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Interfacial and emulsifying properties of Lentil protein isolate

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Abstract

The dynamic interfacial tension (DIFT) at oil-water interface, diffusion coefficients, surface hydrophobicity, zeta potential and emulsifying properties, including emulsion activity index (EAI), emulsion stability index (ESI) and droplet size of lentil protein isolate (LPI), were measured at different pH and LPI concentration, in order to elucidate its emulsifying behaviour. Sodium caseinate (NaCas), whey protein isolate (WPI), bovine serum albumin (BSA) and lysozyme (Lys) were used as benchmark proteins and their emulsifying property was compared with that of LPI. The speed of diffusion-controlled migration of these proteins to the oil/water interface, was in the following order: NaCas > LPI > WPI > BSA > Lys, while their surface hydrophobicity was in the following order: BSA > LPI > NaCas > WPI > Lys. The EAI of emulsions stabilized by the above proteins ranged from 90.3 to 123.3 m²/g and it was 93.3±0.2 m²/g in LPI-stabilized emulsion. However, the stability of LPI-stabilized emulsions was slightly lower compared to that of WPI and NaCas-stabilized emulsions at the same protein concentration at pH 7.0. The ESI of LPI emulsions improved substantially with decrease in droplet size when protein concentration was increased (20-30 mg/ml). Reduction of disulfide bonds enhanced both the EAI and ESI compared to untreated samples. Heat treatment of LPI dispersions resulted in poor emulsion stability due to molecular aggregation. The stability of LPI-stabilized emulsions was found to decrease in the presence of NaCl. This study showed that LPI can be as effective emulsifiers of oil-in-water emulsions as are WPI and NaCas at ≥20 mg/ml concentrations both at low and neutral pH. The emulsifying property of LPI can be improved by reducing the intra and inter-disulfide bond by using appropriate reducing agents.

Key words: Lentil protein isolate, emulsion, interfacial tension, diffusion coefficient, surface hydrophobicity

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1. Introduction

Lentils are one of the most economic sources of plant proteins and belong to the legume family. The protein content of lentils varies from about 22 to 31% (Adsule et al., 1989). Globulins constitute the major protein fraction in lentil. Lentil proteins have an important role in the structure and texture of foods derived from or containing lentils. The functional properties of proteins reflect the inherent properties of proteins, as well as the manner with which they interact with other components of food. Soy protein is one of the most thoroughly studied plant proteins for its functional properties (Kinsella, 1979). There is increased interest in legume proteins, including lentil proteins, as they can be used as good substitute for animal proteins (Alsohaimy et al., 2007). In this regard, the understanding of factors affecting the functional properties of lentil proteins enables better control of these properties, which will facilitate the novel application of these proteins. The functional properties such as solubility, water and oil absorption capacity, gelation, foam and emulsion formation of lentil protein isolate were studied to some extent previously (Bora, 2002; Kaur et al., 2007; Hsu et al., 1982; Joshi et al., 2011). However, there is lack of fundamental knowledge and supporting data on the interfacial and emulsification properties of lentil proteins. These interfacial and emulsification properties are essential, particularly when the lentil proteins are intended to be used as emulsifiers.

Many naturally occurring and processed foods are in an emulsified state, and emulsions play an important part in structure formation of many foods such as milk, butter, soups, sausages, sauces and ice-cream. Emulsions are in a thermodynamically unstable state, produced by the dispersion of two mutually immiscible liquids into one another (McClements, 2005). The formation of an emulsion requires input of large amount of energy and the interface active emulsifiers are required for stability of emulsions (McClements, 2004). Proteins are amphiphilic molecules and can be used as emulsifiers to stabilize emulsions (Damodaran, 1996). Proteins are surface active and preferentially migrate to the oil-water interface and form a protective adsorption layer around the oil droplets which prevents them coalescing. As emulsifiers, proteins lower interfacial tension at oil-water interface, and hence, enhance emulsion stability. The effectiveness of proteins as emulsifiers depends on the speed with which they migrate to the oil-water interface and their stability at the interface.

Proteins are known to be effective emulsifiers and hence are commonly used in commercial food emulsions. The formation and stability of the protein-based emulsions are principally related to the surface-activity of proteins at fluid/fluid interfaces (Dagleish, 2004). The amphiphilic nature of the protein molecule allows it to attach with surfaces of different polarity. The adsorption of protein at fluid-fluid interface occurs in sequence starting with diffusion of protein molecule from bulk into an oil/water interface, penetration and attachment into the adsorbed layer followed by molecular rearrangement of the adsorbed protein molecules. As the proteins are macromolecules, their diffusion and consequently their adsorption are relatively slow processes (Lefebvre & Relkin, 1996). Therefore, study on the kinetic aspects of adsorption is very important in order to broaden the applicability of proteins as effective emulsifiers. Depending on the rigidity of protein molecule and the nature of the interface, proteins can unfold in the interfacial layer or retain their tertiary structure. Lysozyme has a rigid structure with many intramolecular disulfide bonds and retains most of its native conformation when adsorbed in the interface and hence it tends to be a poor emulsifier (Beverung et al., 1999). The proteins which have structurally flexible random coil configuration such as β -casein tend to be more surface-active (Magadassi & Kamishny, 1996).

Proteins carry charge depending on the pH of the solution. Consequently, emulsion droplets may have an electric charge that depends on the type of surface active molecule present and the pH of the aqueous phase. The charge on a droplet is important because it determines the nature of the interactions with other charged species and controls the bulk physicochemical properties of emulsions (McClements, 2005). Kato and Nakai (1980) had demonstrated a strong correlation between surface hydrophobicity, interfacial tension and emulsification properties of proteins. It has been generally accepted that the surface charge of protein molecules, together with hydrophobicity strongly influences their adsorption at fluid-fluid interface (Magadassi & Kamishny, 1996).

Emulsifying and interfacial properties are important functional properties of lentil proteins. The understanding of the interfacial characteristics and emulsion behaviour at fluid/fluid interface at different processing conditions is essential for the application of lentil proteins, particularly the protein enriched lentil protein isolate (LPI) as an emulsifying agent. Therefore, in this study, we have evaluated the emulsifying and interfacial properties of LPI at different processing conditions and compared with the other better characterized food

proteins such as lysozyme (Lys), bovine serum albumin (BSA), sodium caseinate (NaCas) and whey protein isolate (WPI).

2. Materials and methods

2.1 Materials

The seeds of Aldinga cultivar of lentil (*Lens culinaris*) were received from Department of Primary Industries (Horsham, Australia) and protein was isolated from these seeds. Sodium caseinate (NaCas) and whey protein isolate (WPI) with a protein content of 95% (MG 2972, MG Nutritionals, Australia) was used as received. Pure olive oil (Moro Brand, Spain) was purchased from local supermarket, Australia. The naturally occurring surface-active content of this oil was removed using activated charcoal as described in Section 3.2.3. Bovine serum albumin (BSA) and lysozyme were obtained from Research Organics (Australia) and Sigma Aldrich (Australia), respectively.

