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RESEARCH ARTICLE

Preparation and characterization of biodegradable polymer films from cowpea (*Vigna unguiculata*) protein isolate**

Sujatha Hewage* and Sashiprabha M. Vithanarachchi
Department of Chemistry, Faculty of Science, University of Colombo, Colombo 03.

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Abstract: The investigations on a variety of proteins present in corn zein, wheat gluten, milk whey and soya have shown that these proteins possess the ability to form films which can be used in food packaging. We, therefore, selected cowpea (*Vigna unguiculata*), a cereal abundantly available in Sri Lanka, to investigate its polymer film forming ability. Our studies have shown that the choice of a suitable solvent, pH of the medium and the use of a plasticizer were essential to obtain useful films. In this study polyethylene glycol and glycerol were used as plasticizers. The extracted cowpea protein and the plasticizers were dissolved in hot aqueous ethanol to obtain a viscous solution. The casting technique was used to fabricate the films. A series of films were made by varying the pH from 8 to 11. For each pH a series of films was prepared by varying the concentration of polyethylene glycol from 2.5 to 10% (w/v). The fabricated films were tested for transparency, glass transition temperature, tensile strength and water vapour permeability. The pH of the medium and the concentration of the plasticizer were found to have a considerable effect on the physico-chemical properties of the film. The film fabricated at pH 10 with 2.5% (w/v) of polyethylene glycol and 2% glycerol was found to possess the desired tensile strength, water vapour permeability, transparency and glass transition temperature, making the film suitable to be used as an edible food packaging material.

Keywords: Biodegradable polymer film, cowpea protein isolates, plasticizer, polyethylene glycol, *Vigna unguiculata*.

INTRODUCTION

Environmental concerns arising from the heavy use of nondegradable, commercially available plastics have resulted in the search for suitable substitutes. Studies on biodegradable polymers have, therefore, become an important field of research. One of the areas that is being actively investigated is the development of biodegradable films from various biopolymers such as polysaccharides, proteins and lipids. Combinations of these biopolymers are also investigated to obtain suitable polymers. In recent years, the development of degradable films from a variety of protein isolates has drawn much attention[1]. This is due to their ability to form films and also for their abundance and renewable nature. These include soya protein isolate, gelatine, rice bran protein, corn zein, wheat gluten, milk whey protein, casein and collagen. However, only a few proteins have been developed for commercial use[2]. These protein films are ideal to use as a food wrapping material and to replace the plastics used in the food packaging. Such films used in food packaging may function as selective barriers to movement of gases, vapours or solutes. These barrier properties increase the quality, stability and shelf-life of foods[3]. Edible films can also be used as a carrier for flavour[4] and antimicrobials[5]. Films made from proteins are expected to be excellent oxygen barriers because of their hydrogen-bonded network structure.

The parameters that could affect the properties of the film are, plasticizer concentration, heating temperature, pH, thickness and solubility. When protein is subjected to the action of acid, alkali or heat, partially denatured polypeptide chains are obtained. These represent the structures required for film formation. Protein films are formed when these structures associate through noncovalent and covalent bonding to form the protein matrix[6]. Low molecular weight plasticizers such as glycerol, sorbitol and potassium sorbate are added to proteins to improve the flexibility of the polymer by reducing the inter-chain interactions[1].

* Corresponding author (sujatha@chem.cmb.ac.lk)

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The film formation mechanism is believed to occur through the polymerisation of protein and the evaporation of solvent at the interface between film and air. Protein molecules in the film network are associated through disulphide, hydrophobic and hydrogen bonds. The disulphide bonds are primarily responsible for protein polymerisation[7].

So far, cowpea protein isolate has not been investigated for its film forming ability. The main objective of this study, therefore, was to determine the conditions necessary for the formation of polymer films from cowpea protein isolate[8] and to investigate the effect of the concentration of the plasticizer and the pH of the medium on the mechanical and barrier properties of the films fabricated.

METHODS AND MATERIALS

Materials: Cowpea seeds, extracted cowpea protein powder, 0.1M and 0.01 M HCl acid, 0.1 M NaOH, 98% glycerol, polyethylene glycol 20M, distilled ethanol, sodium benzoate, α-alumina (α-Al_2O_3), small porcelain crucibles, dry silica gel and desiccators. The chemicals used are of GPR grade from BDH, England and used without further purification unless otherwise stated.

