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Isolation and development of wheat based gluten edible film and its physico-chemical properties

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Abstract

Two Indian wheat cultivars viz. C-306 and RAJ-3765 were used for gluten extraction and developed gluten based edible film. The wet and dry gluten yield of C-306 cultivar was significantly higher than that of RAJ-3765. Five glycerol concentrations viz. 3, 4, 5, 6 and 7 g were tried for film formation and developed films were then compared. Film thickness was independent on glycerol concentration. Tensile strength decreased and % elongation at break increased with an increase in glycerol concentration. Film prepared from C-306 cultivar required higher force than RAJ-3765 for puncture test. As the concentration of glycerol increased the puncture strength decreased. Water vapor transmission rate increased with increase in concentration of glycerol. Water vapor transmission rate, solubility in water (22.7 to 100%) and HCL (42 to 61%) were lower in C-306 than RAJ-3765 edible film. Results clearly showed that quality of gluten significantly affect the film forming properties.

Introduction

Outcome of several studies conclude that synthetic polymer of food material have several undesirable influence and has problem of disposal and environmental pollution (Malathi *et al.*, 2014). This leads to develop a biodegradable film and increase renewable resources for preparation of packaging materials (Kuorwel *et al*., 2011). Edible and/or biodegradable films are not meant to totally replace synthetic packaging films; however, they do have the potential to replace the conventional packaging in several applications (Mali and Grossmann, 2003). Edible films can be used for versatile food products to reduce loss of moisture, restrict absorption of oxygen, less migration of lipids, improve mechanical handling properties, provide physical protection, or offer an alternative to the commercial packaging materials (Bourtoom, 2009). Biodegradable and edible packaging films from renewable biopolymers have huge opportunity of value addition to agricultural products (Gennadios, 2002) and it also control waste disposal as degraded naturally (De Vlieger, 2003).

The main biopolymers used in the edible films are proteins (Sobral *et al*., 2001). Now a day, packaging research has focused more on edible films which are biodegradable, made from plant and animal edible protein sources such as wheat gluten, corn zein, soy and peanut protein, cottonseed, albumin, gelatin, collagen, casein and whey proteins (Tharanathan, 2003). Among proteins wheat gluten was extensively used for edible film (Guo *et al.*, 2012). Wheat gluten, which constitutes the protein by-product of the starch fabrication, is an interesting raw material for the development of biopolymers, because it is readily available in large quantities and at economical cost (Kaushik *et al*., 2015). Gluten used for biopolymer showed an interesting solution due to its polymer forming potential, quite cheap, abundant, biodegradable and edible (Chen, 1995). Wheat gluten is not a simple thermoplastic material and it has a quite narrow temperature range in the extrusion to produce network structure. Gluten extrudates plasticized by glycerol are solid-like and elastic (Guilbert *et al.*, 2002). Redl *et al*. (1999) have successfully produced glycerol plasticized wheat gluten rods under different operating conditions by twin-screw extruder.

Plasticizers increase film flexibility due to their ability to reduce internal hydrogen bonding between polymer chains while increasing molecular volume (Koskinen *et al.*, 1996). The making of protein-based films generally needs the incorporation of a minimal content of plasticizer to reduce its brittleness. Film plasticizers function by weakening intermolecular forces between adjacent polymer

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chains. This results in an increased film extensibility and flexibility with decreased elasticity, mechanical resistance, and barrier property of the films. The most common plasticizers used are polyols and mono, di and oligosaccharides. Glycerol, as a plasticizer, has been incorporated into most hydrocolloid films (Banker, 1966). Wheat gluten sheet plasticized with glycerol was successfully produced using a twin-screw extruder and the formation of the translucent sheet was found to occur when temperature of the melt was around 137°C (Hochstetter *et al.*, 2006). Gontard *et al.* (1993) reported that glycerin and water plasticized with cast wheat gluten films decreased puncture strength, improved elasticity, and increased extensibility and water vapor transmission rate. Without plasticizer the films are too brittle to handle.

Besides the barrier efficiency, edible films and coatings have to be organoleptically and functionally compatible with foods. Coatings are either applied to or made directly on foods when films are independent structures that can wrap food after their making (Debeaufort *et al*., 1998). As food components, edible films and coatings usually have to be as tasteless as possible in order not to be detected during the consumption of the edible-packaged food product (Contreras-Medellin and Labuza, 1981). When edible films and coatings have a significant or particular taste and flavor, their sensorial characteristics have to be compatible with those of the food (Biquet and Labuza, 1988). Therefore, the present study was conducted with aim to extract gluten from wheat flour and develop gluten-based edible film and to check physico-chemical properties of gluten edible film.

