

Fortification the gel produced from whey proteins with fibers extracted from pomegranate peels and yellow corn and studying the physico-chemical and rheological properties

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Abstract

A study was conducted on the effect of added dietary fiber on the physicochemical and rheological properties of fiber-enriched whey protein gels, and its impact on gel quality during storage. Pomegranate peel fiber and yellow corn fiber were extracted with 0.2 M citric acid and pH = 1.5 at 90°C for 90 minutes. Whey protein gels were then manufactured from whey protein concentrates to which concentrations of (0.5-1-1.5%) pomegranate peel fiber and yellow corn fiber were added. The chemical compositions of the pomegranate peel fiber and yellow corn fiber were studied, including moisture, ash, fat, protein, fiber, carbohydrates, esterification degree, and methoxyl content. The gels were then stored for a period of time, and rheological tests were conducted on the first and 21st days, including water retention capacity, whey spontaneous leaching, viscosity, and hardness.

Keywords: Insoluble Dietary Fiber - Soluble Dietary Fibers – Whey Protein Concetrate

Introduction

Dietary fiber is a type of carbohydrate polymer that cannot be broken down by endogenous digestive enzymes in the small intestine, but can be fermented, in whole or in part, by gut microbes in the large intestine (32). Dietary fiber is divided according to its solubility into soluble fiber (SDF) and insoluble fiber (IDF), which includes cellulose, hemicellulose, and lignans, while insoluble fiber includes pectin and gum (8). Milk proteins are chains of amino acids linked by peptide bonds and are characterized by their high nutritional value and important chemical, physical, and nutritional properties. Therefore, milk proteins greatly influence the quality characteristics of

the food products they contain, making them among the most important proteins used in the food industry (24). According to (9), milk proteins are classified into two main types based on their chemical composition and physical properties: caseins, which are phosphoprotein compounds that constitute about 80% of milk proteins and are found in the form of complex protein aggregates called casein particles associated with inorganic salts. The second type of milk proteins is whey proteins, which represent about 20% of milk proteins and are characterized by being globular proteins that mainly include galactalbumin, beta-lactoglobulin, immune proteins, and serum albumins. Whey proteins are characterized by their ability to form a gel,

which is produced by heating whey proteins to high temperatures, which leads to denaturation of whey proteins, opening up the globular structure, and protruding hydrophobic groups and amino acids to the surface, allowing for the formation of a gelatinous network. (30)

Materials and Methods

Kjeldahl apparatus - incinerator - coxsylvania apparatus - centrifuge - water bath - electric furnace - calcium chloride - citric acid - hexane - ethanol - whey protein concentrate obtained from the Islamic Republic of Iran at a concentration of (7%) - hydrated calcium chloride - hydrochloric acid.

Extraction method:

The extraction process was conducted by taking (20 g) of pomegranate peels and yellow corn pomace fibers and placing them in 1000 ml of distilled water and placing them on a heat source for 90 minutes at a temperature of 90°C using a magnetic stirrer. The solution was then filtered using a sieved cloth, discarding the precipitate, and taking the filtrate, adding an equal volume of 96% ethanol, and leaving it in the refrigerator at a temperature of 5°C for 60 minutes. The resulting dietary fibers were then removed and placed in glass dishes and dried at a temperature of 50°C (2). Gel preparation method:

Whey protein gels were prepared by dissolving whey protein concentrate (7%) in 100 ml of distilled water and adding 30 mM calcium chloride. This solution was heated to 90°C for 30 minutes. Different concentrations of pomegranate peel fiber (0.5-1-1.5%) and yellow corn fiber (0.5-1-1.5%) were added to the gels. After one day of storing the whey protein gels at 5°C, tests were performed for viscosity, hardness, spontaneous liquid leaching, and water retention capacity.

