International Journal of Agricultural Sciences ☞DOI:10.15740/HAS/IJAS/17.2/185-193
Volume 17 | Issue 2 | June, 2021 | 185-193 ■ ISSN : 0973-130X Visit us : *www.researchjournal.co.in*

*RESEARCH PAPER*

# Characterization of radish leaf protein concentrates for biochemical, functional properties, antioxidant activity, mineral content and microbial stability

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**Abstract :** Leaf protein concentrate was extracted from radish leaves using heat coagulation and analyzed for its antioxidant capacity, mineral content, functional properties and microbial stability. Radish leaf protein concentrates (RLPC) constituted 48.3 % protein content and a yield of 38.51% (DW). Glutelins (42.27 %), prolamins (29.07%) and albumins (19.32 %) were found to be three major fractions of protein concentrate, while globulins (9.38%) was a minor component and their apparent molecular weights ranged between 12-60 kDa. Antioxidant activities (FRAP, ABTS and DPPH ) were higher in RLPC as compared to the isolated fractions. Among fractions, globulins and prolamins exhibited highest DPPH and FRAP activity while highest ABTS activity was associated with glutelins, respectively. Functional properties *viz.,* water holding capacity, oil holding capacity, emulsifying capacity and emulsion stability of the RLPC were 545, 347, 51.8 and 49.4%, respectively. The maximum solubility of RLPC was observed at pH 12 (44.64%) and the minimum solubility was observed at pH 4 (28.24%). A considerable amount of minerals were present in the RLPC, Ca and Fe being the most abundant. Microbial load of RLPC remained in acceptable limits up to 35 and 21 days of storage under refrigerated and ambient conditions, respectively. These results indicated that LPC have desirable functional properties, a considerable mineral content, high antioxidant activity and sufficient microbial stability. Thus they could be used as a functional ingredient to be incorporated in food products to supplement diet and combat protein deficiency.

**Key Words :** Functional properties, Heat coagulation, Leaf protein concentrates, Radish leaf

**View Point Article :** Kaur, G. and Bhatia, S. (2021). Characterization of radish leaf protein concentrates for biochemical, functional properties, antioxidant activity, mineral content and microbial stability. *Internat. J. agric. Sci.*, **17** (2) : 185-193, **DOI:10.15740/HAS/IJAS/17.2/185-193.** 

**Article History :** **Received :** 21.02.2021; **Accepted :** 13.03.2021

## INTRODUCTION

India contributes 14.0% of total vegetable production and ranked second in total world production of vegetables. Radish (*Raphanus sativus*) is a popular, quick growing cold season root vegetable belonging to the Cruciferae family which is cultivated widely throughout the world. The entire radish plant is considered

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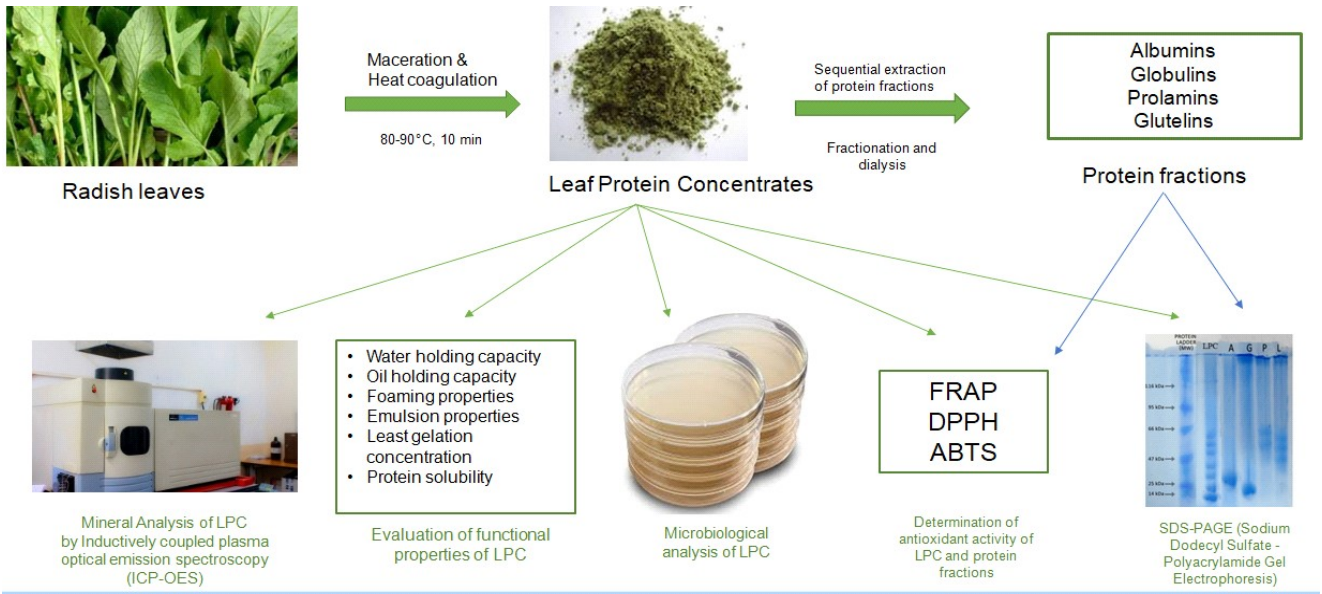


