

Mechanical and Barrier Properties of Multi-Composite Shark Catfish (*Pangasius fungaseous*) Skin Gelatin Films with the Addition of Sorbitol, Clay and Chitosan Using Response Surface Methodology

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Abstract

The effects of four variable factors such as protein, sorbitol, montmorillonite (MMT) and chitosan on the properties of multi-composite shark catfish skin gelatin films were evaluated based on Response Surface Methodology (RSM). Incorporation of chitosan had positive effect on tensile strength, Young's Modulus and water vapor transmission rate ($p < 0.05$). On the other hand, chitosan and MMT had decreased the elongation of the films ($p < 0.05$). The transparency of the gelatin film was increased by the addition of gelatin and chitosan, whereas it was decreased by the addition of MMT. From the present study, it may be concluded that gelatin films with good mechanical and barrier properties could be achieved with 4.50% gelatin, 25% sorbitol, 0.37% MMT and 3.25% chitosan. Thus, multi-composite gelatin films with any desirable properties could be formulated based on the results of the response surface methodology and these films shall be suitable for edible films or coatings for fish and other food products in food processing industries.

Keywords: Fish gelatin; Chitosan; Tensile strength; Solubility; Barrier property

Introduction

Biodegradable edible films from biological materials have gained importance in recent years to reduce the environmental impact caused by synthetic or petroleum based materials. These biopolymer films can be made from proteins, polysaccharides or lipids. Among them, protein based edible films provide nutritional value and possess good mechanical and barrier properties [1]. They also function as protective layers of food products and enhance their shelf-life [2]. Gelatin is the thermally denatured protein, most abundant in connective tissue of animal origin and mainly extracted from land-based mammalian skins. Gelatin is obtained from collagen by acidic or alkaline processes [3]. It has been studied for its film-forming capacity and applicability as an edible film to protect food against dehydration, light and oxygen [4]. Due to its special characteristics such as high biocompatibility and weak antigenicity, it has been widely used in the pharmaceutical, food, healthcare, and cosmetic industries. However, the incidences of diseases such as bovine spongiform encephalopathy (BSE) and foot and mouth disease (FMD) have raised concerns about mammalian gelatin safety [5]. As an alternative, gelatin from fish processing wastes forms a good substitute. Fish gelatin are mainly produced from fish processing wastes such as skins, bones, fins etc. and their extraction procedures have been reported by several authors [6-8].

Gelatin films are generally formed using several plasticizers such as sorbitol, glycerol, ethylene glycol, sucrose, polyethylene glycol, glutaraldehyde, genipin, tannic acid and ferulic acid [9,10]. Sorbitol is a relatively small molecule with a hydrophilic nature that could be easily inserted between protein chains and establish hydrogen bonds with

amide groups and amino acid side chains of proteins [11]. Plasticizers are known to influence the gas and water vapour permeability of films and so they must be added in optimum concentrations to obtain films with improved flexibility without losing the barrier properties [12].

Fish gelatin films do have some limitations such as low tensile strength (TS) and high water solubility. In order to improve these properties, clay nanoparticles have proven to be a promising option [13]. The most common nanoparticles are montmorillonite K10 (MMT), hectorite, saponite, and laponite [14]. Among them, MMT has got a very high elastic modulus and helps to improve the mechanical and physical properties of the films. Chitosan is yet another natural polymer obtained by partial deacetylation of chitin and has been identified as a valuable additive because of its film forming ability, antioxidant and antimicrobial properties [15]. Fish gelatin films can thus be modified with any one or more biomolecules to improve mechanical, physical and barrier properties due to the presence of functional groups.

Few studies have been initiated to develop biodegradable edible films using fish gelatin by incorporation of nanoclay particles and other natural biopolymers like chitosan, starch and cellulose [16,17]. In our earlier study, multi-composite gelatin films were developed with the addition of MMT and chitosan using sorbitol as a plasticizer [18] but the individual effect of these variable factors on the film properties were not assessed. This study was therefore undertaken to examine the effect of four variable factors such as gelatin, sorbitol, MMT and chitosan on the mechanical, barrier and physical properties of the multi-composite films formed from shark catfish gelatin using response surface methodology (RSM).

Materials and Methods

Raw materials

Skins of shark catfish (*Pangasius fongaseous*) obtained from a private fish processing plant, M/s. Britto Seafoods Pvt. Ltd. Tuticorin, South India were used as raw material. They were washed with potable water, cut into small pieces using sharp knives and used for the extraction of gelatin.

