

Effects of Three Different drying Methods on Protein Solubility and Color Parameters of Fenugreek Protein Isolate

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Abstract- The effects of three different drying methods on solubility and color parameters of fenugreek protein isolate were studied. Fenugreek protein isolate was extracted by dissolving in 0.33M NaCl solution, and then adding 1M NaOH solution to reach the pH9.25 along with isoelectric precipitation. The final isolate was subjected to freeze, vacuum oven and oven drying processes. The amount of protein in fenugreek protein isolate was 89%. Except for pH4.5 which is the isoelectric point, in other pHs there were significant differences ($p < 0.05$) in solubility of the protein isolates with different drying methods. At all pHs the solubility of freeze dried protein isolate (FFPI) was higher than two other isolates, more over the solubility of vacuum oven dried fenugreek protein isolate (VOFPI) was higher than oven dried one (OFPI). The results of CIE lab measurements showed the most lightness for freeze drying method. These observations indicate among these three drying methods, freeze drying is more useful, because of less denaturation and damages conducted to proteins, and colorants.

Keywords- color parameters; drying method; fenugreek; solubility; protein isolate.

I. Introduction

Today plant proteins play significant roles in human nutrition, especially in the third world countries where the ingestion of proteins is below the recommended allowance [13, 20]. Due to inadequate supplies of animal proteins, there has been a constant search for new protein sources, for use as both functional food ingredient and nutritional supplements [20].

In recent years, legumes because of their low prices, different varieties and the wide variations, are becoming an important protein source, for use as both functional food ingredients and nutritional supplements [18, 15].

Among different legumes, fenugreek (*Trigonella foenecum graecum*) is considered due to its potential as a protein source with high nutritive value, and also the seeds are used as a cheap source of good quality protein [18]. Fenugreek belongs to the family *Fabaceae*, and is widely cultivated in Mediterranean countries and Asia. It is believed to have originated in southeastern Europe or south-western Asian countries [2, 10, 19]. Fenugreek seeds mainly have between 25 – 38.6% protein [2, 19, 21]. Approximately 52% of total amino acids of fenugreek seeds belongs to essential amino acids, this

amount of amino acids is highest in comparison with wheat, horsbean, barely and sorghum, but less than lupine which has 53.91% essential amino acids [17]. The main protein fractions in fenugreek are albumins (43.8%), globulins (27.2%), glutelins (17.2%) and prolamines (7.4%) respectively [2].

Functional properties are characteristics of the protein, which are defined as the physical and chemical properties which affect the behavior of proteins in food systems during processing, storage, preparation and consumption [6, 7]. These properties are determined by a number of factors: source, procedure employed to obtain seed flours, concentrates and isolates, the naturally occurring matrix of carbohydrate, lipids, and fibers within which the protein occurred; and physiochemical factors like pH, salt and temperature [1, 11, 12], also procedures such as methods and conditions of protein extraction and drying are the factors need to be addressed [9]. Among different functional properties, solubility of protein under varying conditions is one of its important functional properties. This importance is due to the great influence of solubility on other properties, such as emulsification, foaming and gelation. Thus the protein may possess satisfactory properties, e.g. nutritional value, acceptable flavour, odour and texture [7].

The aim of this study was to evaluate the effect of different drying methods on protein solubility, and color parameters of fenugeek protein isolate.

II. Materials and Methods

A. Materials

Fenugreek (*T. foencem graecum*) seeds were obtained from the local market, Mashhad, Iran. The seeds were cleaned, and then ground to a powder using an electrical miller. The fenugreek seed flour (FSF) was defatted with hexane by continuous stirring at a ratio 1:4 for 3 hours at room temperature to produce defatted fenugreek flour (DFF), both FSF and DFF were passed through a 40 mesh sieve.

All chemicals were of analytical grade.

