

The chemical composition and pharmacological properties of many medicinal plants are well studied. Many studies associate the pharmacological action of these plants with the presence of such biologically active substances as steroids, phenolic compounds, terpenes, and alkaloids. However, plants have long been considered as a source of easily digestible forms of amino acids in combination with other biologically active compounds as well.

In order to systematically study the composition of the biologically active substances of corn, it was advisable to conduct a comparative study of the qualitative composition and quantitative content of free and bound amino acids of corn leaves, silk, and roots (variety Svetlana).

MATERIALS AND METHODS

The raw materials of *Zea mays* – leaves, silk, and roots – were harvested in the phase of milky-wax ripeness of the grain. Corn material is grown on research areas of the State Institution “Institute of Plant Industry named for V. Ya. Yuriev UAAN” (Kharkov, Ukraine). Raw materials were collected in July–August 2017–2018 in the Kharkiv region (Ukraine), crushed, and air-dried in shade.

The confirmation of the presence of amino acids in the raw materials of *Z. mays* was performed by qualitative reactions to identify amino acids in the studied raw materials. The free amino acids were determined by the method of paper chromatography (three-fold chromatography) in water extracts from the roots, silk, and leaves of *Z. mays*. The chromatograph paper – Filtrak FN 7 – was used: mobile phase: N-butanol-acetic acid-water (4:1:5).

The chromatogram with the applied substances was placed in a chamber with the indicated solvent system. After the solvent passed one-third of the length of a sheet of chromatographic paper, the chromatogram was removed and dried. The second time, the chromatogram was placed in the same system, with the difference that the solvent passed half of the length of the sheet. The third time, the solvent passed to the front line. A ninhydrin solution (0.1%) and heating to 96°C were used to detect the amino acid spots.^[1]

Quantitative determination of free and bound proteinogenic amino acids in plant materials was carried out by high-performance liquid

chromatography (HPLC). The method is based on the extraction of free amino acids from plant materials, acid hydrolysis, and subsequent analysis of hydrolysates by HPLC.

Chromatographic separation was performed on a liquid chromatograph Agilent 1200 (Agilent Technologies, USA): Column Zorbax AAA (150 mm × 4.6 mm × 3 μm). The precolumn derivatization was performed in an automatically programmed mode, followed by fluorescent detection: mobile phase A – a solution of sodium phosphate dibasic (40 mM) pH 7.8 and mobile phase B – acetonitrile: methanol: water (45:45:10). The separation mode was gradient at constant flow rate of 1.5 ml/min. Column was thermostated at 40°C. The number of experiments during the analysis was five.^[6-8]

Sample preparation and analysis of plant materials: (a) for free amino acids: a portion of powdered raw material was placed in a vial (2 ml), and an aqueous solution of 1 N hydrochloric acid was added and kept in an ultrasonic bath at 50°C for 3 h and (b) for the sum of amino acids: a sample was placed in a vial (2 ml), and an aqueous solution of 6 N hydrochloric acid was added and placed in a thermostat at 110°C. The hydrolysis was carried out for 24 h.

Then, 0.5 ml of the centrifuged extract/hydrolyzate was evaporated on a rotary vacuum evaporator, washing three times with distilled water to remove hydrochloric acid, resuspended in 0.5 ml of distilled water, and filtered through 0.2 μm regenerated cellulose filters.

The preparation of fluorescent derivatives was carried out in an automatic programmed mode before introducing the sample into the chromatographic column. Identification of amino acids was done by comparing the retention times of water extracts with the retention times of a mixture of amino acid standards (Agilent 5061-3334).

The content of bound amino acids was determined by subtracting the content of free amino acids from the total content [Figures 1 and 2].