2.2 Methods

2.2.1 Protein isolate preparation

The lentil protein isolation and purification method previously reported by Joshi et al. (2011) was followed in this study. Briefly, dehulled lentil flour was extracted in alkaline condition (pH 8.0) for one hour using a magnetic stirrer to extract protein followed by centrifugation for 30 min at 15,000 rpm in SS34 Sorvall centrifuge (Sorvall Instruments, Wilmington, DE, USA). The clear protein extract was adjusted to pH 4.5 and the protein was allowed to precipitate. The protein precipitate was recovered by centrifugation, washed with distilled water and subsequently neutralized. The neutralized sample was frozen at -80°C and subsequently freeze dried. The protein content of the freeze-dried lentil protein isolate (LPI) powder was 90.2±1.46% (dwb). The sample was kept in an air tight container at -18°C until it was used.

2.2.2 Protein dispersion preparation

The protein isolate powder was dispersed in phosphate buffer (0.1 M, pH 7.0) by using a magnetic stirrer at 500 rpm for 30 min. All these dispersion experiments were carried out at ambient condition (20°±2°C). The protein dispersion was then centrifuged in a Sorvall centrifuge (SS34) for 30 min at 12,000 rpm to remove the insoluble matter. The clear protein dispersion obtained in this way was used to measure dynamic interfacial tension and

preparation of emulsions. Sodium azide (0.01% w/v) was used as an antimicrobial agent. The protein concentration in dispersions was 10 mg protein/ml, if not mentioned otherwise.

Lentil protein concentration ranging from 0.1 to 30 mg/ml in phosphate buffer (pH 7.0) was used in order to study the effect of protein concentration on the interfacial tension and emulsifying properties. The effect of ionic strength on LPI was studied at different ionic strength (0-1.0 M of NaCl) by mixing NaCl with protein powder (10 mg/ml, pH 7.0) during the preparation of dispersion. Similarly, a reducing agent, dithiothreitol (DTT) was used at 0-30 mM concentration in LPI dispersion (10 mg/ml, pH 7.0) in order to study the effect of disulfide bond formation. LPI powder was dispersed in citrate buffer solution (0.1 M) at different pH (3.0- 6.0) to study the effect of pH.

2.2.3 Charcoal treatment of olive oil

Olive oils are known to contain some natural surface active components. These surface active impurities interfere with the adsorption of the emulsifier at the fluid-fluid interface and can lead to erroneous results. Dopierala et al. (2010) found that different brands of olive oils showed different interfacial tension due to presence of different amount of naturally occurring surface active components.

The olive oil used in dynamic interfacial tension measurements and emulsion studies was purified by charcoal treatment. For this, the charcoal was mixed with oil in 1:10 ratio (w/w) and agitated using a magnetic stirrer overnight. The treated oil was separated from charcoal by centrifugation. The supernatant was filtered through Whatman no. 40 and finally through 0.20µm syringe filter.

The equilibrium interfacial tension attained at olive oil-water interface after one hour of drop formation was 20.59 ± 0.12 mN/m. This interfacial tension value is close to the literature value (23.56 mN/m) for a purified olive oil (Fisher et al., 1985) and ensured that the native surfactants were removed from the oil samples.

2.2.4 Measurement of dynamic interfacial tension (DIFT)

Dynamic interfacial tension at olive oil-water interface was measured by a pendant drop method using a drop profile tensiometer (PAT-1TM, Sinterface Technologies, Germany). In these measurements, the aqueous phase was protein solution and oil phase was olive oil. The

measurement chamber was directly connected to syringe with plunger through screw thread. An oil droplet of 10 mm³ volume was formed manually at the tip of the capillary. The temperature of the test chamber was kept constant at 20±0.5°C by circulating water using a thermostat controlled water bath (Cool Tech 320, Germany). The tensiometer was calibrated at both the air-water (72 mN/m) and oil-water (20.59 mN/m) interface at the same temperature. Deionised water was used throughout these experiments. Each experiment was run at least three times and the representative data was presented in the result and discussion section.

2.2.4.1 Determination of diffusion coefficient

Proteins due to their amphiphilic nature tend to migrate towards oil-water interface, which is thermodynamically favourable. Ward and Tordai (1946) proposed for the first time that the adsorption of amphiphilic molecules from bulk to interfaces is diffusion controlled and the adsorption phenomenon can be quantified by using equation (1) below.

$$\Gamma = 2c_s \left(\frac{D t}{\pi} \right)^{1/2} - 2 \left(\frac{D}{\pi} \right)^{1/2} \int_0^t c_s(t-\tau) d\tau^{1/2} \quad (1)$$

where, Γ is the number of molecules per unit area adsorbed at time t , c is the bulk concentration, c_s is the subsurface concentration and is a function of time, D is the diffusion coefficient and τ is the dummy variable of integration. This equation cannot be solved due to complication to account for back diffusion.

2.2.4.1.1 Determination of diffusion coefficient (D_{eff})

Since it is very difficult to determine diffusion coefficient of the adsorption process using equation (1) even numerically, Fainerman et al. (1994) proposed an asymptotic solution in short time range ($t \rightarrow 0$). The diffusion coefficient determined in this way is called effective diffusion coefficient (D_{eff}) as it not only incorporates molecular diffusion but also other aspects related to protein structural confirmation. In this approach the slope of interfacial tension (σ) versus square root of time ($\sqrt{t}$) curve at early stage of adsorption ($t \rightarrow 0$) is used to determine D_{eff} , by using equation (2) given below (Fainerman et al., 1994). The interfacial tension is time dependent and at $t \rightarrow 0$ is a good indicator of adsorption process.

$$\left(\frac{d\sigma}{dt^{1/2}}\right)_{t \rightarrow 0} = -2nRTc_o \left(\frac{D_{eff}}{\pi}\right)^{1/2} \quad (2)$$

where $n = 2$ for ionic surfactant. In our calculation $n = 2$ is used as proteins are charged in pH other than their isoelectric point and hence can be assumed to be ionic. R is the universal gas constant ($J \text{ mol}^{-1} \text{ K}^{-1}$), T is the absolute temperature (K) and c_o is the bulk concentration (mol m^{-3}). The D_{eff} is the effective diffusion coefficient of protein molecules with a unit of (m^2/s). Equation (2) can be re-written as equation (3) to determine the D_{eff} .

$$D_{eff} = \frac{\pi}{4} \left[\frac{1}{nRTc_o} \left(\frac{d\sigma}{dt^{1/2}}\right)_{t \rightarrow 0} \right]^2 \quad (3)$$

For the asymptotic solution to be applicable the plots of interfacial tension (σ) versus square root of time ($\sqrt{t}$) should yield a straight line. The slope of this line is then used in equation (3) to determine effective diffusion coefficients. In this study we have used the straight line section of (σ) versus the square root of time ($\sqrt{t}$) at $t \rightarrow 0$ to determine the slope. As almost all the σ versus $\sqrt{t}$ plots had a straight line section ($R^2 > 0.98$) (data not shown), the asymptotic solutions is valid in order to determine the effective diffusion coefficient. We require the molecular weight of proteins to solve equation (3). For this purpose, a molecular weight of 55 kDa was chosen based on the molecular weight of the most abundant protein fraction of LPI observed through SDS-PAGE (Joshi et al., 2011).

2.2.5 Emulsion preparation

The oil-in-water emulsion was prepared using the protein dispersion (section 3.2.3) and olive oil. Olive oil was used for emulsion preparation as it is one of the most widely used oil in Australia. The oil fraction of the emulsion was maintained at 0.1 (v/v). The emulsion was prepared using a Microfluidizer (Microfluidics, M-110L, US) and each batch of emulsion was prepared by passing through 5 times at 50 kPa. The emulsions formed in this way were stored at ambient temperature.