Extraction of cowpea protein: The dehulled cowpea seeds were milled using a grinding mill (FRITSCH PUL-14) and the resulting cowpea flour was sieved. The cowpea flour was treated with ten fold amount (w/v) of 0.1 M NaOH. The resulting mixture was stirred for an hour on a magnetic stirrer. Stirred mixture was centrifuged at 2000 rpm for 20 min. The supernatant was collected and isoelectrically precipitated at pH 3.7 using 0.1M and 0.01M HCl. The precipitate was separated by centrifuging at 2500 rpm for 15 min. The supernatant was discarded and the sediment was freeze dried at 20 ^{0}C for 48 h. The freeze dried cowpea protein was ground into a fine powder.

Film fabrication: 3 g of extracted cowpea protein isolate were weighed and dissolved in 50 cm^3 of distilled water. The pH of the solution was adjusted to 9 with 0.1 M NaOH. This solution was heated at 80^{o}C for 10 min with occasional stirring. A mixture of 10 cm^3 of 20% glycerol and 1 g of polyethylene glycol dissolved in 20 cm^3 of distilled ethanol, was added to the hot solution. The heating was continued for a further 30 min. The antifungal agent, 0.1 g of sodium benzoate, was added to the hot solution and stirred to dissolve completely. The hot solution was filtered using a strainer and the filtered solution was immersed in a sonicator for a few minutes to remove air bubbles. Then this homogenized hot solution was spread on a clean dry glass pad to form a thin layer. This was then dried in an oven for 5 h at 80 ± 5^{o}C. The dried film was cooled for about 12 h and peeled off from the glass pad. The film was wrapped in tissue papers as soon as it was peeled off and was dried under room temperature for about five days.

A series of films was fabricated at four different pH values of 8, 9, 10 and 11. For each pH value, another series of films was prepared, using 0.5 g, 1.0 g, 1.5 g and 2.0 g of polyethylene glycol, in order to obtain 2.5%, 5%, 7.5% and 10% (w/v) plasticizer solutions, respectively.

Tensile strength (TS): The film samples were cut into 7.5 cm long dumbbell shaped specimens. For each specimen, three measurements of thickness in the mid portion were taken using a micrometer. The tensile strength of each specimen was measured by using the HOUNDS SIELD H 5000 M tensometer (U.K.) with an extension rate of 500 mm/ min. For each film, tensile strength measurements were replicated five times.

Water Vapour Permeability (WVP): The water vapour permeability of the films was determined gravimetrically at 28^{0}C using a model ASTM standard method E 96 – 80 (ASTM 1980)[9]. The thickness of the cowpea protein films was measured and these films were sealed to porcelain crucibles containing anhydrous silica gel. The exposed surface area of the cowpea protein films was measured and the crucibles were weighed to get the initial weight. Sealed crucibles were placed in desiccators maintained at 100% relative humidity with distilled water. The crucibles were weighed at different time intervals, till the difference of weight between two consecutive intervals became less than 0.0001 g. The experiment was carried out in duplicate. The WVP (g.mm/m^2.h.mm/Hg) of the cowpea protein films was detemined using the following equation:

$$WVP = W.X / A.\ t.\ (P_2 - P_1)$$

where W is the increase in weight of the cup in g , X is the film thickness in mm, A is the exposed film area in m^2, t is the time in hours and $(P_2\text{-}P_1)$ is the vapour pressure difference across the film in mmHg.

Glass Transition Temperature (T_g): This was measured by Differential Thermal Analysis using TAS-100 (Rigaku, Differential Thermal Analyzer – 50 µV, Japan). The samples were cut into tiny pieces and loaded into an aluminium sample pan with a crimped cover to maintain a good thermal contact between the sample and the sample pan. Alumina (α Al_2O_3) was used as the reference material. The glass transition temperature was measured

under normal atmospheric conditions at a heating rate of 5^0C/min. Chart speed was 5 mm/min, Scanning rate was 5 K/min and the target temperature was 200^0C.

Opacity: The film opacity was determined by the modified standard procedure (BSI, 1968) using V 500, UV/ Visible spectrophotometer. The film samples were cut into rectangles and placed on the internal side of the quartz spectrophotometer cell. The absorbance spectrum was recorded for each sample in the wavelength range of 400–800 nm. The film opacity, which is defined as the area under the recorded curve, was determined by an integrating procedure. The opacity was expressed as absorbance units in nanometer (AU. nm).