RESULTS AND DISCUSSION

The paper chromatography method identified the free amino acids in leaves, roots, and silk of *Z. mays* as serine (Ser), Gly, Lys, Thr, glutamic acid (Glu), Val, Asp, Arg, Ala, and proline (Pro). After processing the

Table 1: Quantitative content of identified amino acids of *Zea mays* L. silk

Number	Amino acid	Free		Sum		Bound (μg/mg)
		Retention time	μg/mg	Retention time	μg/mg	
1	Asp	2.57	0.12	2.56	4.08	3.96
2	Ser	4.88	0.11	4.87	4.40	4.28
3	Glu	7.48	0.23	7.47	3.70	3.46
4	His*	-	-	8.30	1.07	1.07
5	Gly	8.75	Traces	8.73	2.99	2.97
6	Thr*	8.93	0.17	8.92	2.97	2.79
7	Ar*	9.45	Traces	9.45	2.70	2.68
8	Ala	10.17	0.24	10.15	3.37	3.13
9	Tyr	-	-	11.26	1.35	1.35
10	Cy	-	-	12.44	13.91	13.91
11	Val*	13.27	Traces	13.25	1.92	1.83
12	Met*	-	-	13.47	0.46	0.46
13	Phe*	14.70	0.18	14.68	2.33	2.15
14	Ile*	-	-	14.88	1.88	1.88
15	Lys*	15.53	Traces	15.50	5.28	5.24
16	Leu*	-	-	-	-	-
17	Pro	19.29	Traces	19.27	3.22	3.17
The sum of essential amino acids		-	0.49	-	18.59	18.10
The sum of nonessential amino acid		-	0.77	-	37.0	36.23
The sum of amino acids		-	1.26	-	55.59	54.33

*Essential amino acid. Asp: Asparagine; Ser: Serine; Glu: Glutamic acid; His: Histidine; Gly: Glycine; Thr: Threonine; Ar: Arginine; Ala: Alanine; Tyr: Tyrosine; Cy: Cysteine; Val: Valine; Met: Methionine; Phe: Phenylalanine; Ile: Isoleucine; Lys: Lysine; Leu: Leucine; Pro: Proline

