Journal of Animal Research: v.12 n.05, p. 641-646. October 2022

DOI: 10.30954/2277-940X.05.2022.5

Hemp Seed Protein and Carrageenan Based Biodegradable Composite Film for Food Packaging Applications

Sabahu Noor, Sunil Kumar, Zuhaib F. Bhat* **and Arvind Kumar**

Division of Livestock Products Technology, F.V.Sc. & A.H., SKUAST-Jammu, R.S. Pura, Jammu, J&K, INDIA

Corresponding author: ZF Bhat; E-mail: zuhaibbbhatvet@gmail.com

Received: 18 Aug., 2022 **Revised:** 20 Sept., 2022 **Accepted:** 23 Sept., 2022

ABSTRACT

A composite biodegradable film was developed using hemp seed protein and carrageenan for food packaging applications. The film was prepared using hemp seed protein, carrageenan and glycerol (plasticizer). Different levels of carrageenan (1.0, 1.4 and 1.8%) and glycerol (10, 15 and 20%) were used to standardise the formulation for the preparation of the basic film. Based on the different physico-mechanical and colour characteristics, 1.4% carrageenan and 15% glycerol were found optimum for development of the basic film with desirable characteristics. Different levels of hemp seed protein (0.75, 1.0 and 1.5%) were incorporated in the basic formulation and the developed composite films were evaluated for different physico-mechanical and colour characteristics. Addition of hemp seed protein increased the thickness (mm) and density (g/ml) whereas decreased the water vapour transmission rate (%). Hemp seed protein also decreased the lightness (L*), redness (a*) and yellowness (b*) values of the film. Based on the results, 1.5% was found optimum for development of the film with desirable characteristics. The film can be used as a carrier of natural bioactive ingredients for improving storage quality of the food products.

HIGHLIGHTS

- Hemp seed protein and carrageenan based biodegradable film was developed for food packaging applications.
- The film with desirable characteristics contained 1.5% hemp seed protein, 1.4% carrageenan and 15% glycerol.
- Hemp seed protein also decreased the lightness (L*), redness (a*) and yellowness (b*) values of the film.

Keywords: Biodegradable film, hemp seed protein, carrageenan, glycerol, physico-mechanical characteristics

Use of biodegradable and edible films as vehicles for bioactive ingredients is a novel way of preserving foods (Mahajan *et al.*, 2022). These films allow controlled release of the bioactive ingredients which are mostly active on the food surface (Kalem *et al.*, 2018). While use of natural bioactive ingredients, such as plant extracts, can improve lipid and microbial stability of the foods (Dua *et al.*, 2015a, b), direct inclusion of bioactive ingredients may affect the sensory quality and other functional properties (Kaur *et al.*, 2015; Singh *et al.*, 2015). Further, processing such as cooking has been reported to affect the efficacy of the bioactive ingredients (Bhat *et al.*, 2019a; Singh *et al.*, 2014a, b). These limitations have led to an increase in the number of the studies related to the development of bioactive edible and biodegradable films.

Being edible and biodegradable in nature, biological ingredients such polysaccharides, proteins, lipids, and their derivatives are used for the preparation of these films and coatings. The importance of these films has increased recently due to the factors such as increased consumer awareness and health consciousness, increased efforts to reduce the environmental impact of plastic packaging and rising concerns about climate change (Sharma *et al.*, 2021a; Jamwal *et al.*, 2015). There has been an ever-increasing emphasis on production of environmentally friendly

How to cite this article: Noor, S., Kumar, S., Bhat, Z.F. and Kumar, A. (2022). Hemp Seed Protein and Carrageenan Based Biodegradable Composite Film for Food Packaging Applications. *J. Anim. Res.,* **12**(05): 641-646.

Source of Support: None; **Conflict of Interest:** None

and less polluting packaging using organically produced or renewable resources (Sharma *et al.*, 2021b). While several proteins have been used for development of the biodegradable and edible films, studies have not reported the use of hemp seed protein for development of the films and needs immediate scientific attention. An attempt was made in the present study to develop a composite film using hemp seed protein and carrageenan. Commonly known as hemp, *Cannabis sativa* L. is a widely grown plant with significant industrial applications. Edestinis the predominant protein in hemp seeds and has gained increasing attention due to its unique amino acid profile (Tang *et al.*, 2006). The hemp protein has been reported to have antioxidant properties (Wang *et al.*, 2009) and may attribute some good characteristics to the films, such as low solubility and high surface hydrophobicity (Yin *et al.*, 2007). However, protein films are often less effective in retaining the moisture content and are therefore combined with other polymers. Formulation of composite films using different polymers helps in utilizing the distinct advantages of each biopolymer (Santhosh *et al.*, 2021).

