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Preparation of Collagen Casings with High Mechanical Properties using Response Surface Methodology

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Abstract: Usages of plastic casing have a number of drawbacks like generation of waste associated with the materials used to package foods and the source of natural casings is limited. So, the interest in the development of biodegradable collagen casing is increasing. For this reason, the present study aimed at developing a new type of collagen casing sprayed with Tea Polyphenols (TP), sodium alginate and sodium pyrophosphate. Box-Behnken Design (BBD) of Response Surface Methodology (RSM) was used to optimize concentrations of TP, sodium alginate and sodium pyrophosphate for the development of collagen casing. The optimization was done on the basis of responses viz. tensile force, tensile strength and elongation at break. The results showed that TP has a significant influence on tensile force and longitudinal tensile strength of collagen casing. Moreover, sodium alginate and sodium pyrophosphate had a significant effect on transverse elongation at break of collagen casing. The optimum level of different parameters resulting in collagen casing with maximum mechanical properties were obtained under conditions of 1.99% TP, 2.90% sodium alginate and 2.99% sodium pyrophosphate concentrations. Further verification test of the optimized conditions revealed a sufficient specific accuracy. The new type of collagen casing exhibited good mechanical properties, so these casings can be utilized for packaging in sausage industry.

Keywords: Collagen Casings, Mechanical Properties, Response Surface Methodology

Introduction

Traditional casings are usually the intestines of animals, such as sheep. While these natural casings still have an important place in the market, their non-uniformity, high costs and growing demand have created greater challenges for the sausage industry (Chen *et al.*, 2019). Collagen casings are edible artificial casings made by cleaning, grinding, acid swelling, homogenization, degassing, extrusion, neutralization and drying of the underlying cowhide (Gómez-Estaca *et al.*, 2009). Collagen casings have the advantages of uniformity, hygiene and flexibility and have been recognized as the most promising casings (Harper *et al.*, 2012). It is estimated that about 80% of sausages require an edible collagen casing, such as hot dogs, Taiwanese sausages (Wang *et al.*, 2015). However, sometimes there will be a series of application problems during the production process. For example, collagen casings can break during filling, deform during fumigation, even fall off the shelves and break or separate from the meat in cooking (Adzaly *et al.*, 2016). It is due to the fact that the collagen molecule loses its triple helix structure to some extent during the extraction process, which affects its mechanical properties (Vergne *et al.*, 2018). In addition, mechanical properties were important during sausage manufacturing because a sausage casing must be tender enough to be pliable during stuffing. Therefore, the mechanical properties (tensile force, tensile strength and elongation at break) of collagen casings need to be improved.

TP is a natural antioxidant of typical flavonoids, which can eliminate reactive oxygen radicals produced by many systems and protect cells from damage (Frei and Higdon, 2003). The main components of TP are Epicatechin (EC), Epicatechin Gallate (EGC), Epicatechin Gallate (ECG) and Epigalate-3-Gallate (EGCG) (Azam *et al.*, 2004). Recent studies have shown that TP protect against oxidative stress-related diseases, including cancer, cardiovascular and degenerative diseases, as well as other biological activities (Higdon and Frei, 2003). In view of the advantages mentioned above as well as low-cost and safety of TP, the application of TP in the development of edible active packaging film has attracted the attention of many researchers in recent years, such as TP as carrier could improve the antioxidant and antibacterial properties of chitosan film (Zhang and Jiang, 2020). Another research reported that great antioxidant and antimicrobial activity as well as mechanical and water-barrier properties were exhibited in bioactive edible packaging films based on pomelo peel flours (Wu *et al.*, 2019). The incorporation of TP in chitosan films obviously enhanced the antioxidant activity and reduced the water vapor permeability (Wang *et al.*, 2013). Sodium alginate, a kind of bio-adsorbent with excellent adsorption properties, is one of the most studied coating materials because of its advantages such as sustainability, biodegradability, biocompatibility and low toxicity (Gao *et al.*, 2017). At present, the sodium alginate films with essential oils had a considerably high antibacterial effect against foodborne pathogenic bacteria and a strong DPPH radical scavenging ability (Mahcene *et al.*, 2020). The biobased alginate/castor oil edible films obviously improved the mechanical properties and displayed a significant inhibitory effect against *S. aureus* and *B. subtilis* (Gram-positive bacteria) (Aziz *et al.*, 2018). In addition, the alginate film was synthesized from turmeric, TP and blackberry extract also has good physical and mechanical properties (Kalaycıoğlu *et al.*, 2017; Dou *et al.*, 2018; Kim *et al.*, 2018). Sodium pyrophosphate is the most widely used functional phosphate in meat processing (Shen and Swartz, 2010). To promote gelation, sodium pyrophosphate is often added to meat processing to help extract myofibrillar protein, which then aggregates and gels during cooking (Jongberg *et al.*, 2015).

Response Surface Methodology (RSM) is an empirical modeling method, which is used to assess the impact of multiple independent variables on the response with the goal of optimizing the response, RSM modeling can provide more accurate and complete data with the least number of experiments (Bagheri *et al.*, 2019). Many studies on optimal film formulation have been investigated by using RSM such as the effects of chitosan, glycerin and drying temperature on the response variables of chitosan food film and the effects of pea starch, chitosan and glycerin on the physical, mechanical and barrier properties of pea starch-chitosan food film (Singh *et al.*, 2015; Thakur *et al.*, 2017). Therefore, the objective of this study was to determine the optimization of collagen casing sprayed with different concentration TP, sodium alginate and sodium pyrophosphate using RSM-BBD.

Materials and Methods

Materials

The collagen casings (from cowhide and the aperture is 25 mm) were offered by Zibo Huanghelong Bioengineering Co., Ltd. (Zibo, China). TP were obtained from Freder Biotechnology Co., Ltd (Suzhou, China). Sodium alginate and sodium pyrophosphate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All other reagents used in this study were analytical grade. Sterile water was used throughout this study.

Experimental Methods

Preparation of Collagen Casings

Collagen casings were cut into 20 cm lengths and then sprayed with 5 mL of food additions solution (sodium alginate, sodium pyrophosphate and TP). After treatment for 10 h at 35°C, collagen casings were kept in a desiccator at 25°C and 50% Relative Humidity (RH) before further analysis.

Mechanical Properties

The films were cut into strips (15×3 cm in the longitudinal direction, 5×3 cm in the transverse direction) and stored at 25°C and 50% RH for 24 h before measurement. The tensile force, tensile strength and elongation at break of the collagen casings were measured at 25°C with a tensile testing machine (XLW; Labthink, China). The tensile tests were performed with a longitudinal gap of 10 cm, a transverse gap of 3 cm and a crosshead speed of 1 mm/s.

Response Surface Experimental Design

In response surface experimental, RSM using BBD was employed to investigate the relationship between three independent variables (X_1, concentration of sodium alginate; X_2, concentration of sodium pyrophosphate; X_3, concentration of TP) that was code at three levels (-1, 0, 1) and six dependent variables (Y_1, longitudinal tensile force; Y_2, transverse tensile force; Y_3, longitudinal tensile strength; Y_4, transverse tensile strength; Y_5, longitudinal elongation at break; Y_6, transverse elongation at break) of collagen casings in this study (Table 1). All the ranges of the parameters in RSM were selected based on the results of single factor experiments according to our previous study (Xie *et al.*, 2020). The concentrations of sodium alginate, sodium pyrophosphate and TP varied from 2.5 to 3.5%, 2.5 to 3.5% and 1.5 to 2.5%, respectively. The uncoded and coded values of the independent variables and the experiment design were depicted in Table 2. The complete design was carried out in a random order and consisted of 17 combinations including 5 replicates at central point and the process flow diagram of collagen casing was shown in Fig. 1.