Extraction of gelatin

Gelatin was extracted as per our method reported earlier [8]. Briefly, skins were first rinsed with tapwater to remove superfluous material and then treated twice with 0.2% NaOH at the ratio of 1:6 (w/v) for 45 min to remove the non-collageneous protein. After thorough washing, they were then treated twice with 0.2% H₂SO₄ at the ratio of 1:6 (w/v) for 45 min to increase swelling as well as to remove the salts. They were then treated with 1% citric acid twice at the ratio of 1:6 (w/v) for 45 min to achieve the lowest degree of turbidity and decalcification. The final extraction was carried out with distilled water at the ratio of 1:1 at 45°C for 24 h. The extract was then filtered through vacuum filter and the filtrate was lyophilized using Lyophilizer (Alpha 2, Martin Christ, Germany).

Preparation of multi-composite gelatin films

The multi-composite gelatin films were made based on the central composite rotatable design. To prepare the films, respective concentration of gelatin (3-5%, w/v) was dissolved in distilled water to obtain the film forming solution (FFS). Sorbitol (20-40%, w/w), chitosan (95% degree of deacetylation) (1-4%, w/v), and MMT K10 (0.25-0.75%, w/v) were then added at the prescribed concentrations to the FFS. Chitosan was previously dissolved in 0.3 M acetic acid then added to the FFS. The FFS was continuously stirred in a magnetic stirrer for 60 min at ambient temperature (25°C) to obtain a homogeneous suspension. The pH of the FFS was kept constant by the addition of 1 N sodium hydroxide or 1 N hydrochloric acid. The FFS (15 ml) was then casted in circular polypropylene plates (63 cm²) and dried at ambient temperature (25°C) for 14-18 h. The dried circular films were manually peeled off from the plates.

Response surface methodology

A central composite rotatable design was formulated using the Design Expert 7.0 software. Thirty blends were designed with different combinations of four independent variable factors such as protein (3-5%, w/v), sorbitol (20-40%, w/v), MMT (0.25-0.75%, w/v) and chitosan (1-4%, w/v) for the preparation of films (Table 1). Seven dependent responses viz. tensile strength, elongation at break, Young's Modulus, thickness, solubility, WVTR and transparency were evaluated using Design-Expert 7.0 (Stat-Ease, Inc. Minneapolis MN, USA).

Blends	Protein (%)	Plasticizer (%)	MMT (%)	Chitosan (%)
1	3.50	25.0	0.38	1.75
2	4.00	30.0	0.50	1.00
3	4.00	30.0	0.50	2.50

4	4.50	25.0	0.38	3.25
5	4.00	30.0	0.50	2.50
6	3.50	25.0	0.63	3.25
7	4.50	35.0	0.63	1.75
8	4.50	25.0	0.38	1.75
9	3.50	25.0	0.63	1.75
10	4.00	30.0	0.25	2.50
11	4.00	40.0	0.50	2.50
12	5.00	30.0	0.50	2.50
13	3.50	35.0	0.63	1.75
14	4.00	30.0	0.50	2.50
15	3.50	35.0	0.38	3.25
16	3.50	25.0	0.38	3.25
17	4.00	20.0	0.50	2.50
18	3.50	35.0	0.38	1.75
19	3.00	30.0	0.50	2.50
20	4.50	25.0	0.63	3.25
21	4.00	30.0	0.50	2.50
22	4.00	30.0	0.75	2.50
23	3.50	35.0	0.63	3.25
24	4.00	30.0	0.50	4.00
25	4.50	35.0	0.38	1.75
26	4.00	30.0	0.50	2.50
27	4.50	35.0	0.63	3.25
28	4.00	30.0	0.50	2.50
29	4.50	35.0	0.38	3.25
30	4.50	25.0	0.63	1.75

Table 1: Quantities of protein, plasticizer, mmt and chitosan used in the formation of multi-composite gelatin films.

Mechanical properties

Mechanical properties such as tensile strength (TS), elongation at break (EAB) and Young's Modulus (YM) were determined as per the standard ASTM method [19] using a Universal Testing Machine (TA plus Texture analyzer, Lloyd instruments, UK). The gelatin films were cut into rectangles of size 25 x 70 mm and fixed on the grips of the device with a gap of 30 mm. They were then pulled apart at crosshead speed of 20 mm/s and preload of 2 N. The TS was calculated by dividing the maximum force at break by cross sectional area of film, and expressed in MPa. The EAB and YM were calculated based on the maximum length extended at rupture and stiffness of the film, expressed in % and MPa, respectively. The thickness of the films were

measured using micro screw gauge (Labtech International, Ambala, Haryana, India) at six random positions as described by the method of Jeya Shakila et al. [18].

