



## Original Article

# Evaluating the Gelling Properties of Red Kidney Beans Protein Isolates with Different Gums

Nighat Raza<sup>1</sup>, Adeel Hakim<sup>1</sup>, Muhammad Shahbaz<sup>1</sup> and Mujahid Farid<sup>2</sup>

<sup>1</sup>Department of Food Science and Technology, MNS-University of Agriculture, Multan, Pakistan

<sup>2</sup>Department of Environmental Science, University of Gujrat, Gujrat, Pakistan

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### \*Corresponding Author:

Nighat Raz  
Department of Food Science and Technology,  
MNS-University of Agriculture, Multan, Pakistan  
[nighat.raza@mnsuam.edu.pk](mailto:nighat.raza@mnsuam.edu.pk)

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## ABSTRACT

Due to the higher consumption, increased demand of animal based hydrocolloids and problems associated with animal based hydrocolloids are religious beliefs and mad cow disease, researchers are looking for alternative sources of hydrocolloids like marine and plant based hydrocolloids. **Objective:** To evaluate the gelling properties of red kidney beans protein isolates with different gums. **Methods:** The gelling powder developed with red kidney bean protein (KPI)-carrageenan (CG) and protein-xanthan (XG) gum with six different concentrations. **Results:** Added protein increased the plasticity of the gel and showed a higher blooms strength and hardness in all treatments except T<sub>1</sub>. KPI-CG gel had bloom strength values 198.67 ±1.53g, 249.67 ±1.53g and 282.33 ±1.56g and respectively KPI-XG gel bloom strength values were 170.33 ±1.6g, 232.67 ±2.08g and 256.67 ±2.52g; while hardness of KPI-CG gel shows 23.5 ±0.5N, 37 ±1N, 42.33 ±1.54N and 22 ±1N, 34 ±1N, 40 ±1N of KPI-XG gel respectively. The lower G'' values than G' indicate that there is gelling ability in all the concentrations. Added carrageenan-protein gelling agent with maximum gum concentration showed the highest gel strength of 1629.99 ±16.12 pa which is double the amount of KPI-XG gel elasticity 878.043 ±8.08 pa. **Conclusions:** These results indicate that the KPI-CG mixed gel has a better gelling strength. The outcomes of this work will be used to provide the groundwork for developing a novel designed plant protein-based gel system and the use of gel in yoghurt, which might increase functionality over protein or gums alone and replace the animal-based gelling component.

## INTRODUCTION

Proteins derived from animal sources like gelatin, ovalbumin, casein and whey, now dominate the protein ingredient sector. However, the replacement with plant derived materials is increased in food industry as customers feel fear of spreading diseases from animal source (e.g. prion), dietary preferences and food choices based on moral, religious beliefs (Muslim and the Jewish communities represent nearly 23 percent of global population) and some traditional restrictions that prohibits the consumption of products derived from animals [1, 2]. Increasing trend of vegetarianism in developed nations and growing demand for food products without soy and wheat protein, red kidney beans (*Phaseolus vulgaris* L.) are

attracting more attention for the development of nutritious foods [3]. Moreover, foods prepared with soy as ingredients attracted the consumers concern due various allergies and off-flavorings. Possible potential substitutes for protein can include oilseed crops and various legumes but they are poor in functional characteristic than the current available proteins. Researchers are trying to improve the functional properties of plant base proteins from many years through enzymes and chemicals means with little achievements [4]. Red beans are known with different names like common bean, "Lal lobia" and "rajma" Haricot bean in English, Haricot commun in French. The red kidney bean, scientifically known as *Phaseolus vulgaris* L., is an excellent



