

EVALUATION OF SOME CITRUS SEEDS FOR THEIR AMINO ACIDS
AND FUNCTIONAL PROPERTIES

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تقييم بعض بذور الموالح من حيث الأحماض الأمينية والخواص الوظيفية

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ملخص البحث

تم دراسة بعض الأحماض الأمينية والخواص الوظيفية للدقيق الناتج من بذور النارج واليوسفى وذلك بغرض التعرف على أنسب استخدام لمثل هذه المنتجات فى مجال الأغذية - وقد أوضحت الدراسة ما يلى :

- ١ - نسبة البروتين الكلى للدقيق بذور النارج كانت أعلى منها فى حالة دقيق بذور اليوسفى .
- ٢ - الأحماض الأمينية المحتوية على الكبريت كانت أعلى فى دقيق بذور اليوسفى بالمقارنة الى بذور دقيق النارج .
- ٣ - محتوى الدقيق فى كلا الحاليتين متساوى فى حمض الليسين الا أن الليسين المتاح كان أعلى فى حالة دقيق بذور النارج .
- ٤ - بالنسبة للخواص الوظيفية وخاصة المقدرة على امتصاص الماء والزيت وتكوين الرغوة وبناتها كانت أعلى بكثير فى حالة دقيق بذور النارج بمقارنتها بدقيق بذور اليوسفى مما يشجع من استخدام مثل هذا الدقيق المنزوع منه المراره فى كثير من الأغراض الغذائية فى منتجات الحبوب وبدائل اللحوم والشوربات .
- ٥ - أما المقدرة الاستحلابية فكانت متساوية فى دقيق كلا البزرتين أيضا منحنى نويان البروتين أوضح أن البروتينات ناثبية عالية فى الوسط الحمضى والقلوى مما يشجع من استخدامها فى الأغذية الحامضية أو الغير حامضية دون أن يؤثر على خواصها ويكون مصدر جيد للبروتين والأحماض الأمينية الأساسية خاصة المحتوية على الكبريت .
- ٥ - أيضا تم دراسة نمونج الهجرة الكهربائية وكان به بروتينات مختلفة فسى أوزانها الجزيئية وسرعتها أثناء الفصل فى المجال الكهربى .

ABSTRACT

Citron and mandarine seeds flour contained 24.14% and 19.25% protein respectively. Sulfur containing amino acids were higher in mandarine seeds than that of citron (6.55 g to 4.39 g/16 gN). Although both seeds were equal in lysine but, citron was richer in tryptophan and available lysine. Water and oil holding capacity and foaming properties of citron seed flour were superior to those of mandarine seed. Emulsification capacity of both seeds was very close (42.5 and 47.75 ml. oil/g flour) for mandarine and citron seeds flour respectively. The electrophoresis pattern showed the presence of 4 (mandarine) and 5 (citron) bands of protein with different relative mobilities.

INTRODUCTION

New protein sources is the major task for food scientists and technologists every where in the world due to the acute shortage in animal proteins and its high prices. Therefore, proteins from oil-seeds, legumes and wastes became an important items. Citrus seed has an excellent amino acids profile as reported by Braddock and Kesterson (1972) and Ory et al. (1978). Grapefruit defatted meal was used to improve the nutritional quality of peanut meal through blending Ory et al. (1978).

In Egypt the seeds remained from citrus industry have no use except those of citron that are mainly used as root stocks. However, the potential application of such seeds in foods should be examined thoroughly. Therefore, the nutritional quality, functional properties and electrophoresis pattern of mandarine and citron seeds flour were described in this study to give an idea about its possible utilization in food and food products.

MATERIALS AND METHODS

Materials:

Preparation of citrus flours: The seeds of ripe mandarine and citron fruits were obtained from the Faculty of Agriculture Farm at Shebin El-Kom during 1986 season. The seeds were taken out from the fruits and air dried at room temperature ($25 \pm 2^{\circ}\text{C}$) for two weeks. After crushing in a blender and removal of the outer fibers by air aspiration, the grits were used for defatting (12 hr of successive extractions) in Soxhlet apparatus using petroleum ether as a solvent. The solvent residues were removed by heating at 45°C overnight in an electric oven, then ground to pass through a 70 mesh (British Standard Screen) sieve. The citrus flours were kept at 4°C and used for analysis.

