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Evaluation of the physicochemical, and mechanical properties of the edible film prepared from soy protein isolate containing the essential oil of the *Ziziphorpa capitata*

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ABSTRACT

Today, the production of edible and biodegradable films is one of the growing technologies in advanced societies. The use of these coatings is due to the replacement with polymeric materials and increasing the storage time of food. The main purpose of this study was to produce an edible film of soybeans protein isolate (3 to 7%), glycerol (1 to 3%) and *Ziziphorpa capitata* essential oil (0 to 1%) in order to create better taste and antimicrobial properties. After preparing the film, the physicochemical and mechanical properties of the film (thickness, density, solubility in water and acid, water vapor permeability, transparency, color changes, elongation percentage and tensile strength) were evaluated. After performing the above tests and investigating the effects of independent variables on the dependent variables, the oral film formulation was optimized. The results showed that the optimal formula can be achieved by using 3.54% soy protein isolate, 1% glycerol and 1% cactus essential oil at the level of desirability of 0.624.

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1. Introduction

Edible packaging is considered a promising alternative to conventional packaging, as it is doubly environmentally friendly: when used as food packaging, it does not produce waste and when it is thrown away , decomposes quickly [1]. Food packaging can be called active or smart. that this packaging can interact with foods and provide technological functions such as the release of antioxidant and antimicrobial compounds to increase the shelf life of the product and remove gases and water vapor that can reduce the quality, nutritional value and preservation of food [2]. One of the possibilities of this technology is adding antimicrobial and antioxidant compounds, preventing the creation of unwanted flavors by microorganisms and even their sticking to the system [3].

Biodegradable coatings can be prepared from all kinds of natural materials such as lipids, polysaccharides and proteins. In addition to being able to increase the nutritional value of food, these coatings are easily biodegradable and environmentally friendly due to their natural nature, and they can be made from various renewable sources as well as agricultural and animal waste. Protein films have better barrier properties against fat and oxygen at low relative humidity, they are widely used to prepare edible films [4].

Basically, the production of edible film using only one type of polymer shows favorable characteristics in some areas, but it will be weak in some areas. One of the ways to improve the properties of the edible film is the combination of biopolymers and the production of composite biofilms [5].

Soy protein is a mixture of globular proteins. Almost 90% of soybean proteins are divided into four categories S2, S7, S11 and S15, which is based on molecular weight and sedimentation coefficient.[1] Is. The two major globular proteins include beta-conglulinin (globulin S7) and glycinin (globulin S11), which are 37 and 31% of soybean proteins, respectively [6].

Packaging coatings of natural origin alone are not able to control the mentioned changes in long-term storage. Therefore, a number of antimicrobial and antioxidant additives are used in the food industry, but most of these chemical additives have toxic and carcinogenic effects. Therefore, nowadays natural additives have been tried to replace chemical additives [7].

Kakuti *They are called clinopodioides*, is a fragrant plant that grows in the Middle East, traditionally used to preserve various types of food during storage. Especially yogurt, minced meat, butter and pickles. This substance has broad antimicrobial and antioxidant activities without having a negative effect on the organoleptic properties of rainbow trout fillets, ground beef and fresh sausages [8].

In recent years, researchers have made new researches on food coatings, such as food packages made of gelatin protein containing green tea extract, fresh sausage coating, films containing green tea extract delayed the lipid oxidation of fresh sausage coated with this film. 9 [.

Other researchers, during a research on modified sweet potato starch and cumin essential oil on fresh pears, showed that the use of edible coating showed changes in color, firmness and destruction of chlorophyll, and reduction of decaying lesions caused by Alternaria bacteria, in addition, it caused a delay In breathing, fruit weight loss and sensory quality improvement [10].

2- Materials and methods

Soybean was procured from Tek Soya Neyshabur Company, then it was ground by an industrial mill and sieved by a 50 mesh sieve to obtain a uniform flour powder for better protein extraction. For degreasing, n-hexane solvent at a ratio of 5 to 1 flour was needed. The mixture was mixed for one hour at laboratory temperature to wash the fat in the flour by hexane. After this step, the mixture was centrifuged and the sediments were used for the next step. At the rate of 1 to 10, the flour obtained from the previous step was mixed with distilled water and circulated at the same time, and we brought the pH of the mixture to 5.10 by adding a normal soda. Then we centrifuged for 5 minutes and poured the liquid on the falcons into the beaker and brought the alkaline solution from the previous step to pH 4.8 (isoelectric point of soybean protein) with 1 normal hydrochloric acid. Then the milky solution was centrifuged at 7400 rpm for 10 minutes and the clear liquid on top of the falcons was discarded. The sediments obtained from the previous step were brought to neutral pH by distilled water and 0.1 normal soda, and in each step it was centrifuged at 7400 rpm for 5 minutes and the supernatant was discarded in both steps. In order to obtain a high-quality isolate, the samples were dried by a freeze dryer made by Martin Crist, Germany, and placed in a freezer at -18 degrees Celsius until use. Kjeldahl apparatus was used to determine protein purity, the purity of protein isolate was measured at 92.8%. Different percentages of protein (3 to 7%), glycerol (1 to 3%) and coconut oil (0 to 1%) were used to prepare the edible film. In order to completely dissolve the compounds, stir the mixture for 1 minute and then bring the pH of the solution to 10.5 with 0.1 normal soda and keep it for 30 minutes at a temperature of 85 degrees Celsius on a water bath that is on the stirrer. It was heated and mixed at the same time. To prepare the film containing essential oil, after the end of this

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step, the essential oil was immediately added to the hot solution and homogenized. The molds containing the film solution were placed at the laboratory temperature for 24 hours to form the films [11].

3-3- Tests

3-3-1- Film thickness measurement

The thickness of the films produced at five different points of each film was measured by a micrometer with an accuracy of 0.01 mm and averaged from them [12].

3-3-2- Film density measurement

To measure the density, the film pieces in dimensions of 1 x 4 cm were measured and cut with a caliper with an accuracy of 0.1 mm, and after measuring the average thickness, it was measured with a digital scale with an accuracy of 0.0001 grams. The density of the film can be measured by dividing the mass by the volume of the cut film piece [13]. $\rho = \frac{m}{IN}$

3-3-3- percentage of substances soluble in water and acid

In this test, two circular pieces with a diameter of 4 cm were separated from each sample and after weighing for 24 hours in 50 ml of distilled water, 1 normal hydrochloric acid was placed on the device. shaker It was placed at a low speed and after this time, the film was removed from the water and after drying in the oven at 105 degrees Celsius for 24 hours, it was weighed again and by calculating the difference in weight, the percentage of solubility of the film in water and acid was obtained [14]. [.