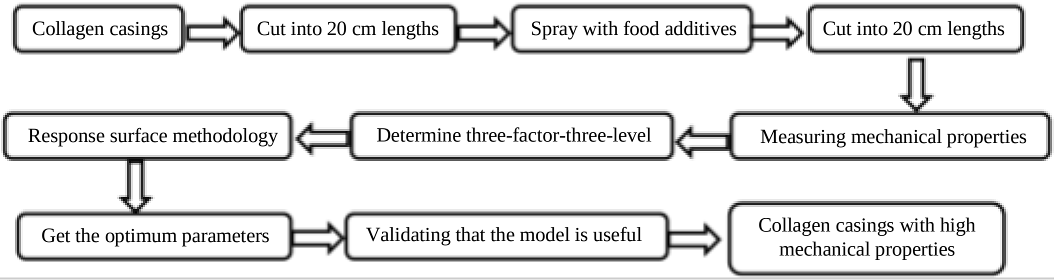

Fig. 1: The process flow diagram of collagen casing with high mechanical properties

Table 1: Independent variables and their code variable levels used for the Box-Behnken design

			Coded levels		
No	Independent variables	Symbol	-1	0	+1
1	Concentration of sodium alginate (%)	X_1	2.5	3.0	3.5
2	Concentration of sodium pyrophosphate (%)	X_2	2.5	3.0	3.5
3	Concentration of TP (%)	X_3	1.5	2.0	2.5

Table 2: Box-Behnken experimental design and corresponding response values

Run order	X_1 (%)	X_2 (%)	X_3 (%)	Y_1 (N)	Y_2 (N)	Y_3 (MPa)	Y_4 (MPa)	Y_5 (%)	Y_6 (%)
1	2.50	3.00	2.50	11.37	6.61	9.02	8.06	24.20	38.83
2	3.50	3.50	2.00	10.49	6.10	8.66	7.92	23.40	45.67
3	3.00	3.00	2.00	12.57	7.60	10.21	9.02	25.90	48.00
4	2.50	3.00	1.50	11.44	5.98	9.53	7.91	25.09	44.83
5	3.00	3.00	2.00	13.01	7.23	10.05	8.40	25.89	48.00
6	2.50	2.50	2.00	11.68	6.68	9.73	8.52	25.00	42.83
7	3.00	3.00	2.00	12.58	7.20	10.11	9.18	26.50	46.52
8	3.50	3.00	1.50	10.02	5.87	8.34	7.82	23.90	45.68
9	3.00	3.50	1.50	10.78	5.99	8.98	7.76	24.52	43.50
10	3.50	2.50	2.00	11.15	5.80	9.29	8.72	24.80	42.33
11	2.50	3.50	2.00	11.68	6.75	9.73	7.95	24.62	43.89
12	3.50	3.00	2.50	10.74	5.89	8.95	7.92	23.35	39.83
13	3.00	3.00	2.00	12.59	7.62	10.30	8.98	26.20	47.67
14	3.00	2.50	2.50	10.89	6.02	9.23	7.68	24.95	39.33
15	3.00	3.00	2.00	12.57	7.90	9.89	8.99	25.08	46.50
16	3.00	3.50	2.50	10.47	6.25	9.56	8.52	23.98	42.00
17	3.00	2.50	1.50	10.56	6.23	8.80	8.43	23.35	42.30

X_1 = Concentration of sodium alginate (%), X_2 = Concentration of sodium pyrophosphate (%), X_3 = Concentration of TP (%), Y_1 = Longitudinal tensile force (N), Y_2 = Transverse tensile force (N), Y_3 = Longitudinal tensile strength (MPa), Y_4 = Transverse tensile strength (MPa), Y_5 = Longitudinal elongation at break (%), Y_6 = Transverse elongation at break (%)

The relationship between dependent variables (responses) and independent variables was evaluated using a polynomial second-degree model given by the following equation (Hayta and İşçimen, 2017):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ii} X_i X_j$$

where, Y is the dependent variable, X_i and X_j are the independent variables and β_0, β_i, β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively.

Statistical Analysis

The results were expressed as the mean values ± standard deviations (n = 3). One-way Analysis Of Variance (ANOVA) was implemented by using Origin 8.0 followed by multiple tests in order to determine the significant difference at $p<0.05$.

Results and Discussion

The Response Surface Methodology Fitting Model

Three-factor-three-level experimental design using RSM and BBD was employed to investigate the influence of variables including sodium alginate, sodium pyrophosphate and TP on the mechanical properties of collagen casings. The design matrix and the corresponding responses were summarized in Table 2. Then the data was used to perform multiple linear regressions analysis by a quadratic polynomial model. The results of fitting quadratic models were presented in

Table 3-5. The result revealed that for the model of six responses (longitudinal and transverse tensile force, tensile strength and elongation at break) p-values were calculated to be <0.0001, 0.0017, 0.0025, 0.0451, 0.0364 and 0.0022, respectively. These data were all significant ($p<0.05$), indicating that the models constructed in this study had significant regression. Moreover, the result showed that p-values for lack of fit were 0.5561, 0.7148, 0.1183, 0.4135, 0.3287 and 0.1111, respectively. These data above were all more than 0.05 and insignificant, specifying that the mathematical models were satisfactory for prediction of mechanical properties of collagen casings involved in this study.

The coefficient of determination (R^2) is the square of the coefficient of correlation and illustrates the adequacy of a model. R^2 ranges from 0 to 1. The higher the value of R^2, the better the fit of the model (Hayta and İşçimen, 2017). As shown in Table 3-5, the values of R^2 for six responses (longitudinal and transverse tensile force, tensile strength and elongation at break) were obtained to be 0.9825, 0.9395, 0.9320, 0.8313, 0.8418 and 0.9347, respectively, implying the close correlation between predicted values and experimental values.

Development of Second Order Polynomial Mathematical Models

The final regression equations in terms of longitudinal and transverse tensile force, tensile strength and elongation at break were developed respectively as follows:

$$Y_1 = 12.66 - 0.47X_1 - 0.11X_2 + 0.084X_3 - 0.17X_1X_2 + 0.20X_1X_3 - 0.16X_2X_3 - 0.60X_1^2 - 0.82X_2^2 - 1.17X_3^2$$

$$Y_2 = 7.51 - 0.30X_1 + 0.045X_2 + 0.087X_3 + 0.057X_1X_2 - 0.15X_1X_3 + 0.12X_2X_3 - 0.61X_1^2 - 0.57X_2^2 - 0.82X_3^2$$

$$Y_3 = 10.11 - 0.35X_1 - 0.015X_1 + 0.14X_3 - 0.16X_1X_2 + 0.28X_1X_3 + 0.038X_2X_3 - 0.47X_1^2 - 0.29X_2^2 - 0.68X_3^2$$

$$Y_4 = 8.91 - 0.0075X_1 - 0.15X_2 + 0.032X_3 - 0.058X_1X_2 - 0.012X_1X_3 + 0.38X_2X_3 - 0.4X_1^2 - 0.23X_2^2 - 0.58X_3^2$$

$$Y_5 = 25.91 - 0.43X_1 - 0.20X_2 - 0.048X_3 - 0.26X_1X_2 + 0.085X_1X_3 - 0.53X_2X_3 - 0.76X_1^2 - 0.70X_2^2 - 1.02X_3^2$$

$$Y_6 = 47.34 + 0.39X_1 + 1.03X_2 - 2.04X_3 + 0.57X_1X_2 + 0.038X_1X_3 + 0.37X_2X_3 - 1.57X_1^2 - 2.08X_2^2 - 3.47X_3^2$$