2.2.5.1 Characterization of emulsions

2.2.5.1.1 Emulsion activity index (EAI) and emulsion stability index (ESI)

The emulsifying activity index (EAI) and emulsifying stability index (ESI) of emulsions were determined by turbidimetric methods (Pearce & Kinsella, 1978). Freshly prepared emulsion was diluted 100 folds with 0.1% (w/v) sodium dodecyl sulfate (SDS) and mixed adequately in a vortex mixer. The absorbance of diluted emulsion was measured using a UV spectrophotometer (Varian Cary® 50, Agilent Technologies) at 500 nm wavelength. The turbidity of emulsion was calculated using equation (4) given below.

$$T = 2.303 \times \frac{A}{L} \times D \quad (4)$$

where, T is the turbidity of emulsion (m^{-1}), A is the absorbance (dimensionless), D is the dilution factor (dimensionless) and L is the light path length (m). The emulsion activity index (EAI, expressed in m^2/g) was calculated as:

$$\text{EAI} = \frac{2 \times T_o}{\Phi \times C \times 1000} \quad (5)$$

where, T_o is the turbidity of fresh emulsion, Φ is the oil volume fraction (dimensionless) and C is the concentration of protein present in the protein dispersion (mg/ml).

Similarly, the emulsion stability index (ESI, expressed in hr) was calculated using equation (6), given below.

$$\text{ESI} = \frac{A_o - A_t}{A_o} \times t \quad (6)$$

where t is the time interval (24 hrs), A_o and A_t are the absorbance at $t = 0$ and after 24 hr, respectively.

2.2.5.1.2 Zeta average size and Zeta potential

A Zetasizer (ZS-90, Malvern instruments Ltd, UK) was used to measure zeta potential and zeta average size of oil-in-water emulsion droplets. The apparatus measures the direction and the velocity of the oil droplets dispersed in an emulsion by applying an electric field and calculates their zeta potential using Smoluchowski model (Kirby & Hasselbrink, 2004). For the sample preparation, the emulsion was diluted 100 times with MilliQ water. Clear disposable zeta potential cuvettes were used in these measurements. Three replicate measurements were made for each sample. Measurements for both the zeta average size and zeta potential were performed within 24 hr of emulsion preparation.

2.2.6 Surface hydrophobicity

The surface hydrophobicity of the LPI was measured using 1-anilinino-naphthalene sulfonate (ANS) as a hydrophobic probe based on the method described by Kato and Nakai (1980). ANS turns into fluorescent upon binding onto hydrophobic sites. In this method, ANS forms a conjugate with hydrophobic groups of protein which are mostly exposed on the surface and its fluorescence intensity (FI) is measured. These hydrophobic groups exposed on the surface play a major role in hydrophobic interactions and is suggested to correlate with the emulsifying and foaming properties of food proteins (Kato & Nakai, 1980). For sample preparation, protein dispersions were serially diluted with phosphate buffer (0.1 M, pH 7.0). Eight micro liters of ANS (8.0 mM in 0.1 M phosphate buffer, pH 7.0) were added to 4 ml of diluted protein dispersion and mixed well. The solution was incubated in dark for 15 minutes before the measurements were made. FI of ANS-protein conjugates was measured with Shimatzu spectrofluorometer (RF 550). The excitation and emission wavelengths used were 390 and 470 nm, respectively. FI for ANS alone was determined by using same amount of ANS in MilliQ water. The relative fluorescence intensity (RFI) for each concentration was calculated using equation (7) given below (Chaudhuri et al., 1993).

$$RFI = \frac{F_s - F_o}{F_o} \quad (7)$$

where, F_s and F_o are fluorescence intensity of protein-ANS conjugate and ANS alone, respectively. The surface hydrophobicity was expressed as the initial slope of the plot of RFI versus protein concentration (mg/ml).

2.2.7 Statistical analysis

Triplicate runs were carried out for each experiment. The data were subjected to statistical analysis, using one way analysis of variance (ANOVA) to evaluate the significance of differences ($p < 0.05$). The above analysis was used for all the emulsion properties. SPSS version 10.0 software (SPSS Co., Chicago U.S.) was used for this purpose. Data are presented as the mean and standard deviation of the triplicates.

3. Results and discussion

3.1 Comparison of interfacial properties of different Proteins at the oil/water interface

3.1.1 Dynamic interfacial tension and the effective diffusion coefficient

The dynamic interfacial tension (DIFT) at olive oil/water interface for the globular proteins, lentil protein isolate (LPI), bovine serum albumin (BSA), lysozyme (Lys) and whey protein isolate (WPI), and the non-globular protein, sodium caseinate (NaCas) at the same concentration (10 mg/ml) and pH 7.0 are presented in Figure 1. As can be seen from this figure, all of these proteins are able to adsorb to the oil/water interface as evidenced by decrease in the interfacial tension of the same interface in the absence of proteins. However, the trend of decrease in the interfacial tension as a function of time is different for globular and non globular proteins. The non-globular protein, NaCas displayed distinctive interfacial behavior with the lowest DIFT at oil-water interface. This protein attained its equilibrium interfacial tension value immediately after the start of the experiment. This high surface activity of the NaCas is attributed to its random coil molecular structure and lack of disulfide bonds both of which promote faster adsorption into the oil-water interface (Beverung et al., 1999).

It can be seen from Figure 1 that the globular proteins show similar trend of decrease in DIFT as a function of time and that they take a longer time to reach their equilibrium interfacial tension values. This interfacial behavior of globular protein is attributed to their tertiary and secondary structures, which take time to unfold and subsequently to lower the interfacial tension compared to the flexible random-coiled proteins such as caseins (Wilde, 2000). All the globular proteins have exhibited two distinct regimes on DIFT curve: a sharp decrease in DIFT (first phase) immediately after the formation of the interface followed by slow decrease in the DIFT (second phase) before reaching the equilibrium value. Depending on the compactness of globular proteins, the rate of decrease of interfacial tension in the first phase is different. It is now generally accepted that the first phase is related to the diffusion and adsorption of protein molecules into the interface and the second phase related to the conformational arrangement of adsorbed protein molecules in the interface. Beverung et al.

(1999) have reported three phases while studying the DIFT in dilute protein solutions. The initial lag or induction regime, usually observed in dilute protein solutions, was not observed in our experiments, most probably due to the high protein concentration used. Among globular proteins, lysozyme (Lys) exhibited the slowest tendency to decrease interfacial tension in the initial stage after drop formation. It could not reach its equilibrium interfacial tension value within 50-second long experimental time. This type of interfacial behavior of lysozyme indicates that this protein has compact and less flexible globular structure (Beverung et al., 1999).

As can be seen from Figure 1, the LPI displayed a typical interfacial behaviour of globular proteins at oil-water interface. The DIFT of LPI decreased rapidly immediately after the formation of the oil-water interface, after which it exhibited much slower decrease in the DIFT values. Among the globular proteins tested, the LPI was the fastest in reducing the DIFT of the interface. The equilibrium interfacial tension value of LPI at oil-water interface was the lowest among the tested globular proteins. In terms of rate of decrease in interfacial tension, LPI was close to whey protein isolate. This time dependent interfacial behavior of LPI can be attributed to its globular structure which takes some time to unfold at the interface. LPI is composed of several protein subunits of different molecular weights in varying amounts and some subunits are linked by disulfide bonds (Joshi et al., 2011). Depending on their molecular properties, some of these protein subunits might selectively and/or differentially adsorb to the oil/water interface.