3-3-4- permeability to water vapor

The water vapor permeability was measured based on the ASTM E96 standard (ASTM 2002). In this way, the supersaturated solution of sodium chloride was poured into the desiccator and small glasses with a diameter of 1.35 cm were selected and 3 grams of anhydrous calcium chloride was poured into them, and the surface of the glasses was closed by film samples and clamps. became. Calcium chloride makes the relative humidity inside the glass closed by the film zero percent. After weighing all the samples, we put them in a desiccator containing supersaturated sodium chloride salt, which creates a humidity equal to 75% at the laboratory temperature [STM].
The weight changes of the samples are measured using a digital scale with an accuracy of 0.0001 grams and the graph of the weight changes is drawn according to time so that the slope of the resulting line is used for calculations.

The steam transfer rate was calculated through formula 1-3.

Formula 1 - WVT water vapor transfer rate $= \frac{G}{t.A}$

WVT: water vapor transfer rate ($g/m^2.s$)
A: The cross section of the glasses (m^2)
G/t: the slope of the average weight change line during the test period
Formula 2-3 was used to measure the permeability to water vapor.
Formula 2- Water vapor permeability = WVP $\frac{WVT}{P(R_1-R_2)}$. X
WVP: water vapor permeability (g/m.pa.s)
P: Vapor pressure of pure water at 25 degrees Celsius (Pa)
X: film thickness (m)
R_1 : The amount of humidity inside the desiccator (75 percent)
R_2 : The amount of humidity inside the glass (0 percent)

3-3-5- Transparency

A spectrophotometer was used to measure the transparency of the prepared films. The test method was such that pieces of the film in the dimensions of 0.9 × 4 cm was cut from the film and it was fixed inside the spectrophotometer cell and in one of the two transparent walls of the cell, and according to the sources for comparison, the other empty spectrophotometer cell was placed inside the spectrophotometer to compare between the most transparent material (the empty cell has no light absorption and its absorption is almost equal to zero) and the produced films are done [15].

3-3-6- color measurement

Hunter Lab device was used to measure the color. The inner diameter of the tube of the Hunter Lab device was measured by a caliper (4.52 mm) and the film samples were cut into circles of the same diameter. First, the device was calibrated with white tiles and then with black tiles The film samples were placed so that the bottom of the Hunter cell was completely covered by it. The test was performed in 6 repetitions and the results were expressed as an average. Indicators L* standard index of transparency, a* standard index of greenness and redness, b* The standard index of yellowness and blueness and factors L, a and b were related to the test samples [16].

3-3-7- Mechanical test

A texture device was used to perform the mechanical test. For this purpose, parts of the film in dimensions 9×2 cm of the film was prepared and placed in a desiccator containing saturated sodium chloride with a relative humidity of 75% for conditioning for 24

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hours. Then the film pieces were closed inside the jaws of the machine. The distance between the two jaws before the test was 4 cm. The upper jaw began to move away at a rate of 40 mm/min. The moment the film was torn, the test ended. The tensile strength was calculated through formula 4-3 [17].

Formula 3- Tensile strength

$$TS = \frac{Maximum\ load}{Original.minimum.section.area} = \frac{F}{A}$$

TS : Tensile strength in megapascals (MPa)

F: the maximum force required to tear the film (N)

A: The area of the film involved in the jaw of the device (m^2)

The percentage of elongation until rupture was also calculated from formula 5-3.

Formula 4- percentage of elongation until rupture

$$\%AND = \frac{Extension\ at\ moment\ of\ rupture}{Intial\ gage\ length} = \frac{\Delta L}{L}$$

L: amount of elongation until the moment of rupture

L: The value of the initial length of the film (the distance between the two jaws of the device)

3-4- optimization and statistical analysis

3-4-3- The process of optimization by RSM method

Design Expert version 7.0.0 software was used to perform statistical design and analysis of test results. In this research, a central composite design with three independent variables at three levels and six repetitions (around the central point) was used to optimize the above-mentioned increase. After entering the results of the answers into the software, data fitting was done with the help of quadratic dependent model and the results of analysis of variance and simultaneous effects diagrams of the two relevant variables were drawn in three dimensions. After specifying the goals The optimization process was done using the numerical optimization technique [18].

3- Results and Discussion

4-1-1- Thickness test

As seen in Figure 1, increasing the protein concentration led to an increase in the thickness of the film samples. On the other hand, the effect of increasing the concentration of glycerol and kakuti essential oil to medium concentrations (in the used range) similarly caused an increase and by increasing from a critical limit, it led to a decrease in the thickness of the film samples ($P<0.05$).