Morphology: A selected cowpea protein film sample, prepared at pH 10 with 2.5% polyethylene glycol plasticizer concentration, was examined for surface characteristics using the scanning electron microscope (SEM), model SEM - TOPCAN, ABT-32 (Japan). The samples were attached to stainless steel stubs with double side cellophane tape, coated with a 10 nm thick layer of gold-palladium alloy. The samples were examined using an accelerating voltage of 15 kV. The lower surface, upper surface and a transverse section of the cowpea protein film were scanned and photographed.

RESULTS AND DISCUSSION

The films obtained were homogeneous, transparent, clear, flexible and strong. The transparency of the film is comparable to that of low density polyethylene. The film is sealable and printable. The film has a slightly yellowish appearance and the colour darkens with increase in film thickness. Attempts to prepare films at pH 7 resulted in a brittle film and it was not possible to strip off the film from the glass pad. Above pH 11, although the films obtained were of good quality, the colour of the films was brown. Therefore, low and high pH values were found unsuitable to prepare films from cowpea protein. Hence pH 8 -11 were used in film fabrication. High pH favours the solubility of proteins which results in the unfolding of the protein chains favouring the film formation.

The films fabricated using polyethylene glycol alone as a plasticizer were relatively brittle and difficult to remove from the glass pad as an unbroken film. Addition of 2% glycerol gave a flexible film that could be easily removed from the glass pad without breaking. The plasticizers were added to reduce the brittleness of polymeric material. The addition of plasticizers decreases the interactions between the protein chains. This results in the increased mobility of the protein molecules. Thus, plasticizers improve the flexibility of films. Polyethylene glycol as a plasticizer was found to be not very effective in reducing the brittleness of the film and hence a mixture of polyethylene glycol and glycerol was needed to improve the flexibility of the cowpea protein film.

The effect of pH on opacity is shown in Table1 and Figure 1. Opacity is inversely proportional to transparency. The film opacity values were used to assess the transparency of the film. With increasing pH, opacity of the film decreased resulting in the increase in transparency of the film at high pH. At higher pH values, solubility of the cowpea protein is high and the protein chains are dispersed evenly, enabling the formation of a homogeneous film. Therefore, more light penetrates through the film giving lower opacity values.

Table 2 and Figure 2 show the effect of plasticizer concentration on opacity. The film opacity increased with the increasing plasticization. When the plasticizer concentration is increased the homogeneity of dispersed cowpea protein chains are disrupted by bulky polyethylene glycol molecules. The loss of homogeneity ultimately leads to high opacity values and reduction in transparency[10].

The effect of pH on the tensile strength of the cowpea protein film is given in Table 3 and Figure 3. The tensile strength of the cowpea protein film increased with the increasing pH of the film forming solution. As the pH of the film forming solution increases, the solubility of the cowpea protein also increases exposing more thiol groups (SH) in the cowpea protein. The thiol groups get oxidized into disulphide bonds (S-S) which are the main associative forces that make cross linkages between the protein polymers[11, 12]. Therefore, at higher pH values, the number of cross linkages in the polymer is higher and that increases the strength of the polymer network leading to higher tensile strength.

The variation of the tensile strength with the plasticizer concentration is shown in Table 4 and Figure 4. The tensile strength decreased with the increasing plasticizer concentration. The plasticizer molecules reside in between the protein chains thereby reducing the extensive intermolecular interactions among the protein chains. Therefore, when the plasticizer concentration is increased, the number of plasticizer molecules that reside between the protein chains are also increased, thus reducing the interactions between protein polymer chain networks resulting in the lowering of the tensile strength. Also, as the plasticizer molecules used are hydrophilic, these can absorb water vapour from the surrounding and cause swelling of the polymer film. This also reduces the interaction between the protein polymer chains.

Table1: Effect of pH on opacity of cowpea protein film

pH	Opacity (A.U. nm)
8	417.46
9	134.95
10	93.06
11	57.70

Table 2: Effect of PEG plasticizer concentration on opacity of cowpea protein film

PEG plasticizer (%)	Opacity (A.U. nm)
2.5	93.06
5.0	129.89
7.5	228.54
10.0	258.79

Table 3: Effect of pH on TS of cowpea protein film (concentration of PEG, 2.5% w/v)

pH	Thickness (mm)[a]	TS (Nmm^{-2})[b]
8	0.07 ± 0.01	2.1 ± 0.47
9	0.17 ± 0.01	9.7 ± 0.97
10	0.09 ± 0.01	18.3 ± 2.00
11	0.12 ± 0.01	23.3 ± 2.11

a – Average and the standard deviation of the film thickness in (n=3) measurements

b – Average and the standard deviation of the tensile strength in (n=5) measurements

