Received: 12 July 2021 | Revised: 22 August 2021 | Accepted: 28 August 2021

DOI: 10.1111/jfpp.15926

Journal of Food Processing and Preservation | Institute of Food Science + Technology | WILEY

ORIGINAL ARTICLE

Optimization of nanocomposite films based on quinoa protein isolate incorporated with cellulose nanocrystal and starch

Mahdieh Safar Razavi Zade[1] | Mohammad Hossein Aghkhani[1] | Mohammad Hossein Abbaspour-Fard[1] | Fereshte Hosseini[2] | Zeinab Qazanfarzadeh[3]

[1]Department of Biosystems Engineering, Faculty of Agriculture, Ferdowsi University of Mashhad (FUM), Mashhad, Iran

[2]Department of Food Additives, Iranian Academic Center for Education, Culture and Research (ACECR), Mashhad, Iran

[3]Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

Correspondence
Mohammad Hossein Aghkhani, Department of Biosystems Engineering, Faculty of Agriculture, Ferdowsi University of Mashhad, Azadi square, Mashhad, Iran.
Email: aghkhani@um.ac.ir

Funding information
Nanotechnology Initiative Council (INIC); Ferdowsi University of Mashhad (FUM)

Abstract

This research aimed to prepare quinoa protein isolate (QPI) based nanocomposite films reinforced with starch and cellulose nanocrystals (CNC). The range of QPI, starch, and CNC contents were chosen as 60%–80%, 10%–30%, and 2.5%–7%, respectively, by mixture design approach. The effect of CNC and starch on physicochemical, mechanical, and barrier properties of the films were then investigated. The obtained results showed that the addition of starch and CNC improved the mechanical properties of films. The incorporation of CNC reduced the film water vapor permeability (WVP). In addition, transparency of the films increased with increasing protein and starch content and reducing CNC content. Furthermore, the optimal formulation of the composite was determined as 78.55% protein, 18.28% starch, and 3.17% CNC. Fourier transform infrared (FTIR) spectroscopy of the film prepared according to optimal formulation demonstrated the interaction of the film's constituents, and its scanning electric spectroscopy (*SEM*) image was in agreement with the physical and mechanical properties the nanocomposite film.

Novelty impact statement: Protein extracted from quinoa seeds using alkaline method with high purity (83%). The optimal formulation obtained with 78.55% protein, 18.28% starch, and 3.17% CNC. The optimal film is recommended for packaging of aromatic foodstuff.

1 | INTRODUCTION

Today, the petroleum-based plastics used in the packaging industry is one of the most important environmental concerns. Thus, many researches have focused on preparing the biodegradable and renewable polymers from different biomass sources (Qazanfarzadeh et al., 2020; Souza & Fernando, 2016; Souza et al., 2018). The biopolymers used in preparing the film and coating often include polysaccharides, lipids, proteins, and their combinations (Jahed et al., 2017; Sukhija et al., 2016).

Proteins are the biopolymers made of different amino acids by peptide bonds and are defined as a stable polymeric network strengthen by hydrophobic, hydrogen, disulfide, and electrostatic interactions. Protein-based films can be prepare from some biological resources such as plant seed proteins (e.g., soybean, pea) (Han, 2001; Zheng et al., 2016) and animal proteins (e.g., gelatin, whey protein) (Mondragon et al., 2014; Sothornvit et al., 2009). Among these biological resources, the plant seed proteins are widely used due to their advantages such as edibility, combinability with other components, non-toxicity, healthy, more abundance and low cost (Kalia, 2016).

Quinoa (*Chenopodium quinoa*) is an old plant that belongs to Chenopodiaceae, and was domesticated around 5,000 years ago. It is cultivated in the Andes Mountains of Bolivia, Chile, and Peru. Quinoa seeds are rich in protein, dietary fiber, B-vitamins, and dietary minerals (Fe, Mn, and P) (Martínez et al., 2015). Nutritional evaluations indicated that quinoa seed protein content ranged from 12% to 23%, which is higher than rice, corn, and barley protein content (Ruiz et al., 2016). Therefore, quinoa as a rich source of protein can be considered to protein extraction and used as a source

of protein for bio-degradable films preparation. In general, protein-based films are a good barrier to gases, lipid, and odor. However, due to their hydrophilic nature, they are sensitive to moisture and have shown poor water vapor permeability (WVP) and mechanical properties, which limit their applications (Azevedo et al., 2015; Rhim & Ng, 2007). One possible method to improve the properties of protein based films is mixing with other polymers such as starch to prepare composite films as recent studies have shown that the preparing composite films might improve their mechanical and barrier properties than those prepare from one polymer alone(Gennadios, 2002). Starch is one of the most important natural polymeric plants that is abundantly available at a low cost (Savadekar & Mhaske, 2012). The presence of high hydroxyl groups in both protein and starch enhances their ability to fusion by possible hydrogen bonds formation and improves the properties of final composite films.

Another method of improving the properties of protein-based films is incorporation of nano-filler compounds into the films as nanocomposite films. These components can improve the mechanical, thermal, and barrier properties of nanocomposites compare to polymer alone. The high interfacial area, aspect ratio, and ability of dispersion of nanoparticles lead to strong interactions between the nano-fillers and polymer, so that improves the properties of the nanocomposites (Fortunati et al., 2017; Li et al., 2011; Lu & Hsieh, 2010, 2012; Oymaci & Altinkaya, 2016; Paralikara & Simonsenb, 2008; Shruthy et al., 2020). Among the different nano-fillers, cellulose is well-known to improve the barrier and mechanical properties of edible film and coatings. The inherent features of cellulose nanocrystals (CNC) include nanoscale size, high surface-to-volume ratio, renewability, safety, compatibility with biopolymers, excellent morphology, biodegradability, and high chemical and thermal stabilities attracted the attention of many researchers in the area of cellulose nanocrystals. They have low density and as a filler they may significantly increase the strength and rigidity of the polymer and, reduce the weight of the nanocomposites network (Gindl & Keckes, 2005; Kristo & Biliaderis, 2007; Chen et al., 2012).

So far, a few studies have been done on finding the optimal formulation of biodegradable films. Therefore, this present study tries to provide an appropriate formulation of a biodegradable film using the mixture design approach. Mixture design is used to study relationships between different variables and responses (Chen et al., 2010). It can establish the surface model of continuous variables, estimate every element in the mixture and their interactions, and optimize the component elements following the target to determine the best ratio of ingredients (Zhou et al., 2007). Nowadays, this statistical method is extensively used for formulation in food industries (Laneuville et al., 2005; Ryland et al., 2010; Yang & Vickers, 2004). Thus, the main objective of this research was to extract quinoa protein isolate (QPI), find the optimal formulation of the biodegradable bio-nanocomposite consisted of QPI, starch and CNC using mixture design method, preparing the bio-nanocomposite film, and investigate the physical, mechanical, and barrier properties of the bio-nanocomposite films.

