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Alkali Extraction of Dietary Fiber from *Trigonella-foenum graecum* L. Seeds (Dietary Fiber of Fenugreek Seeds)

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ABSTRACT

In this study, alkali dietary fiber extraction method was evaluated to obtain soluble and insoluble dietary fiber from *Trigonella-foenum graecum* L. (fenugreek) seeds. The process conditions of alkali extraction method ensuring the highest total dietary fiber yield were investigated by response surface methodology. Furthermore, some physicochemical and functional properties of extracted soluble and insoluble dietary fiber from fenugreek seeds such as water retention capacity, oil adsorption capacity, swelling capacity, glucose adsorption index and α-amylase inhibition capacity were determined. Total dietary fiber yield was 78% at 52.50 g/L of sample: NaOH ratio and 1.01 M NaOH concentration as the optimum process conditions. Furthermore, insoluble dietary fiber gave better results than soluble dietary fiber when physicochemical and functional properties were compared.

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Introduction

Recently, consumption and production of foods having functional properties have been increased because of their disease preventative effects. The consumers are searching for the food products suitable for a balanced diet and nutraceutical foods having ability to prevent chronic diseases such as obesity, hypertension and diabetes (Tejada-Ortigoza et al., 2016). Both in literature and in the food industry, some compounds having therapeutical properties have gained importance, and one of these compounds can be considered as dietary fiber.

The World Health Organization defined dietary fiber (DF) as 'intrinsic plant cell wall polysaccharides' (Cummings and Stephen, 2007). DF is defined as the 7th vital nutrient in organism, and it has important biological activities such as reduction of cholesterol, preventing diseases such as heart attack, colon cancer and diabetes (Ma et al., 2015; Wang et al., 2021). DF can be categorized as soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) (Du et al., 2021). In literature, it is reported that fruits have higher content of SDF than IDF; however, cereals, seeds, legumes and vegetables have higher IDF content than SDF (Tejada-Ortigoza et al., 2016). SDF can be totally hydrolyzed in the colon and it can inhibit the lipid transportation, so that SDF obtained from different plant materials can be considered as fat substitutes (de Moraes Crizel et al., 2013). Moreover, SDF can scavenge some of the free radicals, can support the growth of the intestinal probiotics and can prevent cardiovascular diseases (Huang et al., 2016). On the other side, IDF mainly includes cellulose, hemicellulose and lignin which have high swelling, water retention and oil adsorption capacity (Wang et al., 2020). IDF has cation exchange capacity and due to this ability, they can be used for detoxification (Jiménez-Escrig and Sánchez-Muniz, 2000). Moreover, IDF can prevent chronic diseases, can increase the amount of fecal bulk and reduces transit period of the gastrointestinal system (Elleuch et al., 2011). The functional properties of a DF are mainly associated with its SDF and IDF percentage, and matrix structure (Ma et al., 2015). Therefore, the extraction method and extraction conditions of DF are important topics to produce high-quality DF and to evaluate its physicochemical activities (Wang et al., 2021).

Extraction is a unit operation to separate bioactive materials or molecules from their matrices (Vilkhu et al., 2008). Extraction of DF has various techniques such as enzymatic extraction, acid extraction and alkaline extraction (Wang et al., 2021). The treatment of the alkaline solution to food matrix for obtaining dietary fiber is a common process and it breaks cell walls by hydrolyzation of ester bounds between hemicelluloses, resulting in lignin and silica production (Jacquemin et al., 2012). Some polysaccharides situated in the cell wall can be released to the extraction media by disruption of covalent and hydrogen bonds (Tejada-Ortigoza et al., 2016). By the usage of alkaline extraction method, high DF yields can be achieved and this method can be considered as a cheaper method than enzymatic extraction (Chanliaud et al., 1995). In literature, alkaline extraction method offered as a better extraction technique than acid extraction of DF (Wang et al., 2021).

In literature, fenugreek seeds are reported as a possible source of DF. Fenugreek (*Trigonella-foenum graecum* L.) is a medicinal plant and it was originated from western Asia and southeastern Europe (Khoja et al., 2021). Fenugreek seeds are used for the treatment of the diseases such as fever, high blood pressure and diabetes, and they have antioxidant and antibacterial properties (Naidu et al., 2011; Riasat et al., 2018; İnanan and Kanyılmaz, 2020). Fenugreek seeds are also reported as a good source of soluble and insoluble DF (Hooda and Jood, 2005; Krishnakumar et al., 2012). More than 50% dry weight of seeds of fenugreek is DF and DF of the fenugreek seeds were reported as stable compounds withstanding to processes such as frying, baking, cooking and freezing (Srinivasan, 2006). It was reported that intake of 30 g of fenugreek seed DF in a day combined with proper exercises can help losing weight (Chadra, 1985).