Table 4: Effect of PEG plasticizer concentration on TS of cowpea protein film at pH 10

PEG plasticizer (%)	Thickness (mm)[a]	TS (Nmm^{-2})[b]
2.5	0.09 ± 0.01	18.3 ± 2.0
5.0	0.06 ± 0.01	17.5 ± 3.0
7.5	0.13 ± 0.01	15.9 ± 1.2
10.0	0.03 ± 0.01	6.6 ± 2.6

a – Average and standard deviation of the film thickness in (n=3) measurements

b – Average and standard deviation of the tensile strength in (n=5) measurements

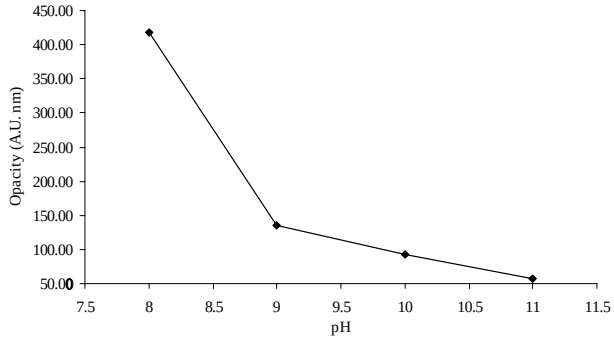

Figure 1: Effect of pH on opacity of cowpea protein film

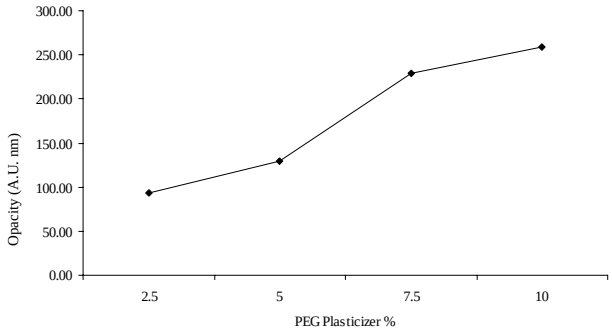

Figure 2: Effect of PEG plasticizer concentration on opacity of cowpea protein film

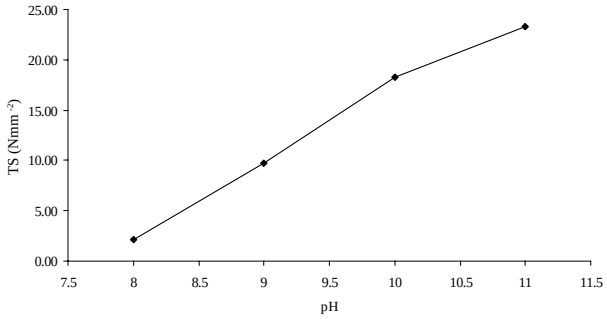

Figure 3: Effect of pH on TS of cowpea protein film (concentration of PEG, 2.5% w/v)

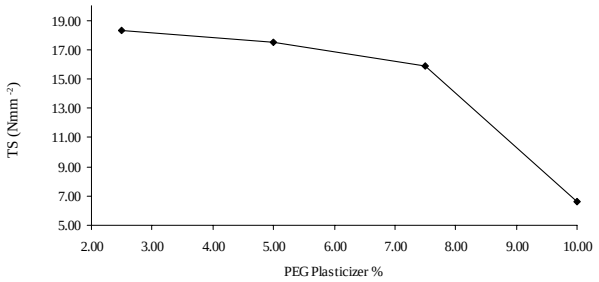

Figure 4: Effect PEG plasticizer concentration on TS of cowpea protein film at pH 10

Table 5: Effect of pH on WVP of cowpea protein film (concentration of PEG, 2.5% w/v)

pH	Thickness (mm)[a]	WVP (g.mm./m^2.h.mmHg)
8	0.03 ± 0.01	0.0040 ± 0.0013
9	0.06 ± 0.01	0.0070 ± 0.0012
10	0.09 ± 0.01	0.0110 ± 0.0014
11	0.12 ± 0.01	0.0170 ± 0.0016

a – Average and standard deviation of the film thickness in (n=3) measurements

b– Average and standard deviation of the WVP in (n=5) measurements

Table 6: Effect of PEG plasticizer concentration on WVP of cowpea protein film at pH 10

