Abstract

Sunflower meal, a by-product obtained after sunflower oil extraction, is a rich source of proteins. In the present study, sunflower protein isolates were prepared from sunflower meal and were evaluated for various physiochemical, structural and functional proteins. The modification of protein was carried out to enhance the functional properties. Three techniques were used for modification i.e. gamma irradiation, ultrasound and thermal treatment. The study was carried out in four stages. In first stage, protein isolates were prepared from different fraction of sunflower meal with aim to enhance the yield and improve colour and minimum polyphenols. In second stage, protein isolates were modified by gamma irradiation to enhance the functional properties. In third stage, ultrasound was used to modify the protein isolates and in fourth stage, protein isolates were heat treated at different pH values.

Sunflower protein isolates were prepared from both dephenolized and undephenolized meals of seed and kernel by isoelectric precipitation to investigate the effect of polyphenol on functional, structural, thermal and rheological properties. Protein isolates from dephenolized meals had higher percentage of proteins than protein isolates prepared from meal with polyphenols. Concentration of proteins in protein isolates obtained from kernel was higher as compared to protein isolates obtained from seeds. The colour of kernel protein isolates improved considerably after removal of polyphenols, however, colour of seed protein isolates did not improve to a larger extent. Higher solubility, emulsifying activity, emulsion stability, dispersibility, foaming capacity and foaming stability were found in protein isolates obtained from dephenolized meal. Lowest values of these properties were obtained at isoelectric point and highest at alkaline pH. Water binding capacity was higher for
protein isolates with higher phenolic content and further increased toward alkaline pH. Polyphenols in protein isolates decreased their available lysine content and reduced both the storage modulus and loss modulus of protein dispersions and formed more viscous and less elastic gels. The structural and thermal changes in protein upon phenolic interaction were studied using circular dichroism, differential scanning calorimetry, thermal gravimetric analysis, X-ray diffraction, sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE), and Fourier Transform Infrared (FT-IR) spectroscopy. Presence of phenolic compounds in proteins decreased the ordered structure content with parallel increase in unordered structure content. Denaturation temperature was higher for protein isolates with phenolic compounds while, enthalpy decreased upon phenolic interaction. In the presence of phenolic compounds, higher mass loss was observed upon heating. Crystalinity and crystal size got increased after removal of phenolic compounds. Protein isolates from kernels had higher percentage of crystalinity and crystal size as compared to seed protein isolates. Phenolic compounds resulted in rough surface with aggregation in protein isolates. Presence of polyphenols reduced the hydrophobicity as well the sulphhydryl content and increased the particle size of proteins.

The effect of gamma irradiation on physicochemical, antioxidant, functional and structural and thermal properties of sunflower protein isolates was investigated. Protein isolates were irradiated at dose level of 0, 10 20, 30, 40 and 50 kGy. Protein solutions obtained from irradiated protein isolates were found to be more turbid and had a higher particle size. Surface hydrophobicity was increased while sulphhydryl content was reduced indicating the conformational changes in protein isolates. Surface hydrophobicity was increased from 122.73 to 139.67 and free sulphhydryl content was decreased from 7.60 to 7.22 µmol/g and total sulphhydryl content from
78.79 to 52.26 µmol/g. Available lysine content decreased from 3.30 to 3.21 g/100 g. Lightness of protein isolates was reduced with increase in yellow-brown colour indicating the formation of Maillard reaction products. DPPH radical scavenging of protein isolates was increased from 5.79 to 19.46 % and total antioxidant capacity was increased from 7.54 to 27.50 %. Solubility, oil binding capacity, emulsion properties and foaming properties were improved, while water binding capacity was impaired. Secondary structure of proteins was disrupted with decrease in α-helix content and concomitant increase in β-sheet content. The change in fluorescence spectra was observed indicating the alteration in tertiary structure of protein molecules mainly due to the conformational changes especially aggregation and crosslinking of protein molecules. Increase in thermal stability of protein after irradiation was found as determined by differential scanning calorimetry and thermal gravimetric analysis. Molecular weight of protein was increased in a dose dependent manner due to protein-protein crosslinking. Storage (G') and loss modulus (G'') of protein dispersion was increased after gamma irradiation. Gamma irradiation treatment can be used to change the conformation of proteins, which could improve their functionality and widen the application area in food systems.

