Ultrasound-assisted extraction of zein from corn

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INTRODUCTION

Corn is an abundant and renewable resource, which is processed into many food and industrial products [1]. Zein, a protein found in maize endosperm is a major co-product of the bio-fuel industry with different application in (i) biomedicine as drug-delivery compound, drug capsules that dissolve in the body [2], (ii) in industrial production of bioplastic, paper coating, and food products [3]. Zein was classified according to the structure of the amino acid sequence, solubilization and molecular weight into 4 fractions, α, β, γ and δ-zeins. The α-zein is found in the largest amount and consists in two peptides with 19 kDa and 22 kDa and is the extraction is usually perform with ethanol. Due to the high complexity of the starting material and to the extraction conditions, analysis of protein compositions requires a combination of modern analytical methods such as SDS-PAGE, 2D gel electrophoresis and mass spectrometry [4,5].

Here, we investigate, by using analytical methods, the efficiency of zein extraction from corn flours using 65-95% aqueous ethanol under ultrasound conditions from dry-ground whole corn as well as meals with different grain sizes. Moreover, the extracted zein and the commercial zein protein were used for different conjugates synthesis. We have applied modern methods such as MALDI ToF mass spectrometry, SDS-PAGE electrophoresis and FT-IR spectroscopy to characterize the extracted zein.

METHODOLOGY

Zein extraction

Maize seeds from the inbred KWS 3381 were ground with a tripod portable mill (MB03, 1500 rpm, produced by IPEE, Romania). An amount of 20 g of the resulting flour was defatted with petroleum ether for 5 hours using a Soxhlet extractor. The flour was allowed to dry for 24 hours in a laboratory oven at 100°C. The flour was further ground using a laboratory electric mill (SAMAP F100, Andolsheim, France) until particles with the size lower than 100 µm were obtained. 20 g of commercial flour was defatted and dried as described above. For separating the flour particles with different sizes, sieves of 710 µm, 500 µm and 250 µm, were employed using a sieve shaker. For the extraction of the zein 150 mg of defatted flour that has selected particle size was mixed with 1.5 mL 65% or 95% ethanol in water for 30 minutes in an ultrasound bath. The samples were centrifuged for 10 minutes at 16,000 rpm using a Hettich Mikro 22R and then precipitated using acetone (4:1) for 2 h at – 20 °C. The proteins were centrifuged for 10 minutes at 16,000 rpm. The commercial zein was purchased form Sigma-Aldrich.
The extracted samples were characterized by MALDI-TOF mass spectrometry, SDS-PAGE electrophoresis, HPLC and FT-IR spectroscopy.

Mass spectrometry analysis

For MALDI-TOF mass spectrometric analysis, a solution of DHB (2,5-Dihydroxybenzoic acid) in acetonitrile: 0.1% trifluoroacetic acid in water (2:1 v/v) was prepared. The extracted zein was mixed with the supernatant of the matrix solution. Afterwards, the samples were placed (0.5 µL of each sample-matrix solution) on a 384-spot target plate of the MALDI-ToF instrument using the dried-droplet method and allowed to dry for 1 hour. MALDI-ToF MS analysis was performed on a Bruker UltraflexMALDI ToF/ToF mass spectrometer operated in positive reflectron mode and equipped with a pulsed nitrogen UV laser.

SDS PAGE analysis of zein

SDS–PAGE gel electrophoresis was performed according to Laemmli protocol using non-denaturing and denaturing conditions for sample preparation. 15 µL of sample were dissolved in a buffer containing 4% SDS, 25% glycerin, 6 M urea and 50 mM Tris, before electrophoresis. All SDS–PAGE data was evaluated using the theoretical molecular weight taken from UniProt Knowledgebase. The Coomassie stained gels were destained, immediately scanned and kept in water at 4°C for further workup.

FT-IR spectroscopy

The infrared spectra were recorded in solid KBr using a Shimadzu 8400S FTIR spectrophotometer (Shimadzu, Japan) from 4000 to 400 cm^{-1}.

RESULTS

1. Zein extraction

P04698- α-zeină 22 kDa
[1]MATKILSLLALLALFASATNASIIPQCSLAPSSIIPQF
LPPVTSMAFEHPAVQAYRLQQAIAASVLQQPIAQL
QQQSLAHLTIQTIATQQQQQQFLPALSHLAMVNPV
AYLQQQLLASNPLALANVVANQQQQQLQQFLPAL
SQLAMVNPAAYLQQQQLLSSSPLAVANAPTYLQQ
ELLQQIVPALTQLAVANPVAYLQQLLPFNQLTMSNS
VAYLQQRQQLLNPLAVANPLVAAFLQQQQLLPYNR
FSLMNPVLSRQQPIVGGAIF[267]

Table 1. The efficiency of zein extraction from corn flour with different solvents

