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# Solubility Pattern of Simmondsins, Proteins and Phenolics of Defatted Jojoba Meal

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#### ABSTRACT

 ${
m Jojoba~defatted~meal~contains~toxic~compounds~mainly~simmondsin~and~simmondsin~2-ferulate.}$ The aim of the present investigation was to study the solubility pattern of simmondsin, simmondsin 2- ferulate, protein, non-protein nitrogen and phenolic compounds present in Jojoba meal at pH ranging from 1-12. Both the supernatant and precipitate resulting after extraction at a certain pH were analyzed for the above mentioned components. The simmondsins were identified and quantified by thin layer chromatography. The antimicrobial activities of the 12 pH extracts were evaluated. Results revealed that the precipitate containing lowest simmondsin 0.55, 0.55 and 0.74 g/100 g meal was achieved at pH 1, 2 and 12, respectively. Meanwhile simmondsin 2-ferulate amounted to 0.67, 0.72 and 0.72 g/100 g meal, at pH 9, 1 and 2, respectively. Isoelectric point of jojoba meal protein showed to be between pH 3-4 with least solubilized protein 14.26-14.08% and highest precipitated protein in the residue 17.74-17.92% protein. Non-protein nitrogen ranged between 2-3.5% in supernatant and between 7-8.6% in the precipitate. Phenolic compounds extracted in the supernatant increase with increasing pH except at pH 4 and 8 where they exhibited some decrease. Normally, the phenolic compounds in the residue followed an opposite trend. Extract at pH 1 inhibited the growth of the five examined bacteria strains. Extracts of jojoba meal resulting from pH 2, 5, 6 and 8 showed inhibition of only one of the five bacterial strains. In conclusion, simmondsins can be effectively removed from the jojoba meal at pH 1, 2 and 12. Jojoba extract at pH 1 exhibited good antibacterial activity.

**Key words:** Jojoba meal, simmondsin(s), solubility pattern, protein, phenolic compounds, antibacterial activity

# INTRODUCTION

The jojoba (Simmondsia chinensis L.), an evergreen shrub native to the Sonoran desert, yield 50-60% of a liquid wax ester commonly referred to as jojoba oil. The oil is mainly used in cosmetics and lubricants (Van Boven et al., 2001). The meal resulting after oil removal comprises other 50% of the seed which represent a potential amendment for animals/or humans. Defatted jojoba meal contains between 28-30% protein, carbohydrates of which 50% are pentosans, some minerals and vitamins. The factor that hinders the use of jojoba meal for human food is the presence of simmondsin and its derivatives (Holser and Abbott, 1999; Kolodziejczyk et al., 2000).

At the moment seven simmonds in derivatives have been identified and quantified these include: Simmondsins, 5-demethylsimmondsins, 4-demethylsimmondsins, simmondsin 2`-trans-

ferulates, 5-demethylsimmondsins 2`-trans-ferulates, 4-demethylsimmondsins 2`-trans-ferulates and didemethylsimmondsins (Kolodziejczyk et al., 2000; Benzioni et al., 2005).

It has been established that simmonds and simmonds in 2'-ferulated are the most prevalent of the simmonds in jojoba meal. Ingestion of both compounds produce reduction in food intake leading to weight loss ending in death of rats (York et al., 2000; Cokelaere et al., 2000; Lievens et al., 2003). Thus can consequently, serve as an appetite suppressant, when used in certain doses (Cokelaer et al., 1996; Bellirou et al., 2005).

Medina and Trejo-Gonzalez (1990) established new methodology to remove the toxic compounds present in jojoba (*Simmondsia chinensis*) seed and meal. They reported the testa of jojoba seed to contain 6.5% phenolics, whereas the seed without testa contained only 1.1%.

Abdel Rahman et al. (2006) found that defatted jojoba meal contained 2.67% phenolic compounds. It is worthy to mention that the polyphenols are effective antioxidants in a wide range of chemical oxidation systems. They are capable of scavenging peroxyl radical, alkyl radicals, superoxide, hydroxyl radical, nitric oxide and peroxynitrite in aqueous and organic environments (Duthie and Crozier, 2000). Phenolic compounds and their subclasses, such as coumarins, flavonoids, tannins, saponins and essential oil, have antimicrobial function (Kubo et al., 1993). Moreover, Teague et al. (2005) reported that jojoba meal extract has been found to be useful as a dietary supplement for use in weight control regiment in humans. It can also be used as a component of functional food, a food additive, a medical food or as a therapeutic agent.

