JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2011, *17*(4), 402-404

Journal of Agroalimentary Processes and Technologies

Obtaining and characterization of crude protein extract from unhulled sesame seeds

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Received: 18 November 2011; Accepted: 07 December 2011

Abstract

The crude protein extract was obtained from unhulled sesame seed, as they are, and washed with concentrated and diluted sodium hypochlorite. The protein content of each crude extract was analyzed with Bradford assay and SDS-PAGE. Crude protein extracts obtained showed no antimicrobial activity against *Escherichia coli*.

Keywords: crude protein extract, unhulled sesame seeds, protein concentration, antimicrobial activity

1. Introduction

Antimicrobial resistance to antibiotics is now a serious health problem [1-3]. Currently the focus is on identifying new structures with antimicrobial activity. The researchers have turned their attention to antimicrobial peptides, low molecular weight structures, usually positively charged, amphipathic with antimicrobial activity against bacteria, fungi, viruses, even protozoa [4,5].

In this paper, crude protein extracts obtained from unwashed or washed unhulled sesame seeds with dilute or concentrated sodium hypochorite, were characterized. Antimicrobial activity of these crude extracts was tested against *Escherichia coli*.

2. Materials şi method

*Materials.*Unhulled sesame seeds from ecological culture, from India; All reagents were analytical grade (Sigma, Roth, Fluka Analytical) and specific for analysis; To test the antimicrobial activity of *Escherichia coli* culture was used.

Method. The proteins were extracted from unhulled sesame seeds using the method described by Costa F.T. et al. [6], with slight modifications. Unhulled sesame seeds were used as such (WS3) and washed with concentrated (WS1) or diluted sodium hypochlorite (30% sodium hypochlorite and 70% distilled water) (WS2). After washing the seeds with sodium hypochlorite (concentrated or diluted) for 5 minutes, stirring continuously, and then with distilled water under the same conditions, seeds were dried under vigorous air flow at room temperature for 48-72 hours. Crude extracts were obtained according to the operating block diagram shown in Figure 1. Ground seeds were subjected to extraction with a solution containing 0.6M NaCl and 0.1% HCl, in a proportion of 1:3 (w/v), overnight at 4°C, with stirring continuously. After filtration and centrifugation at 4000g for 2 hours at 4°C, the supernatant is filtered through filter paper again. The supernatant (crude protein extact) is collected.

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To measure the protein concentration, Bradford assay was used. Bovine serum albumin was used as standard solution

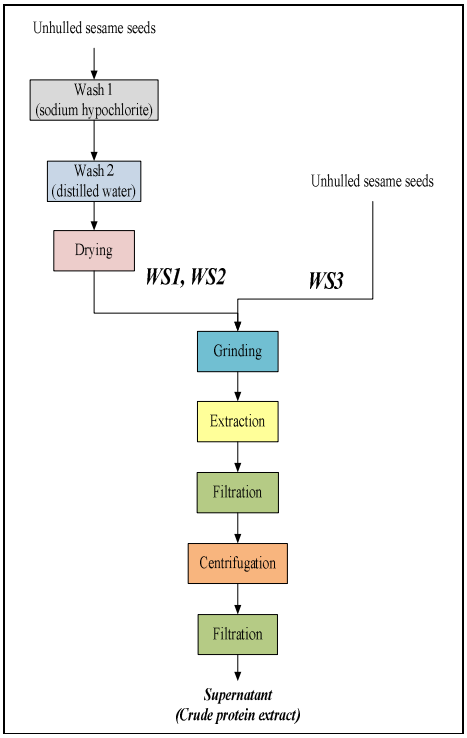

Figure 1. Block diagram for obtaining the protein fractions from unhulled sesame seed

Moleculare mass analyses. Protein fractions were analyzed by 18% SDS-PAGE (sodium dodecyl sulfate – polyacrylamide gel electrophoresis) gel. In the gel pockets, 10 µL mixture consisting of 5 µL crude protein extract (WS1, WS2, WS3) and 5µL sample buffer was applied. The gel was stained with Coomassie Brilliant Blue G250 dye.

Antimicrobial activity was tested against *Excherichiei coli* on solid culture medium prepared from triptych soy broth and agarose. From each protein extract 10 µL was applied on *E. coli* plate. Plates were incubated at 37°C for 24 hours.