Materials and Methods

Materials

Two Indian wheat cultivars viz. C-306 and Raj-3765 were obtained form wheat breading farm, Haryana Agriculture University, Hisar (India). Airtight plastic containers were procured for grain and flour storage. Parad tablets (Himalya, India) (enclosed in cloth) for protection of wheat grains were put into grain containers.

Chemicals

All chemicals used were of analytical grade and were purchased from Rankem (Ranbaxy, New Delhi, India). Glycerol, ethanol (95%), ascorbic acid, HCl were used during gluten edible film development.

Apparatus

During research work magnetic stirrer with hot plate, oven (Narang Scientific Works Pvt. Ltd., Delhi, India), Texture analyzer TAXT2I (Stable MicroSystem, Godalming, and Surrey, U.K), Digital weighing balance (Precesia, Model), Laboratory Flour Mill (Max Egger Laborgerte, Austria) Gluten Washer (Max Egger Laborgerte, Austria), Digital vernier caliper (Mitutogo Corporation Model), Lypohilizer (Scope Enterprises, New Delhi, India) were used.

Milling

Wheat cultivars were tempered to 16.5% moisture by adding required amount of water. The wheat cultivars were tempered at ambient temperature for 24 h to equilibrate moisture content. The above wheat cultivars were milled in roller-mill (Chopin Laboratory CD-1 mill, France). Five kilogram tempered wheat cultivars were milled and the flours were collected. In order to ensure the purity of the roller-milled flour samples from each lot, mechanical and manual cleaning of the roller-mill, including air blasting, were applied between milling of each of the wheat samples (Kumar *et al*., 2013).

Gluten extraction and yield

The extraction was carried out using procedure as described in Kaushik *et al.* (2013). The extracted gluten was then freeze dried (-81°C and 0.05 psi) and its yield was determined.

Wheat gluten film preparation

Films were prepared by film-forming solutions using the formula of Gennadios and Testin (1993). 15 g freeze dried gluten was dispersed in solution of ethanol (72 ml) and glycerol (3, 4, 5, 6, 7 g) and stirring for 10 min on a magnetic stirrer hot plate and slowly adding 48 ml of distilled water and 12 ml of 6N ammonium hydroxide. The glycerol was added as a plasticizer. Heating rate was adjusted so that temperature of the solutions was 75±1°C at the end of preparation.

Casting of wheat gluten film

Film-forming solutions were filtered through cheese cloth to cease bubbling before casting on a film casting surface. Four film casting surfaces used were glass, plastic, aluminium foil and Teflon. A thin-layer spreader bar was used to spread the film forming solutions onto a film casting surface. Each casting plate was taped on either side to restrain movement during casting. Plates with cast wheat gluten solutions were placed in an air circulating oven maintained at 32°C. After 15 h, the plates were removed from the oven; dried films were peeled from

the casting surface and stored at 25°C for 48 h for further use.

Film thickness

Film thickness was measured using a vernier caliper. The thickness of individual film samples was determined as a random average of five measurements.

Tensile strength and % elongation at break

Film Tensile strength and % elongation at break film tensile strength (TS) were determined using a texture analyzer TAXT2I (Stable Micro System, UK), operated according to the ASTM Standard Method. Three specimens (40 mm width) of each film were measured. The peak loads and extension at break were recorded for tested film specimens. The tensile strength and per cent elongation at break were calculated according to the ASTM method. The each test piece was placed centrally on the sample platform of Keiffer with the extension hook previously positioned beneath.

$$\text{Elongation (\%)} = \frac{\text{Distance Sample Stretched}}{\text{Original length of sample}} \times 100$$

Puncture strength

Puncture test was conducted to determine force (g) and deformation (mm) using a texture analyzer (TAXT2I). Samples with diameters of 40 mm were fixed on the plate of the equipment containing central hole of 20 mm with the help of a tape. A cylindrical probe of 3 mm diameter was moved perpendicularly to the film surface at a constant speed of 1mm/s until the probe passed through the film. Force-deformation curves were recorded. At rupture point, deformation force was determined.

