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EXPLORING THE POTENTIAL OF RED KIDNEY BEANS (*PHASEOLUS VULGARIS* L.) TO DEVELOP PROTEIN BASED PRODUCT FOR FOOD APPLICATIONS

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ABSTRACT

Protein isolate was prepared from red kidney beans and its functional properties were evaluated at different pH levels to access its suitability for food applications. Carbohydrates, crude protein, crude fiber, crude fat and ash contents of red kidney bean seeds were found to be 53.02±1.14%, 25.78±0.77%, 6.82±0.31%, 1.92±0.15% and 4.34±0.20%, respectively. Magnesium, calcium, sodium, potassium and iron were observed as macro elements in red kidney bean seeds. Protein solubility, emulsification, gelation as well as foaming properties of the bean protein isolate were significantly ($P \leq 0.05$) affected by different pH levels. The solubility, emulsifying activity and stability as well as foam capacity of the protein isolate were dependant with minimal values observed at pH 4 while maximum at pH 10. Contrarily, the stability of foam was highest at pH 4 while a decreasing trend in foam stability was observed with increase of pH. Gelation properties improved at acidic pH with maximum gelation capacity observed at pH 4 while these properties decreased at alkaline conditions. Conclusively, red kidney beans can be utilized to prepare protein isolate whose functional properties can be modified by changing the pH of the environment for better utilization in the food formulation systems.

Key words: Kidney bean, protein isolate, protein solubility, emulsification, gelation, foaming.

INTRODUCTION

Grain legumes are an important source of nutrients such as proteins, carbohydrates as well as certain minerals and vitamins (Boye *et al*., 2010). Legumes are renowned as poor man's meat due to higher protein content and have the potential to combat the problems of protein energy malnutrition, especially in developing countries (Qayyum *et al*., 2012). Red kidney bean is also an important legume crop which is extensively produced in Asian, South American and African regions (Wani *et al*., 2010). The botanical and linguistic evidences clearly manifested that red kidney beans have been originated in America and belong to the family phaseoleae (Bressani, 1993). Beans are mostly consumed as dry seeds but their utilization as green shelled seeds and green pods is also possible. These are also utilized as staple foods in Maxico, South American and African countries with per capita consumption upto 40 kg per year (Tang *et al.*, 2009). Although red kidney beans contain high amounts of dietary fiber, starch, vitamins, minerals as well as an extensive array of phytochemicals but the most important component of nutritional significance is their high protein content which is 2-3 times that of cereal grains (Mundi and Aluko, 2012). Red kidney bean proteins are the superior source of certain indispensable amino acids particularly lysine which is undersupplied by the cereals. Therefore, the combined consumption of beans and cereals can ensure a balanced protein diet due to the nutritional complementation of essential amino acids. (Tang *et al*., 2009). Due to this nutritional complementation, red kidney beans can be utilized as an economical source of dietary proteins, especially for the people of low income group in the developing countries (Yin *et al*., 2010). However, red kidney beans are also reported to contain different antinutritional factors such as trypsin inhibitor, lectins and heamagglutinins which impede proteolytic enzymes resulting in reduced digestibility and absorption of proteins (Kumar *et al*., 2013). Therefore, it is imperative to eliminate these anti-nutritional factors to improve the digestibility and bioavailability for efficient utilization of proteins. This can be achieved by extracting the proteins to produce isolates or concentrates which will not only contain high protein contents but also have better protein digestibility due to the elimination of anti-nutritional factors. The protein isolate can be utilized as a promising food ingredient in the industry to improve the functional properties of the products as well as to enhance the nutritional status of the diets.

The most widely used method for the preparation of protein isolates on commercial scale involve alkaline extraction of proteins, following by which the extracted proteins are precipitated at their isoelectric point (Kaur and Sing, 2007). The plant

proteins should possess certain desirable characteristics for their successful applications in different food products. These characteristics are often designated as functional properties which can influence the performance of protein in different food formulations (Moure *et al*., 2006). The solubility of the protein is an important functional attribute to access its suitability as food ingredient because the solubility can also influence foaming, emulsifying and gelling properties of the proteins. (Ragab *et al*., 2004).

