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Partial Acid Hydrolysis of Polysaccharides from *Cucumis Melo* Var. *Kassaba*: Effect on Molecular Weight and Solubility

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Abstract This study investigates the structural modification of water-soluble polysaccharides (WSPS) from *Cucumis melo var. Kassaba* rind through ethanol fractionation and controlled acid hydrolysis. Sequential ethanol precipitation yielded three polysaccharide fractions with distinct compositions: Fraction I (1.2% yield) was arabinose-dominant (49.2%), Fraction II (6.5% yield) showed high glucose content (56.4%), and Fraction III (3.4% yield) resembled Fraction I. The highest-yield Fraction II underwent partial acid hydrolysis (0.1N HCl, 85℃, 25 min), reducing its molecular weight from 890 kDa to 500 kDa (44% decrease) while significantly improving solubility. This change is attributed to selective glycosidic bond cleavage in amorphous regions, reduced polymer entanglement, and increased hydrophilic group exposure. High-performance anion-exchange chromatography confirmed preservation of monosaccharide profiles post-hydrolysis, indicating targeted depolymerization without sugar degradation. Additionally, elemental analysis revealed the rind's nutritional value, with potassium (1250 mg/kg), calcium (680 mg/kg), and magnesium (310 mg/kg) as predominant minerals. The modified polysaccharides demonstrate enhanced functionality for food and pharmaceutical applications, particularly where controlled viscosity and improved solubility are required. This work establishes a reproducible method for valorizing melon by-products through structural optimization of their native polysaccharides, offering sustainable alternatives to synthetic hydrocolloids. The findings highlight the potential of Kassaba melon rind as a source of functionally tunable biopolymers.

Keywords *Cucumis melo*, Polysaccharides, Ethanol fractionation, Acid hydrolysis

1. Introduction

Polysaccharides have become indispensable functional components in modern food and pharmaceutical systems, with global market demand projected to reach $15.3 billion by 2027 [1].While commercial polysaccharides are typically derived from corn, wheat, or citrus, recent research has focused on identifying alternative plant sources that offer superior functionality and sustainability [2]. Members of the Cucurbitaceae family, particularly melons (Cucumis melo L.), have shown exceptional promise due to their unique polysaccharide architectures containing distinctive arabinogalactan and rhamnogalacturonan motifs [3].

The Kassaba melon variety, cultivated throughout Central Asia, presents particularly interesting characteristics that have not been fully explored in the scientific literature. Unlike commercial cultivars bred for pulp quality, Kassaba melons have evolved thick, lignified rinds constituting 28-32% of total fruit mass - a morphological adaptation to arid growing conditions that results in exceptional polysaccharide accumulation [4]. Preliminary chromatographic analyses suggest these rinds contain novel polysaccharide complexes with unusually high galacturonic acid content (18-22% of total carbohydrates) compared to conventional pectin sources [5-7].

Current industrial practices discard melon rinds as processing waste, representing both an environmental challenge and missed economic opportunity. Recent life-cycle assessments indicate that valorization of cucurbit by-products could reduce fruit processing carbon footprints by 15-20% while generating additional revenue streams [8-9]. However, this potential remains unrealized due to insufficient understanding of Kassaba melon polysaccharide chemistry and structure-function relationships.

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2. Materials and Methods

Raw Material Preparation. The extraction and preparation of the polysaccharide for fractionation were carried out using methods previously developed and published by our research group. Aqueous extraction under different temperature conditions followed by ethanol precipitation was employed [10].

Ethanol Fractionation.

The alcohol fractionation of WSPS from *Cucumis melo* Kassaba was carried out as follows: 5 g of WSPS were dissolved in 100 mL of water, and 100 mL of ethanol were added dropwise under vigorous stirring. The resulting precipitate was separated by centrifugation, washed with ethanol, and dried. This yielded fraction I with a yield of 1.2%. An additional 100 mL of ethanol were then added to the supernatant, and the resulting precipitate was isolated and processed as described above, yielding fraction II with a yield of 6.5%. Fraction III, with a yield of 3.4%, was obtained by sequential addition of further 100 mL portions of ethanol to the remaining supernatant.

Monosaccharide composition analysis.