source of a variety of nutrients, including proteins, carbs, minerals, and vitamins [5]. These are well-known for having exceptionally high levels of dietary fiber, minerals, and protein respectively. They are also good sources of several vitamins (riboflavin, thiamine, folic acid, niacin and vitamin B6) and minerals Mg, K, Zn, Cu, Fe, P, and Ca [6]. Non-nutritional components, such as antioxidants and phenolics, are also abundant in kidney beans [7-9]. A protein extract of red kidney beans has been found to gel better than those from mung or other beans because of the unique vicilin feature [10]. This bean's principal storage protein, vicilin (also known as phaseolin), has a unique mechanical characteristic, e.g. minimum vulnerable to trypsin digestion, and also higher subunit uniformity, in contrast to other vicilin constituents [10]. An oligomeric protein with molecular weights ranging from 43 to 53 kDa, it contains 2 or 3 polypeptide subunits, based on the generic type [11]. In contrast to soy bean protein, the protein composition of *Phaseolus* family legumes are more homogeneous (having major vicilin content about 83 to 86 percent, compared to the collective legumin and vicilin), therefore these protein isolates can be easily processed to act as functional components. The gelling ability of the protein isolate from kidney bean was found to be the best of the three *Phaseolus* legume protein isolates evaluated [10]. Depending on the protein-polysaccharide ratio, several effects can be observed. There are two possibilities for how gum content affects the final product. As the amount of gum added increases, the thermodynamic incompatibility between proteins and gums tends to increase because the uncertainty of the structure occurs when the quantity of water is deficient, leading to struggle for hydration particles, which in turn leads to higher interaction among the biopolymers and the resulting repulsive interactions [12]. Due to the nature and density of their charge, if gums are compatible with the protein, compatibility tends to increase as the gum content increases. A depletion process occurs when these big hydrophilic macromolecules begin to combine, which causes gum to flocculate. This behavior, however, is only seen up to a particular concentration [13, 14]. As a result, it is advantageous to cross-link the biopolymers within the particles in order to enhance their stability. It's possible to crosslink biopolymers using either chemical or enzymatic methods, depending on the unique properties of the biopolymers involved [15, 16]. Therefore, this study was conducted to evaluate gelling properties of red kidney beans.

## METHODS

Red kidney beans were purchased from the local grain market of Multan. The required chemicals to be used in this study was of Merck and Sigma-Aldrich were procured from

a local scientific store of Multan. Analysis of red kidney beans samples for crude protein, moisture, crude fat, crude fiber and ash were done conferring to the protocols mentioned in AACC (2000). The moisture content of red kidney bean sample was analyzed using a hot air oven according to the standard procedure of AACC (2000). A clean petri-dish already dried at 98 °C for 60 minutes contained about 2 to 10 grams of sample. A hot air oven at 100°C was used to dry the sample for 2 to 3 hours. The % of moisture in the sample was determined by comparing the starting weight of the sample to the final weight after drying (final weight).

$$\text{Moisture \%} = \frac{\text{Initial weight of sample} - \text{final weight of dried sample}}{\text{initial weight of sample}} \times 100$$

Crude protein value of the malted and un-malted flour was calculated according to (Method no. 46-30) prescribed in AACC (2000). In this method kjeldahl's apparatus was used to calculate the crude protein content. Two to three gram of sample was taken in the digestion tube that was placed in distillation unit for 3 to 4 hours with 1 digestion tablet and 30ml of sulphuric acid until the yellowish or transparent color will appear. After distillation, the sample was cool down and placed in the volumetric flask (500 ml) and mark up to 250ml by using distilled water. Took 10ml solution from the sample which was diluted and place it in the digestion flask and pour 15 ml of 40% solution of sodium hydroxide in it. Ammonia started releasing from the sample and comes in the flask with 4% of boric acid solution while methyl red was used as an indicator. Moreover, the resultant solution was then titrated against the 0.1 N sulphuric acid. Protein calculation was done by using the following formula:

$$\text{Nitrogen \%} = \frac{\text{Volume of 0.10N H}_2\text{SO}_4 \text{ used} \times 0.0014 \times \text{volume of dilution (250)}}{\text{Weight of sample} \times 10} \times 100$$