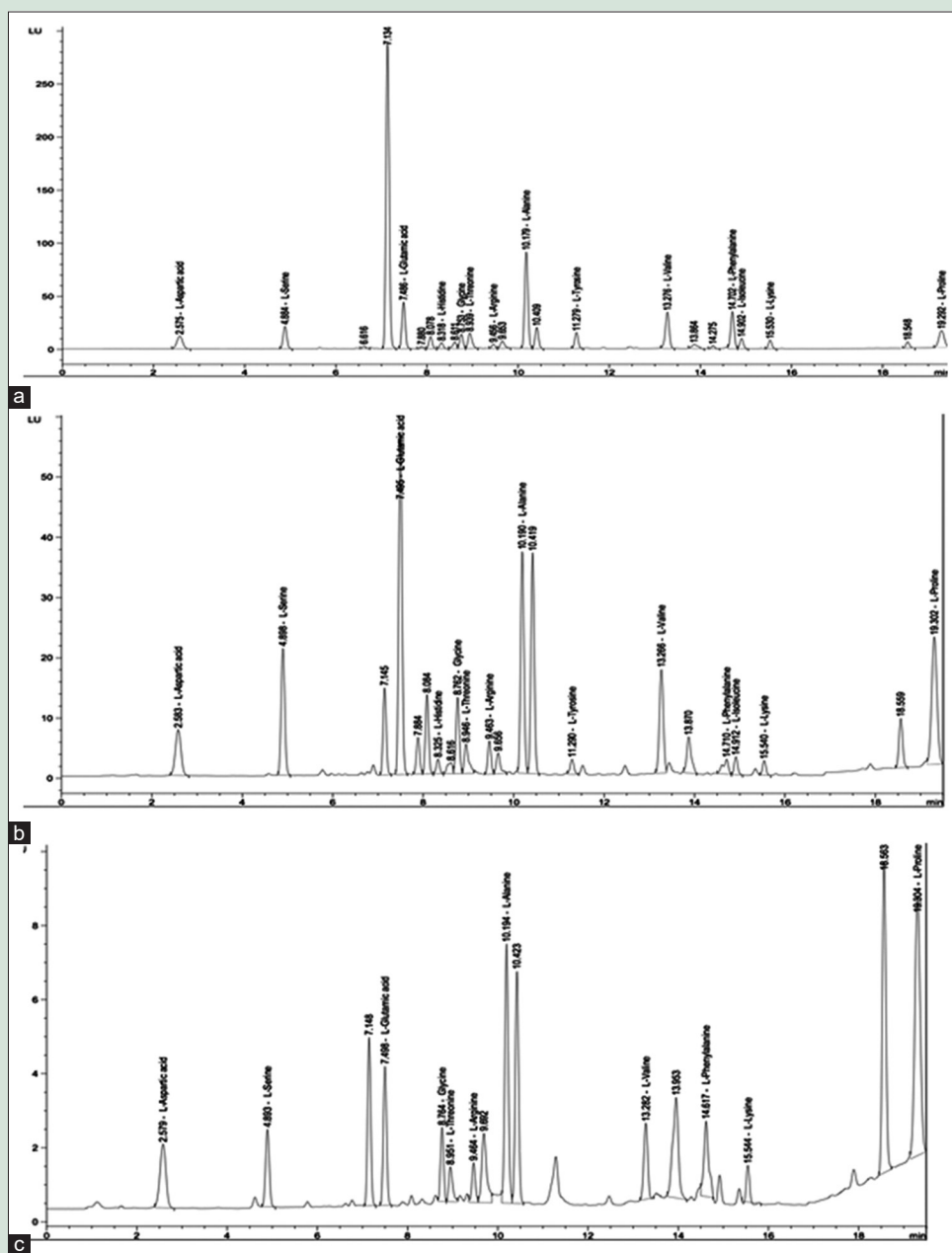


Figure 1: Chromatograms of free amino acids of *Zea mays* raw materials: (a) silk; (b) leaves; (c) roots

chromatogram, Ser, Val, Glu, Lys, Arg, Thr, and Ala gave purple spots; Gly – red-violet; Asp – blue-violet; and Pro – yellow.^[1]

HPLC identified 11 free amino acids in *Z. mays* silk [Table 1], *Z. mays* leaves [Table 2], and *Z. mays* roots [Table 3]: Asp, Ser, Glu, Gly, Thr, Arg, Ala, Val, Phe, Lys, and Pro. The quantitative content of free amino acids was not significant in all types of raw materials of *Z. mays*. The sum of amino acids in the leaves exceeds the sum of amino acids in the roots and silk. The smallest amount of free amino acids was found in the roots of *Z. mays*.

Glu ($0.23 \pm 0.01 \mu\text{g}/\text{mg}$) and Ala ($0.24 \pm 0.01 \mu\text{g}/\text{mg}$) are indicated in the greatest amount in *Z. mays* silk; Glu ($1.97 \pm 0.07 \mu\text{g}/\text{mg}$), Ala ($1.28 \pm 0.05 \mu\text{g}/\text{mg}$), and Pro ($1.19 \pm 0.04 \mu\text{g}/\text{mg}$) are indicated in the greatest amount in *Z. mays* leaves. These free essential amino acids have been found in corn leaves, silk, and roots in small amounts: Thr, Arg, Val, Phe, and Lys.

HPLC identified 16 bound amino acids in *Z. mays* silk [Table 1], *Z. mays* leaves [Table 2], and *Z. mays* roots [Table 3]. There was a trend of a significant increase in the amount of amino acids after hydrolysis. The sum of amino acids after hydrolysis was $54.33 \mu\text{g}/\text{mg}$ in silk, $34.79 \mu\text{g}/\text{mg}$ in leaves, and $16.89 \mu\text{g}/\text{mg}$ in roots.

