Production and Properties of Edible Film from Mung Bean and Red Bean Proteins

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ชื่อวิทยานิพนธ์	การผลิตและสมบัติของฟิล์มบริโภคได้จากโปรตีนถั่วเขียวและถั่วแดง
ผู้เขียน	นางสาวเสาวนิตย์ คีรีเกษตร์สุข
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บทคัดย่อ

ฟิล์มบริโภคได้จากโปรตีนถั่วเขียวและถั่วแดงได้ถูกพัฒนากระบวนการวิธีการมาจากงานวิจัยก่อนหน้านี้ จากการศึกษาผลของพีเอช อุณหภูมิและเวลาในการให้ความร้อนต่อสมบัติต่างๆ ของฟิล์มบริโภคได้จากโปรตีนถั่วเขียวและถั่วแดง ซึ่งวิเคราะห์โดยการใช้โปรแกรมทางสถิติ (Response Surface Methodology) พบว่าพบว่าปัจจัยหลักที่มีผลต่อสมบัติต่างๆ ของฟิล์มอย่างมีนัยสำคัญ ($p< 0.05$) คือพีเอชและอุณหภูมิในการให้ความร้อนแก่สารละลายฟิล์ม ขณะที่เวลาในการให้ความร้อนแก่สารละลายฟิล์มมีผลน้อยที่สุด ค่าการต้านทานแรงดึงสูงสุดและค่าร้อยละการยืดตัวเมื่อขาดมีค่าเพิ่มขึ้น เมื่อพีเอช อุณหภูมิและเวลาในการให้ความร้อนแก่สารละลายฟิล์มเพิ่มขึ้น ในขณะที่ค่าการซึมผ่านไอน้ำ ค่าการละลายของฟิล์มและค่าการละลายของโปรตีนจากฟิล์มมีค่าลดลง นอกจากนี้แผ่นฟิล์มที่ได้จะให้สีที่เข้มขึ้นเมื่อพีเอชและอุณหภูมิในการให้ความร้อนแก่สารละลายเพิ่มขึ้น และจากการศึกษาผลของความเข้มข้นของโปรตีนต่อสมบัติต่างๆ ของฟิล์มบริโภคได้จากโปรตีนถั่วเขียวและถั่วแดง พบว่า เมื่อความเข้มข้นของโปรตีนเพิ่มขึ้นค่าการต้านทานแรงดึงสูงสุดและค่าร้อยละการยืดตัวเมื่อขาดของฟิล์มมีค่าเพิ่มขึ้น ในขณะที่ค่าการซึมผ่านไอน้ำ ค่าการละลายของฟิล์มและค่าการละลายของโปรตีนฟิล์มที่ได้จะให้ค่าที่ลดลง นอกจากนี้พบว่าชนิดและปริมาณของพลาสติไซเซอร์ส่งผลอย่างมีนัยสำคัญต่อสมบัติต่างๆ ของฟิล์มบริโภคได้จากโปรตีนถั่วเขียวและถั่วแดง โดยพบว่าฟิล์มที่ใช้ซอร์บิทอลเป็นพลาสติไซเซอร์จะให้ค่าการต้านทานแรงดึงของฟิล์มสูงที่สุดแต่ฟิล์มที่ได้จะมีลักษณะเปราะ อย่างไรก็ตามฟิล์มที่ได้ก็จะให้ค่าการซึมผ่านไอน้ำที่ลดลง ซึ่งตรงกันข้ามกับฟิล์มที่เติมกลีเซอรอลและพอลิเอทิลีนไกลคอลเป็นพลาสติไซเซอร์ที่มีลักษณะโครงสร้างของฟิล์มที่ยืดหยุ่น ขณะที่ค่าการต้านทานแรงดึงสูงสุดของฟิล์มที่ได้มีค่าต่ำ ส่วนค่าการซึมผ่านไอน้ำมีค่าสูง และเมื่อพิจารณาผลของปริมาณของพลาสติไซเซอร์ต่อสมบัติของฟิล์ม พบว่า เมื่อปริมาณพลาสติไซเซอร์เพิ่มขึ้น ค่าการต้านทานแรงดึงสูงสุดมีค่าลดลง ในขณะที่ค่าร้อยละการยืดตัวเมื่อขาดและค่าการซึมผ่านไอน้ำเพิ่มขึ้น จากการทดลองพบว่าฟิล์มที่ใช้ซอร์บิทอลเป็นพลาสติไซเซอร์จะให้ค่าการละลายของฟิล์มและค่าการละลายของโปรตีนจากฟิล์มมีค่าสูงกว่าฟิล์มใช้กลีเซอรอลและพอลิเอทิลีน ไกลคอลเป็นพลาสติไซเซอร์ จากการศึกษาพบว่าค่าสีของฟิล์มขึ้นอยู่กับชนิดของพลาสติไซเซอร์มากกว่าปริมาณของพลาสติไซเซอร์ที่เติมลงไป เมื่อศึกษา

