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CHEMICAL COMPOSITION AND LIGNINS OF TOMATO AND POMEGRANATE SEEDS

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Tomato and pomegranate seeds were analyzed quantitatively. Lignin hydrochloride was isolated from their seeds and characterized. Lignocarbohydrate complexes were isolated by base hydrolysis. Their elemental composition, IR spectra, and molecular weights were determined.

Key words: tomato and pomegranate seeds, lignin hydrochloride, lignocarbohydrate complex.

The tomato [*Lycopersicon esculentum* Mill. (Solanaceae)] is an annual that is cultivated for its food value.

The pomegranate (*Pinica granatum* L.) is a perennial that has both food and decorative value.

The chemical compositions of seeds from tomato and pomegranate, wastes of the food industry, must be studied in order to determine pathways for their recycling.

The defatted seeds have different chemical compositions (Table 1). The content of Komarov lignin in tomato seeds is 11.51%, in pomegranate seeds, 21.44%. This is characteristic of herbaceous plants. However, they contain much more cellulose than herbaceous plants. In comparison with tomato seeds, pomegranate seeds are rich in polysaccharides.

The studied samples were unusual raw materials with respect to the study of their lignins. Therefore, we considered this factor in further analyses. Thus, tannin-like substances must be removed before the content of Komarov lignin, cellulose, and polysaccharides is determined because they cause an increase in the results for these components.

Lignin hydrochlorides, so-called Willstatter lignins, were isolated for the comprehensive lignin study. These, in contrast to lignin sulfates, are less condensed, especially if the acid treatment is very brief. The yield of lignin hydrochloride from pomegranate seeds was 25.71% of the Komarov lignin or 5.4% of the plant weight; from tomato seeds, 22.59 and 5.2%, respectively. The isolated lignins, the elemental and functional composition of which are given in Table 2, are dark pink and brown amorphous powders that are soluble in dioxane:water (9:1), DMSO, and weakly basic solutions.

The studied samples are not highly lignified materials. Therefore, they contain small amounts of carbon and relatively high amounts of oxygen. The elemental composition of the seeds has not been reported. Therefore, we could not compare our results with previous ones.

Lignin in lignified plants is considered not to contain nitrogen. However, some of the nitrogen that is still in the formation stage in the immature plant may be found in amino acids bound to the lignin. It is known that methionine acts as a methylating agent in the biosynthesis of lignin [1]. Also, the alkaloid hordenine, which is a tyrosine derivative, is converted to lignin as young barley shoots grow and develop [2].

Lignins of tomato and pomegranate seeds contain the principal functional groups typical of lignins (Table 2). The presence of carboxyls distinguishes them from lignins of certain herbaceous plants, e.g., cotton and althaea lignins [1, 2].

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TABLE 1. Chemical Composition of Tomato and Pomegranate Seeds, % of Absolute Dry Plant

Seeds	Lignin (Komarov)	Cellulose	RHPS*	DHPS**
Tomato	11.51	15.33	7.87	20.04
Pomegranate	21.44	18.71	13.05	31.36

*Readily hydrolyzed polysaccharides.

**Difficultly hydrolyzed polysaccharides.

TABLE 2. Elemental and Functional Composition of Lignin Hydrochlorides of Tomato and Pomegranate Seeds, %

Seeds	C	H	N	OH_{tot}	CO	COOH
Tomato	46.09	10.77	1.16	8.17	2.61	0.13
Pomegranate	48.76	11.10	0.41	1024	3.52	0.18

The IR spectrum of tomato-seed lignin contains the following absorption bands (cm^{-1}): 3343, 3306 (ν phenolic and alcoholic hydroxyls included in H-bonds); 2941, 2882 (ν C–H bonds); 1651 (ν carboxyls); 1542 (aromatic skeletal vibrations); 1429, 1370, 1319 (δ C–H bonds); 1263, 1162, 1111 (ν C–O–C, C–C, and C–O bonds). The IR spectrum of pomegranate seeds has the characteristic bands (cm^{-1}): 3354 (ν phenolic and alcoholic hydroxyls included in H-bonds); 2940, 2894 (ν C–H bonds); 1736, 1656, 1639 (ν carboxyls); 1510 (aromatic skeletal vibrations); 1464, 1426, 1371, 1334 (δ C–H bonds); 1266, 1232, 1161, 1113 (ν C–O–C, C–C, and C–O bonds). It should be noted that frequencies in the range 1500-1610 cm^{-1} are typical only of lignins.