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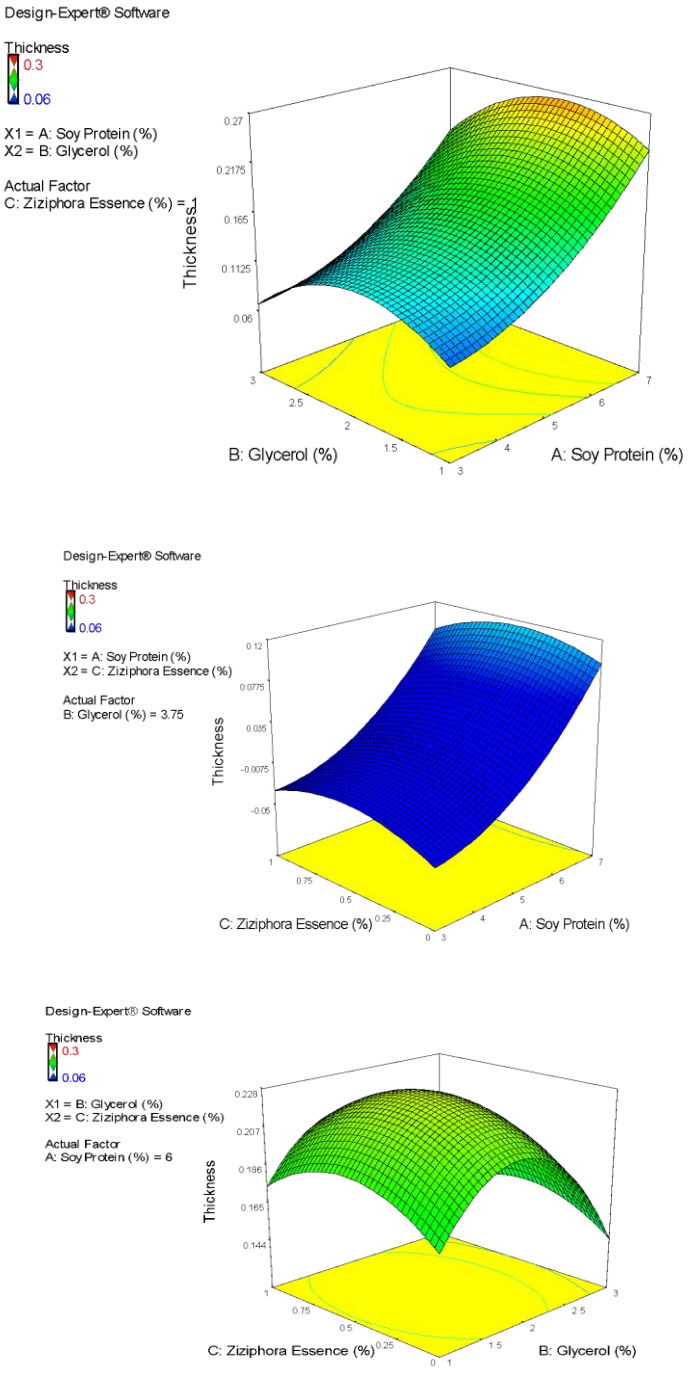

Figure 1- Showing a three-dimensional diagram of the simultaneous effect of independent variables on the density of films

Kokoszaka et al. (2009) investigated the effect of soy protein isolate percentage on the thickness of production film and concluded that by increasing the protein isolate percentage from 6% to 9%, the film thickness increased from 0.0526 mm to 0.836 mm. which was similar to the thicknesses measured in our research and the results were similar to our results. It was announced that increasing the percentage of soy protein isolate at a fixed concentration of glycerol had a significant effect ($P<0.05$) on increasing the thickness of the production film. Also, at a constant concentration of protein, with an increase in the percentage of glycerol, they observed a slight decrease in the thickness of the films, but its effect on the thickness was not significant ($P<0.05$), and the

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results of this research were similar to the results of the current research [19].
Also, our results were similar to the results of He et al. (2005) who discussed the increase of ferulic acid on the properties of the film prepared from soy protein isolate. In this research, the thickness of the produced films was between 65 and 80 micrometers [20].
Gunga et al. (2007) during a study on edible films prepared from whey protein isolates reached the conclusion that increasing the percentage of protein isolate has a significant effect ($P<0.05$) on the thickness of the prepared film. But the thickness of the produced films was not similar to our results, which was due to the difference in the edible film production process and the type of molds used [21].
Cao et al. (2007) obtained similar results by investigating the effect of adding ferulic acid and tannic acid on edible gelatin films and stated that the thickness of the edible film increases with the increase of ferulic acid and tannic acid [22].

4-2- L color index test

Table 1- Analysis of variance of data obtained from color test (L, a, b)

Source	df	P-value L	P-value a	P-value b
Model	9	<0.0001	<0.0001	<0.0001
A- I am Protein (%)	1	<0.0001	0.3842	0.0020
B- Glycerol (%)	1	<0.0001	0.0188	0.0019
C- Ziziphoria Essence (%)	1	<0.0001	0.0418	0.3419
AB	1	<0.0001	0.0002	<0.0001
AC	1	0.0303	0.1222	0.0462
BC	1	0.3174	1.0000	0.0054
A^2	1	0.0021	0.9800	<0.0001
B^2	1	0.0002	0.0038	0.0003
C^2	1	<0.0001	0.0171	0.3035

Increasing the concentration of protein and glycerol led to a decrease in the L color index in the film samples (Table 1). Kakuti essential oil up to medium concentrations (in the range used) has caused an increase, and with an increase from a critical limit, it has led to a decrease in the L color index in the film samples. But in general, it can be seen that the effect of kakuti essential oil led to a decrease in this index.
With the increase in temperature during the film production process, in protein films containing lysine, glycerol reacts with the amino acid lysine and darkens its color. Probably because acidic conditions and high pH cause the destruction of the protein structure and the formation of free amino acids, and as a result, it creates the conditions for the browning reaction between amino acids and the few carbohydrates that remain in the isolate during the production of protein isolate. The color becomes darker with the increase of protein.
Sivaruban et al. (2008) investigated edible films prepared from soy protein isolate and stated that the values of L, a and b for their samples were 95.4, -0.2 and 4.7 respectively; Compared to the edible films prepared from soy protein isolate, it is much whiter than our results, but the yellowness and redness of the films are almost the same [23].
One of the reactions that occurs during film production is the release of pigments caused by the denaturation of proteins and the browning of Maillard. Denaturation of proteins led to the release of most of the remaining pigments in the protein isolate. During this reaction, a large amount of amino acids such as lysine, which is the dominant amino acid of legumes and soybeans, reacts with the carbohydrate part left in the protein isolate as a result of the reaction and produces a brown color.

4-3- Color index test a

As can be seen in Table 1, increasing the protein concentration led to an increase in the color index a in the film samples. On the other hand, the effect of increasing the concentration of glycerol led to the decrease of this index ($P<0.05$). Kakuti essential oil up to medium concentrations (in the range used) has caused an increase, and with an increase from a critical limit, it has led to a decrease in the color index a in the film samples.

4-4- color index test b

The linear and quadratic effect of soy protein and glycerol concentration on the b index of the samples has become significant ($P<0.01$); However, the linear and quadratic effect of the concentration of kakuti essential oil on this index was not significant (Table 1). Increasing the protein concentration led to a decrease in the b color index in the film samples. On the other hand, the effect of increasing the concentration of glycerol and kakuti essential oil led to the increase of this index.