The functional attributes of the protein based ingredients are not only dependent upon type of preparation, kind of protein and method of production but are also affected by different environmental factors (Sakamoto *et al*., 1994). The pH or ionic strength can influence the electrostatic and hydrophobic interactions within the matrix of protein molecules. Moreover, the hydrodynamics of proteins in the food formulations is also affected by the prevalent pH (Aluko and Yada, 1995). Therefore, by manipulating the pH, the functional properties as well as structural conformation of the proteins can be modified for better utilization in the food formulations. The objectives of this study were to explore the potential of underutilized red kidney beans to produce value-added protein product and to evaluate the functional properties of the value-added product under different pH conditions to envisage its compatibility in different food systems.

MATERIALS AND METHODS

Raw material collection and preparation: The seeds of indigenous cultivar of red kidney beans were obtained from NARC, Islamabad. The seeds were screened and rinsed with deionized water to remove the extraneous material. After drying the seeds in an oven at 50^{o}C, these were ground and passed through a screen to get uniform size flour, which was stored in air tight containers until used for analysis. The analytical grade chemicals were used during the analysis.

Chemical analysis of red kidney bean seeds: The proximate analysis of red kidney bean seed flour for moisture, crude fat, crude fiber, ash protein and carbohydrate contents was carried out in triplicate by adopting the standard procedures of AOAC (2000). The moisture content was determined by oven dry method by drying the sample in hot air oven at 130^{o}C till constant weight was attained. For crude protein content, kjeldhal method was used to determine the nitrogen content which was then multiplied with factor (6.25). The estimation of the fat content of the samples was carried out in soxhlet apparatus by using petroleum ether. The estimation of ash contents was carried out by using muffle furnace at a temperature of 550 ^{o}C for 8 to 10 hours. Carbohydrate content of the seed flour calculated by subtracting the percentages of the above mentioned parameters on moisture free basis from 100. The gross energy was calculated by multiplying percentages of fat, protein and carbohydrates with their Atwater factors.

For mineral analysis, the digestion of seed flour sample was carried out by using concentrated nitric acid and perchloric acid at 180-200^{o}C while the digested sample was subjected to atomic absorption spectrophotometer (GBC-932, Australia) by following the standard procedure of AOAC (2000).

Preparation of Protein Isolate: By using hexane, red kidney bean seed flour was defatted under continuous shaking for 12 hours, following by which the removal of hexane was carried out by the decantation process. The defatted flour was dried in air and used for the preparation of protein isolate. The proteins from defatted flour of red kidney beans were isolated by alkaline extraction and isoelectric precipitation method as described by Kaur and Sing, (2007) with slight modifications. Different steps involved in the preparation of protein isolate are depicted Figure 1.

Protein Solubility Profile: The solubility of the protein isolate was determined by mixing 1 g of the sample in 25 ml of distilled water and adjusting the pH of dispersions in the range of 2-10 by either using 1N HCl or NaOH solutions. After stirring the dispersions for 1 hour and centrifuging at 8000 g for 15 minutes, supernatants were separated and their protein contents were determined (Sai-Ut *et al*. 2009). Following equation was used to calculate the solubility percentage of the isolate:

$$\text{Solubility \%} = \frac{\text{Protein content of supernatants}}{\text{Protein content of sample}} \times 100$$

Emulsification Properties: The method of Neto *et al*. (2001) was followed to evaluate the emulsification properties of protein isolate. The determination of emulsifying activity was carried out by blending 3.5 g of the isolate sample with 50 ml of water for 30 seconds and adjusting the pH (2-10) either by using 1N HCl or NaOH solutions. After the addition of 5ml of the canola oil, the suspensions were again blended. The emulsions were then centrifuged at 2000g for 5 minutes and the heights of the total contents and that of emulsified layer in the tubes were measured. Following equation was used to calculate the emulsion activity:

$$\text{EA\%} = \frac{\text{Height of emulsified layer in the tube}}{\text{Height of the total contents in the tube}} \times 100$$

For the determination of emulsion stability, emulsions were heated at 80^{o}C for 30 minutes and then cooled under running tap water for 15 minutes prior to centrifugation. Emulsifying stability was calculated as

$$\text{ES\%} = \frac{\text{Height of the emulsified layer after heating}}{\text{Height of the total contents in the tube}} \times 100$$

Gelation Properties: The method of Lawal *et al*. (2005) was followed to determine the gelation properties of the

protein isolate. The isolate sample was suspended in distilled water at different concentrations (2-20%) and the pH of suspensions was adjusted to the desired value (2-10). Each sample suspension (10 ml) was taken into the test tube and heated for 1 hr. in boiling water bath and then cooled rapidly in cold water as well as further cooled at 4^{o}C for 2 hrs. The test tubes were then inverted and analyzed for gelling ability. The concentration at which the sample did not slip or fall from the inverted test tubes was expressed as least gelation concentration.

