The Pharma Innovation Journal 2018; 7(3): 530-535

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(3): 530-535

www.thepharmajournal.com
Received: 20-01-2018
Accepted: 21-02-2018

Paul SK
Department of Agricultural Engineering, Assam University, Silchar, Assam, India

Sarkar S
Department of Agricultural Engineering, Assam University, Silchar, Assam, India

Sethi LN
Department of Agricultural Engineering, Assam University, Silchar, Assam, India

Ghosh S
Department of Chemistry, Assam University, Silchar, Assam, India

Correspondence
Sarkar S
Department of Agricultural Engineering, Assam University, Silchar, Assam, India

Effect of variation in composition on water vapor resistance and solubility properties of chitosan based edible film

Paul SK, Sarkar S, Sethi LN and Ghosh S

Abstract
This study was undertaken to analyze the effect of variation in composition on water vapor resistance and solubility properties of chitosan based edible film. The experiments were carried out in Central Composite Rotatable Design (CCRD) and total 14 film solutions were formulated by varying chitosan and glycerol concentration from 0.5 to 3% (w/w) and 0 to 3% (w/w) respectively. Films were analyzed individually for water vapor transmission rate (WVTR) and percentage water solubility (PWS) after conditioning. In this study it was observed that changes in chitosan and glycerol concentration has significant effect on both the responses. It was revealed that film with lower concentration of chitosan (0.5%, w/w) and higher concentration of glycerol (1.5%, w/w) showed WVTR and PWS at the higher range (0.12 g/m^2.24 hrs and 13.04% respectively). However, higher concentration of chitosan with minimum proportion of glycerol (2.63%, w/w and 0.44%, w/w respectively) demonstrated optimum value for both the responses (0.04 g/m^2.24 hrs and 11.25% respectively) in Response Surface Method (RSM) of optimization. Comparison test further verified the adequacy of fitted model for prediction of optimized values.

Keywords: CCRD, experimental validation, film conditioning, optimisation, percentage solubility, WVTR

1. Introduction

Edible films and coatings are generally formed by renewable natural biopolymers, which are being used as a barrier to lipid, water vapor, gas and flavor for fresh and processed food products to maintain the quality and extension of shelf-life [1-7]. For the past 10 years, researches on edible films and coatings in foods is driven by the high demand of consumers and processors for longer shelf–life and better quality of fresh foods, as well as by their interest in environmentally and health friendly packaging [5-7]. Many materials have been used for film and coating formulations such as carbohydrates, proteins, lipids, or combination of these.

Chitosan is a highly non-toxic polysaccharide with antimicrobial properties. It has been successfully used as a food wrap or coating because of its film-forming properties [8], biocompatibility and biodegradability [9]. The functionality and performance of edible films or coating mainly depend on their barrier, mechanical and optical properties, which in turn primarily depend on film composition and its formation process.

Permeability and solubility of edible film are considered as very important key factors for maintaining the quality of the products during storage and extension of the shelf-life, which determine the efficacy of the film or coating [10]. Chemical composition and structure of the film-forming polymers, composition of film-forming solution, interactions between the sorbent and solvent, coating or film thickness are mainly responsible for the barrier properties and solubility of the film or coating [11-12].

Though different researchers have applied chitosan on various fruits and vegetables like-strawberry, citrus, banana, grapes and grape fruits [13-19], and also in developing edible film to achieve certain goals [20-22] but the effect of plasticizer composition with chitosan on water vapor transmission rate (WVTR) and solubility can rarely be found.

However, few researchers including Singh *et al.* [23] attempted to optimize the concentration of chitosan and glycerol for the film formulations along with other independent parameters including drying temperature. Drying temperature of 40 ºC is already established for different kinds of edible films and coatings with thorough investigations [5-7]. The range of chitosan and glycerol concentration adopted by Singh *et al.* [23] was also not always sufficient, as beyond their ranges good quality films can also be produced [14, 20]. Further, Singh *et al.* [23] predicted the optimized values based on several other responses like- thickness, moisture, color profile, penetrability, density and transmittance, in addition to solubility and WVTR. It is known that more numbers of responses may lead to impractical optimum values and increase the error in experimental validation. Viewing upon the above facts and considering the importance of WVTR and solubility of packaging films, in the present work an attempt has been made to study the effect of variation in chitosan and glycerol composition on WVTR and percentage water solubility of produced edible film.