4-5- Density test

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The increase in protein concentration led to an increase in the density of the film samples. On the other hand, the effect of increasing the concentration of glycerol led to the decrease of this index; Kakuti essential oil up to medium concentrations (in the used range) has caused an increase and by increasing from a critical limit it has led to a decrease in the density in the film samples, which according to the analysis of variance table, these changes were not significant. The increase in density has a direct relationship with the thickness of the produced film; Because the parts were cut and measured in the same sizes to perform this test, the thickness of the film had the greatest effect on the density of the sample, in cases where the density value does not match with its thickness, probably due to the replacement of the hydrophobic groups of glycerol and coconut oil in the building envelope. It is a film that has caused a decrease in density and a porous structure in the film structure [11].

Table 2- Analysis of variance of data obtained from density test

Source	Sum of square	df	P-value
Model	2.145×10^5	9	<0.0001
A- I am Protein (%)	24632.03	1	<0.0001
B- Glycerol (%)	22681.660	1	<0.0001
C- Ziziphoria Essence (%)	1819.10	1	0.0.642
AB	186.89	1	0.5200
AC	489.11	1	0.3061
BC	57.79	1	0.7185
A^2	4391.66	1	0.0090
B^2	20221.99	1	<0.0001
C^2	2438.80	1	0.0368
Lack of fit **0.3028**		Mean	1113.92
R – Sqaured **0.9808**		Adj – R –sqaured	0.9635

4-6- water vapor permeability test

Increasing the concentration of protein and glycerol led to an increase in water vapor permeability of the film samples; Increasing the concentration of kakuti essential oil in the range used has led to a decrease in permeability to water vapor in the film samples.

The presented model is a quadratic polynomial (regression) equation, which is presented after removing meaningless coefficients and at a 95% confidence level. The magnitude of the coefficients of the variables in this polynomial, regardless of whether they are positive or negative, shows the importance of the corresponding coefficients in the response changes (infiltration of water vapor).

Water vapour permeability(g/m.pa.s) =
-4.35655
+2.07816*Soy Protein (%)
-0.15230*Glycerol (%)
+0.93595*Ziziphora Essence (%)
-0.21250*Soy Protein (%) * Ziziphora Essence (%)
-0.14000*Glycerol (%) * Ziziphora Essence (%)
$-0.11534*\text{Soy Protein (\%)}^2$
$+0.17864*\text{Glycerol (\%)}^2$

Kokoszaka et al. (2009), by examining the dry film produced from soy protein isolate, concluded that with the increase in the thickness of the produced film, the rate of water vapor transmission increases linearly, and at low relative humidities of edible films, there is little effect in controlling the permeability to It has oxygen, which was similar to our research; Also, at a constant protein concentration with an increase in the percentage of glycerol ,**oxygenpermeability increased, but the effect of protein on permeability is greater than that of glycerol**]19[.

Hamaguchi et al. (2007) in their study on the effect of pH on the properties of the edible film prepared from fish spear muscle protein achieved similar results and stated that unlike hydrophilic compounds that show good properties against water vapor permeability. Proteins are weak compounds for inhibiting water vapor transfer. Films prepared from spearfish had relatively lower permeability to water vapor than our films [24].

Yang and Paulson (2000) during the study of edible films prepared from gellan stated that with increasing percentage of glycerol, water vapor permeability increases and with increasing chain length and saturation level, water vapor permeability decreases [25].

Gontard et al. (1993) also obtained similar results by examining edible films prepared from wheat gluten. In their research, the permeability increased with an increase in the percentage of glycerol [26].

McHugh et al. (1994) also stated in their reports that the permeability to water vapor increases with the increase of glycerol percentage, which was similar to the present results [27]. Tavin Burtom (2008) investigated the edible films prepared from rice starch

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and chitosan and obtained similar results. He stated that the permeability to water vapor increases with the increase in plasticizer resistance [28]. Ozdemiro Floros (2007) by examining his protein films, came to the conclusion that protein and wax decreased the permeability to water vapor, which was against many of our studies and results. However, with the increase in the softener concentration, they reported an increase in permeability in the films, which was similar to the results of this research [29]. Choi and Han (2001) concluded that the percentage of glycerol does not significantly affect the permeability to water vapor [30]. However, in the research of Mashkani et al. (2009) on chickpea protein isolate, increasing the percentage of glycerol caused an increase in permeability to water vapor [18].

Kao et al. (2007) evaluated the permeability of films containing ferulic acid and tannic acid on gelatin films and stated that increasing these factors had little effect on reducing the permeability to water vapor, which was similar to the results of the present research [24]. Tanaka et al. (2000) during their research, while investigating the effect of oleic, linoleic and linolenic acid on the permeability of edible films prepared from soluble fish proteins, concluded that with the increase in the length of the unsaturated fatty acid chain, the permeability to water vapor increases. 31 [.

As can be seen, the film prepared from soy protein isolate has a high permeability to water vapor, which is due to the presence of many hydroxyl (-OH) groups in the structure of these films, which increases the hydrophilic properties in the film and causes a decrease in the barrier properties in the film. equal to the transfer of water vapor.

Soybean protein is a hydrophilic protein, because the main proteins of legumes are globulins and albumins, which are hydrophilic in nature.

4-7- Solubility test in water

Only the linear effect of glycerol concentration on the water solubility of the samples was significant ($P<0.01$); But the quadratic expressions of protein and glycerol concentration have also become significant ($P<0.01$). On the other hand, the term of interaction between the concentration of soy protein and glycerol (AB) and the term of the interaction between the concentration of glycerol and coconut oil (BC) have also been significant on the solubility of the samples ($P<0.05$). However, the term of interaction between soy protein concentration and coconut essential oil (AC) was not significant ($P>0.05$) and indicated the absence of synergistic or antagonistic effects on water solubility. Increasing the concentration of protein, glycerol and coconut oil in the range used led to an increase in the water solubility of the film samples.

Ozdemir and Floros (2007) obtained results similar to ours by examining edible films with whey protein origin and stated that increasing the percentage of protein and beeswax decreased water-soluble substances, but sorbitol as a softener increased water-soluble substances. Movies became edible. This means that with the increase of protein and wax, more water in the film structure causes more water to be retained in the film structure, and increasing the amount of sorbitol causes less water to remain in the film structure [29].

Choi and Han (2001) in the investigation of the amount of total soluble matter of edible films prepared from concentrated pea protein stated that the amount of total soluble matter of these films is about 40% [30].

Perez-Gago et al. (1990) also stated that the amount of total soluble material of edible films prepared from proteins is about 20%. These results show lower values compared to our results [32].