Analytical Methods:

Crude protein content was determined by micro-Kjeldahl method (A.O.A.C., 1980). The factor of 6.25 was used to calculate total protein. Amino acids methionine, lysine and cystine were determined microbiologically in the acid hydrolysate according to the method of Barton-Wright (1952). Alkaline hydrolysis was performed for tryptophan determination; Leuconostoc citrovorum was used for Systeine and Leuconostoc mesentroids used for both methionine and lysine estimation. Available lysine was determined according to the method of Carpenter (1960).

Polyacrylamide gel electrophoresis (PAGE) pattern of mandarine and citron seed flour proteins was identified by the method of Davis (1964) in 0.01 M sodium phosphate buffer of pH 7.8 and 7.5 gel. Amido black (0.5% in 7.5% acetic acid) was used to stain the resolved protein bands. However, acetic acid was used for washing and removal of the free dye.

Functional properties of citrus seeds flour:

Protein solubility profile: The standard method of A.O.A.C. (1980) was followed using 1 g flour sample in 40 ml solution. A solutions of 0.1 M of HCl and NaOH were used to adjust the pH of the extraction solution.

Water and oil absorpction properties: The methods of Sosulski (1962) and Sosulski et al. (1976) were used to measure water and oil absorption capacity respectively. Distilled water and refined corn oil were used. The values were expressed as grams of water or oil retained by 100 grams of flour sample. The density of corn oil was considered as 0.9 gm/cm³.

Emulsification capacity: The method of Yasumatsu et al. (1972) was used using 2 gm of flour sample and refined corn oil. The emulsification capacity values were expressed as ml emulsified oil per gram flour.

Foaming properties: The method of Huffman et al. (1975) was performed. Two grams of flour sample in 50 ml distilled water were mixed for five minutes in a waring blender and then poured completely into 100 ml measuring cylinder. The percentage increase in volume was recorded as foam capacity while foam volume only (Total volume-liquid volume) at the particular time recorded as foam stability.

All determinations were done in duplicates and the mean value was recorded.

RESULTS AND DISCUSSION

Protein content and amino acids composition:

The crude protein content of mandarine and citron seed flour was 19.25% and 24.14% respectively (Table 1). Citron seed flour

Table (1) : Total protein and some essential amino acids content of mandarine and citron seeds flour .

Amino Acid g/16 gN	Citrus seed flour of	
	Mandarine	Citron
Cystine	4.47	3.02
Methionine	2.08	1.28
Total sulfur A.A.	6.55	4.30
Tryptophan	2.60	3.02
Lysine	4.42	4.88
Available lysine	3.69	4.47
Total E.A.A. [⊗]	13.57	12.20
Total protein ^{⊗⊗} %	17.25	24.14

⊗ Total estimated essential amino acids .

⊗⊗ Calculated on moisture free basis .

Table (2) : Some functional properties of mandarine and citron seeds flour .

Functional property	Citrus seed flour of	
	Mandarine	Citron
Water absorption g H ₂ O/100 g flour	202.0	234.5
Oil absorption g oil/100 g flour [⊗]	148.5	234.5
Emulsification capacity ml oil/g fl.	42.5	47.75
Foam capacity (% volume increase)	3.5	39.0

⊗ The density of oil considered as 0.9 g/cm³ .

protein content is higher than mandarine seed flour by 1.3 times. However, the nutritional quality of any protein depends upon its essential amino acids content. Comparing both seeds flour it could be seen that mandarine seed flour had more sulfur containing amino acid (cystine + methionine) than citron seed flour. Meanwhile, citron seed flour was higher in tryptophan, lysine and available lysine than mandarine seed flour. It is seen that both seeds flour had almost an equal amounts of estimated essential amino acids (13.57 g for mandarine seed flour and 12.20 g for citron seed flour). It is interesting to note that more than 90% of citron seed flour lysine was in the available form compared to 83% found for mandarine seed flour. Generally these data agree well with those of Braddock and Kesterson (1972), and Ory *et al.* (1978), who reported that citrus seeds flour has an excellent amino acid profile particularly sulfur containing amino acids. Based on these data flour of mandarine and citron seeds could be used to improve the amino acids pattern of oil seeds and legumes in food products. The blends of peanut and grapefruit seeds flour was reported by Ory *et al.* (1978). Also, Mostafa (1987) used orange seed flour with peanut and wheat flour.