2. Materials and methods

Chitosan (medium molecular weight), glycerol (87%) and other chemicals including reagents used were of analytical grade (A.R.). Chitosan (medium molecular weight), glycerol (87%) were from Hi-media (Mumbai) and other chemicals including reagents used were Merck (Germany) make. All glassware used were sterilisable and borosilicate (Borosil, Kolkata).

2.1 Experimental design and preparation of edible film formulation

Variation in composition of edible film formulations were designed by adopting two factor central composite rotatable design (CCRD) in two blocks (block 1 and block 2). In the experimental design chitosan and glycerol concentrations (% w/w) were taken as independent variable. The variation in water vapor transmission rate or WVTR (g/m^2.24 hrs) and water Solubility (%) with the changes in independent variable were analyzed. The levels of independent variables used in this design are listed in Table 1. One center point (C) with six replications was used in this design (Table 2) to calculate the repeatability of the method [24].

Table 1: Levels of independent variable used in experimental design by CCRD

Independent variable	Levels		
	Low (-α)	**Centre (0)**	**High (+α)**
Chitosan concentration (% w) /w	0.5	1.75	3
Glycerol concentration (% w/w)	0	1.5	3

Table 2: Central Composite Rotatable Design (CCRD) with independent variables and experimental composition

Run	Block	*Independent variables*	
		Chitosan% (w/w)	**Glycerol% (w/w)**
3	Block 1	0.87	0.44
6	Block 1	2.63	0.44
2	Block 1	0.87	2.56
4	Block 1	2.63	2.56
5 (C)	Block 1	1.75	1.50
1(C)	Block 1	1.75	1.50
7(C)	Block 1	1.75	1.50
9	Block 2	0.50	1.50
8	Block 2	3.00	1.50
11	Block 2	1.75	0.00
13	Block 2	1.75	3.00
12(C)	Block 2	1.75	1.50
14(C)	Block 2	1.75	1.50
10(C)	Block 2	1.75	1.50

The experimental range was decided based on the range adopted by different previous researchers for the production of chitosan based films and subsequent pre-experimental trials to obtain a suitable edible film. Jafarizadeh *et al.* [14] and Osman *et al.* [20] adopted 0.5-2.5% (w/w) of chitosan and 0-2% (w/w) of glycerol. They obtained 2.02% and 2.30% of chitosan and 0.18% and 0.25% of glycerol as optimum concentration respectively for their desired goal. Similarly, Tongchai and Ratiporn [25] considered 1-2% (v/v) chitosan for their experiment and 1% was obtained as optimum. Further, Zhong and Xia [22] and Rhim *et al.* [21] reported 2% (w/v) as most suitable concentration of chitosan in the edible film formulation. These previous reports suggest 0.5-2.5% (w/w) of chitosan and 0-2% (w/w) of glycerol are the broadest ranges adopted for chitosan based edible film. However, our pre-experimental trials revealed that almost similar edible films can be produced with slight increase in the previously reported concentration of chitosan and plasticizer up to 3% (w/w). Hence, in the experimentation concentration of chitosan and glycerol was varied from 0.5-3% (w/w) and 0-3% (w/w) respectively.

Edible film formulations were prepared by following the method of Jafarizadeh *et al.* [14] and Rhim *et al.* [21] with slight modifications. In this method, 14 combinations of film compositions were formulated by dissolving them in 0.5% glacial acetic acid and stirred overnight at 30 ºC on a heating plate with magnetic stirrer (SPINT™-4220, Tarson, Kolkata, India). The pH of the solutions was adjusted to pH 5.6 with 0.1 M sodium hydroxide and the solution was sieved through a mesh of 50 µ pore size. Edible films were produced by casting 35 mL for each combination of film formulation in triplicates on Teflon coated plate of 9 cm diameter (pre standardized) and subsequent drying at 40 ºC for 48 hours and then peeled off for analysis. The mesh size of sieve, casting volume and drying temperature for the film preparation was decided based on the preliminary trials and consulting with different literatures to produce uniform and good quality edible film [5, 21, 23].