Table 3: Analysis of variance for the fit quadratic model of tensile force

Indicators	Sources of variation	Sum of squares	df	Mean square	F value	P value
Y_1	Model	13.47	9	1.500	43.72	<0.0001[a]
	X_1	1.78	1	1.780	51.91	0.0002[a]
	X_2	0.092	1	0.092	2.70	0.1443
	X_3	0.056	1	0.056	1.64	0.2412
	X_1X_2	0.11	1	0.110	3.18	0.1177
	X_1X_3	0.16	1	0.160	4.56	0.0701
	X_2X_3	0.10	1	0.100	2.99	0.1273
	X_1^2	1.51	1	1.510	44.03	0.0003[a]
	X_2^2	2.80	1	2.800	81.86	<0.0001[a]
	X_3^2	5.80	1	5.800	169.33	<0.0001[a]
	Residual	0.24	7	0.034		
	Lack of fit	0.090	3	0.030	0.80	0.5561
	Pure error	0.15	4	0.037		
	Cor total	13.71	16			
		$R^2 = 0.9825$				
Y_2	Model	7.31	9	0.810	12.07	0.0017[a]
	X_1	0.70	1	0.700	10.34	0.0147[a]
	X_2	0.016	1	0.016	0.24	0.6387
	X_3	0.061	1	0.061	0.91	0.3719
	X_1X_2	0.013	1	0.013	0.20	0.6709
	X_1X_3	0.093	1	0.093	1.38	0.2782
	X_2X_3	0.055	1	0.055	0.82	0.3951
	X_1^2	1.55	1	1.550	22.99	0.0020[a]
	X_2^2	1.37	1	1.370	20.42	0.0027[a]
	X_3^2	2.81	1	2.810	41.68	0.0003[a]
	Residual	0.47	7	0.067		
	Lack of fit	0.12	3	0.041	0.48	0.7148
	Pure error	0.35	4	0.087		
	Cor total	7.78	16			
		$R^2 = 0.9395$				

[a] Significant difference with $p<0.05$, X_1 = Concentration of sodium alginate (%), X_2 = Concentration of sodium pyrophosphate (%), X_3 = Concentration of TP (%), Y_1 = Longitudinal tensile force (N), Y_2 = Transverse tensile force (N), df = Degree of freedom

Table 4: Analysis of variance for the fit quadratic model of tensile strength

Indicators	Sources of variation	Sum of squares	df	Mean square	F value	P value
Y_3	Model	5.10	9	0.5700	10.660	0.0025[a]
	X_1	0.96	1	0.9600	18.050	0.0038[a]
	X_2	1.80E-003	1	1.80E-003	0.034	0.8592
	X_3	0.15	1	0.1500	2.900	0.1324
	X_1X_2	0.099	1	0.0099	1.870	0.2140
	X_1X_3	0.31	1	0.3100	5.900	0.0454[a]
	X_2X_3	5.62E-003	1	5.62E-003	0.110	0.7544
	X_1^2	0.93	1	0.9300	17.580	0.0041[a]
	X_2^2	0.35	1	0.3500	6.600	0.0371[a]
	X_3^2	1.95	1	1.9500	36.760	0.0005[a]
	Residual	0.37	7	0.0530		
	Lack of fit	0.27	3	0.0910	3.720	0.1183
	Pure error	0.098	4	0.0250		
	Cor total	5.47	16			
		$R^2 = 0.9320$				
Y_4	Model	3.35	9	0.3700	3.830	0.0451[a]
	X_1	4.50E-003	1	4.50E-003	4.63E-003	0.9476
	X_2	0.18	1	0.1800	1.850	0.2156
	X_3	8.45E-003	1	8.45E-003	0.087	0.7766
	X_1X_2	0.013	1	0.0130	0.140	0.7231
	X_1X_3	6.25E-004	1	6.25E-004	6.43E-003	0.9383
	X_2X_3	0.57	1	0.5700	5.870	0.0459[a]
	X_1^2	0.68	1	0.6800	7.050	0.0327[a]
	X_2^2	0.23	1	0.2300	2.360	0.1685
	X_3^2	1.43	1	1.4300	14.750	0.0064[a]
	Residual	0.68	7	0.0970		
	Lack of fit	0.32	3	0.1100	1.210	0.4135
	Pure error	0.36	4	0.0890		
	Cor total	4.03	16			
		$R^2 = 0.8313$				

[a] Significant difference with $p<0.05$, X_1 = Concentration of sodium alginate (%), X_2 = Concentration of sodium pyrophosphate (%), X_3 = Concentration of TP (%), Y_3 = Longitudinal tensile strength (MPa), Y_4 = Transverse tensile strength (MPa), df = Degree of freedom

In the equations above, the positive signs in front of the terms (X_1, X_2 and X_3) symbolized synergistic effect and the negative signs indicated antagonistic effect. Moreover, values in front of the terms higher, impact of the coefficient greater.

Effect of Independent Variables on Tensile Force

Longitudinal and transverse tensile force of collagen casings ranged from 10.02 to 13.01 N and 5.8 to 7.9 N in the RSM experiments (Table 2), respectively. As shown in Table 3, coefficients of X_1, X_1^2, X_2^2 and X_3^2 were significant based on a 95% confidence level in affect longitudinal and transverse tensile force of collagen casings, it implied that sodium alginate (X_1) was the significant variable and had higher impact on longitudinal tensile force and transverse tensile force than sodium pyrophosphate (X_2) and TP (X_3). The interaction effects of the independent variables on the tensile force of collagen casings were presented in Fig. 2 and 3. As illustrated in Fig. 2A and Fig. 3A, the longitudinal and transverse tensile force increased firstly and then decreased as the concentration of sodium alginate increased, which was consistent with our previous research (Xie *et al.*, 2020). We also found that longitudinal tensile force reached the lowest value and transverse tensile force reached highest value when concentration of TP was 1.5 and 1.99%, respectively (Fig. 2C and 3C). However, no significant interaction effects on the tensile force of collagen casings were illustrated between variables (sodium alginate, sodium pyrophosphate and TP) (Table 3, Fig. 2 and 3).

Effect of Independent Variables on Tensile Strength

In the RSM experiments, longitudinal and transverse tensile strength of collagen casings ranged from 8.34 to 10.3 MPa and 7.68 and 9.18 MPa, respectively (Table 2). As illustrated in Table 4, coefficients of X_1, X_1X_3, X_1^2, X_2^2 and X_3^2 were found to be significant ($p<0.05$) in affect longitudinal tensile strength of collagen casings, illustrating that sodium alginate (X_1) exhibited higher impact on longitudinal tensile strength than sodium pyrophosphate (X_2) and TP (X_3) and significant interaction effects on longitudinal tensile strength of collagen casings were demonstrated between sodium alginate and TP (Fig. 4B); to transverse tensile strength of collagen casings, the coefficients of X_2X_3, X_1^2 and X_3^2 were significant ($p<0.05$) which demonstrated that the effects of three factors on the transverse tensile strength were not significant but the interaction between sodium alginate and TP (X_2X_3) had a significant influence on transverse tensile strength (Fig. 5C). Moreover, the highest longitudinal and transverse tensile strength values were obtained at 2.90% of sodium alginate concentration and 2.99% sodium pyrophosphate, respectively. The results from Fig. 4 and 5 also showed that the longitudinal and transverse tensile strength first increased and then decreased with the increase of sodium alginate, sodium pyrophosphate and TP concentrations.

Effect of Independent Variables on Elongation at Break

The longitudinal and transverse elongation at break of collagen casings varied from 23.35 to 26.5% and 38.83 to 48% in the RSM experiments, respectively (Table 2). As shown in Table 5, coefficients of X_1^2, X_2^2 and X_3^2 were significant based on a 95% confidence level in affect longitudinal elongation at break of collagen casings, showing that three variables had no significant influence on longitudinal elongation at break and the coefficients of X_2, X_3, X_1^2, X_2^2 and X_3^2 were significant ($p<0.05$) in affect transverse elongation at break of collagen casings, implying that sodium pyrophosphate (X_2) and TP (X_3) were the significant variable and had higher impact on transverse elongation at break. These results above as well as results from Fig. 6 and 7 also indicated that there were no significant interaction effects between variables on the elongation at break of collagen casings.

Determination and Validation of Optimized Compositions

The desirability function was used for simultaneous optimization of the multiple responses. This function enables a combination of independent variables that simultaneously optimizes the requirement for each response in the design. The aim was to maximize six mechanical properties. Therefore, these responses were considered to study the possibility of choosing one formulation which optimizes the mechanical properties of studied collagen casings. The maximum, minimum and average values of these variables experimentally achieved in the Box-Behnken design (Table 2) were applied for calculation of the desirability function.

