Article AR2070b

DOI: HTTPS://DOI.ORG/10.23910/1.2020.2070b

International Journal of Bio-resource and Stress Management

August 2020

Crossref

Print ISSN 0976-3988

Online ISSN 0976-4038

IJBSM 2020, 11(4):335-344

Research Article *Natural Resource Management*

Optimization of Process Variables and Validation of The Models for Nano-Particle Embedded Biodegradable Polymers for Packaging

Rakesh Babu Gautam[1]*, Satish Kumar[2] and Anu Kalia[3]

[1]Dept. of Processing and Food Engineering, A.N.D.University of Agriculture and Technology, Kumarganj, Ayodhya, U.P. (224 229), India

[2,3]Dept. of Processing and Food Engineering, Section Nano Technology, Punjab Agricultural University, Ludhiana, (141 004), India

Corresponding Author

Rakesh Babu Gautam

e-mail: rksh_gautam@yahoo.co.in

Citation: Gautam et al., 2020. Optimization of Process Variables and Validation of The Models for Nano-Particle Embedded Biodegradable Polymers for Packaging. International Journal of Bio-resource and Stress Management 2020, 11(4), 335-344. HTTPS://DOI.ORG/10.23910/1.2020.2070b.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

Abstract

The aim of this work was to develop antimicrobial biodegradable polymeric films based on whey and soy protein isolate and titanium dioxide nano powder. Five levels of sonication 20%, 40%, 60%, 80% and 100%.When TiO2 was incorporated into a polymer matrix of a packaging material, it provided protection from food borne microorganism as well as odour staining deterioration and allergens due to presence of radiation of relative low wavelength near the ultraviolet region. The TiO2 film exhibits excellent mechanical and chemical durability. Experimental design and data analysis: Statistical package Design-Expert Version 7.0 (Statease Inc., Minneapolis, MN, USA) was used to design the experiment. The central composite design CCRD for three independent variables was performed. The independent variables considered were pH (A) Sonication levels (B). The levels of each variable were established based on the results of earlier experiments and preliminary trials A rotatable, central composite design was employed to determine the film formation conditions. Effect of sonication on color and strength of biopolymers film was examined. The predicted values and experimental values were analysed by using different models. The experiments were repeated at optimized values of process variables. The film was then tested for differential scanning calorimetric (DSC), scanning electron microscopy (SEM), film forming and melting temperature (Tm) etc. The predicted and experimental values were statistically analysed for model validation. The film was used for individual packaging and storage of guava and tomato under ambient and cold storage conditions.

Keywords: Nano-particle, nano-imaging, validation, development of models and sonication

1. Introduction

Development of a new type of bio-packaging materials to replace petroleum-based non-biodegradable plastics has become a growing field of interest since synthetic plastics lead to serious environmental concerns as a result of their non-biodegradability and depletion of natural resources (Ferreira et al., 2016). The packaging requirements of biological systems, including foodstuffs, are more diverse and complex than those for other products (Gallocchio et al., 2015). In recent years, edible films and coating materials are being increasingly used for food packaging to improve the food quality by serving as mass transfer barriers between

Article History

RECEIVED in 26th January 2020 RECEIVED in revised form 10th June 2020 ACCEPTED in final form 03rd July 2020

food components and the surrounding environment (Ashley, 1994; Cha and Chinnan, 2004). Food package should protect the products against physical hazards and environmental elements such as water vapour, gases, and odours; a combination of structural design and material selection should meet the requirements. An adequate selection of packaging materials can prevent food quality loss by providing barrier, or otherwise protective features (Campose et al., 2011), and enhancing food quality and shelf life (Gennadios, 2002).

Biodegradable whey protein isolate, glycerol, titanium dioxide blend films were developed and characterised as a function of different levels (0, 20, 40, 60, 80 and 100). Results were showed that water vapour permeability, moisture content, moisture absorption and water solubility decreased with increasing sonication level. Mechanical tests revealed the plasticising effect of nano particles on bio-film. Application of sonication to whey protein isolate films solution significantly increased tensile strength and elongation at break (Gautam and Kumar, 2016).

Environmentally friendly thermoplastic nanocompositewere successfully developed using a colloidal suspension of chitin whiskers as a filter to reinforce soy protein isolate plasticsthe chitin whiskers, having lengths of 500±50 nm and diameter of 50±10 nm on average, were prepared from commercial chitin by acid hydrolysis. The dependence of morphology and properties on the chitin whiskers content in the range from 0 to 30 wt % for the glycerol plasticized SPI nanocomposite was investigated by dynamic mechanical thermal analysis, SEM, swelling equipment, and tensile testing. The result indicate that the string interaction between filters and SPI matrix play an important role in reinforcing the composite without interfering with their biodegradability. The SPI / chitin whisker nanocomposite at 43% relative humidity increased in both tensile strength and young's modulus from 3.3 Mpa for the SPI sheet to 8.4 Mpa and 26 Mpa for the SPI sheet to 158 Mpa, respectively. further, incorporating chitin whiskers into the SPI matrix leads to an improvement in water resistance for the SPI based nanocomposite (Lu et al., 2004).

Research and development of bio-nanocomposite materials for food applications such as packaging and other food contact surfaces is expected to grow in the next decade with the advent of new polymeric materials and composites with inorganic nano-particles. This article reviews different types of new bio-based materials, such as edible and biodegradable nanocomposite films, their commercial applications as packaging materials, regulations and future trends. Special emphasis is given to the advantages of nanotechnology application in order to improve the mechanical and oxidation stability, the barrier properties, and eventually the biodegradability of conventional polymeric matrices. (Sorrentino et al., 2007).

