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Research Article
Influence of Reduced Phenolics and Simmondsins Contents on Protein Quality of Defatted Jojoba Meal

Suzanne M. Wagdy, F.S. Taha and Salma S. Omar

Department of Fats and Oils, National Research Centre, 33 Bohouth St. Dokki, Giza, Egypt

Abstract

Objective: The objectives of this study were to reduce the toxic compounds and polyphenolic compounds in jojoba meal by (1) One solvent with one concentration (100%) and different techniques, (2) Different solvents and different concentrations with the same technique and (3) Illustrate its effection on native and protein bands. **Methodology:** The jojoba press cake was defatted in a soxhelt apparatus, the dried defatted meal was extracted with different solvents and different techniques to reduce both phenolic and simmondsins content. **Results:** The results showed that maximum simmondsins and phenolic extractes were 14% and 18.5 mg g^{-1} meal, respectively. The best achieved condition to extract simmondsins was soxhlet extraction for 30 h whereas, in case of phenolic compounds, shaking for 15 min, then soaking for 72 h was the best condition. The examination of the dried residue by electrophoresis (native and SDS-PAGE) analysis showed that five major polypeptide bands (10, 15, 20, 38 and 48 kDa) were predominant with similar ratios in almost all used techniques, no reduction in protein bands with 100% solvent, whereas variations in solvent concentrations caused reduction in protein bands and the protein mobility increased with increasing the effect of microwave time. **Conclusion:** The protein analysis revealed that there is no relation between the simmondsins content and the protein quality.

Key words: Defatted jojoba meal, solvent extraction, microwave-assisted extraction, ultrasound assisted extraction, native, SDS-PAGE electrophoresis

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Corresponding Author: Suzanne M. Wagdy, Department of Fats and Oils, National Research Centre, 33 Bohouth St. Dokki, Giza, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jojoba (*Simmondsia chinens*) is now commercially cultivated in many countries. The main compositions of the seed is up to 50% oil, 25% protein and 30% carbohydrate[1,2]. Jojoba is good solution for plantation in Saudi Arabia and Egypt, because lesser possibilities for infection and it requires heat, low fertilizers and salt tolerance.

Simmondsins are a group of cyclohexcyanomethylene glycosides isolated from jojoba seed and meal[3]. Jojoba meal contains up to 10% simmondsins and Williams and Price[4] reported that simmondsins alone in jojoba meal was not causing the death of mice but phytate, phenolics, trypsin inhibitors and tannins acts as synergists with simmondsins and responsible for the weight loss and death for the laboratory mice and experimental animals and jojoba meal are high enough to warrant which caused food intake depression and decreased growth in rats[5]. Defatted jojoba meal contained 30-35% crude protein and up to 15% compounds related to simmondsins[1,6]. This relatively high protein content makes jojoba meal a potential source of protein for animals and humans[7]. Total jojoba proteins are composed of 65% albumins, 21% globulins, 8% prolamines and 6% glutelins[8]. There are no specific data on the effect of reducing phenolic and simmondsins content in the protein quality or polypeptides of jojoba meal. Chemical and physical treatments such as mechanical pressure, sonications, temperature, microwave heating, osmotic and chemical lysis can be extracts of proteins from cell walls[9]. Aqueous alcohols or aqueous acetone were the best organic solvents used to reduce phenolic compounds from most of food sources and widely used on a commercial scale and removal of simmondsins from jojoba meal has been attempted in several studies[10-12]. In order to improve the high quality of the jojoba meal, several treatments used to reduce the toxicity compounds[13,14]. Water washing, solvent extraction, chemical treatment, heat and microbial methods resulted in reduced simmondsins levels[11,12,15] reported 8.1% total polyphenolic content in defatted jojoba meal. Although, the presence of tannins in jojoba seeds has been established[15] however, their nutritional significance has not been studied. Jojoba meal proteins have been extracted, characterized and evaluated for amino acid content[8] and functionality[16] but data on digestibility are not available.

The extraction with aqueous alcoholic solvents, change in the protein structures, quality, can be coagulated and reduced its functional properties. To avoid these problems thermal and mechanical treatments are used to obtain protein concentrated or isolated with good functionality[17,18].