ผลของชนิดและปริมาณของไขมันต่อสมบัติต่างๆ ของฟิล์ม พบว่า ค่าการต้านทานแรงดึงสูงสุดและค่าการซึมผ่านไอน้ำของฟิล์มบริโภคได้จากโปรตีนถั่วเขียวและถั่วแดงมีค่าลดลงเมื่อเติมไขมันลงไปในแผ่นฟิล์ม ในขณะที่ค่าร้อยละการยืดตัวเมื่อขาดมีค่าที่เพิ่มขึ้น และการเติมไขมันลงไปในสารละลายฟิล์มจะมีผลต่อค่าสีของฟิล์มอย่างมีนัยสำคัญ โดยที่พบว่าเมื่อปริมาณไขมันเพิ่มขึ้นแผ่นฟิล์มที่ได้จะมีสีที่เข้มขึ้น โดยพบว่าการเติมกรดโอเลอิคในฟิล์มจะให้ค่า L* และ b* ที่สูงกว่าและให้ค่า a* ที่ต่ำกว่าเมื่อเทียบกับกรดเสตียริคและน้ำมันปาล์ม และพบว่าพื้นผิวหน้าของฟิล์มที่เติมกรดโอเลอิคลงไปจะเรียบกว่าและฟิล์มที่ได้จะให้ค่าการต้านทานแรงดึงสูงสุดและค่าร้อยละการยืดตัวเมื่อขาดที่สูงกว่า ขณะที่ค่าการซึมผ่านไอน้ำที่ต่ำกว่าฟิล์มที่เติมไขมันชนิดกรดเสตียริคและน้ำมันปาล์ม ตามลำดับ

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ABSTRACT

Edible film from mung bean and red bean proteins were developed. The effect of pH, heating temperature and heating time on the properties of edible film from mung bean and red bean proteins were investigated using Response Surface Methodology (RSM). The effect of pH and heating temperature of film solutions were more significant, overall, on the film's properties than heating time. Tensile strength (TS) and elongation at break (%E) were increased when pH and heating temperature and heating time of film solutions increased, while water vapor permeability (WVP), film solubility (FS) and protein solubility (PS) decreased. Film color was darker and more yellowish with increase in the pH and heating temperature of film solution. The effect of protein concentration on the properties of edible film from mung bean and red bean protein were determined. The results showed that increasing of the protein concentration resulted in a higher TS and %E, but lowered of WVP, FS and PS. Type and concentration of plasticizer significantly ($p<0.05$) affected the mechanical and barrier properties of the protein films. Sorbitol (SOR) plasticized films were the most brittle and TS was the highest; however, its effect on WVP was low. In contrast, glycerol (GLY) and polyethylene glycol (PEG) plasticized films exhibited flexible structure, even though, the TS was low, resulting in increased WVP. As plasticizer concentration increased, TS decreased concomitant with increase in %E and higher WVP. SOR plasticized films, showed higher FS and PS compared to GLY and PEG plasticized films. Increasing the plasticizer concentration, overall, resulted in both higher FS and PS. The color of films was more affected by the nature of the plasticizer used than by its concentration. TS and WVP of edible film from mung bean and red bean proteins decrease with the addition of lipids, where as %E was in increased in these films. Addition of lipids significantly increased film yellowness for protein films. The results showed that films added with oleic acid gave higher L* and

b* values but lower a* value than stearic acid and palm oil. Oleic acid incorporated films provided the films with smoother surface and higher both TS and %E but lower WVP than stearic acid and palm oil respectively.