The interfacial behavior of proteins at oil-water interface is important as it is associated with their emulsifying property. During emulsion, oil phase breaks down into numerous oil droplets in the continuous aqueous phase. In high pressure and high shear systems such as microfluidizer the formation of emulsion takes place very rapidly and occurs within a timescale of milliseconds (Jafari et al., 2008). The role of an emulsifier especially in these rapidly occurring processes is important in stabilizing the emulsions by preventing the droplets from coalescing. Protein performs these functions by adequately lowering the interfacial tension at oil-water interface of newly formed droplets which facilitates formation and smaller droplets and their stability against coalescence by covering the droplets with thin layer of protective coating (McClements, 2004)). Therefore, the quicker the protein adsorbs to the surface of the droplets during homogenization or emulsification process, the smaller the droplets produced and the better the stability of the emulsions. In this regards, the DIFT

values of proteins immediately after the formation of new interface are more relevant to the emulsification property.

Table 2 lists the effective diffusion coefficients D_{eff} for different proteins. Bulk concentration of protein solution was expressed as mol m^{-3} . For the calculation purpose, molecular weight of LPI was considered as 55 kDa, which is the most abundant subunit band in non-reducing SDS-PAGE (Joshi et al, 2011), however, the contribution of other minor proteins present in the LPI can also be expected. Among the proteins, LPI has the highest D_{eff} ($1.22 \times 10^{-13} \text{ m}^2/\text{s}$) closely followed by BSA. The D_{eff} for sodium caseinate (NaCas) was the lowest ($3.98 \times 10^{-15} \text{ m}^2/\text{s}$) in contrast to this, the lowest IFT was observed in the case of NaCas (Figure 1). This means that NaCas adsorbed extremely fast into the interface as a consequence the interfacial tension reaches almost to its equilibrium value (which is lowest) within less than 1 sec. At these relatively higher protein concentrations the oil-water interface was almost saturated by the NaCas by the time the first data as recorded during the IFT tests. Wüstneck et al (1996) have found D_{eff} value of $1.62 \times 10^{-8} \text{ m}^2/\text{s}$ and $9.42 \times 10^{-9} \text{ m}^2/\text{s}$ for β -lactoglobulin (0.02 mol/m^3) and β -casein (0.01 mol/m^3), respectively. The highest (0.7305 ± 0.01) and the lowest (0.1445 ± 0.01) gradient value at $t \rightarrow 0$ were found for Lys and NaCas respectively. This again signifies that interfacial tension change was still ongoing for compact globular proteins due to slow adsorption and conformation change. This has great impact on the emulsion application of proteins as the emulsion process is very short and occurs within few seconds breaking down oil into very small oil droplets and if the emulsifier used is not able to cover the newly formed interface then oil droplets start to coalesce leading to formation of large oil droplets which results into poor emulsion stability.

3.1.2 Emulsification properties of different proteins

The emulsion activity index (EAI) of emulsions stabilized by various proteins is presented in Figure 2 (a). EAI of these emulsions ranged from 90 to $123 \text{ m}^2/\text{g}$. The highest EAI value was obtained in BSA stabilized-emulsion. On the other hand, the highest stability (ESI) was displayed by NaCas-stabilized emulsion. The ESI value of LPI-stabilized emulsion was significantly lower than the ESI values of NaCas and WPI-stabilized emulsions ($p < 0.05$). The mean droplet size of oil droplets in LPI stabilized-emulsion was $0.585 \pm 0.027 \text{ }\mu\text{m}$ which is larger than the droplet size in NaCas, WPI and BSA-stabilized emulsions. As can be seen from Figure 2 (b), the mean size of oil droplets of all the emulsions were below $1 \text{ }\mu\text{m}$ except

in the case of Lys stabilized-emulsion ($8.04 \pm 1.37 \mu\text{m}$). According to Stoke's law, the creaming or the phase separation rate (terminal velocity) in oil-in-water emulsions is directly proportional to the square of the size (diameter) of the oil droplets. Therefore, the droplet size of oils in oil-in-water emulsions is one of the critical factor for the stability of the emulsion against creaming (Nir et al., 1994).

The surface hydrophobicity values for all the tested proteins are compared in Figure 3. Among all these proteins, BSA had the highest surface hydrophobicity (1935.0 ± 1), while Lys had the lowest (5.7 ± 0.2). The relative intensity of surface hydrophobicity for BSA and Lys are similar to those reported in the literature by other workers (Haskard & Li-Chan, 1998; Chaudhuri et al., 1993). The surface hydrophobicity value of LPI (392.1 ± 0.5) is higher than WPI (56.8 ± 2.7) while it is close to that of NaCas. The surface hydrophobicity of proteins is found to follow BSA>LPI>NaCas>WPI>Lys order. The lowest surface hydrophobicity of Lys helps to explain the reason for the slowest reduction of DIFT at the oil-water interface (Figure 1). Surface hydrophobicity influences the intermolecular interactions such as protein-protein or protein-lipid interactions. This supports the theory that initial protein adsorption is induced by hydrophobic interactions between the oil and hydrophobic protein patches at the exterior of the protein configuration (Beverung et al., 1999).

To elucidate the relation between surface charge of proteins and the stability of oil droplets in protein stabilized-emulsion, zeta potential values of the emulsions were also measured. The zeta potential values of the emulsions stabilized by all the proteins except Lys (Figure 2 c) were negatively charged due to protein coatings on surface of oil droplets. As can be seen from this Figure, the magnitude of zeta potential value of the LPI stabilized-emulsion is slightly lower ($-43.3 \pm 0.6 \text{ mV}$) than those of NaCas, WPI and BSA stabilized-emulsions (-55.0 to -56.1 mV). Lys stabilized-emulsion is found to have the lowest magnitude of zeta potential value ($9.0 \pm 0.9 \text{ mV}$). Zeta potential provides a measure of the net surface charge on the particle and potential charge distribution at the interface. It is a good indicator of magnitude of the repulsive interaction between colloidal particles, which is one of the forces governing stability of emulsion (Li & Tian, 2007). These imply that relatively lower stability of LPI stabilized-emulsion and very unstable Lys stabilized-emulsion is related to surface charge property of protein-stabilized emulsion.

3.2 Effect of LPI protein concentration

3.2.1 Interfacial properties and effective diffusion coefficients

The effect of LPI protein concentration (0.1 to 30 mg/ml, pH 7.0) on the DIFT values at the oil-water interface is illustrated in Figure 4. As can be seen from this Figure, the protein concentration had marked effect on the lowering of the interfacial tension. At a given time and within the protein concentration of 0.1-10 mg/ml, the higher the protein concentration the greater was the reduction in interfacial tension. The DIFT values of emulsions having 10 mg/ml and 30 mg/ml protein in the bulk were not significantly different ($p>0.05$). This means that the oil-water interface gets saturated when the bulk protein concentration is at or above 10 mg/ml. The fall in DIFT values immediately after the formation of the fresh interface is greater in the case of 30 mg/ml protein concentration compared to 10 mg/ml. This can be attributed to the shorter induction time due to higher diffusion rate of protein molecules when protein concentration is higher in the bulk. However, when the critical micelle concentration (or saturation concentration) is reached, further decrease in interfacial tension does not take place (Shrestha et al., 2008). The role of an emulsifier is to reduce the interfacial tension by coating the surface of the newly formed oil droplets with protective film and stabilize these droplets in the aqueous continuous phase. To achieve this emulsion stabilisation, the concentration of emulsifier is one of the main factors. When the concentration of the emulsifier is low in the bulk solution, then the speed of decrease in the DIFT will be slow and the equilibrium interfacial surface tension will be relatively high. This means that the time for the formation of the protective film around the oil droplets will be relatively longer and the resultant film will be thinner. This means that the lower LPI concentration (lower than 10 mg/ml) will not be adequate in stabilizing the oil-in-water emulsions.