Cys was found in corn silk in the greatest amount: $13.91 \mu\text{g}/\text{mg}$. The amount of other amino acids is significantly less: Lys – $5.24 \mu\text{g}/\text{mg}$, Ser – $4.28 \mu\text{g}/\text{mg}$, Asp – $3.96 \mu\text{g}/\text{mg}$, Glu – $3.46 \mu\text{g}/\text{mg}$, Pro – $3.17 \mu\text{g}/\text{mg}$, Ala – $3.13 \mu\text{g}/\text{mg}$, Gly – $2.97 \mu\text{g}/\text{mg}$, Thr – $2.79 \mu\text{g}/\text{mg}$, Arg – $2.68 \mu\text{g}/\text{mg}$, Phe – $2.15 \mu\text{g}/\text{mg}$, Ile – $1.88 \mu\text{g}/\text{mg}$, Val – $1.83 \mu\text{g}/\text{mg}$, tyrosine (Tyr) – $1.35 \mu\text{g}/\text{mg}$, His – $1.07 \mu\text{g}/\text{mg}$, and Met – $0.46 \mu\text{g}/\text{mg}$. His, Tyr, Cys, Met, and Ile were identified only after hydrolysis. Leu was absent.

The contents of Cys ($9.12 \mu\text{g}/\text{mg}$), Asp ($5.69 \mu\text{g}/\text{mg}$), Lys ($4.25 \mu\text{g}/\text{mg}$), Ser ($3.74 \mu\text{g}/\text{mg}$), and Phe ($2.25 \mu\text{g}/\text{mg}$) were the greatest in *Z. mays*

