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Factors Affecting Protein Extractability of Defatted Karkade (*Hibiscus sabdariffa*) Seed Flour*

Hamza M. Abu-Tarboush
Food Science Department, College of Agriculture,
King Saud University, Riyadh, Saudi Arabia

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Abstract. Karkade seed flour was defatted by two different methods. The cold method was better than the hot method in terms of protein extractability. Protein extractability was studied under various conditions of pH (2-12), time (30, 45, 60 min), salt type ($CaCl_2$, NaCl), solvent: flour ratio (10:1, 15:1, 20:1) and salt concentrations (0.2, 0.4, 0.6, 0.8 and 1 M). Results showed that protein extractability was dependent on pH, type of salt and salt concentration. Higher yield was obtained with $CaCl_2$ than NaCl. Highest protein yield was obtained at pH 11 and at 0.4 M $CaCl_2$.

Introduction

The need for diversification of protein sources is especially imperative in developing countries. Plant protein is needed in such countries and could play significant roles in human nutrition. Several novel plant protein sources have been suggested [1], but cultural food selection, among other factors, has minimized the use of these sources. Use of plants for high protein production should help improve the protein intake of such population.

Roselle (Karkade) is the most widely known name for *Hibiscus sabdariffa,* a plant belonging to the family Malvaceae. It is a popular plant in some Middle East countries. It is mainly used for its fiber and the calyces of the flower which are used as cold and warm beverages. The seeds have good potentiality as a new source of protein and oil. They contain considerable amounts of oil [2] and protein [3].

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Aqueous extraction of the protein from legume and oilseeds defatted flour as well as the effects of many factors in the protein extractability were discussed by many investigators [4-8]. Effects of salt type and concentration, pH, solvent to flour ratio and other factors were studied by El-Tinay *et al.* [4, 5] for cottonseed protein extraction. The same study was conducted for conophor seeds by Ekpenyong [7]. Parakash [8] studied the effect of NaCl on the extractability of protein from sesame seed. Shehata and Thannoun [6] studied the effect of pH and several salts in the extractability of nitrogenous constituents from Iraqi mung bean.

The objective of this study was to investigate the effects of pH, time, salt type, salt concentration and solvent to flour ratio on protein extractability of defatted Karkade seed flour by hot and cold extraction methods.

Materials and Methods

Source of seed

Karkade seeds of the variety *El-Rahad* were obtained from El-Nohoud region in Western Sudan. Plant debris, stones and dust were removed and the seeds were ground by an electric grinder to pass a mesh size of 425 um.

Extraction of oil

Two methods were used to extract oil, namely, the hot and cold methods. In the cold method, Karkade seeds were soaked in n-hexane for two days. The extraction procedure was repeated three times during this period with solvent: flour ratio of 10:1 after filtration. A meal containing about 1.2% fat was obtained. This defatted meal was kept at room temperature for two days to evaporate the solvent. In the hot method, Soxhlet apparatus and petroleum ether (40-60°C) were used as solvent. Extraction was run for 16 h. A meal containing about 1% fat was collected and dried. Karkade defatted flour prepared by one of the two methods were separately kept in air tight glass containers at refrigeration temperature (4°C) until needed.

Extraction of protein

Extraction of protein was conducted according to the procedure described by El-Tinay *et al* [5]. Extraction periods tested were 30, 45 and 60 min. protein extraction using either $CaCl_2$ or NaCl was carried out with 0.2, 0.4, 0.6, 0.8 and 1 M salt concentrations for 10 min. The solvent: flour ratio were 10:1, 15:1 and 20:1. A pH range of 2-12 with 1 pH unit interval was assigned for protein extractability.

Protein extraction

Distilled water (25 ml) was added to 0.5 g of the defatted flour and the suspension was shaken for 10 min. The desired pH was maintained by the addition of 1 N HCl or 1 N NaOH and the sample was shaken for 30 min at room temperature (25°C). The suspension was separated from insoluble residue by centrifugation (Tlettich

EBA3S, Germany) at 350 g for 15 min. A volume of 2 ml of the clear supernatant solution was taken for nitrogen determination according to the AOAC [9] micro Kjeldahl method.