2.2 Film conditioning

Films were conditioned at 50% relative humidity at 25±1ºC for at least 48 hours in a control humidity chamber (Scientech instruments, India) prior to measurement of different properties. This helped to maintain water activity (a_w) of all the films at 0.5 during measurement [26, 45]. This was confirmed by measuring the water activity (a_w) of film samples in a digital water activity meter (Hygrolab C1, Rotronic Instrument Corp-USA).

2.3 Analysis of the effect of chitosan and glycerol concentration on film properties

Films produced from 14 different formulations were separately analyzed for WVTR and water solubility (%) to assess the effect of variation in independent parameters on individual response factor.

2.4 Water vapor transmission rate (WVTR)

WVTR of the edible film was determined by placing silica gel in a cup and covering the mouth of the cup with the film. The film was sealed to the cup using grease. The cup was stored in laboratory desiccators for 24 hours. Initial weight was recorded (W_1). The cup was then placed in a hot air oven with a tray filled with water for further 24 hours at 70°C. The weight of the cup was measured (W_2). The WVTR of the film was determined as follows [27]:

$$\mathrm{WVTR} = \frac{\mathrm{W_2} - \mathrm{W_1}}{\mathrm{A\ T}}$$

where, $W_2 - W_1$ is the weight of water absorbed in the cup, A is the area of film, T is the time for weight change.

2.5 Percentage water solubility

Percentage water solubility was determined by following the method of Rhim *et al.* [21] and Farayde *et al.* [28] with slight modification. About 1 g of film strip (at least 10 samples) was soaked in 10 mL water and stirred gently for 30 minutes. The water was removed by centrifugation at 3000×g for 20 min. The residue of the film strip was recovered and dried in an oven at 105 °C. Moisture content of drying samples was measured at each 15 minutes interval after 2 hours of continuous drying by bringing out a sample from the oven. The drying and estimation of moisture content was continued till the film reaches its initial moisture content level having no significant difference at $p \leq 0.05$. Solubility was calculated as the percent weight loss of the film strip during soaking.

2.6 Optimization of film formulation and statistical analysis

Both numerical and graphical optimizations were performed from the experimental data to determine the optimum concentrations (% w/w) of independent variables (chitosan and glycerol) [29]. Three dimensional response surface plots were generated from the fitted models for each response to better visualize the interaction effects of chitosan and glycerol concentrations on the dependent parameters. A numerical optimization was performed by the response optimizer for determining the exact optimum concentrations of independent variables leading to the overall optimum condition. The terms statistically found non-significant were dropped from the initial models and experimental data were refitted only to significant effect of ($P \leq 0.05$) independent variable to obtain the final reduced model [30]. The Design Expert-8 statistical software (Stat Ease Inc, USA) was used for design of experiment, optimization and statistical analysis.

All the analytical experiments of each film types (from 14 types of films produced by CCRD) were carried out in triplicates (n=3). Mean and Standard deviation (SD) were evaluated for all parameters and the data were represented in the form of mean±standard deviation (SD). Significance level of $p \leq 0.05$ was used to compare the mean of same parameter of different samples for all the analysis.

2.7 Experimental validation

Verification experiments were performed at the optimum concentrations of the chitosan and glycerol, and the experimental values of studied responses were compared with predicted response values by Duncans multiple range test ($p \leq 0.05$). It is performed to verify the adequacy of the final response models developed by the optimizer during optimization. The optimization and comparison procedures were carried out using 'Design Expert-8' software.

3. Results and discussion

Interesting outcomes were achieved when the effect of chitosan and glycerol concentration (% w/w) of the developed experimental films on WVTR and percentage water solubility were assessed.