The physicochemical properties of nano-materials, including size, shape (Kinnear et al., 2017) structure (Zhang et al., 2015), rigidityand surface modification, are closely linked to the delivery efficiency and the release profile of drugs. Organic macromolecular compounds obtained by polymerisation, polycondensation, polyaddition or any similar process from molecule with a lower molecular weight or by chemical alteration of natural macro-molecular compounds. (Kirwan et al., 2015).

The coating surfaces with active nanomaterials to provide protective functions (*e.g.* AgNPs for antibacterial packaging) (Carbone, 2016) fillers to improve mechanical properties of packaging (*e.g.* nanoclays) (Majeed et al., 2013); sensing materials to develop methods for in situ monitoring of food quality and safety, ideally attached to or included in packaging (*e.g.* TiO_2 for O_2 sensing)

Gelatin films were cross-linked, respectively, by ferulic acid and tannin acid obtained from plant kingdom. Influences of concentrations of cross-linking agents and pH values of film-forming solution on the mechanical properties, swelling properties and water vapor permeability of gelatin films were investigated in details. And influence of storage time on the properties of treated gelatin films was studied as well. UV-Vis absorption spectra and scanning electron microscopy (SEM) were applied to observe the changes of gelatin films after modified in order to explore primarily cross-linking mechanism (Cao et al., 2007).

Novel citric acid (CA) modified starch-carboxymethyl cellulose (CMC)-montmorillonite (MMT) bio-nanocomposite films were prepared by casting method. X-ray diffraction (XRD) test showed that the 001 diffraction peak of nanoclay was shifted to lower angles in the bio-nanocomposites and it may be implied that the clay nanolayers formed an intercalated structure. However, completely exfoliated structure formed only in the pure starch-MMT nanocomposites (without CA and CMC). At the level of 7% MMT, the composite films showed the lowest solubility (7.21%). The MMT addition at content of 7% (w/w), caused to increase in ultimate tensile strength (UTS) by more than threefold in comparison to starch-CMC biocomposites (Almasi et al., 2009).

Chitosan-based composites play an important role in food packaging applications and can be used either as films or as edible coatings. Due to their high costs and lower performance (i.e., lower barrier against water vapour, thermal, and mechanical properties) when compared to the traditional petroleum-based plastics, the use of such biopolymers in large-scale is still limited. Several approaches of chitosan composites in the packaging industry are emerging to overcome some of the disadvantages of pristine polymers. Thus, this work intends to present the current trends and the future challenges towards production and application of chitosan composites in the food packaging industry (Souza et al., 2020).

Concerns on environmental waste problems caused by non-biodegradable petrochemical-based plastic packaging

materials as well as consumers' demand for high quality food products has caused an increasing interest in developing biodegradable packaging materials using annually renewable natural biopolymers. However, inherent shortcomings of natural polymer-based packaging materials, such as low mechanical properties and low water resistance, are causing a major limitation for their industrial use. The recent advent of nanocomposite technology raised renewed interest in the use of natural biopolymers in food packaging applications. Polymer nanocomposites, especially natural biopolymer-layered silicate nanocomposites, exhibit markedly improved packaging properties due to their nanometer size dispersion. (Liu et al., 2010) These improvements include increased mechanical strength, gas barrier, and water resistance without sacrificing the biodegradability and optical clarity of the packaging material. Additionally, biologically active ingredients can be added to impart the desired functional properties to the resulting packaging materials. Such biopolymer-based nanocomposite packaging materials with bio-functional properties have huge potential for application in the active food packaging industry (Sothornvit et al., 2009).

The modification of soy protein isolate (SPI) with different amounts of a naturally occurring cross-linking agent (genipin, Gen) and glycerol used as plasticizer was carried out in this work. The films yielded were cast from heated and alkaline aqueous solution of SPI, glycerol and Gen and then dried in an oven. Total soluble matter, water vapor permeability and mechanical properties were improved by adding small amounts of Gen. These properties were not significantly affected (P P 0.05) by additions exceeding 2.5% (w/w of SPI). The opacity and cross-linking degree were linearly increased with the addition of Gen, whereas the swelling ratios in water were decreased. All the films were submitted to degradation under indoor soil burial conditions and the weight loss of the films was measured at different times. This study revealed that the film biodegradation time can be controlled or modified from at least 14 to 33 days. The tests performed showed the potential of Gen to improve the SPI film properties, in which the possibility of employing such new films as biodegradable food packaging was raised (Gonzalez et al., 2011).