Water solubility

Water solubility of the gelatin films was determined as per the procedure of Gómez-Estaca et al. [20]. Film portion of size 4 cm² was initially dried at 105°C for 24 h, weighed and placed in a beaker with 15 ml distilled water, gently shaken at 22°C for 15 h and then filtered through the Whatman filter paper No 1. The residue collected on the filter paper was dried in a vacuum oven at 105°C for 24 h. The solubility of the film was calculated by the following formula:

$$\text{Solubility} = (W_0 - W_f / W_0) \times 100$$

Where, W₀ - Initial weight of the sample; W_f – Weight of the undissolved desiccated film residue

Light transmission

The light barrier properties of gelatin films were measured by exposing the films to light absorption at wavelengths at 600 nm using a UV/Vis Spectrophotometer (Model V-530, Jasco, Japan) according to the method of Gomez-Guillén et al. [21]. The transparency of the films was calculated by the equation $T = \text{Abs}_{600}/x$.

Where, T -transparency of the film, Abs₆₀₀ - value of absorbance at 600 nm, x - the film thickness in mm.

Water vapour transmission rate (WVTR)

The WVTR of the gelatin films was measured as per the standard ASTM (American Society for Testing and Materials) method [22] as described by Jeya Shakila et al. [18]. The gelatin film was cut into 0.90 x 0.90 cm pieces and each piece was put onto a permeability cup. The cup was previously filled with fused calcium chloride. The cup was then sealed with a cover and put into a humidity chamber at 25°C and 90% RH for 24 h. The weight of the sealed cup was measured at the beginning and at 1 h interval. The WVTR of the films was calculated using the following equation.

$$\text{WVTR} = (10,000 \times Q) / A \text{ g/m}^2/\text{day @ 90\% RH at 25}^\circ\text{C}$$

Where, Q = Quantity of water vapor passed through the test material; A=Area of test material

Results and Discussion

The multi-composite fish gelatin films produced from different blends with high concentration of chitosan and MMT were slightly opaque, homogenous and tough to break. Mechanical, physical and barrier properties of multi-composite gelatin films are given in Table 2.

Blends	TS (MPa)	EAB (%)	YM (MPa)	Thickness (mm)	Solubility (%)	Light Transmission	WVTR
1	13.8 ^a	65.5 ^h	139 ^a	0.064 ^a	46.0 ^b	0.92 ^f	677 ^h
2	13.5 ^a	73.6 ⁱ	132 ^a	0.075 ^a	57.5 ^d	0.84 ^e	632 ^g
3	27.0 ^d	71.9 ^j	222 ^b	0.072 ^a	46.1 ^b	0.49 ^b	646 ^g
4	33.3 ^e	41.9 ^c	545 ^e	0.089 ^f	44.4 ^b	0.25 ^a	679 ^h
5	27.0 ^d	71.9 ^j	222 ^b	0.072 ^a	46.1 ^b	0.49 ^b	646 ^g
6	34.9 ^e	36.9 ^c	602 ^f	0.096 ^d	32.9 ^a	1.43 ^h	707 ⁱ
7	13.2 ^a	75.4 ^j	118 ^a	0.09 ^c	53.3 ^d	0.81 ^e	706 ⁱ
8	16.8 ^a	67.1 ^h	185 ^b	0.098 ^b	51.3 ^c	0.87 ^f	663 ^h
9	20.5 ^b	43.9 ^d	338 ^c	0.086 ^a	43.8 ^b	1.05 ^g	608 ^g
10	21.6 ^c	88.7 ^j	192 ^b	0.098 ^c	45.1 ^b	0.55 ^c	621 ^g
11	31.3 ^e	64.0 ^h	415 ^d	0.093 ^d	61.7 ^e	1.19 ^g	632 ^g
12	37.7 ^h	49.5 ^f	639 ^f	0.081 ^d	56.1 ^d	0.16 ^a	342 ^a
13	15.4 ^a	53.2 ^f	204 ^b	0.081 ^a	54.7 ^d	1.83 ⁱ	532 ^e
14	27.0 ^d	71.9 ^j	222 ^b	0.072 ^a	46.1 ^b	0.49 ^b	646 ^g
15	34.3 ^e	54.5 ^f	373 ^c	0.083 ^d	60.8 ^e	0.49 ^b	502 ^e
16	45.1 ⁱ	37.0 ^c	794 ^h	0.069 ^c	56.2 ^d	1.05 ^g	526 ^e
17	63.7 ^l	18.1 ^a	1684 ^l	0.076 ^e	58.2 ^e	0.64 ^c	432 ^c
18	35.4 ^f	55.5 ^f	551 ^e	0.084 ^c	59.0 ^e	0.28 ^a	483 ^d
19	34.9 ^e	42.8 ^d	556 ^e	0.090 ^b	57.3 ^d	0.71 ^d	551 ^f