Statistical analysis

Extraction procedure and other determination were carried out in duplicate. Data were statistically analyzed using the analysis of variance [10] and the differences among the means were determined for significance at 5% level using Duncan's New Multiple Range Test and SAS computer programs [11].

Results and Discussion

The chemical composition of whole Karkade flour and defatted flours are reported in Table 1. Results show no significant differences between the two methods of lipid extraction. The oil constitutes a substantial portion of the kernel weight (20.30%). Whole mature Karkade seeds were found to have 20% oil [2] and 25.2% protein [3].

Table 1. Chemical composition of whole Karkade flour and defatted flour obtained by the hot and cold methods of extraction (g/100g).

Sample	moisture	oil	protein	ash	carbohydrate
Whole flour	5.3±*0.191	20.3±0.042	25.00±0.311	4.8±0.003	44.5±0.224
Defatted flour (cold extraction)	6.5±0.017a	1.2±0.071a	34.8±0.121a	6.2±0.38a	51.3±0.302a
Defatted flour (hot extraction)	5.7±0.004a	1.0±0.071a	34.9±0.266a	5.6±0.032a	52.8±0.172a

* Mean ± sd

The effect of extraction time on protein extraction is shown in Table 2. Most of the extractable proteins were solubilized during the first 10 min (Fig. 1). This result agreed with the finding of Bello and Okezie [12] and El-Tinay *et al.* [5]. Beradi *et al.* [13] and Martinez *et al.* [14] indicated that time above 30 min was not important to optimum protein extraction if the extracting solvent contained sufficient ions for maximum nitrogen solubility.

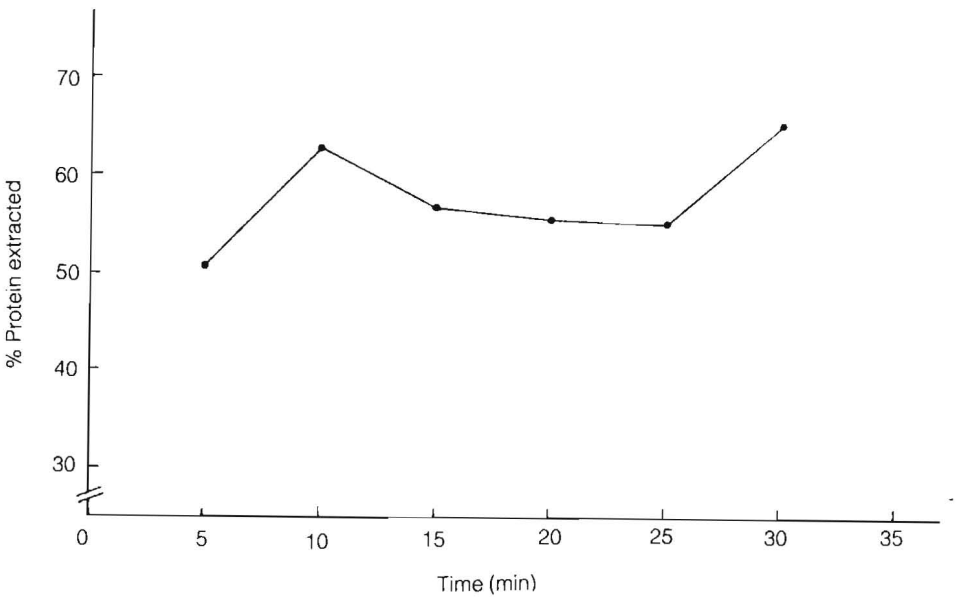

Fig. 1. Effect of time on the extractability of protein from the defatted karkade flour.