Table 5: Analysis of variance for the fit quadratic model of elongation at break

Indicators	Sources of variation	Sum of squares	df	Mean square	F value	P value
Y_5	Model	13.11	9	1.46	4.18	0.0364[a]
	X_1	1.50	1	1.50	4.29	0.0770
	X_2	0.31	1	0.31	0.89	0.3757
	X_3	0.018	1	0.018	0.10	0.8265
	X_1X_2	0.26	1	0.26	0.75	0.4164
	X_1X_3	0.029	1	0.029	0.083	0.7818
	X_2X_3	1.14	1	1.14	3.28	0.1129
	X_1^2	2.44	1	2.44	7.01	0.0330[a]
	X_2^2	2.05	1	2.05	5.87	0.0460[a]
	X_3^2	4.35	1	4.35	12.49	0.0095[a]
	Residual	2.44	71	0.35		
	Lack of fit	1.32	3	0.44	1.57	0.3287
	Pure error	1.12	4	0.28		
	Cor total	15.55	16			
		$R^2 = 0.8418$				
Y_6	Model	132.36	9	14.71	11.14	0.0022[a]
	X_1	1.22	1	1.22	0.93	0.3676
	X_2	8.55	1	8.55	6.48	0.0384[a]
	X_3	33.29	1	33.29	25.22	0.0015[a]
	X_1X_2	1.30	1	1.30	0.98	0.3542
	X_1X_3	5.62E-003	1	5.62E-003	4.26E-003	0.9498
	X_2X_3	0.54	1	0.54	0.41	0.5427
	X_1^2	10.43	1	10.43	7.90	0.0261[a]
	X_2^2	18.29	1	18.29	13.85	0.0074[a]
	X_3^2	50.74	1	50.74	38.43	0.0004[a]
	Residual	9.24	7	1.32		
	Lack of fit	6.88	3	2.29	3.89	0.1111
	Pure error	2.36	4	0.59		
	Cor total	141.60	16			
		$R^2 = 0.9347$				

[a]Significant difference with $p<0.05$, X_1 = Concentration of sodium alginate (%), X_2 = Concentration of sodium pyrophosphate (%), X_3 = Concentration of TP (%), Y_5 = Longitudinal elongation at break (%), Y_6 = Transverse elongation at break (%), df = Degree of freedom

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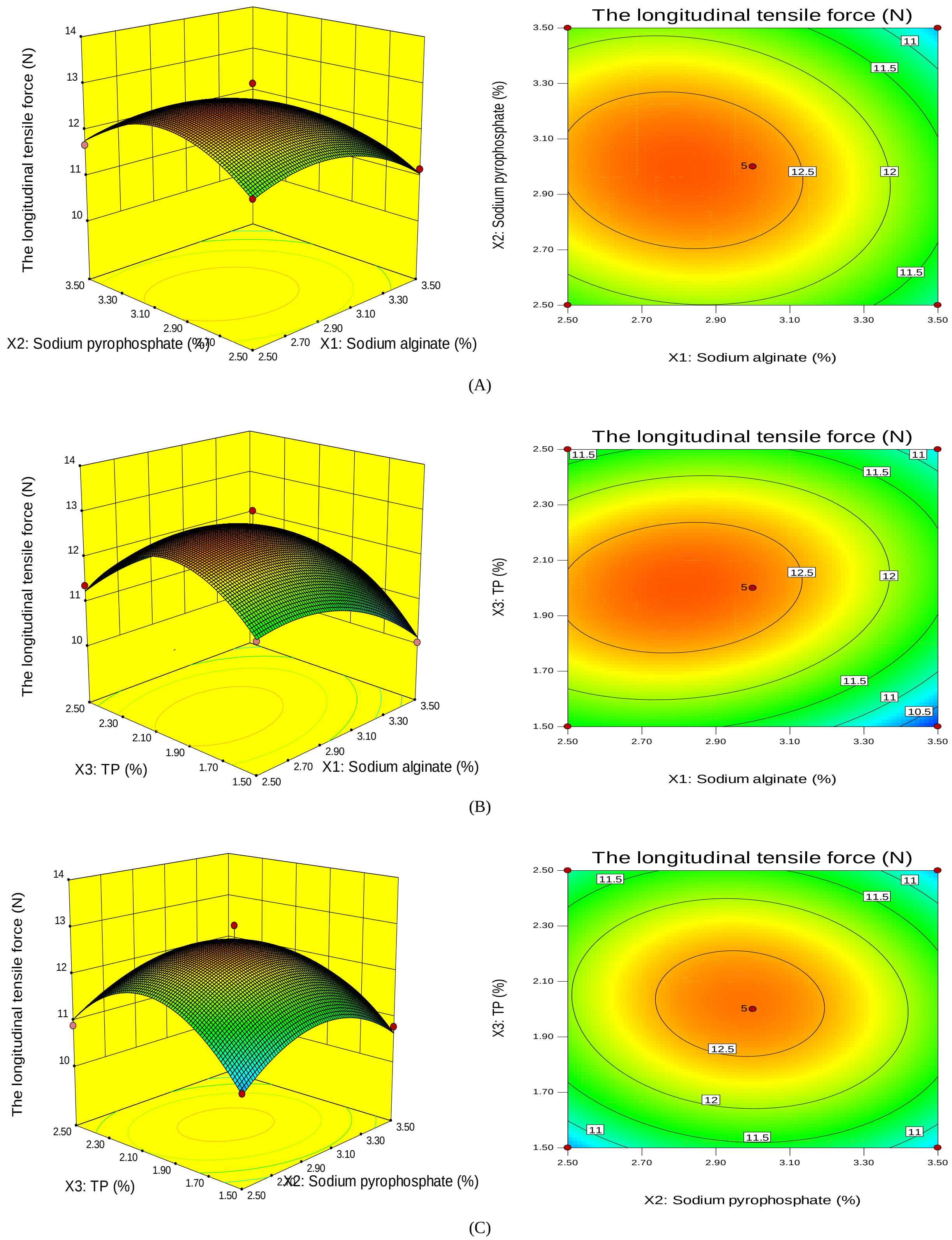

Fig. 2: Response surface plots (3D) and contour plots of longitudinal tensile force as a function of significant interaction between factors. (A) Concentration of sodium alginate and sodium pyrophosphate; (B) Concentration of sodium alginate and TP; (C) Concentration of sodium pyrophosphate and TP