2 | MATERIALS AND METHOD

2.1 | Materials

Quinoa seed (*Red Carina variety*) was supplied from the Seed and Plant Improvement Institute (SPII) in Karaj, Iran. CNCs were purchased from Nanotechnology Laboratory Network, Tehran University, Iran. The starch powder was purchased from the retail market in Mashhad, Iran. All the chemicals used for analysis, including hexane, hydrochloric acid, sodium hydroxide, glycerol, and trypsin enzyme, were purchased from Merck (Germany).

2.2 | Extraction of quinoa seed protein

The quinoa seed protein was extracted according to the method of Ruiz et al. (2016). The quinoa seeds were soaked in cold water several times to remove the dust and then air-dried in ambient temperature. The cleaned seeds were then milled using an electrical mill and sieved under 60 mesh. The obtained flour was defatted with hexane with a sample-to-solvent mass ratio of 1:5 for 2 hrs to remove the oily components. The resulting flour was stored in a refrigerator at 4°C until used. The defatted flour was suspended in deionized water (10% w/v), and the pH was adjusted to 11 by 2 N NaOH. The resulting mixture was stirred for one hour at room temperature and stored at 4°C for 24 hr and then centrifuged for 10 min at 6,000 × g to remove non-proteinaceous compounds. The supernatants were acidified to pH 4.5 by 2 N HCl and centrifuged at 6,000 × g for 10 min. The remaining sediment was collected and freeze-dried (FD 10, Iran) for 48 hr.

2.3 | Determination of the chemical composition of quinoa seed

The chemical composition of the samples (flour and QPI) was measured by the standard methods of AOAC (2000). The moisture, lipid, ash, and protein contents were determined by oven drying at 105°C, soxhlet method at 550°C, and Kjeldahl method, respectively. The carbohydrates content was calculated through the fraction of the percentage of all compounds from 100. All tests were performed with three replications.

2.4 | Preparing of the protein/starch/CNC bio-nanocomposite film

The control protein film was prepared by dissolving 3g of QPI in 100 mL distilled water through constant stirring for 30 min. The pH of the suspension was then adjusted to11 using 2 N NaOH to better dissolution of protein. Subsequently, glycerol as a plasticizer was added as 50% (w/w of QPI), and the solution was heated at 80°C for 20 min. After cooling down, ultrasonic treatment was performed

to remove the possible air bubbles in the film forming solution. The film forming solutions were cast on Teflon-coated glass plates (25 cm × 25 cm) and dried in ambient temperature for 24 hr (Bamdad et al., 2006).

In order to prepare the bio-nanocomposite films, different amounts of CNC, starch, and protein were used according to Table 1. For this purpose, CNCs were dissolved in 100 ml distilled water and subjected to ultrasonic treatment for 15 min. Then, QPI powder was added to the CNC suspension and after adjusting the pH, the specified amounts of starch were added to the film forming solution. The next steps were performed according to the method mentioned for the control film. After drying the peeled films were kept in a desiccator containing sodium carbonate at 20% relative humidity and 25 ± 2°C for 48 hr before the WVP and mechanical properties tests.

2.5 | Characterization of films

2.5.1 | Solubility in water

The film samples (4 cm × 4 cm) were dried in an oven at 105°C for 24 hr to obtain the initial dry weight of samples (W_i). Then, the film samples were immersed in distilled water (25°C for 24 hr) and subsequently oven-dried (at 105°C for 24 hr) again to determine the final dry weight of the samples (W_f). Finally, water solubility was determined according to the following equation (Abdollahia et al., 2013):

$$\text{Water solubility}\,(\%) = \frac{W_i - W_f}{W_i} \times 100. \quad (1)$$

2.5.2 | Water vapor permeability (WVP)

The WVP of films was determined according to the ASTM E96 method (2001). For this purpose, glass cups (2.5 cm diameter and 8 cm height) containing 2 g CaSO4 were used and the top of the cups was covered with a piece of film. The cups were weighed with their contents and placed in a desiccator containing NaCl solution with 75% relative humidity. The cups were weighed every 4 hr for two days. The WVP was determined according to the following equation:

$$\text{WVP} = \frac{m}{t}\frac{L}{A\Delta P}, \quad (2)$$

where m/t is the water vapor transmission rate (g/day), L (mm) is the film thickness, A (m^2) is the exposed area of the film, and ΔP (kPa) is the partial vapor pressure difference of the outside and inside of the cups.

TABLE 1 The various formulations used for preparing bio-nanocomposite films (all data in percent)

Formulation	Protein (x_1)	Starch (x_2)	CNC (x_3)
1	75	20	5
2	71.25	25	3.75
3	67.50	30	2.5
4	62.50	30	7.5
5	78.75	15	6.25
6	68.75	25	6.25
7	87.50	10	2.5
8	82.50	10	7.5
9	81.25	15	3.75

Abbreviation: CNC, cellulose nanocrystals.

2.5.3 | Film opacity

Film transparency was measured at 600 nm wavelength using a UV-Vis spectrophotometer (CAMSPEC M550, England). The thickness of film samples (4 cm × 1 cm) was measured at five random positions, and then their absorption was determined (Siripatrawan & Harte, 2010) (Guilbert & Cuq, 1992). Their transparency was determined using the following equation:

$$\text{Opacity} = \frac{A600}{X}, \quad (3)$$

where A600 is the absorption at 600 nm and X is the film thickness (mm).

2.5.4 | Mechanical properties of films

Tensile strength (TS) and elongation at break (EB) of films were determined according to the standard method (ASTM & D882-02, 2002) using a texture analyzer (H5 KS, England). The thickness of film samples (80 mm × 25 mm) was measured at five random positions. The distance between the grips and cross-head speed was set to 33 mm and 50 mm/min, respectively. The TS and %EB of the film were determined according to the following equations:

$$\text{TS} = \frac{F_{max}}{x \times y}, \quad (4)$$

$$\text{EB} = \frac{\Delta L}{L_0}, \quad (5)$$

where F_{max} is the maximum force applied to the film (N), x is the film width (mm), y is film thickness (mm), ΔL is the absolute value of the elongation at break (mm), and L_0 is the initial length of the sample (mm) between the grips.

2.5.5 | Scanning electron microscopy (*SEM*)

The bio-nanocomposites microstructure (surface and cross-section) were analyzed using *SEM* (VP1450, Germany). For cross-sectional analysis, the film's pieces were cry-fractured in liquid nitrogen. Then, the films pieces were fixed to an aluminum base using silver glue

and were gold-sputter-coated for several minutes (Mao et al., 2002). The *SEM* images of the film samples were observed at different magnifications.

2.5.6 | Fourier transform infrared spectroscopy (FTIR)

In order to investigate the chemical interactions between polymers and the other components as well as identifying the new functional groups, FTIR spectroscopy (370 Avatar, USA) was performed at the wavelengths from 400 cm^{-1} to 4,000 cm^{-1} at 4 cm^{-1} resolution (Shujun et al., 2006).