Crude protein value of the malted and un-malted flour was calculated according to (Method no. 46-30) prescribed in AACC (2000). In this method kjeldahl's apparatus was used to calculate the crude protein content. Two to three gram of sample was taken in the digestion tube that was placed in distillation unit for 3 to 4 hours with 1 digestion tablet and 30ml of sulphuric acid until the yellowish or transparent color will appear. After distillation, the sample was cool down and placed in the volumetric flask (500 ml) and mark up to 250ml by using distilled water. Took 10ml solution from the sample which was diluted and place it in the digestion flask and pour 15 ml of 40% solution of sodium hydroxide in it. Ammonia started releasing from the sample and comes in the flask with 4% of boric acid solution while methyl red was used as an indicator. Moreover, the resultant solution was then titrated against the 0.1 N sulphuric acid. Protein calculation was done by using the following formula:

Protein % = Nitrogen percentage  $\times$  6.25

Determination of fat content of the sample was done according to the method No (30-25) of AACC (2000). In this method solvent extraction method was applied to calculate the total amount of fat and n-hexane was used as a solvent extraction. Took 5g sample (Pre-dried) and warped in a filter paper to make thimble and the weight the thimble. The thimble was then placed in the soxhlet apparatus. 250 ml of petroleum ether was added in the receiving flask. Set the rate 1 to 2 drops after condensation. The fat content that present in the sample were removed and then placed the sample in the dried oven for 10 to 15 minutes at 65 to 75°C and then weigh the sample. Fat was calculated by using the formula:

$$\text{Crude Fat \%} = \frac{\text{weight of sample} + \text{Thimble} - (\text{Weight of dried fat free sample})}{\text{Weight of sample}} \times 100$$

Method No (08-01) of AACC (2000) was used to estimate the ash % in red kidney beans. In this method sample were placed in the crucible for ashing and burned by using hot flame. After charring the sample, it was employed in the muffle furnace at a temp about 550°C for a time duration of 5 hours until ash of grey color appeared. At the end the sample was placed in the desiccator and allowed them to cool. By using the formula, we can determine the ash content.

$$\text{Crude Ash \%} = \frac{\text{final weight}}{\text{Weight of sample}} \times 100$$

Fiber content was calculated according to the method of (Method no. 32-10) of AACC (2000). 1.25% concentrated sulphuric acid was used to boil the 5g of red kidney beans sample for about 25 to 30min. The boiled sample was than filtered and washed two to three times to remove to acid content present in the sample. The residue sample was again boiled with 200 ml of sodium hydroxide (1.25) % for 25- 30 minutes. Filter the sample and washed with distilled water. Place the sample in hot air oven at 100°C for about 24 hours. Then the sample was burned in muffle furnace for 5 hours at 550°C. After that, sample was weighed. Fiber content was determined by using this formula:

$$\text{Fiber \%} = \frac{\text{Weight of oven dried sample (g)} - \text{Weight of sample after ashing(g)}}{\text{Weight of sample (g)}} \times 100$$

Nitrogen Free Extract (NFE) value of sample was obtained by subtracting the proximate analysis of flour from 100. NFE was calculated according to the following expression: NFE = 100 - (% Crude Protein + % Crude Fiber + % Crude Fat + % Ash) Anti-nutrients were inactivated in red kidney beans according to the method adopted by Shimelis and Rakshit. Red kidney beans were sifted to remove the broken beans, dust and other foreign particles. Soaking and cooking were the processing methods utilized to remove the anti-nutritional elements. Further the samples were freeze dried, grinded and sieved [17]. Red kidney bean sample of

