

Short Communication

The extraction of proteins from the neem seed (*Indica azadirachta A. Juss*)

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Techniques for maximizing the extraction of protein from the neem seed (*Indica azadirachta A. Juss*) were investigated. Extractants used were sodium chloride and sodium sulphate solutions of varying concentration and pH. Maximum extractions of 17.86 g of extractable protein was obtained from 1 kg of crude protein, using 0.5 M NaCl solution at pH of 7.5. All the extracts were devoid of the usual neem smell and its bitter taste. As the pH increased from 7.0 to 7.5 there was steady increase in the quantity of extractable protein by sodium chloride solutions. However a decrease in the quantities of extractable proteins was observed at pH of 8.0 to 10 with sodium chloride solution. As the pH increased from 7.0 to 7.5 on the other hand, the quantities of the extract with sodium sulphate solutions decreased. While at pH of 8.0 to 9.5 the quantity of extractable protein increased, and the least quantity was obtained at pH of 10. 0.5 M NaCl at pH of 7.5 was found to be a better extractant for neem seed protein.

Key words: Neem seed, protein, extraction, detoxification.

INTRODUCTION

Interest in newer sources of protein has grown due to protein shortage in developing countries. As part of the quest for newer sources, some lesser-known oil seeds have been evaluated for their nutritional qualities in India (Udayashekhara and Belavady, 1979; Udayashekhara, 1985, 1987, 1991, 1994; Rukmini and Udayashekhara, 1986; Vijayakumari et al., 1994). In Nigeria, locally available but unusual proteins containing foodstuffs have been evaluated for their nutritional potentials, aiming at reducing dependence and competition between livestock and man for the consumable sources (Fetuga et al., 1974; Mba et al., 1974; Oyenuga and Fetuga, 1975).

Information available for the evaluated oil seeds and foodstuffs revealed that their proteins have nutritive values comparable with commonly used oil seeds and nuts (Udayashekhara and Belavady, 1979; Udayashekhara, 1985; Rukmini and Udayashekhara, 1986). However, the presence of antinutrients in these oil

seeds often causes their inferior nutritional qualities, and hence limits their use as food for livestock and man. However, it is possible to utilize the seeds for food by either removing the antinutrients and/or isolate the proteins contents and subsequently use them in food processing industries. Timothy and Michael (1988) prepared protein concentrates from rapeseed meal, and Klockeman et al. (1997) isolated protein from canola seed, while albumin and globulin have been extracted from detoxified *Thevetia peruviana* seed cake by Usman et al. (2003).

It has been established by Udayashekhara (1987) that neem seed has a high nutritional potential for livestock but its bitter taste and foul odour prevented its use as food. Sulphur containing compounds such as nimbin, nimbidin and nimbosterol, though having insecticidal properties, have been established as been responsible for the bitter taste (Karkar, 1976). Tignic acid is the principle believed to be responsible for the distinctive odour of neem seed (Raman and Santhanagopalan, 1979). Meanwhile Regional Research Laboratory Hyderabad in India has developed a simple extraction process of removing toxic compounds. When

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Table 1. Extraction of protein by aqueous NaCl at the various pH

| NaCl Concentration(M) | Amount of extractable seed protein (g/kg of seed protein) | | | | | | |
|-----------------------|---|-------------|-------------|------------|------------|------------|------------|
| | pH 7.0 | pH 7.5 | pH 8.0 | PH 8.5 | pH 9.0 | pH 9.5 | pH 10.0 |
| 0.1 | 1.96 ± 1.10 | 7.68 ± 1.0 | 6.50 ± 0.2 | 6.25 ± 0.7 | 6.05 ± 0.4 | 6.02 ± 0.2 | 5.04 ± 0.1 |
| 0.2 | 2.86 ± 0.9 | 6.25 ± 1.2 | 6.20 ± 1.4 | 4.64 ± 0.2 | 3.46 ± 0.5 | 3.14 ± 0.3 | 2.61 ± 0.1 |
| 0.3 | 3.21 ± 0.8 | 10.54 ± 1.2 | 8.40 ± 0.4 | 3.21 ± 0.2 | 2.90 ± 0.2 | 2.73 ± 0.2 | 2.10 ± 0.2 |
| 0.4 | 6.21 ± 0.1 | 13.57 ± 0.9 | 8.45 ± 0.4 | 2.86 ± 0.3 | 2.40 ± 0.1 | 2.32 ± 0.2 | 1.72 ± 0.4 |
| 0.5 | 15.00 ± 2.6 | 17.68 ± 1.0 | 10.50 ± 0.5 | 2.32 ± 0.4 | 1.80 ± 0.2 | 1.50 ± 0.3 | 1.01 ± 0.4 |

Values are means of 3 determinations ± S. D.

Udayashekara (1987) employed this process on neem seed, the seed was devoid of the bitter taste and its usual foul smell while retaining its nutritional value.

As an alternative to the simple extraction process of the bitter principles, which of course involves the use of expensive organic solvents, this work is aimed at isolating the protein from defatted neem seed cake. As a first step towards achieving this objective, the solubility of the seed protein was investigated using two different extractants, sodium chloride and sodium sulphate, to determine the most effective extraction procedure.

MATERIALS AND METHODS

Preparation of seed cake

The seeds were obtained from ripe fruits harvested from different locations in Ilorin, Nigeria. The exocarp and pulp of the riped fruit was removed and the endocarp was oven dried at 60°C. The dried endocarp was cracked to obtain the seed. The seeds were milled using magic blender (SHB – 515 model made by Sorex Company Limited, Seoul Japan) to obtain the seed cake. Standard Official and Tentative Method of Oil Chemists Society procedure was used to defat the seed cake (AOCS, 1979). The defatted seed cake was dried and kept for analysis.