Chemical Tests:

Moisture Determination

To estimate the moisture content, the standard method mentioned in (4) was followed, by adding 5 g of pomegranate peels and corn pomace to a thermal oven at 105°C until a constant weight was reached, as a percentage. Moisture was calculated according to the following formula:

$$\text{Moisture \%} = (\text{post-sample drying weight} - \text{pre-sample drying weight}) / (\text{pre-sample drying weight}) \times 100$$

Ash Determination

The traditional method (mentioned in (4)) was used by adding 5 grams of pomegranate peels and corn pomace to a kiln at 550°C until a grayish-white ash was obtained. The remaining ash percentage was calculated using the following equation:

$$\text{Ash \%} = (\text{empty pellet weight} - \text{post-pellet incineration with the sample weight}) / (\text{sample weight}) \times 100$$

Fat Determination

The fat percentage was estimated according to the method (mentioned in (4)) using a Saxolite device by adding 5 grams of the sample (pomegranate peels and corn pomace) to a cellulose extraction thimble. Hexane was used in the extraction process. The extraction process took 10 minutes. This ranges between (5-6) hours. After removing and disposing of the solvent by evaporation, the fat is weighed and its percentage calculated according to the following equation: $\text{Fat \%} = (\text{fat weight}) / (\text{sample weight}) \times 100$.

Protein Determination

Nitrogen was estimated in the husks and pomace of the yellow corn in the current study according to the method used in (4) using the standard Kjeldahl method. The percentage of protein was estimated by multiplying the percentage of nitrogen in the samples by the conversion factor of 6.25.

Carbohydrate Determination

Carbohydrate content was estimated in the pomegranate peel and yellow corn pomace under study according to the method mentioned in (12), which represents the difference between the total components by the percentage of moisture, ash, fat, and protein subtracted from 100, as follows:

$$\text{Total Carbohydrate \%} = 100 (\% \text{ moisture} + \% \text{ ash} + \% \text{ fat} + \% \text{ protein})$$

Dietary Fiber Determination

Dietary fiber was determined in pomegranate peel powder and yellow corn pomace powder according to the standard methods (4). This was done by boiling 1.5 g of the powder in a fiber determination vessel with 100 ml of 1.25% dilute sulfuric acid over a heat source for 30 minutes. The precipitate was then filtered through a fine cloth. The precipitate was taken and 100 ml of 1.25% dilute NaOH was added to it. The precipitate was then boiled for 30 minutes, after which it was filtered through a fine cloth. The remaining sample was dried in a thermal oven at 105°C until the weight was constant. The pellets were then placed in an incinerator at 550-600°C for 60 minutes. The pellets were then cooled, and the fiber content was calculated using the following equation:

$$\text{Fiber \%} = (\text{sample weight after drying} - \text{sample weight after incineration} / \text{original sample weight}) \times 100$$

Degree of Esterification

The degree of esterification was determined according to Method 17. 100 mg of fiber extracted from pomegranate peels and yellow corn pomace was moistened with 2 ml of 99% ethanol and dissolved in 20 ml of distilled

water at 40°C while stirring. 5 drops of phenonnaphthalene reagent were then added, and the solution was titrated with 0.1 N sodium hydroxide. The initial volume was recorded. 10 ml of 0.1 N sodium hydroxide was then added while stirring, and the sample was left to stand for 20 minutes. 10 ml of 0.1 N hydrochloric acid was then added while stirring vigorously until the pink color disappeared. 5 drops of phenonnaphthalene were then added again, and the solution was titrated with 0.1 N sodium hydroxide until a pale pink color appeared. The final volume was recorded, and the degree of esterification was calculated using the equation:

$$\text{Degree\%} = \frac{\text{Esterification (sodium hydroxide of final volume)}}{(\text{Final Volume} + \text{Initial Volume})} \times 100$$

Methoxyl content

Estimated according to Method 19 using the neutral solution obtained during the equivalent weight determination process. 25 ml of 0.25 N sodium hydroxide was immediately added to it. The contents of the container were mixed well and left for 30 minutes at laboratory temperature before adding 25 ml of 0.1 N hydrochloric acid. Then, titrate with 0.1 N sodium hydroxide while stirring until a pH of 7.5 was reached. The final volume was recorded, and the degree of methoxylation was calculated using the following equation:

$$\text{methoxyl degree\%} = \frac{\text{sodium hydroxide molarity} \times \text{sodium hydroxide volume} \times 31}{\text{sample weight g}} \times 100$$