The effective diffusion coefficients at different protein concentration showed that as the protein concentration increased D_{eff} was found to decrease (Table 2). Wüstneck et al. (1996) reported a similar tendency for the decrease in effective diffusion coefficients as a function of increasing protein concentration in the case of β -lactoglobulin and β -casein. According to these authors at low protein concentration, the area per protein molecule strongly increases at the interface due to rearrangement of molecules as the adsorption starts, however, at high concentration it is hindered by further adsorbing molecules and higher concentration complete unfolding or re-orientation does not occur due to overcrowding of protein molecules at the interface (Fainerman & Miller, 1998). The surface/interfacial area available

per protein molecule strongly influences effective diffusion coefficient values. This fact can further be confirmed by decrease in protein gradient when the bulk protein concentration increased (Table 2).

Furthermore, as we have reasoned in Section 4.1.1, the protein adsorption process (at the interface) is extremely fast. This means that the time the instruments such as PAT-1 take their first reading, the interface almost gets saturated especially at higher bulk protein concentrations. This means that the diffusion process of the protein to the interface gets greatly slowed down in the time frame of experiments. The lower diffusion coefficients at higher protein concentrations is another confirmation that higher the protein concentration in the bulk, faster the instantaneous protein adsorption during emulsion process.

3.2.2 Emulsion properties

The effect of lentil protein concentrations (0.1 to 30 mg/ml) on the turbidity (m^{-1}) and ESI of LPI stabilized-emulsions is presented in Table 1. As can be seen from these tabulated data, the increase in the LPI concentration from 10 mg/ml onwards had enhanced the emulsion properties as indicated by the high turbidity and ESI values along with the smaller oil droplet size. At low protein concentration (up to 5.0 mg/ml), the turbidity was much lower and increased nearly three fold when protein concentration increased to 10 mg/ml. These results corroborate with the DIFT results and suggest that that prior knowledge of DIFT helps in formulating stable emulsions. The highest turbidity values were obtained in emulsions when 20 and 30 mg/ml LPI concentration was used. ESI was also found to increase by more than three fold when protein concentration increased from 10 to 30 mg/ml, indicating an improved stability at higher protein concentration. This effect can be attributed to the formation of multilayered protein shell around oil droplet which successfully prevents the coalescence of oil droplets. The above observations highlights the importance of LPI concentration in the stability of LPI-stabilized emulsions. The emulsion prepared by using ≥ 10 mg/ml LPI was fairly stable for few days at room temperature without visible phase separation.

The effect of LPI protein concentration on the mean droplet size of oil phase is listed in Table 1. These results show that the higher the protein concentration (within 0.1-30 mg/ml range), the smaller is the mean size of the oil droplets in the emulsion. At 10 mg/ml of LPI, the average oil droplet of emulsion was 0.585 ± 0.027 μm . On the other hand, when the LPI concentration was increased above 5 mg/ml, the increase in the LPI concentration in the bulk

could not decrease the average droplet size significantly ($p>0.05$) indicating that the LPI bulk concentration of 10 mg/ml is adequate in providing stable coating around the oil droplet. This finding also corroborates with the DIFT data and suggests that the oil-water interface is saturated by protein molecules when the LPI concentration is 10 mg/ml. In contrast to this, at low LPI concentration (<5 mg/ml) the oil droplet size (>1 μm) was much larger, which produced an unstable emulsion. These findings agree well with McClements (2004)'s suggestions that the droplet size in emulsions is controlled by the concentration of emulsifier, because insufficiently covered emulsion droplets will coalesce, whereas at excess concentration the droplet size is independent of emulsifier concentration. The data on the effect of protein concentration on the size of oil droplets also correlated well with ESI outcome, as the emulsions having smaller droplet size had higher ESI values.

The increase in the LPI concentration did not show marked effect on zeta potential value and was fairly constant as can be seen from data in Table 1. There was no significant difference among the zeta potential values of emulsion containing LPI concentration except at 30 mg/ml ($p>0.05$). The zeta potential values for the LPI stabilized-emulsion are in the range of -40.7 to -43.3 mV with slight decrease in the magnitude of zeta potential at high protein concentration. These results on zeta potential values indicate that the higher stability of emulsion at higher LPI concentration is primarily due to steric repulsion mechanism (Li & Tian, 2007) due to formation of multilayer at excessive LPI concentration rather than the extent of surface charge.

3.3 Effect of Reducing agent: Dithiothreitol (DTT)

3.3.1 Interfacial properties and effective diffusion coefficients

The effect of reduction or absence of disulfide bonds on the interfacial behavior of LPI was studied by treating the LPI with dithiothreitol (DTT) at 5 to 30 mM concentration. The effect of variation of DTT concentration on dynamic interfacial tension of LPI dispersions (10 mg/ml, pH 7.0) is shown in Figure 5. The DIFT of LPI dispersion at the same concentration without DTT is taken as a control for comparison. As can be seen from the plot of DIFT, LPI dispersions treated with DTT showed slightly higher reduction in interfacial tension than the control in the initial regime (<5 sec), however, there was no noticeable distinction between treated and untreated protein dispersions afterwards. Kim & Kinsella (1987) had reported similar results regarding the effect of reduction in disulphide bonds in soy protein. They

found that the treatment of glycinin with DTT had reduced all the disulfide bonds. The DTT cleaved both the intra and inter-molecular disulfide bonds making protein molecules more flexible which permitted faster diffusion into oil-water interface. This result indicates that the reduction of interfacial tension is diffusion controlled in the early stage of formation of emulsion. In this early stage, the prevention of protein aggregation can be helpful to enhance the diffusion of protein molecules to the oil-water interface. Slow diffusion is one of the limiting factors for the globular proteins in their application as emulsifier. There was no noticeable difference in DIFT of LPI when the DIFT concentration was increased from 10 mM to 30 mM. This indicates that DTT concentration as low as 10 mM is adequate to prevent the formation of intra and inter molecular disulfide bonds. Similar effect of DTT concentration on soy protein was reported by Kim & Kinsella (1987).

DTT has found to lower D_{eff} of LPI dispersion compared to that of the control. This is in line with the previous results that the presence of either more molecules per unit volume or lower number of disulphide bonds (less aggregation) would favor more rapid adsorption and hence lower diffusion coefficient due to surface saturation. Likewise, the gradient values of σ versus $\sqrt{t}$ curve for DTT treated samples at the beginning of the experiment ($t \rightarrow 0$) were also found to be lower than the control.