2.5.7 | Statistical analysis

In this work, the formula optimization was performed by mixture design method, using MINITAB software version 17.3.1. To determine the treatments, the ranges of compounds including protein, starch, and CNC were defined as 60%–80%, 10%–30%, and 2.5%–7%, respectively. The number of central points was nine, and the design was considered extreme vertices. After the preparation of treatments and performing the analysis, to determine the best model describing the variables, some linear models, including quadratic, Special quartic (correspond to Equations 6–8), were examined for data processing. The ANOVA test was applied to determine the significance of each equation at the 5% level with the following equations.

$$Y = b_1X_1 + b_2X_2 + b_3X_3, \quad (6)$$

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_1b_2X_1X_2 + b_1b_3X_1X_3 + b_2b_3X_2X_3, \quad (7)$$

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_1b_2X_1X_2 + b_1b_3X_1X_3 + b_2b_3X_2X_3 + b_1b_2b_3X_{12}X_2X_3 + b_1b_2b_3X_{22}X_1X_3 + b_1b_2b_3X_{32}X_1X_2, \quad (8)$$

where *Y* is the predicted dependent variable (or response including water solubility, WVP, transparency, and mechanical properties), b is the equation coefficient, and X is the formulation components.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of quinoa flour and CNC

Table 2 shows the chemical compounds of the quinoa flour and QPI. The results showed the protein extraction process from quinoa seed was well performed and contained more than 83% protein. This amount is 7.22% higher than that of reported by Abugoch et al. (2008) for QPI. In addition, QPI was included higher protein content compared to the protein found in other cereals such as barley (11%) and rice (7.5%) (Ruiz et al., 2016). Therefore, quinoa can be considered as a rich source of protein. The amount of quinoa seed oil was obtained about 22% higher than that of reported by Elsohaimy et al. (2015). The amount of both ash and carbohydrates of QPI was less than quinoa flour due to the partial removal of the crust and endosperm as well as the heavy component of flour in the steps of extraction because the crust and endosperm contain a significant amount of the carbohydrate compounds and also there is a major part of ash in the crust. According to other studies, starch constitutes the main part of the quinoa carbohydrate (Abugoch, 2009).

3.2 | Characterization of the bio-nanocomposite film

As shown in Table 3, WVP and TS were fitted by quadratic models, and other characteristics were fitted by special quartic models with a high correlation coefficient. According to these models, it was observed that the predicted fitting models for all of the bio-nanocomposites properties have a coefficient of determination above 80%. Therefore, these models were able to predict the changes in the parameters very well.

The film properties including water solubility, WVP, opacity, TS, and EB are presented in Table 4. The content of this Table is discussed in more detail through the following sections.

3.2.1 | Water solubility

Water solubility results of films showed (Table 4 and Figure 1) that the CNC and starch incorporations had a significant effect on the films solubility. All the films kept their integrity after 24 hr of immersion in water. As shown in Figure 1, the solubility of bio-nanocomposite films reduced by increasing and decreasing the amounts of CNC and starch, respectively. These results may be due to the possible hydrogen bonds between hydroxyl groups of CNC and carboxylic and amine groups of QPIS, so that by increasing the amount of CNC, these free groups with potential to bind to water molecules reduced and water solubility decreased. It could also be due to the low diameter of CNCs that were well distributed in the protein structure and occupied the free spaces of the protein network (Kristo & Biliaderis, 2007). Increased with increasing the starch contents. In

TABLE 2 The chemical compounds of the quinoa full flour and quinoa protein isolate

Quinoa (%)	Moisture (%)	Ash (%)	Crude protein (%)	Crud fat (%)	Carbohydrates (%)
Full flour	7.16 ± 0.288	2.5 ± 0.2	12.615 ± 0.466	9.16 ± 1.647	77.78 ± 2.662
Protein isolate	6.16 ± 0.288	1.66 ± 0.577	83.07 ± 1.442	1.165 ± 0.233	13.93 ± 2.62

TABLE 3 Regression coefficients and correlation of the fitted model for various properties of the bio-nanocomposite films

Factor	Water solubility	WVP	Opacity (AU/mm)	TS (MPa)	EB (%)
x_1	−3*	4.96*	3*	14.1*	−288*
x_2	−140*	45.59*	−84*	−185.6*	−444*
x_3	−8081*	−92.92*	2,330*	622.7*	−152189*
x_1x_2	582ns	−64.91*	147*	291*	1989ns
x_1x_3	8553ns	97.90*	−2582*	−694*	172,124*
$x_1^2x_2x_3$	−1894ns	—	−1869*	—	−5117ns
$x_2^2x_1x_3$	6784ns	—	−6634*	—	459,092*
$x_3^2x_1x_2$	152422ns	—	−10365*	—	1,326,042*
R^2	95.24	91.24	98.89	89.19	87.67

Note: The components include protein percentage (x_1), starch percentage (x_2), and CNC percentage (x_3).

Abbreviations: EB, elonation at break; TS, tensile strength; WVP, water vapor permeability.

*Indicates significant ($p < .05$), and ns indicates non-significant.

TABLE 4 Various properties of the bio-nanocomposite films

Bio-nanocomposites	Water solubility (%)	WVP (g.mm/m^2/d/kpa)	Opacity (AU/mm)	TS (MPa)	EB (%)
1	31.28	2.251	2.53	23.05	35.91
2	32.28	2.383	2.88	20.07	50.45
3	39.48	3.272	3.23	16.67	62.44
4	32.97	2.295	2.37	21.90	41.74
5	28.03	2.235	2.41	21.97	42.23
6	32.1	1.861	2.18	22.29	42.24
7	25.88	3.02	2.97	19.73	53.84
8	20.35	2.312	2.47	21	40.31
9	29.02	2.444	2.91	21.03	51.26

Abbreviations: EB, elonation at break; TS, tensile strength; WVP, water vapor permeability.

contrary, starch due to high water-absorbent feature and hydrophilic nature increased the water solubility of bio-nanocomposite films (Cao, Chen, Chang, Muir, et al., 2008a).

3.2.2 | WVP

As shown in Figure 2 and Table 4, increasing the amount of CNC in the bio-nanocomposite film decreased WVP and on the other hand, increasing the protein and starch contents increased WVP. Moreover, the impact of increasing starch content was more than that of increasing protein content on the WVP of films. This may be due to more hydrophilicity of starch than protein, which increased free hydrophilic groups to bind to water vapor molecules. The improvement of the barrier properties of bio-nanocomposite using CNC nano-fillers was mainly caused by indirect penetration of the water vapor pathway in the material network due to dispersed impenetrable crystalline particles. These particles increased the effective diffusion and path length by moving the penetrating molecules around the particles in random paths. Thus, the water vapor molecules are disseminated and created a maze in their pathway and hence reduced the WVP of the bio-nanocomposite films. CNC can be well distributed in the protein network at lower levels, so it blocks the path of water vapor transmission. In contrary at the high amount of nano fillers, because of their aggregation and creating the pores in film network WVP decreases (Chang et al., 2010; Pereda et al., 2011). In this way, Abdollahi et al. (2013) reported that the WVP of the alginate-based films significantly decreased by adding 5% of cellulose nano-crystal and nano-clay.