**Fig. A : Graphical abstract**

to be edible and can be eaten in the raw form. Despite of having incredibly good amount of protein and being a rich source of essential vitamins and minerals, the radish leaves are not utilized to their full potential (Ankita and Prasad, 2015). World production of radish is reported to be 7 million tonnes per year. Leaves constitute 30-50% of the total weight of a radish plant indicating that several tonnes of leaves are wasted every year since they are discarded. The high nutritional value associated with leafy portions of the radish plant paves a way in making it a more valuable food source in order to fight against the protein energy malnutrition problems prevalent all across the globe. In the present day world, around one billion people are combating with the major problems of protein deficiency and malnutrition. The outcome of protein malnutrition is adverse since it causes detrimental diseases like marasmus and kwashiorkor (Ghaly and Alkoaik, 2010). So, collective efforts need to be put by the researchers to find economically inexpensive sources of good quality protein to save people from protein malnutrition.

Leaf protein concentrate (LPC) has been recognized as an unconventional source of protein. The use of protein from the leaves is possible if the leaf is subjected to certain processes which can considerably eliminate toxic, anti-nutritional and fibrous parts of the tissue, practically enabling humans to consume enough quantities of leaves. LPCs can be prepared by employing various methods like heat coagulation (Fasuyi and Aletor, 2005), chemical extraction (Jiamyangyuen *et al*., 2005) and enzyme-assisted extraction (Tang *et al.*, 2003), among various others. The present research focuses on extraction of LPCs by the method of heat coagulation since it involves extraction in an economical and environment friendly way without the involvement of any chemicals/ organic solvents enabling by-product utilization in a sustainable manner.

Development of value-added products from protein concentrate and its subsequent use as an alternative protein requires information on its functional properties. These functional properties affect the organoleptic characteristics and quality of the food they are incorporated into (Ogunwolu *et al.*, 2009). It is also important to evaluate other properties such as antioxidant capacity, mineral content and microbial stability of the protein concentrates to understand their nutritive value, health benefits and storage life. At present, to the best of researchers' knowledge, no information on the extraction of LPC from radish leaves or their characterization is available in literature. Prompted by these facts, the present investigation was undertaken with the objective of extracting protein from radish leaves and evaluating its functional properties, antioxidative activity, mineral content and storage stability in order to ascertain its suitability to use as functional food ingredient.

## MATERIAL AND METHODS

The present investigation was carried out on the leaves of radish variety- Punjab Safed Mooli, cultivated

by taking into consideration the recommended cultural and agronomic practices. The crop was grown in the fields of Department of Vegetable Science, Punjab Agricultural University, Ludhiana. The leaves were separated manually from their stalks and washed under running tap water. Excess water was drained and the leaves were dried in a tray dryer at $50^0$C for 8-10 h. The dried samples were ground into fine powder using a grinder and the leaf powder was stored in airtight containers at room temperature for further experimentation. Chemical reagents used in this study for various experiments were of analytical grade and were purchased from Molychem, Pvt. Ltd. (Mumbai, India), Sisco Research Laboratories Pvt. Ltd. (Mumbai, India) and MP Biomedicals, Pvt. Ltd. (Mumbai, India). A high range protein marker (14-220 kDa, catalogue number 99625, Sisco Research Laboratories Pvt. Ltd., Mumbai, India) was used as the molecular weight marker for electrophoresis.