The bio films were prepared using fixed level of TiO_2 (5%), SPI/WPI (5%) and glycerol contents, (1.5%). The TiO_2 was first dispersed into distilled water (100 ml) and stirred using a magnetic stirrer overnight to reach complete hydration/ swelling. Subsequently 5 g of SPI/ WPI was added to obtain an aqueous solution, followed by addition of 1.5 g of glycerol. The film solutions were heated at 90 °C for 15 min in a waterbath cooled to room temperature and degassed using a bath-type ultrasound sonicator. (Gautamand Kumar, 2016; Kadam et al., 2013). The film forming solutions were then spread onto levelled Teflon-coated sheet (24×30 cm^2). The process conditions for development of film were optimized using numerical optimization techniques. (Gautam and Kumar, 2017) The main criteria for constraints optimization were minimum weight of film, thickness of film, moisture absorbance, moisture content, water solubility & WVTR and maximum possible, viscosity of film solution, area of film, tensile strength, and elongation at break and GTR of the film(Gautam and Kumar, 2015, Kumar et al,2010). In order to optimize the process conditions for development of bio-polymeric film by numerical optimization techniques, equal importance of '3' was given to process parameters (viz. pH levels and sonication levels) and responses (i.e. value of film thickness, area of film, weight of film, L value, a* value, b* value, viscosity of film solution, moisture absorbance, moisture content, water solubility, tensile strength, elongation at break, WVTR and GTR). (Gennadios et al., 1993b) The conditions were experimentally verified with deviation of + 0.1%. The optimum conditions for pH and sonication level for both films were 8.5 and 60 respectively. Corresponding to these value of process variables of SPI based film, the value of responses viz. weight of film, thickness of film, area of film, L value, a* value, b* value , viscosity of film solution, moisture absorbance, moisture content, water solubility, tensile strength, elongation at break, WVTR and GTR were 13.8005 g, 0.0418 mm, 400.0000 cm^2, 84.8846, -4.2000, 9.36154, 31.8353 mpa-s, 7.0602%, 22.0738%, 47.0915%, 4.6500, 90.3346, 165.7970×10^{-11} g/m^2s and 2.9300×10^{-8} m cc m^{-2} day respectively. The overall desirability for SPI based film was 0.955. Similarly the value of responses of WPI based film viz. weight of film, thickness of film, area of film, L value, a* value, b* value, viscosity of film solution, moisture absorbance, moisture content, water solubility, tensile strength, elongation at break, WVTR and GTR were 10.77 g, 0.0325 mm, 412.465 cm^2, 89.7854, -4.47855, 6.76785, 32.9019 mpa-s, 5.23059%, 18.575%, 45.8256%, 4.1880 Mpa, 49.8250%,167.2270×10^{-11} g/m^2s and 2.6307×10^{-8}m cc m^{-2} day respectively. The overall desirability of WPI based film was 0.923. Thus, biopolymers fulfil the environment concerns but they show some limitations in terms of performance like thermal resistance, barrier and mechanical properties, associated with costs. Moreover, this kind of packaging materials needs more research in the form of value addition like the introduction of smart and intelligent molecules Duncan, 2011). Biodegradable packaging was prepared which was found very much environment friendly and easily available protein were used. These types of packaging may be set a future for the human being.

2. Materials and Methods

Experimental design and data analysis: Statistical package Design-Expert Version 7.0 was used to design the experiment. The central composite design CCRD for three independent variables was performed. The independent variables considered were pH (A) Sonication levels (B). The levels of each variable were established based on the results of earlier experiments and preliminary trials a rotatable, central composite design was employed to determine the

film formation conditions. Experiments were randomized in order to minimize the systematic bias in observed responses due to extraneous factors. Dependent variables were specific film thickness, area of film, moisture content, moisture absorption and viscosity of film forming solution. Response surface methodology was applied for experimental data, for generation of contour plots and for statistical analysis of experimental data. The analysis of variance (ANOVA) tables were generated for each of the response functions. When TiO2 was incorporated into a polymer matrix of a packaging material, it provided protection from food borne microorganism as well as odour staining deterioration and allergens due to presence of radiation of relative low wavelength near the ultraviolet region. The TiO2 film exhibits excellent mechanical and chemical durability in the visible and near infrared region. Incorporation of TiO2 into synthetic plastic matrix to increase the bio-degradability has been investigated. Procedure for development of bio-film: The WPI films were prepared using fixed level of TiO2 (5%) WPI (5%) and glycerol contents, (1.5%). The control film solution was prepared by dissolving 5 g of WPI in 100 ml distilled water with 1.5 g of glycerol. In the preparation of WPI film solutions, TiO2 was first dispersed into distilled water (100 ml) and stirred using a magnetic stirrer overnight to reach complete hydration/swelling. Subsequently 5 g of WPI was added to obtain an aqueous solution, followed by addition of 1.5 g of glycerol. The film solutions were heated at 90°C for 15 min in a water bath cooled to room temperature and degassed using a bath-type ultrasound sonicator. The cast films were dried onto levelled Teflon-coated sheet (24×30 cm^2) at an ambient temperature (20–25 °C, 50 ± 5% RH) for 2 days and then peeled off from surface and stored for further analysis. Determination of film characteristics Film solution parameters: Temperatures of the solution were recorded by mercury thermometer after sonication process and just before spreading the solution on Teflon-coated glass plates. Electronic balance (FX400 FCOSET) was used to measure weight of film solution as well as films so formed. Its least count was found to be 0.001g. Viscosity of solution: Viscosity of WPI based film was determined by using Bohlin 100 Rheometer. Two ml of sample was placed between the plates of rheometer and the gap between the plates was adjusted to 100 µm. The viscosity of the solution was obtained at the temperature 30 °C. For viscosity determination shear rate of 37 $1s^{-1}$ was taken. Thickness and area of film: Thickness of the developed packaging film was measured with the help of digital micrometer with sensitivity of ±0.001 mm. The measurements of thickness were carried out on different location each of film and average values were determined. After drying of film the length and width were measured by scale and area was determined. Moisture content: The moisture content (MC) of films so formed was determined by measuring the weight of films before and after drying in a laboratory oven (Shimaz, Iran) at 105±1 °C.

The film was dried until constant weight was reached. The observations were replicated thrice MC = {(Wi-Wf)/Wi}×100

Where, Wi= initial weight of film, Wf= final weight of film Moisture absorption: Moisture absorption (MA) was measured according to the modified method. The dried sheets of 20×20 mm^2 were first conditioned at 0% RH (calcium sulphate) for 24 h. After weighing, they were conditioned in a desiccators containing calcium nitrite saturated solution at 25 °C to ensure a RH of 55%. The samples were weighed at desired intervals until the equilibrium state was reached. The MA of the samples was calculated by using the relationship

