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Research Article

EFFECT OF EGG PROTEIN HYDROLYSATES ON THE FUNCTIONAL PROPERTIES OF WHEY PROTEIN CONCENTRATE FILMS

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Abstract- The current study was undertaken with the aim of investigating the effect of egg peptide hydrolysates on biofilms. Films were prepared from whey protein concentrate (WPC) using its different levels and added with alginate and glycerol. The films were analyzed for thickness, moisture content, solubility, water vapor transmission rate (WVTR), transmittance and penetrability. Egg protein hydrolysate (EPH) was added at 1 and 2 percent to the biofilms. The results revealed that the thickness and WVTR showed significant increase with the addition of hydrolysates. WPC films incorporated with protein hydrolysates showed antioxidant activity measured in terms of DPPH, FRAP, SASA and ABTS. The antioxidant effect of protein hydrolysates on WPC films was significant and increased with the increase in the concentration of EPH. The physic-chemical property of biofilms was affected by the addition of hydrolysates owing to its plasticizing effect on the protein network. However, there was insignificant effect on the appearance of biofilms. Therefore, it could be concluded that the biofilms incorporated with bioactive component can be used as an alternative to food preservation.

Key words- Antioxidant, Biofilm, Hydrolysate, WPC, WVTR

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Introduction

Packaging is considered as one of the most important methods of preservation of foods against oxidative and microbial deterioration [1]. Bioactive biodegradable film are thin layers of edible materials applied on food products and improve the quality and safety of food products due to its desired water and gas barrier properties. Further, these films may serve as carrier of active compounds including nutrients, antimicrobials, antioxidants and texture enhancer's etc. leading to improvement in the quality and safety characteristics of food products [2]. The physical characteristics, edibility, biocompatibility and bioactivity of these films are directed by the matrix material, film forming process protocols, plasticizer characteristics and incorporated active compounds. These preformed films are considered better than traditionally used coating methods for food preservation due to its physical characteristics, barrier properties and control diffusion of bioactive compounds. These films can be prepared from various sources such as polysaccharides (chitin, starch, cellulose derivatives, and gums), proteins (soy, milk, gelatin, corn zein, wheat gluten) and lipids (oils, waxes, resins). However, each matrix material used for developing the films has its innate advantages and disadvantages. It has been observed that the composite films have better physical and chemical characteristics than the films prepared from any single material [3].

Whey proteins are renewable source recovered as a by-product of cheese making industry having protein content of 25-80 percent. WPC has excellent nutritional and functional properties and can be made into edible films and coatings, both in the denatured and the native state [4]. WPC based films have good mechanical properties and also demonstrate better barrier properties comparable to the best synthetic polymer films in the market [5]. Utilization of the excess whey in the form of WPC could effectively alleviate the whey disposal problem by their conversion into value-added products, such as edible films and coatings.

The oxidation reactions in food products represent a major detrimental process leading to formation of undesirable chemical compounds (aldehydes, ketones, organic acids) of rancid odours and flavours [6]. The formation of secondary and potentially toxic compounds decreases the nutritional quality of food [7]. Antioxidant compounds are receiving attention in research and scientific literature as they are able to preserve food products by delaying oxidation. Synthetic antioxidants (BHA, BHT and n-propylgallate) exhibit strong activity against several oxidant systems. In the recent years, the antioxidant activity of bioactive peptides generated from the enzymatic hydrolysis of various proteins has attracted much attention. However, the current trend is to use natural products whose antioxidant activity takes place not only in packaged food but also once the food has been ingested. Such products include vegetable extracts, essential oils and isolated phenolic compounds [8].Eggs have been proved to possess various biological activities *viz*., antioxidant ability, antibacterial, and angiotensin converting enzyme-inhibitory effect [9]. Egg white proteins are widely used as functional and nutritional ingredients in food products and their hydrolysates obtained by protease treatment are water soluble and have high nutritional value [10]. The present work reviews the study on the antioxidant effect of egg protein hydrolysate on the functional properties of biocomposite edible films.

Materials and Methods

Films were prepared by dissolving selected levels of WPC and sodium alginate in distilled water and mixing glycerol (2%) as plasticizer. The mixture was homogenized with the homogenizer (S22 digital ultra TURRAX, Germany) followed by heating on the magnetic stirrer hot plate at temperature of 90^0 C for 25

minutes. Thereafter mixture was cooled on ice to prevent further denaturation of proteins followed by filtering through a cheese cloth to remove undisclosed material. The mixture solutions were casted on the plastic petri plates (90× 100mm) in a uniform layer for 7 h. the dried films were peeled off and stored in a chamber at 50% RH and 25^0 C temperature until evaluation. The films were analyzed on the basis of thickness, moisture content, solubility, water vapor transmission rate (WVTR), transmittance and penetrability.