PEG plasticizer (%)	Thickness (mm)[a]	WVP (g.mm./m^2.h.mmHg)
2.5	0.06 ± 0.01	0.0008 ± 0.0043
5.0	0.09 ± 0.01	0.0120 ± 0.0014
7.5	0.12 ± 0.01	0.0170 ± 0.0076
10.0	0.12 ± 0.01	0.0170 ± 0.0076

a – Average and standard deviation of the film thickness in (n=3) measurements

b – Average and standard deviation of the WVP in (n=5) measurements

Table 7: Effect of PEG plasticizer concentration on T_g of the cowpea protein film at pH 10

PEG plasticizer (%)	T_g (^{0}C)
2.5	62.5
5.0	56.0
7.5	51.5

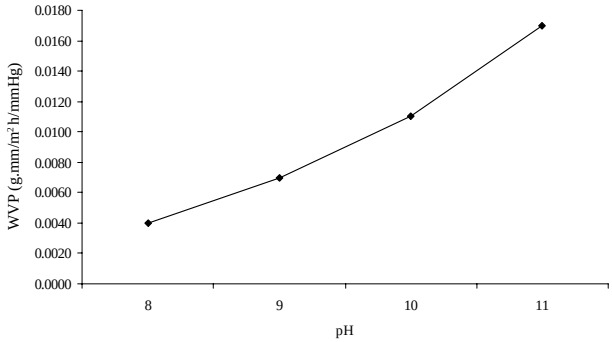

Figure 5: Effect of pH on WVP of cowpea protein film (concentration of PEG, 2.5% w/v)

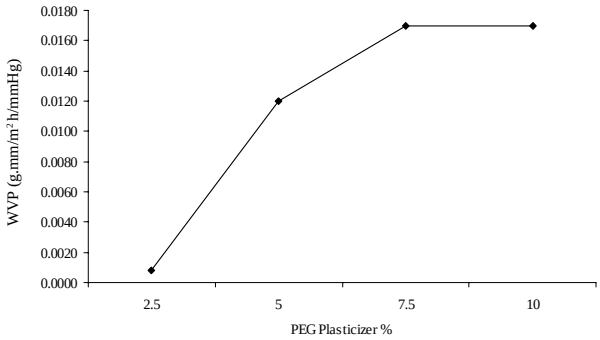

Figure 6: Effect of PEG plasticizer concentration on WVP of cowpea protein film at pH 10

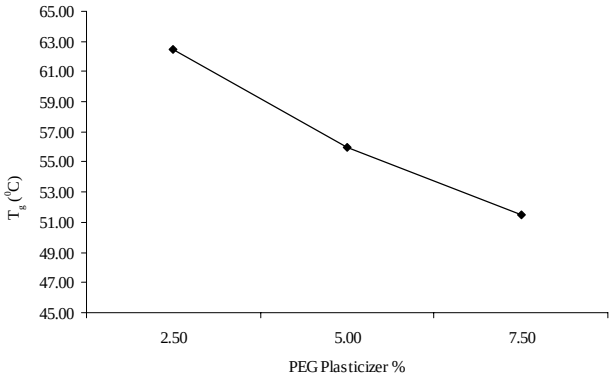

Figure 7: Effect of PEG plasticizer concentration on T_g of the cowpea protein film at pH 10

The variation of the WVP with the pH of the film forming solution is shown in Table 5 and Figure 5. The WVP increased with the increasing pH of the film forming solution. The WVP is dependent on the number of polar groups present in the polymer film. Denaturation of cowpea protein at higher pH values exposes the hydrophilic residues on the protein surface. That enhances the sorption of migrating water molecules to polar groups, facilitating the transport of water and causing the penetration of more water vapour through the film[13].

The WVP of the cowpea protein film increased with the increasing plasticizer concentration as shown in Table 6 and Figure 6. The plasticizer molecules increase the intermolecular spacing between the protein polymer chains, thus allowing the water molecules to go through the films[14].