Even though having high amount of DF, extraction of DF from fenugreek seeds was not investigated in detail in literature. To best of our knowledge, there is no study which applied alkaline dietary fiber extraction technique on fenugreek seeds. In this study, alkaline extraction method was performed to obtain IDF and SDF from fenugreek seeds. The conditions of the alkali extraction technique namely sample:NaOH ratio and NaOH concentration were also optimized by a central composite design. Moreover, some physicochemical and functional properties of IDF and SDF produced at different extraction conditions were investigated.

Materials and Methods

Material

Fenugreek seeds were bought from a local market in Tokat, Turkey. The seeds were harvested in 2020 and stored for five months. The seeds were combed out to purge and after that they were grained using a rotary grinder (Sinbo SHB 3020, Turkey). Powdered seeds were then sieved using a sieve having 630 μm pore diameter, and the powder under the sieve collected and used as samples. The moisture content of the final sample was 4.51% (wet base).

Preparation of the Defatted Fenugreek Powder

The powdered fenugreek seed samples having 16.15±1.02% of fat content was subjected to defatting before DF extraction processes to avoid fat interaction. The fenugreek seed powder was mixed with hexane (n-Hexane Ultra-Pure >96%, Tekkim, Turkey) (1:4, w/v) at 400 rpm with a magnetic stirrer for two hours at room temperature, and this procedure was repeated thrice with fresh hexane at each time. After defatting process, the hexane layer was discarded and the residual hexane in the defatted fenugreek seed powder removed at 50°C overnight.

Extraction of Soluble and Insoluble Dietary Fiber and Experimental Design

The extraction of SDF and IDF of defatted fenugreek seeds were carried out using alkali extraction process. The sample:NaOH ratio (20-100 g/L) and NaOH (Sigma-Aldrich, Germany) concentration (0.5-2 M) were chosen as dependent variables and all boundary values were determined with preliminary tests. A full-type face centered central composite design having 15 points (with 3 center points) was used. Slightly modified version of alkali SDF and IDF extraction method by Wang et al. (2021) was applied to the defatted fenugreek samples. The prepared solutions were mixed for two hours at 450 rpm and at room temperature (25°C). The SDF, IDF and total DF (TDF) yields were calculated using Equation (1), Equation (2), and Equation (3), respectively. The detailed chart of extraction of SDF and IDF was shown in Figure 1 and the experimental design was given in Table 1.

$$\text{SDF (\%)} = \frac{\text{weight of dried SDF (g)}}{\text{weight of defatted fenugreek seed (g)}} \tag{1}$$

$$\text{IDF (\%)} = \frac{\text{weight of dried IDF (g)}}{\text{weight of defatted fenugreek seed (g)}} \tag{2}$$

$$\text{TDF (\%)} = \frac{\text{weight of dried SDF (g)+weight of dried IDF (g)}}{\text{weight of defatted fenugreek seed (g)}} \tag{3}$$

Physicochemical Properties of the IDF and SDF

Water Retention Capacity

The extracted IDF and SDF were mixed with distilled water and the samples were left at room temperature for one hour and centrifugation was applied for 20 minutes at 3000 g. After discarding the supernatants, the precipitates were weighed and water retention capacity (WRC) of the samples was calculated as gram water for per gram dry sample (Sun et al., 2018).

Oil Adsorption Capacity

Similar steps were applied as WRC to determine oil adsorption capacity (OAC) of the samples, only virgin olive oil (Extra Virgin Olive Oil, Kırlangıç, Turkey) was used instead of distilled water. The OAC was defined as gram virgin olive oil for per gram dry sample (Sun et al., 2018).

Swelling Capacity

A certain amount of IDF and SDF were weighed and hydrated with distilled water for 18 hours. The initial volume of the sample and the volume of the sample prior to hydration were recorded, and the swelling capacity (SC) of the IDF and SDF was calculated using Equation (4).

$$SC\ (mL/g)= (v_1\text{-}v_0)/w_0 \qquad (4)$$

where;

SC: is swelling capacity

v_1: is volume of the sample after hydration

v_2: is volume of the sample prior to hydration

w_0: is the initial weight of the sample (Ma and Mu, 2016).

Functional Properties of the IDF and SDF

Glucose adsorption capacity

Glucose adsorption capacity (GAC) of the samples were determined using the method of Chu et al. (2019). IDF and SDF were mixed with 100 mmol/L glucose (D (+)-Glucose Monohydrate, Merck, Germany) solution and incubated in a shaker water bath at 37°C for 6 hours. After that, the samples were centrifuged at 3219 g for 15 minutes. DNS (3-5 dinitro salicylic acid, CDH, India) method (540 nm) was used to determine the glucose content of the supernatants and the GAC was calculated using Equation (5).

$$GAC\ (mmol/g)= (G_1\text{-}G_2)/W\times V \qquad (5)$$

where;

G_1: is the glucose concentration before adsorption (mmol/g)

G_2: is the glucose concentration after adsorption (mmol/g)

W: is the weight (g) of the samples

V: is the supernatant volume in mL.