Gel chromatography on Sephadex G-75 was used to determine the molecular weights of the isolated lignins. The average numerical (M_n) and average mass (M_w) molecular weights of tomato and pomegranate seed lignans are 2700 and 4100, and 5800 and 11000, respectively. The dispersivity of these lignins (M_w/M_n) was 1.51 and 1.90, respectively.

Therefore, lignin hydrochloride of pomegranate seeds not only has greater molecular weight but also has higher dispersivity than that of tomato lignin. The lignin molecular weight depends on the plant species from which it is isolated. Lignins of tomato and pomegranate seeds are similar to those of herbaceous plants in molecular-weight distribution and value.

Natural seed lignin was hydrolyzed under basic conditions to investigate more fully the lignins of the studied samples. Base hydrolysis under mild conditions led to cleavage of the ester bond between the lignin and the phenolic acids. For example, *p*-coumaric acid esterified with lignin was first identified in wheat straw lignin [5]. Furthermore, *p*-coumaric acid is known to be bound to lignin [6-8] whereas ferulic acid is bound mainly to hemicelluloses [9] and can be identified in many non-lignin tissues [10].

The investigations found that 44.8% of pomegranate seeds and 73.8% of tomato seeds of the plant sample are hydrolyzed by base. We isolated the total phenolic acids and suspended particles (Table 3).

Suspended particles formed during acidification by H_2SO_4 of the basic hydrolysate of the studied seed samples are dark brown amorphous powders that are insoluble in water and organic solvents and poorly soluble in alkali. Apparently the suspended particles can be formed by hydrolysis of the lignocarbohydrate components and, therefore, are their small fragments. The lignin component of the seeds can be dissolved as both small particles and large particles of a high-molecular-weight polymer or colloid.

The chemical nature of the suspended particles was studied by acid hydrolysis in H_2SO_4 (2 N) for 48 h at 100°C. Carbohydrates were determined in the hydrolysates using phenol sulfates. The qualitative composition of the hydrolysates, in particular, that of glucose, xylose, arabinose, and galactose, was determined by paper chromatography.

Thus, the hydrolyzed part of the suspended particles contains carbohydrates. The presence of lignin in the suspended particles was established using a color reaction with phloroglucinol (red color) [11]. Therefore, the suspended particles isolated from base hydrolysis of tomato and pomegranate seeds are fragments of their lignocarbohydrate complex. The colloidal state of these fragments is stabilized by the protective action of the polysaccharides [12]. Upon acidification, as in our instance, or cryogenic treatment, the colloidal state of the suspended particles changes [13] and they precipitate.

TABLE 3. Base Hydrolysis of Tomato and Pomegranate Seeds

Seeds	Solid, g	Suspended particles, g	Total phenolic acids, g
Tomato	1.3072	0.31.96	0.0432
Pomegranate	2.7595	1.1612	0.0224

Plant weight: 5.0 g.

TABLE 4. Elemental Composition and Molecular Weights of Suspended Particles of Tomato and Pomegranate Seeds

Suspended particles	C, %	H, %	N, %	MM*
Tomato seeds	50.77	8.31	3.85	83400
Pomegranate seeds	30.64	5.18	0.27	79800

*Molecular weights were determined by viscometry using an Ostwald viscometer.

The IR spectra of the suspended particles from tomato and pomegranate contain absorption bands typical of lignins and carbohydrates. The absorption bands for suspended particles of pomegranate seeds are given in parentheses (cm^{-1}): 3752, 3737, 3713, 3650, 3287 (3421), ν of phenolic and aliphatic hydroxyls involved in H-bonds; 2926, 2854 (2922), ν C–H bonds; 1716, 1654 (1717, 1654), ν of carbonyls and carboxyls; 1543, 1517 (1542, 1508), aromatic skeletal vibrations; 1458 (1458), δ C–H bonds; 1147 (1122), ν C–C, C–O, C–O–C bonds; 1051 (1044), pyranose skeletal vibrations. In contrast with the IR spectra of the isolated lignins, those of the suspended particles are broader owing to the overlap of the lignin and carbohydrate signals. This is evident in the broadening of the OH absorption bands in the ranges 3200-3600 and 3100-3700 cm^{-1}.