Foaming Properties: The determination of the foaming properties of the protein isolate was carried out by following the method of Lawal *et al*. (2005). For this purpose, 3% sample dispersions were prepared in distilled water and their pH was adjusted in the range of 2-10 either with HCl or NaOH solutions. After vigorous whipping of the dispersions in blender for 2 minutes, foaming activity was calculated according to the following equation:

$$FC\% = \frac{\text{Vol. after whipping} - \text{vol. before whipping}}{\text{Vol. before whipping}} \times 100$$

For the determination of foam stability, the volume of the foam was recorded after 30 minutes time interval up to 90 minutes.

Statistical Analysis: Results of three replications were articulated as mean±standard deviation. The data regarding the effect of pH on functional properties was subjected to analysis of variance (ANOVA) techniques while the comparison of the means was performed by using Duncun,s multiple range test as described by Steel *et al*. (1997).

RESULTS AND DISCUSSION

Chemical analysis of red kidney bean seeds: The results regarding the proximate composition of bean seeds are depicted in Table 1. Moisture content of red kidney beans was found to be 8.12±0.53%. Moisture content is an important factor that dictates storage stability as high moisture content in seeds may lead to deterioration and spoilage through bacterial and fungal attack. The red kidney bean seeds having low moisture content can be stored for a longer period of time. The moisture content of red kidney bean seeds is comparable to the values (8.9%) reported by Olaofe *et al.* (2010) for common beans. The results regarding protein content revealed red kidney beans to be an excellent protein source containing 25.78±0.77% protein. Dietary proteins are essential for synthesis and repairing of body tissues, hormones, enzymes as well as other substances required for healthy functioning. Present results regarding the protein content corroborated the findings of Rui *et al*. (2011) who reported protein content of 22.36-28.50% in different bean varieties. The carbohydrate content calculated as nitrogen free extract by difference accounted for 53.02±1.14% in the red kidney bean seeds. Carbohydrates are the main source of energy which are stored as glycogen and act as reservoir for glucose. The carbohydrate value observed in this study is in conformity with the values of 51.5, 57.3 and 59.62% for tirga beans, pinto beans and white kidney beans, respectively (Amir *et al.*, 2006). The crude fiber content of red kidney beans was observed to be 6.82±0.31%. Although the crude fiber has little food value but it plays an essential role in the control of different diseases by exerting certain physiological functions. The fiber content of red kidney beans is adequate in relation to diet. These findings are in accordance to Costa *et al*. (2006) who observed crude fiber content of 6.26-8.98% in pea, chickpea and common beans. The crude fat content of red kidney bean seeds was observed to be fairly low (1.92±0.15%) which was within the range of 1-3% for most of the legumes (Costa *et al*., 2006). The ash content of bean seeds was 4.34±0.20% which falls within the range of 4-5% as reported for different varieties of beans (Rui *et al*., 2011). The energy value of red kidney bean seeds calculated by the use of Atwater factors was found to be 332.48 Kcal/100g indicating these to be a fairly good source of energy. Previously, Khattab *et al*. (2009) reported a caloric content range of 375.32-383.91Kcal/100g for Canadian and Egyptian cowpea and kidney beans.

The results regarding mineral composition of red kidney bean seeds are depicted in Table 2. These results elucidated red kidney bean flour to be a good source of minerals containing magnesium, calcium, sodium, potassium and iron in relatively higher amounts while phosphorus, nickel, zinc, manganese and copper in smaller quantities. Magnesium was found to be most abundant mineral in the raw flour (762.7±6.61 mg/100g) which contradicts the findings of Olaofe and Sanni (1998) who reported potassium to be the abundant element in agricultural products. The results regarding the mineral analysis substantiated the findings of Audu and Aremu (2011), however, slight variations may be due to varietal difference and geographic conditions.