The objective of the present research was to study the solubility pattern of some constituents of jojoba meal at pH range 1.0-12.0. The studied constituents were simmondsin, simmondsin 2 ferulate, protein, non-protein nitrogen and phenolic compounds. This study is a simple economic method to extract the toxic simmondsins from the meal rendering the meal nutritionally appropriate. The extracted simmondsins can be concentrated and used medicinally. The phenolic compounds can also be extracted at its pH of maximum solubility and used as a functional food ingredient. The extracts will be also tested for their antimicrobial activity on five food borne pathogenic bacteria.

## MATERIALS AND METHODS

**Materials:** Defatted jojoba press cake was obtained from Egyptian Natural Oil Co. (NATOTL), Ismailia Branch and Farm Factory, 10th of Ramadan City, Egypt. The pressed cake (crop of year 2010) was then defatted in a soxhlet apparatus using n-hexane to give jojoba meal which was then spread to dry at room temperature and sieved to pass 80 mesh screen.

Microorganisms: E. coli o157: H7 ATCC 51659 (E. o157), Staphylococcus aureus ATCC 13565 (St), Bacillus cereus EMCC 1080 (Bc), Listeria monocytogenes EMCC 1875 (Lis) and Salmonella typhimurium ATCC25566 (Sa) were bought from the Microbiological Resources Center, Cairo (MIRCEN), Faculty of Agriculture, Ain Shams University.

#### Methods

Extraction of simmonds and simmonds are 2-ferulate from jojoba meal: Simmonds and simmonds 2-ferulate were extracted from defatted jojoba meal according to Elliger *et al.* (1973). Compounds were identified by Thin Layer Chromatography (TLC) using authentic samples.

Quantitative determination of simmonds in and simmonds in 2-ferulate using TLC: The TLC plates were developed with ethyl acetate: Ethanol (7:3,v/v), simmonds in has Rf~0.35 and

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simmondsin 2-freulate has Rf~0.68. Both compounds char well with a 10% sulphuric acid spray followed by heat then quantitative determination of the two compounds was carried out according to Grady *et al.* (1973).

Solubility pattern of simmondsins, protein, non-protein nitrogen and total phenolic compounds: Defatted jojoba meal (5 g) were mixed well with 50 mL distilled water for 5 min, the pH of the solution was then adjusted to the desired pH from pH 1 to 12 using 6N-HCl or 0.01-N-NaOH and stirring continued for 10 min on a magnetic stirrer. The solution was centrifuged for 30 min at 4000x g. The filtrate was separated from the residue which was dried in a draft air oven at 50°C.

Both the supernatants and residues were analyzed for their content of simmondsin, simmondsin 2-ferulate, protein, non-protein nitrogen and total phenolics.

Assay of phenolic compounds on microorganisms: Extraction of jojoba meal at pH 1-12, subjected to centrifugation yielded 12 supernatant extracts. The 12 extracts were assayed on five chosen food borne pathogenic bacteria. The antibacterial activity was measured by the disc diffusion method (Kotzekidou *et al.*, 2008). All the tests were run in triplet for each sample and means of inhibition zones were used to assess the activity.

Chemical analysis: Total protein and non-protein nitrogen were determined in meal, supernatants and residual materials according to the methods described in AOAC (2000). Whereas, total phenolic compounds was determined in the supernatant and residues using Folin-Denis reagent according to the method of Martinez-Valverde *et al.* (2002). Total phenols were calculated as gallic acid equivalents from a standard curve.

**Statistical analysis:** The results are represented as average and standard deviation, calculated on Excel program, Microwsoft 2007.

#### RESULTS AND DISCUSSION

Table 1 gives the proximate composition of jojoba seed and jojoba defatted meal. Values in Table 1 are self-explanatory and are within those reported in the literature. Jojoba meal originally contained 4.55 and 1.55 g simmondsin and simmondsin 2-ferulate/100 g meal, respectively. The values reported in Table 1 for simmondsin and simmondsin 2-ferulate in the meal are higher than those reported by Verbiscar and Banigan (1978) possibly due to difference in genotype and cultivation circumstances. Toxicity studies involving both purified simmondsin and defatted jojoba meal have shown that toxicity is species specific (Booth *et al.*, 1974).

Solubility pattern of simmondsin and simmondsin 2-ferulate throughout the range 1.0-12.0: Various attempts for the detoxification of jojoba meal have been reported. Solvent extraction, water washing, heat, chemical treatments and microbial methods resulted in reduced simmondsin levels (Verbiscar *et al.*, 1980, 1981).