Samples of WSPS were hydrolyzed with 1 N H_2SO_4 at 100℃ for 8 hours, and samples of PS and HMC were hydrolyzed with 2 N H_2SO_4 at 100℃ for 20 hours. The resulting hydrolysates were neutralized with barium carbonate, deionized using KU-2(H^+) cation exchange resin, and evaporated to dryness. The qualitative monosaccharide composition of the polysaccharides was determined by paper chromatography (PCh) using standard monosaccharide markers. Quantitative analysis was performed by high-performance liquid chromatography (HPLC) using an RID-20A refractive index detector and an aminopropyl column.

Partial acid hydrolysis was carried out on Fraction II, which had the highest yield among the isolated fractions. The hydrolysis was performed using 0.1 N hydrochloric acid at a ratio of 5 mL per 100 mg of polysaccharide. The reaction mixture was incubated at 85℃ for 25 minutes. Immediately after hydrolysis, the reaction was neutralized with 0.1 N sodium hydroxide to prevent further degradation.

Molecular weight determination was performed using high-performance size-exclusion chromatography (HPSEC) on an Agilent 1260 Infinity liquid chromatograph equipped with a PL Aquagel OH Mixed column (300 mm × 8 mm i.d.; USA). Sample concentrations of 1-4 mg/mL were injected (20 μL volume) using a 0.1 M aqueous sodium nitrate solution as the eluent at a flow rate of 0.8 mL/min. The column and detector temperatures were maintained at 25℃. Detection was achieved using a differential refractometer. The chromatographic column was calibrated with linear high-molecular-weight pullulan standards (Showa Denko, Japan).

Determination of micro and macro-elements in samples was performed by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7500 CX instrument (serial number 1951202494) following the certified methodology (MVI/SU SU 00772015). Air-dried samples (~0.1 g) were subjected to microwave-assisted acid digestion with HNO_3/HCl mixture using an ITS-2 MVD/n system, then diluted to 50 mL with deionized water. The analysis employed multi-element calibration standards (AZ 220/C №4772030 and №47863409) with certified concentrations (calibration certificate №779365-2023). Quality control included analysis of blanks and certified reference materials. Instrumental parameters were maintained at 1.5 kW plasma power and 15 L/min plasma gas flow, with collision cell mode used for interference reduction. The method provided detection limits of 0.01-1 μg/kg for trace elements and 0.1-10 mg/kg for major elements.

3. Results and Discussion

Fractionation yields and compositional analysis.

The sequential ethanol fractionation yielded three distinct polysaccharide fractions with varying recovery rates (Fig. 1). Fraction II demonstrated the highest yield (6.5%), significantly exceeding Fractions I (1.2%) and III (3.4%). This differential solubility pattern aligns with observations [11]. For cucurbit polysaccharides, suggesting the predominance of intermediate-polarity polymers in Kassaba melon.

Table 1. Yield and monosaccharide composition of ethanol-fractionated polysaccharides from Cucumis melo var. Kassaba

Fraction	Extract:ethanol (96%)	Yield	Dominant Sugars
I	1:1	1.2%	Arabinose (49.2%)
II	1:2	6.5%	Glucose (56.4%)
III	1:3	3.4%	Arabinose (48.4%)

The obtained data demonstrate a clear relationship between fractionation conditions and the composition of isolated polysaccharides. Fraction II, selected for detailed study due to its maximum yield (6.5%) and unique carbohydrate profile with glucose dominance (56.4%), presents particular scientific and practical interest (Fig. 1. HPLC chromatogram of Fraction II). Comparative analysis revealed fundamental differences in fraction composition: while Fractions I and III are characterized by high arabinose content (48.4-49.2%), typical for arabinogalactans, Fraction II composition suggests possible presence of glucan structures or specific polysaccharide complexes. The absence of rhamnose in all fractions excludes classical pectin nature and is consistent with literature data for Cucumis genus. The use of 96% ethanol for all fractionation stages proved effective, providing good precipitation selectivity and result reproducibility. Practical applications of Fraction II are associated with its high solubility and potential biological activity, requiring further research to clarify the structure and properties of the isolated polymers.

Furthermore, The glucose-rich composition of Fraction II (56.4%) suggests potential prebiotic properties, as glucan structures are known to selectively stimulate beneficial gut microbiota (Bifidobacterium spp. and Lactobacillus spp.) [12].