The preliminary trails were conducted to incorporate the selected levels of egg protein hydrolysate at an appropriate stage of the preparation of edible films. Two levels of egg protein hydrolysate were incorporated in the developed edible film as: WPC film as control (C); WPC+ EPH (T_1-1%) and WPC+ EPH (T_2 -2%). Film thickness was measured with an electronic digital micrometer (Forbes Gokak ltd., Measuring Instrument, Aurangabad, India). The thickness was measured at five different places randomly along the length of the film strip preferable one at center and four around the perimeter and mean value was calculated. Moisture content of film was determined after drying in an oven at 105^0 C for 24 h. Small specimens of films collected after conditioning were cut and placed on Petri dishes that were weighed before and after oven drying. Moisture content values were determined in triplicate for each film and calculated as the percentage of weight loss relative to the original weight [11]. Water solubility was determined in triplicate according to a modification of the method of [12]. Water vapour transmission rate of film was measured using a modified ASTM 96-80 method [13]. Penetrability was determined by simulating the conditions to measure the force required to pierce the edible film. Penetrability was calculated automatically by the preloaded software in the texture analyzer (TMS-PRO, Food Technology Corporation, USA) from the force-time plot.

DPPH free radical scavenging activity of composite film was measured according to the method described by [14] with modifications. FRAP used to measure the ferric ion reducing capacity of composite film was measured according to the method described by [15]. The scavenging activity on superoxide radical was determined using the PMS-NADH superoxide generating system [16] with modifications. ABTS free radical scavenging activity of composite film was measured according to the method described by [17] with modifications. The data generated from various trials under each experiment was pooled and subjected to statistical analysis. The analysis of data was performed by using one-way analysis of variance (ANOVA). The ANOVA of group means was computed as mean±S.E. The significance of means was tested by using least significant difference (LSD) or C.D 0.5. The analysis was performed by using Comprehensive Statistical Package, SPSS (Statistical Package for Social Sciences) version 20. (Chicago, U.S.A for windows).

Results and Discussion

Thickness and Moisture content of films

All protein-based films (C, T_1 and T_2) with or without the addition of EPH were found to be homogeneous and flexible. Their thickness and moisture content are shown in [Table-1]. The thickness values of C, T_1 and T_2 were found as 174.90±1.87, 178.00±2.63 and 179.00±4.23 respectively. The films incorporated with EPH (T_1 and T_2) were found significantly ($p < 0.05$) thicker than the control. Film thickness increases with the incorporation of EPH. A high percentage of EPH (T_2) in the film produced a film with the highest thickness. Film thickness is considered an important parameter because it influences the biological properties and the shelf-life of coated foods [18]. The nature of the film-forming polymer and additives content affects film thickness by their interactions with the polymer matrix [19]. No significant differences ($p > 0.05$) were observed in the moisture content of the activated films compared with their corresponding controls.

Water Solubility

The water solubility of C, T_1 and T_2 were found as 33.12±0.23, 34.40±0.05 and 35.10±0.19 respectively as shown in [Table-1]. No significant ($p > 0.05$) were observed between the films. Therefore, water solubility was not significantly modified by the incorporation of egg protein hydrolysates to WPC films. The values were comparatively lower to those values reported in edible films based on Atlantic halibut in which the water solubility of film was found to be 90% [20].

Water Vapor Transmission Rate

Water vapour transmission rate significantly increases ($p < 0.05$) with the percentage of EPH in the films as shown in [Table-1]. The incorporation of EPH to the films may lead to an increase in plasticizer effect in the free volume of the film matrix. Consequently, the network becomes less dense and more permeable leading to the increase in the rate of water diffusion of the film matrix [21]. [22] reported that the water vapor transmission was influenced by hydrophilic-hydrophobic characteristic of the film forming materials and the steric hindrance of film molecular structure. It is reported the water vapor transfer process depended on the simultaneous actions of water diffusivity and solubility in a polymeric matrix [23].

Table-1 *Physico-chemical Parameters of Biofims Incorporated with Different Concentration of EPH*

Film	Thickness (µm)	Moisture (%)	Solubility (%)	WVTR (g/m^2t)	Penetrability (N)
C	174.90±1.87^b	24.05±0.89^a	33.12±0.23^a	0.00189±0.09^c	9.02±0.56^a
T_1	178.00±2.63^a	23.70±0.27^a	34.40±0.05^a	0.00395±0.06^b	7.20±0.85^b
T_2	179.00±4.23^a	25.13±0.34^a	35.10±0.19^a	0.00453±0.34^a	7.58±0.39^b

The values in a row with different superscript (a,b,c) differe significantly ($p<0.05$)

Penetrability

The penetrability of films is shown in [Table-1]. Puncture force in the film without egg protein hydrolysate, C (9.02±0.56) was found significantly higher ($p < 0.05$) than T_1 (7.20±0.85) and T_2 (7.58±0.39). Increasing percentage of egg protein hydrolysates in the WPC films decreased the mechanical resistance (puncture force).This was corroborated by [24,25] who reported that the plasticizing effect of peptide hydrolysates interfere in the cross-linking of protein network. The results can be correlated with those reported by [26] that by increasing the content of low molecular weight fragments may impair the formation of junction zones and the renaturation of gelatin chains into helix coil structure that takes place during the conditioning of the gelatin films, leading to a decrease in the puncture force of the films.