Solubility in water and HCL

The percentage of initial dry matter of each film was determined at 105°C for 24 h. Two discs of film (2 cm diameter) were cut, weighed, immersed in 50 mL of distilled water and 0.1N HCL and slowly agitated for 24h at 25°C. The pieces of film were then taken out and dried (105°C for 24h) to determine the weight of dry matter insoluble in water.

$$\text{Solubility} = \frac{\text{Initial weight of film- Final weight of film}}{\text{Initial weight of film}} \times 100$$

Water vapor transmission rate

Water vapor transmission rate of gluten films was determined by a static method. Here wide-open mouth vials containing anhydrous $CaCl_2$ were closed with coated with gluten edible film. Wax was used, to make it an airtight unit. All the prepared vials were then kept in desiccators containing saturated KNO_3 solution (for maintaining 90% RH). Further, these desiccators were kept at 38°C in the incubator. WVTR was expressed as gm/m2.d by observing the change in weight per unit time.

$$WVP = (WVTR \times L)/Ap$$

Where,

WVTR = Water vapor transmission rate

L = mean film thickness (mm)

Ap = partial water vapor pressure difference (Pa) across the two sides of the film

Statistical analysis

Means (n=3), standard error mean (SEM), linear regression analysis and 95% confidence intervals were calculated using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA) (Kaushik *et al.* 2014). Data was subjected to a single way analysis of variance (ANOVA) to determine (CD) value.

Results and Discussion

Film formation mechanisms of wheat gluten (WG)

A film formation mechanism is proposed for WG films. Heating (with alkaline conditions) of film-forming solutions denatures WG proteins, reducing existing S-S bonds, and revealing previously unexposed SH groups. Upon drying, covalent S-S bonds formed by air oxidation cross-link protein molecules. Besides formation of S-S bonds from free SH groups, most likely, sulfhydryl-disulfide interchange reactions also contribute to protein cross-linking by S-S bonds. Similar film-formation mechanisms involving cross-linking by S-S bonds have been postulated for cast films from other sulfur-containing proteins such as soy protein, whey protein isolate and egg albumen (Gennadios *et al.*, 1996). Also, besides cast films, protein polymerization through S-S bonds has been implicated in film formation on the surface of heated soymilk and WG or soy protein solutions (Rocha *et al*., 2013).

The gluten film was not formed over aluminum foil, whereas hard film formed over glass surface, film formed but struck on surface of teflon coated surface. When film formed over plastic surface, best film formed and peeled easily from surface. Different concentrations (0 to 7%) of glycerol were tried for film formation. Upto 2% glycerol, no film was formed, whereas brittle film formed by 3% glycerol. 4 to 7% glycerol concentration leads to film formation

and extensibility increased with increase of glycerol concentration

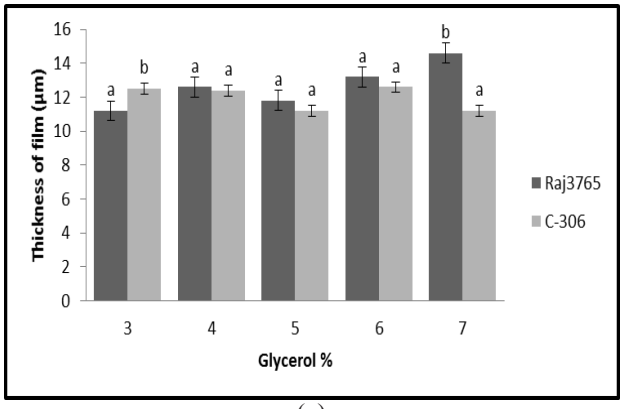
Film thickness

Thicknesses of films varied from 11.25 to 12.6 µm and 11.2 to 14.6 µm for C-306 and RAJ-3765 cultivars, respectively for different glycerol concentrations (Figure 1a). 3 and 7% glycerol when used non significant difference (P>0.05) was observed between film thickness of two wheat cultivars, whereas no difference (P<0.05) was observed when 4, 5 and 6% glycerol was used. Gennadios *et al.* (1993) studied the various properties of wheat gluten film. They reported that thickness of wheat gluten film varies from 6.6 to 10.2 µm. Hochstetter *et al.* (2006) determined average thickness of gluten film 5 µm.