In some treatments, ultrasound waves may possibly destroy the antioxidant compounds and reduce the extraction yield[19]. Microwave-assisted extraction acts directly on molecules[20]. Water within the plant matrix absorbs microwave energy, which also allows the separation of compounds from the matrix and temperatures allow fast and efficient extraction[21].

Electrophoresis separates mixture of proteins and used as an analytical tool[22,23], native gels can be sensitive to detecting things such as chemical treatments, degradation and to recover proteins in their native state after the separation[24].

The objectives of this study was to reduce the toxic compounds and polyphenolic compounds in jojoba meal by (1) One solvent with one concentration (100%) and different techniques, (2) Different solvents and different concentrations with the same technique and (3) Illustrate its effection on native and protein bands.

MATERIALS AND METHODS

Materials: All chemicals were obtained from Sigma Chemical Co. Defatted jojoba press cake was obtained from Egyptian Natural Oil Co. (NATOTL), Cairo, Egypt.

Equipment and methods: In this study, the bioactive compounds (phenolics and simmondsins) of defatted jojoba meal were reduced by 21 treatment methods: Shaker, stirring, ultrasound-assisted extracting, microwave assisted extraction, with three different times on dry meal, soaking with solvent and aqueous solvents were compared.

Preparation of defatted jojoba meal: The pressed cake was extracted with n-hexane in a soxhlet extractor apparatus for 30 h. The defatted jojoba meal was allowed to air-dry in a fume hood to remove residual hexane. This defatted jojoba meal was ground in a coffee mill to obtain a finely divided material ($\leq$100 mesh) suitable for extraction studies.

Solvent extraction: Dried powder of defatted jojoba meal (1.0 g) was mixed with 90 mL of each solvent and extracted as illustrated in Table 1 and 2. The extracts were filtered through filter paper (Whatman No. 1) and completed with the same solvent to the 90 mL and were stored at -20°C until testing.

Microwave-assisted extraction: Heating using the microwave was carried out using a L.G. microwave oven generating 1450 W power at 2450 MHZ according to the method of Yoshida and Kajimoto[25]. Defatted Jojoba meal was placed as a single layer in a pyrex petridish and then heated with microwave for 1, 2 and 3 min as described in Table 1.

Ultrasound-assisted extraction: Dried powders of samples (1 g) were mixed with 90 mL of solvent (1:90). All the samples

were placed in bottles with narrow necks and mixed for 1 h. In crest ultrasonic water bath of 35°C and at 38.5 kHz. The extracts were filtered and stored at -20°C until testing.

Determination of total phenolic extract: The content of phenolic compounds in the extracts was determined according to the method of McDonald *et al.*[26]. Briefly, 0.5 mL of extract was mixed with 2.5 mL of 10-fold-diluted Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. Then, the mixture was shaken for 1 min and allowed to stand at room temperature for 15 min. Absorbance of the solution was measured at 765 nm using a spectrophotometer (T80 UV-vis spectrophotometers). The concentration of phenolic compounds was estimated using a calibration curve traced with gallic acid in distilled water at concentrations of 0.1 g L^{-1} as a polyphenol reference. Results were expressed as milligram per gram gallic acid equivalent milligram per gram meal. Each test was repeated three times and the results were averaged.

Determination of crude simmondsins extract: The extraction of simmondsins and other related compounds was carried out using the reported methods of[27,28] with slightly modification. All the aqueous extracts described previously evaporated using a rotary evaporator. The concentrated extracts were stored in a freezer[29]. The concentrate extracts above were re-dissolving in the same solvent but 100% and were filtered through filter papered (Whatman No. 1), evaporated and weighed. Results were expressed as gram crud simmondsins per gram meal[6].

Electrophoresis

A-Sodium Dodecyl Sulphate Polyactylamide Gel Electrophoresis (SDS-PAGE): The SDS-PAGE was performed according to the method of Laemmli[30].

The residue of defatted jojoba meal was dried. About 0.01 g of the defatted meal residue was extracted by adding 0.5 mL of tris HCl extraction buffer (0.125 M, pH 6.8) and was carried out (analyzed) in polyacryalmide gels vertical type using a Mini-Protein II Electrophoresis cell unit. About 1.0 mm thick, 10×10 cm notched glass plates. Using 12% separating gel and 5% stacking gel according to the methods prescribed by Jensen and Lixue[31] and Laemmli[30].