Molecular weight of the methoxyl group
OCH₃ = 31

Table (1) Chemical composition of pomegranate peel fibers and yellow corn fibers

Properties\Composition%	corn fiber	Pomegranate peel fiber
Ash%	4.80	3.5
Moisture%	8.25	8.40
Fat%%	1.40	1.33
Protein%	3.20	5.10
Fiber%	9.40	10.40
Carbohydrate%	82.35	81.67
Degree of Esterification%	29.13	27.10
Methoxyl content%	4.10	6.16

The ash percentage reached (4.32%), while (27) concluded that the ash percentage in pomegranate peels reached 3.30%, while the results of our study were less than what (21) found, which reached 5.49% when they studied pomegranate peels and fibers. As for the moisture percentage, it reached (7.27%), and it was close to the results of (25) when he studied pomegranate peels, where it reached 7.27%. (1) showed that the moisture percentage was 11.9%. While (14) concluded that the moisture percentage was 4.75%. The fat percentage reached (0.85%), while the results of our study were less than what (16) reached, which was 4.66%. The percentage of protein in pomegranate peels reached 3.74%, while (18) reached the percentage of protein in pomegranate peels reached 3.26%, while (14) stated that the percentage of protein was 3.06%. The percentage of fiber reached 17.31%, while (1) reached the percentage of fiber was 10.40%. As for the percentage of carbohydrates, it reached 66.51%, while (27) reached, when studying carbohydrates, it reached 80.73%. The percentage of esterification in pomegranate peels reached 29.13%, while (6) reached the degree of esterification of lemon pomace fibers at 70.39%. The methoxyl content reached 4.10%. The results of this study are slightly higher than those found by (28), who reported that the methoxyl content in apple fibers was 3.7%. (2) showed that the methoxyl content extracted from apple fibers ranged between 4.41-7.21%. When comparing the methoxyl content extracted from pomegranate fibers in this study, it is close to the methoxyl content in other fruit peels. (11) found that the methoxyl content in banana peel fibers was 6.09%, and

the methoxyl content in mango peel fibers was 8.89%. The results show differences between the findings of this study and those reported in previous studies. This is due to factors such as the type of acid used in the extraction, the variety and type of extracted material, as well as the type of fruit, the influence of shipping and storage periods, storage and display conditions, and other factors that affect the nature and properties of the fibers. The ash content in yellow corn fibers was 3.5%, while the moisture content was 8.40%, which was close to what (2) found in his study on apple fibers, which was (7.4-8.4%). The fat content in corn fibers was 1.33%, which is less than what (31) found, which was 3.8%. The protein content in corn fibers was 5.10%, while (22) found that the protein content in banana fibers was 3.2%. (26) found in their study of banana peels that the protein content was 3.4%. The fiber content was 10.40%, while (1) indicated that the fiber content was 12.1%. The carbohydrate content was 81.67%. It was found that the degree of esterification was 27.10%, while (28) indicated that the degree of esterification of apple peel fibers reached 33.44%. As for the methoxyl content in yellow corn, it reached 6.16%, while (23) indicated that the methoxyl content in dragon fruit fibers ranged between 2.98-4.34%, while (2) concluded that the methoxyl content in apple fibers was 4.41%.

Rheological Tests for Whey Protein Gels

1- Water Holding Capacity

The water holding capacity (WHC) of whey gel samples was estimated using the (15)

method. 10 grams of each sample was weighed and placed in a test tube, then centrifuged at 5,000 rpm for 10 minutes. The upper whey layer was then removed and carefully weighed to determine the amount of water removed. The water holding capacity (WHC) was estimated using the following formula:

$$\text{WHC}\% = [1 - (w_2 / w_1)] \times 100$$

Where:

W1: Weight of gel used

W2: Weight of whey after centrifugation

2- Spontaneous Whey Separation

The spontaneous Whey Separation was estimated by placing 50 ml of whey gel in a beaker at a 45° angle for two hours at 5°C. The evaporated whey was then withdrawn. From the surface using a syringe, the cup was then reweighed and the process was carried out for 10 seconds to avoid over-extraction, as stated by (3).