500 (g) were dipped in water at ambient temperature for 12 hours for hydration, water having a pH of 6.9 and 0.05%, solution of sodium bicarbonate having a pH of 8.2. The solution to seed ratio was 3:1 (w/v). The extra unabsorbed water was removed, and rinsing of seeds was done twice with distill water [17]. After 12 hour of hydrations red kidney bean seeds were washed with distill water and then boiled/cooked (double the amount of water than the weight of soaked red kidney beans). The cooking of hydrated red kidney beans was done at 97°C. After boiling the extra water was removed and red beans were washed with distill water twice. Further, freeze drying was done using a bench top freeze drier at 60 °C under a pressure of vacuum 0.03 bar. The freeze dried red kidney bean samples were than grinded using 60mesh size and until further use it is stored in air tight jar at temp 4°C [17]. Red kidney bean protein isolates (KPI) were extracted using the process of Kusumah et al., (2020) with little modifications. The freeze dried and grinded red kidney bean powder is defatted by solvent extraction method by with n-hexane. The sample was wrapped in the thimble and placed in the soxhlet apparatus and set 1 to 2 drop after condensation. Repeated washings were done for complete removal of fat from the samples. After complete washing of fat, the sample were removed from the soxhlet apparatus and oven dried for 10 to 15 minutes at 65 to 75°C for the complete removal of n-hexane. The red kidney bean protein isolation was done in alkaline environment and continues settlement process of protein at isoelectric point. The 100g red kidney bean defatted flour solution was made with distill water in a ratio 1:10. Then stirred about 10mins and checked for pH. Then the solution pH was regulated to 9 by careful addition of 0.5N NaOH solution with a dropper and continuous stirring was done for 20min. Then the solution was loaded in the centrifuge and first centrifugation was to extract the supernatant. The first centrifugation was done at 4500rpm at 10°C for 30min. The supernatant was free from all the excess materials like carbohydrates and fiber and concentrated with protein [18]. To separate the protein from the supernatant second centrifugation was done but before that pH was calibrated to 4.5 by adding HCl of 1N to reach the isoelectric pH. The supernatant was then centrifuged at 4500rpm at 4°C for 30min. The sludge stucked to the bottom of the falcon tube is protein free from all the other impurities. The obtained protein was then freeze dried for 2 hours at 50°C. The wet protein was then converted in to powdered form [18]. The protein isolates were then stored in air tight container for further use. Hua et al., (2003) method was used to prepare the KPI and gums gel according to the treatment plan shown in Table 1.

**Table 1:** Treatment plan for gel preparation

Treatments	Protein isolates (%)	Carrageenan gum (%)	Xanthan gum (%)
T0	0	50	50
T1	75	25	0
T2	50	50	0
T3	25	75	0
T4	75	0	25
T5	50	0	50
T6	25	0	75

Lopes-da-Silva and Monteiro, (2019) methodology was used to measure the rheology and viscosity of gels. Gelation was examined utilizing a controlled-stress rheometer and oscillatory rheological experiments performing small deformation amplitudes with a plate to plate geometry (20 mm, gap 1mm). The operating conditions of instrument for oscillation frequency are, 1HZ and a strain of 0.3%. Solutions were directly transferred on to the plate at 25°C (room temperature). Heating of samples for 5min at 90°C and then again cooled to 25 °C and kept at this temp for 20 min. The rise and fall of temperature was done at a frequency of 1°C/min. Gel samples were covered with castor oil to avoid the evaporation at high temperature while measurement. To fit the size of the geometries, the samples were sliced with a sharp knife; a spatula was used to shift the samples of weak gel on the plate. The sample was carefully examined to confirm that the gel was free of air bubbles. All samples were taken in triplicates and results mentioned are mean of the replicates [19]. Gel strength was determined by the bloom strength by using the method of Hafidz *et al.*, and Sarbon *et al.*, (2013). The different samples of gel were prepared and poured in the bloom jar and the jar was covered. Gel in the bloom jar was placed in the refrigerator to cool at 10 °C overnight about (16 to 18 hour) to mature and stabilize the gel for further testing. Texture analyzer was used with standard cylinder of 1.25mm diameter. The bloom jar was placed underneath the plunger in the center and when the power was applied and the plunger penetrated 4mm deep in to the gel. The maximum force (the resistance to penetration) applied to penetrate the cylinder in the gel was noted as the Bloom Strength (g) of the gel. All the samples were analyzed in triplicate and readings were note [20, 21]. All the samples were prepared in triplicates and analysis of variance (ANOVA) two-way factorial design was applied for the determination and evaluation of results.

