Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 317-321
Received: 11-01-2019
Accepted: 15-02-2019

Suman Rai
Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Amrita Poonia
Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence
Suman Rai
Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Formulation and characterization of edible films from pea starch and casein

Suman Rai and Amrita Poonia

Abstract
The aim of the study was to developed edible film from pea starch and casein with good physical, mechanical and water vapour permeability properties. The films were prepared from dissolving of 5% pea starch and casein in different ratios 1:0, 1:1, 1:3, 3:1 into the water with Glycerol (2.5 %) and were analysed for determine its physical and mechanical properties. The film from starch/casein ratio 1:3 was better in film thickness(0.204 mm), solubility (52.72%), tensile strength(20.9 MPa), elongation at break (9.7%) and water vapour permeability (12.09 g $m^{-1}s^{-1}Pa^{-1}$). Increasing concentrations of casein was cause high moisture content in film with higher transparency. Scanning electron microscopy (SEM), FTIR spectrum and X-ray diffraction of T_2 sample was showed smooth surface without any fracture, interaction between of hydroxyl and amino group of pea starch / casein and revealed an amorphous-crystalline structure of film, respectively.

Keywords: edible film, pea starch, casein, mechanical properties, water vapour permeability

Introduction
Packaging is the most appropriate technique to protect as well as preserve the food component from the manufacturing to its consumption. Commercially, synthetic polymers are mostly chosen because of their superior mechanical and barrier properties. These synthetic films have big drawback are non-biodegradable and impossible to recycle it that cause toxicity and environment pollution. Therefore, need to develop Eco-friendly packaging materials that are nontoxic and biodegradable in environment. Edible films are primary packaging types that are natural thin layer of nontoxic material and they could still contribute to the reduction of environmental pollution. The main advantage of edible films over synthetics polymer are directly consumed with food and naturally degraded in the environment (Guilbert *et al*., 1996) [4]. It has protected the food component by its oxygen and moisture barrier properties as well as improved the quality of food by limiting the movement of moisture, lipids, flavour and colour between food components (Krochta, 1992) [12]. Different types of natural components such as hydrocolloids (polysaccharides, proteins, and alginate) and lipids (fatty acids, waxes and acyl-glycerol) were used to developed edible film (Donhowe and Fennema, 1993) [1]. The composite edible films also developed by combination of two or more these ingredients which can provide the strength to their properties (Hernandez-Izquierdo and Krochta, 2008) [7].
Polysaccharides are generally hydrophilic nature which contributes the poor barrier properties to oxygen and water vapour that is affected its mechanical properties and stability (Garcia *et al.*, 2011) [2]. It provides strong hydrogen bonding which can be used to bind the functional additives such as colors, flavors and nutrients. Therefore, other biopolymer materials, plasticizers (glycerol) and essential oil are used to improve its physical, mechanical and barrier properties (Kester and Fennema, 1989) [11]. The high amylose corn starch (71% amylose) films had no detectable oxygen permeability at relative humidity less than 100% reported by Mark *et al*. (1966)[15]. Protein based edible films have good mechanical strength which can be used to reduce the mechanical injuries during transportation (Saucedo-Pompa *et al.*, 2009; Miller and Krochta, 1997; Larotonda, 2005; Janjarasskul and Krochta, 2010) [18, 16, 13, 9]. Casein has gained importance due to its flexibility, transparency and flavourless film properties as well as possesses brilliant nutritional value. In additional, it serves as carrier of additives such as antimicrobial and antioxidants agents.

Materials and Methods
Raw materials
Raw materials used in this research were glycerol and casein obtained from Department of Food Science and Technology, IAS, BHU. Pea starch was obtained as by-product from wet milling of yellow peas.

Scanning Electron Microscope (SEM) and X-Ray Diffraction (XRD) were performed in Instrumentation Lab, IIT, BHU. Fourier Transform Infrared Spectrometer (FTIR) was performed in Department of Chemistry, Faculty of Science, BHU.