3.1 Effect of chitosan and glycerol concentration on WVTR

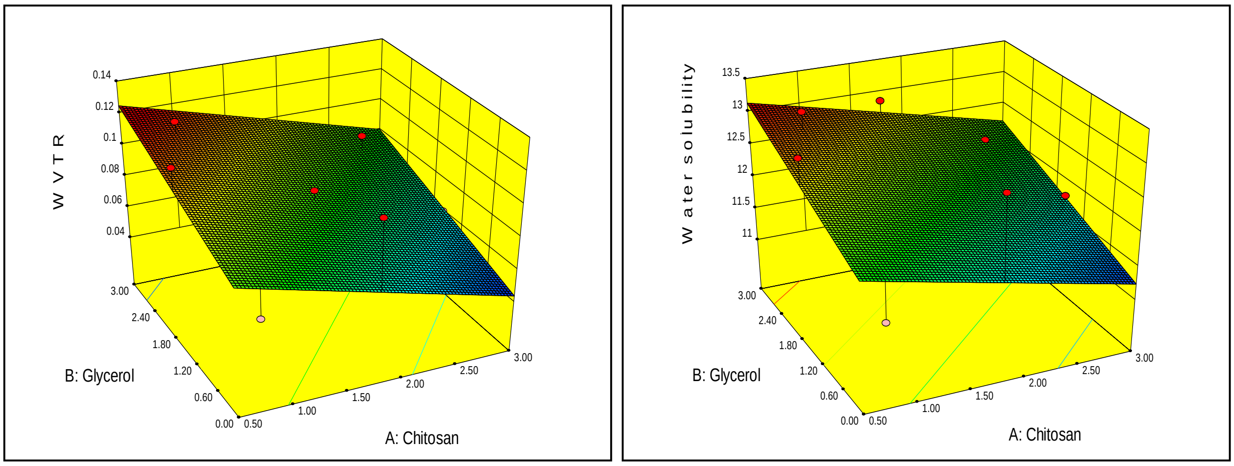
Water vapor permeability of the edible film is useful to understand possible mass transfer mechanisms, and solute and polymer interactions in the film. Figure 1(a) shows the changes in WVTR of the films as function of chitosan and glycerol concentrations. As clearly observed that at lower concentrations of glycerol with an increase in the concentration of chitosan reduced the WVTR of edible film. Increasing trend of WVTR was found with increasing concentrations of glycerol beyond certain proportion (depending upon chitosan ratio) in the film formulation. The results also revealed that the ratio of chitosan to glycerol in the film formulation played more significant role in the variation of WVTR of films, rather the concentration of individual component.

This result can be explained by the facts that hydroxyl group on each carbon atom of glycerol binds with hydrogen in water leading to the transmission of water molecules through the film [31, 32], and installation of glycerol molecules inside the chitosan network increases the intermolecular spacing in the film matrix leading to the entry of water [7, 33]. But, at lower concentration glycerol with increase in chitosan concentration might strengthened the intermolecular cross linkages and increased the resistance to water vapor. The results are in agreement with the findings of the previous works on the films and coating made using various hydrophilic substances including starch, carrageenan and chitosan with or without plasticizers like- glycerol and sorbitol [34-38].

3.2 Effect of chitosan and glycerol concentration on percentage water solubility

Water solubility of polysaccharide film mainly depends upon the extent of intermolecular cross-linking, hydrophilic-hydrophobic ratio and crystalinity of the film matrix [39, 40].

Figure 1(b) revealed that the lower concentration of glycerol with higher fraction of chitosan produced lower water solubility. This can be justified with the fact that the formation of sufficient inter-molecular hydrogen cross linkages between chitosan, glycerol and/or water molecules in the film matrix at lower concentration of glycerol with sufficient chitosan during the gel processing stage reduced the solubility of the produced film [39-42]. On the other hand, higher solubility was observed in those films produced with formulation having higher fraction of glycerol with lower concentration of chitosan. Similar results of variation in film solubility with the change in base components of film matrix were also advocated and strengthened the present outcome by some other researchers [28, 43, 44].