Solubility profile of red kidney bean protein isolate: The results regarding the solubility profile of the protein isolate are illustrated in Figure 2. It is obvious from the results that the protein solubility was experiential to be pH-dependant with minimum solubility (7.5%) was observed at pH 4-5, beyond that subsequent change in pH on either side resulted in the progressive increase in protein solubility. The protein solubility was higher in the alkaline region with maximum solubilization (92%) was observed at pH 10. Similar variations in solubility with pH were previously reported for pigeon pea and lentil proteins (Mwasaru *et al*. 2000; Bora. 2002). Protein solubility may be influenced by the ratio of hydrophilic or hydrophobic amino acids. The low protein solubility at

the isoelectric region is ascribed to the balance of negative and positive charges resulting in the reduction of electrostatic repulsive interactions. High solubility of the red kidney bean protein isolate beyond isoelectric point may be attributed to large net charges and electrostatic repulsion due to the prevalence of cation III and anion II, respectively. The solubility is a valuable indices for the suitability of the isolate for various food applications as low protein solubility over a wide range of pH indicate severe protein denaturation and insolubilization which may also influence other functional properties. High protein solubility of the isolate signify its potential for industrial application to develop high protein drinks and beverages. Moreover, high protein solubility of the isolate can be advantageously utilized to develop infant formulas, spray dried products and milk replacers.

Emulsification properties of red kidney bean protein isolate: The influence of pH on emulsification properties of protein isolate is shown in Figure 3 and 4, respectively. Minimum value of emulsion activity was observed at pH 4 which increased with increasing the pH with maximum value (70.4%) was observed at pH 10. The emulsification activity was pH-dependant with alkaline pH improved this property more than acidic pH. The results regarding emulsion stability also followed similar pattern with minimum value was observed at pH 4 while maximum emulsion stability was observed at pH 10. Different factors such as protein conformation, interfacial tension, droplet size as well as net protein charge can influence the values of emulsion stability There is a direct relationship between emulsification properties and protein solubility. Low values of the emulsification properties at the isoelectric region may be ascribed to low protein solubility due to minimum electrostatic repulsion among the particles which promotes coalescence and flocculation (Hung and Zayas, 1991). At extremes of the acidic and alkaline regions, unfolding of proteins exposes majority of the obscured lipophilic functional groups with inadequate interfacial energy between aqueous and oil phases resulting in improvement of the emulsification properties (Chavan *et al*. 2001). The emulsification properties play an imperative role for the stability of high fat food products by reducing the rate of oxidative rancidity. The emulsification properties of the protein isolate make it a promising food ingredient for utilization in cake batters, bread, meat sausages, salad dressing, frozen desserts and dairy products.

Gelation Properties of red kidney bean protein isolate: The results regarding the gelling potential of the protein isolate are depicted in Table 3. The least gelation concentration (LGC) was used as an indicator of gelling ability as lower values of LGC indicate better gelation capacity. At pH 4 minimum value (8% w/v) of LGC was observed which indicate optimum gelling ability of the protein isolate at this pH, beyond that subsequent change of pH on either side resulted in an increase of LGC. At pH 10 highest value (16%) of LGC was observed which indicate poor gelling potential of the isolate at alkaline conditions. The value of LGC (12%) in the present study, at pH 7 acting as control, was lower as compared to mucuna bean and lupin proteins (Sathe *et al*., 1982; Adebowale and Lawal, 2003) signifying red kidney bean proteins to be better gelling food hydrocolloid than these legumes. Variations in gelation capacity of the protein isolate at different pH levels may be ascribed to the prevalence of surface charge on protein molecules. The balance of positive and negative charges at the isoelectric region minimizes repulsive interactions which promote stronger interactions of protein molecules resulting in an improvement of gelation capacity. Conversely, at pH levels beyond the isoelectric region, the interaction among protein molecules weakens due to the prevalence of repulsive forces which prevents the formation of a well-organized protein network (Elofsson *et al*., 1997). The gelation properties of the protein isolate are technologically advantageous for baked and pasta products, simulated ground meats as well as for dairy products such as curds and cheese.

Foaming properties of red kidney bean protein isolate: The results regarding the foaming properties of the isolate at different pH levels are depicted in Table 4. These results clearly indicated that foaming properties were pH-dependant. Minimum value (33%) of foam capacity was found at pH 4 , which increased gradually by changing the pH on either side with maximum values (84%) was found at pH 10. Acidic pH also favor the foaming capacity of the extracted protein isolate. The increased net charge on the protein molecules at the acidic and alkaline regions weakens the hydrophobic interactions, allowing quick spreading of proteins at the air-water interface as well as encapsulation of air particles resulting in increased foam formation (Chau *et al*. 1997). Foam stability of all samples decreased with time at all pH levels. Contrary to foam capacity, highest foam stability was found at the isoelectric region while subsequent change in pH on either side decreased stability with minimum values observed at pH 10. Highest foam stability in the acidic regions may be ascribed to minimum repulsive interactions leading to the development of viscous film as well as stable molecular layer at the interface, thus imparting stability to the foam. Similar variations in foaming properties were previously reported for sesame and locust bean proteins (Inyang and Idhu 1996; Lawal *et al*. 2005). Foaming properties of the protein isolate can be advantageously utilized in whipped toppings, angel cakes, confectionary and chiffon mixes.