Proteins extracted from Karkade seed flour by the hot and cold methods with two different salts of various concentrations are shown in Fig. 2. Protein extractability increased significantly with increased NaCl and $CaCl_2$concentration from 0.2 (27.34 and 43.44%, respectively) to 0.6 M (50.11 and 52.96%, respectively) by the hot method (Fig. 2). Increasing the concentrations beyond 0.6 M did not result in a significant increase in protein extractability. In fact, protein extractability decreased slightly when the salts concentration increased to 1 M (in case of $CaCl_2$). Protein extractability in the cold method increased as NaCl concentration increased from 0.2 M to 0.8 M and $CaCl_2$ concentration increased from 0.2 to 0.4 M. In the view of economical factor, it is better therefore to extract protein of Karkade seed flour with $CaCl_2$. Moreover, data in Fig. 2 show that protein extraction with $CaCl_2$ by the cold method was more efficient than with NaCl. Preliminary data showed that the protein extraction were 66.20% and 55.90% with $CaCl_2$ and NaCl, respectively. These findings did not agree with the results of El-Tinay *et al.* [5] who found that NaCl was more efficient for extraction of protein from cottonseed flour than $CaCl_2$. Also, data in Fig. 2 indicated that extractable protein from cold defatted Karkade seed flour (61.97%) was significantly higher compared to hot method of extraction (45.57%). Heat treatment of Karkade seed flour may have resulted in protein denaturation.

Solvent to flour ratio had no effect on protein extractability by either the two methods of extraction or with the two different solvents (Table 3). These results agree with the findings of El-Tinay *et al.* [5] on glanded cottonseed flour.

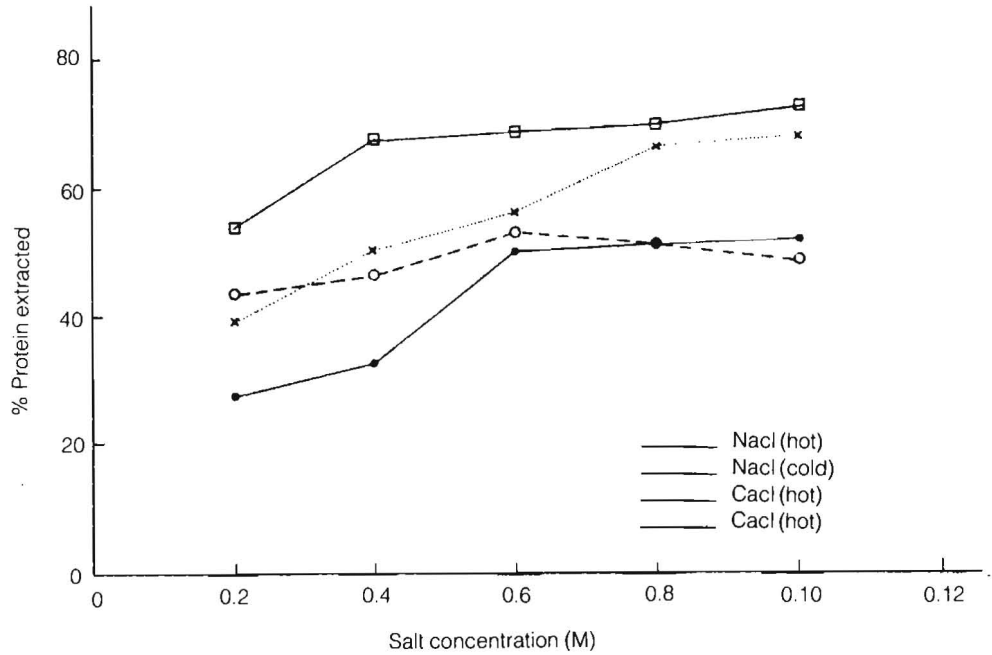

Fig. 2. Percent protein extractability from hot and cold defatted harkade flour as a function of salt and salt concentrations.