(a) (b)

Fig 1: Effect of chitosan and glycerol concentration on (a) WVTR and (b) Percentage water solubility of edible film

3.3 Optimization and experimental validation

For optimization, the effects of chitosan and glycerol concentration on WVTR and percentage water solubility of experimental films were assayed. In order to meet the desirable goal the independent parameters were optimized for minimum WVTR and water solubility of the developed film.

The results of numerical and graphical RSM optimization indicated that films with chitosan and glycerol concentration of 2.63% (w/w) and 0.44% (w/w) respectively showed optimum value of WVTR and percentage water solubility within the experimental ranges. The RSM predicted values of studied responses under optimum condition were represented in Table 3.

Table 3: Predicted and experimental values of responses at optimum concentrations of chitosan and glycerol

Response variables	Predicted value	Experimental value
WVTR (g/m^2.24 hrs)	0.04^a	0.040 ± 0.001^a
Percentage water solubility (%)	11.25^b	11.520 ± 0.020^b

*Values in a row with different letters indicate significant differences at $P\leq0.05$

**Experimental value is represented as mean±SD, n=3.

Verification experiments were performed at the optimum concentrations of the chitosan and glycerol, and the experimental values of studied responses are given in Table 3. All the dependent experimental parameters were analyzed with triplicate samples for each experiment. Comparison test demonstrated that there was no significant ($p\leq0.05$) difference between predicted and experimental values for all response variables. The closeness between these values of responses verified the adequacy of final reduced models fitted by RSM.

4. Conclusion

Composition of film formulation was found to have direct relationship with WVTR and percentage water solubility of the film. Very high concentration of glycerol with lower chitosan fraction in the film formulation showed lesser effectiveness in controlling the response parameters, whereas higher concentration of chitosan with presence of lesser glycerol fraction showed very good resistance to water vapor and solubility of the film. Optimization process revealed that films with chitosan and glycerol concentration of 2.63% (w/w) (or above) and 0.44% (w/w) respectively showed maximum control of WVTR and percentage water solubility. From the outcome of experimental validation of the predicted value, it is recommended that the chitosan/glycerol ratio of 2.63% (w/w)/0.44% (w/w) will be one of the best film formulations for producing chitosan-glycerol edible film with maximum resistance to water vapor and solubility. It is also expected that the effectiveness of the optimum film formulation can probably be further improved by the addition of certain functional additives for the enhancement of structural and mechanical properties. Hence, this study strengthens the scope for further research in the domain of tailoring the alternative packaging materials with desired and effective functionalities.

5. References

1. Park HJ, Chinnan MS. Gas and water vapor barrier properties of edible films from protein and cellulosic materials. Journal of Food Engineering 1995; 25:497-507.
2. Butler BL, Vergano PJ, Testin RF, Bunn JM, Wiles JL. Mechanical and barrier properties of edible chitosan films as affected by composition and storage. Journal of Food Science. 1996; 61:953-955.
3. Kaya S, Kaya A. Microwave drying effects on properties of whey protein isolate edible films. Journal of Food Engineering. 2000; 43:91-96.
4. Ou S, Wang Y, Tang S, Huang C, Jackson M. Role of ferulic acid in preparing edible films from soy protein isolate. Journal of Food Engineering. 2005; 70:205-210.
5. Das DK, Dutta H, Mahanta CL. Development of a rice starch-based coating with antioxidant and microbe-barrier properties and study of its effect on tomatoes stored at room temperature. LWT- Food Science and Technology, 2011; 50:272-278.
6. Paul SK, Sarkar S, Sethi LN, Sujit KG. Study on the effect of chitosan and glycerol composition on respiration rate and optical parameters of edible coated tomato (*Lycopersicon Esculentum* Mill) to extend shelf life during storage. International Journal of Agriculture and Food Science Technology. 2014; 5(7):727-740.
7. Begum N, Paul SK, Prasanna KGV, Jatindra KS, Saiyyad AH. Development of tulsi impregnated starch-based edible coating to extend the shelf-life of tomatoes. The Pharma Innovation Journal. 2017; 6(9):249-255.
8. Shahidi F, Arachchi JKV, Jeon Y. Food applications of chitin and chitosan. Trends in Food Science and