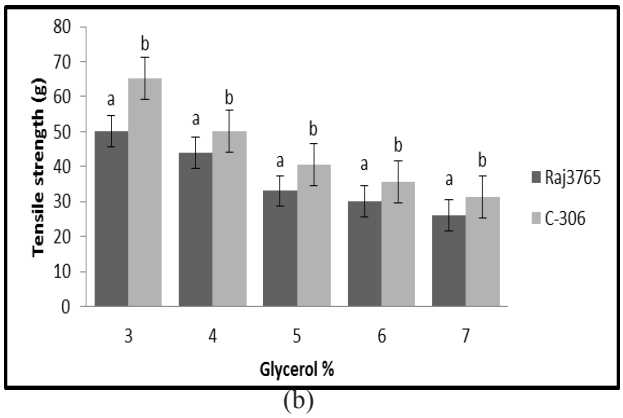
Mechanical properties

An edible film should be resistant in order to withstand the manipulation during its application and to maintain its integrity and also its barrier properties. Tensile strength decreased and per cent elongation at break increased with an increase in glycerol concentration. Non-plasticized gluten film has low flexibility due to highly cooperative protein-protein interaction, mainly because of the high glutamine content which is responsible for numerous hydrogen bonds between protein chains. Glycerol is a small hydrophilic molecule which could be inserted between protein chains. With glycerol in the protein network the distance between protein chains are increased and direct interactions are reduced.

Films prepared with gluten from C-306 wheat cultivar and low concentrations of glycerol had greater tensile strength (55 g) than films from RAJ-3765 wheat cultivar. The tensile strength of film prepared from C-306 was significantly higher (P<0.05) in comparison to RAJ-3765 cultivar at all levels of glycerol used (Figure 1b), whereas percent elongation of film prepared from RAJ-3765 was significantly higher (P<0.05) in comparison to C-306 cultivar at all levels of glycerol used (Figure 2a). All films with low amounts of glycerol had higher tensile strength and lower percent elongation at break. Parris *et al.* (1995) reported that the tensile strength of alginate edible films decreased exponentially with increasing sodium lactate. The mechanism to form a resistant film could involve a higher number or a better localization of bonds between protein chains. During the drying of the film-forming solution, ethanol and ammonium hydroxide were evaporated, allowing the formation of bonds between protein chains. During this stage, the proximity of protein chains induced



(a)



(b)

Figure 1. Characteristics of gluten film (a) Thickness of wheat gluten films (b) Tensile strength values (g) of gluten films

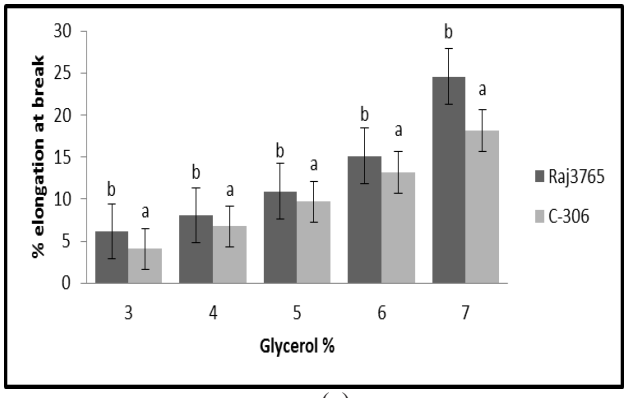
[a-b]Samples represented with different letters are significantly different (p<0.05) from each other

Error bars show the variations of three determinations in terms of standard error of mean

by high gluten concentrations could form such cross-bonds. During film formation, polymerization occurs through intermolecular disulfide, hydrophobic and hydrogen bondings. An alkaline environment and heating are necessary to denature the gluten protein, thus disrupting protein structure, breaking existing disulfide intramolecular bonds and exposing sulphydryl and hydrophobic groups, making them available for bonding. The cleavage of disulfide bonds results in polypeptide chains with lower molecular weights, destroying elasticity and cohesiveness of gluten casting and drying, sulphydryl groups reform disulfide bonds by air oxidation, which leads the film structure (Gennadios *et al.*, 1993).