**Preparation of Radish leaf protein concentrates:**

The radish leaf protein concentrate (RLPC) was prepared using the heat coagulation method (Fasuyi and Al 2005). Fresh radish leaves (1 kg) were separated from their stalks and immediately washed under running tap water. The leaves were macerated in a grinder to obtain leaf juice which was filtered using the filtration apparatus. The pulp was discarded and the juice so obtained was heated at temperature ranging between 80 - $90^0$C for 10 min in a water bath. The heat coagulated juice was then centrifuged at 3000 g for 30 min. The residue (RLPC) was washed with distilled water, freeze dried and stored for further analysis.

**Determination of yield of RLPC and protein content:**

Yield of protein (%) in RLPC was calculated as the percentage of RLPC obtained (g) from the total amount of fresh radish leaves (g). Crude protein content of RLPC was determined by the standard Kjeldahl method (AOAC, 2000). For the determination of nitrogen, Kelplus Nitrogen Estimation System (Pelican Equipments, Chennai, India) was used. The nitrogen content was multiplied by conversion factor 6.25 to obtain protein content.

**Isolation of protein fractions from RLPC and determination of molecular weight:**

The protein fractions namely, albumins, globulins, prolamins and glutelins were isolated from RLPC by modifying the sequential extraction method (Adebiyi and Aluko, 2011) depending upon their solubilities in different solvents. The isolated protein fractions were freeze dried in a lyophilizer and stored for analysis. Protein content of fractions were determined by method of Lowry *et al.* (1951). Electrophoretic profiling of the proteins in RLPC and their protein fractions was done using SDS-PAGE in order to determine the apparent molecular size of the proteins. The method of Laemmli (1970) was adopted to separate the proteins and obtain the banding pattern on the gel. Molecular weights were determined by comparing them to the molecular ladder which was run simultaneously along with the samples.

**Determination of antioxidant properties of LPC and protein fractions:**

Antioxidative activities of radish leaves as well as isolated fractions were estimated. ABTS radical scavenging activity was measured at 30 min by method of (Re *et al.*, 1999). DPPH radical scavenging activity (30 min) was measured according to method of Lin *et al.* (2009). Method of Benzie and Strain (1996) was employed for the estimation of FRAP activity.

**Functional Properties of RLPC:**

Protein solubility (PS) was estimated by method of (Yu *et al.,* 2007). Methods of Lin *et al.* (1974) was used for estimation of water holding capacity (WHC) and oil holding capacity (OHC). Foaming capacity (FC) and foaming stability (FS) were measured by method employed by Yasumatsu et al. (1972). Method of Kaushik *et al.* (2016) was used for determining the Emulsifying Capacity (EC) and Emulsion Stability (ES). Least Gelation Concentration was estimated by method of Huda *et al.* (2001).

**Mineral analysis of RLPC:**

LPC (0.5 g) was digested 10 ml of conc. $HNO_3$ and $HClO_3$ (v/v 2:1) in Kjeldahl infra digestion system using temperature profile- 150°C for 1 h and 250°C for 2 h (until clear solution was obtained) followed by addition of 10 ml of double distilled water and filtration (Pedler *et al.*, 2000). Presence of different minerals was quantitatively determined using inductively coupled plasma optical emission spectroscopy (ICP-OES). The mineral composition of the RLPC was recorded as mg of mineral/100g of RLPC.

**Microbiological analysis of RLPC during storage:**

RLPCs were stored in low density polyethylene bags (100 gauge) at ambient (Temperature: 30.1-40.6$^0$, Relative Humidity: 32-82%) and refrigerated (Temperature : 5±1$^0$, Relative Humidity: 90%) conditions for 42 days. The microbial analysis of the stored RLPC was done at regular weekly intervals. For estimation of the total yeast and mould count Potato Dextrose Agar (PDA) media was prepared and sterilized at pressure 15 psi for 15min. Serial dilutions ($10^{-2}$) were made and microbial count of this dilution was analyzed where 1ml of the dilution was taken in a petri plate on to which 15-20 mL media was poured. Plates were then left for incubation at 27! for 48-72 h. The colonies were counted and the results were expressed in log CFU/ml. The total plate count of the LPC was estimated using Nutrient Agar (NA) media. The NA media was prepared and sterilized at pressure 15 psi for 15min. Serial dilutions