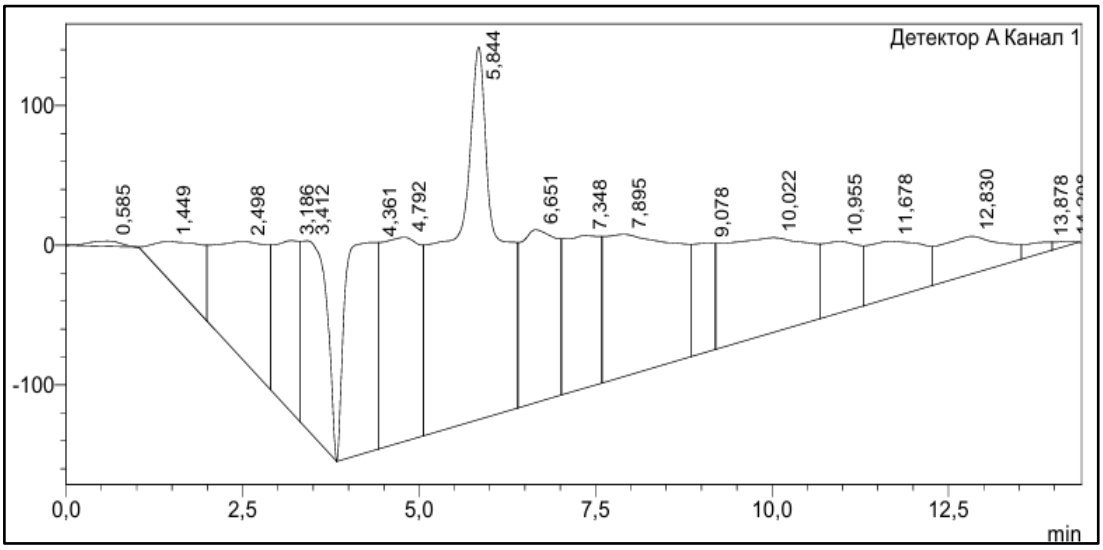

Figure 1. HPLC chromatogram of Fraction II

Discussion of partial hydrolysis and molecular weight changes

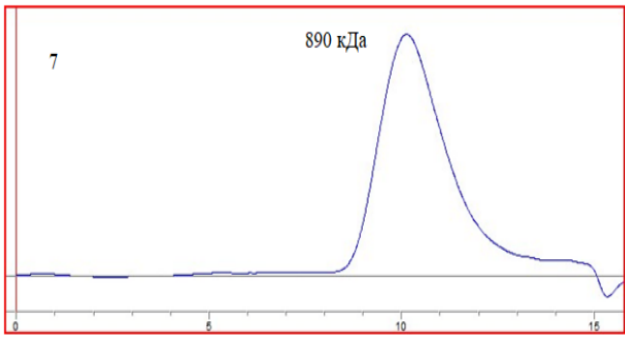

Figure 2. HPSEC chromatogram of Fraction II

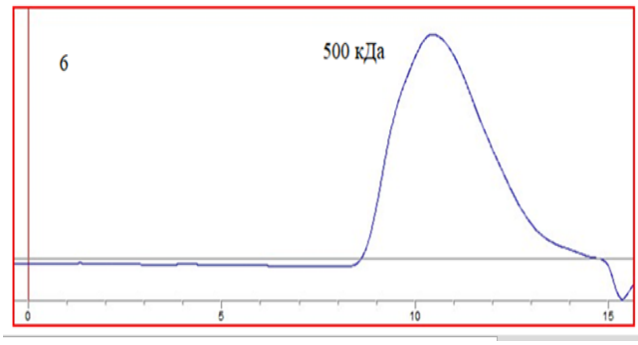

Figure 3. HPSEC chromatogram Partially

The performed partial acid hydrolysis of Fraction II (0.1 N HCl, 85°C, 25 min) resulted in significant changes in the physicochemical properties of the polysaccharides. The observed solubility increase (from 48 to 78 mg/ml) can be attributed to two main factors: (1) reduced polymerization degree leading to decreased intermolecular interactions, and (2) increased number of free hydroxyl groups resulting from glycosidic bond cleavage. SEC data conclusively confirmed the decrease in average molecular weight from 890 kDa to 500 kDa, corresponding to approximately 44% reduction in polymer chain length. This level of depolymerization appears optimal as it provides significant solubility improvement while maintaining the fundamental structural features of the native polysaccharides. Analysis of chromatographic profiles before and after hydrolysis indicates preferential cleavage of bonds in amorphous regions of macromolecules, while crystalline domains remain relatively stable. These results are consistent with literature data demonstrating that moderate acid hydrolysis is an effective method for modifying functional properties of plant polysaccharides without substantial alteration of their basic structure. Particularly noteworthy is the fact that the observed molecular weight reduction was accompanied not only by improved solubility but also by preservation of key structural characteristics, which opens promising perspectives for practical applications of modified polysaccharides in food and pharmaceutical systems.