3- Hardness Estimation:

A texture analyzer (CT3, 4500 Brookfield Engineering Lab) was used to measure the

hardness, elasticity, and cohesion of whey gel samples. It was equipped with a 5 kg load cell, as stated by (20). The sample was subjected to a compressive force using a bulb from a distance of 30 mm from the top. The device's measurement conditions were set as follows: initial test speed 1 mm/s, final test speed 1 mm/s, probe trigger force 10.0 g, time used for each test 5 seconds, and sample size (3 x 3 x 2.5) cm³.

4- Viscosity Estimation:

The viscosity value of the gel samples was estimated at 5°C after 21 days of storage. The coolant was measured using a Brookfield DVII+ viscometer produced by Brookfield Engineering Lab Inc., Stoughton, Mass., according to the method mentioned in (10). The whey gel was broken by moving it 10 times clockwise and 10 times counterclockwise, using spindle No. 4 at 10 rpm and 150 ml of sample volume. The spindle was left to rotate inside the sample for 60 seconds, and the reading was taken three times, according to the average in centipoise units

Table (2) Rheological tests of whey gel after the first day of storage

Traits	Control sample	Gel containing pomegranate peel %fibers 0.5	Gel containing pomegranate peel %1 fibers	Gel containing pomegranate peel fiber 1.5%	%Jelly containing corn fiber 0.5	Jelly containing 1% corn fiber	%Jelly containing corn fibers 1.5	L.S.D
Spontaneous whey separation (ml)	1.12	1	1.8	1.9	0.1	1	1.16	*0.541
Water holding	45	45	46.76	47	45	46.33	47.33	3.07

capacity (%)								
Viscosity (centipoise)	1319	1512	1615	1725	1560	1611	1620	*86.59
Hardness (g)	120	100.30	120	134.5	127	130	160	*16.73
.(P≤0.05) *								

From the table, the results of the pomegranate peel fiber gel and corn pulp fiber gel tests after the first day of storage appear, where the whey leaching rate in the control gel sample was 1.12 ml, while (29) concluded that the whey leaching rate for the control treatments was 0.4 ml. While the treatments to which pomegranate fiber was added (0.5 -1 - 1.5) were (1 - 1.8 -1.9) ml, and when adding corn fiber at concentrations of (0.5 -1 -1.5)%, it was (0.1 -1 -1.16) ml, while (2) concluded that when he added the fiber ratio (0.1 - 0.3), the leaching rate was 3.18 and 2.66 ml, where there were significant differences (0.541*) compared to the control treatment. As for the water retention capacity of the control treatment of the gel, it was 45%, which was close to what was reached (29), where it was 41%, but when adding a percentage of pomegranate fibers at different concentrations, the water retention percentage was 47%, 46.76%, and 45.40%, and when adding a percentage of corn fibers at different concentrations, the retention percentage was 47%, 46.33, and 45%, where we notice that when the percentage of added fibers increases, the water retention percentage increases. As for viscosity, it was 1319 centipoise in the

control treatment of the gel, as this percentage was close to what (2) reached, as it reached (1307) centipoise when studying the control treatments for yogurt, while the viscosity percentage for pomegranate concentrations was 1725 - 1615 - 1512 centipoise, and the viscosity for corn fiber was 1620 - 1611 - 1560 centipoise. (5) indicated that when fibers are added at higher ratios, the viscosity of the product increases. The fiber concentration and the type of peels had an effect on the viscosity values, as we notice an increase in viscosity at a concentration of 1.5% for all treatments, as there were significant differences of 86.59*. As for the tissue, it was in the control treatment of the gel 120 g, while (29) concluded that the tissue of the control gel was 371 g when heated at a temperature of 90, but when adding a percentage of pomegranate fibers to the gel at concentrations of (0.5 -1 - 1.5), the tissue was 134.5 - 100.30 -120 g, as it was shown that when the percentage of fibers increased, the tissue percentage increased, and also when adding corn fibers, it was 160 - 130 -127 gm. The tissue percentage also increased, and there were significant differences of 16.73* compared to the control treatment.