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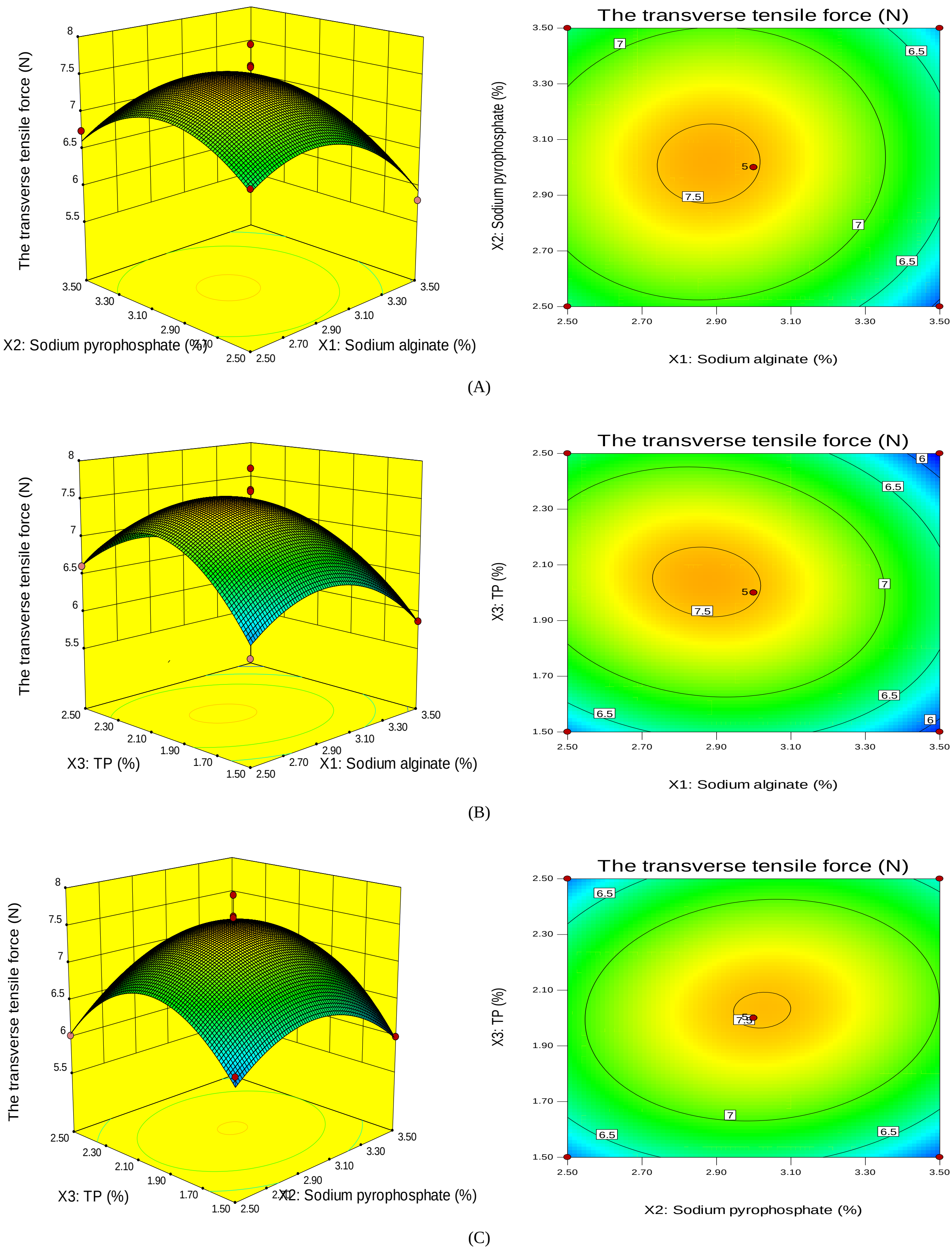

Fig. 3: Response surface plots (3D) and contour plots of transverse tensile force as a function of significant interaction between factors. (A) Concentration of sodium alginate and sodium pyrophosphate; (B) Concentration of sodium alginate and TP; (C) Concentration of sodium pyrophosphate and TP

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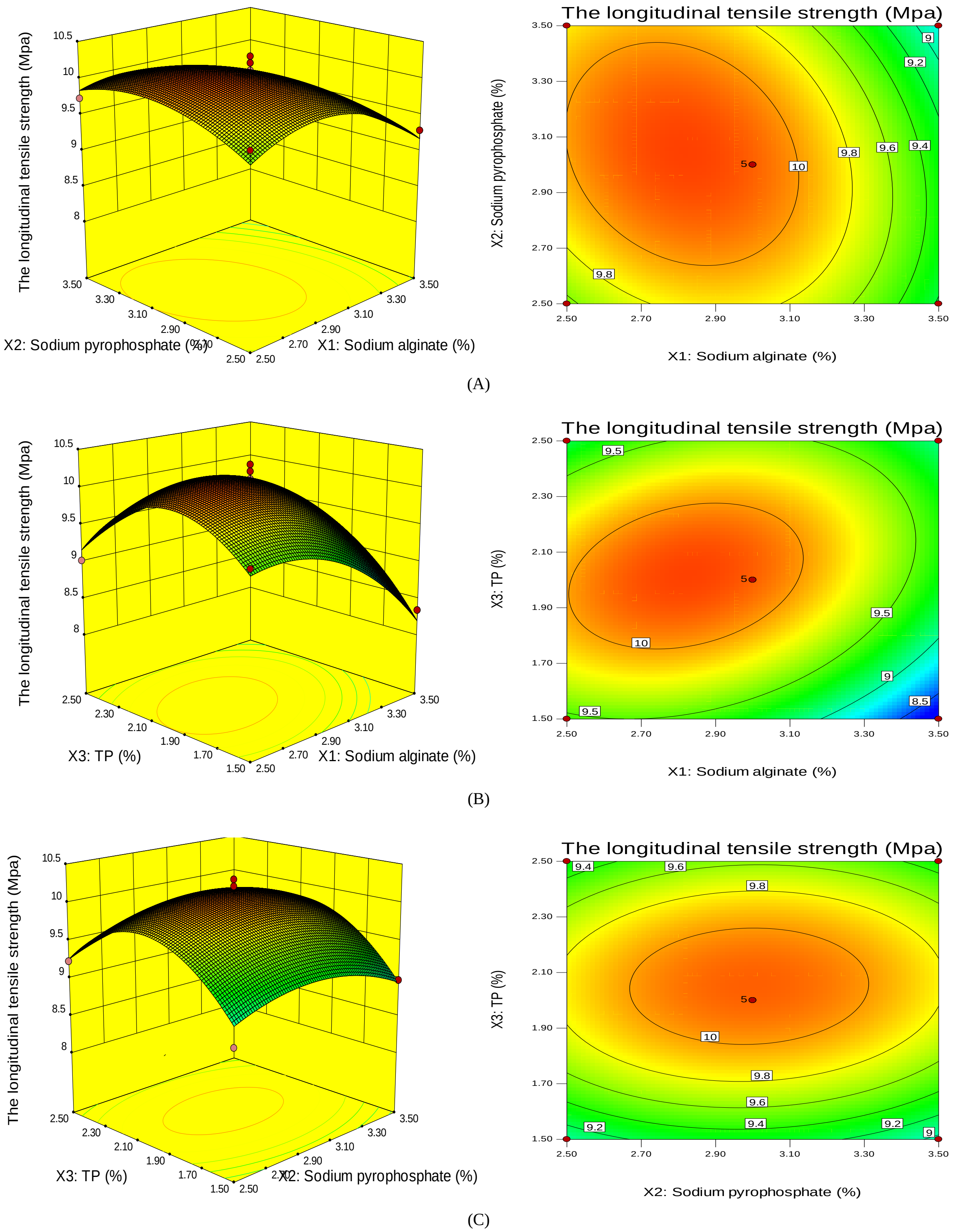

Fig. 4: Response surface plots (3D) and contour plots of longitudinal tensile strength as a function of significant interaction between factors. (A) Concentration of sodium alginate and sodium pyrophosphate; (B) Concentration of sodium alginate and TP; (C) Concentration of sodium pyrophosphate and TP