Preparation of edible films

Edible films were prepared by dissolving of 5% pea starch/casein in different ratios 1:0, 1:1, 1:3, 3:1 into the water. Glycerol (2.5 %) was added into the solution after proper mixing of starch and casein. Edible film solution was stirred at 75°C for 40 minutes for gelatinization. Afterward, it was cooled to 45°C and sieved through 0.05 cm diameter for removing of air bubbles. Each solution was spread onto Teflon plate in a thin layer and kept for drying at 50°C for 16 hours into the tray drier. It were cooled at room temperature and removed for further analysis.

Physical properties

Moisture

The moisture content of composite pea starch/casein edible films were determined by the gravimetric method.

Film thickness

The thickness of the edible films was measured with a digital micrometre from the average of five measurements at different points of the film.

Film solubility

Water solubility of composite pea starch/casein edible films were determined according to the method described by Gontard *et al.* (1994) [3] with some modification. The film were cut into 2 cm diameter and kept in an oven at 105°C for the determination of initial dry mass. These films were placed in beaker with 100 mL of distilled water. Then, it kept into incubator shaker at 100 rpm (25°C for 24 h). After incubation period, the un-dissolved films were removed and dried at 105°C for determination of the final dry mass. Solubility (%) was calculated based on weight difference as shown in Eq. 1.

$$solubility\ (\%) = \frac{Initial\ dry\ mass - Final\ dry\ mass}{Initial\ dry\ mass} \text{ X } 100 \ldots. (1)$$

Mechanical properties

Tensile strength

Tensile strength (TS) was measured according to the method described by Shevkani & Singh (2015) [19] with some modification. The texture analyzer (TA/XT2-plus; Stable Micro Systems, Surrey, UK) with a 50-kg load cell and crosshead speed at 60 mm /min was used to measure TS of composite pea starch/casein edible films. The four film specimens were cut into 10 X 1 cm for the measurement. The specimens were fixed in 2 grips separated by 100 mm. The maximum force (N) and extension of film (mm) were recorded to calculate tensile strength (TS) and percent elongation at break (% E) at break of the films using a tensile test. Each trial per film was taken in four replicate measurements and the results of tensile and elongation tests were expressed in MPa and percentage (%), respectively.

Water vapour permeability (WVP)

The water vapour permeability (WVP) of film was determined by the method described by Saberi *et al*. (2017) [17] with some modification. The test cell was containing anhydrous calcium chloride desiccant was covered with the developed film and sealed it with help of paraffin. The test assembly was weighted and incubated at 25 ºC and 75% relative humidity (saturated NaCl solution) for 7 days. The cells were weighed after 24 h intervals over a 7-day period to determine the amount of moisture transferred through the sample into the desiccant. The water vapour transmission rate (WVTR) was calculated through the slope of the straight line (g/s) divided by the test area of film (m^2). After the permeation tests, the film thickness was measured and WVP was calculated by multiplication of WVTR with thickness.

Scanning electron microscopy (SEM)

Morphology of composite pea starch/casein edible films was examined by using ZEISS Supra-40 Scanning electron microscope (SEM) at an accelerating potential voltage of 20 kV under 500 x magnifications.

X-ray diffraction (XRD)

X-ray patterns of film were analyzed for the relative crystallinity state of composite pea starch/casein edible films by using a Rigaku Mini Flex 300/600 X-ray diffractometer (XRD). Film was cut into 4 x 4 cm^2 and it exposed in the diffractometer chamber for measurement at 40 KV, 15 mA over an angular range of 2 Θ = 3-50° with a speed of 5° 2θ /min.

FT-IR spectroscopy

FTIR analysis of composite pea starch/casein edible films was carried out using PerkinElmer, Spectrum version 10.4.3. FTIR spectrum was used to determine presence of functional group in edible film. In this procedure thin films were applied directly onto ATR cell. The FTIR spectra of the film were recorded at a resolution of 4 cm^{-1} and the range from 4000 to 600 cm^{-1}.

Results and Discussion

Physical properties

Physical properties regarding the moisture, thickness and solubility of composite pea starch/ casein edible film are given in Table 1. The moisture content was found low in T_0 and high in T_2 sample. The lowest film thickness was found for sample T_2 while highest thickness of film found in T_0 pea starch film. The thickness of starch film high could be due to the gelatinization of starch that increases the film viscosity. Solubility of edible film is ability to dissolve in water. It was found high for T_2 film and low for starch film. Solubility increased with increasing the content of casein in film. The result of physical properties of film was shows that the T_2 sample having high solubility with thin transparent film.