Functional properties:

Fig. (1) illustrates the protein solubility index of mandarine and citron seeds flour at different pH values. Proteins of mandarine seeds were highly soluble at extreme acidic pH (81% at pH 1.3). However, the solubility at pH 9.5 was 67% which is fairly lower than that at acidic pH. The minimum solubility (31% soluble protein) was observed at pH 5.0. The general shape of mandarine solubility curve was almost similar to that of other vegetable proteins (Rahma, 1979). Citron seed proteins solubility was also higher in acidic side than in alkaline region. There was no sharp minimum solubility pH, but this was noticed in the range from pH 4 to pH 6. At the isoelectric point only 22.5% of protein was in solution. From the solubility

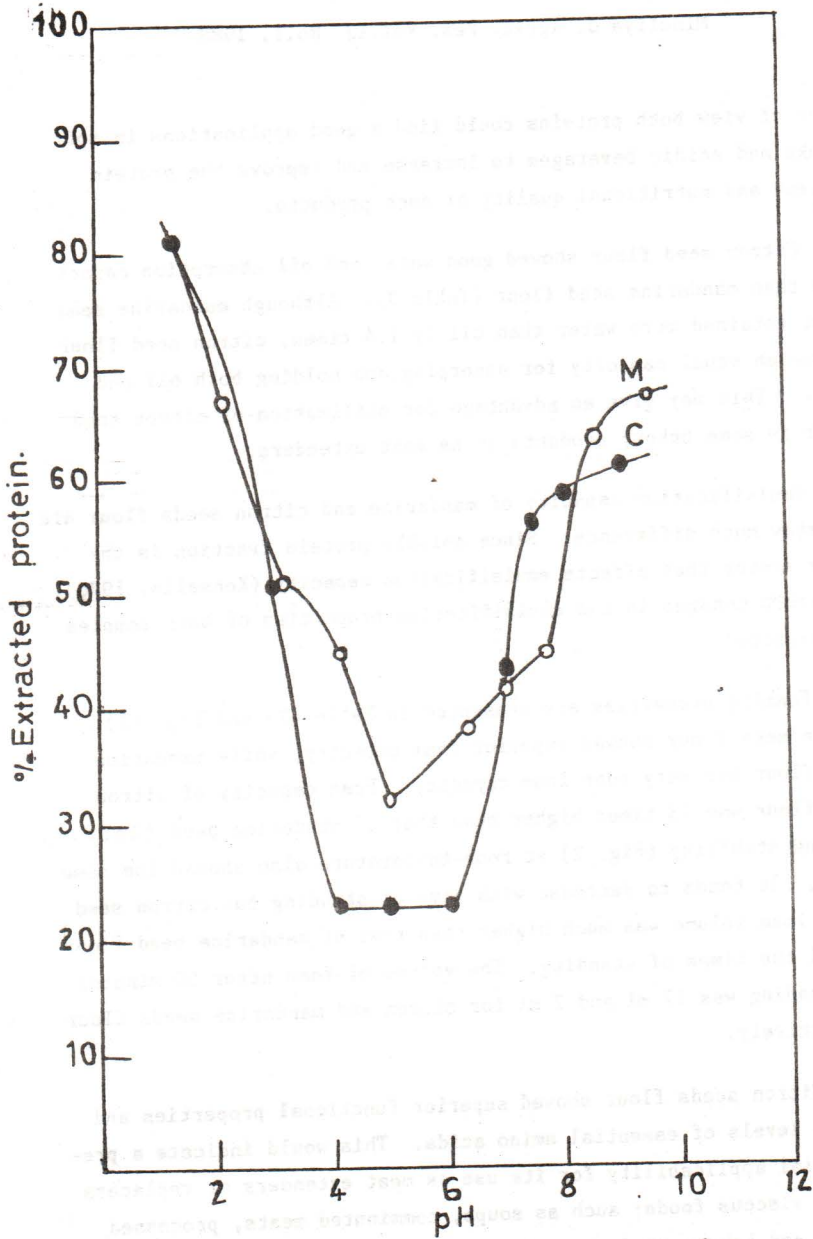


Fig. (1) Protein solubility curves of citron and mandarin seeds flour (C, citron M, mandarin).

point of view both proteins could find a good applications in soft drinks and acidic beverages to increase and improve the protein content and nutritional quality of such products.