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals were purchased from standard firms and were of analytical grade. Carrageenan (GRM1576-100G) and glycerol (GRM1027) were purchased from Hi-Media (Mumbai, India) and hemp seed protein was purchased from Health Horizons (U.P., India).

Preparation of the film

Three different levels of carrageenan (1.0, 1.4 and 1.8%) and glycerol (10, 15 and 20%) were used to optimize the concentration for preparation of the basic film with desirable characteristics. Based on the film characteristics, 1.4% carrageenan and 15% glycerol were found optimum. Hemp protein was incorporated at three different levels (0.75, 1.0 and 1.5%) in the basic formulation to standardize the composite film. The aqueous solution of carrageenan was continuously stirred [at 350 rpm at 80 °C on a magnetic stirrer (Glassco laboratory Pvt. Ltd., India)] for 20 min and after addition of hemp protein and glycerol, the solution was again stirred for 10 min. The solution was allowed to cool to 70 °C and casted on glass plates (10 × 20 cm) and dried at 50 °C for 5 h and were peeled off and stored in low density polyethylene pouches at 4 °C.

Physico-mechanical characteristics of the film

Different physico-mechanical properties of the film were determined using methods described by Mahajan *et al.* (2021a). A micrometre was used to measure film thickness (Swastik Scientific Company, Mumbai, India). The water vapour transmission rate (WVTR) of the film was estimated using a test cell that was sealed with film and contained 15 ml of distilled water and was allowed to dry for 24 hin a desiccator. The flotation method was used to measure the density of the film (1.5 × 1.5 cm) using carbon tetrachloride (1.5935 g/ml) and heptane (0.71 g/ml) as solvents. The transmittance of film strips (3 cm x 1 cm) placed in a water-filled cuvette was measured at a wavelength of 660 nm using a UV-VIS spectrophotometer. Drying of the film (500 mg)at 105±1 °C for 24 h allowed the gravimetric measurement of its moisture content (%).

Colour analysis

The various colour parameters of the film viz. lightness (L*), redness (a*) and yellowness (b*) were determined using a colorflex colorimeter manufactured by Hunterlab (Hunter Associated laboratory Inc., VA, the USA) (Bhat *et al.*, 2019b).

STATISTICAL ANALYSIS

The experiments were replicated six times (n=6). The data was collected and analysed using SPSS-20.0 (SPSS Inc. Chicago IL,USA) and the results are presented as Mean ± S.E. Data was analysis by one-way Analysis of Variance (ANOVA)and multiple range tests (DMRT) by Duncan were used to determine the significance between means at 0.05 level of significance.

RESULTS AND DISCUSSION

Physico-mechanical properties

Different concentrations of carrageenan (1.0, 1.4 and 1.8%) and glycerol (10, 15 and 20%) were used to optimize the basic formulation of the film. The mean values of various physico-mechanical characteristics of the basic film are presented in Tables 1 and 2. Different levels of hemp protein (0.75, 1.0 and 1.5%) were added to the basic formulation to develop a composite film. The mean values of various physico-mechanical characteristics of the composite film are presented in Table 3.

Table 1: Effect of carrageenan on the characteristics of the film.

Parameters	Treatments		
	T_1	T_2	T_3
Thickness(µm)	140.83±2.15^{c}	148.16±2.76^{b}	159.83±1.83^{a}
Moisture (%)	28.24±0.20^{c}	31.08±0.25^{b}	34.21±0.26^{a}
Density(g/ml)	0.86±0.01	0.88±0.007	0.92±0.03
WVTR (mg/m^{2}t)	1.46±0.20^{c}	2.06±0.01^{b}	2.58±0.25^{a}
Transmittance (%)	90.32±0.06^{a}	87.32±0.25^{b}	85.45±0.14^{c}
Lightness(L*)	86.42±0.13^{a}	84.50±0.13^{b}	82.66±0.19^{c}
Redness (a*)	1.05±0.02^{a}	0.95±0.01^{b}	0.82±0.01^{c}
Yellowness (b*)	3.31±0.07^{a}	3.02±0.09^{ab}	2.81±0.11^{b}

Mean ± S.E. with different superscripts row-wise differ significantly ($P<0.05$); n = 6 (replications), T_1 = films containing 1.0% carrageenan, T_2 = films containing 1.4% carrageenan, T_3 = films containing 1.8% carrageenan, All the films contained 15% glycerol, WVTR = Water vapour transmission rate.