α-amylase inhibition capacity

The method of Ma and Mu (2016) was used to determine the α-amylase (α-amylase from *Aspergillus Oryzae*, ~1.5 U/mL, Sigma-Aldrich, Germany) inhibition capacity (AAIC) of the IDF and SDF. The α-amylase, 4% (w/v) potato starch (Merck, Germany) solution and IDF and SDF sample were mixed, and the mixtures were incubated at 37°C for 30 minutes. Following that, the solutions were incubated in a water bath at 100°C for 5 minutes, and the samples were centrifuged for 30 minutes at 3219 g. The supernatants were collected, and the glucose content of the supernatants were determined by DNS method. A sample only without DF was prepared and this sample was used as the blank sample. AAIC of the samples were determined according to the Equation (6).

$$AAIC\ (\%)= (A_2\text{-}A_1)/A_2\times 100 \qquad (6)$$

where;

A_1: is the absorbance of the supernatant with the samples containing DF

A_2: is the absorbance of the supernatant with the samples without DF.

$$\text{Total DF Yield (\%)}$$

$$= \beta_0+\sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{k}\beta_i X_i^2+\sum_{i=1}^{k\text{-}1}\sum_{j=1+1}^{k}\beta_{ij} X_{ij} \qquad (7)$$

$$k=1, 2$$

where;

β_0: indicates the constant

k: is the number of independent variables

X_i: is the *i*th independent variable

β_{ij}: is the *j*th coefficient of *i*th observation

X_{ij}: indicates the *j*th independent variable of *i*th observation.

One-sample t-tests and comparison of the analysis results for the samples were carried out using the SPSS 22.0 (IBM, USA) package program. The regression analysis which was used to determine the effects of the independent process variables on the responses, response surface graph and optimization study were done using the Design Expert 7.0 (Stat-Ease Inc., USA) package program. According to the mathematical models, significant terms in the model for total DF values were determined by variance analysis.

Results and Discussion

The IDF, SDF and TDF yield results are given in Table 1. Here, the effects of the sample:NaOH ratio ratio and NaOH concentration on DF extracted from fenugreek seeds are shown. According to the results, the highest TDF yields were obtained at the 60 g/L of solid to liquid ratio. On the other hand, lowest results for TDF yields were at the 100 g/L of solid to liquid ratio (Table 1). Generally, the extraction of biological compounds can be enhanced with higher amount of solvent (Ding et al., 2019).