Solvent used for zein extraction	Concentration (mg/mL)	
	Hybrid KWS 710 µm	Hybrid KWS 100 µm
Ethanol absolute	0.147	0
70% Ethanol	3.040	6.471
50% Ethanol	1.427	1.524
30% Ethanol	1.004	1.056
10% Ethanol	1.113	0.998
Methanol absolute	0.148	0.358
70% Methanol	0.712	0.774
50% Methanol	0.415	0.405
30% Methanol	0.861	0.687
10% Methanol	0.979	0.728
Isopropanol absolute	0	0
70% Isopropanol	0.966	1.043
50% Isopropanol	2	3.926
30% Isopropanol	0.612	0.628
10% Isopropanol	0.638	0.712

2. Zein characterization by mass spectrometry and HPLC

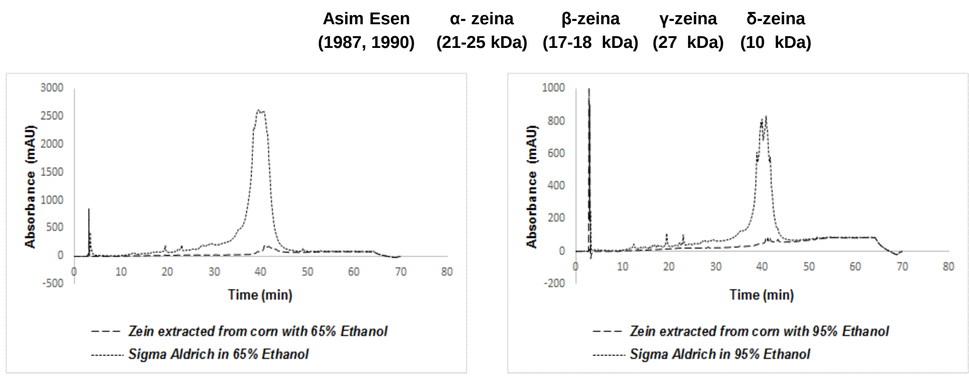

Figure 3. HPLC chromatogram of of commercial and extracted zein from corn with 65% (a) and 95% ethanol (b) using ultrasound

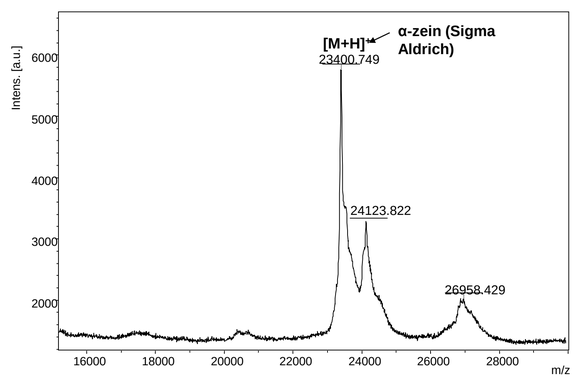

Figure 4. Mass spectra of comercial α-zein from Sigma Aldrich using DHB (2,5-Dihydroxybenzoic acid) as matrix

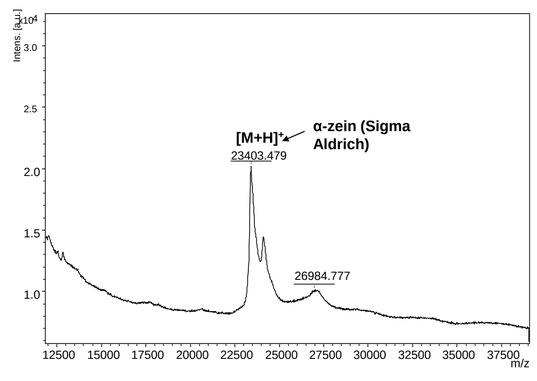

Figure 5. Mass spectra of comercial α-zein from Sigma Aldrich using super-DHB (2,5-Dihydroxybenzoic acid and 2-hydroxy-5-methoxybenzoic) as matrix