3.3.2 Emulsion properties

The effect of DTT (0, 5, 10, 20 mM) treatment on EAI and ESI of LPI stabilized-emulsion (10 mg/ml protein at pH 7.0) is given in Table 1. As can be seen from these data, compared to the control (without DTT at same protein concentration), the emulsions stabilized by DTT-reduced LPI have higher EAI values. Nevertheless, no statistically significant effect on EAI value was observed when the concentration of DTT was increased from 5 mM to 30 mM. These results corroborate well with DIFT results (Figure 5), which showed that the mere addition of 5 mM DTT reduced the disulphide bonds which might have helped the LPI molecules to diffuse faster into the oil-water interface. It can also be seen from Table 1 that the stability of emulsion is enhanced in the presence of DTT up to 10 mM. However, at higher concentration of DTT (20 and 30 mM), the ESI values were found to decrease instead of increasing. A similar effect of reducing agents such as DTT, sulfite and β -mercaptoethanol on the partial or full reduction of disulfide bonds of soy glycinin was reported previously (Kella et al., 1989; Kim & Kinsella, 1987; Nir et al., 1994). The improvement in the emulsion

properties of proteins when they are treated with DTT could be attributed to the dissociation of subunits due to cleavage of disulfide bonds, unfolding of subunits and increase in solubility due to less aggregation. The reason for lower emulsion stability at higher DTT concentration (>20 mM) may be due to excessive dissociation of proteins and subsequent generation of small fractions which do not help stabilizing the oil-water interface. The presence of excess amount of DTT may also have weakened the intermolecular interactions (such as hydrophobic and electrostatic) thereby negatively impacting the emulsion stability. These results show that the intermolecular interactions (hydrophobic and electrostatic) which are responsible for the formation of strong interfacial film can be affected by very high concentration of DTT. Although the effect of DTT treatment on the interfacial tension values was observed only in the first few seconds, nevertheless the implication of the DTT treatment is unequivocal in the formation and stability of LPI-stabilized emulsions.

The surface hydrophobicity of DTT treated and untreated LPI dispersions (Figure 6) was not found to be significantly different ($p > 0.05$) except at 30 mM concentration ($p < 0.05$). In contrast to this, some researchers found increase in surface hydrophobicity for soy protein and whey proteins when treated with reducing agent (Kim & Kinsella, 1987; Nir et al., 1994). Likewise, average oil droplet size of DTT treated and untreated LPI-stabilized emulsions were not significantly different ($p > 0.05$) (Table 1). The zeta potential value of LPI stabilized emulsion (Table 1) with 5 mM DTT was found to be lower than that of untreated emulsion. The zeta potential value increased with increase in DTT concentration at and above 10 mM of DTT.

3.4 Effect of Ionic strength (NaCl)

3.4.1 Interfacial properties and effective diffusion coefficient

The variation of interfacial tension at oil-water interface of LPI dispersion (10 mg/ml, pH 7.0) in the presence of NaCl (0 to 1.0 M) is presented in Figure 7. LPI dispersion without salt and having the same protein concentration was used as a control for comparison. It can be seen from this graph that at low concentration (0.1M) of NaCl, the rate of decrease of the DIFT at oil-water interface is slightly higher compared to that of the control. The DIFT values of the LPI-stabilized emulsions containing 0.1M NaCl are consistently lower compared to those of the control. This can be attributed to the salting-in effect of proteins resulting into high solubility in the presence of low concentration of salt. According to Damodaran & Kinsella

(1981), salt concentrations up to 0.1 M enhance solubility because hydrated salt ions especially the anions weakly bind to the charged group of proteins. However, when the salt concentration increases above 0.15 mM, it can rather reduce the protein solubility. It can also be seen from Figure 7 that the decrease in the DIFT at higher salt concentration (>0.5 M) is lower and that the DIFT values throughout the experiments were higher compared to those of the control. This can be attributed to the protein aggregation due to lower electrostatic repulsion in the presence of higher salt concentration. It has been previously reported that as the ionic strength increases, the net charge of protein progressively decreases due to preferential binding of ions (Damodaran, 1996). This promotes protein-protein interaction and protein aggregate formation, which ultimately results into slow diffusion of protein molecules into oil-water interface at high salt concentration. Furthermore, it has also been suggested that salt at its high concentrations can compete with protein for water to ionize itself. This competition effectively reduces the availability of water and increases the protein dehydration (Smith & Culbertson, 2000). The salt-induced protein dehydration enhances protein-protein interaction and promotes protein aggregation. However, at low protein concentration, the binding of salt ions to protein does not affect the hydration shell of the charged group of proteins. At lower salt concentration the increased solubility of proteins comes from the water bound with the ions (Kinsella & Whitehead, 1989).

The effective diffusion coefficient (D_{eff}) was found to decrease in the presence of salt compared to its value in the control. However, with the increase in salt concentration, both D_{eff} and gradient value ($\sigma - \sqrt{t}$) was found to increase. The protein molecules are amphiphilic in nature and carry negative or positive charge depending on pH. Salt has charge screening effect and affects its solubility and other functional properties depending on the concentration used. On the other hand, charge shielding can help to reduce electrostatic repulsion between protein molecules and help in adsorption.

3.5.2 Emulsification properties

Protein stabilized emulsions are sensitive to ionic concentration. The effect of NaCl on the emulsifying properties (EAI, ESI and droplet size) of LPI-stabilized emulsion is presented in Table 1. These data show that as the concentration of NaCl increased, the EAI of emulsion decreased first, then started to increase. For example, the EAI values of the emulsion at 0.5 M and 1.0 M NaCl were $94.77 \pm 1.60 \text{ m}^2/\text{g}$ and $107.87 \pm 0.51 \text{ m}^2/\text{g}$, respectively both of which are

much lower than the EAI value of the control ($111.32 \pm 1.57 \text{ m}^2/\text{g}$). A similar trend was observed in the case of ESI values, which decreased when the concentration of the salt increased. After a certain salt concentration the ESI increased slightly. However, all the ESI values in the presence of salt were much lower than the ESI value of the control. Zhang et al. (2009) also found similar results regarding the effect of NaCl on chickpea protein stabilized emulsions. This can be attributed to the electrostatic screening of charges of proteins adsorbed in droplets by salt ions. The salt ions reduce the electrostatic repulsion forces that help to maintain emulsion stability. The electrostatic repulsive force helps the proteins to overcome various attractive forces, for example, van Der Waals, hydrophobic or depletion (McClements, 2005). These results are also corroborated by surface hydrophobicity (Figures 8) and zeta potential measurements carried out as a function of ionic strength (Table 1). The magnitude of zeta potential value of emulsions decreased as the concentration of salt increased. When salt concentration in protein dispersion increased from 0 to 1.0 M NaCl, the absolute value of zeta potential of emulsion was found to decrease from 43.3 to 33.1 mV. The Na^+ and Cl^- ions can reduce the zeta potential of charged surface asymptotically to zero (Hunter, 1981). Similarly, the average droplet size diameter slightly increased with increase in the ionic strength (Table 1). McClements (2004) have reported a similar trend regarding the effect of monovalent ions on emulsion droplet size with more pronounced effect. The author had reported that oil droplet size of the emulsion increased by 10 folds or more when KCl concentration in whey protein-stabilized emulsion (7% w/w soybean oil) was increased from 0.1 to 0.5M.