TS = F/L×W

Where TS = tensile strength (MPa), F = Tension at break, L and W represents the length (mm) and with width (mm) of film sample respectively (Quing et al., 2009) Eb(%) = lb/lo× 100 Where, Eb = represents the Elongation at break (%) lb = measured elongation at break (mm) lo = Original specimen length (mm), Water vapour permeability : Water vapour permeability (WVP) is a proportional constant assumed to be independent of the water vapour pressure gradient applied across the film. WVP was determined using Gravimetric method as per ASTM E 96 using aluminium cups (5). About 50 cm^2 dia sample were sealed on the aluminium cups containing highly hygroscopic fused $CaCl_2$. Samples surface areas of film was sealed using hot wax. Before placing the samples in the humidity chamber, samples were kept at room temperature and weight was noted down. The sealed cups were kept in chamber at a temperature 38 ±1 °C at 90% RH. Water vapour transmission rate (WVTR)=Slope x area of sample (gm/m2 / day) Gas transmission rate: The packaging film samples were cut in to circular shape using the templates supplied with permeability cell. Oxygen transmission rate (OTR) of the films was determined according to ASTM-D 1484 standard using permeability cell (Model CS-135; C, Custom Scientific Instruments). The samples to be tested for OTR were placed between the disc using six equally spaced bolts after placing filter as support on the upper disk and a rubber gasket to fit the pressure tightly. The cell consisted of a glass capillary in a vertical position to an opening in the centre of the upper disc. Oxygen was supplied from a cylinder at a constant pressure to the bottom inlet of the cell. A short plug of mercury, contained in the capillary, was displaced upward by the permeating gas through the packaging material. An electro-mechanical vibration was used to avoid friction to the movement of plug. Optical characteristics: Hunter colorimeter was used for the measurement of color (Optical properties) of the developed packaging film. The three dimensional scale L*, a* b* was used. L* is the lightness coefficient, ranging from 0 (black) to 100 (white), a* represents greenness (-ve) and redness (+). While b* represents yellowness (+ve) and blueness (-ve), C represents Chroma H represents Hue angle. The sample was placed in the sample cup. The deviation of the color of the sample to standard was also observed and recorded in the computer interface.

The measurements were replicated thrice. E, C and H were determined using the following relationship

DE=(DL 2+Da 2+Db 2)

Where DE=Color change, DL= L control film–L treated film Da=a* control film-a* treated film, Db=b* control film -b* treated film. C=a*2+b*2 H=Arctan (a*/b*) Where H is denoted hue angle and C Thermal characteristics : Thermal properties of the composite films were measured using differential scanning calorimetry (DSC) equipment (Mettler Toledo, DSC1 Star System). A 10 mg film sample was cut into small pieces and placed into a sample pan of DSC equipment. An empty aluminium pan as reference was used. Samples were scanned at a heating rate of 10 °C min^{-1} between temperatures ranging from 0 to 250 °C. The nitrogen gas was used as the purge gas at a flow rate of 20 ml min^{-1}. The melting point (Tm) was calculated as the temperature where the peak of the endotherm occurs. The Tm of the each film was determined in triplicate and the results were averaged. Statistical analysis: Design Expert 7.0 was used for optimization of process parameters and prediction of optimised values of responses. Duncan's multiple ranges test were used to compare the difference among mean values of film specimens' properties at the level of (0.05).

3. Results and Discussion

3.1. Validation of model

The predicted values (optimum values of responses given by RSM) were compared with experimental values (experimental conducted at optimum process parameters with pH: 8.5 and sonication level: 60% using statistical technique as per data given in Table 1 to 3. There was non-significant difference in thickness, weight, 'b' value (color), moisture absorbance, moisture content tensile strength, elongation at break and GTR of both the film at 5% level of significance where as significant difference was observed in water solubility and WVTR of both the films. There was non significant difference in area of the film; viscosity of film solution in SPI based film and 'a' value in WPI film. There was significant difference in 'a' value in SPI based film and area of film, viscosity of film solution in WPI film.

3.2. Characterization of the bio-polymeric film

3.2.1. Thermal characteristics

The T_m (C) of films decreased with increase in pH and sonication levels. The value of T_m (°C) of the developed film at optimized input variable (pH: 8.5 and sonication level: 60%) was 70 and 67 (°C) for SPI and WPI based films respectively. The Tm of the control film (without Tio2 and sonication) was much higher (85 °C) compared with developed film; thereby resulting in easy and early degradation of the film. In another study, thermo grams demonstrate partial miscibility of nano-TiO_2 and SPI at the molecular level for any given concentration ratio. No phase separation was observed (separation of Tg or melting peaks) because the miscibility of nano-TiO_2 and SPI remained homogenous during heating cycle. If mixtures of polymer and nano-TiO_2 were immiscible, the mixture would exhibit two Tg and Tm corresponding to the two pure phases (Ghasemolou et al., 2011)

3.2.2. Bio-degradability

Titanium dioxide embedded SPI and WPI films were taken for test of bio-degradability. The effect of TiO_2 in weight loss was found non significant. The biodegradability was lower and gradually decreasing in first 40 to 60 days as shown figure 1. Therefore bio-degradability was high. Most of the film parts digested after 140 days. Polymer-based products are required biodegrade on a controlled way: natural polymer (like rubber, lignin, and humus) and synthetic polymer like polyolefin biodegrade following an oxo-biodegradation mechanism (Arvanitoyannis, 1999) and consequently cannot satisfy the rapid mineralization criteria required for standard biodegradation. Here this film fits into criteria of biodegradability.

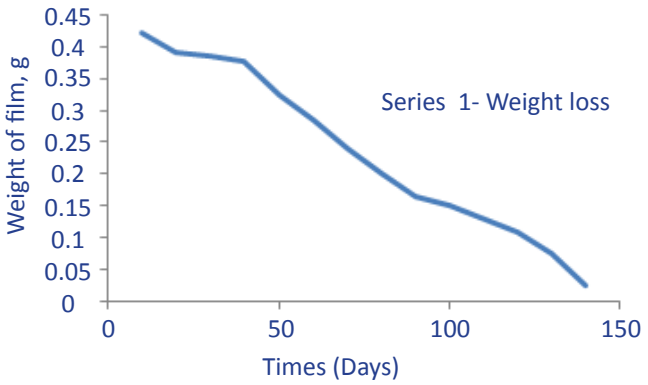

Figure 1: Biodegradability of nano –particle embedded bio-polymers films

3.2.3. Nano-imaging (SEM)

Scanning electron microscopy showed the strength and structure (compactness). The structure was homogeneous and showed scattering of particle throughout the film. A strong bond between molecule and fine structure developed was observed due to sonication.