Table 2: Effect of glycerol on the characteristics of the film

Parameters	Treatments		
	T_1	T_2	T_3
Thickness (µm)	131.42± 0.16^{c}	148.22±0.41^{b}	171.32±0.43^{a}
Moisture (%)	26.08±0.26^{c}	29.45±0.13^{b}	34.83± 0.15^{a}
Density (g/ml)	1.03±0.02	1.05±0.02	1.09±0.07
WVTR (mg/m^{2}t)	1.32±0.03^{c}	2.62±0.06^{b}	3.91±0.16^{a}
Transmittance (%)	93.04±0.25^{a}	89.51±0.20^{b}	86.58±0.25^{c}
Lightness (L*)	84.05±0.01^{c}	82.23±0.18^{b}	81.50±0.08^{a}
Redness (a*)	0.95±0.02^{a}	0.90±0.16^{a}	0.83±0.01^{b}
Yellowness (b*)	3.05±0.01	3.02±0.01	2.94±0.02

Mean ± S.E. with different superscripts row-wise differ significantly ($P<0.05$); n = 6 (replications), T_1 = films containing 10% glycerol, T_2 = films containing 15% glycerol, T_3 = films containing 20% glycerol; All films contained 1.4% carrageenan, WVTR = Water vapour transmission rate.

Table 3: Effect of hemp seed protein on the characteristics of the film

Parameters	Treatments		
	T_1	T_2	T_3
Thickness (µm)	148.50±2.29^{b}	150.66 ±2.81^{ab}	156±1.31^{a}
Moisture (%)	31.40±0.07	31.19±0.07	30.09±0.24
Density (g/ml)	0.92±0.01^{c}	1.03±0.06^{b}	1.16±0.04^{a}
WVTR (mg/m^{2}t)	2.34±0.06^{a}	1.93 ±0.62^{b}	1.64±0.11^{c}
Transmittance (%)	92.06±0.69^{a}	88.41±0.42^{b}	85.83±0.71^{c}
Lightness (L*)	79.36±0.23^{a}	78.26±0.26^{b}	76.11±0.02^{c}
Redness (a*)	1.33±0.08^{a}	1.03±0.10^{b}	0.96±0.02^{c}
Yellowness (b*)	3.05±0.07^{a}	2.26±0.25^{b}	1.67±0.11^{c}

Mean ± S.E. with different superscripts row-wise differ significantly ($P<0.05$); n = 6 (replications), T_1 = films containing 0.75% hemp seed protein, T_2 = films containing 1.0% hemp seed protein, T_3 = films containing 1.5% hemp seed protein; All the films contained 1.4% carrageenan and 15% glycerol, WVTR = Water vapour transmission rate.

Film thickness

Thickness is a crucial factor that determines the suitability of the edible films as a packaging material for food products (Galusand Lenart, 2013). Thickness of a film can have an impact on a number of physicochemical characteristics including transmittance, water vapour permeability, and mechanical properties (Zhang *et al.*, 2020). A significant ($P<0.05$) increasing pattern was observed in the thickness of the film with increasing concentration of both carrageenan and glycerol. The highest values were recorded for the films containing highest concentration of carrageenan and glycerol. Increase in viscosity and total dissolved solids of the film forming solutions may be the cause of the increased film thickness with increasing carrageenan level (Mahajan *et al.*, 2021b). Carrageenan is a marine polysaccharide and has been widely studied for the production of the edible films. Its ability to produce a solid gel with a melting point much above the initial gelation temperature makes it an excellent choice as the basic material for the preparation of edible films (Lacroix and Tien, 2005). Film thickness may have increased with glycerol concentration due to an increase in dry matter content and the molecular volume that plasticizers contribute (Nemet *et al.*, 2010). Glycerol is the most commonly used plasticizer for the preparation of edible and biodegradable films due to its high stability and compatibility with the hydrophilic biopolymer chains (Cevera *et al.*, 2004). Its hydrophilic nature allows more permeability to water vapour and attributes a flexible and less brittle properties to the films while maintaining the mechanical characteristics and appearance during storage (Oses *et al.*, 2009; Maran *et al.*, 2013).

A significant increasing trend was also observed in the thickness of the composite film with increasing hemp concentration. This might be attributed to the gel-forming properties of the hemp seed protein. Mahajan *et al.* (2019) reported an increase in thickness of the film with increasing protein concentration that increased the viscosity of the film forming solution.