Table 1. Proximate analysis of red kidney bean seeds.

Parameters	Composition (g/100g)
Moisture	8.12±0.53
Crude fat	1.92±0.15
Crude protein	25.78±0.77
Crude fiber	6.82±0.31
Ash	4.34±0.20
Carbohydrate	53.02±1.14
Energy value (Kcal/100g)	332.48

Results of three determinations were reported as means ±SD

Table 2. Minerals profile of red kidney bean seeds.

Minerals	Composition (mg/100g)
Magnesium	762.7±6.61
Calcium	51.4±1.04
Sodium	39.2±0.25
Potassium	19.6±0.62
Iron	14.2±0.40
Phosphorous	4.4±0.70
Nickel	3.76±0.08
Zink	3.62±0.04
Manganese	2.71±0.05
Copper	0.92±0.05

Results of three determinations were reported as means ±SD.

Table 3. Gelation properties of red kidney bean protein isolate at different pH levels.

Sample concentration (%)	pH 2	pH 4	pH 6	pH 7	pH 8	pH 10
2	(−)	(−)	(−)	(−)	(−)	(−)
4	(−)	(±)	(−)	(−)	(−)	(−)
6	(−)	(±)	(±)	(−)	(−)	(−)
8	(±)	(+)	(±)	(±)	(−)	(−)
10	(±)	(+)	(+)	(±)	(±)	(−)
12	(+)	(+)	(+)	(+)	(±)	(±)
14	(+)	(+)	(+)	(+)	(+)	(±)
16	(+)	(+)	(+)	(+)	(+)	(+)
18	(+)	(+)	(+)	(+)	(+)	(+)
20	(+)	(+)	(+)	(+)	(+)	(+)
LGC	12	8	10	12	14	16

Gelation levels: (+) gelation; (−) No gelation; (±) partial gelation

Table 4. Foaming properties of red kidney bean protein isolate at different pH levels.

pH	Initial volume (ml)	Volume after whipping (ml)	Foam capacity (%)	Foam stability (%) After time (minutes)		
				30	**60**	**90**
2	50	85.5±0.5	71±1^{c}	82.14±1.03^{e}	71.82±0.40^{d}	61.96±0.54^{d}
3	50	76.5±1	53±2^{e}	84.28±0.63^{d}	77.38±1.03^{c}	64.76±0.65^{c}
4	50	66.5±0.5	33±1^{g}	90.89±0.27^{a}	84.83±0.46^{a}	72.70±0.82^{a}
5	50	67.5±0.5	35±1^{g}	88.56±0.32^{b}	79.93±0.57^{b}	68.55±0.90^{b}
6	50	74±1	48±2^{f}	86.81±0.72^{c}	76.38±0.60^{c}	59.7±0.89^{e}
7	50	78±1	56±2^{d}	82.12±0.63^{e}	71.4±1.02^{d}	54.74±0.90^{f}
8	50	86.5±0.5	73±1^{c}	76.70±0.32^{f}	56.15±0.60^{e}	36.97±0.86^{g}
9	50	88.5±0.5	77±1^{b}	72.71±0.35^{g}	49.34±0.66^{f}	29.85±0.91^{h}
10	50	92±1	84±2^{a}	66.65±0.79^{h}	40.06±0.90^{g}	19.03±0.73^{i}

Results of three determinations were reported as means ±SD. Means sharing different alphabetic letters within the columns are significantly ($P \leq 0.05$) different.