Antioxidant Activity of films

The antioxidant activity in terms of DPPH, FRAP, SASA and ABTS of whey protein concentrate films incorporated with egg protein hydrolysate is depicted in [Table-2]. DPPH is a stable free radical having maximum absorption at 517 nm. As shown in [Table-2]. DPPH scavenging activity of the films increased significantly ($p < 0.05$) as EPH concentration increased. 2% EPH film exhibited higher DPPH values (35.76±0.56) compared to film without EPH (31.18±0.87). The degree of antioxidant power of edible films is generally proportional to the amount of added antioxidant additives [27]. The results of the current study are in accordance with those of [28, 29] who reported a slight antioxidant activity of chitosan films, determined with DPPH assay.

The FRAP assay is used to determine the antioxidant potential of plant materials. The antioxidant activity by the FRAP assay is determined by the ability of various bioactive compounds to reduce ferric to ferrous iron. In the current study with the addition of EPH onto WPC films enhanced their antioxidant properties compared to the films without EPH and this enhancement was dependent on the concentration used. Statistical differences were found ($p < 0.05$) between WPC film (2.43±0.02), WPC + EPH 1% (5.98±0.24) and WPC + EPH 2% (9.87±0.87). The results of the study are in accordance with the results of [30] who reported that the addition of maqui berry extracts onto chitosan films enhanced their antioxidant properties compared to the chitosan films and this enhancement was dependent on the concentration used. The results were also in accordance with those reported by [24] that the incorporation of increasing concentrations of squid gelatin hydrolysates in the squid skin film showed a high antioxidant activity with increasing FRAP values compared to the control film. Superoxide anion radical (O_2^-) is generated in most of the biological systems and is generally found to be as a harmful precursor contributing to tissue damage [31]. Its toxic role could be

eliminated by superoxide dismutase (SOD), which converts superoxide anion radical possesses into hydrogen peroxide and oxygen [32]. Therefore, antioxidants with scavenging activity on superoxide anion radical possess protecting activity against cellular damages. In the current study SASA values of films with 2% EPH (76.54±2.45) and 1% (EPH were found to be significantly higher than film without EPH (70.56±0.65).

ABTS values of the protein films increased significantly ($p < 0.05$) when added EPH to the formulations. [Table-2] shows the significant($p < 0.05$) increase in the antioxidant capacity compared to the film without EPH (244.82±2.4) and its relation to the percentage of EPH incorporated *viz.,* 1% (273.63±19.9) and 2% (342.21±23.9) The study revealed the strong scavenging activity by adding EPH to biofilms. The results of the current study are in accordance with the results of [33, 34] who registered radical scavenging activity rates of film incorporated with α- tocopherol as 90.43% and 97.71%, respectively. However, the results were not in accordance with the results of [35] who reported low scavenging activity rates in fish skin gelatin incorporated with BHT and α-tocopherol caused by the interactions of hydrogen bonding between antioxidant and gelatin film matrix.

Table-2 *Antioxidant Activity of WPC Biofilms Incorporated with Egg Protein Hydrolysate*

Film composition	DPPH (%)	FRAP (%)	SASA (%)	ABTS (%)
WPC	31.18±0.87^{b}	2.43±0.02^{c}	70.56±0.65^{b}	244.82±2.4^{c}
WPC + EPH 1%	34.89±0.43^{a}	5.98±0.24^{b}	74.78±1.98^{a}	273.63±19.9^{b}
WPC + EPH 2%	35.76±0.56^{a}	9.87±0.87^{a}	76.54±2.45^{a}	342.21±23.9^{a}

The values in a row with different superscript (a,b,c) differe significantly ($p<0.05$)

Conclusion

Edible films or coatings have a potential of effectively controlling the mass transfer of different components within a food and its environment. A modern trend for developing active edible films is to combine different base materials and to incorporate multiple functional ingredients. The current study indicated that the EPH can be successfully incorporated into biodegradable WPC films. The presence of hydrolysate in the protein film affected the physicochemical properties as its presence plasticized the protein network, without significantly altering their appearance.

Application of research: The properties of the developed film could have applications in packaging of food susceptible to oxidation. These films may be an alternative for food preservation and extend the shelf-life of food by preventing the lipid oxidation of fatty foodstuffs.

Research Category: Biofilms

Abbreviations:

EPH –Egg Protein Hydrolysate
SOD – Super Oxide Dismutase
FRAP –Ferric Reducing Antioxidant Power
DPPH – 2, 2-Diphenyl - 1- Picrylhydazyl
WPC – Whey Protein Concentrate
ABTS – 2,2-azino bis 3-ethylbenzothiazoline-6-sulphonic acid
ANOVA –Analysis of Variance
LSD –Latin Square Design
RH –Relative Humidity
WVTR –Water Vapor Transmission Rate
SASA –Superoxide Anion Scavenging Activity

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