20	58.4 ^k	49.6 ^f	863 ^h	0.057 ^f	56.7 ^d	0.96 ^f	366 ^b
21	27.0 ^d	71.9 ^j	222 ^b	0.072 ^a	46.1 ^b	0.49 ^b	646 ^g
22	36.3 ^f	29.6 ^b	825 ^h	0.097 ^b	50.9 ^c	1.84 ⁱ	554 ^f
23	34.6 ^e	47.5 ^e	649 ^f	0.096 ^b	53.2 ^d	1.89 ^j	405 ^c
24	56.1 ^k	42.5 ^d	1043 ^j	0.081 ^d	42.8 ^b	0.47 ^b	339 ^a
25	20.8 ^c	60.7 ^g	339 ^c	0.086 ^c	59.1 ^e	0.84 ^e	486 ^d
26	27.0 ^d	71.9 ^j	222 ^b	0.072 ^a	46.1 ^b	0.49 ^b	646 ^g
27	49.4 ⁱ	37.5 ^c	978 ⁱ	0.076 ^e	56.0 ^d	1.04 ^g	420 ^c
28	27.0 ^d	71.9 ^j	222 ^b	0.072 ^a	46.1 ^b	0.49 ^b	646 ^g
29	57.4 ^k	24.8 ^b	1508 ^k	0.067 ^d	46.4 ^b	0.36 ^a	429 ^c
30	19.6 ^b	29.5 ^b	739 ^g	0.082 ^d	56.2 ^d	0.35 ^a	473 ^d

Different superscripts in the same column indicate significant differences (P<0.05).

Table 2: Mechanical, physical and barrier properties of multi-composite gelatine films according to the blends.

Mechanical properties

The ANOVA for response surface linear model on the mechanical properties such as TS, EAB and YM of multi-composite gelatin films are given in Table 3. The results indicated that chitosan had significant effect (p<0.01) on TS of the films. Increase in the TS with the addition of chitosan has been reported by Pierro et al. [23] in ovalbumin films and in tuna skin gelatin films [20]. But, increase in protein (3-5%), plasticizer (20-40%) and MMT (0.25-0.75%) concentrations did not influence the TS (p>0.05). A decrease in the TS of gelatin films with the addition of sorbitol has been observed by some workers [18,24] due to reduced intermolecular bonds between gelatin chains [25].

	p-value Prob>F						
	TS	EA B	YM	Thicknes s	Solubilit y	Transparenc y	WVT R
Gelatin	0.40	0.93	0.29	0.01	0.66	0.01	0.23
Plasticizer	0.33	0.10	0.24	0.66	0.02	0.33	0.53
MMT	0.70	0.05	0.43	0.75	0.87	0.01	0.50
Chitosan	<0.01	0.02	0.01	0.01	0.16	0.90	0.03

Table 3: ANOVA for response surface linear model on the properties of multi-composite gelatin films.

There are few earlier reports stating that the TS of gelatin films were influenced by the protein and chitosan concentration [26,27]. Also, many of the earlier reports suggested that the TS of gelatin films increased with an increasing MMT concentration [13,18]. It was also further evident that the incorporation of MMT within the gelatin molecules leads to unfold the gelatin molecule by hydrogen bonding [28]. But, our results did not show any significant effect by MMT as the ANOVA predicted the combination effect of all the four variable factors, which could not be assessed otherwise by simple experiments.

On the other hand, the chitosan had influenced the TS of the films as there were strong interactions between anionic gelatin and cationic chitosan by electrostatic/ hydrogen bonding during film formation.

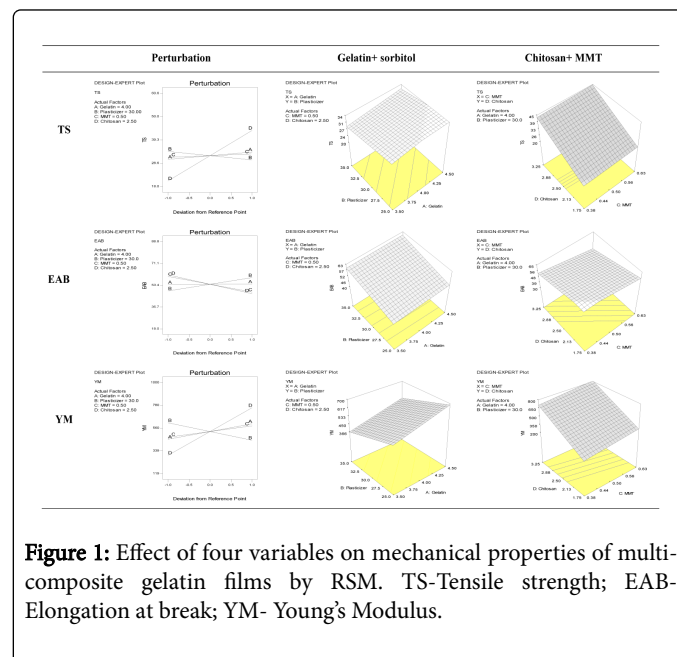


Figure 1: Effect of four variables on mechanical properties of multi-composite gelatin films by RSM. TS-Tensile strength; EAB- Elongation at break; YM- Young's Modulus.