A new simple approach which can help in the removal or reduction of simmonds in in jojoba meal was thought to be investigated. This approach included studying the solubility pattern of simmonds in jojoba meal at the whole pH range 1.0-12.0. Results of this investigation are represented in Table 2.

Table 1: Proximate composition of jojoba seed and defatted meal

Composition	Seed	Defatted meal
Moisture (%)	5.63±0.65	6.03±0.49
Oil (%)	50.26±0.48	1.01±0.67
Protein (%)	$18.57 \pm 0.52$	32.33±0.59
Ash (%)	$2.01 \pm 0.73$	4.13±0.43
Crude fiber (%)	$4.29 \pm 0.62$	10.30±0.38
Nitrogen free extract (%)	$19.24 \pm 0.66$	$46.23 \pm 0.55$
Simmondsin (g/100 g)	$2.34 \pm 0.29$	$4.55\pm0.61$
Simmondsin 2-ferulate (g/100 g)	0.75±0.51	1.55±0.21

Values are Mean±SD calculated from values of four replicates

Table 2: Effect of pH on the solubilization of simmonds and simmonds in 2-ferulate present in jojoba meal (g/100 g meal)

	Supernatant		Residual meal	
pН	Simmondsin	Simmondsin 2-ferulate	Simmondsin	Simmondsin 2- ferulate
1	4.00±0.21	0.83±0.15	0.55±0.019	0.72±0.54
2	4.01±0.33	$0.83 \pm 0.43$	$0.55 \pm 0.46$	$0.72 \pm 0.61$
3	2.68±0.29	0	$1.87 \pm 0.43$	$1.55 \pm 0.42$
4	$1.83 \pm 0.41$	$0.31 \pm 0.39$	$2.72\pm0.57$	$1.25\pm0.52$
5	$2.73\pm0.39$	0.66±0.58	$1.82 \pm 0.62$	$0.95 \pm 0.62$
6	$3.20 \pm 0.48$	$0.58\pm24$	$1.35\pm0.33$	0.97±0.66
7	$2.97 \pm 0.53$	$0.64 \pm 0.51$	$1.58\pm0.28$	$0.91 \pm 0.28$
8	$3.08 \pm 0.52$	0.51±0.34	$1.47 \pm 0.51$	$1.05 \pm 0.41$
9	$2.63 \pm 0.46$	0.88±0.55	$1.92 \pm 0.55$	0.67±0.56
10	$3.35 \pm 0.55$	0	$1.20\pm0.44$	$1.55 \pm 0.19$
11	$2.91 \pm 0.57$	$0.49\pm0.19$	$1.64 \pm 0.48$	$1.06 \pm 0.54$
12	$3.81 \pm 0.61$	0	$0.74\pm0.39$	$1.55\pm0.63$

Results are average of triplicate analysis with Mean±SD

Results reveal that highest removal of simmondsin can be achieved at very acidic or alkaline pH values. At pH values 1.0 and 2.0, 4.0 simmondsin/100 g meal were solubilized, next highest removal was at pH values 10.0 and 12.0 where 3.35 and 3.81 g simmondsin/100 g meal were extracted corresponding to the least amount of simmondsin remaining in the meal. Resulting residues (or meal) from extraction at pH values 1.0, 2.0 and 12.0 contained 0.55, 0.55 and 0.74 g simmondsin/100 g meal. Highest simmondsin 2-ferulate removed was at pH values 9.0, 1.0 and 2.0 where 0.88, 0.83 and 0.83 g simmondsin 2-ferulate/100 g meal were removed, respectively while 0.67, 0.72 and 0.72 g simmondsin 2-ferulate/100 g meal remained in the residual meal, respectively.

Figure 1 indicates the % simmondsin and simmondsin 2-ferulate removed from the meal. Highest% removal of simmondsin was achieved at pH values 1.0, 2.0 and 12.0 with 87.9, 87.9 and 83.7% removal of simmondsin. Highest removal of simmondsin 2-ferulate was achieved at pH values 9.0, 1.0 and 2.0 with 56.77, 53.5 and 53.5% removal of simmondsin 2-ferulate. No literature was found to report on the relation between pH and the simmondsins in jojoba meal. Verbiscar *et al.* (1980) reported that water extraction reduced the simmondsin and simmondsin 2 ferulate contents of jojoba meal to 0.08%. While, Banigan and Verbiscar (1980) pointed out that the most effective chemical detoxification process was using ammonium hydroxide and hydrogen peroxide. These chemicals reduced simmondsin and simmondsin 2-ferulate to levels to 0.19%.