3.2.3 | Opacity

As shown in Table 4, the incorporating nanoparticles into biocomposites affects the transparency of the films. This may be due to the occupying the empty spaces of the protein network by nanoparticles, which is caused by heating and protein denaturation. This phenomenon blocked the light path through the film network and reduced the transparency of the bio-nanocomposite films. In addition, the samples containing high protein content absorbed some of the incident beams due to the presence of tyrosine and tryptophan in the protein structure. These amino acids have optical activity and

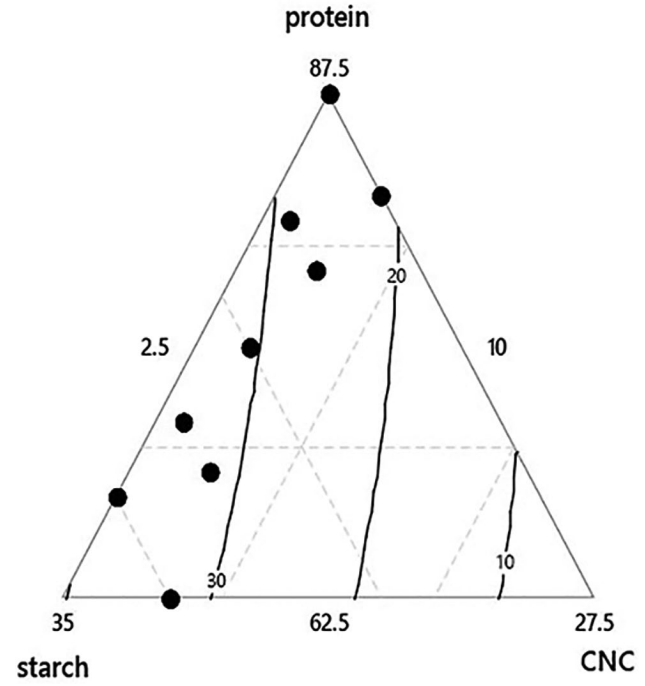


FIGURE 1 Contour line presentation of the effect of various components on the water solubility of bio-nanocomposite films

absorption capacity due to the presence of a benzene ring in their structure (Li et al., 2011). Figure 3 indicates that by increasing the protein and starch contents and reducing the CNC amount the transparency of the bio-nanocomposite films. The most transparent film was obtained from the treatment containing the highest contents of starch and protein. This may be due to the homogeneous structure of the two polymers, which provided sufficient pathways for light transmission. Also, some information about the dispersion degree of particles in the polymer network can be found by transparency analysis. So, the particles larger than the visible wavelengths blocked the light paths and increase the film's opacity (Kampeerapappun et al., 2007).

3.2.4 | Mechanical properties

The mechanical properties of bio-nanocomposite films, including TS and EB, are also present in Table 4. The TS of bio-nanocomposite films was obtained between 16.67 MPa (for formulations 3) and 23.05 (for formulations 1). These results showed that the mechanical properties of films differed according to their formulation. As shown in Figure 4a, the starch addition had a decreasing effect on TS value. However, the incorporation of CNC increased the TS value of bio-nanocomposite films especially at the lowest amount of starch. The increase of TS value can be attributed to the strength and rigidity nature of the CNC chains due to the high intermolecular hydrogen bonding, the uniform distribution of nano-fillers in the polymeric network, and the high compatibility between nanoparticles and polymeric networks because of the high ratio of the nanoparticles.

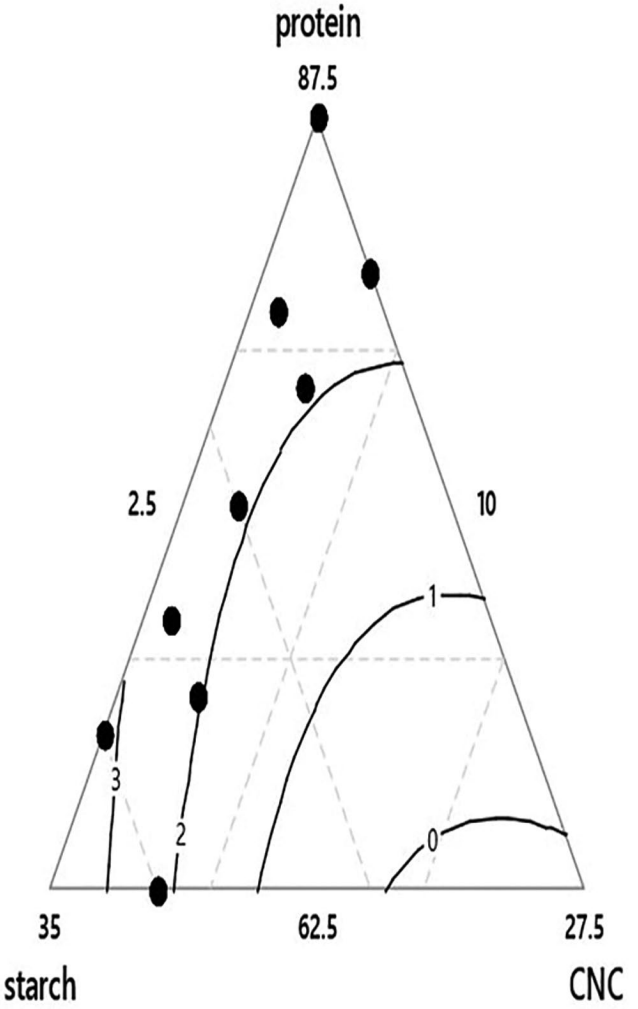


FIGURE 2 Contour line presentation of the effect of various components on WVP of bio-nanocomposite films

Cho and Park (Cho & Park, 2011) investigated the tensile properties of nano-cellulose / polyvinyl alcohol nanocomposite films. They reported that by adding 5% of nano-cellulose, the TS value of nanocomposites increased about 28% and then showed a decreasing trend.

Figure 4b shows the effect of incorporating starch, protein, and CNC on the EB value of the bio-nanocomposite films. As can be seen, increasing the amount of starch in the film caused an increase in the EB value of the bio-nanocomposite. At the lowest amount of starch, increasing the amount of CNC and decreasing the amount of protein resulted in a decrease in EB value. Hence, the Bio-nanocomposite films became more fragile when the amount of CNC increased. Reducing the EB value can be due to the rigid nature of the filler. In fact, the addition of CNC limited the mobility of protein chains in film network due to the strong interactions between the filler and the biopolymer network (Cao, Chen, Chang, Stumborg, et al., 2008b). In this way, Qazanfarzadeh and Kadivar (2016) reported the whey protein isolate / CNC bio-nanocomposite films showed at the highest TS and the lowest EB values at the 5% (w/w) concentration of CNC.