Puncture strength

Puncture test measured the force required to push a puncture and probe into a food or food products. The gluten film from C-306 wheat cultivar had high value of puncture strength in comparison to wheat gluten film prepared from Raj-3765 cultivar (Figure 2b). Gluten film from C-306 cultivar showed significantly higher (P<0.05) puncture strength to



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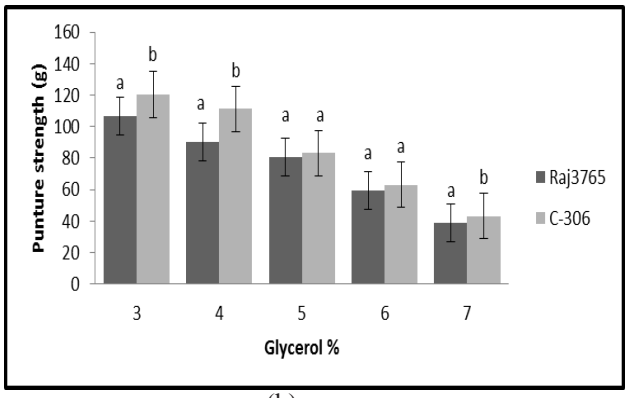


(b)

Figure 2. Textral properties of gluten film. a) Describes percent elongation b) Puncture strength

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.

Error bars show the variations of three determinations in terms of standard error of mean.

gluten film prepared from Raj-3765 cultivar at 3, 4 and 7% glycerol, whereas no difference ($P>0.05$) was observed at 5 and 6% glycerol. As the concentration of glycerol was increased the puncture strength decreased. The films prepared from low concentration of glycerol had higher values of puncture strength in comparison to higher glycerol concentration.

The effect of plasticizer on reduction of the puncture force is well known and its explanation is found in the literature (Bourtoom, 2008). Gontard *et al.* (1993) observed a linear reduction of the puncture force in gluten films from 1.9 to 0.3 N, between 16 and 33 g glycerol/100g dry matter. According to the classic polymer science, the plasticizers weaken the inter molecular forces between the chain of adjacent macromolecules, increasing the free volume and causing a reduction of Tg of the system (Jastrzebski, 1987). Thus the increase in the plasticizers concentration caused a reduction of the puncture force due to the decrease in the intermolecular interactions and causes an increase of the puncture force due to the increase in the mobility of the micromolecules.

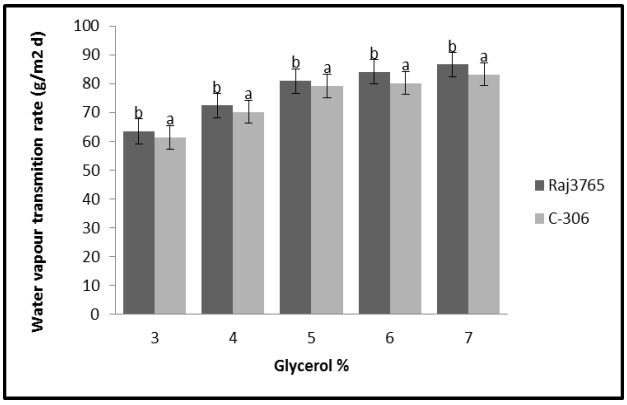


Figure 3. Water vapor transmission rate (WVTR) of gluten films prepared from wheat varieties C-306 and Raj-3765

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.

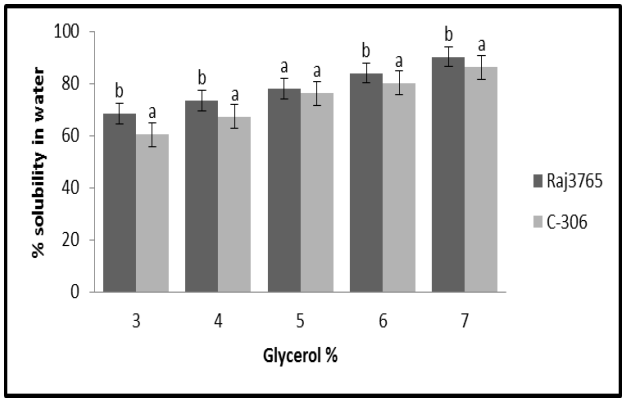
Error bars show the variations of three determinations in terms of standard error of mean.