B. Methods

Protein extraction

Protein extraction was according to the method of Horax, Hettiarachchy, Kannan, & Chen [23], with slight modification. Protein from the defatted fenugreek flour (DFF) was extracted, using sodium chloride solution (DFF:NaCl solution was 1:20 w/v) at concentration of 0.33M and pH 9.25 (using 1 N HCl and 1 N NaOH) to increase the solubility of

protein. The stirring period was about 2 hours. The peptized liquor was centrifuged at 4500×g for 30 min. After centrifugation of the suspensions, supernatants were adjusted to pH 4.5 (isoelectric pH of Fenugreek protein) and centrifuged at 4500×g for 20 min to precipitate the protein. The protein precipitate was washed twice, using deionized water, followed by centrifugation for 5min at 4500×g, re-solubilized by adjusting the pH to 7.2 using 1 N NaOH and then the isolate was dried using one of three drying methods.

Drying methods of Fenugreek protein isolate

The isolate obtained from above procedure was dried either by freeze, vacuum oven or oven dryer. For freeze drying method the isolate was in freeze dryer for about 2 days, at pressure of 700mmHg, at -30°C, and in vacuum oven drying, isolate was directly dried overnight at 50°C and pressure of 800mmHg, for oven drying method the isolate was in oven 80°C for overnight. After all three drying methods the dried fenugreek protein isolate was ground and stored in refrigerator until use. Protein content of the isolates was determined using Kjeldahl method [3].

Determination of protein isolate solubility

The protein solubility of isolates at three different pHs (4.5, 7, and 9) was determined using Biuret method [24]. The preparation of protein solutions were according to Bera and Mukherjee method with slight modification [14], Protein dispersions were made using 1.5g of the protein isolates in 100 ml of deionized water. The dispersions were treated with either 0.5 M HCl or 0.5 M NaOH to obtain various pHs (4.5, 7, 9), and stirred for 30 min at room temperature, then the slurry was centrifuged at 5000×g for 15min to separate the supernatant. After centrifugation at 5000×g for 15 min, protein content was determined by the Biuret method using RayLight, UV-2601 spectrophotometer, all absorbances were read at 540nm.

Color parameters

A digital colorimeter (Chroma meter CR-410, Sensing, INC. Japan) was used to measure the color of fenugreek protein isolates and color scales L*a*b* values were recorded. A cylindrical plastic dish (58 mm in diameter and 15 mm in depth) containing the same quantity of samples was placed at the light port (50 mm in diameter). The instrument was initially calibrated with a white standard plate. h and C value of all three different drying methods were calculated using equations below [22]:

$$C = ((a^*)^2 + (b^*)^2)^{1/2} \quad (1)$$

$$h = \tan^{-1} \{a^*/b^*\} \quad (2)$$

Statistical analysis

The data reported in the tables are an average of duplicate observations and were subjected to analysis of variance (ANOVA) using SPSS Statistical Software version 16. Duncan procedure was used to compare means, and for the significance among different dried fenugreek protein isolates at a 5% significance level ($p < 0.05$).

iii. Result and discussion

Protein solubility

Protein solubility is the most important functional property because it influences other functional properties [9, 26]. The nitrogen solubility as a function of pH and drying method is shown in Fig. 1. The data showed in every three methods of drying, the solubility at pH4.5, which is the iso electric point of fenugreek protein isolate, was the minimum. This result is the same as results of Abdel-Aal, Shehata, El-Mahdy, & Youssef [5], El-Hawwary[17], El Nasri & El Tinay[18]; who worked on fenugreek protein extraction, concentration and isolation. Also it can be observed that with increasing pH the solubility increases too, this is due to the negative charges which protein acquires at alkaline pHs and causes the repulsion of molecules and thereby increases the solubility of the protein [25].