Table 1: Physical properties of composite pea starch- casein edible films (Mean ± SD)

Treatment (Pea starch: Casein)	Moisture (%)	Film thickness (mm)	Film solubility (%)
T_0 (1:0)	12.60±0.81	0.351±1.23	41.10±0.56
T_1 (1:1)	14.93±0.45	0.277±2.09	48.69±0.99
T_2 (1:3)	17.37±0.75	0.204±1.91	52.74±1.0
T_3 (3:1)	13.09±0.59	0.301±2.56	43.02±0.61

Mechanical properties

Mechanical properties regarding the tensile strength (TS), elongation at break (%E) and water vapour permeability (WVP) of composite pea starch/ casein edible films are given in Table 2. Tensile strength and WVP were obtained high in T_0 starch film and low in T_2 sample. Tensile strength and

WVP were high could be due to the high amount of starch in film. Similar study had been reported by Hassan and Norziah (2012) [6] for combination of starch-gelatine. Elongation at break (%E) was found high in T_2 sample could be due to the high flexibility of film because it contain high amount of protein. The result of mechanical properties of film was shows that the T_2 sample having good tensile strength (MPa), elongation at break (%E) and WVP characteristics.

Table 2: Mechanical properties and WVP of composite pea starch- casein edible films (Mean ± SD)

Treatment (Pea starch: Casein)	Tensile strength (MPa)	Elongation at break (%E)	WVP 10^{-10} g $m^{-1}s^{-1}Pa^{-1}$
T_0 (1:0)	33.6±2.1	6.1±1.9	23.86
T_1 (1:1)	24.1± 2.8	8.2±2.1	16.32
T_2 (1:3)	20.9±1.6	9.7±1.0	12.09
T_3 (3:1)	28.3±2.4	7.5±2.8	18.15

Scanning electron microscopy (SEM)

SEM morphograph of the surface edible film sample T_2 is shown in figure 1. Surface morphology of edible film was decided using SEM with 500x magnification. The micrograph shown smooth surface without any fractures and is homogenous. The presence of white peaks shows dispersed casein particles inside starch matrixes, which means not all casein is dissolved in the solution. Also, the more casein added, that increase the solubility with flexibility had been appeared.

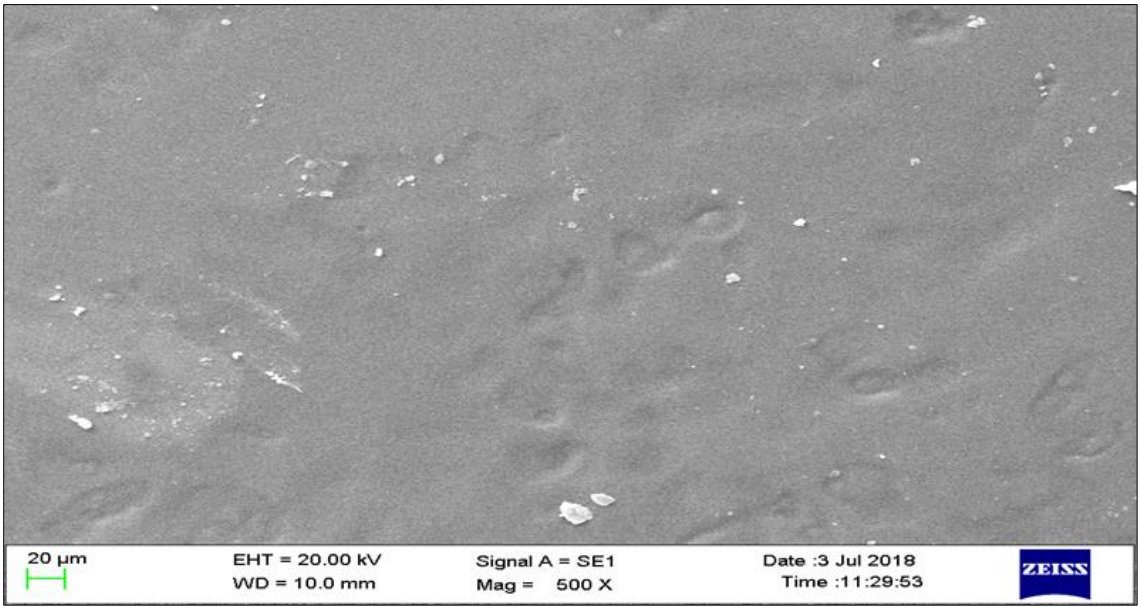

Fig 1: scanning image (500X) of the surface of the composite pea starch/ casein edible films T_2.