Moisture (%) and film density

While no significant impact of hemp seed protein was observed on the moisture content of the composite film, both carrageenan and glycerol increased ($P<0.05$) the moisture content of the basic film and highest values were observed for highest concentrations. A higher moisture content favours the microbial growth and affects the storage quality of the films and the products stored within. Both carrageenan and glycerol are hygroscopic in nature and have high tendency to bind with moisture. Like other hydrophilic plasticizers, glycerol loosens the film structure and a positive correlation has been established between the concentration of glycerol and the moisture content of hydrocolloid-based films such as those made of whey protein and chitosan (Leceta *et al.*, 2013). The moisture content of the composite films observed in our study was lower than those reported for pectin, alginate or whey protein films in the literature (Guidotti *et al.*, 2018). This might be attributed to the hydrophobic nature of hemp seed protein (Asli *et al.*, 2021).

While no significant ($P>0.05$) effect of carrageenan and glycerol were observed on the density of the basic film, addition of hemp seed protein increased ($P<0.05$) the density of the composite film and highest density was observed for the film containing highest concentration (1.5%) of hemp protein. Density (g/ml) is considered as an indicator of film weight and particularly affects the behaviour of the packaging in case of high moisture food products such as meat and meat products (Noor *et al.*, 2018). The higher the density, the heavier the film and vice versa. Increase in the density of the composite film with hemp seed protein might be attributed to its higher weight/density and increased dry matter content. Mahajan *et al.* (2019) reported a similar increase in the film density with increasing zein protein concentration.

Water vapour transmission rate (WVTR)

The ability to preserve the moisture content of food is directly influenced by the permeability of a film to allow moisture transfer between the environment and food. Addition of both glycerol and carrageenan resulted in a significant ($P<0.05$) increase in the WVTR of the films. The number of polar groups in a film change as carrageenan and glycerol concentration is altered. When glycerol concentration is increased, the film matrix undergoes a number of changes,such as increased inter-chain spacing and biopolymer mobility and decreased hydrogen bonding, which increases the diffusion of water molecules (Mahajan *et al.*, 2021a). Studies have reported an increase in the WVTR with increased starch and glycerol concentrations (Basiak *et al.*, 2018). The hydrophobic nature of hemp seed protein might be attributed to the lower WVTR of the composite film observed in our study (Asli *et al.*, 2021).

Transmittance (%)

Transmittance determines the transparency which is an aesthetic consideration in the marketing of the edible films. In general, higher transmittance results in better product visibility. Transmittance also indicates the light barrier properties of the films which can affect the lipid oxidation in foods and is an important attribute from the consumer's perspective (Leceta *et al.*, 2013). The transmittance of the films showed a significant ($P<0.05$) decrease with increasing concentration of carrageenan, glycerol, and hemp seed protein and might be attributed to the increased density and thickness of the film (Qian *et al.*, 2020). Similar findings were reported by Yan *et al.* (2012) in corn starch films.

Instrumental colour analysis

The mean colour values (L*-lightness, a*-redness and b*-yellowness) of the basic film containing different levels of carrageenan and glycerol are presented in Tables 1 and 2. The mean colour values of the composite film containing hemp seed protein are presented in Table 3. A significant

(P<0.05) diminishing pattern was observed in all the colour values (L*, a* and b*) of the films with increasing concentration of glycerol. This could be due to the dilution effect caused by the addition of glycerol, which is colourless, at increasing concentrations. The colour values of starch-based edible films have been observed to decrease as glycerol concentration increased (Basiak *et al.*, 2018). A significant (P<0.05) diminishing pattern was also observed in the colour values of the films with increasing concentration of carrageenan and hemp seed protein and could be attributed to the increased thickness and decreased transmittance of the film.

CONCLUSION

The present study showed a successful development of a composite biodegradable film using hemp seed protein and carrageenan for food packaging applications. Different levels of carrageenan (1.0, 1.4 and 1.8%) and glycerol (10, 15 and 20%) were used to standardise the formulation of the basic film. Based on the results, 1.4% carrageenan and 15% glycerol were found optimum for development of the basic film with desirable characteristics. Hemp seed protein was incorporated (0.75, 1.0 and 1.5%)in the basic formulation to develop a composite film with improved characteristics. Based on the results, 1.5% was found optimum for development of the film with desirable characteristics. The composite film can be used as a vehicle for controlled release of natural bioactive ingredients for developing bioactive packaging for the food products.

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