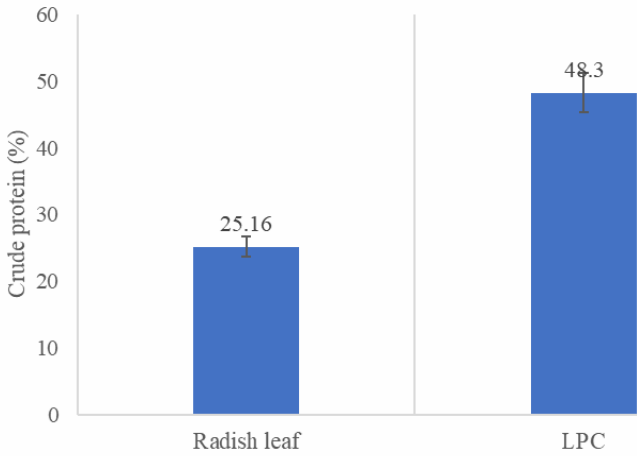


**Fig. 1 : Crude protein content (%) of radish leaf and its Leaf Protein Concentrate (LPC)**

($10^{-4}$) were prepared and microbial count of the dilutions was analyzed by taking 1ml of the dilution pouring on to the petri plate. Plates were then incubated at 37$^0$ for 24 h, the colonies were counted and the results were expressed in log CFU/mL.

**Statistical analysis:**

All the experiments mentioned above were performed in triplicate and the data was expressed as mean ± standard error.

## RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized

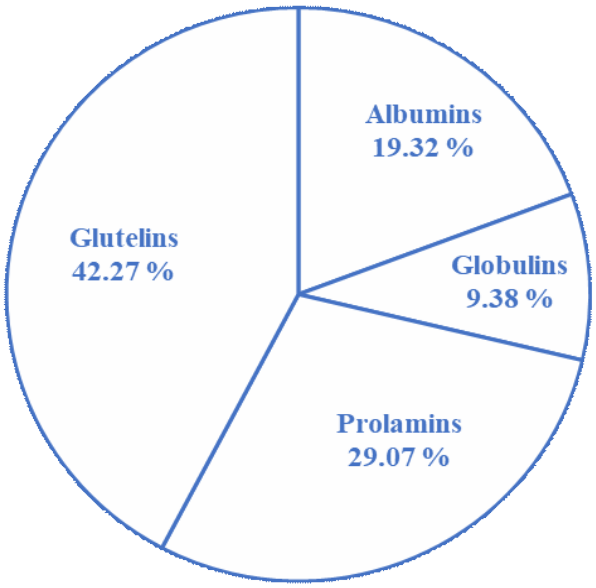


**Fig. 2 : Proportion of protein fractions (%) : albumins, globulins, prolamins and glutelins in the LPC**

under following heads :

**Protein content and yield of RLPC:**

The yield of the extracted RLPC was observed to be 38.51% on dry weight basis. The protein content of the radish leaf and that of the RLPC extracted from them was 25.16% and 48.3%, respectively. The extraction method enhanced the crude protein content by 23.14% as shown in Fig. 1. RLPCs extracted from the leaves of four leafy vegetables species: *Vernonia amygdalina* (Bitter leaf), *Solanum africana*, *Amaranthus hybridus* (Green tete) and *Telfaria occidentalis* (Fluted pumpkins contain 35.1-54.9 % crude protein (Aletor *et al.*, 2002). which is comparable to the results observed in case of RLPC. The value of radish RLPC is also higher than 39.13 % reported for moringa LPC (Sodamade *et al.*, 2013). The presence of significant quantity of crude protein in RLPC means that they could be used as nutritionally valuable and a healthy ingredient to improve protein deficiency of human and animal diet.