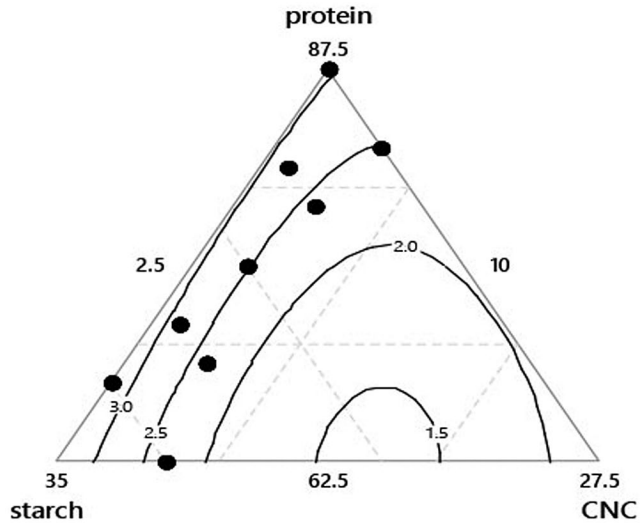


FIGURE 3 Contour line presentation of the effect of various components on transparency of bio-nanocomposite films

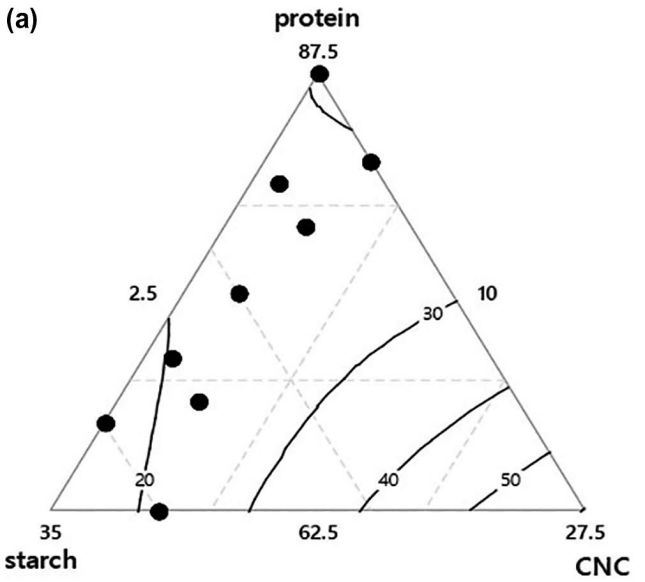
3.2.5 | Optimization of composite formulation

After the evaluations of bio-nanocomposite film's properties, regarding to the desirable characteristics of the final film, including lowest water solubility and WVP as well as highest transparency and TS, an optimal formulation was proposed by determining the acceptable range for each ingredient using the software. The optimal formulation was obtained as 78.55% protein, 18.28% starch, and 3.17% CNC with desirability value of 94%. The FTIR and *SEM* analysis were then performed for optimal bio-nanocomposite film.

3.2.6 | FTIR analysis

Figure 5 Shows the FTIR spectra of CNC particles, the control film, and the optimal bio-nanocomposite films. The peak observed at the range of 3000–3500 cm^{-1} in all spectra was related to the C-H and O-H groups. Another peak at the range of 1500–1600 cm^{-1} was due to C = C bonds in the aromatic rings of bio-nanocomposite film. Also, the peaks in control and the optimal films at 1739 and 1748 cm^{-1} wavelengths corresponded to the C-H and C-O groups.

There were several peaks at the wavelengths range of 800 to 1,800 cm^{-1}. These peaks are used for protein analysis because the groups that form the amide bonds (C-N and C-O) are located at this range. By incorporating CNC into the protein film, some new peaks were observed at the wavelengths of 1,040, 1,110, and 11,640 cm^{-1}, which indicated a good combination of the ingredients. The peak at 1,040 cm^{-1} can be related to the C-O stretching bound in the cellulose, while the peak at 1,110 cm^{-1} was related to the structural vibrations, including C-O stretching bound in the β (1 → 4) glycoside bond of β-D-Glycopyranosyle units in cellulose structure. The peak at the range of 1,590 to 1,690 cm^{-1} corresponded to amide-1 vibrations that are common in proteins and as well as to water molecules absorbed in CNC. However, the presence of this peak in the CNC



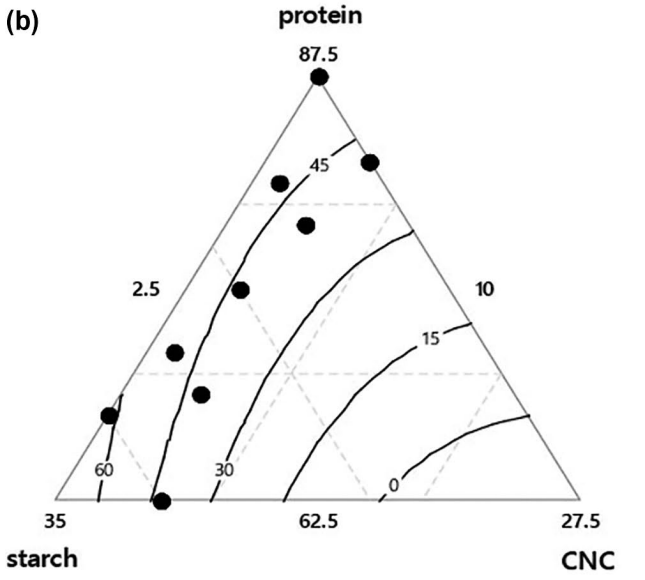


FIGURE 4 Contour line presentation of the effect of various components on (a) TS and (b) EB of bio-nanocomposite films

spectrum confirmed its crystallinity. The spectrum of the optimal bio-nanocomposite film indicated a good combination of the film ingredients. These peaks were also reported by Pereda et al. (2011).

3.2.7 | *SEM* analysis

The *SEM* images of both surface and the cross-section of the control and the optimal bio-nanocomposite film are shown in Figure 6. The surface microstructure of both films was smooth without any

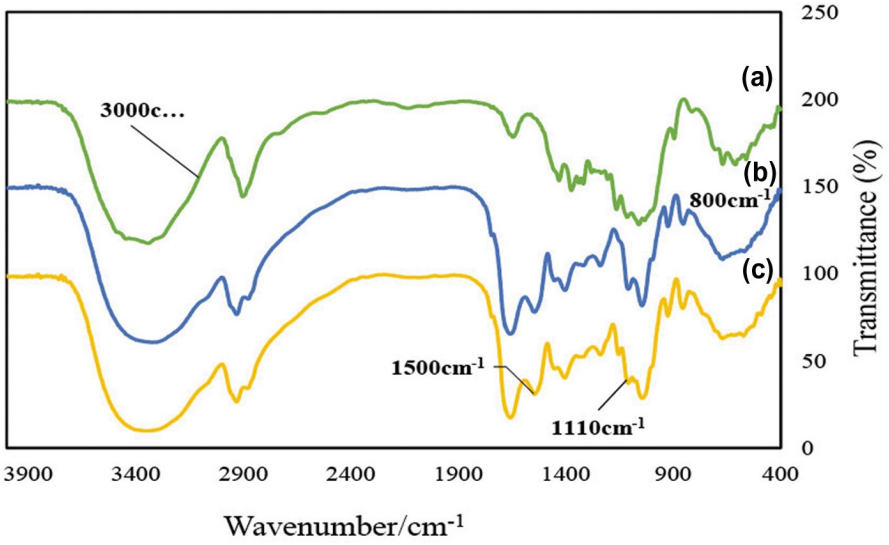


FIGURE 5 The FTIR spectra of (a) CNC particles, (b) the control film, and (c) the optimal bio-nanocomposite films