Conte et al. (1997) have also reported the percentage of soluble substances between 40 and 30 percent in the study of films made from soy protein, while reaching the same results as our research.]33[. Tavin (2008) during his research on the edible film prepared from rice starch and chitosan stated that the solubility of the film increases with the increase in the softener percentage. Probably, the decrease in the solubility of the edible film with the increase in the protein percentage is due to the denaturation of the protein during the film production process and the change in the nature of the protein structure, which creates a strong network with strong disulfide bonds in the protein network structure and prevents the collapse of the network and its solubility. can be And this building is able to keep some fatty acid molecules in it, which by being in water has caused the building to open more and help to dissolve it more [28].

8-4- Solubility test in acid

Only the linear effect of protein concentration on the degree of solubility in the acid of the samples is significant ($P<0.01$); The quadratic expressions of protein and glycerol concentration have also become significant ($P<0.01$). Other linear and quadratic expressions were not significant ($P>0.05$). On the other hand, only the term of interaction between the concentration of soy protein and glycerol (AB) on the degree of solubility in acid of the samples is also significant ($P<0.05$). However, the interaction term between the concentration of glycerol and coconut oil (BC) and the interaction term between the concentration of soybean protein and coconut oil (AC) is not significant ($P>0.05$) and indicates the absence of synergistic or antagonistic effects on the dissolution rate. were acceptable in acid.

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Increasing the protein concentration in the range used led to a decrease in the acid solubility of the film samples. However, increasing the concentration of glycerol and coconut oil in the range used led to an increase in the solubility in acid of the film samples.
Arabi et al. (2015), during their research on the effect of glycerol percentage on the solubility of edible films prepared from wheat gluten, achieved similar results to ours and stated that with increasing glycerol percentage, the edible film dissolves more in acid.
Because kakuti essential oil in the composition of the edible film acts somewhat similar to the softener, as a result, by increasing the amount of these two, the amount of disulfide and hydrogen bonds between and within the protein chain is reduced, and as a result, the structure of the film becomes more open and the film becomes more soluble] 34 [. But the increase in protein has increased the hydrogen bonds between protein and water molecules and has helped to maintain the structure of the film in the acid. Also, by decreasing the pH, the solubility of the protein decreased and as a result, the solubility in acid was lower than the solubility in water and alkali [11].

4-9- Transparency test

Increasing the protein concentration in the range used led to a decrease in the transparency of the film samples. But increasing the concentration of glycerol and kakuti essential oil in the range used led to an increase in the transparency of the film samples.
Hamaguchi et al. (2007) by examining the edible film prepared from spearfish, stated that the average transparency of the prepared films was 1±It is 6.6 and they are suitable for packages that need the material inside the package to be visible, which produced more transparent films compared to the results in this research [24]. Fernandez et al. (2006) during a study on the effect of saturated and unsaturated fatty acids on the film prepared from whey proteins, stated that saturated fatty acids reduce transparency more than unsaturated fatty acids, and the addition of oleic acid causes The turbidity of films increases, but this effect is not significant [35]. Limpan (2009) during research on edible films prepared from fish myofibril protein concluded that the transparency of produced films increased in the range between pH 3 and 11. Acidic films had transparency similar to our results, but alkaline films had more transparency than our results [36]. The range of transparency of produced films shows that the results of our research were similar to the research of Shiko et al. (2003) [37]. Probably, the decrease in transparency due to the increase in the percentage of protein is due to the increase in solid materials in the film structure, which with the increase in pH, the proteins are more dissolved in the solution and more pigments enter the solution. The effect of glycerol in increasing transparency is probably due to its diluting property, but this increase in transparency has less effect than increasing the percentage of protein, and in higher protein percentages, the release of pigments and the creation of turbidity in the film cannot show its effect well. Also, the increase in transparency is due to the increase in the molecular distance of proteins, which prevents the creation of a closed network structure and increases the transparency [11].

4-10- Length increase percentage test

The increase in protein concentration and glycerol concentration in the range used led to an increase in the percentage of film samples. However, increasing the concentration of kakuti essential oil in the range used led to a decrease in the percentage of elongation in the film samples. The results of this research were similar to the research of Fernandez et al. W protein, oleic acid increased the elongation percentage, but this effect was not significant; It is likely that the effect of oleic acid was not significant in their research due to the low percentage of oleic acid used. In the researches of Klein et al. (2002) and Ractoruriraini (2001) on the properties of decorative edible films containing oleic acid, they came to the conclusion that In addition to influencing the barrier properties, oleic acid also plays the role of a softener [39 and 38]. Mundaro et al. (2008) obtained similar results by examining films made from soy protein isolate and different percentages of oleic acid and beeswax. They stated that by increasing the amount of wax in low concentrations (0.25, 0.5 and 75), the increase in length has little changes, but in high concentrations of wax due to the opening of the protein network and the replacement of hydrophobic groups instead of protein molecules with an open structure. and the percentage of elongation decreased [40].