The ANOVA indicated that the protein and sorbitol concentrations did not significantly affect the EAB (p>0.05) (Table 3), but perturbation studies showed positive effect on EAB by the sorbitol (Figure 1). An increase in EAB was observed by few workers with the increase in the plasticizer concentration in wheat gluten films, in brown and bigeye red snapper skin gelatin films and in chitosan films [25,27,29]. The increase in EAB was attributed to the formation of hydrogen bonding with gelatin chain by the sorbitol resulting in a reduction of intermolecular interactions [11]. In multi-composite gelatin films, however the effect of sorbitol on elongation of film was masked due to interference of chitosan-MMT-gelatin interactions resulting in the reduction of intermolecular spaces between gelatin

molecules ($p < 0.05$). A decrease in EAB by incorporation of chitosan was reported by some workers in food grade gelatin and tuna skin gelatin films [16,20]. In addition, the effect of MMT was also noticed in decreasing the EAB of the films ($p < 0.05$).

The response surface linear model ANOVA indicated that the YM was not significantly influenced ($p > 0.05$), but increased by chitosan concentration ($p < 0.01$) (Table 3). Perturbation studies, however, showed some increase of YM by the protein and MMT concentrations and negative effect by sorbitol (Figure 1). Our earlier study also showed an increase in YM of the redsnapper and grouper bone gelatin films with incorporation of chitosan and MMT [18]. The increase was caused by the gelatin-chitosan interactions that interfere with the gelatin network formation [30].

Physical properties

The ANOVA indicated that the protein and chitosan greatly increased the thickness of the gelatin films ($p < 0.01$), while sorbitol and

MMT did not exhibit any influence (Table 3) and the same was exhibited in the perturbation chart (Figure 2). The increase in thickness of films with the increase in protein concentration has been reported in bigeye and brownstripe red snapper skin gelatin films due to more protein aggregation [25,26]. Similarly, the influence of chitosan on the thickness of tuna skin gelatin-chitosan films was reported by Gomez-Estaca et al. [20]. The interaction of gelatin with the high molecular chitosan could be responsible for the increased thickness [18]. Earlier reports claimed an increase in thickness with increasing plasticizer concentration in cuttlefish skin gelatin films and pigskin gelatin films [31,32]. In the present study, the sorbitol effect was insignificant due to the prominence of protein-protein and protein-chitosan interactions. The MMT did not influence the thickness as they tend to uncoil the triple helical chains of gelatin molecules. Besides, sorbitol and MMT are smaller molecules than gelatin and chitosan to greatly influence the thickness of the films.