Water vapor transmission rate (WVTR)

Water vapor permeability should be as low as possible since an edible film or coating should retard moisture transfer between the food and the environment, or between two components of a heterogeneous food product. The films prepared from gluten extracted from wheat cultivars Raj 3765 using higher concentration of glycerol, showed higher values for WVTR (85.74 g/m^2 d) as compared to films prepared from C-306 (Figure 3). WVTR values of films were lower at lower glycerol concentration. Plasticizers are added to films to reduce brittleness, increase toughness, strength, tear and impact resistance and impart flexibility. Usually, the addition of a plasticizer increases the permeability of gas, water vapor and solute and decreases the tensile strength of the films. For the films with different glycerol concentration, the WVTR values of films were higher for variety RAJ-3765 as compared to the variety C-306. Gas and water vapor barrier properties of an edible film and coatings vary greatly with composition, and presence of bubbles and pinholes of the films (Pascat, 1986). McHugh, Aujard and Krochta (1994) determined that water vapor permeability of gluten films plasticized with glycerin at 25°C, varied linearly with the plasticizer concentration with very good correlation coefficient. The same behavior can be observed in the work of Gontard *et al.* (1993). Sobral *et al.* (2001) reported the water vapor permeability increased with increase in plasticizer concentration.

Solubility in water

Water resistance is an important property of edible films particularly for food applications. For all the films tested, the higher solubility in water was observed at higher concentration of glycerol used.



(a)

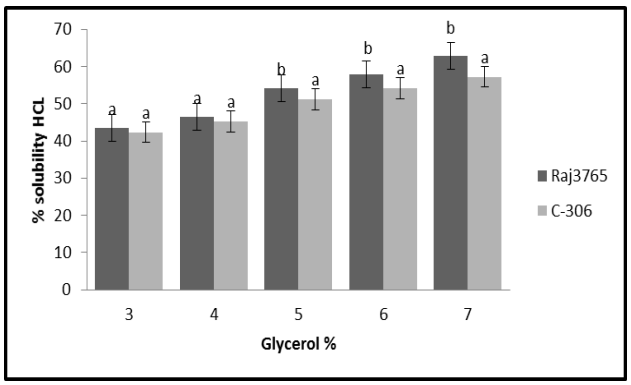


Figure 4. Solubility of gluten film a) Water Solubility of gluten films b) HCL Solubility of gluten films
[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Fakhouri and Palmu (2004) studied the solubility of wheat gluten and cellulose acetate phthalate films in water and reported the values in the range from 22.7 to 100%. Films prepared with glycerol at 7% showed higher values for solubility in water and the films of variety C-306 had lower values. Increasing the amount of plasticizer, especially glycerol, caused an increase in moisture and WVTR of the WPI (whey protein isolate) films (figure 4a). This is probably due to the plasticizer that disrupts intermolecular interactions between polymer molecules. Polyols seem to have ability to locate between polymer molecules, disrupting intermolecular polymer associations, being this effect attributed to their ability to associate with water. Considering that glycerol is infinitely soluble in water, differences in solubility is noticed due to the difference in the intensity of the polymer association. At lower concentration of glycerol, the solubility of the films was lower and there was no significant difference among the films tested, indicating that the polymer matrix was able to partially accommodate and protect the polyol loss through the solution. Edible films with high water solubility may be required to contain premeasured portions which will be dissolved in water or in hot food. Besides, the increase in the plasticizer concentration increases the moisture content of the film because of its high hydroscopic character which is also contributes to the reduction of the forces between the adjacent macromolecules (Sobral *et al*., 2001).

Solubility in HCL

The solubility of films in HCL increased as the concentration of glycerol for film preparation was increased. The gluten films of variety C-306 showed lower HCL solubility values when compared with films prepared from variety Raj-3765 (figure 4b). Fakhouri and Palmu (2004) reported the solubility of wheat gluten and cellulose acetate phthalate film in HCL from 30.5 to 100%.

Conclusions

Wet and dry gluten yield of C-306 was higher than Raj-3765 cultivar. Gluten was frieze dried and used for preparation of gluten film. Glycerol was used as plasticiser. Five glycerol concentrations viz. 3, 4, 5, 6 and 7 g were tried during film formation and compared. Film thickness was independent on glycerol concentration. Tensile strength decreased and % elongation at break increased with an increase in glycerol concentration. The film prepared from C-306 cultivar required higher force than Raj-3765 for puncture test. As the concentration of glycerol was increased the puncture strength decreased. Water vapor transmission rate was increased with increase in concentration of glycerol. Water vapor transmission rate, solubility in water and HCL was lower in case of C-306 in comparison to Raj-3765. From above results, it can be concluded that wheat gluten properties affect the gluten film properties. With higher addition of glycerol decrease tensile strength and force required during puncture test and increase % elongation of gluten film. Water vapor transmission rate and solubility in water and dilute acid were decreased with increase in glycerol concentration.

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