Gel documentation and analysis: Molecular weight of different bands were compared with (FISHER BIO reagents) a mixture of standard protein markers include range in size from 10-200 kDa when analyzed by SDS-PAGE and stained with coomassie blue.

B-native electrophoresis

Sample preparation: About 0.01 g of the dried residue samples were mixed with 0.5 mL of sample buffer. Gel preparation and sample loading were similar as the running conditions, according to the methods prescribed by Jensen and Lixue[31] and Laemmli[30]. Except that SDS was not the part of sample and running buffers. The concentrate separating gel were prepared 8% (Fig. 1) and 10% (Fig. 2).

Statistical analysis: All results were carried out in triplicates and values were expressed as Means±Standard Deviation (SD). Significant statistical differences of investigated parameters were determined and analyzed using one way analysis of variance (ANOVA PC-STAT, 1985 version IA copyright, University of Georgia).

RESULTS AND DISCUSSION

Simmondsins and penolics extraction: The results of all the extractions are listed in Table 1 and 2. The results in Table 1

Table 1: Extraction of phenolics and simmondsins compounds from defatted jojoba meal with 100% acetone and different techniques

Treatments: (1.0 g meal+90 mL 100% acetone)	Phenolics extract (mg g^{-1} meal)	Crude simmondsins (g/100 g meal)
Stirring three times, each time 30 mL with acetone for 15 min	1.90±0.03^{g}	1.30±0.2^{h}
Stirring for 15 min then soaking for 72 h	5.64±0.04^{b}	2.90±0.4^{g}
Sonication for 1 h	3.77±0.07^{e}	4.50±0.04^{f}
Sonication for 1 h then soaking for 72 h	5.10±0.3^{c}	6.30±0.4^{d}
Microwave heating of the dry meal for 1 min then soaking for 72 h	4.48±0.1^{d}	8.50±0.2^{c}
Microwave heating of the dry meal for 2 min then soaking for 72 h	4.57±0.17^{d}	9.55±0.05^{b}
Microwave heating of the dry meal for 3 min then soaking for 72 h	5.10±0.2^{c}	9.80±0.3^{b}
Microwave heating of the dry meal for 1 min then sonicating with acetone for 1 h	3.26±0.04^{f}	5.90±0.5^{e}
Microwave heating of the dry meal for 2 min then sonicating with acetone for 1 h	3.40±0.4^{f}	6.60±0.1^{d}
Microwave heating of the dry meal for 3 min then sonicating with acetone for 1 h	3.40±0.3^{f}	6.80±0.4^{d}
Shaking mixture of meal and acetone for 6 h then soaking for 24 h	4.90±0.1^{c}	5.93±0.1^{e}
Extraction of meal with acetone by soxhlet at 50°C for 30 h	6.32±0.02^{a}	14.00±0.4^{a}
LSD (5%)	0.2909	0.5065

Different letter(s) in each column indicates significant differences at $p<0.05$, ±SD

Table 2: Extraction of phenolics and simmondsins compounds from defatted jojoba meal with different solvents and deferent concentrations under the same technique (condition)

Treatments: Shaking for 15 min then soaking for 72 h (1 g meal+90 mL solvent)	Phenolic extract (mg g^{-1} meal)	Crude simmondsins (g/100 g meal)
Acetone (100%)	5.60±0.2^{f}	6.36±0.24^{c}
Acetone (70%)	18.50±0.4^{a}	3.60±0.1^{g}
Acetone (50%)	17.70±0.3^{b}	5.20±0.2^{e}
Methanol (100%)	14.30±0.3^{c}	6.00±0.1^{c}
Methanol (70%)	13.30±0.1^{d}	10.40±0.3^{a}
Methanol (50%)	12.66±0.04^{e}	5.80±0.4^{d}
Isopropanol (100%)	4.00±0.1^{g}	4.72±0.05^{f}
Isopropanol (70%)	13.62±0.3^{d}	10.10±0.5^{a}
Isopropanol (50%)	12.50±0.1^{e}	8.01±0.1^{b}
LSD (5%)	0.405	0.459

Different letter(s) in each column indicates significant differences at $p<0.05$, ±SD