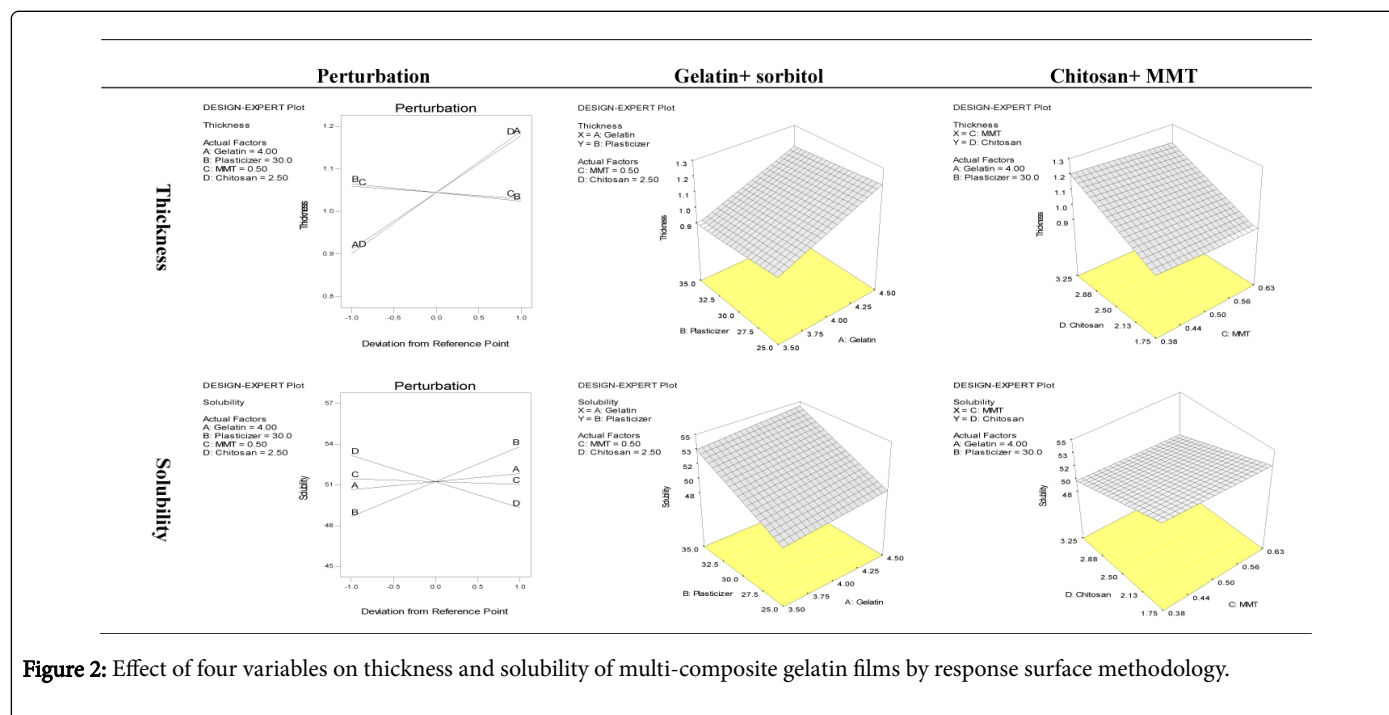


Figure 2: Effect of four variables on thickness and solubility of multi-composite gelatin films by response surface methodology.

The ANOVA indicated that the water solubility of the gelatin films was greatly increased by sorbitol ($p < 0.05$) and the other factors did not exhibit any influence ($p > 0.05$) (Table 3). The collective hydrophilic nature of gelatin and sorbitol had contributed for the increase in the water solubility [33]. However, perturbation chart showed some effect by chitosan in reducing the solubility (Figure 2). A decrease in the water solubility with the addition of chitosan has been reported in bovine-hide, and in tuna skin gelatin films, due to the cross-linking of proteins with chitosan through electrostatic interaction [20].

Barrier properties

Transparency and WVTR are important barrier properties that make an edible film suitable for food packaging applications. The

ANOVA indicated that the protein and chitosan exhibited lower value of light transmission, while sorbitol did not exhibit any significant effect ($p > 0.05$) (Table 3). The lower degree of light transmission with chitosan and protein indicated that these molecules improve the light barrier properties of gelatin film.

On the other hand, the MMT increased the films transparency value ($p < 0.01$) as clearly seen in chart (Figure 3). The gelatin molecule tends to unfold as they adsorb MMT at the interlayer surface and their maximum contact with the surface lead to decreased light barrier property of the gelatin films. Hoque et al. [31] reported that a decrease in transparency increases the light barrier property of the gelatin films.