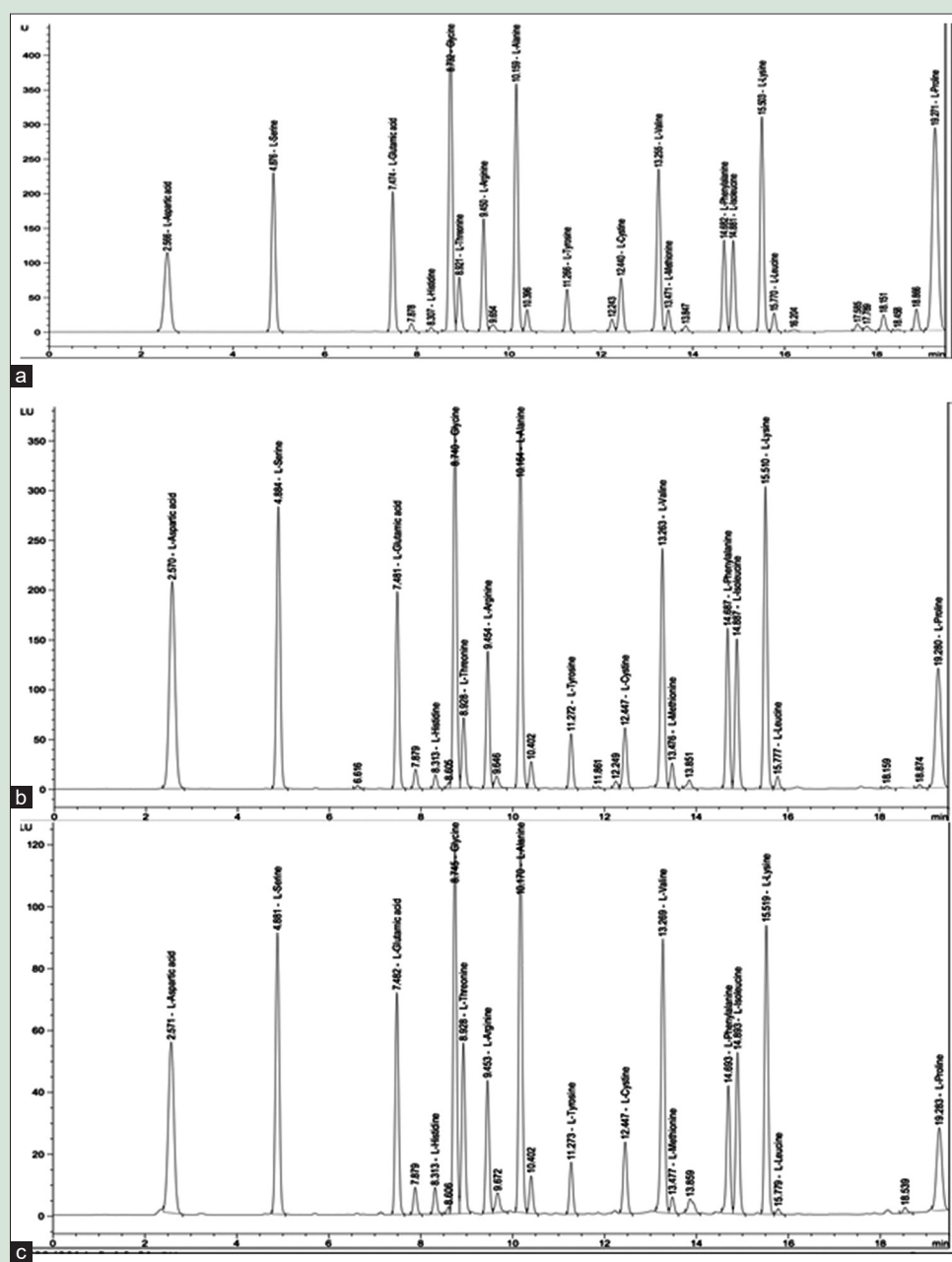


Figure 2: Chromatograms of free and bound amino acids of *Zea mays* raw materials: (a) silk; (b) leaves; (c) roots

leaves. Gly, Ile, Leu, Lys, and Pro were present in amounts <2 µg/mg. Pro was present in the least amount – 0.13 µg/mg. Leu was absent. His, Tyr, Cys, Met, and Ile were identified only after hydrolysis.

The contents of bound Cys (3.54 µg/mg), Thr (1.73 µg/mg), Asp (1.67 µg/mg), Ser (1.50 µg/mg), Lys (1.37 µg/mg), His (1.24 µg/mg), and Glu (1.13 µg/mg) have been found in the corn roots after hydrolysis. Other amino acids, such as Gly, Arg, Ala, Tyr, Val, Met, Phe, Ile, and Pro, were present in amounts <1 µg/mg. Leu was absent. Some amino acids were found only in bound form: His, Tyr, Cys, Met, and Ile.

Bound amino acids from *Z. mays* silk, leaves, and roots included seven essential amino acids (His, Thr, Arg, Val, Met, Phe, Ile, and Lys). The content of Met was the least in all samples.

One of the indicators of biological value of mixtures of amino acids is the content of essential amino acids in them. It should be 45%–50% of

the sum of all amino acids in order to show the high biological value of the plant raw material.^[9] The analysis of the content of essential amino acids of corn silk, leaves, and roots did not show high biological value (<40%).

The highest sum of amino acids was in silk (55.59 µg/mg). The sum of corn leaves was 49.77 µg/mg. The roots showed the smallest sum (17.33 µg/mg).

It is noted in the agricultural literature that corn silage and green fodder has low protein level.^[10,11] The results obtained for the composition and content of amino acids confirm this statement.

Nevertheless, it is necessary to note the high content of Cys in all studied samples of *Z. mays*. Cys is an amino acid that is known as a strong antioxidant. Cys also binds to toxic metals, plays an important role in the formation of skin tissues, and promotes rapid regeneration after surgery