3.6 Effect of heat treatment

3.6.1 Interfacial properties and effective diffusion coefficient

The effect of partial heat denaturation of LPI dispersion (10 mg/ml, pH 7.0) below its denaturation temperature (50°, 60°, 70°C for 10 min) was studied previously by Joshi et al. (2011). The DIFT as a function of time, presented in Figure 9, clearly show that heat treatment at 50 and 60°C enhanced the decrease in the interfacial tension. This can be attributed to the partial unfolding of protein molecules which makes proteins more flexible and facilitates their faster adsorption into interface. The faster adsorption of proteins subsequently gets reflected in the rapid decrease in the interfacial tension (Sikorski, 2001). However, heat treatment at higher temperature (70°C) showed less rapid reduction in interfacial tension compared to the control and the samples which were treated at lower

temperatures (50° and 60°C). This can be attributed to the aggregation of proteins caused by increased exposure of hydrophobic groups at higher temperature which otherwise remain buried inside native protein confirmation. The increased exposure of hydrophobic moieties of proteins at higher temperature favours the protein-protein interaction which leads to protein aggregation. The aggregation of proteins prevents them from diffusing into the interface within experimental time frame. Both diffusion coefficient D_{eff} and gradient of σ - $\sqrt{t}$ plot at early stage of adsorption for heat treated protein solution was much lower than untreated sample as shown in data in Table 2 supporting the explanation given above.

3.6.2 Emulsification properties

The Effect of heat treatment (50-70°C) of LPI protein dispersions (10 mg/ml LPI at pH 7.0) on (EAI) and (ESI) is presented in Table 1. The heat treatment at 50 and 60°C was found to slightly reduce EAI whereas at higher temperature (70°C) a substantial decrease in its value was observed. Similar results were observed for ESI except at 50°C at which an increase in ESI was observed. These observations might have been resulted due to substantial denaturation of LPI molecules at higher temperature which favours protein-protein interaction and results into greater protein aggregation. On the other hand, at 50°C only partial denaturation of LPI occurred which helped in increasing stability of emulsion. The reason for the loss of emulsifying capability of proteins subjected to high temperature heat treatment may be the result of the loss of solubility and formation of larger aggregates. As suggested in preceding sections, the excessive exposure of hydrophobic groups due to heat treatment at higher temperature leads to greater protein-protein interaction and results into large protein aggregates. These large protein aggregates are disadvantaged where the diffusion to the interface is concerned. These results are also reflected into the lower magnitude of zeta potential values when proteins were subjected to higher heating temperatures (Table 1). The average size of the oil droplets in emulsion stabilized by LPI when it was subjected to 70°C was very large (>3.6 μm). This result once again supports the fact that the emulsion stabilizing capability of LPI subjected to higher temperature is very poor. In contrast to this, Nir et al. (1994) reported that the heat treatment of soy proteins at 85°C resulted into formation of smaller emulsion droplet size. This implies that different globular proteins exhibit different emulsifying propensity when they are subjected to higher temperature.

3.7 Effect of pH

3.7.1 Interfacial properties and effective diffusion coefficient

The effect of pH on the DIFT of LPI is presented in Figure 10. It is interesting to note that the sensitivity of pH to the surface active behaviour at different pH is different. It can be seen from this figure that the rate of decrease in the interfacial tension is maximum at pH 6.0 closely followed by pH 7.0. It can further be observed that, at low pH 3.0, the interfacial tension is much higher than at the proximity of isoelectric point (pH 5.0) indicating that a slow adsorption of LPI takes place at low pH. It can also be seen from Table 2 that the pH has a strong effect on the diffusion coefficient (D_{eff}) and gradient values (σ vs. $\sqrt{t}$ plot) with low values near isoelectric pH and high values at both acidic and neutral pH (Table 2). The highest D_{eff} value was found for pH 7 while the highest gradient was observed for pH 3. The effects of pH may be related to the conformational characteristics and surface charge properties LPI. Similar effect of pH on the interfacial behaviour of globular protein (β -lactoglobulin) was observed by Das & Kinsella (1989). According to these authors, at lower pH the structure of β -lactoglobulin is rigid while in alkaline region its molecular structure becomes more flexible.

3.7.2 Emulsification properties

As can be seen from the data presented in Table 1, LPI stabilized-emulsions have high EAI both at low (pH 3.0) and high pH (pH 7.0) except at the proximity of its isoelectric point (pH 5.0). The isoelectric point for LPI is 4.5 (Bamdad et al., 2009). This is attributed solely to the electrostatic effect. The surface charge concentration of protein molecules is the lowest at their isoelectric point and hence maintaining the pH of the protein dispersion at about their isoelectric point favours the aggregation of protein molecules due to the absence of the repulsive electrostatic forces.

The effect of pH on the size of oil droplet and zeta potential value of LPI-stabilized emulsions is given in Table 1. The data presented in this table show that pH can have strong influence on both droplet size and zeta potential. The small size of oil droplet in emulsion is associated with better stability of emulsion. The size of the oil droplet was much smaller ($<1 \mu\text{m}$) at pH away from isoelectric point compared to when the pH was close to the LPI's isoelectric point. The largest oil droplets were obtained at pH 5.0 ($1.31 \pm 0.17 \mu\text{m}$)

accompanied by very low ESI value (Table 1). Similar results were reported by Wang et al. (2010) for soy protein isolate stabilized emulsions. It appears that the zeta potential values of LPI are dependent on pH of the emulsion. At pH above isoelectric point (> 4.5), emulsion carries negative zeta potential while at pH below isoelectric point it carries positive zeta potential values. The highest magnitude of zeta potential (-43.3 mV) was observed at pH 7.0 and lowest at pH 5 (-12 mV). The higher the zeta potential value, the greater is the magnitude of the repulsive power amongst the particles and the emulsion becomes more stable (Li & Tian, 2007). The above results indicate that the magnitude of the electrostatic repulsion between the emulsion droplets in LPI- stabilized emulsion is strongly affected by change in pH.

4. Conclusions

When lentil protein isolate (LPI) was used as emulsifier in oil-in water emulsions, its surface properties, such as dynamic interfacial tension (DIFT), surface hydrophobicity and surface charge were found to affect its emulsifying properties. LPI was found to have better ability in lowering the DIFT at oil-water interface and higher surface hydrophobicity compared to that of whey protein isolate. However, the stability of LPI-stabilized oil-in-water emulsion was poorer compared to the stability of sodium caseinate and whey protein isolate stabilized emulsions. This can be attributed to the poor surface charge and resultant low electrostatic repulsion in the case of LPI-stabilized emulsions. The LPI was found to effectively stabilize the oil-in-water emulsions at and above 10 mg/ml and the emulsion stability was found to increase within 20-30mg/ml LPI concentration. The presence of NaCl was found to improve the emulsion activity index (EAI) but the emulsion stability index (ESI) decreased due to charge shielding effect of ions. The heat treatment of the LPI dispersion improved the emulsion stability within moderate temperature of 50°C above which the emulsion stability was negatively impacted due to greater aggregation. The molecular flexibility of LPI can be improved by reducing the intra and intermolecular disulfide bonds by using appropriate reducing agents which helps to improve emulsifying property.

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Table 1. Effect of protein concentration, dithiotrethiol (DTT), ions (NaCl), pH and heat treatment c dispersion on emulsifying activity index (EAI), emulsifying stability index (ESI), average oil droplet size potential of lentil protein isolates (LPI)-stabilized emulsions.