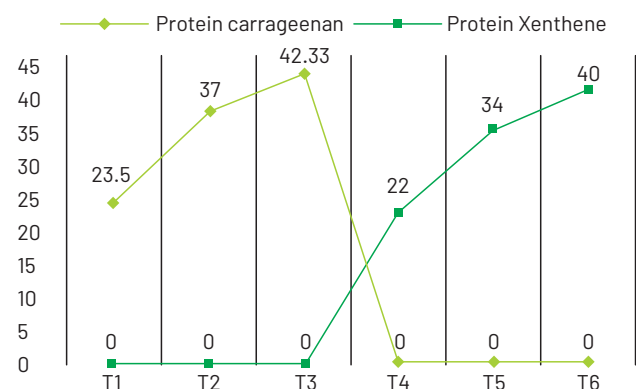
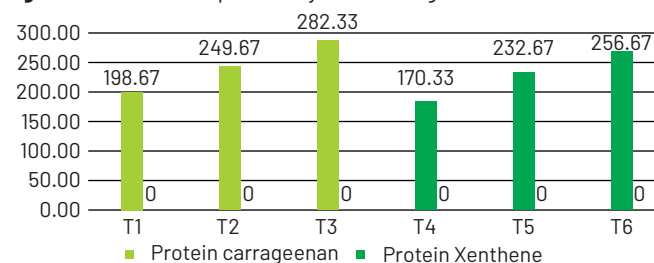
## RESULTS

The proximate compositions of red kidney beans are shown in Table 2. The crude protein, ash, moisture, crude fiber, crude fat and carbohydrate quantity were described in % of dry weight.

**Table 2:** Proximate analysis of red kidney beans

Chemical Parameter	Mean ± SD
Crude Fat%	2.27 ± 0.15
Ash	4.03 ± 0.10
Crude Fiber%	4.1 ± 0.11
Moisture Content %	10.03 ± 0.15
Crude Protein %	25.21 ± 0.20
NFE %	64.22 ± 0.24

The gelation characteristics of KPI and hydrocolloids were evaluated as a characteristic of concentration of CG and XG in Figure 1 and 2. The bloom strength and hardness two parameters are evaluated; a clear increase can be observed in gel hardness as the hydrocolloid concentration increase. The KPI- CG gel shows a higher gel hardness than KP-XG gel at all concentrations. Same trends can be observed in the bloom strength of KPI-CG and KPI-XG gel. At minimum concentration KPI and CG gel shows a hardness of 23.5±0.5 (N) and with the increase in gum concentration hardness shows a clear increasing trend. The protein-XG gel also shows a growing trend with the increase in gum concentration and shows a maximum hardness of 40 ±1 (N) at maximum concentration, which is lower than the KPI-CG gels maximum hardness 42.33±1.54 (N).

**Figure 1:** Hardness of protein hydrocolloid gel**Figure 2:** Bloom strength protein hydrocolloid gel

The KPI-CG and KPI-XG samples were examined through frequency sweep tests and results are shown in Table 3. All samples, showed a gel-like structure. The G' elastic modules was higher than the G'' which indicates the proper gelling in all the formulations. The KPI-CG G' was higher than the KPI-XG formulations. At lower concentration of

KPI-CG the storage modulus were low as well as in the case of KPI-XG gel.

**Table 3:** Rheological properties ( $G'$ ,  $G''$ ) of red kidney bean protein-carrageenan and protein-xanthene gum mixtures under varied conditions

Treatment	T gel (°C)	$G'$ pa	$G''$ pa
T0	45.20 ±1.06	910.67 ±5.62	135.03 ±2.04
T1	38.83 ±1.04	133.69 ±6.16	137.75 ±7.20
T2	44.17 ±1.26	1647.2 ±16.12	234.96 ±6.47
T3	45.43 ±1.01	1629.99 ±13.54	182.95 ±6.07
T4	73.5 ±0.5	584.17 ±10.39	132.09 ±8.60
T5	71 ±1	1483.79 ±11.41	208.75 ±6.74
T6	74 ±1	878.043 ±8.08	182.46 ±6.95