FTIR spectra

The FTIR spectra of edible film sample T_2 is shown in Figure 2. The absorption peak at 3301.50 cm^{-1} showed –OH stretching vibration and the C–H stretching band at around 2918 cm-1 in the film. Similar – OH stretching and C–H aliphatic stretching bands at absorption 3300 cm-1 and 2925.00 cm-1 had been reported by Kaya *et al*, (2018) [10] for P. terebinthus' leaf, seed, and stem extracts. The bend of C=O stretching (amide I) and NH bending (amide II) were detected at detected at 1644 cm-1 and 1543 cm-1,respectively. Similar study were reported by Zhong and Xia (2008) [20] for chitosan, cassava starch, gelatin, chitosan/cassava starch, and chitosan/cassava starch/gelatin composite films absorption band for C=O stretching (amide I) and NH bending (amide II) at 1630–1660 and 1540–1570 cm–1, respectively. Accoerding to Haq *et al*., (2016) [5] absorption bands at 1635 and 1544 cm−1 are characteristic of protein amide I (C=O) and amide II (N–H and C–N). The band at 999.50, 1016.50, 1151 and 1240.16 cm-1 are attributes of C–O stretching vibration. According to Hou *et al*. (2014) [8] C–O stretching vibration bands from 1250 to 950 cm−1. The result of FTIR spectra of blend film was shows the interactions between the hydroxyl groups and the amino groups of pea starch and casein, respectively.

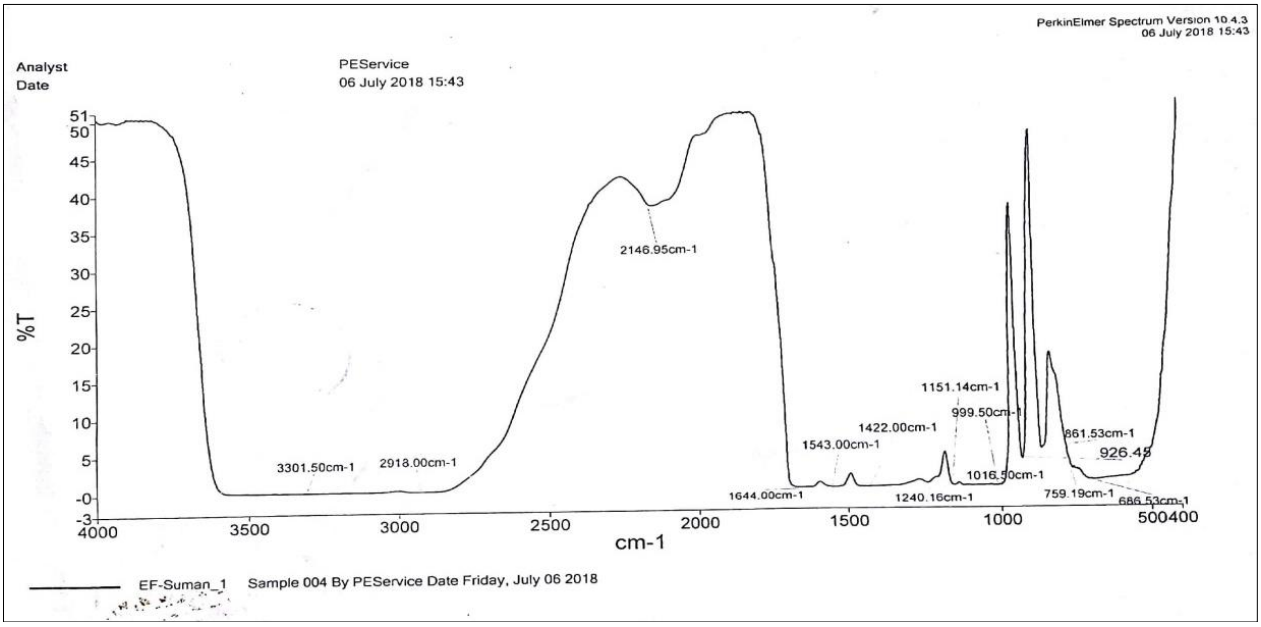

Fig 2: FTIR spectra of the composite pea starch/ casein edible films T2

X-ray diffraction (XRD)