Citron seed flour showed good water and oil absorption capacities than mandarine seed flour (Table 2). Although mandarine seed flour retained more water than oil by 1.4 times, citron seed flour showed an equal capacity for absorbing and holding both oil and water. This may give an advantage for utilization of citron seed flour in some bakery products or as meat extenders.

Emulsification capacity of mandarine and citron seeds flour did not show much difference. Since soluble protein fraction is the major factor that affects emulsification capacity (Kensella, 1976), no marked changes in the emulsification properties of both samples are expected.

Foaming properties are presented in Table (2) and Fig. (2). Citron seed flour showed superior foam capacity, while mandarine seed flour has very poor foam capacity. Foam capacity of citron seed flour was 13 times higher than that of mandarine seed flour. Foaming stability (Fig. 2) at room-temperature also showed the same trend. It tends to decrease with time of standing but citron seed flour foam volume was much higher than that of mandarine seed flour at all the times of standing. The volume of foam after 60 minutes of standing was 17 ml and 2 ml for citron and mandarine seeds flour respectively.

Citron seeds flour showed superior functional properties and higher levels of essential amino acids. This would indicate a preferential applicability for its use as meat extenders or replacers and in viscous foods; such as soups, comminuted meats, processed cheese and bakery products. Also, data reported here on solubility

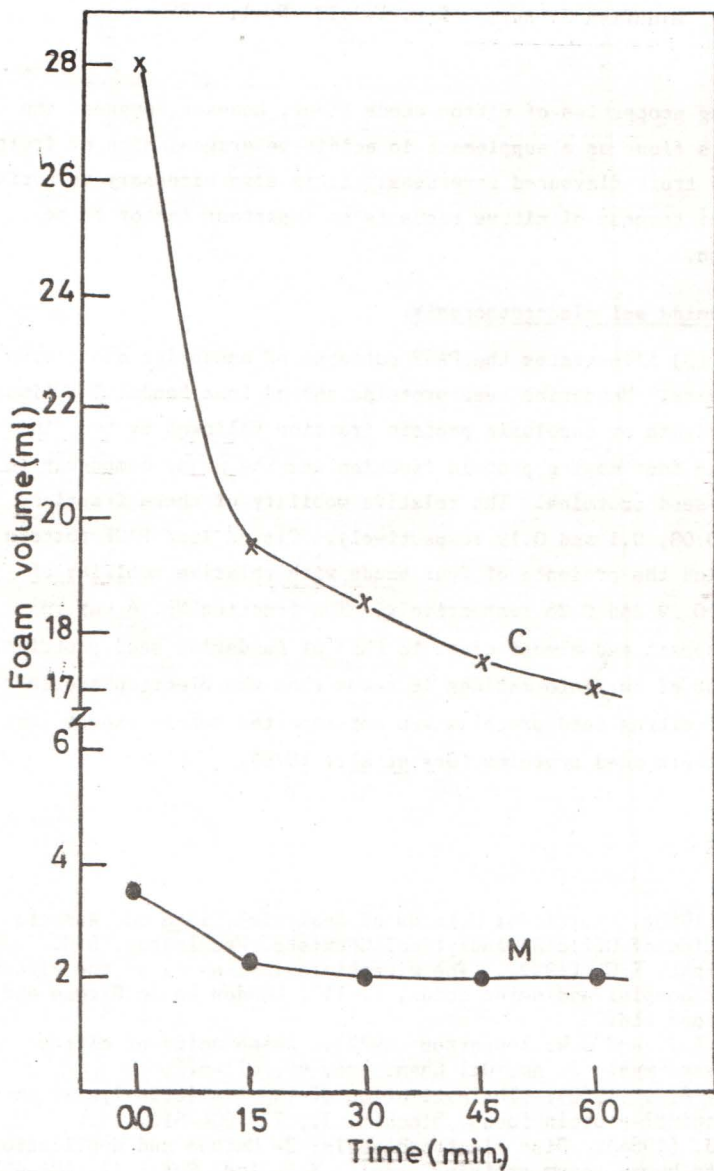


Fig. (2) Foam stability (ml) patterns of citron and mandarine seeds flour(C,citron M,mandarine)