Extraction of proteins

The extraction technique employed was similar to those used by Balogun and Odutuga (1981) to extract protein from the West African locust bean seed. Extractants used were sodium chloride and sodium sulphate solutions at concentrations of 0.1 to 0.5 M, and pH of 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0. Extractions were carried out at ambient temperature (29°C), using a mechanical shaker. All suspensions were centrifuged at 3,000 rpm for 1 h. The supernatant liquids obtained were dialyzed against distilled water for 48 h. Triplicates of the dialyzed extracts (concentrates) were taken for analysis.

Analysis

The samples were analyzed for crude protein using the methods of AOAC (1970). Protein concentrates were determined by Biuret method of Gornall et al. (1949). All data collected for the concentrates were subjected to analysis of variance, ANOVA. Means were compared using Duncan's multiple range tests.

RESULTS AND DISCUSSION

The quantity of crude protein obtained was 56 ± 2%. Protein extracts obtained from the neem seed cake, irrespective of the ionic strength of the extractants and the pH of the medium used for extraction, were devoid of the usual neem smell and bitter taste. This implies that what constituted the smell and the taste was not extracted along with the proteins.

Table 1 shows a steady increase in the quantity of extractable proteins from the seed at pH of 7.0 to 8.0 as the concentration increases. The solubility increases with increase in concentration of the salt. This may either be due to non-existence of most of the extractable protein at their isoelectric pH, or low ionic strength of the salt solution at concentrations used for the extraction. Maximum extraction of 17.86 g/kg of the seed protein was obtained at 0.5 M concentration and at pH of 7.5. At pH of 8.5, there was a steady decrease in the quantity of extractable protein as the concentrations increase. This is attributable to the existence of most of the extractable protein at their isoelectric pH, hence least solubility at pH of 8.5.

It has been reported that protein solubility in neutral salt solutions depends on ionic strength and pH of the medium used for the extraction (Charles and Guy, 1999). Increase in ionic strength of any salts solution may reduce the solubility of protein fractions. The effect of pH on the solubility of protein depends on whether the desired protein is at its isoelectric pH. Some protein fractions are at their minimum solubility at isoelectric pH, while some are completely insoluble at this pH. Effects of ionic strength on the solubility of locust bean protein in sodium chloride solutions as reported by Balogun and Odutuga (1981) indicate that the solubility of the proteins increases with increase in ionic strength. The solubility of canola seed protein also followed the same trend as observed by Klockeman et al. (1997).

Results of the extractable protein for aqueous sodium sulphate are presented in Table 2. There is a steady decrease in the quantity of extractable protein as the concentration of the salt (Na₂SO₄) solution increases at pH of 7.0 and 7.5. The decrease might be due to the increases in ionic strength as the concentration of the

Table 2. Extraction of protein by aqueous Na₂SO₄ at the various pH.

| Na ₂ SO ₄ Concentration (M) | Amount of extractable seed protein in g/kg of seed protein | | | | | | |
|---|--|-------------|-------------|--------------|------------|------------|------------|
| | pH 7.0 | pH 7.5 | pH 8.0 | PH 8.5 | pH 9.0 | pH 9.5 | pH 10.0 |
| 0.1 | 9.11 ± 1.50 | 3.21 ± 0.04 | 2.50 ± 0.6 | 2.68 ± 0.02 | 2.58 ± 0.1 | 2.60 ± 0.1 | 2.20 ± 0.1 |
| 0.2 | 6.25 ± 0.7 | 3.86 ± 0.07 | 2.60 ± 0.4 | 2.86 ± 0.01 | 2.70 ± 0.1 | 2.81 ± 0.1 | 2.40 ± 0.2 |
| 0.3 | 1.61 ± 0.2 | 2.68 ± 0.01 | 3.61 ± 0.3 | 3.21 ± 0.02 | 2.75 ± 0.2 | 2.90 ± 0.2 | 2.60 ± 0.2 |
| 0.4 | 1.07 ± 0.2 | 2.32 ± 0.02 | 4.00 ± 0.1 | 7.68 ± 0.04 | 3.20 ± 0.4 | 4.21 ± 0.2 | 2.85 ± 0.2 |
| 0.5 | 0.89 ± 0.1 | 1.9 ± 0.01 | 6.01 ± 0.02 | 13.39 ± 0.06 | 5.40 ± 0.2 | 6.10 ± 0.1 | 3.00 ± 0.4 |

Values are means of 3 determinations ± S. D

extractant increases. Normally, high ionic strength reduces protein solubility (Charles and Guy, 1999).

In contrast to these observations, the quantity of extractable protein increases at pH of 8.5 with increase in the concentration of sodium sulphate solution. The increases might be due to the non-existence of the extractable proteins at their isoelectric pH. Hence, their solubility increases with increase in ionic strength. Maximum extraction of 13.39 g/kg of the seed protein using Na₂SO₄ was obtained at 0.5 M and at pH of 8.5.

Mean values of various quantities of the protein extracts were compared by the Duncan multiple range test. The results revealed that, the mean values of the protein extracts obtained by different concentration of aqueous sodium chloride are significantly different ($p < 0.05$). The difference may be due to the existence of the extractable protein in different forms at different pH. The same result was obtained for the extracts obtained from aqueous sodium sulphate. However the quantity of extractable proteins obtained from using NaCl is significantly higher ($p < 0.05$) especially at pH 7.5 than those obtained using Na₂SO₄. This may be due to the difference in ionic strength of the two extractants. From these results, we conclude that 0.5 M NaCl at pH of 7.5 is a better extractant for neem seed protein. Extraction at higher meal : solvent ratio is currently being investigated. The fractionation of the extracts and the nutritive potential is also being investigated.

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