X-ray diffractograms of edible film sample T_2 is shown in Figure 3. The XRD patterns showed molecular miscibility and an interaction between starch, casein and glycerol. The broad peaks are showed amorphous-crystalline structure of composite film with six main diffraction peaks are given in Table 3. Similar study had been reported by Zhong and Xia (2008) [20] for chitosan-based film. According to Lu *et al*. (2002) [14] glycerol affect the crystallinity of starch film by inter into macromolecular structure.

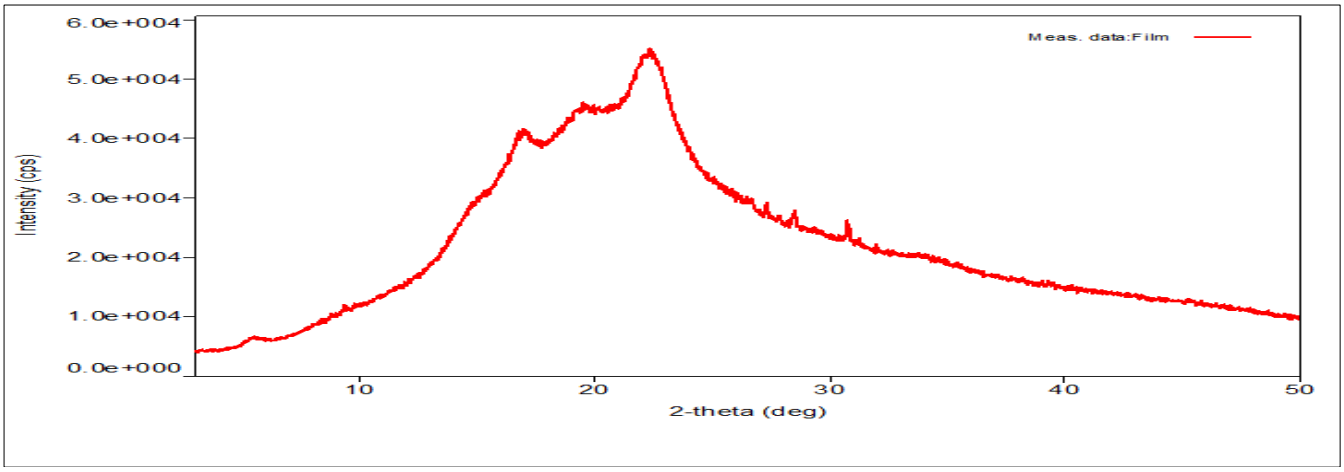

Fig 3: X-ray diffractograms of the composite pea starch/ casein edible films T_2

Table 3: The diffraction peaks for the composite pea starch/ casein edible films T_2

Peak No.	2θ (Deg)	D (Ang.)	Height (cps)	FWHM (Deg)	Size (Ang.)
1	5.43(4)	16.26(12)	811(58)	0.83(4)	100(5)
2	9.4(4)	9.4(4)	392(40)	2.0(4)	42(8)
3	16.87(5)	5.253(16)	1684(84)	1.2(2)	73(14)
4	22.42(5)	3.962(9)	6822(169)	1.60(5)	52.9(15)
5	28.40(9)	3.140(10)	994(64)	0.33(15)	257(118)
6	30.734(13)	2.9068(12)	3338(118)	0.09(2)	947(235)

Conclusion

In study of developed edible films from starch/casein (T_2) ratio 1:3 was better physical (Thickness and solubility), mechanical (tensile strength and elongation at break) and Water-vapour permeability properties than other films. The increase of the casein content in the film was produced flexible film with lower film thickness. X-ray diffraction of T_2 sample confirmed an amorphous-crystalline structure of edible film. SEM morphograph was showed the smooth surface of film without any fracture. An FTIR spectrum of T2 sample was showed interaction of hydroxyl and amino group of pea starch and casein, respectively.