Technology, 1999; 10:37-51.
9. Majeti NV, Ravi K. A review of chitin and chitosan applications. Reactive and Functional Polymers, 2000; 46:1–27.
10. Zactiti EM, Kieckbusch TG. Potassiom sorbate permeability in biodegradable alginate film: Effect of the antimicrobial agent concentration and crosslinking degree. Journal of Food Engineering. 2006; 77:462-467.
11. Phan The D, Péroval C, Debeaufort F, Despré D, Courthaudon J, Voilley A. Arabinoxylan-lipids-based edible films and coatings: Influence of sucroester nature on the emulsion structure and film properties. Journal of agricultural and food chemistry. 2002; 50:266-272.
12. Valérie M, Debeaufort F, Geneviève B, Martine C, Andrée V. Factors Affecting the Moisture Permeability of Lipid-Based Edible Films: A Review. Critical Reviews in Food Science and Nutrition. 2002; 42(1):67-89.
13. Chien PJ, Sheu F, Lin HR. Coating citrus (*Murcott tangor*) fruit with low molecular weight chitosan increases postharvest quality and shelf life. Food Chemistry. 2007; 100:1160-1164.
14. Jafarizadeh Malmiri H, Osman A, Tan CP, Abdul Rahman R. Development of an edible coating based on chitosan-glycerol to delay 'Berangan' banana (*Musa sapientumcv*. *Berangan*) ripening process. International Food Research Journal. 2011; 18(3):989-997.
15. Laura S, Clara P, María V, Amparo C, Chelo G, Maite C. Effect of hydroxypropyl methylcellulose and chitosan coatings with and without bergamot essential oil on quality and safety of cold-stored grapes. Postharvest Biology and Technology. 2011; 60:57-63.
16. Meng X, Li B, Liu J, Tian S. Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. Food Chemistry. 2008; 106:501-508.
17. Pilar H, Eva A, Valeria DV, Dinoraz V, Rafael G. Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria ananassa*) quality during refrigerated storage. Food Chemistry. 2008; 110:428-435.
18. Vargas M, Albors A, Chiralt A, González-Martínez C. Quality of cold-stored strawberries as affected by chitosan–oleic acid edible coatings. Postharvest Biology and Technology. 2006; 41(2):164-171.
19. Bico SLS, de Jesus Raposo MF, de Morais RMSC, de Morais AMMB. Chemical dips and edible coatings to retard softening and browning of fresh-cut banana. International Journal of Postharvest Technology and Innovation. 2010; 2(1):13-24.
20. Osman A, WanSekeran SNC, Tan P, Jafarizadeh MH. Optimization of an edible coating formulation based on chitosan on 'Sekaki' papaya (*Carica papaya* cv. Sekaki) to reduce water loss and delay changes in pH, TSS and firmness. Empowering Science, Technology and Innovation Towards a Better Tomorrow, 2011, 235-241.
21. Rhim JW, Hong SI, Park HM, Ng PKW. Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. Journal of Agriculture and Food Chemistry. 2006; 54(16):5814-5822.
22. Zhong Q, Xia W. Physicochemical properties of edible and preservative films from chitosan/cassava starch/gelatin blend plasticized with glycerol. Food Technology and Biotechnology, 2008; 46(3):262-269.
23. Singh TP, Chatli MK, Sahoo J. Development of chitosan based edible films: process optimization using response surface methodology. Journal of Food Science and Technology. 2015; 52(5):2530-2543.
24. Mirhosseini H, Tan CP, Hamid NSA, Yusof S. Effect of Arabic gum, xanthan gum and orange oil contents on ζ-potential, conductivity, stability, size index and pH of orange beverage emulsion. Colloids and Surfaces 2008; 315:47-56.
25. Tongchai P, Ratiporn H. Formulation of chitosan-oleic acid coating for kiew wan tangerine by response surface methodology. Kasetsart J. (Nat. Sci.). 2010; 44:462-470.
26. American Society for Testing and Materials (ASTM 1995). Standard test methods for water vapor transmission of materials: Annual book of ASTM standards. Philadelphia, PA: American Society for Testing and Materials, 1995.
27. Kamper SL, Fennema ON. Water vapor permeability of an edible fatty acid, bilayer films. Journal of Food Science. 1984; 49:1482-1485.
28. Farayde MF, Silvia MM, Larissa CB, Fabio Y, Lúcia H, Innocentini M, Fernanda PCQ. Edible films made from blends of manioc starch and gelatin-Influence of different types of plasticizer and different levels of macromolecules on their properties. LWT - Food Science and Technology, 2012; 49:149-154.
29. Quanhong L, Caili F. Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. Food Chemistry, 2005; 92(4):701-706.
30. Mirhosseini H, Tan CP, Nazimah SAH, Salmah Y, Boo HC. Characterization of the influence of main emulsion components on the physicochemical properties of orange beverage emulsion using response surface methodology. Food Hydrocolloids, 2009; 23(2):271-280.
31. Alves VD, Mali S, Beléia A, Grossmann MVE. Effect of glycerol and amylose enrichment on cassava starch film properties. Journal of Food Engineering. 2007; 78:941-946.
32. Martelli SM, Moore G, Paes SS, Gandolfo C, Laurindo JB. Influence of plasticizers on the water sorption isotherms and water vapor permeability of chicken feather keratin films. LWT - Food Science and Technology, 2006; 39:292-301.
33. Fundo JF, Andrea CG, Ivonne D, Cristina LMS, Mafalda ACQ. The effect of polymer/ plasticiser ratio in film forming solutions on the properties of chitosan films. Food Biophysics, 2015; 10:324.
34. Henrique CM, Teófilo RF, Sabino L, Ferreira MMC, Cereda MP. Classification of cassava starch films by physicochemical properties and water vapor permeability quantification by FT-IR and PLS. Journal of Food Science. 2007; 72(4):184-189.
35. Laohakunjit N, Noomhorm A. Effect of plasticizers on mechanical and barrier properties of rice starch film. Starch/Stärke 2004; 56:348-356.
36. Mali S, Grossmann MVE, Garcia MA, Martino MN, Zaritzky NE. Microstructural characterization of yam starch films. Carbohydrate Polymers, 2002; 50:379-386.
37. Mali S, Grossmann MVE, García MA, Martino MN, Zaritzky NE. Barrier, mechanical and optical properties of yam plasticized starch films. Carbohydrate Polymers, 2004; 56:129-135.
38. Sobral PJA, Menegalli FC, Hubinguer MD, Roques MA. Mechanical, water vapor barrier and thermal properties of