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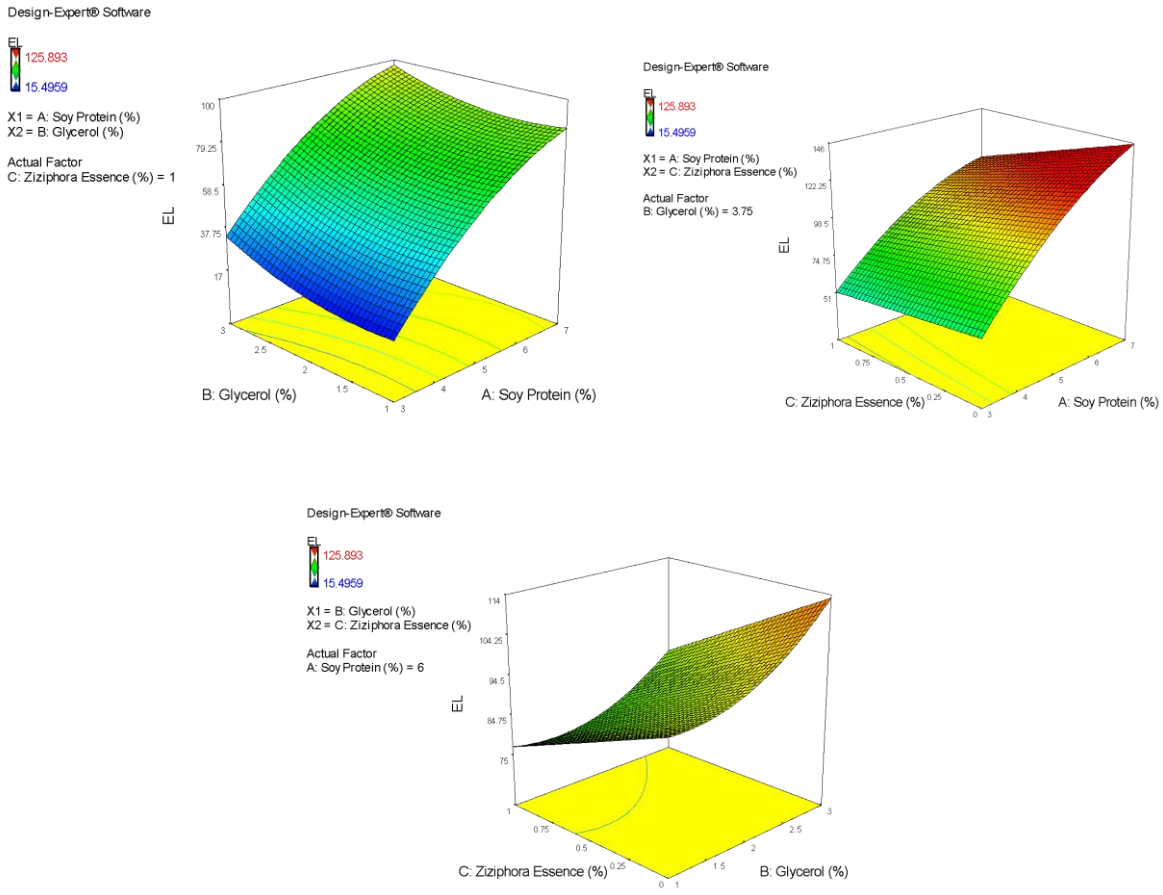

Figure 2- Showing a three-dimensional diagram of the simultaneous effect of independent variables on the percentage of elongation of the oral film

4-11- Tensile strength

The linear effect of soy protein isolate concentration and the linear and quadratic effect of glycerol concentration on the tensile strength of the samples are significant (P<0.05); However, none of the interaction terms were significant and this indicated the absence of synergistic or antagonistic effects of these two variables on the tensile strength.

Table 3- Analysis of variance of data obtained from tensile strength test

Source	Sum of square	df	P-value
Model	72.93	9	<0.0001
A- I am Protein (%)	5.19	1	<0.0001
B- Glycerol (%)	0.89	1	0.0092
C- Ziziphoria Essence (%)	0.12	1	0.2551
AB	0.060	1	0.4208
AC	0.24	1	0.1228
BC	0.035	1	0.5360
A^2	0.010	1	0.7336
B^2	0.48	1	0.0387
C^2	0.17	1	0.1883
Lack of fit **0.0600**		Mean	7.33
R – Sqaured **0.9884**		Adj – R –sqaured	0.9779

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Increasing the protein concentration in the range used led to an increase in the tensile strength of the film samples. However, increasing the concentration of glycerol and coconut oil in the range used led to a decrease in the tensile strength of the film samples.
Meshkani et al. (2009) obtained similar results by examining the film prepared from chickpea protein isolate and stated that the tensile strength of the films decreased significantly (P<0.01) with an increase in the percentage of glycerol (P<0.01). Glycerol has been attributed to reducing hydrogen bonds between protein chains and increasing intermolecular spaces. Also, in other results, with the increase in the amount of protein, the tensile strength increased, which is caused by the interaction between protein molecules in alkaline conditions, caused by the alkaline condition of the film or protein denaturation at high temperature [18].

Arabi et al. (2015) in a study on edible films prepared from wheat gluten stated that increasing the amount of glycerol in concentration (1.5 to 3.5%) caused a decrease in the tensile stress of the film. Munoz et al. (2003) also investigated edible films made from glutenin and gliadin during a research and stated that increasing the percentage of glycerol decreased the tensile strength and this effect was more effective in the case of films made from glutenin [34].
Choi and Han (2001) and Crocetta (2002) by examining the films prepared from different proteins (soy, whey, pea and wheat gluten) on the tensile strength of edible films, measured the tensile strength values that the films prepared from Khlor protein isolate It has the same tensile strength as peas, 7.3 megapascals (the highest amount of tensile strength) and its tensile strength was higher than the films made from whey protein isolate, soy and gluten [41 and 30].