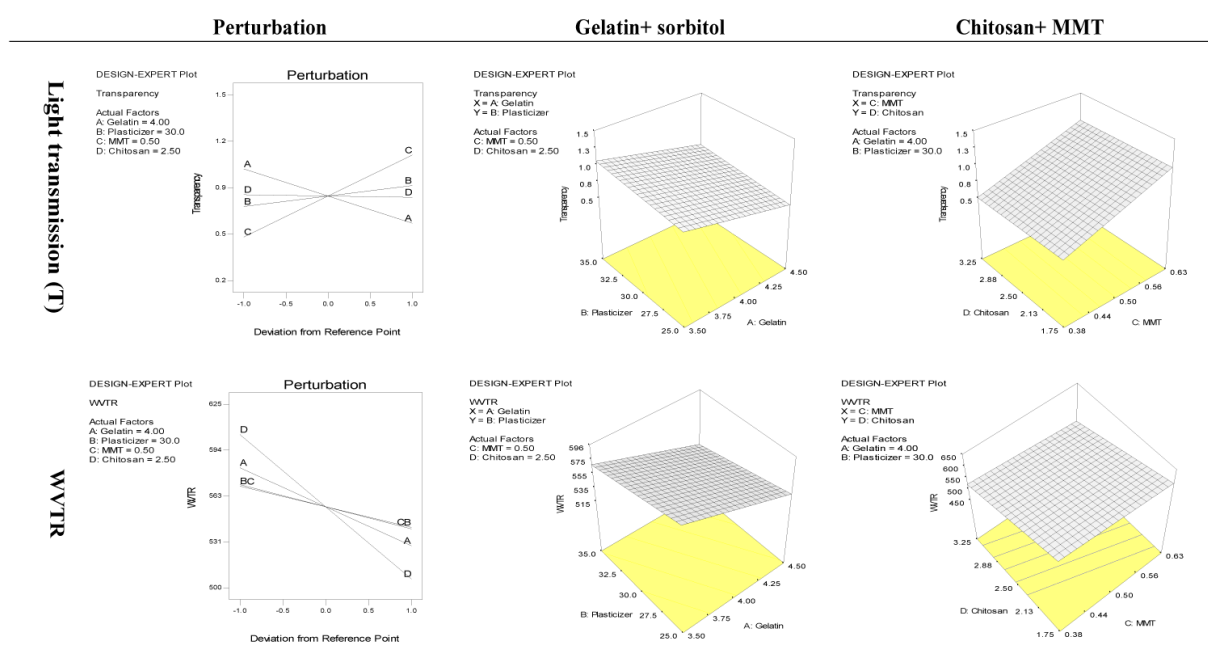


Figure 3: Effect of four variables on light transmission and water vapor permeability of multi-composite gelatin films by response surface methodology.

The ANOVA indicated that the effect of chitosan was significantly high in reducing the WVTR of the gelatin films ($p < 0.05$) than the other variable factors ($p > 0.05$) (Table 3). The chart showed that all the variable factors did have some influence on the WVTR (Figure 3), but the effect was more pronounced with chitosan. Earlier, researchers have observed that an increase in plasticizer concentration had increased the WVTR in rice starch films and chitosan films [33,34]. On contrary, Kowalczyk and Baraniak [35] found that an increase in sorbitol concentrations did not affect the WVTR of pea protein isolate films, in support to our findings. The effect of chitosan on WVTR has been reported earlier in grouper and red snapper gelatin films and in bovine-hide, tuna skin gelatin films [18,20]. The protein-chitosan interaction decreases the free volume between the gelatin polymer matrixes and reduces the WVTR of the films.

Conclusion

From the above results, the response surface methodology applied to the model enabled to understand the behavior of different variable factors on the mechanical, physical and barrier properties of the gelatin films. Also, the effect of every individual factor was influenced by the interaction of other variable factor in multi-composite gelatin films properties. Predictive models indicated that gelatin films with high TS, EAB and YM could be formulated with gelatin 4.40%, sorbitol 29.0%, MMT 0.38% and chitosan 3.25%. This property is adequate to prepare gelatin films with strength and flexibility. Gelatin films with low WVTR and solubility could be made with the blend that possess protein 3.50%, sorbitol 25.0%, MMT 0.60% and chitosan 3.25%. Opaque gelatin films with good light barrier properties can be made with minimum amounts of sorbitol 30.0% and MMT 0.50% and maximum amounts of protein 5.0% and chitosan 2.50%. In conclusion, the prepared multi-composite fish gelatin films with desirable

properties could be suitable biomaterials in biomedical and pharmaceutical industries as alternatives to mammalian gelatin films.

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References

1. Ou SY, Kwok KC, KANG YJ (2004) Changes in in-vitro digestibility and available lysine of soy protein isolate after formation of film. J Food Eng 64: 301-305.
2. Gennadios A, Weller CL, HANNA MA (1997) Soy protein/fatty acid films and coatings. Inform 8: 622-623.
3. Arvanitoyannis IS (2002) Formation and properties of collagen and gelatin? Ims and coatings. In A. Gennadios (ed.). Protein-Based Films and Coatings 275-304.
4. Ledward DA (1986) Gelation of gelatin, functional properties of food macromolecules. London: Elsevier Applied Science Publishers 171-201.
5. Sobral PJA, Menegalli FC, Hubinger MD, Roques MA (2001) Mechanical, water vapor barrier and thermal properties of gelatin based edible films. Food Hydrocolloid 15: 423-432.
6. Gomez-Guillen MC, Turnay J, Fernandez-Diaz MD, Ulmo N, Lizarbe MA, et al. (2002) Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocolloid 16: 25-34.
7. Muyonga JH, Cole CGB, Duodu KG (2004) Characterization of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chem.85: 81-89.