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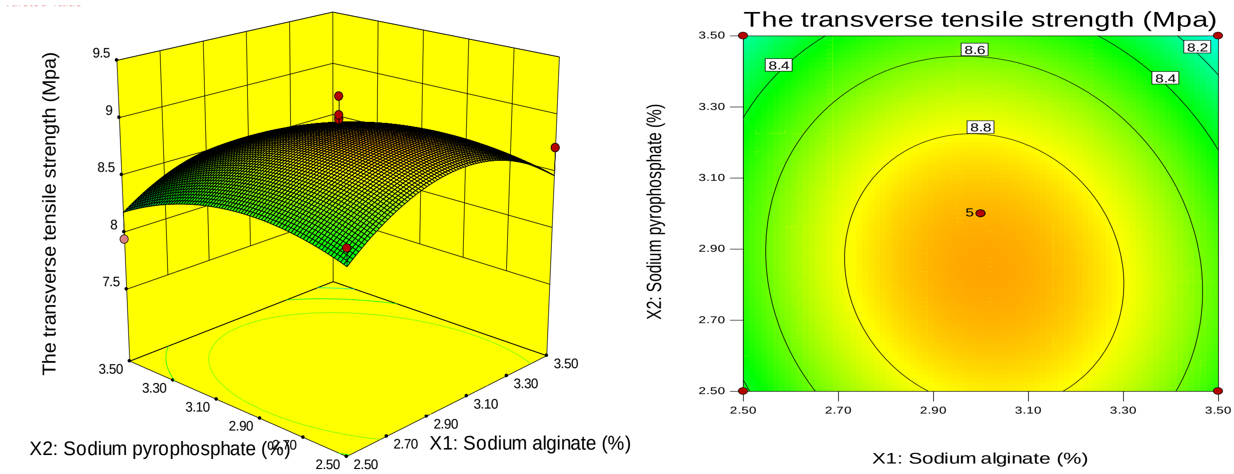
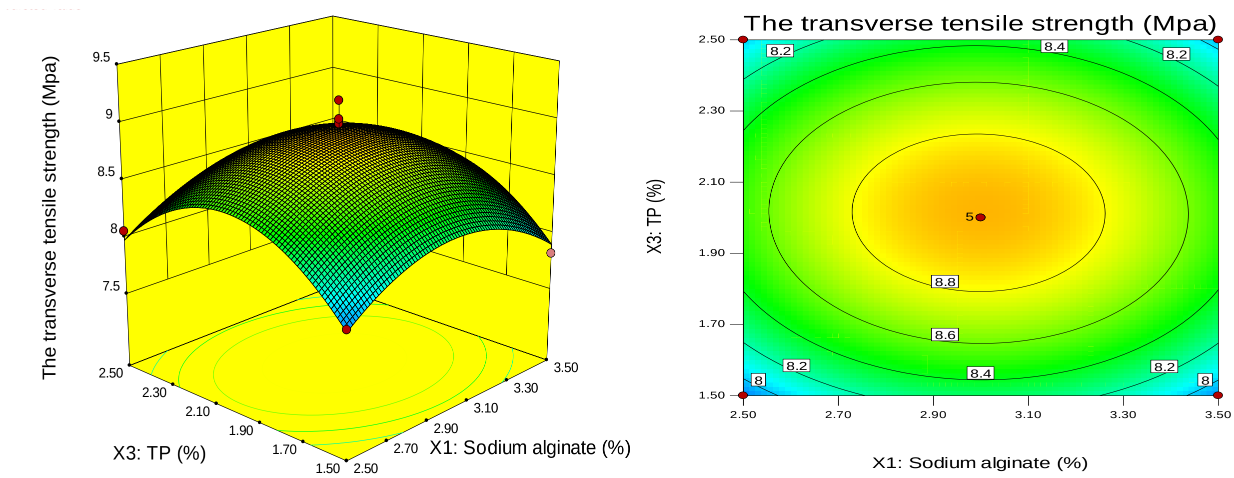
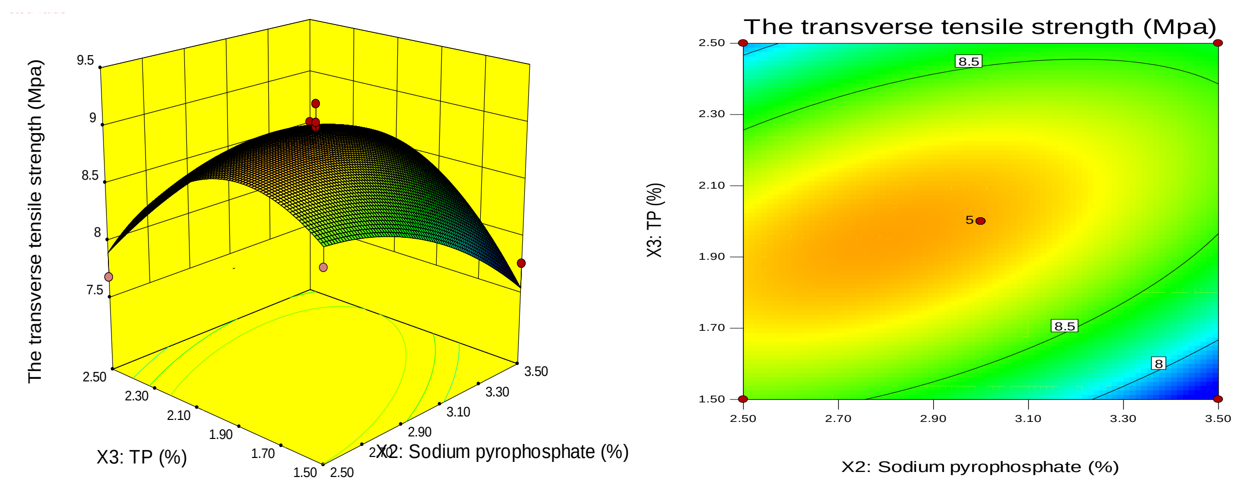


(A)



(B)



(C)

Fig. 5: Response surface plots (3D) and contour plots of transverse tensile strength as a function of significant interaction between factors. (A) Concentration of sodium alginate and sodium pyrophosphate; (B) Concentration of sodium alginate and TP; (C) Concentration of sodium pyrophosphate and TP