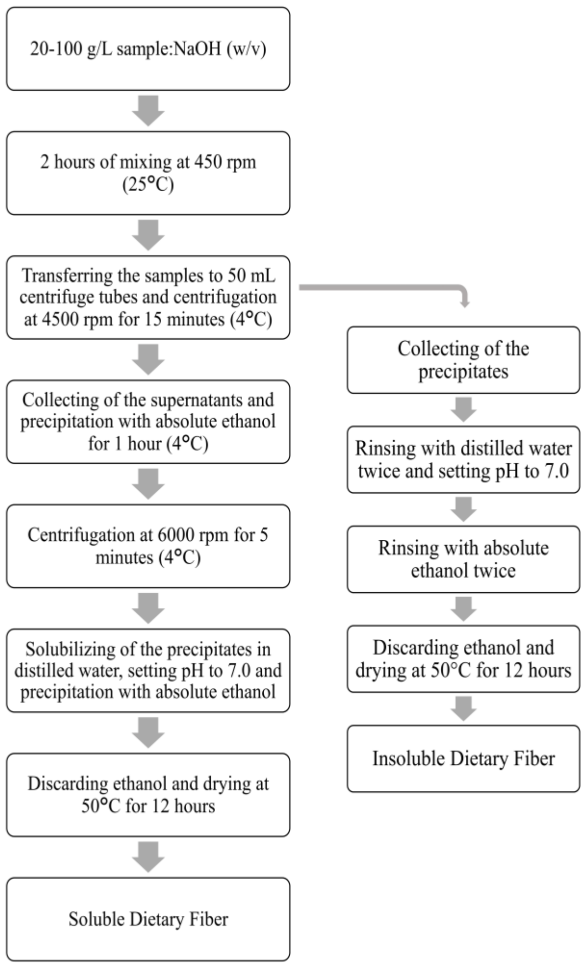
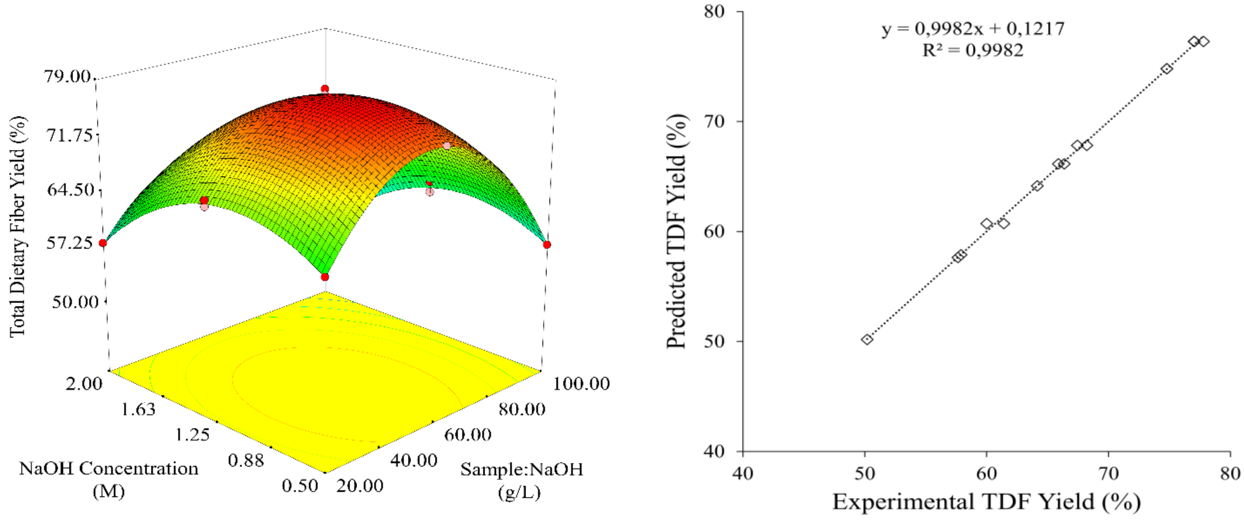


Figure 1. Flow chart of the production of soluble and insoluble dietary fiber



(a) (b)

Figure 2. Response surface graph at the optimum point (a) and the relationship between predicted and experimental total dietary fiber yields (b)

Table 1. The experimental design and DF yields

Run	Sample:NaOH ratio (g/L) (X_1)	NaOH (M) (X_2)	SDF (%)	IDF (%)	TDF (%)
1	20	1.25	21.50	46.71	68.21
2	20	0.50	18.34	45.81	64.16
3	100	2.00	15.84	34.36	50.19
4	60	2.00	19.60	46.29	65.89
5	60	2.00	19.72	46.66	66.38
6	60	0.50	17.08	57.72	74.80
7	60	1.25	20.15	56.88	77.02
8	100	1.25	15.94	44.06	60.00
9	100	0.50	13.47	44.18	57.65
10	60	0.50	18.67	56.11	74.78
11	100	1.25	17.29	44.12	61.41
12	60	1.25	18.27	58.77	77.05
13	20	1.25	21.37	46.07	67.44
14	20	2.00	15.77	42.13	57.89
15	60	1.25	22.03	55.76	77.79

SDF: Soluble Dietray Fiber, IDF: Insoluble Dietary Fiber, DF: Dietary Fiber

Table 2. ANOVA table and statistical parameters for alkali extraction process

Source	DF	Sum of Squares			F Value			p - Value		
		IDF	SDF	TDF	IDF	SDF	TDF	IDF	SDF	TDF
Model	7	684.51	72.46	980.57	73.93	5.95	548.53	< 0.0001	0.0157	< 0.0001
X_1	1	5.30	23.21	50.69	4.01	13.34	198.50	0.0854	0.0082	< 0.0001
X_2	1	108.94	3.18	74.89	82.36	1.83	293.24	< 0.0001	0.2184	< 0.0001
X_1X_2	1	9.40	6.10	0.36	7.11	3.51	1.39	0.0322	0.1033	0.2766
X_1^2	1	442.49	16.21	628.09	334.53	9.31	2459.46	< 0.0001	0.0185	< 0.0001
X_2^2	1	74.02	20.41	172.15	55.96	11.72	674.08	0.0001	0.0111	< 0.0001
$X_1^2X_2$	1	6.80	1.79	1.61	5.14	1.03	6.31	0.0578	0.3444	0.0403
$X_1X_2^2$	1	2.88	2.92	1.30×10^{-4}	2.18	1.68	5.07×10^{-4}	0.1833	0.2361	0.9827
Residual	7	9.26	12.18	1.79						
Lack of Fit	1	3.06	2.94	1.21×10^{-3}	2.96	1.91	4.05×10^{-3}	0.1359	0.2163	0.9513
Pure Error	6	6.20	9.24	1.79						
Total	14	693.77	84.65	982.36						