**Protein fractions isolated from RLPC and SDS-PAGE profiles:**

Fig. 2 represents the proportion of fractions (%) in the LPC. The alkali soluble fractions (glutelins) is the major fraction (42.27%) followed by the ethanol soluble prolamins (29.07 %). The water soluble fractions

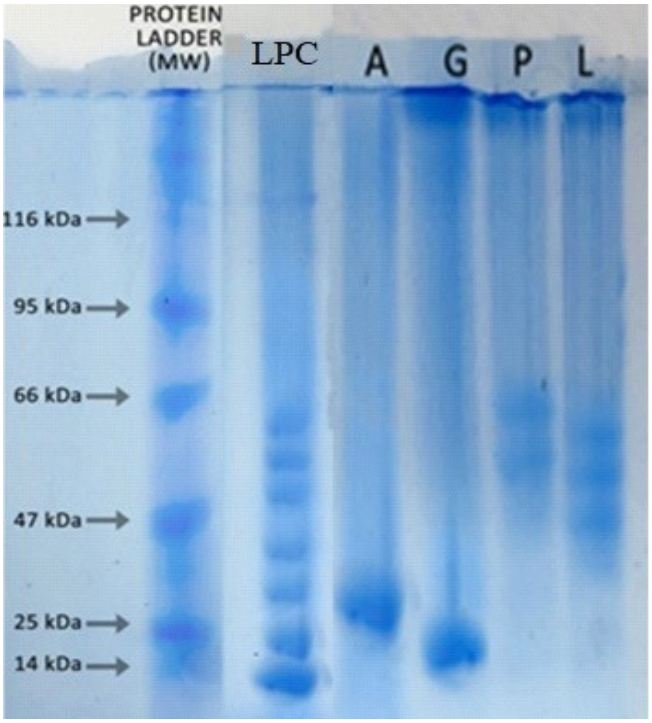


**Fig. 3 : SDS-PAGE profile of leaf protein concentrate (LPC) and its isolated protein fractions (A- albumins, G- globulins, P-prolamins and L- glutelins)**

(albumins) constituted 19.32% whereas the salt soluble globulins were only 9.38 % of the total protein content of the LPC. In case of deoiled rice bran protein concentrate, albumin (37.23%), globulin (20.27%) and glutelin (46.82%) are reported to be three major fractions, while prolamin (1.18%) is a minor component (Mann *et al.,* 2016).

The banding pattern on the electrophoretogram determined the apparent molecular weight distribution of various protein subunits. In lane 1 of the profile, seven protein bands were quite clearly observed with approximate molecular weights of 12, 20, 32, 50, 56 and 60 kDa indicating the presence of seven abundant proteins in the RLPC (Fig. 3). These results are comparable to sour cherry kernel protein concentrates where the apparent molecular weights of proteins varied from 14 to 66 kDa under reducing and denaturing conditions (Çelik *et al.*, 2019). Albumins (25-30 kDa) and globulins (14-25 kDa) isolated from the RLPC show a low molecular weight range, while prolamins (50-66 kDa) and glutelins (40-60 kDa) show a higher molecular weight range. The molecular weights of rice bran albumin, globulin, glutelin and prolamin are in the range 10-100, 10- 150, 33-150 and 25-100 kDa, respectively (Hamada, 2000). The differences might be due to the heterogeneous nature of polypeptides in RLPCs and protein fractions (Adebiyi *et al.*, 2009).

**Antioxidative properties of RLPC and protein fractions:**

The results indicate that DPPH radical scavenging activity for the RLPC (40.33 %) and the corresponding values for the protein fractions albumins, globulins, prolamins and glutelins are 25.85 %, 6.69 %, 4.36 % and 9.96 %, respectively (Table 1). The isolated fractions have a lower antioxidant activity than the RLPC indicating that the RLPC have a greater ability to quench DPPH and they possibly contain certain substrates that can react with free radicals and convert them into stable products (Xie *et al.*, 2008). The sequential fractionation for the isolation of protein fractions involves various steps which might be responsible for the dilution of free radical scavengers or the substrates important for imparting antioxidant activity to the fractions. The FRAP value for the RLPC is 48.08 mg/100 g which is higher than the values observed for the protein fractions (Table 1). Among the protein fractions, prolamins show maximum FRAP (37.22 mg/100 g of fraction) whereas albumins show the minimum activity (12.60 mg/100g). Higher electron donating power of the LPC leads to the higher reducing power, which can be attributed to lower molecular weight peptides and higher protein solubility (Galla *et al.*, 2012). Maximum ABTS radical scavenging activity for RLPCs is 59.43 % (Table 1). Among different protein fractions, glutelins show the maximum activity whereas the prolamins show the least activity. These