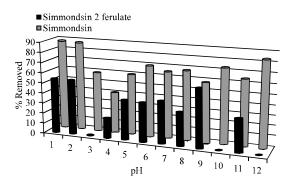


Fig. 1: % Removed simmondsin and simmondsin 2 ferulate in defatted jojoba meal

Verbiscar et al. (1981) again studied microbial treatment as a detoxification process. They found that selected strains of Lactobacillus acidophilus and Lactobacillus bulgaricus and Saccharomyces cerevisae grow well on jojoba meal and reduced the levels of simmondsin and simmondsin 2-ferulate. Growth of Lactobacillus acidophilus B 629 in defatted jojoba meal reduced total toxicants to 95 and 98% (Verbiscar et al., 1981).

In our opinion removal of both simmondsin and simmondsin 2-ferulate by the pH extraction reported above is much better than using ammonium hydroxide and hydrogen peroxide (both are chemicals) while in our method we use only water and adjust pH. Generally, water has been shown to non selectively extract simmondsin compounds from defatted jojoba meal and a pilot scale process has been developed to the recovery and concentration of simmondsin from such an extract, using membrane separation techniques which adds to the cost of the operation (Abbott *et al.*, 1991, 1996; Erhan *et al.*, 1997). Bellirou *et al.* (2005) reported repeated extraction of both simmondsin and oil with water at 90°C from ground jojoba seeds. This method removed both the oil and simmondsin together in one step. It seems that extraction of simmondsin and simmondsin 2-ferulate at their pH of maximum solubility is the simplest and most economic method.

Solubility pattern of protein and non-protein nitrogen throughout the pH range 1-12: When the aim was to prepare protein products free or low in simmondsin and simmondsin 2-ferulate, it was necessary to study the solubility of jojoba meal protein together with the solubility of the simmondsins over the whole pH range. Table 3 represent solubilized protein and non protein nitrogen (NPN) in the supernatants and the residual protein and non protein nitrogen remaining in the jojoba meal after extraction at different pH values from 1.0-12.0.

Knowing that jojoba meal contains 32.33 g protein/100 g meal (Table 1) it can be clearly seen that the highest solubilized protein was at alkaline pH values starting from pH 8.0 to pH 12.0. Highest protein solubility was achieved at pH 12.0 resulting in 31.78 g protein/100 g meal solubilized, in parallel to this result least amount of protein remains in the residual meal leveling to 0.22 g protein/100 g meal. Highest solubilization of oilseed proteins is well achieved at alkaline pH (Taha et al., 1981, 1986, 1987; Abbasy et al., 1981). Least solubilized protein 14.26 and 14.08 g protein/100 g meal were at pH values 3 and 4, extraction at these same pH values resulted in residual meal with highest protein content reaching~18 g protein/100 g residual meal. From this result the IEP of jojoba meal protein can be said to be pH 4.0. Wolf et al. (1988) reported IEP of jojoba dehulled defatted meal to be pH 3.0 while (Cardoso and Price, 1982) reported IEP to be pH 3.0-4.0.

Table 3: Effect of pH on solubility of protein and nonprotein nitrogen present in jojoba meal (g/100 g meal)

	Supernatant		Residual meal	
pН	Total protein	Nonprotein nitrogen	Total protein	Nonprotein nitrogen
1	20.94±0.51	3.51±0.56	11.06±0.43	7.33±0.31
2	20.94±0.46	3.39±0.75	11.06±0.39	7.70±0.29
3	$14.26 \pm 0.49$	$2.73 \pm 0.42$	$17.74 \pm 0.62$	7.95±0.41
4	$14.08 \pm 0.55$	$2.78 \pm 0.68$	17.92±0.55	$7.93\pm0.71$
5	$15.71 \pm 0.67$	$2.54 \pm 0.71$	$16.29\pm0.71$	8.18±0.49
6	$20.76 \pm 0.71$	$2.54 \pm 0.59$	$11.24 \pm 0.43$	8.18±0.65
7	$17.69 \pm 0.48$	$2.54 \pm 0.49$	14.31±0.57	8.18±0.81
8	$24.19 \pm 0.63$	2.06±0.63	$7.81 \pm 0.67$	8.66±0.52
9	$25.09\pm0.72$	2.30±0.46	$6.91 \pm 0.72$	8.42±0.29
10	$25.27 \pm 0.44$	$2.30\pm0.52$	6.73±0.26	7.92±0.46
11	26.36±0.58	2.30±0.57	$5.64 \pm 0.74$	7.82±0.76
12	$31.78 \pm 0.61$	2.30±0.68	$0.22 \pm 0.41$	7.26±0.65