R^2: 0.9982, adj- R^2: 0.9964, Adequate Precision: 73.41, PRESS: 5.25, C.V. (%): 0.76, X_1: Sample:NaOH ratio (g/L), X_2: NaOH Concentration (M), IDF: Insoluble Dietary Fiber, SDF: Soluble Dietary Fiber TDF: Total Dietary Fiber, DF: Degrees of Freedom, Adj- R^2: Adjusted R^2, PRESS: Predicted residual error sum of squares, C.V. (%): Coefficient of variation

On the other hand, excessive or lower dispersion of the sample may reduce the TDF extraction yield (Cheung and Wu, 2013). It was concluded that 60 g/L solid to liquid ratio gave better results than 20 and 100 g/L. For TDF, another extraction parameter was NaOH concentration. The results showed that the extracts obtained with 1.25 M of NaOH solution gave the best results at all different solid to liquid ratios (Table 1). 1.25 M NaOH solution resulted higher yields than 0.5 M NaOH solution and this phenomenon can be related with the hydrolyzation of ester bonds between lignin and hemicellulose at higher NaOH concentrations (Chaturvedi and Verma, 2013). On the other hand, the lowest yields were determined when NaOH concentration was 2 M. Some authors reported that NaOH concentrations beyond 1.0 M reduced the DF yield (Ding et al., 2020). Similar results were obtained for the IDF and SDF of the defatted fenugreek seeds, the highest yields were at 60 g/L solid to ratio and 1.25 M NaOH concentration. The possible increment of SDF yield at 1.25 M may be explained with the higher solubility of DF due to proper hydrolyzation at this level of NaOH concentration (Chaturvedi and Verma, 2013; Ding et al., 2020).

ANOVA results for IDF, SDF and IDF of defatted fenugreek seeds are shown in Table 2. All generated quadratic models were significant ($P<0.05$) and unsignificant lack of fit values were obtained ($P>0.05$). Results showed that the linear effect of both sample:NaOH ratio and NaOH concentration was significant for the TDF yield ($P<0.05$). Moreover, quadratic effect of both independent variables was significant on IDF, SDF and TDF yields ($P<0.05$). Linear interaction of the independent variables significantly affected the IDF yield ($P<0.05$); however, that of SDF and TDF were not statistically significant ($P>0.05$).

Table 3. Physicochemical and functional properties of IDF extracted at different conditions

Run	Sample:NaOH ratio (g/L)	NaOH (M)	WRC (g/g)	OAC (g/g)	SC (mL/g)	GAC (mmol/g)	AAIC (%)
1	20	1.25	6.81(±0.02)^{f}	2.56(±0.22)^{c}	10.06(±0.19)^{c}	12.44(±0.06)^{de}	11.29(±0.44)^{de}
2	20	0.50	5.79(±0.13)^{g}	2.27(±0.01)^{d}	10.79(±0.07)^{b}	12.96(±0.18)^{bc}	11.91(±0.43)^{b}
3	100	2.00	2.13(±0.14)^{j}	1.28(±0.02)^{g}	6.09(±0.17)^{e}	7.15(±0.30)^{i}	6.74(±0.21)^{j}
4	60	2.00	3.01(±0.06)^{i}	1.56(±0.01)^{f}	6.07(±0.16)^{e}	7.35(±0.12)^{i}	7.05(±0.20)^{ij}
5	60	2.00	2.94(±0.02)^{i}	1.54(±0.03)^{f}	6.58(±0.17)^{e}	8.14(±0.06)^{h}	7.37(±0.22)^{i}
6	60	0.50	8.18(±0.02)^{d}	2.80(±0.00)^{b}	11.08(±0.23)^{b}	13.42(±0.06)^{a}	11.76(±0.22)^{bc}
7	60	1.25	8.62(±0.30)^{bc}	3.03(±0.05)^{a}	11.07(±0.13)^{b}	13.21(±0.30)^{ab}	11.13(±0.23)^{de}
8	100	1.25	4.76(±0.12)^{g}	2.01(±0.02)^{e}	9.07(±0.22)^{d}	10.71(±0.24)^{fg}	9.56(±0.12)^{h}
9	100	0.50	7.33(±0.13)^{e}	2.48(±0.04)^{c}	9.06(±0.14)^{d}	10.32(±0.06)^{g}	10.34(±0.01)^{g}
10	60	0.50	8.35(±0.10)^{cd}	2.79(±0.01)^{b}	12.08(±0.94)^{a}	13.25(±0.06)^{ab}	12.70(±0.21)^{a}
11	100	1.25	4.84(±0.21)^{h}	1.97(±0.05)^{e}	8.44(±0.33)^{d}	10.76(±0.29)^{f}	9.25(±0.22)^{h}
12	60	1.25	9.46(±0.29)^{a}	2.99(±0.04)^{a}	10.54(±0.12)^{bc}	12.70(±0.24)^{cd}	11.44(±0.14)^{bcd}
13	20	1.25	6.80(±0.07)^{f}	2.56(±0.02)^{c}	10.53(±0.29)^{bc}	12.09(±0.23)^{e}	10.82(±0.23)^{fg}
14	20	2.00	2.29(±0.10)^{j}	1.34(±0.02)^{g}	6.10(±0.16)^{e}	6.53(±0.18)^{j}	6.74(±0.22)^{j}
15	60	1.25	8.79(±0.08)^{b}	2.98(±0.07)^{a}	11.05(±0.23)^{b}	12.31(±0.06)^{de}	13.01(±0.22)^{a}
OP	52.50	1.01	9.26(±0.07)	2.99(±0.04)	10.29 (±0.25)	12.56(±0.13)	12.59(±0.50)