## DISCUSSION

Total moisture content of raw sample of red kidney beans was found to be 10.3±0.15%. In other studies, moisture content of raw sample of red kidney beans were found to be in a range of 10.12% which is quite similar to the moisture content assessed in this study [22]. Results of some other studies show similarly moisture content of kidney beans 13.30 per 100 g [23]. Ash content in raw sample of red kidney beans was 4.03±0.10 % respectively. Rui et al., reported the similar ash content ranged from 4.25% to 5.09% which can be correlated with present findings [24]. Whole red kidney beans had 3.57 % ash [18]. Red kidney beans had lower fat content 2.27±0.15 % which is slightly higher than the 1.97% previously reported by Kusumah et al., [18] and lower than the 3.84 % reported by Moreno et al., [25]. Fat content of the kidney bean is small making these beans a foodstuff with positive nutritional implications. Fiber content of red kidney beans was 4.1±0.11%. Almost similar results of fiber content 4.6% can be seen in literature [26]. A wide range of protein content in the red kidney bean has been reported in the literature like 28.31% which is more than the observed results by Roy et al., [27] and 22.36 % less than the obtained values by Rui et al., [24]. 24.25% protein was reported by Olanipekun et al., [28]. Amount of ash, fat, protein, moisture, and fiber is normally subtracted from 100 to determine nitrogen-free extraction. In current situation the NFE readings were 64.22±0.24% in raw red kidney bean. These results have similarity with the results of Rui et al., [24]. Previous studies have reported the use of polysaccharides such as gellan gum to increase the gel strength and functional properties of animal protein [29]. Bloom strength results shows an increase in the gelling strength of KPI-CG gel with the highest value 282.33±1.56 (g) and the same concentration of KPI-XG exhibits a value of 256.67±2.52 (g) which is much lower than the former one. KPI-CG gel seemed more firm, while KPI-XG gel had moderate hardness. Similar results were observed when fish gelation-xanthene and fish gelatin-gum arabica gel

hardness was checked gelatin xanthene gum gel showed the less hardness and bloom strength [30]. Studies showed that kappa-carrageenan and other hydrocolloids improved the gel strength and texture. Results showed that composites protein polysaccharide gel increase the gelation up to certain concentration and shows minimum syneresis [31]. While the  $G'$  follows an increasing trend with KPI-CG and KPI-XG concentration up to a certain limit. Researchers found greater  $G'$  values by increasing the concentration of polysaccharide [32]. The  $G''$  modules also flows the same trend in viscoelastic behavior. The Gelling temp also increase with concentration of CG and XG. Similar results were reported in different studies that shows the gelling temp become high when protein and kappa-carrageenan concentration increased in the gel [33]. Literature shows that whey protein and xanthan exhibit similar condition of gelling with the increase in gum concentration [34]. Protein-carrageenan and protein-xanthene shows a maximum  $G'$  and  $G''$  value respectively at when the protein concentration is medium and gums molecular weight is not high. Comparably, it is evaluated that gelatin and CG shows that the higher the amount of CG used, the gel's  $G'$  was enhanced because electrostatic interactions between the two biopolymers led to the creation of junction zones [35]. When the CG and XG amount cross the certain limit, the  $G'$  and  $G''$  show a reverse phenomenon and  $G'$  decrease up to certain limit. The  $G'$  and  $G''$  of KPI-XG gel is much lower than the KPI-CG gel. Similar results suggest that gum concentration effect the preparation of protein-polysaccharide gels. In this situation it is observed that high amount of XG in egg white gel makes it less coarse [36]. Despite these findings, a zeta potential analysis demonstrated that at greater gum concentrations, the gel formed a sheet-like microstructure with less protein-gum interactions. As the gum content increased, the density of the network at gelatin-tara gum increased, becoming coarser, indicating an excessive weighting of polysaccharide in the gel [37].

## CONCLUSIONS

Gel formation via regulated protein-polysaccharide interactions may provide a method for increasing their function as components without requiring enzymatic or chemical treatment. The outcomes of this work will be used to provide the groundwork for developing a novel designed plant protein-based gel system and the use of gel in yoghurt, which might increase functionality over protein or gums alone and replace the animal-based gelling component.

## Authors Contribution

Conceptualization: NR, AH

Methodology: AH, MS

Formal Analysis: MF

Writing-review and editing: NR

All authors have read and agreed to the published version of the manuscript.

### Conflicts of Interest

The authors declare no conflict of interest.

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