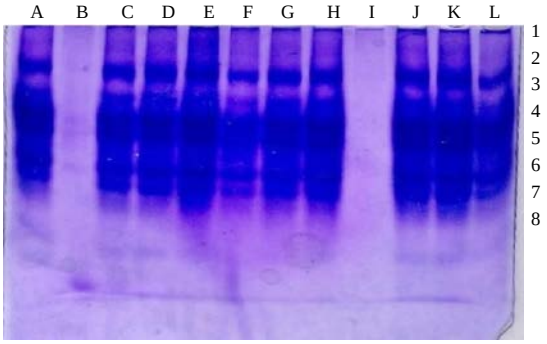

Fig. 1: Eight percent native-PAGE electrophoresis proteins of defatted jojoba meal residue, lane A: Microwave heating of the dry meal for 1 min, then soaking for 72 h, lane B: Protien marker, lane C: Defatted jojoba meal, lane D: Extraction of meal with acetone by soxhlet at 50°C for 30 h, lane E: Microwave heating of the dry meal for 3 min, then soaking for 72 h, lane F: Microwave heating of the dry meal for 3 min, then sonicating with acetone for 1 h, lane G: Sonication for 1 h, then soaking for 72 h, lane H: Sonication for 1 h, lane I: Sample buffer, lane J: Stirring for 15 min, then soaking for 72 h, lane K: Stirring three times, each time 30 mL with acetone for 15 min and lane L: Microwave heating of the dry meal for 2 min then sonicating with acetone for 1 h

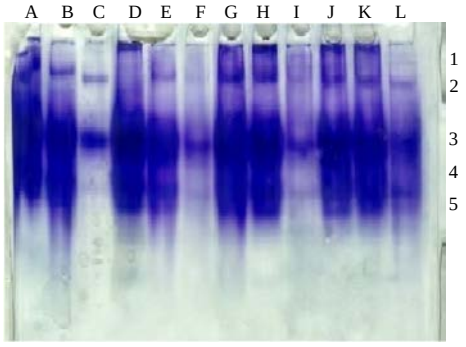

Fig. 2: Ten percent native-PAGE bulk electrophoregrams proteins of jojoba residue meal seed, lane A: Acetone 100% shaking for 15 min, then soaking for 72 h, lane B: Acetone 70% shaking for 15 min, then soaking for 72 h, lane C: Acetone 50% shaking for 15 min, then soaking for 72 h, lane D: Methanol 100% shaking for 15 min, then soaking for 72 h, lane E: Methanol 70% shaking for 15 min, then soaking for 72 h, lane F: Methanol 50% shaking for 15 min, then soaking for 72 h, lane G: Isopropanol 100% shaking for 15 min, then soaking for 72 h, lane H: Isopropanol 70% shaking for 15 min, then soaking for 72 h, lane I: Isopropanol 50% shaking for 15 min, then soaking for 72 h, lane J: Extraction of meal with acetone by soxhlet at 50°C for 30 h, lane K: jojoba meal and lane L: Microwave heating of the dry meal for 3 min then sonicating with acetone for 1 h

showed that, the best techniques in extracting the highest yield of simmondsins and most phenolics compounds (14%, 6.32 mg g^{-1}, respectively) were carried out using soxhlet apparatus for 30 h and 50°C. This result is agreeing with Holser and Abbott[27] and Zaher *et al.*[28] and soxhelet was the best technique for phenolic extraction rates from guava seed and seeds of three wild grapevines[32,33]. The second best conditions were microwave heating, in which the results after 3 min and soaking was better than after 2 min and soaking and 1 min. and soaking, respectively. In addition, in all the treatments, soaking gave best result compared to ultrasound only; because the solvent penetrate rapidly into the plant cell and prevents degradation of phenolics compounds[34,35].

Table 2 shows that 70% methanol was the best solvent in extracting the highest yield of simmondosin in the defatted jojoba meal than other solvents and the lowest one was 70% acetone. The second best solvent was 70% isopropanol as reported by Medina *et al.*[36] followed by 50% isoprpanol, 00% acetone, 100% methanol, 50% acetone, 100% isopropanol and 70% acetone. This can be explained as follow, each solvent possesses various degrees of polarity that resulted in different extraction strengths and detoxification of jojoba meal which may be reduced using different methods and reduced with 70% isopropanol[36] with different solvent systems namely acetone, isopropanol, methanol, ethanol and its different concentrations as described previously[37], with methanol or water the simmondsins and the toxicity of the protein should also decrease[38].