WRC: Water retention capacity, OAC: Oil adsorption capacity, SC: Swelling capacity, GAC: Glucose adsorption capacity, AAIC: α-amylase inhibition capacity, OP: Optimum point. $^{a\text{-}j}$ Means with uncommon superscripts within a column are significantly different ($P<0.05$).

Table 4. Physicochemical and functional properties of SDF extracted at different conditions

Run	Sample:NaOH ratio (g/L)	NaOH (M)	WRC (g/g)	OAC (g/g)	SC (mL/g)	GAC (mmol/g)	AAIC (%)
1	20	1.25	3.40(±0.30)^{b}	1.92(±0.01)^{d}	5.11(±0.20)^{cd}	6.05(±0.06)^{d}	5.17(±0.66)^{cd}
2	20	0.50	2.95(±0.18)^{cd}	1.63(±0.06)^{e}	5.58(±0.11)^{ab}	6.61(±0.23)^{bc}	5.49(±0.22)^{bcd}
3	100	2.00	1.70(±0.06)^{e}	1.03(±0.13)^{g}	2.86(±0.19)^{f}	3.36(±0.42)^{h}	2.98(±0.28)^{i}
4	60	2.00	1.99(±0.04)^{e}	1.17(±0.12)^{fg}	2.86(±0.17)^{f}	3.78(±0.18)^{g}	3.61(±0.22)^{gh}
5	60	2.00	1.95(±0.11)^{e}	1.21(±0.04)^{f}	3.09(±0.17)^{f}	3.60(±0.24)^{gh}	4.08(±0.44)^{fg}
6	60	0.50	3.81(±0.22)^{a}	2.17(±0.01)^{c}	4.83(±0.14)^{d}	6.58(±0.12)^{bc}	6.11(±0.22)^{ab}
7	60	1.25	4.03(±0.23)^{a}	2.41(±0.06)^{ab}	5.82(±0.23)^{a}	6.86(±0.06)^{ab}	6.27(±0.44)^{ab}
8	100	1.25	2.80(±0.20)^{d}	1.69(±0.02)^{e}	4.85(±0.19)^{d}	5.42(±0.30)^{e}	5.02(±0.45)^{cde}
9	100	0.50	3.26(±0.03)^{bc}	1.96(±0.05)^{d}	4.48(±0.02)^{e}	5.20(±0.12)^{ef}	5.64(±0.46)^{bcd}
10	60	0.50	3.83(±0.07)^{a}	2.31(±0.16)^{bc}	5.23(±0.03)^{bc}	7.17(±0.12)^{a}	6.74(±0.02)^{a}
11	100	1.25	2.76(±0.05)^{d}	1.68(±0.01)^{e}	4.23(±0.02)^{e}	4.92(±0.18)^{f}	4.23(±0.12) ^{efg}
12	60	1.25	4.06(±0.05)^{a}	2.52(±0.06)^{a}	5.59(±0.17)^{ab}	6.33(±0.18)^{cd}	5.80(±0.22)^{bc}
13	20	1.25	3.26(±0.03)^{bc}	1.99(±0.06)^{d}	5.09(±0.17)^{cd}	6.15(±0.06)^{d}	4.86(±0.68)^{def}
14	20	2.00	1.75(±0.09)^{e}	1.01(±0.06)^{g}	2.86(±0.19)^{f}	3.82(±0.06)^{g}	3.45(±0.84)^{gh}
15	60	1.25	4.08(±0.09)^{a}	2.44(±0.01)^{ab}	5.36(±0.18)^{bc}	6.40(±0.06)^{cd}	6.74(±0.22)^{a}
OP	52.50	1.01	4.20(±0.09)	2.44(±0.05)	5.22(±0.24)	6.70(±0.10)	6.53(±0.33)

WRC: Water retention capacity, OAC: Oil adsorption capacity, SC: Swelling capacity, GAC: Glucose adsorption capacity, AAIC: α-amylase inhibition capacity, OP: Optimum point. $^{a\text{-}h}$ Means with uncommon superscripts within a column are significantly different ($P<0.05$).