Phan et al. (2005) observed that casting SPI and WPI films were transparent, homogenous and flexible with light yellow and white colour. The surface was compact structure without any pores and cracks. This may results from formation of strong and homogeneous surfaced SPI and WPI films with TiO_2 particles without sonication had different structure compared to control films. There were coagulation and some big particles were collected at different places, which mean a weak structure. This showed that the strength and structure of film get stronger and compact with sonication treatment. Zolfi et al. (2014) observeda uniform distribution of TiO_2 nanoparticles at 1 and 3% weight loading levels as shown by using scanning electron microscopy (SEM) micrographs.

Table 1: Equation predicting responses (Characterisctics) as affected by pH and sonication levels

Sl. No.	Film Characteristics	Equations	R^2
Soy protein isolate film			
1	Weight (g)	13.80-0.66×P-1.23 x S+0.94×P*×S*	0.6687
2	Film thickness (mm)	0.042-4.268E-004×P-0.013×S-2.500E-003×P×S-2.025E-003x +4.475E-003×S^2	0.9870
3	Area (cm^2)	400.00-4.25×P+43.67×S+1.50×P×S+24.63×P2+3.63×S^2	0.8797
4	L value	109.09079-1.92530×P -0.13068×S	0.7382
5	a* value	-7.62509+0.37831×P +3.49112E-003×S	0.1011
6	b* value	2.53047+0.62332×P+0.022598×S	0.3489
7	Viscosity of film solution (mpa-s)	31.84+0.25×P+4.19×S	0.6071
8	Moisture absorbance (%)	7.06-1.90×P-0.81×S+0.39×P×S+1.13×P^2-0.40×S^2	0.9105
9	Moisture content (%)	22.07-2.22×P-2.71×S	0.5344
10	Water solubility (%)	69.55823-1.82486×P-0.11592 S	0.8325
11	Tensile srength (Mpa)	-3.05183+1.48472 × P +0.016709×S+5.00000E-004×P×S - 0.079444 ×P^2 -1.21094E-004×S^2	0.9730
12	Elongation at break (%)	41.06112+5.48045×P+0.044828×S	0.6641
13	WVTR (×10^{-11} g m^{-2}s)	184.90720+0.64217×P -0.40948×S	0.4679
14	GTR (×10^{-8} m cc m^{-2} day)	0.76080+0.17143×P +0.011868×S	0.6518
Whey protein isolate film			
1	Weight (g)	12.65-0.22×P-1.30×S+1.10×P×S	0.5692
2	Film thickness (mm)	0.035-4.571E-004×P-0.012×S-3.000E-003×P×S-4.250E-004×P^2 +8.075E-003×S^2	0.9753
3	Area (cm^2)	392.00+18.71×P+38.71×S+15.00×P×S-9.75×P^2 +0.25×S^2	0.6514
4	L value	104.06124-1.27890×P-0.046766×S	0.5244
5	a* value	-8.90924+0.62273×P+0.091389×S-0.012500×P×S	0.2633
6	b* value	6.48-0.41×P+0.70×S+0.15×P×S-0.62×P^2-0.31×S^2	0.8068
7	Viscosity of film solution (mpa-s)	29.06-0.82×P+3.30×S	0.4841
8	Moisture absorbance (%)	6.09-0.20×P-1.29×S+1.17×P×S-0.48×P^2+1.28×S^2	0.7584
9	Moisture content (%)	20.15-2.05×P-2.93×S	0.6166
10	Water solubility (%)	64.61974-1.45832×P-0.10664×S	0.7766
11	Tensile srength (Mpa)	2.45873+0.17522×P+4.00574E-003×S	0.7605
12	Elongation at break (%)	35.62643+1.05867×P +0.086786×S	0.6884
13	WVTR (×10^{-11} g m^{-2}s)	247.76721-6.54248×P-0.41548×S	0.5649
14	GTR (×10^{-8} m cc m^{-2} day)	- 0.44487+0.28755×P+0.010524×S	0.9217

*P: pH; S: Sonication levels

3.3. Effect of bio-polymeric film on shelf life and quality of fruits and vegetables

3.3.1. Physiological loss in weight

The films resulted in significant decreased in PLW in uni-packed tomato and guava fruits compared to unpack especially under cold storage conditions. This was due to combined effect of film and temperature. PLW was 13.24 and 3.54% in control and SPI packed tomato after 16 days of cold storage. The corresponding values in guava were 14.67 and 4.05%. The WPI film has lower effect on PLW compared to SPI as indicated by PLW of 15.72 and 7.34% in tomato and 16.24 and 7.85% in guava in control and packed fruits as shown in Figure 2. The effect of film on reducing PLW under ambient condition was quite lower as indicated by PLW of about 17 to 23% in both