About 70% acetone was the best solvent reduced phenolic compounds in the jojoba meal amongst all solvents and the lowest one was 100% isoprpanol. The second best solvent was 50% acetone, followed by 100% methanol, 70% isoprpanol, 70% methanol, 50% methanol, 50% isoprpanol, 100% acetone and 100% isoprpanol. These differences could be due to the properties of each solvent and sample matrix.

Results showed that maximum simmondosins extracted was 14% and phenolics extracted was 18.5 mg g^{-1} in defatted jojoba meal. The extracted simmondsins and phenolics of this study was higher than those extracted by Booth *et al.*[3], perhaps due to using soxhlet for simmondsins and soaking for phenolic with longer time.

Protein quality of defatted jojoba meal residue using native and SDS-PAG electrophoresis

Native electrophoresis: Figure 1 and 2 show an example with proteins that have been reduced by solvent extractions. In Fig. 1, lanes A-L correspond to the same proteins processed differently. Five lanes (E, G, H, J and K) show higher mobility than the others. By comparing lane D, meal without simmondsins, to lane C, defatted meal with simmondsins, it is obvious that there is not any significant changes in the protein patterns. This indicates that there is no binding between simmondsins and protein. This agrees with Wiseman and Price[15] and Wagdy *et al.*[39]. By comparing lane C, defeated jojoba meal, with lanes A, L and E which represent the effect of microwave after 1, 2 and 3 mines, it can noticed that by increasing time the protein mobility increases. Previous studies show that the simmondsins was co-extracted with the protein and simmondsins also remained with the protein concentrates[15].

Figure 2 represents the same amount of protein of the meal residue that was treated with different solvent and different solvent concentrations and examined for the degree of aggregation and degradation. Figure 2 shows that the mobility of the protein bands is greater than those in Fig. 1, this may be due to the presence of more charges than the starting protein at lane K (defatted meal). As shown by the native gel 10% concentrations, the lanes corresponding to 100% solvent (A, D G, lanes) and 70% solvent (B, E, H, lanes) of acetone, methanol and isopropanol, respectively, showed the highest mobility and also more concentrate. However, 50% solvent (C, F and I lanes) shows very weak aggregates which is different with different solvents indicating that under these condition aggregation of the protein mixture reduced.

Generally, degradations is highly and dependent on the type of solvent, technique as well as solvent concentrations.

The native gel approved that lane J (starring and soaking), K (stirring) and lane D (soxhlet) are like lane C (meal) in Fig. 1 and the protein structures can be reduced during extraction with aqueous alcohols[17,18] (Fig. 2). In addition, the SDS-PAGE gel approved that in Fig. 3 lane H (stirring), lane J (meal) and in lane H (soxhelt) and lane J (meal) show the same protein separation pattern. Therefore, one can use electrophoresis to screen the best conditions.

The SDS-PAGE in Fig. 3 shows results of analysis of the proteins extracted at various techniques, from defatted jojoba meal (residue). The main protein bands from 50-15 kDa gave essentially the same pattern and agreed with results reported by Wolf *et al.*[40], bands were reported to be detected in the range of 10-120 kDa with major components at 15-20 and 47 kDa and lesser bands from 10-15 kDa, the heavy band at 20 kDa appeared to be a doublet in most gel. Figure 3 shows that the low molecular weight proteins around 10 kDa increased in lanes A, C, F and H. In addition, in all lanes some

of the bands in the residuals meals, between 25-40 kDa have been disappeared. In lane E, a more dense bands between 10-15 kDa appeared compared to the other lanes.

In Fig. 4, it is obvious that there are significant differences in the proteins extracted by 100% solvent and the defatted meal. In Fig. 4, the protein pattern is less intense than the