For TDF yield, a second-order polynomial model was generated and the model equation of TDF yield is given in Equation (8). The response surface graph representing the effects of the independent variables on TDF yield and the relation between experimental and estimated TDF yields are also given in Figure 2. In Figure 2(a), it was observed that 60 g/L of solid to liquid ratio and 1.25 M of NaOH concentration gave the best yield results. The experimental and predicted TDF yield values were plotted and it was concluded that values were close to each other which proved that appropriateness of the generated quadratic model (Figure 2b). The suitability of the model was also investigated with statistical results such as R^2, adjusted R^2 (adj-R^2), adequate precision, predicted residual error sum of squares (PRESS) and coefficient of variation C.V. (%) (Table 2). High R^2 value of the model and closeness of the R^2 adj-R^2 values showed the goodness of the model and it was a proof that only statistically significant terms were included in the generated model (Table 2).

$$\text{TDF} (\%) = 37.51+1.01X_1+27.83X_2-0.10X_1X_2-0.01X_1^2 -12.12X_2^2+7.48\times10^{-4}X_1^2X_2+3.58\times10^{-4}X_1X_2^2 \quad (8)$$

For the alkali extraction process of DF from defatted fenugreek seeds, a numerical optimization study was carried out to determine the optimum point in terms of sample:NaOH ratio and NaOH concentration. Nine different but relatively similar solutions were calculated by the package program having desirability values of 1.0. The optimum conditions of the extraction process were chosen as 52.50 g/L of sample:NaOH ratio and 1.01 M NaOH concentration. For these conditions, the predicted TDF yield was 78.15%, and according to the optimum point verification results (done in triplicate), the experimental TDF yield was 78.52±1.76% (10.36 g/13.19 g sample). IDF percentage was 59.36±1.08% (7.83 g/13.19 g sample) and SDF percentage was 19.16±1.58% (2.53 g/13.19 g sample) for this optimum point. There was no significant difference between experimental and predicted values of TDF yield according to the single sample t-test ($P>0.05$).

Physicochemical properties of IDF and SDF for different extraction conditions are given in Table 3 and Table 4, respectively. WRC of a DF is the ability to retain water when centrifugation application was carried out (Ma and Mu, 2016). The highest result for the WRC of IDF was obtained for the 60 g/L solid to liquid ratio and 1.25 M of NaOH concentration. On the other hand, the results were the lowest for 2 M NaOH concentration. These results indicated that 2 M of NaOH concentration might have affected the structure of IDFs and WRC of the samples were reduced (Ding et al., 2020). Similar results have been observed for the SDF of the defatted fenugreek seed samples (Table 4). OAC is another important physicochemical for DF and it can be defined as the ability of adsorbing fat (Navarro-González et al., 2011). OAC of the IDFs are varied between 1.28 and 3.03 g oil/g sample. The lowest values were observed for the 2 M NaOH concentration, which might have affected the surface characteristics of IDF (Dong et al., 2019). On the other hand, 1.25 M of NaOH solution gave the best results when 60 g/L of solid to liquid ratio was used (Table 3). Some of cellulose, lignin and hemicellulose can be removed by intense alkali treatment and more functional groups can be exposed which may enhance the oil retention of the DF samples (Zhang et al., 2020). Same results were given for the buckwheat straw IDF obtained by alkali extraction (Meng et al., 2019). Same as IDF, SDF of the samples had the lowest results for 2 M NaOH solution (Table 4). In literature, there are few studies about DF extraction from fenugreek seeds and properties of fenugreek seed DF. A study presented by Krishnakumar et al. (2012) produced SDF from fenugreek seeds and they reported that soluble fibers of fenugreek seeds were composed of linear chains of (1 → 4) linked β-d-mannopyranosyl residues to which α-d-galactopyranosyl groups are attached via (1 → 6) glycosidic linkages. Moreover, their study revealed that SDF of fenugreek seeds had 20 mL/g WRC and 3 mL/g OAC, and OAC results were consistent that of our study. SC can be defined as the ratio of the volume of DF after applying excess amount of water (later then the equilibrium state) to the actual weight of DF (Ma and Mu, 2016). It was also reported that alkaline treatment may improve the SC of DFs due to disrupting cellulose chains and forming porous DF structures (Meng et al., 2019). In this study, SC values of IDF were measured between 6.07 and 12.08 mL/g, and these values were higher than that of peas and chickpeas (Tosh and Yada, 2010). On the other hand, Raghavarao et al. (2008) obtained IDF from coconuts and they reported that SC of coconut IDF was in the range of 17 to 20 mL/g, which was relatively higher than our SC results (Table 3). These results may be associated with the extraction properties such as pH, solid to liquid ratio, and drying temperature which may lead to an increase or decrease of SC of the DF samples (López et al., 1996). Our findings also showed that both WRC and SC values had minimum values at the 2 M of NaOH concentration and high concentration of NaOH may affect adversely the surface characteristics (Ding et al., 2020).