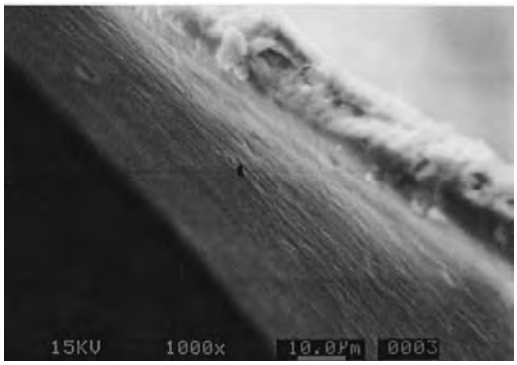

Figure 8: A SEM image of the lower surface of the cowpea protein film

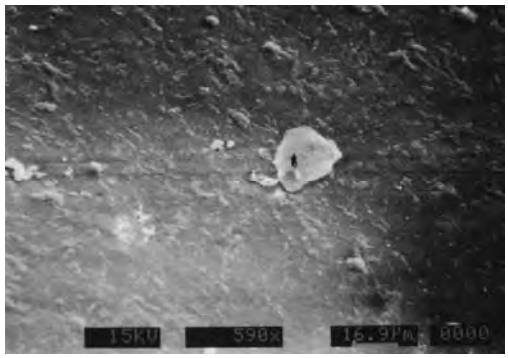

Figure 9: A SEM image of the upper surface of the cowpea protein film

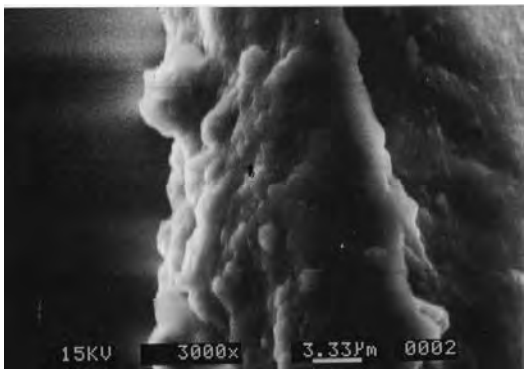

Figure 10: A SEM image of a transverse section of the cowpea protein film

The Table 7 and Figure 7 show the variation of T_g with the plasticizer concentration. According to the results obtained, the T_g of the cowpea protein film decreased with the increasing plasticizer concentration. The T_g of the pure protein lies in the region of 155-257 ^{0}C. When plasticizers are added they reside in between the protein polymer chains and space them from one another reducing the interaction between the polymer chains. Therefore, the T_g of the plasticized protein film is less than that of the pure protein.

Figures 8 to 10 show scanning electron microscopy images of cowpea protein film prepared at pH 10 with 2.5 % polyethylene glycol. The SEM image of the lower side of the film, facing the glass pad, is very smooth with uniform deposition of the film components. The reverse side the side exposed to the atmosphere, shows globules in an irregular fashion. The transverse section shows the globular arrangement.

The cowpea protein films are very soluble in water. When kept inside a desiccator between tissue papers or between polythene sheets the films were unaffected and the properties of the films were preserved well. When the films were exposed to air, they absorbed moisture from the atmosphere and became more flexible. After several days fungal growth was observed. The fungal growth can be prevented by using antifungal agents. When a lesser amount of plasticizer was used, the films became brittle, and began to fragment and then disintegrate. The films made out of cowpea protein and the plasticizers added, polyethylene glycol and glycerol, in the film formation are known to biodegrade completely[1].

CONCLUSION

The films obtained from cowpea protein isolate have a good appearance and are homogeneous, transparent and flexible. When polyethylene glycol was used as the plasticizer the cowpea protein film obtained was brittle. Flexibility of the film increased when a mixture of polyethylene glycol and glycerol was used as plasticizers. An antimicrobial agent, sodium benzoate was used to control the growth of microbes (fungi and mold) on the film. The conditions used in the film formation, such as the pH of the film forming solution and the plasticizer concentration, have a considerable influence on the properties of the polymer film.

Our studies have shown that the best pH for the fabrication of cowpea protein film is pH 10. At this pH, a film which has the highest tensile strength and lower opacity was formed. However, the water vapour permeability is relatively high at pH 10. When the concentration of the plasticizer, polyethylene glycol, is 2.5% (w/v), the properties observed of the fabricated films were the best having the highest tensile strength, the lowest opacity and the lowest water vapour permeability. From our studies, we can conclude that the best conditions to prepare cowpea protein films are to use a mixture of 2% glycerol and 2.5% of polyethylene

glycol as plasticizers and to maintain the pH of the film forming solution at 10. The films fabricated under these conditions are suitable to be used as a food packaging material.

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