Table 2: Optimum values of process parameters and responses

Parameters	Goal	Lower limit	Upper Limit	Importance	Optimization level
A. pH Level	In range	7.0	10.0	3	8.5
B. Sonication level	In range	20	100	3	60
Responses (SPI based film)					
Weight (g)	Minimum	13.1000	16.4300	3	13.8005
Film thickness (mm)	Minimum	0.0400	0.0460	3	0.0418
Area (cm^2)	Maximum	379.2900	450.7300	3	400.0000
L value	In range	82.9200	92.2400	3	84.8846
a^* value	In range	-5.1600	-0.6000	3	-4.2000
b^* value	In range	8.2400	13.5400	3	9.3615
Viscosity of film solution (mpa-s)	Maximum	29.9700	38.8200	3	31.8353
Moisture absorbance (%)	Minimum	6.2100	9.1400	3	7.0602
Moisture content (%)	Minimum	20.2700	28.8400	3	22.0738
Water solubility (%)	Minimum	48.4300	52.0900	3	47.0915
Tensile srength (Mpa)	Maximum	4.5700	4.8500	3	4.6500
Elongation at break (%)	Maximum	87.0300	102.7100	3	90.3346
WVTR ($\times 10^{-11}$ g $m^{-2}s$)	Minimum	156.1300	201.9800	3	165.7970
GTR ($\times 10^{-8}$ m cc m^{-2} day)	Maximum	2.8400	3.2600	3	2.9300
Responses (WPI based film)					
Weight (g)	Minimum	9.0500	14.0900	3	10.7700
Film thickness (mm)	Minimum	0.0290	0.0390	3	0.0325
Area (cm^2)	Maximum	380.6900	482.7500	3	412.4650
L value	In range	87.2100	95.4900	3	89.7854
a^* value	In range	-6.2500	-1.0600	3	-4.4785
b^* value	In range	5.9800	8.2600	3	6.7678
Viscosity of film solution (mpa-s)	Maximum	29.3600	40.7300	3	32.9019
Moisture absorbance (%)	Minimum	3.4100	8.6600	3	5.2305
Moisture content (%)	Minimum	15.7200	24.8800	3	18.5750
Water solubility (%)	Minimum	44.4000	51.1400	3	45.8256
Tensile srength (Mpa)	Maximum	4.0900	4.5500	3	4.1880
Elongation at break (%)	Maximum	48.4100	55.1400	3	49.8325
WVTR ($\times 10^{-11}$ g $m^{-2}s$)	Minimum	176.5900	202.2600	3	167.2270
GTR ($\times 10^{-8}$ m cc m^{-2} day)	Maximum	2.3900	3.5400	3	2.6307

of the film. Physiological loss in weight in wrapped tomato significantly decreased and fruits were more firm than non wrapped tomatoes (Shetty et al., 1989 and Risse et al., 1985). However Shahnawaj et al. (2012) stated tomatoes wrapped in polyethylene bags have better quality in terms of weight loss, color and vitamin C content at red ripe stage

3.3.2. Microbial load

It was observed that microbial load increased with the duration of storage in control as well as packed fruits. The microbial load in bio-film packed fruits was much lower compared with control. Again microbial load was much lower in packed fruits kept under cold storage condition. This was due to combined effect of film and temperature. The microbial load in guava at the end of 16th day of storage was 23.13 and 17.33 CFU/g under ambient condition and 9.33 and 7.00 CFU/g under cold storage condition in control and packed film respectively as shown in Figure 3. Similar effect was observed in WPI based film and the values were quite closer to SPI based film. The nanoparticles restricted microbial

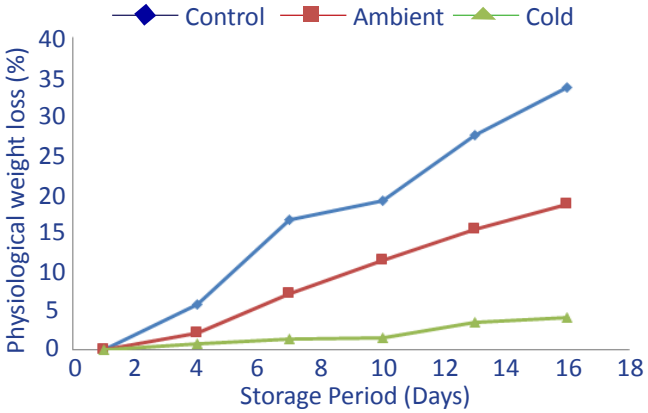

Figure 2: Physiological weight loss(5) during storage

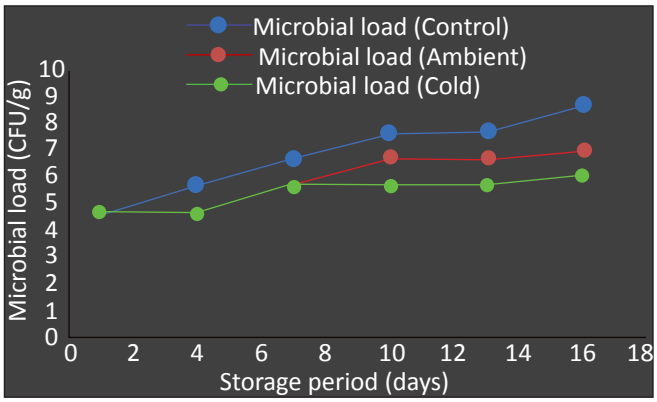

Figure 3: Microbial load during storage

growth in stored product (Sothornvit et al., 2009). In bio-film packed and stored tomato relatively lower microbial load was observed. This may be due to types of crops. It showed the effect of packaging film and temperature on microbial growth. However the effect on WPI based film was higher compared to SPI based film. For example under cold storage condition microbial load of control and packed after 16 days of storage was 8.66 and 6.00 CFU/g and 8.66 & 6.66 CFU/g in SPI and WPI based film respectively.