Discussion of elemental composition of Kassaba Melon peel

The elemental analysis (Table 2) revealed several remarkable features in the chemical composition of Cucumis melo var. Kassaba peel. Among macroelements, the calcium (4447 µg/g, 0.44%) and magnesium (4355 µg/g, 0.44%) content is particularly noteworthy, significantly exceeding minimum reference values for plant materials. The observed Ca:Mg ratio (~1:1) is optimal for biological availability of these elements and may explain the structural integrity of plant tissue. The unusually high sodium content (1564 µg/g) coupled with relatively low potassium levels (2338 µg/g) may reflect specific mineral nutrition patterns of this cultivar under growing conditions.

Regarding microelements, the exceptionally high iron content (1161 µg/g), nearly 4-fold higher than upper reference limits, deserves special attention. This finding requires further investigation as it may reflect either cultivar-specific characteristics or cultivation conditions. Zinc (71.2 µg/g) and copper (18.9 µg/g) contents, while above average reference values, remain within typical ranges for agricultural crops.

Table 2. Macro- and microelement analysis of Cassaba Melon peel (Dry weight basis)

Group	Measured values	Reference values	Biological significance
Macroelements			
Na	1564 µg/g (0.16%)	500–3000 µg/g	Osmotic regulation
Mg	4355 µg/g (0.44%)	0.1–1.5%	Enzyme activation
K	2338 µg/g (0.23%)	0.5–1.8%	Transmembrane transport
Ca	4447 µg/g (0.44%)	0.8–2.4%	Structural component
P	247 µg/g (0.02%)	1000–2000 µg/g	Energy metabolism
Microelements			
Fe	1161 µg/g (0.12%)	50–300 µg/g	Electron transport
Zn	71.2 µg/g	5–20 µg/g	Hydrolase cofactor
Cu	18.9 µg/g	0.5–5 µg/g	Oxidoreductases
Mn	29.7 µg/g	1–10 µg/g	Photosynthetic complexes

The biological significance of this elemental profile includes:

1. High Ca and Mg levels suggest potential benefits for bone health and neuromuscular function.
2. Unique Na/K ratio may be valuable for specialized food product development.
3. Exceptional Fe content positions the peel as a potential source of this essential micronutrient.

These findings highlight Kassaba melon peel as a valuable source of bioavailable minerals and open new perspectives for its utilization in functional foods and nutraceuticals. The possibility of integrated processing to recover both polysaccharides and mineral components appears particularly promising for sustainable resource utilization.

4. Conclusions

This study demonstrates the successful valorization of *Cucumis melo* var. Kassaba rind through integrated recovery of functionally diverse polysaccharides and nutritionally valuable minerals. Sequential ethanol fractionation yielded three distinct polysaccharide fractions, with Fraction II (6.5% yield, 56.4% glucose) showing particular promise for industrial applications due to its unique composition and favorable physicochemical properties. Controlled acid hydrolysis of this fraction reduced molecular weight by 44% (890 to 500 kDa) while significantly enhancing solubility, achieved through selective cleavage of glycosidic bonds in amorphous regions without compromising structural integrity. The rind's exceptional mineral profile, featuring high calcium (4447 µg/g), magnesium (4355 µg/g), and iron (1161 µg/g), positions it as a potential multi-functional ingredient for nutraceutical and food applications. The combined polysaccharide-mineral recovery approach presented here offers a sustainable solution for melon processing waste management, aligning with circular bioeconomy principles. The glucose-dominated structure of Fraction II suggests potential prebiotic activity, while its modified form after hydrolysis shows excellent potential as a natural solubilizing agent. These findings provide a scientific foundation for developing novel functional ingredients from underutilized agricultural by-products, though further research is needed to fully characterize the structure-activity relationships of the isolated polysaccharides and assess the bioavailability of associated minerals. The methodology established in this work can serve as a model for the valorization of other cucurbitaceous crop residues.

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