Table 2: Quantitative content of identified amino acids of *Zea mays* L. leaves

Number	Amino acid	Free		Sum		Bound (µg/mg)
		Retention time	µg/mg	Retention time	µg/mg	
1	Asp	2.58	0.45	2.57	6.13	5.69
2	Ser	4.89	0.81	4.88	4.56	3.74
3	Glu	7.49	1.97	7.48	3.03	1.06
4	His*	-	-	8.31	1.90	1.90
5	Gly	8.76	0.44	8.74	2.08	1.64
6	Thr*	8.94	0.22	8.92	2.20	1.98
7	Ar*	9.46	0.19	9.45	1.90	1.72
8	Ala	10.19	1.28	10.16	2.83	1.54
9	Tyr	-	-	11.27	1.02	1.02
10	Cy	-	-	12.44	9.12	9.12
11	Val*	13.26	0.72	13.26	1.60	0.88
12	Met*	-	-	13.47	0.31	0.31
13	Phe*	14.71	Traces	14.68	2.39	2.25
14	Ile*	-	-	14.88	1.81	1.81
15	Lys*	15.54	Traces	-	4.33	4.25
16	Leu*	-	-	-	-	-
17	Pro	19.30	1.19	19.28	1.06	0.13
The sum of essential amino acids		-	1.35	-	12.3	10.95
The sum of nonessential amino acid		-	6.14	-	30.08	23.94
The sum of amino acids		-	7.49	-	49.77	34.79

*Essential amino acid. Asp: Asparagine; Ser: Serine; Glu: Glutamic acid; His: Histidine; Gly: Glycine; Thr: Threonine; Ar: Arginine; Ala: Alanine; Tyr: Tyrosine; Cy: Cysteine; Val: Valine; Met: Methionine; Phe: Phenylalanine; Ile: Isoleucine; Lys: Lysine; Leu: Leucine; Pro: Proline

Table 3: Quantitative content of identified amino acids of *Zea mays* L. roots

Number n/n	Amino acid	Free		Sum		Bound (µg/mg)
		Retention time	µg/mg	Retention time	µg/mg	
1	Asp	2.57	Traces	2.57	1.69	1.67
2	Ser	4.89	Traces	4.88	1.51	1.50
3	Glu	7.49	Traces	7.48	1.15	1.13
4	His*	-	-	8.31	1.24	1.24
5	Gly	8.76	Traces	8.74	0.70	0.70
6	Thr*	8.95	Traces	8.92	1.74	1.73
7	Ar*	9.46	Traces	9.45	0.61	0.61
8	Ala	10.19	Traces	10.17	0.97	0.96
9	Tyr	-	-	11.27	0.32	0.32
10	Cy	-	-	12.44	3.54	3.54
11	Val*	13.28	Traces	13.26	0.61	0.60
12	Met*	-	-	13.47	Traces	Traces
13	Phe*	14.61	Traces	14.69	0.66	0.65
14	Ile*	-	-	14.89	0.65	0.65
15	Lys*	15.54	Traces	15.51	1.37	1.37
16	Leu*	-	-	-	-	-
17	Pro	19.30	Traces	19.28	0.24	0.22
The sum of essential amino acids		-	-	-	6.92	6.88
The sum of nonessential amino acid		-	-	-	10.45	10.06
The sum of amino acids		-	0.123	-	17.33	16.89

*Essential amino acid. Asp: Asparagine; Ser: Serine; Glu: Glutamic acid; His: Histidine; Gly: Glycine; Thr: Threonine; Ar: Arginine; Ala: Alanine; Tyr: Tyrosine; Cy: Cysteine; Val: Valine; Met: Methionine; Phe: Phenylalanine; Ile: Isoleucine; Lys: Lysine; Leu: Leucine; Pro: Proline

and burns; it also accelerates the process of fat burning and muscle formation.^[5,12,13]

CONCLUSIONS

The analysis of the qualitative composition and quantitative content of free and bound amino acids of *Z. mays* leaves, silk, and root (variety Svetlana) was carried out using paper chromatography and HPLC.

At least 11 compounds were found among the free amino acids and at least 16 amino acids among the bound ones.

The content of bound amino acids significantly exceeds the content of free amino acids in all types of plant materials in this study.

Corn leaves, silk, and roots contain Cys in a bound form in the largest quantity.

The roots have the lowest content of amino acids, compared to leaves and silk.

The composition and content of amino acids of *Z. mays* aspartic acid leaves, silk, and roots show low biological value; however, they can be used to produce complex drugs and functional food supplements which include amino acid complexes.

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Conflicts of interest

There are no conflicts of interest.

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