3.3.3. Optical characteristics

Colour is an important attribute of fruits and vegetables. Colour attributes viz. L*, a* and b* were recorded over the storage period. The colour was observed in terms of overall colour change (ΔE) and intensity of colour (ΔL). It was observed that bio film resulted in reduction in overall colour change under both storage conditions. Further the colour change in SPI based bio-film packed and stored under cold storage conditions was minimum compared with ambient conditions. This was due to combined effect of film and temperature. The overall colour change (ΔE) of guava at the end of 16th day was 18.754 and 9.531 under ambient condition 10.351 and 7.258 under cold storage condition in control and packed film respectively. Similar effect was observed in WPI based film and the values were quite closer to SPI based film. Further in comparisons to guava, lower effect in overall colour change

Table 3: Predicted and experimental values of responses

Film characteristics	Predicted value	Experimental value	Significance difference ($p \leq 0.05$)
Soy protein isolate based film			
Weight (g)	13.8005	13.9400	N
Film thickness (mm)	0.0418	0.0410	N
Area (cm^2)	400.0000	406.6600	N
L value	84.8846	86.6620	Y
a* value	-4.2000	-4.3210	Y
b* value	9.3615	9.4500	N
Viscosity of film solution (mpa-s)	31.8353	30.7290	N
Moisture absorbance (%)	7.0602	7.1900	N
Moisture content (%)	22.0738	21.9200	N
Water solubility (%)	47.0915	45.8246	Y
Tensile srength (Mpa)	4.6500	4.6460	N
Elongation at break (%)	90.3346	90.3560	N
WVTR ($\times 10^{-11}$ g m^{-2}s)	165.7970	187.6260	Y
GTR ($\times 10^{-8}$ m cc m^{-2} day)	2.9300	2.8800	N
Responses (WPI based film)			
Weight (g)	10.7700	11.0180	N
Film thickness (mm)	0.0325	0.0346	N
Area (cm^2)	412.4650	403.6600	Y
L value	89.7854	88.4400	Y
a* value	-4.4785	-4.3120	N
b* value	6.7678	7.0210	N
Viscosity of film solution (mpa-s)	32.9019	29.8960	Y
Moisture absorbance (%)	5.2305	5.3860	N
Moisture content (%)	18.5750	19.010	N
Water solubility (%)	45.8256	47.0945	Y
Tensile srength (Mpa)	4.1880	4.2630	N
Elongation at break (%)	49.8325	49.4730	N
WVTR ($\times 10^{-11}$ g m^{-2}s)	167.2270	181.065	Y
GTR ($\times 10^{-8}$ m cc m^{-2} day)	2.6307	2.6500	N

Where, N: No; Y: Yes

(ΔE) in bio-film packed and stored tomato under ambient and cold storage conditions was observed. This may be due to types of crops.

The colour observed in terms of intensity of colour (ΔL) also showed the effect of packaging film and temperature. However the effect in WPI based film was higher compared to SPI based film. For example under cold storage condition value of ΔL after 16 days of storage was 6.60 and 3.93 in SPI and WPI based film respectively. Another finding was that the difference between ΔL values of control and packed was slightly lower compare to change in colour on ΔE basis. Coordinate L*, representing the lightness of colour, significantly ($p \leq 0.05$) decreased during storage period for the all the samples.

3.3.4. Overall acceptability

The overall acceptability was determined using hedonic scale for sensory evaluation of quality attributes viz appearance, colour, texture and flavour. It was observed that overall acceptability decreased with time in control as well as packed fruits under both storage conditions. The film resulted in retaining OA. The overall acceptability in SPI based bio-film packed and stored under cold storage conditions was higher compared with ambient conditions. This was due to combined effect of film and temperature. The OA in guava at the end of 16th day was 3.33 and 6.66 under ambient condition and 5.66 and 7.00 under cold storage condition in control and packed film respectively. Similar effect was observed in WPI based film and the values were quite closer to SPI based film. Further in comparison to guava high OA in bio-film packed and stored tomato was observed. This may be due to types of crops. The film had almost equal effect on retention of OA. Acceptance **o**f a food is basically the result of the interaction between food and man at a certain moment (Shepherd, 1989).

4. Conclusion

The present study was under taken for development of nano-particle embedded biodegradable polymers for packaging and storage of fruits and vegetables. Design of experiment was done by central composite rotatable design method using RSM methodology. Two process variables viz. pH (7-10) and sonication levels (0-100%) were taken and 12 responses were obtained. The predicted values (optimum values of responses given by RSM) were compared with experimental values (experimental conducted at optimum process parameters with pH: 8.5 and sonication level.

5. References

Arvanitoyannis, I., 1999. Totally-and-partially biodegradable polymer blends based on natural and synthetic macromolecules: preparation and physical properties and potential as food packaging materials. Journal of macromolecular Sciences-Reviews in macromolecular chemistry and physics C39, 205–271.

Almasi, H., Ghanbarzadeh, B., Entezami, A.A., 2009. Physicochemical properties of starch-CMC-nanoclay biodegradable films. International Journal of Biological Macromolecules 46, 1–5.

Ashley, A.J., 1994. Permeability and plastic packaging, Polymer permeability, Comyn, Journal. (Ed.), Chapman and Hall, London, 269–308.

Campose, C.A, Gerschenson, L.N., Flores, S.K., 2011. Development of edible films and coatings with antimicrobial activity. Food and Bioprocess Technology 4, 849–875.

Cao, N., Fu, Y.H., He, J.H., 2007. Preparation and physical properties of soy protein isolate and gelatin composite films. Food Hydrocolloids 21, 1153–1162.

Carbone,M., 2016. Silver nanoparticles in polymeric matrices for fresh food packaging. Journal of King Saud University Science 28, 273−279.

Cha, D.S., Chinnan, M.S., 2004. Biopolymer-based antimicrobial packaging: a review. Critical Reviews in Food Science and nutrition 44, 223–237.

Duncan, T.V., 2011. Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. Journal of Colloid and Interface Science 363(1), 1−24

Ferreira, A.R.V., Alves, V.D., Coelhoso, I.M., 2016. Polysaccharide-based membranes in food packaging applications. Membranes (Basel) 6(2),22. doi: 10.3390/membranes6020022.