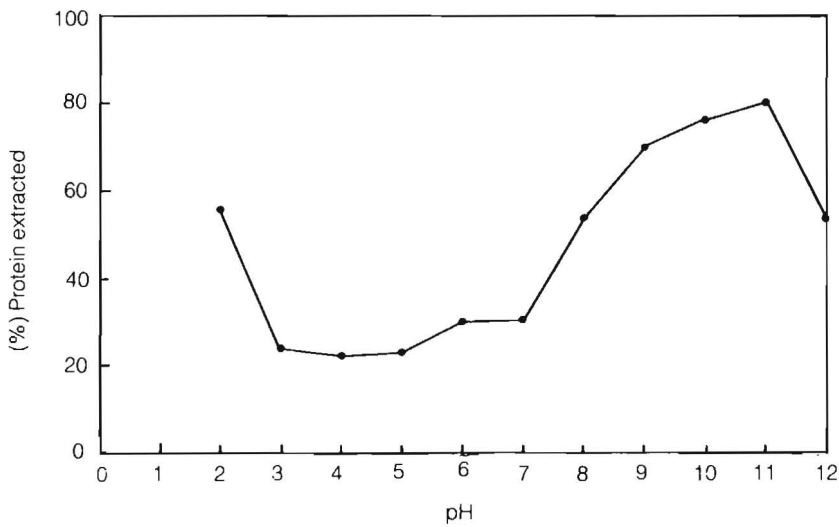

Fig. 3. Effect of pH on the extractability or protein from the defatted karkade flour.

The effect of $CaCl_2$ at different pH values in protein extractability is shown in Fig. 3. In the acidic range, the highest and the lowest protein extractability were observed at pH 2 and at pH 3-5, respectively. Data in Fig. 3 therefore suggested that most Karkade seed flour proteins have their isoelectric points through the pH range of 3 to 5. These findings agree with data given by other investigators. Hang *et al.* [15] reported that several bean proteins, namely, mung bean, pea bean and red kidney bean had a common point of minimum dispersion at pH 4.0. Ekpenyong [7] foiund that the minimum dispersion for conopher seeds occurred at pH 4.0. In the alkaline range, highest protein extractability was observed at pH 11. In general, extractability of protein increased as the pH increased from 7.0 to 11. At pH 12, the extractability of protein decreased significantly compared to the protein extracted at pH 11.

According to this study, the extraction of Karkade seed proteins flour depended on the type and salt concentration as well as the pH during extraction. Highest yield of protein extracted from the seeds was obtained at pH 11 and $CaCl_2$ concentration of 0.4 M.

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العوامل المؤثرة على استخلاص بروتين بذرة الكركديه المنزوعة الدهن

حمزة محمد أبو طربوش

قسم علوم الأغذية، كلية الزراعة، جامعة الملك سعود،

الرياض، المملكة العربية السعودية

(قدم للنشر في ١٨/٤/١٤١٤هـ؛ وقُبل للنشر في ١٠/١٠/١٤١٤هـ)

ملخص البحث. استخدمت طريقتان لنزع دهن زيت دقيق بذرة الكركديه وكانت طريقة الاستخلاص البارد أفضل من طريقة الاستخلاص الساخن فيما يتعلق باستخلاص البروتين. وجرت دراسة استخلاص بروتين بذرة الكركديه تحت ظروف مختلفة من الأس الهيدروجيني (٢ ـ ١٢) والوقت (٣٠، ٤٥ و٦٠ دقيقة) ونوعية الملح (كلوريد الكالسيوم وكلوريد الصوديوم) ومعدل المذيب إلى الدقيق (١ : ١٠ و ١ : ١٥ و ١ : ٢٠) وتركيز الأملاح (٠,٢ و ٠,٤ و ٠,٦ و ٠,٨ و ١,٠ مولر).

وخلصت نتائج الدراسة إلى أن استخلاص بروتين بذرة الكركديه يعتمد على الأس الهيدروجيني ونوعية وتركيز الأملاح. وكان كلوريد الكالسيوم أفضل من كلوريد الصوديوم في الناتج المتحصل عليه من البروتين حيث كان أعلى ناتج مستخلص من البروتين عند أس هيدروجيني ١١ وتركيز ٠,٤ مولر من كلوريد الكالسيوم.