3. SDS-PAGE electrophoresis

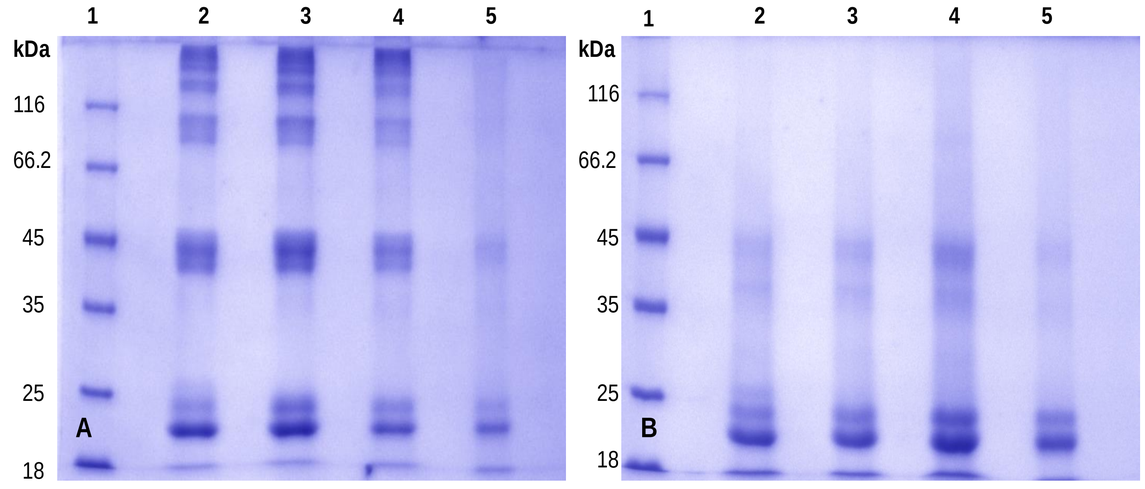

Figure 6. SDS-PAGE electrophoresis of zein under ultrasound conditions (A) ethanol extraction and (B) acetonitrile extraction with DTT; 1-prestained protein ladder; 2-KWA 710 µm; 3-comercial flour 710 µm; 4- comercial flour 250 µm; 5-standard zein

4. FT-IR spectroscopy

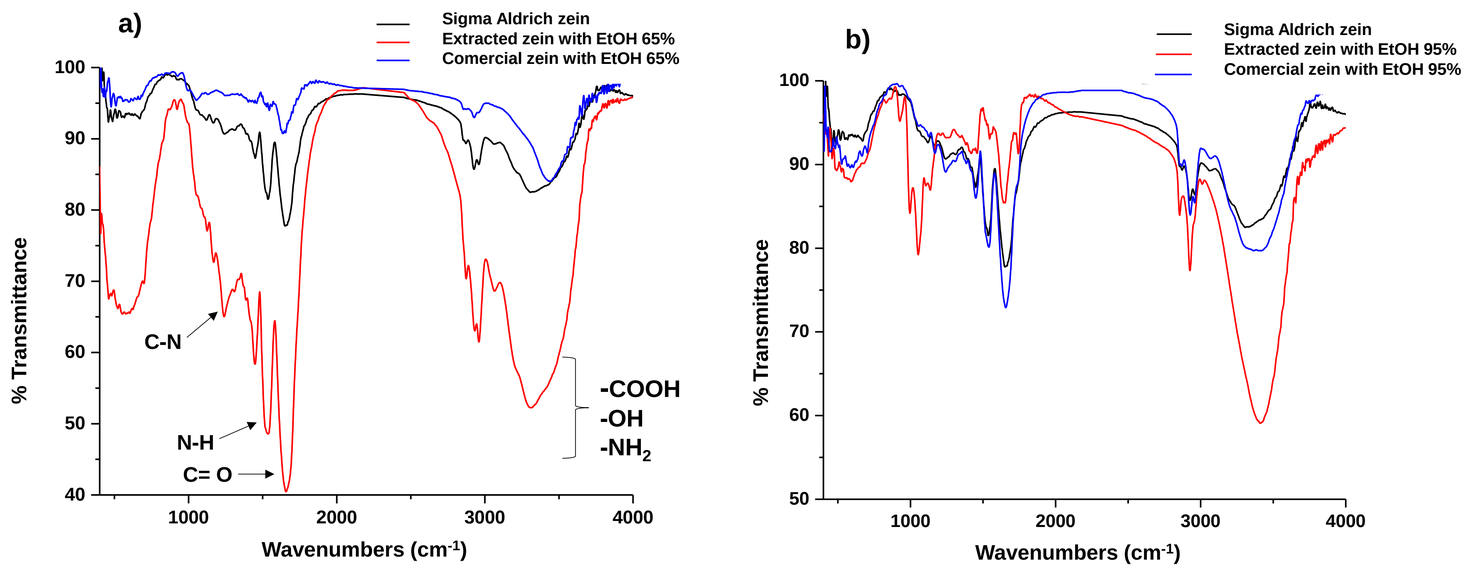

Figure 7. FT-IR spectra of commercial and extracted zein from corn with 65% (a) and 95% ethanol (b) using ultrasound conditions

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Conclusions

- ✓ MALDI-TOF mass spectrometry, SDS-PAGE electrophoresis, FT-IR spectroscopy and HPLC were successfully used for rapid analysis of comercial and extracted zein from corn using ethanol;
- ✓ Extraction of zein from corn was investigated with different solvents. The optimal solvent is ethanol of approximate 70% concentration;
- ✓ MALDI-ToF mass spectrometry showed extraction only of α-zein by using 95% ethanol.

References

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