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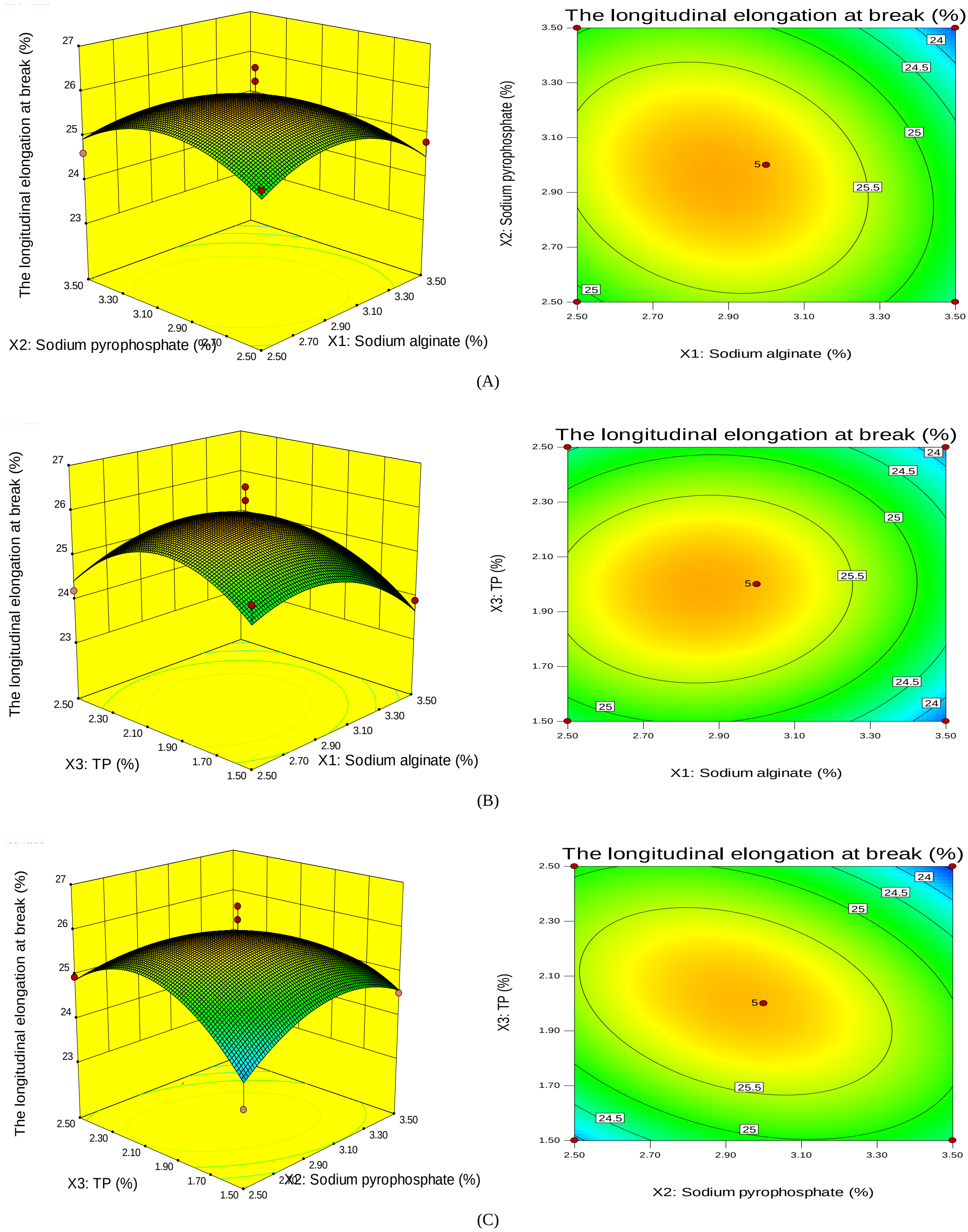
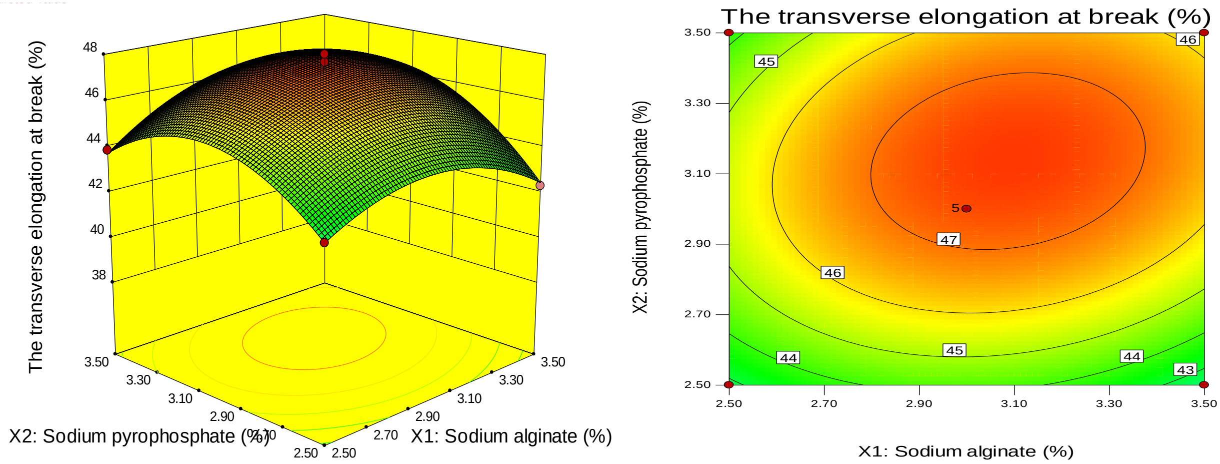
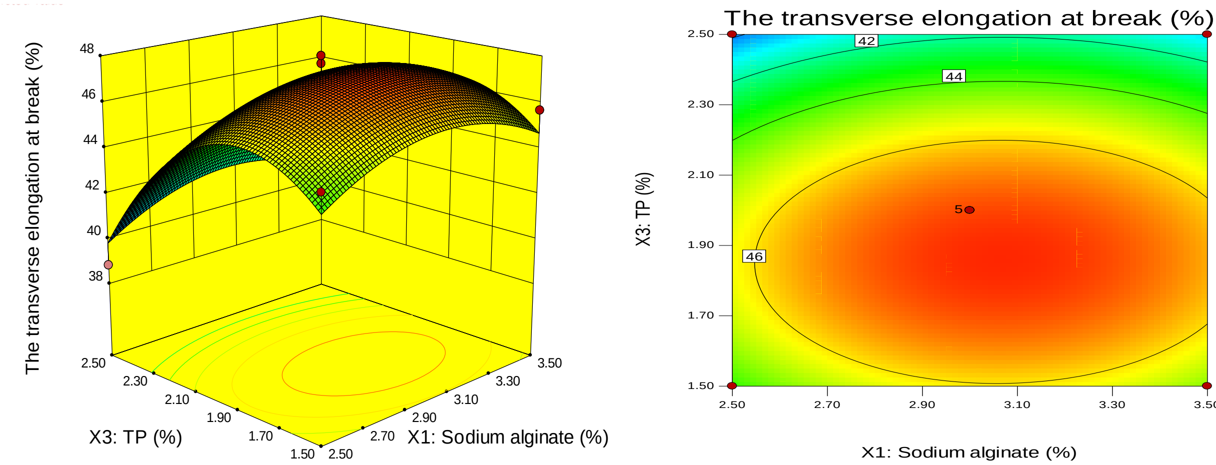


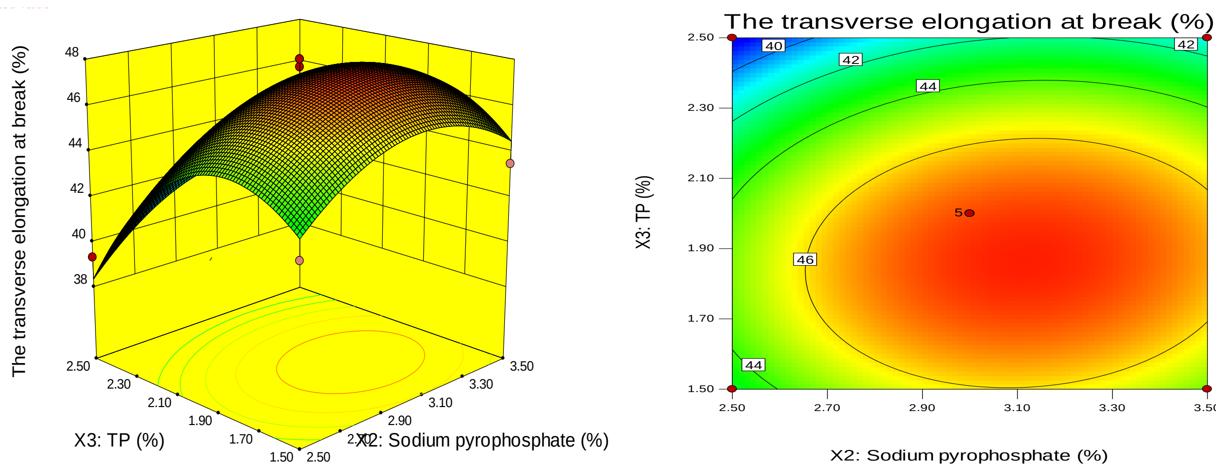
Fig. 6: Response surface plots (3D) and contour plots of longitudinal elongation at break as a function of significant interaction between factors. (A) Concentration of sodium alginate and sodium pyrophosphate; (B) Concentration of sodium alginate and TP; (C) Concentration of sodium pyrophosphate and TP



(A)



(B)



(C)

Fig. 7: Response surface plots (3D) and contour plots of transverse elongation at break as a function of significant interaction between factors. (A) Concentration of sodium alginate and sodium pyrophosphate; (B) Concentration of sodium alginate and TP; (C) Concentration of sodium pyrophosphate and TP

Table 6: Predicted and experimental response values at optimum conditions

Response	Predicted value	Experimental value
Y_1 (N)	12.73390	12.65±0.0608
Y_2 (N)	7.54078	7.52±0.1015
Y_3 (MPa)	10.15980	10.21±0.0916
Y_4 (MPa)	8.90120	8.86±0.0435
Y_5 (%)	25.97350	26.05±0.1000
Y_6 (%)	47.22120	47.16±0.0793

Y_1 = Longitudinal tensile force (N), Y_2 = Transverse tensile force (N), Y_3 = Longitudinal tensile strength (MPa), Y_4 = Transverse tensile strength (MPa), Y_5 = Longitudinal elongation at break (%), Y_6 = Transverse elongation at break (%)

The optimum level of different parameters by applying the methodology of desired function was obtained under condition of sodium alginate of 2.90%, sodium pyrophosphate of 2.99% and TP of 1.99% with an overall desirability of 0.87. As shown in Table 6, the collagen casings were prepared experimentally using the optimized compositions in triplicate and the mechanical properties of these samples were determined to be 12.65±0.0608 N of longitudinal tensile force, 7.52±0.1015 N of transverse tensile force, 10.21±0.0916 MPa of longitudinal tensile strength, 8.86±0.0435 MPa of transverse tensile strength, 26.05±0.0435% of longitudinal elongation at break and 47.16±0.0793% of transverse elongation at break, respectively and no significant differences were found between the predicted values and the experimental values of six responses, demonstrating the validity of the optimized model.