8. Jeya Shakila R, Jeevithan E, Varatharajakumar A, Jeyasekaran G, Sukumar D (2012) Functional characterization of gelatin extracted from bones of redsnapper and grouper in comparison with mammalian gelatin. Food Sci Technol 48: 30-36.
9. Bigi A, Borghi M, Cojazzi G, Fichera AM, Panzavolta S, et al. (2000) Structural and mechanical properties of cross-linked drawn gelatin films. J Therm Anal Calorim 61: 451-459.
10. Cao N, Fu Y, He J (2007) Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. Food Hydrocolloid 21: 575-584.
11. Gontard N, Guilbert S, Cuq JLZ (1993) Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. J Food Sci 58: 206-211.
12. Sothornvit R, Krochta JM (2000) Plasticizer effect on oxygen permeability of beta-lactoglobulin films. J Agric Food Chem 48: 6298-6302.
13. Abdollahi M, Alboofetileh M, Behrooz R, Rezaei M, Miraki R (2013) Reducing water sensitivity of alginate bio-nanocomposite film using cellulose nanoparticles. Int J Biol Macromol 54: 166-173.
14. Singa Ray S, Okamoto M (2003) Polymer/layered silicate nanocomposites: a review from preparation to processing. Prog Polym Sci 28: 1539-1641.
15. Jeon YJ, Kamil JY, Shahidi F (2002) Chitosan as an edible invisible film for quality preservation of herring and atlantic cod. J Agric Food Chem 50: 5167-5178.
16. Arvanitoyannis I, Psomiadou E, Nakayama A, Aiba S, Yamamoto N (1997) Edible films made from gelatin, soluble starch and polyols. Food Chem 60: 593-604.
17. Bae HJ, Darby DO, Kimmel RM, Park HJ, Whiteside WS (2009) Effects of Transglutaminase - induced cross-linking on properties of fish gelatin-nanoclay composite film. Food Chem 114: 180-189.
18. Jeya Shakila R, Jeevithan E, Varatharajakumar A, Jeyasekaran G, Sukumar D (2012) Comparison of the properties of multi-composite fish gelatin films with that of mammalian gelatin films. Food Chem 135: 2260-2267.
19. ASTM (1999) Standard test method for tensile properties of thin plastic sheeting. Standard designation D882-97. In: Annual book of ASTM standards. Philadelphia: ASTM 163-171.
20. Gomez-Estaca J, Gomez-Guillen MC, Fernandez-Martin F, Montero P (2011) Effects of gelatin origin, bovine-hide and tuna-skin, on the properties of compound gelatin chitosan films. Food Hydrocolloid 25: 1461-1469.
21. Gomez-Guillen MC, Ihl M, Bifani V, Silva A, Montero P (2007) Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae* Turcz). Food Hydrocolloid 21: 1133-1143.
22. ASTM (1987) Standard methods for water vapor transmission of materials (E 96-80). Annual book of ASTM Standards. Philadelphia, PA: American Society for Testing and Materials.
23. Pierro DP, Chico B, Villalonga R, Mariniello L, Masi P, et al. (2007) Transglutaminase catalyzed preparation of chitosan-ovalbumin films. Enzyme Microb Technol 40: 437-441.
24. Lim L, Mine Y, Tung MA (1999) Barrier and tensile properties of transglutaminase cross-linked gelatin films as affected by relative humidity, temperature and glycerol content. J Food Sci 64: 616-622.
25. Jongiareonrak A, Benjakul S, Visessanguan W, Tanaka M (2006) Skin gelatin from bigeye snapper and brownstripe red snapper: chemical compositions and effect of microbial transglutaminase on gel properties. Food Hydrocolloid 20: 1216-1222.
26. Limpisophon K, Tanaka M, Weng W, Abe S, Osako K (2009) Characterization of Gelatin films prepared from under-utilized blue shark (*Prionace glauca*) skin. Food Hydrocolloid 23: 1993-2000.
27. Zhong QP, Xia WS (2008) Physicochemical properties of edible and preservative films From chitosan /cassava starch/ gelatin blend plasticized with glycerol. Food Technol Biotech 46: 262-269.
28. Rhim JW, Hong SI, Park HM, Ng PK (2006) Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. J Agric Food Chem 54: 5814-5822.
29. Mangata I, Bauduin G, Boutevin B, Gondard N (2001) New plasticizers for wheat gluten films. Eur Polym J 37: 1533-1541.
30. Sionkowska A, Wisniewski M, Skopinska J, Kennedy CJ, Wess TJ (2004) Molecular interactions in collagen and chitosan blends. Biomaterials 25: 795-801.
31. Hoque S, Benjakul S, Prodpran T (2011) Properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin incorporated with cinnamon, clove and star anise extracts. Food Hydrocolloid 25: 1085-1097
32. Park JW, Whiteside WS, Cho SY (2008) Mechanical and water vapor barrier properties of extruded and heat-pressed gelatin films. Food Sci Technol 41: 692-700.
33. Lamim R, Freitas RA, Rudek EI, Wilhelm HM, Cavalcanti OA, et al. (2006) Films of chitosan and N-carboxymethyl chitosan. Part II: effect of plasticizers on their physico-chemical properties. Polymer Int 55: 970-977.
34. Laohakunjit N, Noomhorm A (2004) Effect of plasticizers on mechanical and barrier properties of rice starch film. Starch 56: 348-356.
35. Kowalczyk D, Baraniak B (2011) Effects of plasticizers, pH and heating of film-forming solution on the properties of pea protein isolate films. J Food Eng 105: 295-305.