As it is shown in Fig.1 drying method had significant effect on protein solubility except at pH4.5 which was not significant difference ($p < 0.05$) between the protein solubility of oven dried and vacuum oven dried isolate, either vacuum oven dried and freeze dried isolate. These results are in contrast with the results of Amza, Amadou, zhu, & Zhou [26], their results about effect of vacuum oven and freeze drying methods on ginger bread pulm seed protein isolate solubility at various pH implied that the drying methods did not considerably affect the protein isolates' solubility. Yu, Ahmedna, and Goktepe [9], dried peanut protein isolate with spray drier and vacuum oven at 70°C, they reported at all pHs (2- 10) the solubility of spray dried peanut protein isolate is significantly higher than vacuumed oven ones. This observation contributed to the speed of spray drying. This procedure is a very fast drying method during which protein molecules are only subjected to a few seconds of heating, which minimizes their denaturation. In contrast, protein molecules were subjected to excessive denaturation during vacuum oven drying.

In our study the solubility of freeze dried isolate was the highest in general. This fact shows the amount of major changes in the secondary, tertiary, and quaternary structures without cleavage of backbone peptide bonds, which are regarded as

denaturation, in freeze drying method is less than vacuum oven. In addition in vacuum oven dryer we deal with less denaturation in comparison with oven because of long period and high temperature, 50°C and 80°C respectively, which are conducted in two latest methods.

Color parameters

One important factor about protein isolates is their colors. As protein isolates are used in food formulation they affect food colors.

Fenugreek seeds contain five flavonoids include vitexin, tricetin, naringenin, quercetin and tricetin-7-O-b-D-glucopyranoside [16].

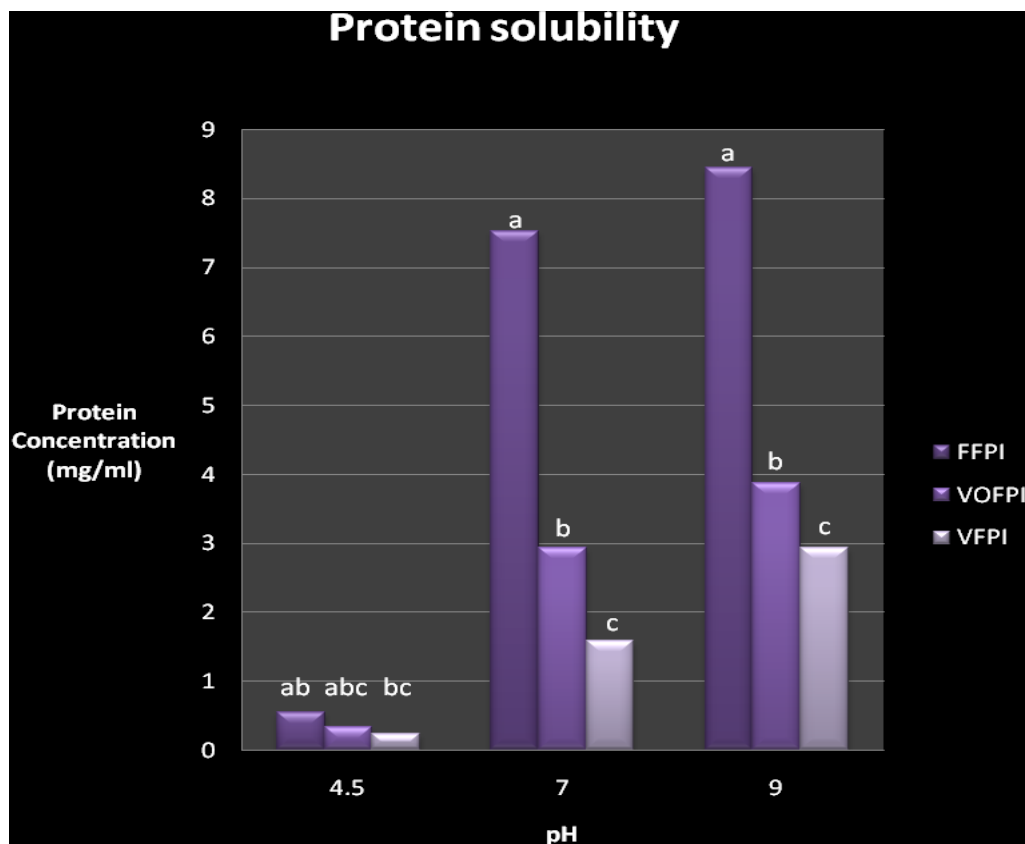


Figure 1. Effects of drying methods and pH on the solubility of FFPI, VOFPI, and VFPI.