**Table 1: Antioxidant properties of LPC and protein fractions**

| Sample | DPPH (%) | FRAP (mg/100g) | ABTS (%) |
|---|---|---|---|
| LPC | 40.33±0.35 | 48.08± 1.26 | 59.43± 0.52 |
| Albumins | 25.85 ±0.37 | 12.60 ± 0.27 | 58.09±0.61 |
| Globulins | 6.69 ±0.11 | 14. 27 ± 0.21 | 15.59±0.31 |
| Prolamins | 4.36 ± 0.33 | 37.22 ± 0.11 | 2.51±0.12 |
| Glutelins | 9.96 ± 0.18 | 27.98 ± 0.13 | 84.21±1.43 |

The values represent mean ±SE

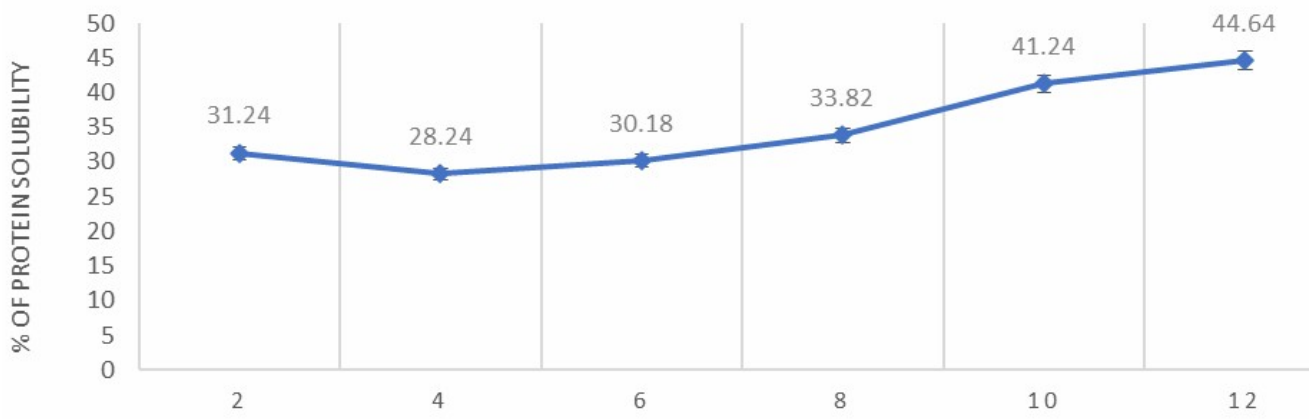


**Fig. 4 : Protein solubilities (%) of LPC as a function of pH**

**Table 2: Functional properties of RLPC**

| Functional Property | Percentage (%) |
|---|---|
| Water holding capacity | 545.00± 11.09 |
| Oil holding capacity | 347.00± 7.06 |
| Foaming capacity | 18.40± 2.12 |
| Foaming stability (at 10 min) | 29.00± 0.76 |
| Emulsifying capacity | 51.80±2.10 |
| Emulsion stability | 49.40± 1.75 |
| Least gelation concentration | 9.00± 0.00 |

The values represent mean ±SE

results suggest that the RLPC extracted from the radish leaves possess high antioxidant activity and can potentially protect foods and humans against oxidative damage caused by free radicals such as hydroxyl, peroxyl, and superoxide radicals.

## Functional properties of RLPC:

The value of water holding capacity for the radish RLPC is observed to be 545.00% (Table 2) which is much higher than the corresponding values of the protein concentrates of different varieties of cassava leaves which range between 118-200% (Fasuyi and Aletor, 2005). WHC is critical for the preparation of various types of soups, sauces and gravies along with baked products and confectionery (Adeyeye *et al.*, 1994). Oil holding capacity plays a vital role in flavor retention, stabilization and food formulation, especially in case of sausages, salad dressings, soups, etc. Oil holding capacity value of RLPC is 347.00% which is higher than 207.00% in sunflower flour (Lin *et al.*, 1974). Foaming capacity of RLPC (18.4%) is closer to those reported for *Talium triangulare* (22.1 %), *Amaranthus cruentus* (18.9%) and *Telfeira occidentalis* (19.2%) protein concentrates (Fasuyi, 2006). The foaming stability of the RLPC (29.00%) is lower than 76.90 % reported for mung bean protein isolate (Garcia-Moreno *et al.,* 2011). Both these foaming properties are important for the use of various food products as whipping agents and foaming ingredients in ice creams, aerated drinks and bakery products. The production and stabilization of fat emulsions is critically