Based on the elemental analyses of the suspended particles, the studied samples differ in content of carbon and hydrogen (Table 4). The comparatively low C and H content indicates that the studied suspended particles are not only ligninaceous in nature. Pure lignin preparations typically have a higher carbon content (58-62%).

Thus, lignins of tomato and pomegranate seeds are most similar to those of herbaceous plants. Their quantitative chemical composition gives the same conclusion.

EXPERIMENTAL

IR spectra of lignin hydrochlorides and lignocarbohydrate complexes were recorded on a Perkin—Elmer model 2000 Fourier spectrophotometer in KBr disks.

Raw Material Preparation. The alcohol—benzene extracts of tomato and pomegranate seeds were defatted beforehand by extraction with hexane and $CHCl_3$ according to the TAPPI-6m-59 method [11]. The extracted seeds were ground to a powder in a Cyclone laboratory grinder.

Elemental Composition. The C, H, and N content of the lignin hydrochloride in the lignocarboyhdrate complex was determined on a CHN-analyzer (CSFR).

Functional Composition. The contents of hydroxyls, carbonyls, and carboxyls in the lignins were determined by the literature methods [14].

Basic Components. The lignin content was established by a modified Komarov method using H_2SO_4 (72%) [11]; cellulose content, using Kirsner and Hoffer methods based on the use of an alcoholic solution of HNO_3 [11]. The contents of readily and difficultly hydrolyzed polysacharides were determined using an ebullioscope [11].

Lignin sulfates (Willstatter lignins) were isolated as before [1].

Base Hydrolysis of Seeds. Powder of tomato (or pomegranate) seeds extracted by alcohol—benzene (1:2) was treated with NaOH (8%) at 100°C for 1 h. The solid was separated from the hydrolysate. The hydrolysate was acidified with conc. H_2SO_4 until the pH was 1. This produced a precipitate of the lignocarbohydrate components (suspended particles) that was

washed with water and saved for further study. The hydrolysate was extracted with ether. The ether extract was treated with saturated $NaHCO_3$ solution. The resulting aqueous solution was again extracted with ether to give the phenolic-acid fraction.

Gel chromatography of lignin hydrochlorides was performed using an analytical column packed with Sephadex G-75 with elution by freshly distilled DMSO. The column was charged with lignin in DMSO (0.5 mL, 0.5%). Fractions of 1.0-1.5 mL were collected. The optical density was measured on a SF-26 spectrophotometer at 280 nm. The gel chromatography plots had the ratio of total eluent volume to total gel volume ($V/V_1 = V_{con}$) along the abscissa. This made it possible to compare these plots. The resulting gel-chromatograms were recalculated using the equation $K_{av} = 2.9 - \log M$ 0.65 [15, 16] in integral and differential forms and defined the average mass M_w and numerical M_n molecular weights of the lignin preparations according to the literature method [16].

Paper chromatography was performed in butanol—pyridine—water (6:4:3) with development by acidic anilinium phthalate using FN 11 paper.

Acid hydrolysis of suspended particles was carried out according to the literature [13]. Particles (0.1 g) in H_2SO_4 (4.0 mL, 2 N) in sealed ampuls were heated on a water bath for 48 h at 100°C. The ampuls were opened. The hydrolysate was centrifuged. The centrifugate, the hydrolyzed part of the suspended substances, was evaporated to 1 mL.

Phenol-Sulfate Samples. Diluted hydrolysate (0.5 mL) of suspended particles was treated with phenol (0.5 mL, 0.5%) and conc. H_2SO_4 (2.5 mL) to give a brown color.

A **color reaction** for the presence of lignin in the suspended particles was performed using phloroglucinol [11].

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