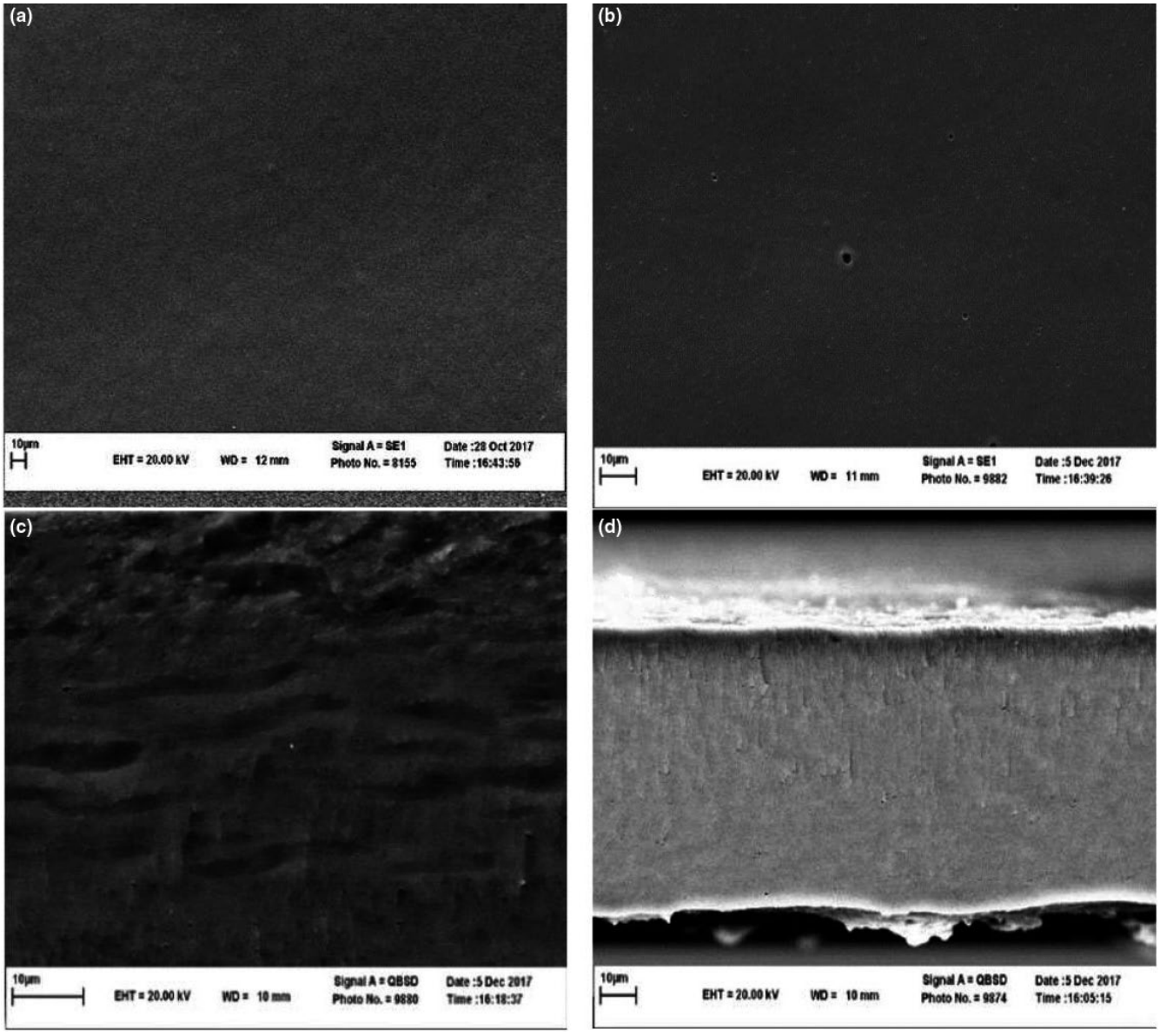


FIGURE 6 The *SEM* images of bio-nanocomposite film: the surface images of (a) the control film and (b) optimal film, and the cross-section images of (c) the control film and (d) optimal film

cracks and voids. A heterogeneous cross-sectional image was observed for the control film (Figure 6c) probably due to the presence of non-protein compounds during QPI extraction (Kowalczyk & Baraniak, 2011). However, the bio-nanocomposite film showed a smooth, homogenous and dense cross-section image probably due to good interactions between CNCs, starch and protein in optimal film network.

It is worthy to note that regarding the results of the FTIR spectrum (Figure 5), starch and CNC exhibited a broad peak of OH groups compared to protein. Therefore, they can interact together by hydrogen bonds more that protein. As a result, the formation of strong bonds between hydrophilic compounds during film drying may cause a compact and ordered film's matrix.

4 | CONCLUSION

Protein extraction from quinoa seeds has been successfully performed using the alkaline method with protein purity of about 83% and used to prepare bio-nanocomposite film. The addition of starch and CNC to the protein film significantly changed its properties. Some changes had a positive effects on bio-nanocomposite film properties. The CNC was incorporated to QPI-based to improve its mechanical properties, however, the barrier and physical properties of the films were determined. The mechanical properties of the QPI-based films were improved by adding CNC and reducing starch contents, and the highest TS value were obtained for the film prepared based on formulations 1 (5% CNC and 20% starch). However, CNC negatively affected the film's elongation because of its stiff nature. The improvement of mechanical properties can be due to the uniform distribution of nanoparticles in the polymer network. The WVP and water solubility of films were reduced by increasing the CNC content. The optimal formulation was obtained as 78.55% protein, 18.28% starch, and 3.17% of CNC with a desirability of 94%. The FTIR spectrum and *SEM* analysis of the optimal bio-nano composite film indicated a great compatibility of the film ingredients and confirmed the obtained results. In conclusion, this study suggested a potential for the QPI/starch/CNC bio-nanocomposite film in the preparation of edible film and coatings for food packaging to overcome the drawbacks of protein-based materials.

ACKNOWLEDGMENTS

The authors appreciatively acknowledge the financial support of the Research Affairs Division, Ferdowsi University of Mashhad (FUM), Mashhad. Furthermore, financial support from Iran Nanotechnology Initiative Council (INIC) is also gratefully acknowledged.

AUTHOR CONTRIBUTION

Mahdieh Safar razavi zade: Methodology; Writing-original draft. **Mohammad Hossein Aghkhani:** Writing-review & editing. **Mohammad Abbaspour-Fard:** Writing-review & editing. **fereshte hosseini:** Writing-review & editing. **Zeinab Qazanfarzadeh:** Methodology.

DATA AVAILABILITY STATEMENT

The data set(s) supporting the conclusions of this article is(are) included within the article.