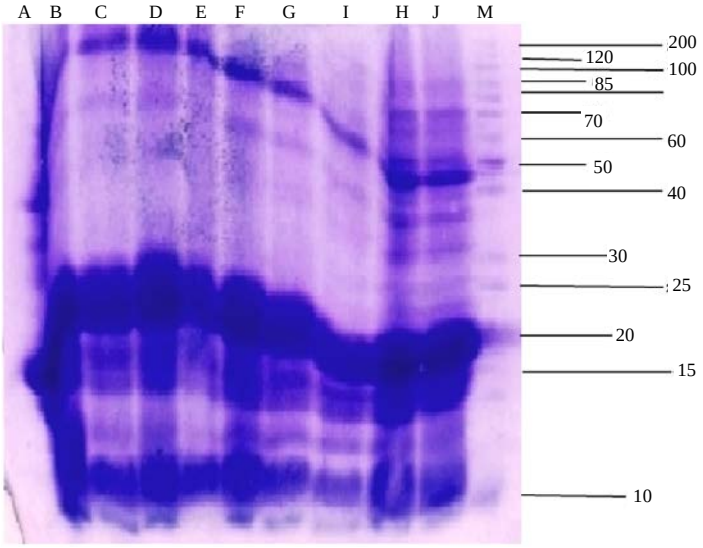

Fig. 3: SDS-PAGE electrophoresis proteins of jojoba residue meal seed, lane A: Extraction of meal with acetone by soxhlet at 50°C for 30 h, lane B: Microwave heating of the dry meal for 3 min, then sonicating with acetone for 1 h, lane C: Microwave heating of the dry meal for 3 min, then soaking for 72 h, lane D: Methanol 50% shaking for 15 min, then soaking for 72 h, lane E: Sonication for 1 h, then soaking for 72 h, lane F: Sonication for 1 h, lane G: Stirring for 15 min, then soaking for 72 h, lane H: Stirring three times, each time 30 mL with acetone for 15 min, lane J: Jojoba meal and lane M: Marker

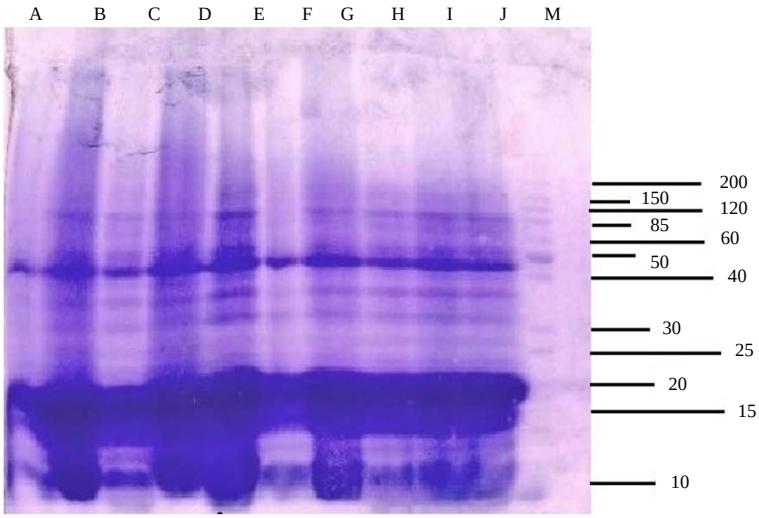

Fig. 4: SDS-PAGE electrophoresis proteins of jojoba residue meal seed, lane A: Isopropanol 50% shaking for 15 min, then soaking for 72 h, lane B: Isopropanol 70% shaking for 15 min, then soaking for 72 h, lane C: Isopropanol 100% shaking for 15 min, then soaking for 72 h, lane D: Methanol 70% shaking for 15 min, then soaking for 72 h, lane E: Methanol 100% shaking for 15 min, then soaking for 72 h, lane F: Acetone 50% shaking for 15 min, then soaking for 72 h, lane G: Acetone 70% shaking for 15 min, then soaking for 72 h, lane H: Acetone 100% shaking for 15 min, then soaking for 72 h, lane I: Extraction of meal with acetone by soxhlet at 50°C for 30 h, lane J: Jojoba meal and lane M: Marker

defatted meal (lane J) at 100 and 50% acetone concentration (lane H and F) and at 100 and 50% isopropanol concentration (lane C and A). However, the protein pattern is more condensed than the defatted meal (lane J) at 70% isopropanol and acetone (lane B and G). In addition, both 70 and100% methanol concentration (lane D and E) showed very high intense protein bands compared to the other lanes.

CONCLUSION

Results of this study showed that the phenolics contents and simmondsins were reduced compared to the defatted jojoba meal in all of the treatments used.

Extraction using soxhlet was the best method for reducing simmondsins, however the polyphenolic content was reduced more in 70% acetone concentrations and shaking for 15 min, then soaked with long times. The SDS-PAGE and native electrophoresis also showed that protein quality of the defatted jojoba meal residue was affected by reduction of phenolics and simmondsins compounds in some treatments.

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