Functional properties of IDF and SDF for different extraction conditions (Namely GAC and AAIC) are also given in Table 3 and Table 4, respectively. GAC is used to determine the impact of DFs on dietary carbohydrates as an *in vitro* index and it is one of the mechanisms of the hypoglycemic action (Chau et al., 2003; Jia et al., 2020). In this study, GAC of the IDF samples gave the peak point at 60 g/L solid to liquid ratio, 0.5 M (13.42±0.06 mmol/g) and at 60 g/L solid to liquid ratio, 1.25 M (13.21±0.30 mmol/g) and the difference between these results were statistically not significant ($P>0.05$) (Table 3). Moreover, GAC of the SDF samples was ranged between 3.07 and 7.08 mmol/g (Table 4). In literature, Begum and Deka (2019) extracted IDF from banana bracts and they reported the GAC for their IDF as 7.95±0.13 mmol/g when alkali extraction method was applied. Wang et al. (2021) extracted DF from kiwifruit with 5% NaOH solution (1.25 M) and according to their results, IDFs (2.04±0.03 mmol/g) had more GAC than SDF samples, and these results supported our results. Dong et al. (2020) reported relatively higher GAC results (162.40 mmol/g for alkali extraction technique) for their SDF obtained from coffee peel. The results of GAC for IDF and SDF found in literature showed that GAC values can vary due to the extraction method, extraction conditions and core material which was used for the extraction of DF.

Alpha-amylase is an essential enzyme for the formation of monosaccharides from oligosaccharides and suppressing α-amylase can prevent converting of blood glucose and can retard the increment of the blood glucose levels (Hua et al., 2020). Hence, it is important to provide inhibition of α-amylase to treat type 2 diabetes (Im and Yoon, 2015). In this study, both IDF and SDF of defatted fenugreek seeds had α-amylase inhibitory effect. IDF samples had higher AAIC levels than SDF samples. AAIC may be affected by functional groups (such as hydroxyl groups), glucose content and molecular weight (Jiang et al., 2021). Moreover, DF concentration, surface area, and particle size may affect the AAIC of different samples (Ma and Mu, 2016). Begum and Deka (2019) reported similar results for the IDF of defatted banana bract (AAIC value of 18.32%).

DF with different properties can be used as ingredients for the different food formulations. DF having high WRC and SC improves viscosity, and DF having high OAC may reduce fat loss during processing of foods due to binding cholesterol and bile acid (Hassan et al., 2011). In this study, WRC, OAC and SC values of the defatted fenugreek seed DFs produced at the optimum extraction conditions were higher than those of orange peel (Wang et al., 2015), millet bran (Dong et al., 2019), carrot (Chau et al., 2007) and *Nannochloropsis oceanica* (Ding et al., 2020). These results may be associated with the different extraction conditions, sample origin and surface characteristics of the samples. The high values of the physicochemical properties of defatted fenugreek seed samples can also be explained with the application of alkaline extraction technique. Because of breaking the hydrogen bonds between hemicellulose by alkaline treatment, the number of hydrophilic groups of DF might be increased and the hydration ability of the DF can be enhanced (Zhang et al., 2020). Functional properties namely GAC and AAIC of IDF and SDF obtained at the optimum conditions were the best among the extracts obtained from the given conditions of the experimental design. For the optimum extraction point, both physicochemical and functional properties of IDF were higher than that of SDF. Moreover, highest results were obtained at the optimum extraction conditions. Similar results were reported by Ding et al. (2020) and Dong et al. (2019).

Conclusion

In this study, IDF and SDF were obtained using alkali extraction method from fenugreek seeds and optimum extraction conditions ensuring the maximum TDF were determined via response surface methodology. Moreover, some physicochemical and functional properties of the IDF and SDF produced at different conditions were also determined. According to the results, high yields were obtained for both IDF and SDF after optimization of the extraction parameters. The WRC, OAC, SC, GAC and AAIC values of the DFs obtained at the optimum conditions exhibited the high quality and potential of the fenugreek seed as DF source. The present research revealed that alkali extraction method, which is cheap and easy-to-use can be applied to obtain high-quality DF from fenugreek seeds and for further studies, these DFs can be used in the different food formulations because of its potential health benefits.

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