ORCID

Mahdieh Safar Razavi Zade https://orcid.org/0000-0002-5891-3510

REFERENCES

Abdollahi, M., Alboofetileh, M., Rezaei, M., & Behrooz, R. (2013). Comparing physico-mechanical and thermal properties of alginate nanocomposite films reinforced with organic and / or inorganic nano fillers. *Food Hydrocolloids*, *32*, 416–424. https://doi.org/10.1016/j.foodhyd.2013.02.006

Abdollahia, M., Alboofetileha, M., Behroozb, R., Rezaeia, M., & Miraki, R. (2013). Reducing water sensitivity of alginate bio-nanocomposite film using cellulose nanoparticles. *International Journal of Biological Macromolecules*, *54*, 166–173. https://doi.org/10.1016/j.ijbiomac.2012.12.016

Abugoch, L. E. (2009). Quinoa (Chenopodium Quinoa Willd.): composition, chemistry, nutritional, and functional properties. *Advances in Food and Nutrition Research*, *58*, 1–31.

Abugoch, L. E., Romero, N., Tapia, C. A., Silva, J., & Rivera, M. (2008). Study of some physicochemical and functional properties of quinoa (Chenopodium Quinoa Willd) protein isolates. *Journal of Agricultural and Food Chemistry*, *56*, 4745–4750. https://doi.org/10.1021/jf703689u

AOAC (2000). *Official methods of analysis*. Association of Official Analytical Chemists.

ASTM (2002). Standard test methods for tensile properties of thin plastic sheeting, D882–91. In *Annual book of ASTM* (pp. 1–9). Philadelphia, PA: American Society for Testing and Materials.

ASTM (2001). Standard methods of test for water vapor transmission of materials in sheeting, E 96–00. In *Annual book of ASTM* (pp. 1048–1053). Philadelphia, PA: American Society for Testing and Materials.

Azevedo, V. M., Dias, M. V., Borges, S. V., Costa, A. L. R., Silva, E. K., Medeiros, E. A. A., & Soares, N. F. F. (2015). Development of whey protein isolate bio-nanocomposites: Effect of montmorillonite and citric acid on structural, thermal, morphologicaland mechanical properties. *Food Hydrocolloids*, *48*, 179–188. https://doi.org/10.1016/j.foodhyd.2015.02.014

Bamdad, F., Goli, A. H., & Kadivar, M. (2006). Preparation and characterization of proteinous film from lentil (Lens Culinaris) edible film from lentil (Lens Culinaris). *Food Research International*, *39*, 106–111. https://doi.org/10.1016/j.foodres.2005.06.006

Cao, X., Chen, Y., Chang, P. R., Muir, A. D., & Falk, G. (2008a). Starch-based nanocomposites reinforced with flax cellulose nanocrystals. *Express Polymer Letters*, *2*, 502–510. https://doi.org/10.3144/expresspolymlett.2008.60

Cao, X., Chen, Y., Chang, P. R., Stumborg, M., & Huneault, M. A. (2008b). Green composites reinforced with hemp nanocrystals in plasticized starch. *Journal of Applied Polymer Science*, *109*, 3804–3810. https://doi.org/10.1002/app.28418

Chang, P. R., Jian, R., Zheng, P., Yu, J., & Ma, X. (2010). Preparation and properties of Glycerol Plasticized-Starch (GPS)/ Cellulose Nanoparticle (CN) composites. *Carbohydrate Polymers*, *79*, 301–305. https://doi.org/10.1016/j.carbpol.2009.08.007

Chen, D., Lawton, D., Thompson, M. R., & Liu, Q. (2012). Biocomposites reinforced with cellulose nanocrystals derived from potato peel waste. *Carbohydrate Polymers*, *90*, 709–716. https://doi.org/10.1016/j.carbpol.2012.06.002

Chen, R., Zhang, Z., Feng, C., Hu, K., Li, M., Li, Y., Shimizu, K., Chen, N., & Sugiura, N. (2010). Application of simplex-centroid mixture design

in developing and optimizing ceramic adsorbent for as (V) removal from water solution. *Microporous and Mesoporous Materials*, *131*, 115–121. https://doi.org/10.1016/j.micromeso.2009.12.010

Cho, M. J., & Park, B. B. (2011). Tensile and thermal properties of nanocellulose-reinforced poly (Vinyl Alcohol) nanocomposites. *Journal of Industrial and Engineering Chemistry*, *17*, 36–40. https://doi.org/10.1016/j.jiec.2010.10.006

Elsohaimy, S. A., Refaay, T. M., & Zaytoun, M. A. M. (2015). Physicochemical and functional properties of quinoa protein isolate. *Annals of Agricultural Sciences*, *60*, 297–305. https://doi.org/10.1016/j.aoas.2015.10.007

Fortunati, E., Gigli, M., Luzi, F., Dominici, F., Lotti, N., Gazzano, M., Cano, A., Chiralt, A., Munari, A., Kenny, J. M., Armentano, I., & Torre, L. (2017). Processing and characterization of nanocomposite based on poly (Butylene / Triethylene Succinate) copolymers and cellulose nanocrystals. *Carbohydrate Polymers*, *165*, 51–60. https://doi.org/10.1016/j.carbpol.2017.02.024

Gennadios, A. (2002). *Protein-based films and coatings*, 1st ed. (Vol. 24; p. 773). CRC Press.

Guilbert, S., & Cuq, J.-L. (1992). Edible wheat gluten films: Influence of the main processvariables on film properties using response surface methodology. *Journal of Food Science*, *57*, 190–196. https://doi.org/10.1111/j.1365-2621.1992.tb05453.x

Han, W. (2001). Physical and mechanical properties of pea-protein-based edible films. *Food Engineering and Physical Properties*, *66*, 319–322.

Jahed, E., Alizadeh Khaledabad, M., Almasi, H., & Hasanzadeh, R. (2017). Physicochemical properties of carum copticum essential oil loaded chitosan films containing organic nanoreinforcements. *Carbohydrate Polymers*, *164*, 325–338. https://doi.org/10.1016/j.carbpol.2017.02.022

Kalia, S. (2016). *Biodegradable green composites* (pp. 80–117). John Wiley & Sons.

Kampeerapappun, P., Aht-ong, D., Pentrakoon, D., & Srikulkit, K. (2007). Preparation of cassava starch/montmorillonite composite film. *Carbohydrate Polymers*, *67*, 155–163. https://doi.org/10.1016/j.carbpol.2006.05.012

Kowalczyk, D., & Baraniak, B. (2011). Effects of plasticizers, pH and heating of film-forming solution on the properties of pea protein isolate films. *Journal of Food Engineering*, *105*, 295–305. https://doi.org/10.1016/j.jfoodeng.2011.02.037

Kristo, E., & Biliaderis, C. G. (2007). Physical properties of starch nanocrystal-reinforced pullulan films. *Carbohydrate Polymers*, *68*, 146–158. https://doi.org/10.1016/j.carbpol.2006.07.021

Laneuville, S. I., Paquin, P., & Turgeon, S. L. (2005). Formula optimization of a low-fat food system containing whey protein isolate-xanthan gum complexes as fat replacer. *Journal of Food Science*, *70*, 513–519. https://doi.org/10.1111/j.1365-2621.2005.tb11527.x

Li, Y., Jiang, Y., Liu, F., Ren, F., Zhao, G., & Leng, X. (2011). Food hydrocolloids fabrication and characterization of TiO_2/whey protein isolate nanocomposite Fi Lm. *Food Hydrocolloids*, *25*, 1098–1104. https://doi.org/10.1016/j.foodhyd.2010.10.006

Lu, P., & Hsieh, Y. L. (2010). Preparation and properties of cellulose nanocrystals: Rods, spheres, and network. *Carbohydrate Polymers*, *82*, 329–336. https://doi.org/10.1016/j.carbpol.2010.04.073

Lu, P., & Hsieh, Y. L. (2012). Preparation and characterization of cellulose nanocrystals from rice straw. *Carbohydrate Polymers*, *87*, 564–573. https://doi.org/10.1016/j.carbpol.2011.08.022

Mao, L., Imam, S., Gordon, S., Cinelli, P., & Chiellini, E. (2002). Extruded cornstarch – glycerol – polyvinyl alcohol blends : mechanical properties, morphology, and biodegradability. *Journal of Polymers and the Environment*, *8*, 1–7.