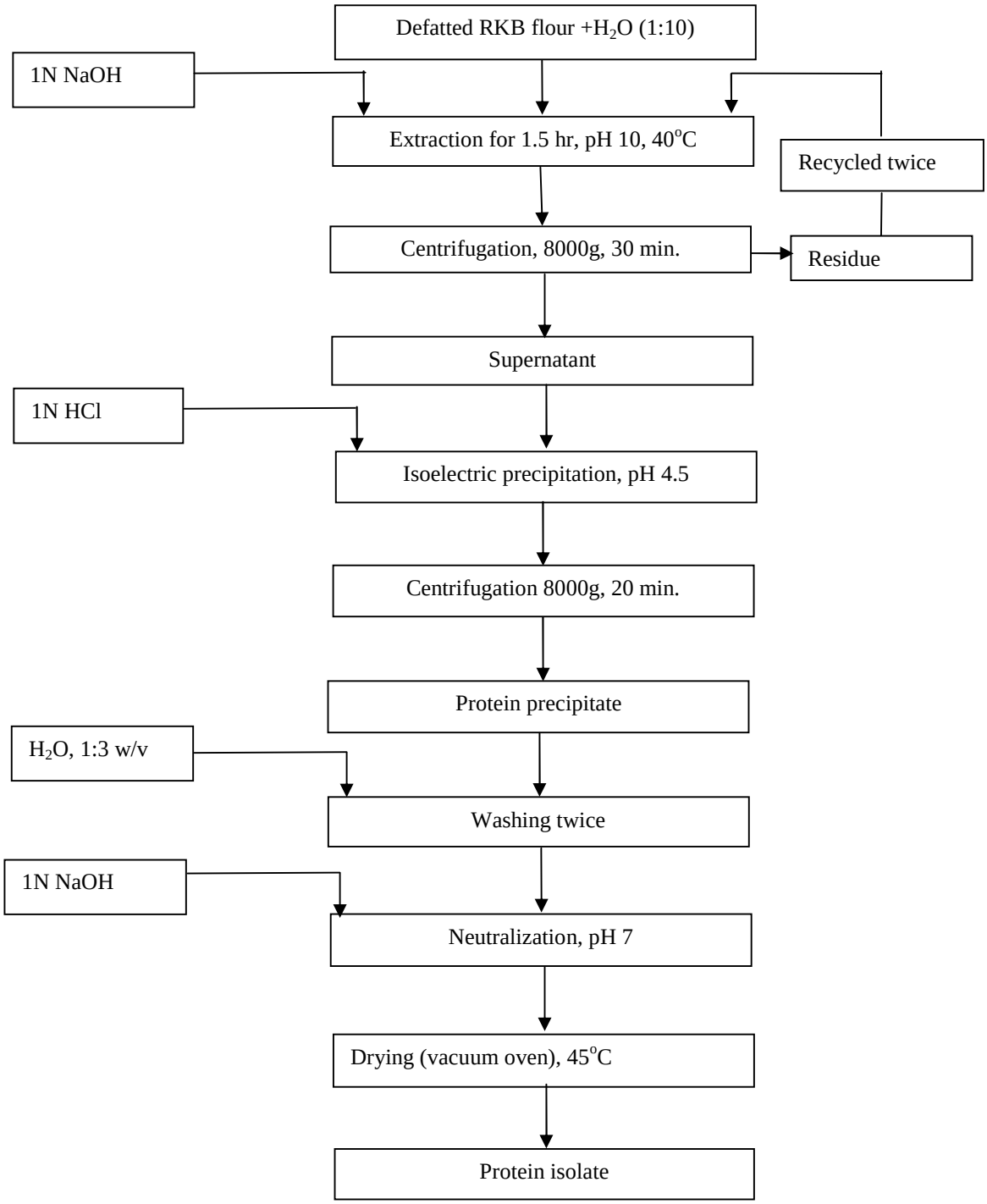

Figure 1: Different steps involved in the preparation of red kidney bean protein isolate