**Table 3: Enumeration of Yeast /Mould count (log CFU/ml) and total plate count (log CFU/ml) in LPC during storage under different environmental conditions**

| Storage period (Days) | Yeast/ mould count (log CFU/ml) | | Total plate count (log CFU/ml) | |
|---|---|---|---|---|
| | Ambient | Refrigerated | Ambient | Refrigerated |
| 0 | Not detected | Not detected | Not detected | Not detected |
| 7 | 2.65± 0.01 | 2.17±0.13 | 2.89± 0.05 | Not detected |
| 14 | 2.9±0.02 | 2.54±0.04 | 3.41±0.13 | 2.81±0.18 |
| 21 | 3.07±0.01 | 2.74±0.16 | 4.66±0.16 | 3.17±0.21 |
| 28 | 3.41±0.13 | 2.87±0.21 | 5.13±0.23 | 4.34±0.25 |
| 35 | 3.72±0.02 | 3.02±0.27 | 5.87±0.20 | 5.01±0.31 |
| 42 | 4.12±0.17 | 3.37±0.15 | 6.14±0.33 | 5.21±0.38 |

The values represent mean ±SE
FSSAI Specification, 2018 for dehydrated vegetable products
Yeast/mould count: Acceptable up to 4 log CFU/ml
Total plate count: Acceptable up to 5 log CFU/ml

determined by properties such as emulsifying capacity and emulsion stability (Adeyeye *et al.*, 1994). The emulsifying capacity of RLPC (51.8%) is higher than 37.00 % observed in the *Moringa oleifera* (Sodamade *et al.*, 2013) and 47.8% in *Telfeira occidentalis* (Aletor *et al.*, 2002). Similarly, the emulsion stability of RLPC (49.4 %) is higher than the corresponding values reported for wheat flour (Lin *et al.,* 1974). These results indicate that the RLPC can be used as additives for the stabilization of emulsions in the production of various food products. Least gelation concentration is a property that determines the production of matrix for holding water, sugars, flavors and other ingredients during gel formation. The lower the least gelation concentration of the protein isolate, the better is its gelation characteristics (Celik *et al.*, 2019). The value for RLPC is 9.00% which is lower than the values reported for soybean protein isolate which was 16% (Fernández-Quintela *et al*., 1997) and for chickpea protein concentrate ranging between 14-16 % (Ghribi *et al*., 2015). As the RLPC demonstrated superior gelling properties, it could be useful as an additive in food products for gel formation. The solubility profile of heat coagulated RLPC is shown in Fig. 4 where the solubility is reduced as the pH increases until it reaches the isoelectric point; this is followed by progressive increase in solubility with further increase in the pH. Similar observation is reported for winged bean and Chickpea (Sanchez-Vioque *et al.*, 1999; Sathe *et al.,* 1982). The solubility results indicate that leaf protein concentrates might find good use in both acidic and alkaline foods.