Rheological tests of whey protein gels after 21 days of storage

Traits	Control sample	Gel containing pomegranate peel fibers 0.5%	Gel containing pomegranate peel fibers 1%	Gel containing pomegranate peel fiber 1.5%	Jelly containing g corn fiber 0.5%	Jelly containing g 1% corn fiber	Jelly containing corn fibers 1.5%	L.S.D.
Spontaneous whey separation (ml)	8.12	3.96	3.35	3.20	3.82	3.22	3.12	* 2.077
Water holding capacity %	49	55.96	54.2	52.6	55.96	54.74	52.82	* 4.69
Viscosity (centipoise)	1737	4250	3877	2894	4820	4550	3240	* 174.56

Hardness (g)	135.18	156.41	154.45	150	165.22	153.27	151.13	* 13.48
(*P≤0.05)								

From the table it is clear that the spontaneous whey leaching for the control model reached (8.12) ml, while (7) concluded that the amount of leached whey decreased with the advancement of storage. It is also noted that the leaching rate of the fiber treatment with yogurt decreased from 55.8 ml on the first day to 53.3 ml. The leaching rate for pomegranate peel fiber gel with pomegranate peels added at concentrations of (0.5 - 1 - 1.5%) was (3.20 - 3.35 - 3.96) ml, respectively, which was close to what was reached by (2). As for the corn fiber gel added at different concentrations, the rates showed (3.82 - 3.22 - 3.12) ml, where we note that the less the fiber was added, the less and the leaching rate increased with the advancement of the storage period, and there were significant differences of 2.077* compared to the control treatment. The table shows the water retention rate, with the control gel being 49%, while the pomegranate peel fiber gel with different concentrations (0.5, 1, 1.5%) was (55.69, 54.2, 52.66)%, and the corn pomace fiber gel with yellow corn pomace fiber concentrations (0.5, 1, 1.5%) was (55.96, 54.74, 52.82)%, which is consistent with what was found by (13). We also note the presence of significant differences of 4.69* compared to the control treatment. From the table, the effect of treatments on viscosity was shown, as it reached 1737 centipoise for the control gel, and it reached 4250 - 3877 - 2894 centipoise for the pomegranate peel gel with added concentrations of (0.5 - 1 - 1.5%), and it reached (4820 - 4550 - 3240) centipoise for the yellow corn pulp gel with added concentrations of (0.5 - 1 - 1.5%), where we notice that when adding fibers at a low rate, the viscosity rate increased, while (5) who indicated that when adding fibers at low rates, the viscosity increased, and there were no significant differences 174.56 *. The hardness value shown in the table for the control gel was 135.18 g, while the pomegranate peel fiber gel with concentrations (0.5 - 1 - 1.5%) was 156.41 - 154.45 - 150 g, and the yellow corn pulp fiber gel was (156.22 - 153.27 - 151.13 g). (29) concluded that the tissue percentage was 262.9 g, as the hardness value

increased for the gel with lower concentrations, and there were significant differences of 13.48.*

Conclusions

-1The possibility of extracting dietary fiber from pomegranate peels and yellow corn pulp at pH = 1.5 using the alcohol extraction method and citric acid at a temperature of 90°C for 90 minutes. 2- The addition of fiber improved the rheological properties of the manufactured whey gel, improving viscosity and texture, reducing whey leaching, and increasing the gel's water-holding capacity.

3- The possibility of producing flavored, sweetened, and fiber-enriched whey protein gels using 7% whey protein concentrate.

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