The influence of high intensity ultrasound (HIUS) on physicochemical, functional, structural and thermal properties of sunflower protein isolates was investigated. Protein solutions (10 % w/v) were treated with ultrasound probe (20 kHz) and ultrasound bath (40 kHz) for 5, 10, 20 and 30 min. Thermal stability of protein isolates was reduced as indicated by differential scanning calorimetry. Minimum thermal stability was observed at 20 min of sonication and increased further with increase in treatment time indicating aggregation at prolonged sonication. SDS-PAGE profile of proteins showed a significant reduction in molecular weight.
Further, surface hydrophobicity and sulfhydryl content increased after HIUS treatment indicating partial unfolding of protein and reduction in the intermolecular interactions. The particle size analysis showed that HIUS treatment reduced the particle size. Less turbid solution were observed largely due to reduction in particle size. HIUS decreased the available lysine content in protein isolates. Solubility, emulsifying capacity, emulsion stability, foaming capacity, foam stability and oil binding capacity were improved significantly, while as, water binding capacity was decreased. The effect of ultrasound on rheological properties was examined by the monitoring the changes in storage modulus (\(G'\)), loss modulus (\(G''\)) and loss tangent. Storage modulus and loss modulus increased, while loss tangent decreased, indicating the formation of stronger gel after ultrasound treatment. Change in tertiary and secondary structure was observed by intrinsic fluorescence and circular dichroism, respectively. The conformation of protein was altered with decrease in \(\alpha\)-helical content and concomitant increase in \(\beta\)-sheet content. Ultra-sonication changed the surface morphology considerably with more heterogeneous and disordered surfaces and showed the formation of clumps with different sizes and shapes. The regular confirmation of proteins was altered along with reduction in crystalinity in HIUS treated samples as measured by X-ray diffraction. Thermal gravimetric analysis showed that ultra-sonicated protein isolates were more prone to thermal degradation as compared to the untreated protein isolates. Probe ultrasound treatment showed higher effect on the physicochemical, functional, structural and thermal changes in protein isolates as compared to bath ultrasound treatment. The structural changes in protein isolates altered their behavior, which resulted in better functional properties and thereby increased the application in different food systems.
Proteins near isoelectric pH have low surface net charge, which have significant negative effect on their functionality. Application of heat near isoelectric pH can negate the effect of low charge on protein molecules and would subsequently improve their functionality. Protein isolates were subjected to heat treatment at 80°C for 5, 15 and 25 min at three pH values (3.5, 4.5 and 5.5) and were then evaluated for physicochemical, functional, structural and thermal properties. It was observed that surface hydrophobicity and sulfhydryl content of sunflower protein isolate were lower near isoelectric point and was increased with heat treatment indicating the conformational changes in protein structure. Highest particle size was observed at pH 4.5 due to aggregation of proteins and was further increased with heat treatment at all pH values. More turbid solutions were obtained from protein isolate prepared at pH 4.5 and increase in heat treatment time caused more turbid solutions. SDS-PAGE analysis showed that heat treatment near isoelectric point did not cause hydrolysis of protein molecules. Among functional properties, solubility was increased at isoelectric point after heat treatment due to denaturation of protein isolates and exposure of hydrophilic groups. Other functional properties like emulsification and foaming properties were also increased with heat treatment. Water binding capacity was impaired with heat treatment near isoelectric point but oil binding capacity was increased with heat treatment. The strength of gel prepared from treated protein isolates was lower than the gels from native protein isolates and gel strength was increased with increase in temperature treatment. Weak gels were obtained from protein isolates prepared at pH 4.5 compared to the gels obtained from protein isolates prepared at pH 3.5 and 5.5. Higher denaturation temperatures were observed in treated protein isolates than native protein isolates and increased with increase in thermal treatment time. Treated protein isolates showed more resistance against
thermal degradation than native protein isolates as was evident from thermal gravimetric analysis. Protein isolates prepared at pH 4.5 and at 25 min temperature treatment showed highest resistance against thermal degradation. Secondary and tertiary structure determined by circular dichroism and intrinsic fluorescence was significantly altered after thermal treatment. Protein isolates prepared at pH 4.5 had higher α-helix content than protein isolates prepared at pH 3.5 and 5.5 but lower than native protein isolate. α-helix content decreased with concomitant increase in β-sheet content with increase in heat treatment at all three pH values. Lower crystal size along with reduced crystallinity was observed in treated protein isolates than native protein isolates and was further reduced with increase in heating time as was determined by X-ray diffraction.

Amino acid analysis of protein isolates was performed to analyze the effect of polyphenol interaction and three modifications on the amino acid profile and nutritional parameters. Polyphenol interaction and modification techniques showed considerable effect on the amino acid profile and nutritional parameters. Among the modification techniques heat treatment showed more effect on the nutritional quality of protein isolates while as ultrasound treatment showed least effect.