Treatments/ Condition	Concentration	EAI (m ² /g)/ Turbidity* (m ⁻¹)	ESI (hr)	droplet size (μm)	Zeta potential (mV)
1. Concentration	0.1 mg/ml	8860±849 ^a	24±0 ^a	12.577±1.040 ^a	-
	1.0 mg/ml	13588±365 ^{*b}	24±0 ^a	2.906±0.234 ^b	-
	5.0 mg/ml	18378±91 ^{*c}	59±2 ^b	1.102±0.054 ^c	-43.1±0.3 ^a
	10.0 mg/ml	46650±104 ^{*d}	101±4 ^c	0.585±0.027 ^d	-43.3±0.6 ^a
	20.0 mg/ml	62540±13 ^{*e}	145±7 ^d	0.348±0.073 ^d	-42.1±0.5 ^a
	30.0 mg/ml	67690±45 ^{*e}	386±11 ^e	0.398±0.002 ^d	-40.7±0.5 ^b
2. DTT	0 mM	111.3±1.6 ^a	101±4 ^a	0.585±0.027 ^a	-43.3±0.6 ^a
	5 mM	196.4±0.6 ^a	465±32 ^b	0.891±0.037 ^a	-39.7±0.5 ^b
	10 mM	187.9±0.5 ^a	448±10 ^b	0.670±0.001 ^a	-42.5±0.8 ^a
	20 mM	184.1±0.3 ^a	105±8 ^a	0.960±0.102 ^a	-43.7±0.9 ^a
	30 mM	182.8±0.3 ^a	77±1 ^a	1.650±0.110 ^a	-44.2±0.8 ^a
3. NaCl	0 M	111.3±1.6 ^a	101±4 ^c	0.585±0.027 ^c	-43.3±0.6 ^a
	0.1 M	84.2±0.54 ^a	40±1 ^c	0.610±0.038 ^c	-32.5±0.4 ^c
	0.5 M	191.0±0.14 ^b	68±3 ^c	0.695±0.067 ^c	-12.3±2.3 ^c
	1.0 M	216.0±1.1 ^b	52±2 ^c	0.803±0.033 ^c	-40.6±0.1 ^c
4. Heat treatment	20°C	111.3±1.6 ^a	101±4 ^c	0.585±0.027 ^c	-43.3±0.6 ^a
	50°C	76.0±0.1 ^a	563±27 ^c	0.384±0.001 ^c	-43.4±0.4 ^c
	60°C	70.7±0.1 ^a	45±1 ^c	0.545±0.033 ^c	-41.9±0.2 ^c
	70°C	3.9±1.0 ^b	25±1 ^c	3.670±0.160 ^c	-
5. pH	3.0	130.2±0.5 ^a	245±13 ^c	0.270±0.008 ^c	-32.5±0.4 ^c
	5.0	6.1±0.2 ^b	27±1 ^c	1.310±0.174 ^c	-12.3±2.3 ^c
	6.0	83.2±0.8 ^c	92±5 ^c	0.670±0.014 ^c	-40.6±0.1 ^c
	7.0	111.3±1.6 ^a	101±4 ^c	0.585±0.027 ^c	-43.3±0.6 ^a

All the data are expressed as mean ± SD and are the mean of three replicates.

Means with the different superscript letters within the same column for each treatment are significantly different (p<0.05).

~ Concentration of protein used was 10 mg/ml protein concentration and pH was 7.0 (0.1 M phosphate buffer), if otherwise stated and

*Emulsifying effectiveness of of protein concentration is expressed as turbidity.

Table 2. Diffusion coefficients at short time range for lentil protein isolate (LPI) and different proteins at concentrations, pH, ionic strength and heat treatments.

Proteins/ Treatments	Concentration (mol/m ³)/ Condition	Diffusion coefficient D ₀ (m ² /s)	Gradient (mNm ⁻¹ s ^{-1/2})
Lentil protein isolate (LPI)	0.182	1.22 ±0.08 E-13	-0.3499 ±0.011
Bovine Serum albumin (BSA)	0.149	4.94 ±0.77 E-13	-0.1825 ±0.013
Lysozyme (Lys)	0.588	7.32 ±0.58 E-14	-0.7305 ±0.011
Sodium caseinate (NaCas)	0.417	3.98 ±0.31 E-15	-0.1445±0.006
Whey protein isolate (WPI)	0.417	1.91 ±0.04 E-14	-0.2954 ±0.003
Concentration of LPI	0.002	2.03 ±0.12 E-9	-0.4505 ±0.011
	0.018	1.19 ±0.06 E-11	-0.3456 ±0.008
	0.091	4.38 ±0.11 E-13	-0.3310 ±0.004
	0.182	1.22 ±0.08 E-13	-0.3499 ±0.011
	0.546	4.92 ±1.20 E-15	-0.2095 ±0.026
LPI (0.182 mol/ m ³) treated with DTT	5 mM	7.70 ±0.03 E-14	-0.2775 ±0.005
	10 mM	5.22 ±0.04 E-14	-0.2284 ±0.009
	20 mM	5.37 ±0.01 E-14	-0.2317 ±0.003
	30 mM	9.31 ±1.10 E-14	-0.3049 ±0.018
LPI (0.182 mol/ m ³) treated with NaCl	0.1 M	5.43 ±0.30 E-14	-0.2393 ±0.007
	0.5 M	7.26 ±1.08 E-14	-0.2690 ±0.021
	1.0 M	9.68 ±0.80 E-14	-0.3109 ±0.018
LPI (0.182 mol/ m ³) heat treated	50°C	3.18 ±0.20 E-14	-0.1782 ±0.008
	60°C	2.38 ±0.26 E-14	-0.1541 ±0.008
	70°C	2.93 ±0.33 E-14	-0.1711 ±0.009
pH of LPI (0.182 mol/ m ³)	3.0	8.86 ±0.12 E-13	-0.9415 ±0.006
	5.0	2.74 ±0.50 E-13	-0.5228 ±0.048
	6.0	4.00 ±0.70 E-14	-0.1996 ±0.017
	7.0	1.38 ±0.07 E-13	-0.3499 ±0.011

All the data are expressed as mean ± SD and are the mean of three replicates.

Figure 1. Dynamic interfacial tension at olive oil/water interface containing different food proteins (10 mg/ml protein concentration, pH 7.0, $20 \pm 1^\circ\text{C}$): lentil protein isolate (LPI), bovine serum albumin (BSA), lysozyme (Lys), whey protein isolate (WPI) and sodium caseinate (NaCas).

Figure 2 (a)

Figure 2b

Figure 2 (c)

Figure 2 (a) Emulsifying activity index (EAI) and emulsifying stability index (ESI), (b) average oil droplet size (μm) and (c) zeta potential value of oil-in-water emulsion containing different food proteins (10 mg/ml protein, pH 7.0): lentil protein isolate (LPI), bovine serum albumin (BSA), lysozyme (Lys), whey protein isolate (WPI) and sodium caseinate (NaCas) as an emulsifier at $20 \pm 1^\circ\text{C}$.

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Research highlights

- Lentil protein isolate (LPI) has interfacial behavior similar to that of globular proteins
- LPI lowers the dynamic interfacial tension at oil-water interface faster than whey protein isolate
- LPI has comparable hydrophobicity to that of whey protein isolate (WPI)
- The emulsion stability of LPI-stabilized emulsion is slightly lower than that of WPI-stabilised ones
- Emulsifying property of LPI can be improved by using reducing agent and increasing its concentration