, L-tartaric acid; DPPH, 2,2-diphenyl-1-picrylhydrazine; MeOH, methanol.

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