Gallocchio, F., Belluco, S., Ricci, A., 2015. Nanotechnology and food-brief overview of the current scenario. Procedia Food Science 5, 85−88.

Gautam, R.B., Kumar, S., 2015. Effect of drying chamber for nano-particle embedded bio-degradable polymeric films. South Asian Journal Food Technology and Environment 1(3&4), 225−232.

Gautam, R.B., Kumar, S., 2016. Development and characterization of nano-particle embedded whey protein isolate bio-degradable films. Progressive Research anInternational Journal 11(3), 178−183.

Gautam, R.B., Kumar, S., 2016. Development and characterization of nano-particle embedded soy protein isolate bio-degradable films. International Journal of Bio-resource and Stress Management 7(2), 271−279.

Gautam, R.B., Kumar, S., 2017. Development of Protein Based Films with Nanoparticle as Strengthening Material for Biodegradable Packaging – A Review. International Journal of Agriculture Innovations and Research 5(5), 790−805.

Gennadios, A., 2002, Protein based films and coatings, CRC Press. Journal of Engineering and Technology, 672.

Gennadios, A., Park, H.J., Weller, C.L., 1993b. Relative humidity and temperature effects on tensile strength of edible protein and cellulose ether films. Transactions of the ASAE 36, 1867–1872.

Ghasemolou, M., Khodaiyan, F., Oromiehie, A., 2011. Physical, mechanical, barrier and thermal properties of polyol-plasticized bio-degradable edible film made from Kefran. Carbohydrate Polymers 80(1), 477−483.

Gonzalez, A., Strumia, M.C., Igarzabal, C.I.A., 2011. Cross-linked soy protein as material for biodegradable films: Synthesis, characterization and biodegradation. Journal of Food Engineering 106, 331–338.

Kadam, D.M., Thunga, M., Wang, S., Kessler, M.R., Grewell, D., Lamsal, B.,Yu, C., 2013. Preparation and characterization of whey protein isolate films reinforced with porous silica coated titania nanoparticles. Journal of Food Engineering 117(1), 133–140.

Kinnear, C., Moore, T.L., Rodriguez-Lorenzo, L., Rothen-Rutishauser, B., Petri-Fink, A., 2017. Form follows function: nanoparticle shape and its implications for nanomedicine. Chemical reviews 117(17), 11476−11521.

Kirwan M.J., Plant, S., Strawbridge, J.W., 2015. Plastics in Food Packaging. Food and Beverage Packaging Technology 7, 158−212.

Kumar, P., Sandeep, K.P., Alavi, S., Truong, V.D., Gorga, R.E., 2010. Preparation and characterization of bionanocomposite films based on soy protein isolate and montmorillonite using melt extrusion. Journal of Food Engineering 100, 480–489.

Liu, D., Chen, H., Chang, P.R., Wu, Q., Li, K., Guan, L., 2010. Biomimetic soy protein nanocomposites with calcium carbonate crystalline arrays for use as wood adhesive. Bioresource Technology 101, 6235–6241.

Lu, Y., Weng, L., Zhang, L., 2004. Morphology and properties of soy protein isolate thermoplastics reinforced with chitin whiskers. Biomacromolecules 5, 1046–1051.

Majeed, K., Juwaid, M., Hassan, A., Baker, A.A., 2013. Potential materials for food packaging from nanoclay/natural fibres filled hybrid composites, Material and design. 46, 391−410.

Phan, T.D., Debeaufort, F., Luu,D., Voilley, A., 2005. Functional properties of edible agar based and starch based films for food quality preservation. Journal of Agricultural Engineering and Food Chemistry 53, 973−981.

Risse, L.A., Miller, W.R., Ben, S.Y., 1985. Weight loss, firmness, colour and decay development of individual film wrapped tomatoes. Tropical Science 2, 117−121.

Shahnawaj, M., Ade Abhinash, B., Sanghle, M.K., 2012. A Review on Biodegradation of polythene:The Microbial Approach. Journal of Bioremediation and Biodegradation 3, 164.

Shepherd, R., 1989. Factors influencing food preferences and choice. In: Shepherd (ed.), Handbook of the Psychophysiology of Human Eating. John Wiley and Sons Ltd, Chichester, UK. 3–24.

Shetty, R., Raghuveer, B. P., Rao, S.S., Nayak, R., 1989. Taguchi's Journal of the Brazalian . Society of Mechanical sciences and Engineering Technique in Machining of Metal Matrix Composites January-March 2009, Vol. XXXI, No. 1 / 13, 12−20.

Sothornvit, R., Rhim, J.W., Hong, S.I., 2009, Effect of nano-clay type on the physical and antimicrobial properties of whey protein isolate/clay composite films. Journal of Food Engineering 91, 468–473.

Sorrentino, A., Gorrasi, G., Vittoria, V., 2007. Potential perspectives of bio-nano-composites for food packaging applications. Trends in Food Science & Technology 18, 84–95.

Souza, V.G.L., Pires, J.R.A., Rodrigues, C., Coelhoso, I.M., Fernando, A.L., 2020. Chitosan composites in packaging industry-current trends and future challenges. Polymers (Basel) 12(2), 417.

Zhang, L., Feng, Q., Wang, J., Zhang, S., Ding, B., Wei, Y., Dong, M., Ryu, J.Y., Yoon, T.Y., Shi, X., 2015. Microfluidic synthesis of hybrid nanoparticles with controlled lipid layers: understanding flexibility-regulated cell–nanoparticle interaction. ACS Nano 9(10), 9912−9921.

Zolfi, M., Khodaiyan, F., Mousavi, M., Hashemi, M., 2014. Development and characterization of the kefiran-whey protein isolate-TiO_2 nanocomposite films. International Journal of Biological Macromolecules 65, 340–345.