Conclusion

RSM-BBD was successfully employed to optimize the tensile force, tensile strength and elongation at break of collagen casing sprayed with different concentration TP, sodium alginate and sodium pyrophosphate. Results demonstrated that TP has a significant influence on tensile force and longitudinal tensile strength, sodium alginate and sodium pyrophosphate have a significant effect on transverse elongation at break. Based on the model, the optimum condition of the collagen casing was selected with addition of 2.90% sodium alginate, 2.99% sodium pyrophosphate and 1.99% TP. The optimum set of the independent variables was predicted numerically to obtain the desired levels of longitudinal tensile force (12.7339 N), transverse tensile force (7.54078 N), longitudinal tensile strength (10.1598 MPa), transverse tensile strength (8.9012 MPa), longitudinal elongation at break (25.9735%), transverse elongation at break (47.2212%). The corresponding validation responses were longitudinal tensile force (12.65±0.0608 N), transverse tensile force (7.52±0.1015 N), longitudinal tensile strength (10.21±0.0916 MPa), transverse tensile strength (8.86±0.0435 MPa), longitudinal elongation at break (26.05±0.1000%), transverse elongation at break (47.16±0.0793%). In a similar type of study, the optimal film formulation has been investigated by using RSM such as the effects of chitosan, glycerin and drying temperature on the response variables of chitosan food film and the effects of wheat gluten, carboxymethyl cellulose and cellulose nanofiber on the water vapor permeability of new biodegradable nanocomposite films (Singh *et al*., 2015; Bagheri *et al*., 2019). Thus, the development of collagen casing formulation prayed with TP, sodium alginate and sodium pyrophosphate has been successfully optimized and can be exploited to be used as packaging in sausage industry.

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Author’s Contributions

Zhike Xie: Participated in the whole experiment process and also contributed to the interpretation of the results and manuscript preparation.

Ming He and Yuhan Zhai: Participated in part of the experimental design.

Feifei Xin and Shuyan Yu: Data curation.

Shaoxuan Yu: Ameliorated the manuscript.

Huanying Zhao: Contributed to the preparation of collagen casings.

Haifang Xiao: Contributed to the study design, the interpretation of the results and manuscript preparation.

Yuanda Song: Contributed to the guidance of experimental design and ameliorated the manuscript.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Aziz, M. S. A., Salama, H. E., & Sabaa, M. W. (2018). Biobased alginate/castor oil edible films for active food packaging. LWT, 96, 455-460.

Adzaly, N. Z., Jackson, A., Kang, I., & Almenar, E. (2016). Performance of a novel casing made of chitosan under traditional sausage manufacturing conditions. Meat science, 113, 116-123.

Azam, S., Hadi, N., Khan, N. U., & Hadi, S. M. (2004). Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: implications for anticancer properties. Toxicology in vitro, 18(5), 555-561.

Bagheri, V., Ghanbarzadeh, B., Ayaseh, A., Ostadrahimi, A., Ehsani, A., Alizadeh-Sani, M., & Adun, P. A. (2019). The optimization of physico-mechanical properties of bionanocomposite films based on gluten/carboxymethyl cellulose/cellulose nanofiber using response surface methodology. Polymer Testing, 78, 105989.

Chen, X., Zhou, L., Xu, H., Yamamoto, M., Shinoda, M., Tada, I., ... & Yamane, H. (2019). The structure and properties of natural sheep casing and artificial films prepared from natural collagen with various crosslinking treatments. International journal of biological macromolecules, 135, 959-968.

Dou, L., Li, B., Zhang, K., Chu, X., & Hou, H. (2018). Physical properties and antioxidant activity of gelatin-sodium alginate edible films with tea polyphenols. International journal of biological macromolecules, 118, 1377-1383.

Frei, B., & Higdon, J. V. (2003). Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. The Journal of nutrition, 133(10), 3275S-3284S.

Gao, C., Pollet, E., & Avérous, L. (2017). Properties of glycerol-plasticized alginate films obtained by thermo-mechanical mixing. Food Hydrocolloids, 63, 414-420.

Gómez-Estaca, J., Montero, P., Fernández-Martín, F., & Gómez-Guillén, M. C. (2009). Physico-chemical and film-forming properties of bovine-hide and tuna-skin gelatin: A comparative study. Journal of Food Engineering, 90(4), 480-486.

Harper, B. A., Barbut, S., Lim, L. T., & Marcone, M. F. (2012). Microstructural and textural investigation of various manufactured collagen sausage casings. Food research international, 49(1), 494-500.

Hayta, M., & İşçimen, E. M. (2017). Optimization of ultrasound-assisted antioxidant compounds extraction from germinated chickpea using response surface methodology. LWT, 77, 208-216.

Higdon, J. V., & Frei, B. (2003). Tea catechins and polyphenols: health effects, metabolism and antioxidant functions.

Jongberg, S., Terkelsen, L. D. S., Miklos, R., & Lund, M. N. (2015). Green tea extract impairs meat emulsion properties by disturbing protein disulfide cross-linking. Meat science, 100, 2-9.

Kalaycıoğlu, Z., Torlak, E., Akın-Evingür, G., Özen, İ., & Erim, F. B. (2017). Antimicrobial and physical properties of chitosan films incorporated with turmeric extract. International journal of biological macromolecules, 101, 882-888.

Kim, S., Baek, S. K., & Song, K. B. (2018). Physical and antioxidant properties of alginate films prepared from Sargassum fulvellum with black chokeberry extract. Food Packaging and Shelf Life, 18, 157-163.

Mahcene, Z., Khelil, A., Hasni, S., Akman, P. K., Bozkurt, F., Birech, K., ... & Tornuk, F. (2020). Development and characterization of sodium alginate based active edible films incorporated with essential oils of some medicinal plants. International Journal of Biological Macromolecules, 145, 124-132.

Shen, Q. W., & Swartz, D. R. (2010). Influence of salt and pyrophosphate on bovine fast and slow myosin S1 dissociation from actin. Meat science, 84(3), 364-370.

Singh, T. P., Chatli, M. K., & Sahoo, J. (2015). Development of chitosan based edible films: process optimization using response surface methodology. Journal of Food Science and Technology, 52(5), 2530-2543.

Thakur, R., Saberi, B., Pristijono, P., Stathopoulos, C. E., Golding, J. B., Scarlett, C. J., ... & Vuong, Q. V. (2017). Use of response surface methodology (RSM) to optimize pea starch–chitosan novel edible film formulation. Journal of food science and technology, 54(8), 2270-2278.

Vergne, D. M. C., Vasconcelos, A. C. P., Batista, R. A., Freitas, M. M., Júnior, R. L. C. A., de Freitas, O., ... & Cardoso, J. C. (2018). Collagen modification by Maillard reaction. Journal of Thermal Analysis and Calorimetry, 131(1), 671-679.

Wang, L., Dong, Y., Men, H., Tong, J., & Zhou, J. (2013). Preparation and characterization of active films based on chitosan incorporated tea polyphenols. Food hydrocolloids, 32(1), 35-41.

Wang, W., Zhang, Y., Ye, R., & Ni, Y. (2015). Physical crosslinkings of edible collagen casing. International journal of biological macromolecules, 81, 920-925.

Wu, H., Lei, Y., Zhu, R., Zhao, M., Lu, J., Xiao, D., ... & Li, S. (2019). Preparation and characterization of bioactive edible packaging films based on pomelo peel flours incorporating tea polyphenol. Food Hydrocolloids, 90, 41-49.

Xie, Z., Yu, S., He, M., Yu, S., Zhao, H., Xiao, H., & Song, Y. (2020). Preparation of Antimicrobial Collagen Casings with High Mechanical Properties. American Journal of Biochemistry and Biotechnology, 16(3), 407-416.

Zhang, W., & Jiang, W. (2020). Antioxidant and antibacterial chitosan film with tea polyphenols-mediated green synthesis silver nanoparticle via a novel one-pot method. International journal of biological macromolecules, 155, 1252-1261.