Results are average of triplicate analysis with Mean $\pm$ SD. Non protein nitrogen in jojoba meal = 10.37%

Based on results from Table 3, protein isolates prepared by alkali solubilization then IEP of the protein are not advisable first because during the alkali solubilization most of the simmondsin and simmondsin 2-ferulate will be solubilized with the protein. Second, during precipitation of the protein at IEP pH 4.0, 14% protein will be still solubilized therefore wasted. This is in accordance with the findings of Wolf *et al.* (1988), who reported that 40% of the total protein nitrogen was solubilized at pH 3.0, compared to soybean meal where only 10% of the protein remains soluble at its IEP. Wolf *et al.* (1994) in another study indicated that this high solubility in the apparent IE region of the proteins suggests the presence of a large fraction of acid soluble protein (s) and/or high NPN content.

Thus investigation of the NPN at the tested pH range was carried out and results represented in Table 3. NPN in the meal was 10.37%. It can be deduced from the results that NPN is extracted more at highly acidic pH values and alkaline pH values. The (N) of the (CN) group of simmonds in compounds is considered among the NPN compounds (Abbott *et al.*, 1988). Results in Table 3 confirm this fact, highest solubilization of NPN corresponds to highest solubilization of simmonds in at pH values 1.0, 2.0, 10.0, 12.0, with values of NPN removed in the supernatant 3.51, 3.39, 2.30, 2.30 g NPN/100 g meal. Abbott *et al.* (1988) estimated that the simmonds compounds contributed about 36% of the N in the NPN fraction. The remainder of the NPN fraction is likely to be free amino acids and peptides. Wolf *et al.* (1994) reported NPN of jojoba meal to be 21-30% which they found rather very high compared to other oilseeds, such as rapeseed, sunflower and soybean which contain 6.9, 5.0 and 2.5% NPN, respectively. Bhatty and Finlayson (1973) found a difference in NPN values of jojoba meal and related it to be due to difference in genotypes.

Solubility pattern of total phenolic compounds throughout the pH range 1-12: Phenolic compounds are found in abundance in the plant kingdom. They are considered functional food ingredients or nutraceuticals. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia et al., 1997; Manach et al., 2005; Puupponen-Pimia et al., 2001; Samman et al., 1998). A drawback of the

Table 4: Effect of pH on solubility of phenolic compounds present in jojoba meal (g/100 g meal)

pН	Supernatant	Residual meal
1	0.517±0.25	0.363±0.36
2	0.519±0.44	0.361±0.46
3	0.607±0.51	0.273±0.39
4	$0.588 \pm 0.46$	0.293±0.61
5	0.654±0.28	0.226±0.33
6	0.604±0.53	$0.276 \pm 0.52$
7	0.631±0.56	0.249±0.47
8	0.525±0.55	0.355±0.28
9	0.755±0.46	0.125±0.43
10	$0.863 \pm 085$	0.044±0.65
11	0.829±0.65	$0.051 \pm 0.21$
12	$0.816 \pm 0.27$	0.064±0.35

Results are average of triplicate analysis with Mean $\pm$ SD deviation. Phenolic compounds in jojoba meal = 0.88 g/100 g

presence of phenolic compounds with proteins is that they can form phenolic acid-protein complexes thus lowering the nutritional value of the protein (Shahidi and Naczk, 1992). Also phenolic compounds may impart astringency and bitterness (Ozawa et al., 1987) and lead to unpalatability. Thus, it was worth studying the solubility pattern of phenolic compounds present in jojoba meal.

Table 4 show the solubility of phenolic compounds present in jojoba meal throughout the whole pH range. Determination of Total Phenolic (TP) compounds in jojoba meal indicated it contained 0.88 g TP/100 g meal. In agreement with this result Medina and Trejo-Gonzalez (1990) reported untreated jojoba meal to contain 1.8% phenolics and defatted untreated flour to contain 0.9% phenolics. Detoxified samples extracted with 70% isopropanol contained no phenolics. Results in Table 4 indicate that at alkaline pH values 9.0-12.0 more of the phenolic compounds are solubilized with the protein and less are present in the residual meal. This result is contrary to what is known that at alkaline pH values the phenolics are oxidized to O-quinones which react with the protein causing discoloration (Loomis and Battaile, 1966). But our result agrees with Cater et al. (1972), who compared the protein solubility of conventional sunflower meal (with chlorogenic and caffeic acid) and sunflower meal (freed from chlorogenic and caffeic acid) at different pH values. They were surprised to find that the solubility of sunflower protein at alkaline pH 9-11 was not improved after the removal chlorogenic and caffeic acids. At the IEP of the protein relatively little TP are solubilized. Table 4 also show that TP are removed in increasing order with increasing pH from 1.0-12.0, except at pH 4.0 and 8.0 where they show slight decrease in solubility. Thus, the study of using different pH values for removal of TP from jojoba meal proved of no help since highest extraction of TP is accompanied with highest extraction of protein which is the valuable part of the meal.