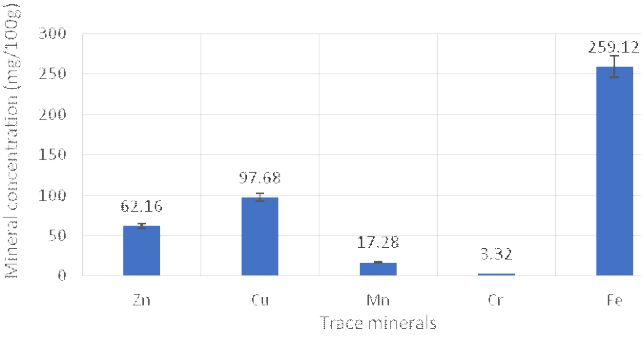


**Fig. 5(a) : Protein solubilities (%) of LPC as a function of pH**

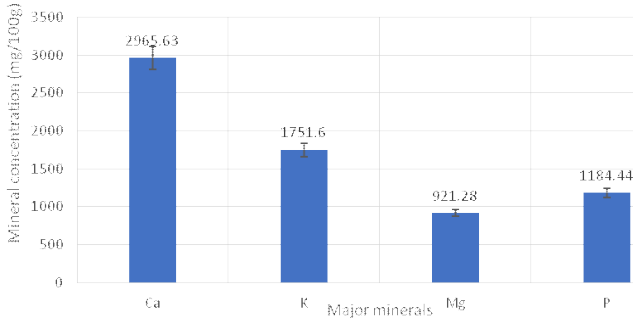


**Fig. 5(b) : Mineral content in LPC. (a) Trace mineral content (mg/100g). (b) Major minerals (mg/100g)**

## Mineral Composition of RLPC:

The mineral composition of the RPLC has been represented in Fig. 5 A (trace minerals) and 5 B (major minerals). Considerably high amount of calcium concentration is obtained in the RLPC (2965.63 mg/100g). The value of calcium is much higher than the recommended daily allowance (RDA) which is 800 mg per day for both adults and children (NRC, 1989). The value of Ca in RLPC is also higher than the reported value in *Moringa oleifera* LPC which is 723.00 mg/100g (Sodamade *et al.*, 2013). RLPC contain 1751.60 mg/100g Potassium and 921.00 mg/100g magnesium. Potassium content is close to the RDA value of 2000 mg and magnesium content is almost thrice the amount of required 350 mg respectively for adults (NRC, 1989). Potassium content in RLPC is much higher than 14.55 mg/100g reported for astragalina leaves (Gafar and Itodo, 2011) and 33.63 mg/100g reported in Moringa leaves (El Sohaimy *et al.*, 2015). Phosphorus is also present in appreciable amounts in RLPC (1184.44 mg/100g) The reported values phosphorus content in the LPCs of *Vernonia amygdalina*, *Solanum africana*, *Amaranthus hybridus* and *Telfaria occidentalis* are 930.40 mg/100g, 640.20 mg/100g, 1300 mg/100g and 1640 mg/100g, respectively (Aletor *et al*., 2002).

The iron concentration of RLPC is 259.12 mg/100g which is higher than 187.00 mg/100 g reported in *Moringa oleifera* LPC (Sodamade *et al.*, 2013). The concentrations of zinc, copper, manganese and chromium in the RLPC are 62.16 mg/100g, 97.68 mg/100g, 17.28 mg/100g and 3.32 mg/100g, respectively. The concentrations of all these trace minerals are observed to be higher than the recommended daily allowance. Since the results indicate that all the minerals in the RLPC are present more than or around the requisite amounts for the normal functioning of the body, they can be used as a potential nutritive ingredient in various food formulations and dietary supplements.

## Microbial analysis of RLPC:

Microbial contamination of the RLPC has been

recorded over a period of 42 days of storage under ambient and refrigerated conditions in order to determine the safety of their consumption (Table 3). While determining the yeast and mould count of RLPC, no growth has been detected after the first week of storage under refrigerated conditions. During the subsequent weeks, the yeast and mould count remained in acceptable limits in case of refrigerated storage conditions up to 42 days of storage whereas the growth is within acceptable range up to 35 days under ambient conditions of storage. The microbial growth increased as the storage period was elongated. A similar trend is seen in case of total plate count of the RLPC which is within the acceptable limits up to 21 days of storage under ambient conditions and up to 35 days of storage under refrigerated conditions.

## Conclusion:

RLPCs prepared by heat coagulation method constitute 48.3% protein content which is 23.14 % higher than the crude protein of raw radish leaf. The RLPC have a high protein concentration, desirable functional properties, considerable mineral content and high antioxidant activity, desirable microbial stability which indicate that they can be used as a promising functional ingredient in food products in order to supplement diet. Other methods for extraction of protein concentrates from radish leaves need to be investigated to further understand the effect of different extraction procedures on protein content, yield and other characteristics of the RLPCs.

## Acknowledgement:

Authors are grateful to Dr Tarsem Singh Dhillon, Associate Director (Seeds), Punjab Agricultural University, Ludhiana for providing radish leaves and Head, Dept. of Food Processing and Engineering, Punjab Agricultural University, Ludhiana for providing facilities for carrying the research experiments.

## Conflict of Interest:

None to declare

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