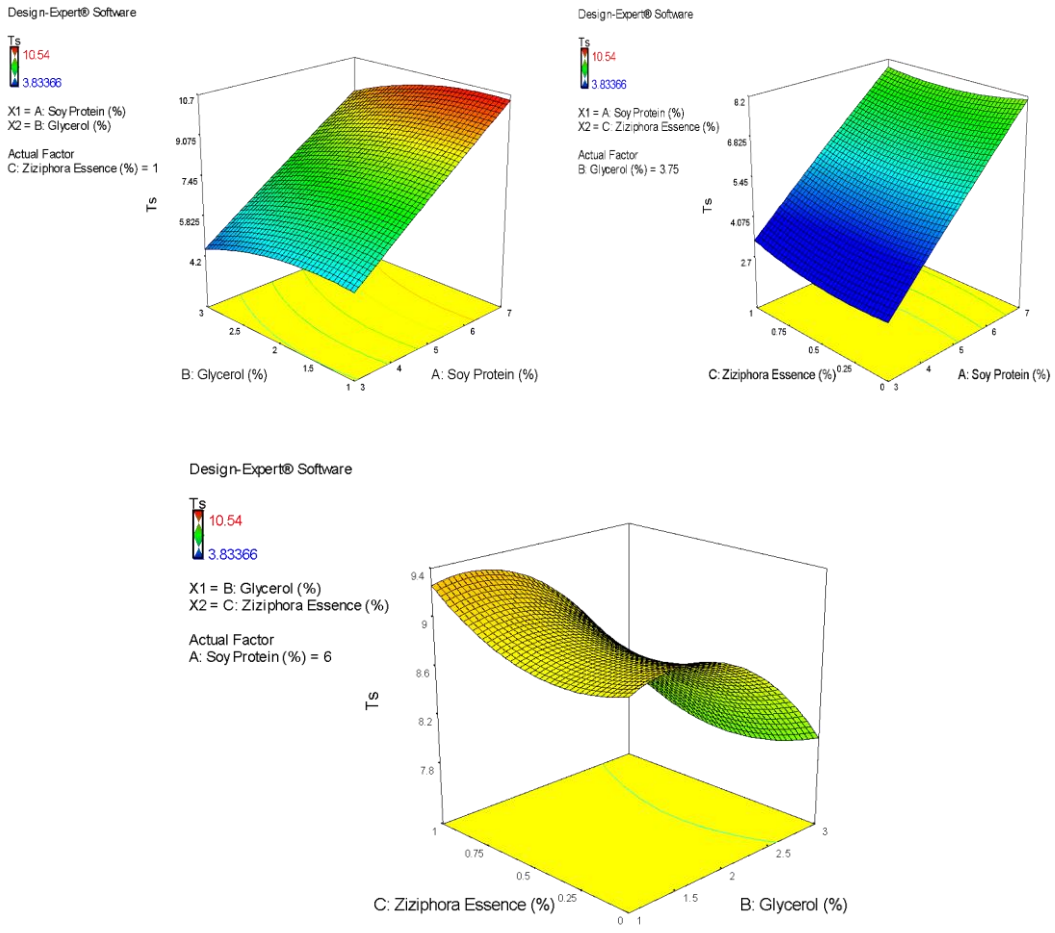

Figure 3- Showing a three-dimensional diagram of the simultaneous effect of independent variables on the tensile strength of an oral film

4-12- Numerical optimization using the response surface method

According to the tests and analyzes carried out in order to find an optimal film and perform subsequent tests on it, we decided to formulate the optimal production film based on all the physicochemical and mechanical tests. As mentioned, an ideal edible film has the lowest thickness, density and permeability to water vapor, maximum color index L, color index a

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close to zero and color index b close to zero, the highest amount of substances soluble in water, acid, transparency, resistance It was tensile and percentage of elongation. In Table 2, these specifications and entries of the software can be seen.

Table 4 - The domain used to optimize the process and the optimization response by the software

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Soy Protein (%)	is in range	3	7	1	1	3
Glycerol (%)	is in range	1	3	1	1	3
Ziziphora Essen	is in range	0	1	1	1	3
Thickness	minimize	0.06	0.3	1	1	3
l	maximize	6.3	13.57	1	1	3
a	minimize	0.02	1.32	1	1	3
b	minimize	0.47	2.74	1	1	3
Density (Kg/m3)	minimize	920.564	1344.92	1	1	3
Water vapour pe	minimize	0.68	5	1	1	3
Solubility in wate	maximize	39.78	80.17	1	1	3
Solubility in HCl	maximize	17.16	74.74	1	1	3
Transparency	maximize	0.57	6.9	1	1	3
EL	maximize	15.4959	125.893	1	1	3
Ts	maximize	3.83366	10.54	1	1	3

Therefore, considering these things, we found the optimal point, which in the conditions where the concentration of soy protein isolate is 3.54%, glycerol is 1%, and the percentage of coconut oil is about 1%, the above indicators are optimized at the desirability level of 0.624. In this case, the thickness is 0.093 mm, the density is 996.827 kg/m3, the water-soluble material is 74.05%, the acid-soluble material is 60.60, the alkali-soluble material is 100%, the permeability ratio is to water vapor $^{10-}$10× 1.35 g/m pascal second, transparency 4.84733, index L equal to 10.94, index a equal to 0.27, index b equal to 1.11, tensile strength 12.6 MPa and elongation 13.32 percentage will be (Table 3).

Table 5 - Design Expert software output for optimizing the composite film production process

Solutions

Number	Soy Protein (%	Glycerol (%)	Ziziphora Esse	Thickness	l	a	b	Density (Kg/m3	Water vapour p	Solubility in wa	Solubility in HCl	Transparency	EL	Ts	Desirability
1	3.54441	1.00002	0.999981	0.0931102	10.9471	0.276442	1.11734	996.827	1.35319	74.0588	60.6035	5.47148	32.1314	6.12185	0.624
2	3.55201	1	0.963349	0.0957335	11.0867	0.294166	1.08995	1000.07	1.3713	73.2873	60.5949	5.48098	32.4333	6.06788	0.623
3	3.5327	1.00003	0.90042	0.0991953	11.3591	0.311579	1.05727	1003.76	1.36806	72.3965	61.0816	5.56187	32.1457	5.93842	0.620
4	3.46754	1	0.890612	0.0987128	11.5076	0.295993	1.07658	1002.08	1.3021	73.0213	62.2326	5.70748	30.5189	5.83572	0.619
5	3.56932	1.00001	0.836279	0.102933	11.5078	0.34063	1.00493	1008.57	1.42205	70.8592	60.538	5.51998	33.275	5.88995	0.617
6	3.56407	1.00002	0.818561	0.103592	11.5699	0.343334	0.996719	1009.17	1.42072	70.629	60.6399	5.54009	33.199	5.85743	0.617
7	3.56671	1	8.94453E-005	0.0795944	11.3961	0.125542	0.72569	964.376	1.50323	61.3745	55.2338	5.54022	36.0182	5.36738	0.612
8	3.49592	1	4.79554E-006	0.0782252	11.5092	0.101815	0.747149	961.356	1.41599	62.1244	56.5558	5.70267	33.8772	5.26172	0.611
9	3.53731	1	0.0668381	0.0852765	11.6494	0.164023	0.741988	972.769	1.46936	62.1238	56.6454	5.63658	34.9126	5.3141	0.610
10	3.55469	1.00002	0.543329	0.108117	12.1015	0.357459	0.865319	1011.52	1.4617	66.6914	60.288	5.65205	33.8485	5.52916	0.608

After optimizing the formula of the composite film produced from soy protein isolate, glycerol and kakuti essential oil, this formula was made in practice and the optimized composite film was made. Then tests were

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done on the final film to evaluate the final film more and with better accuracy.