Martínez, E. A., Francisco, F. F., & Bazile, D. (2015). History of Quinoa : Its Origin, Domestication, Diversification, and Cultivation with Particular Reference to the Chilean.Quinoa: Improvement and sustainable production, pp.19-24.

Mondragon, G., Peña, C., González, A., Eceiza, A., & Arbelaiz, A. (2014). Bio-nanocomposites based on gelatin matrix and nanocellulose. *European Polymer Journal*, *62*, 1–9. https://doi.org/10.1016/j.europolymj.2014.11.003

Oymaci, P., & Altinkaya, S. A. (2016). Improvement of barrier and mechanical propertiesof whey protein isolate based food packaging films by incorporation of zeinnanoparticles as a novel bionanocomposite. *Food Hydrocolloids*, *54*, 1–9. https://doi.org/10.1016/j.foodhyd.2015.08.030

Paralikar, S. A., Simonsen, J., & Lombardi, J. (2008). Poly(vinyl Alcohol)/cellulose nanocrystal barrier membranes. *Journal of Membrane Science*, *320*, 248–258. https://doi.org/10.1016/j.memsci.2008.04.009

Pereda, M., Amica, G., Rácz, I., & Marcovich, N. E. (2011). Structure and properties of nanocomposite films based on sodium caseinate and nanocellulose fibers. *Journal of Food Engineering*, *103*, 76–83. https://doi.org/10.1016/j.jfoodeng.2010.10.001

Qazanfarzadeh, Z., & Kadivar, M. (2016). Properties of whey protein isolate nanocomposite films reinforced with nanocellulose isolated from oat husk. *International Journal of Biological Macromolecules*, *91*, 1134–1140. https://doi.org/10.1016/j.ijbiomac.2016.06.077

Qazanfarzadeh, Z., Kadivar, M., Shekarchizadeh, H., & Porta, R. (2020). Rye secalin characteriation and use to improve zein-based film performance. *International Journal of Food Science & Technology*, *56*, 742–752.

Rhim, J. W., & Ng, P. K. W. (2007). Natural biopolymer-based nanocomposite films for packaging applications. *Critical Reviews in Food Science and Nutrition*, *47*, 411–433. https://doi.org/10.1080/10408390600846366

Ruiz, G. A., Xiao, W., Boekel, M. V., Minor, M., & Stieger, M. (2016). Effect of extraction pH on heat-induced aggregation, gelation and microstructure of protein isolate from quinoa (Chenopodium Quinoa Willd). *Food Chemistry*, *209*, 203–210. https://doi.org/10.1016/j.foodchem.2016.04.052

Ryland, D., Vaisey-Genser, M., Arntfield, S. D., & Malcolmson, L. J. (2010). Development of a nutritious acceptable snack bar using micronized flaked lentils. *Food Research International*, *43*, 642–649. https://doi.org/10.1016/j.foodres.2009.07.032

Savadekar, N. R., & Mhaske, S. T. (2012). Synthesis of nano cellulose fibers and effect on thermoplastics starch based films. *Carbohydrate Polymers*, *89*, 146–151. https://doi.org/10.1016/j.carbpol.2012.02.063

Shruthy, R., Jancy, S., & Preetha, R. (2020). Cellulose nanoparticles synthesised from potato peel for the development of active packaging film for enhancement of shelf life of raw prawns (penaeus monodon) during frozen storage. *International Journal of Food Science & Technology*, *56*, 3991–3999.

Shujun, W., Jiugao, Y., & Jinglin, Y. (2006). Preparation and characterization of compatible and degradable thermoplastic starch / polyethylene film. *Journal of Polymers and the Environment*, *14*, 1–6. https://doi.org/10.1007/s10924-005-8708-9

Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids*, *24*, 770–775. https://doi.org/10.1016/j.foodhyd.2010.04.003

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. https://doi.org/10.1016/j.jfoodeng.2008.09.026

Souza, V. G. L., & Fernando, A. L. (2016). Nanoparticles in food packaging: Biodegradability and Potential migration to food—A review. *Food Packaging and Shelf Life*, *8*, 63–70. https://doi.org/10.1016/j.fpsl.2016.04.001

Souza, V. G. L., Pires, J. R. A., Rodrigues, P. F., Lopes, A. A. S., Fernandes, F. M. B., Duarte, M. P., Coelhoso, I. M., & Fernando, A. L. (2018).

Bionanocomposites of chitosan/montmorillonite incorporated with rosmarinus officinalis essential oil: Development and physical characterization. *Food Packaging and Shelf Life*, 148–156. https://doi.org/10.1016/j.fpsl.2018.03.009

Sukhija, S., Singh, S., & Riar, C. S. (2016). Analyzing the effect of whey protein concentrateand psyllium husk on various characteristics of biodegradable film from lotus(Nelumbo Nucifera) rhizome starch. *Food Hydrocolloids*, *60*, 128–137. https://doi.org/10.1016/j.foodhyd.2016.03.023

Yang, B., & Vickers, Z. (2004). Optimization of cheddar cheese taste in model cheese systems. *Journal of Food Science*, *69*, 229–236. https://doi.org/10.1111/j.1365-2621.2004.tb11010.x

Zheng, T., Yu, X., & Pilla, S. (2016). Mechanical and moisture sensitivity of fully bio-based dialdehyde carboxymethyl cellulose cross-linked soy protein isolate films. *Carbohydrate Polymers*, *157*, 1333–1340. https://doi.org/10.1016/j.carbpol.2016.11.011

Zhou, J. Z., Liu, X. L., Huang, K. H., Dong, M. S., & Jiang, H. H. (2007). Application of the mixture design to design the formulation of pure cultures in Tibetan Kefir. *Agricultural Sciences in China*, *6*, 1383–1389. https://doi.org/10.1016/S1671-2927(07)60187-4

How to cite this article: Safar Razavi Zade, M., Hossein Aghkhani, M., Abbaspour-Fard, M. H., Hosseini, F., & Qazanfarzadeh, Z. (2021). Optimization of nanocomposite films based on quinoa protein isolate incorporated with cellulose nanocrystal and starch. *Journal of Food Processing and Preservation*, 45, e15926. https://doi.org/10.1111/jfpp.15926