Antibacterial effect of supernatants resulting from extractions at pH's from 1 to 12: The supernatants resulting from the extraction of jojoba meal at pH values from 1.0 to 12.0 were assayed against several food pathogenic bacteria strains including, *Escherichia coli* 0157:H7 ATCC 51659, *Staphylococcus aureus* ATCC 13565, *Bacillus cereus* EMCC 1080, *Listeria monocytogenes* EMCC 1875 and *Salmonella typhimurium* ATCC25566, using the disc diffusion method. Table 5 show the inhibitory effect of the only five supernatants that exhibited antibacterial activity. Supernatants resulting from extraction of jojoba meal at pH values 3,4,7, 9, 10, 11 and 12 did not

Table 5: Effect of supernatants resulting at different pH extractions on the growth inhibition of some pathogenic bacteria

Samples	Strains/Inhibi	ition zone diameter (mm)			
	 St*	Sa*	Lis*	E.O157*	B.c*
pH 1	17	19.6	14.4	25	16.5
pH 2		6.3			
pH 5	9				
pH 6	8				
pH 8			10.0		
Acetone		3.8	5.0		

St\*: Staphylococcus aureus ATCC 13565, Sa: Salmonella typhimurium ATCC25566, Lis: Listeria monocytogenes EMCC, 1875, E.O157\*: E. coli o157:H7 ATCC 51659, B.C: Bacillus cereus EMCC 1080

exhibit any antimicrobial activity. Supernatant resulting at pH 1.0 possessed the highest antibacterial activity expressed by inhibiting the five bacterial strains investigated. Inhibition zone diameter exhibited by Staphylococcus aureus, Salmonella typhimurium, Listeria monocytogenes, Escherichia coli, Bacillus cereus was 17, 19.6, 14.4, 25, 16.5 mm, respectively. Supernatant extracts of pH 2.0, 5.0, 6.0 and 8.0 inhibited the growth of Salmonella typhimurium, Staphylococcus aureus, Staphylococcus aureus, Listeria monocytogenes with inhibition zone diameter 6.3, 9, 8, 10 mm, respectively. For comparison defatted meal was extracted with 100% acetone as recommended by Elliger et al. (1973) for the extraction of simmonds and simmonds are 2 ferulate The acetone extract rich in the two simmondsins was also analyzed for phenolic compounds which proved to be little (0.4 TP/100 g). The acetone extract inhibited only Salmonella typhimurium and Listeria monocytogenes with inhibition zone diameter 3.8 and 5.0 mm. Comparing this result with results of supernatant pH 1.0, the inhibition of acetone extract is very poor where zone diameter for Salmonella typhimurium and Listeria monocytogenes were 19.6 and 14.4 mm compared to 3.8 and 5.0 mm, respectively. This result shows that the simmondsins have poor antibacterial activity and perhaps the phenolic compounds in the extracts are probably the cause of growth inhibition of the bacteria. Again we come to the question that all supernatant extracts contained different levels of TP and simmondsins, then why not all supernatant extracts showed growth inhibition power? The antibacterial activity of jojoba meal needs further detailed investigation.

No literature reported antibacterial activity of jojoba meal but that phenolic compounds could have an activating or inhibiting effect on microbial growth according to their constitution and concentration is well documented (Rauha et al., 2000; Reguant et al., 2000; Alberto et al., 2001, 2002; Estevinho et al., 2008; Vaquero et al., 2010).

# CONCLUSION

It is advisable to use the pH method illustrated in this paper for the removal of both simmondsin(s) and phenolic compounds to be further purified and used for medicinal purposes. It is not also advisable to prepare jojoba protein concentrates or isolates from jojoba meal by acid leaching at IEP, nor alkali solubilization and IE precipitation, respectively. But it can be suggested that after removal of the antinutritional compounds the remaining meal can be used for human consumption.

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