3. Results and Discussion

Extract obtained from unwashed seed (WS3) presented a light brown color, while liquid obtained from sesame seeds washed with sodium hypochlorite, presented a yellowish color. The higher the active chlorine concentration was, the lighter the color of extract obtained was.

The amount of protein (Table 1) was calculated using the linear regression equation:

$y = 0.923x + 0.408$ for protein extract obtained from WS1, $y = 0.81 x + 0.428$ for protein extract obtained from WS2, and $y = 0.6854 x + 0.3516$ for protein extract obtained from WS3.

Table 1. Protein concentration (mg/mL) of crude protein extracts (*WS1, WS2 and WS3*)

Sample	*Dilution*	*The average absorbance values at 595 nm*	*Protein concentration (mg/mL)*
WS1	1:5	1.281	4.72
	1:10	1.14166667	7.93
	1:20	0.90733333	10.79
	1:50	0.709	16.26
WS2	1:5	1.113	4.22
	1:10	0.96033333	6.56
	1:20	0.79766667	9.12
	1:50	0.679	15.48
WS3	1:5	0.958	4.31
	1:10	0.76633333	6.20
	1:20	0.7	9.49
	1:50	0.512	14.52

After comparing the obtained results, it was found that protein concentration (mg /mL) is higher for crude protein extract WS1 and lower for WS3 (Figure 2).

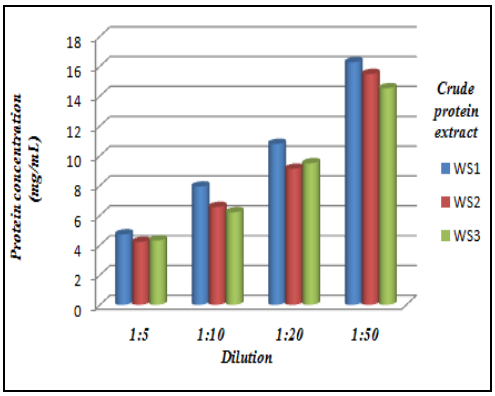

Figure 2. The comparison of protein concentration (mg/mL) of *WS1*, *WS2* and *WS3* crude extracts

Electrophoregram (18% gel) obtained for the crude protein extracts, presented in Figure 3, showes that the intensity of protein bands in series decreases (WS1, WS2, WS3) (WS1> WS2> WS3).

The differences were attributed to the oxidizing efficiency of sodium hypochlorite in sesame seed washing, which removes more impurities when the active chlorine content of sodium hypochlorite is higher.

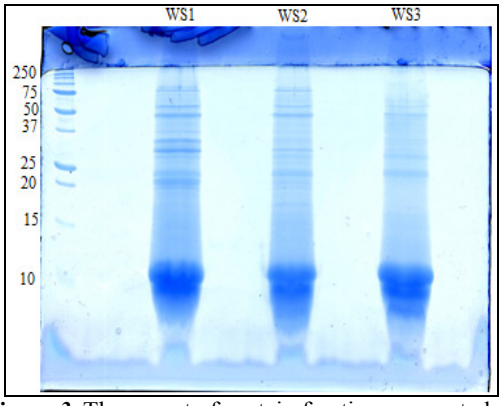

Figure 3. The aspect of protein fractions separated on gel, from crude protein extracts WS1, WS2 and WS3

After 24 hours of incubation at 37°C, crude extracts showed no antimicrobial activity.

4. Conclusion

Obtained in the same working conditions, protein extracts showed different protein concentrations, confirmed also by the intensity of protein bands from the 18% gel. Protein extracts obtained showed no antimicrobial activity against *Escherichia coli*. After testing the antimicrobial activity of crude extract obtained from kernels of white and black sesame seeds against *Escherichia coli* at a concentration of 100 µg /mL, Costa, F.T., et. al. [6], obtained the same result.

Acknowledgment

We wish to thank the Banat's University of Agriculture Sciences and Veterinary Medicine Timişoara, Faculty of Food Technology Products, for a generous helpful of different kinds and financial support by doctoral scholarship programme SOP HRD (Sectoral Operational Programme Human Resources Development) by the Managing Authority of SOP HRD (MA SOP HRD).

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