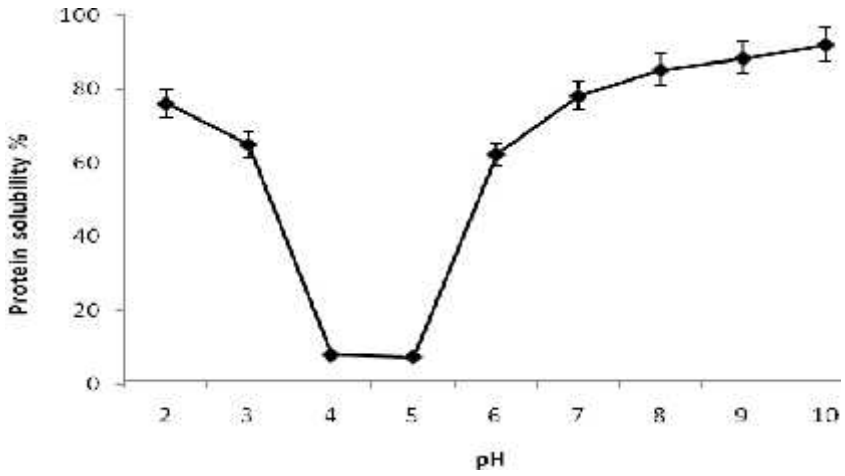

Figure 2: Solubility profile of red kidney bean protein isolate at different pH levels

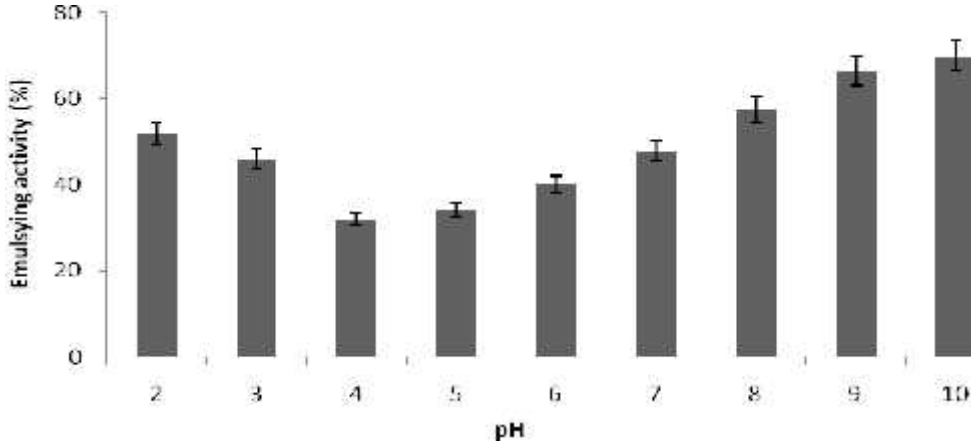

Figure 3: Emulsifying activity of red kidney bean protein isolate at different pH levels

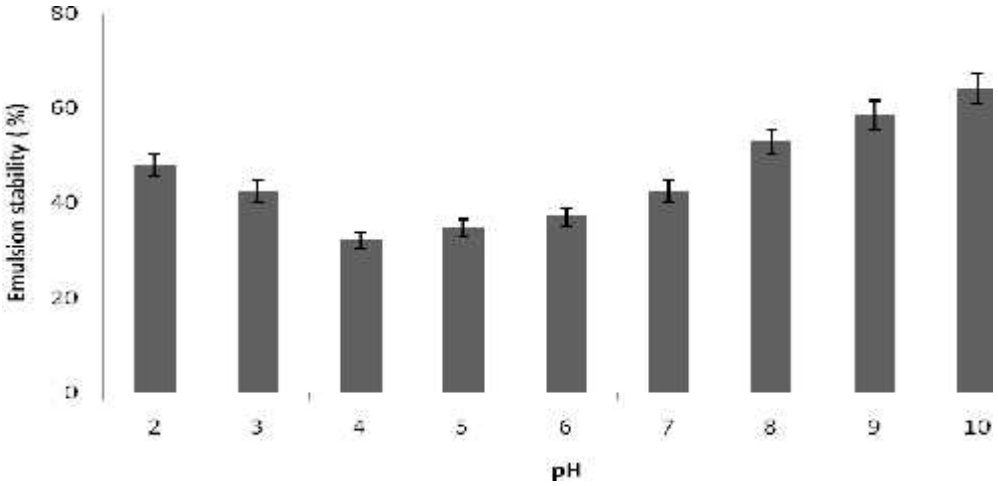

Figure 4: Emulsion stability of red kidney bean protein isolate at different pH levels.

Conclusions: Red kidney beans are an excellent source of proteins, fiber and essential minerals. Being a rich source of proteins, red kidney beans can be utilized for the production of protein isolate for utilization as promising ingredient in the food industry. Variations in pH can exhibit modifications of functional properties. Red kidney bean protein exhibited higher solubility as well as better emulsifying and foaming properties. Moreover, protein isolate was also found to be a better gelling hydrocolloid. Therefore, red kidney bean protein isolate has the potential to be utilized in different food formulation systems.

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