Values represent the means±SD.

Mean values in rows with different letters (a, b or c) were significantly different (Duncan's test); significance at (p<0.05) analysis of variance.

FFPI: Freeze dried fenugreek protein isolate

VOFPI: Vacuum oven dried fenugreek protein isolate

VFPI: Oven fenugreek protein isolate

FFPI appears to be lighter than VOFPI and VFPI, which is an indication that drying methods do have an impact on the final protein isolate physical appearance. These observations were confirmed by color instrumental analysis (Table 1).

The highest amount of L* value among three different methods belongs to FFPI (80.27), but there is no significant difference between L* value of VOFPI and VFPI. These observations implicate oven and vacuum oven drying would have led to reaction

of amine compounds with aldehydes via Maillard reaction to form dark pigments (melanoidins) [4].

It can be seen from Table 1 the a* value of the freeze dried fenugreek protein isolate is lowest which shows more green color, and the b* value of it, is the highest which shows more yellow color in isolate. Both a* and b* value of FFPI are significantly different from the isolates of two other drying methods, it may show in freeze drying method

because of the absence of high temperature the flavonoid compounds of fenugreek seed are stable, and they are less destroyed so the green-yellow native color of seed is sustained.

In other hand the high value of b^* is considered to show maillard reaction [27], slight high amount of b^* value in OFPI in comparison with VOFPI, although it is not significant, can be attributed to this reaction. It also shows during vacuum drying, although there was little oxygen in the early stage and little moisture in the later stage, the maillard reaction could not be totally absent [26], but the low amount of oxygen in procedure like vacuum condition leads to positive effect on the stability of flavonoid compound [8], so beside lower temperature, it can be the reason why a^* value of VOFPI is closer to FFPI than OFPI.

As it can be seen, the h value of FFPI was more than VOFPI and OFPI. This observation is because of minus amount of a^* in FFPI, which is related to the high amount of green color in it. There were no significant difference between h value of VOFPI and OFPI. Both of these isolates have orange – red color which can be contributed to the high temperature and millard reactions.

Chroma (saturation) will increase with increasing pigment concentration, and then decrease

as the sample becomes darker [22]. C value of three isolates were significantly different, among these the C value of FFPI was highest. It shows that the colorant concentration in this isolate is the most, so again it implicates the best drying method for color maintenance is freeze drying.

IV. Conclusion

Three different drying methods were used to investigate the effects of drying methods on protein solubility and color parameters of fenugreek protein isolate. The best drying method in case of solubility was freeze drying. The solubility at pH9 was the most in all three drying methods. All color parameters include L^* , a^* , b^* , h , and C value in FFPI were considerably better in comparison with VOFPI and OFPI. In conclusion the least protein denaturation, and colorant destruction were occurred in freeze drying method, also because of low temperature which is inducted in freeze drying procedure, no maillard reaction occurs. With take these in to account, among these three drying processes, freeze drying should be the best method of drying for fenugreek protein isolate.

Table 1. CIE lab values of Fenugreek protein isolates from different drying methods.^{ab}

Dried protein isolate	L^*	a^*	b^*	C	h
FFPI	80.270±0.53 ^a	-0.195±0.26 ^a	26.920±0.09 ^a	26.921±0.09 ^a	359.58°±0.55 ^a
OFPI	31.515±0.08 ^b	4.845±0.09 ^b	11.470±0.51 ^b	12.451±0.37 ^b	22.90°±0.41 ^b
VOFPI	31.820±0.02 ^b	4.565±0.06 ^b	9.815±0.38 ^c	10.826±0.42 ^c	24.97°±1.59 ^b

a Means followed by same letter within a column do not differ significantly ($P < 0.05$).

b Mean of duplicate analysis ± SD.

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