and foaming properties of citron seeds flour, however, suggest the use of this flour as a supplement in acidic beverages, such as fruit juices and fruit-flavoured beverages. It is also necessary to notice that the bitterness of citrus seeds is an important factor to be looked into.

Polyacrylamide gel electrophoresis:

Fig. (3) illustrates the PAGE patterns of mandarine and citron seed proteins. Mandarine seed proteins showed four bands. The first is an aggregate or insoluble protein fraction followed by two minor bands. The fast moving protein fraction was the major component in mandarine seed proteins. The relative mobility of these fractions are 0.0, 0.08, 0.1 and 0.19 respectively. Citron seed PAGE pattern (2) revealed the presence of four bands with relative mobility of 0.0, 0.1, 0.19 and 0.26 respectively. The fraction No. 4 was the major component and almost close to that of mandarine seed proteins. To the best of our informations it seems that the electrophoresis pattern of citrus seed proteins was not reported before except that of grape fruit seed proteins (Ory et al., 1978).

REFERENCES

- A.O.A.C. (1980). "Official Methods of Analysis", 13th ed. Association of Official Analytical Chemists, Washington, D.C.
- Barton-Wright, E.C. (1952). The microbiological assay of the vitamin B-complex and amino acids, P. 117, London Isaac Pitman and Sons Ltd.
- Braddock, R.J. and J.W. Kesterson (1972). Amino acids of citrus seed meal. J. Am. Oil Chem. Soc. 49, 671-672.
- Carpenter, K.J. (1960). The estimation of the Available lysine in animal-protein foods. Biochem. J., 77: 604-610.
- Davis, B.J. (1964). Disc electrophoresis: 2- Method and application to human serum proteins. Anal. N.Y. Acad. Sci., 121:404-427.
- Huffman, V.K.; C.K. Lee and E.E. Burns (1975). Selected functional properties of sunflower meal (Helianthus annuus). J. Food. Sci., 40: 520-523.

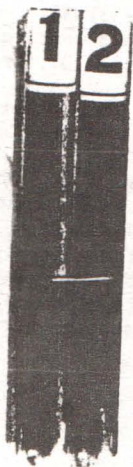


Fig. (3) Polyacrylamide gel electrophoresis patterns of citron and mandarine seeds flour. (1, mandarine 2, citron).

- Kinsella, J.E. (1976). Functional properties of proteins in foods: A survey. *Crit. Rev. Food Sci. Nutr.*, 7: 219-280.
- Mostafa, M.M. (1987). Effect of blending orange seed meal on protein quality of peanut. *J. Agric. Sci. Mansoura Univ.*, (12), 1302-1311.
- Ory, R.L.; E.J. Conkerton and A.A. Sekul (1978). Effect of blending plant materials on protein quality. 1- Peanut and citrus seed proteins. *Peanut Sci.*, 5: 31-34.
- Rahma, E.H. (1979). Characterization and functional properties of the protein of sunflower seed. Ph.D. thesis, University of Mysore, Mysore, India.
- Sosulski, F.W. (1962). The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chem.*, 39: 344-350.
- Sosulski, F.W.; E.S. Humbert; K. Bui and J.D. Jones (1976). Functional properties of rapeseed flour, concentrates and isolates. *J. Food Sci.*, 41: 1349-1352.
- Yasumatsu, K.; K. Sawada; S. Moritaka; M. Misaki; J. Toda; T. Wada and K. Ishii (1972). Studies on the functional properties of food grade soybean products. IV- Whipping and emulsifying properties of soybean products. *Agric. Biol. Chem.*, 36: 517-521.