gelatin-based edible films. Food Hydrocolloids, 2001; 15:423-432.
39. Salmieri S, Lacroix M. Physicochemical properties of alginate/ polycaprolactone-based films containing essential oils. Journal of Agricultural and Food Chemistry. 2006; 54:10205-10214.
40. Vachon C, Yu HL, Yefsah R, Alain R, St-Gelais D, Lacroix, M. Mechanical and structural properties of milk protein edible films cross-linked by heating and gamma irradiation. Journal of Agricultural and Food Chemistry. 2000; 48:3202-3209.
41. Almasi H, Ghanbarzadeh B, Entezami AA. Physicochemical properties of starch-CMC-nanoclay biodegradable films. International Journal of Biological Macromolecules. 2010; 46(1):1-5.
42. Wang L, Khor E, Lim LYS. Chitosan-alginate-$CaCl_2$ system for membrane coat application. Journal of Pharmaceutical Sciences. 2001; 90:1134-1142.
43. Bourtomm T. Edible films and coatings: characteristics and properties. International Food Research Journal. 2008; 15(3):1-12.
44. Davanço T, Tanada-Palmu P, Grosso C. Gelatin, triacetin, stearic and caproic acid composite films: effect of pH and surfactants on film properties. Ciência e Tecnologia de Alimentos, 2007; 27:408-416.
45. Chowdhury T, Das M. Effect of antimicrobials on mechanical, barrier and optical properties of corn starch based self-supporting edible film. International Journal of Food Studies. 2013; 2:212-223.