References

1. Donhowe IG, Fennema OR. The effects of plasticizers on crystallinity, permeability, and mechanical properties of methylcellulose films. Journal of Food Processing and Preservation. 1993; 17:247-257.
2. Garcia NL, Ribbon L, Dufresne A, Aranguren M, Goyanes S. Effect of glycerol on the morphology of

nanocomposites made from thermoplastic starch and starch nanocrystals. Carbohydrate Polymers. 2011; 84(1):203-210.
3. Gontard N, Duchez C, Cuq JL, Guilbert S. Edible composite films of wheat gluten and lipids water-vapor permeability and other physical-properties. International Journal of Food Science and Technology. 1994; 29:39-50.
4. Guilbert S, Gontard N, Gorris LGM. Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. LWT-Food Science and Technology. 1996; 29:10-17.
5. Haq MA, Hasnain A, Jafri FA, Akbar Mf, Khan A. Characterization of edible gum cordia film: Effects of beeswax. LWT - Food Science and Technology. 2016; 68:674-680.
6. Hassan AAA, Norziah MH. Starch-gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. Food Hydrocolloids. 2012; 26:108-117.
7. Hernandez-Izquierdo VM, Krochta JM. Thermoplastic processing of proteins for film formation a review. Journal of Food Sciences. 2008; 73:30-39.
8. Hou L, Feng K, Wu P, Gao H. Investigation of water diffusion process in ethyl cellulose-based films by attenuated total reflectance Fourier transform infrared spectroscopy and two-dimensional correlation analysis. Cellulose. 2014; 21(6):4009-4017.
9. Janjarasskul T, Krochta JM. Edible packaging materials. Annual review of food science and technology. 2010; 1:415-448.
10. Kaya M, Khadem S, Cakmak YS, Mujtaba M, Ilk S, Akyuz L *et al*. Antioxidative and antimicrobial edible chitosan films blended with stem, leaf and seed extracts of Pistacia terebinthus for active food packaging, Royal Society of Chemistry. 2018; 8:3941-3950.
11. Kester JJ, Fennema O. Resistance of lipid films to water vapor transmission. Journal of the American oil Chemists' Society. 1989; 66(8):1139-1146.
12. Krochta JM. Control of mass transfer in foods with edible-coatings and films. In: Singh RP, Wirakarstakusumah MA, editors. Advances in food engineering. Boca Raton: CRC Press, 1992, 517-538.
13. Larotonda FDS, Hilliou L, Sereno AMC, Gonçalves MP. Green edible films obtained from starch-domestic carrageenan mixtures. In Proceedings of the 2nd Mercosur Congress on Chemical Engineering and 4th Mercosur Congress on Process Systems Engineering, ENPROMER, August 14th to August 18th, 2005.
14. Lu YS, Weng LH, Zhang LN. Bio macromolecules. 2002; 5:1046-1051.
15. Mark AM, Roth WB, Mehltretter CL, Rist CE. Oxygen permeability of amylomaize starch films. Food Technology. 1966; 20:75-80.
16. Miller KS, Krochta JM. Oxygen and aroma barrier properties of edible films: A review. Trends of Food Science and Technology. 1997; 8:228-237.
17. Saberi B, Thakur R, Bhuyan DJ, Vuong QV, Chockchaisawasdee S, Golding JB *et al*. Development of edible blend films with good mechanical and barrier properties from pea starch and guar gum. Starch-Stärke. 2017; 69:1-2.
18. Saucedo-Pompa S, Rojas-Molina R, Aguilera-Carbo AF, Saenz-Galindo A, De la Garza H. Edible film based on candelilla wax to improve the shelf life and quality of avocado. Food Research International. 2009; 42:511-515.
19. Shevkani K, Singh N. Relationship between protein characteristics and film-forming properties of kidney bean, field pea and amaranth protein isolates. International Journal of Food Science and Technology. 2015; 50:1033-1043.
20. Zhong QP, Xia WZ. Physicochemical properties of chitosan-based films. Food Technology and Biotechnology. 2008; 46(3):262-269.