4-13- Electron microscope test

Atomic electron microscope (SEM) images are used to investigate the surface properties of the film. In Figure 3, images with different magnifications of the surface of the optimal edible film prepared from the formula obtained during the previous step were examined. The optimal film had a relatively smooth and continuous surface with few peaks and valleys, which shows the approximate uniformity of its matrix structure. The existence of these ups and downs is due to the branching structure of soy protein isolate and as discussed earlier, due to the hydrogen bonds between glycerol molecules and soy protein isolate in combination with coconut oil, whose strands are able to form bonds in different directions. . The tiny white dots in these photos are probably essential oil molecules that are spread randomly in the film structure [42].

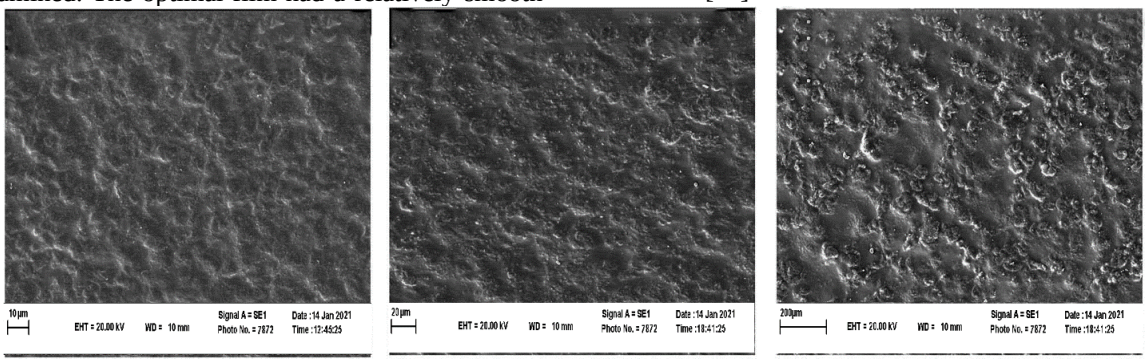

Figure 4 - Electron microscope images at 10, 20 and 200 micrometers magnification of the optimal film produced

4-14- FTIR test

The FTIR test results of the optimal film are shown in Figure 4, two big peaks can be seen in this graph; In the 800-1150 cm region, we can see that the main absorption bands of glycerol are shown here. On the other hand, according to the theories of Pichirili et al. (2019), in the range of 3000-3600 cm, the main absorption peak is attributed to free and limited O-H and N-H groups of protein and water. This region is characterized by a broad absorption band for almost all proteins [37].

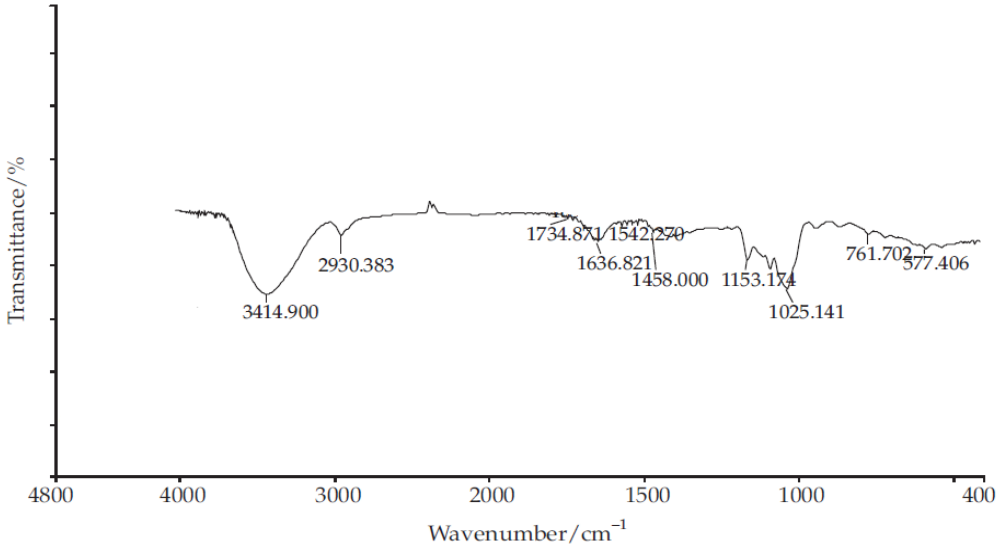

Figure 5- FTIR test performed on the optimal oral film

5-Conclusion

In general, it can be concluded that by using soy protein isolate and using a softener (plasticizer) together with kakuti essence, an edible film suitable for packaging can be produced. Increasing the concentration of protein used in the formulation leads to an increase in thickness, color indices L and a, density, permeability to water vapor, solubility in water, percentage of elongation.

and the tensile strength of the film, but it caused a decrease in the color index b, solubility in acid and transparency of the films. To optimize the film

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formula, it is recommended to use a concentration of 3.54% soy protein isolate, 1% glycerol and 1% coconut oil.

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مقاله علمی_پژوهشی

ارزیابی خصوصیات فیزیکوشیمیایی و مکانیکی فیلم خوراکی تهیه شده از ایزوله پروتئین سویا حاوی اسانس گیاه کاکوتی

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چکیده

امروزه تولید فیلمهای خوراکی و زیست تخریب پذیر یکی از تکنولوژیهای رو به رشد در جوامع پیشرفته است. کاربرد این پوششها به دلیل جایگزینی با مواد پلیمری و افزایش مدت زمان انبار مانی ماده غذایی می باشد. هدف اصلی این پژوهش تولید یک فیلم خوراکی از ایزوله پروتئین سویا (۳ تا ۷ درصد) ، گلیسرول (۱ تا ۳ درصد) و اسانس کاکوتی (۰تا ۱ درصد) به منظور ایجاد طعم بهتر و نیز خصوصیات ضد میکروبی بود. پس از تهیه فیلم ویژگیهای فیزیکوشیمیایی و مکانیکی این فیلم (ضخامت، دانسیته، حلالیت در آب و اسید، نفوذپذیری به بخار آب، شفافیت، تغییرات رنگ، درصد ازدیاد طول و مقاومت کششی) مورد ارزیابی قرار گرفت. پس از انجام آزمونهای فوق و بررسی تاثیرات متغیرهای مستقل بر روی متغیرهای وابسته، فرمولاسیون فیلم خوراکی بهینه سازی شد. نتایج نشان داد با استفاده از ۳/۵۴ درصد ایزوله پروتئین سویا، ۱ درصد گلیسرول و ۱ درصد اسانس کاکوتی در سطح مطلوبیت ۰/۶۲۴ می توان به فرمول بهینه دست یافت.

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