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CHAPTER 1

INTRODUCTION

Packaging is a necessary step for preserving the organoleptic, nutritional and hygienic characteristics of food during storage and commercialization. The wide variety of packaging films can be divided into synthetic and edible or biodegradable. Constant progress in the technology of synthetic film preparation has expanded and supported their utilization in the food industry. However, most synthetic films are petrochemical-based and non-biodegradable, it takes a several hundred years to degrade petroleum-based synthetic plastics, which have caused serious solid waste contamination in the world (Park *et al.* 2001). In contrast, edible films use renewable resources as raw materials and are biodegradable, making them more compatible with the environment. Additionally, other adjuncts such as antimicrobials, antioxidants, nutrients, colorants, etc. are easier to add to edible films, thus further enhancing their protective functions. Edible films can be prepared from protein, polysaccharide and lipid materials (Parris *et al*, 1995). Among them, proteins-based edible films are the most attractive. These films have impressive gas barrier properties compared with those prepared from lipids and polysaccharides. When they are not moist, the O_2 permeability of soy protein-based film was 500, 260, 540 and 670 times lower than that of low-density polyethylene, methylcellulose, starch and pectin respectively (Cuq *et al*, 1998). The mechanical properties of protein-based edible films are also better than that of polysaccharide and fat-based films because proteins have a unique structure (based on 20 different monomers) which confers a wider range of functional properties, especially a high intermolecular binding potential (Cuq *et al.*, 1995). Protein-based edible films can form bonds at different positions and offer high potential for forming numerous linkages (Ou *et al.*, 2004). The interest in the study of plant protein films has increased during the past decade, and research on the properties of such films has been outlined in recent literature including soy proteins (Gennadios and Weller,1991; Gennadios et al., 1994; Stuchell and Krochta, 1994; Kunte et al., 1997; Rhim et al., 2000), corn zein (Yamada et al., 1995; Parris and Coffin, 1997), wheat proteins (Gennadios and Weller, 1990;

Gennadios et al., 1993; Gontard et al., 1992, 1993; Sanchez et al., 1998), cotton seed proteins (Marqui´e et al.,1995), pea proteins (Gu´eguen et al., 1998), peanut protein (Jangchud and Chinnan, 1999), and sunflower proteins (Orliac et al., 2002, 2003). Mung bean and red bean are of interest as a potential component of biopolymeric films because of their high protein content. The whole seed of mung beans and red beans contain approximately 25-30% of protein (Magee, 1996). Mung bean and red bean are the primary crop produced in the Thailand. In industrial mung bean starch-noodle manufacturing process, mung bean starch is washed with alkaline solution to remove proteins to produce a colorless and characteristic of noodle. As results of washing, approximately 20-30% of proteins are loss in the process. Using mung bean protein from cannot only reduce the negative environment impact and costs of waste disposal, but may generate potential profits especially in the form of edible film from mung bean proteins. At the moment, there is very limit information on films produced from mung bean and red bean proteins, their mechanical properties and applications is due to the unavailability of commercial mung bean protein concentrate. It is important to have knowledge of suitable film forming method, the effect of film forming parameters, and film characteristics for further research into their food application.

Review of Literature

1. Edible Films and Coatings

Edible films are defined as thin layer of material which can be eaten by the consumer and provide a barrier to moisture, oxygen and solute movement for the food. The material can complete food coating or can be disposed as a continuous layer between food components (Guilbert, 1986). Edible films can be formed as food coatings and free-standing films, and have potential to be used with food as gas aroma barrier (Kester and Fennema, 1986). However, the technical information is still needed to develop films for food application (Donhowe and Fennema, 1993). The edible films and coatings have received a consideration attention in the recent years because of their advantage over the synthetic films. The advantages of edible films over other traditional synthetic films are summarized below:

1. They can be consumed with the package products. This is obviously of critical importance since it represents the environmentally ideal package.

2. There is no package to dispose of even if the films are not consumed they could still contribute to the reduction of environmental pollution.

3. The films are produced exclusively from renewable, edible ingredients and therefore are anticipated to degrade more readily than polymeric materials.

4. The films can enhance the organoleptic properties of packaged foods provided that various components (flavorings, colorings, sweeteners).

5. The films can supplement the nutrition value of the foods. This is particular true for films made from proteins.

6. The films can be used for individual packaging of small portion of food, particularly products that currently are not individually packaged for practical reasons such as pears, beans, nuts and strawberries.

7. The films can be applied inside heterogeneous foods at the interfaces between different layers of components. They can be tailored to prevent deteriorative intercomponent moisture and solute migration in foods such as pizzas, pies and candies.

8. The films can function as carriers for antimicrobial and antioxidant agents. In a similar application they also can be used at the surface of food to control the diffusion rate of preservative substances from the surface to the interior of the food.

9. The films can be very conveniently used for micro encapsulation of food flavoring and leavening agents to efficiently control their addition and release into the interior of foods.

10. Another possible application for edible films could be their use in multilayer food packaging materials together with non edible films. In this case, the edible films would be the internal layers in direct contact with food materials.

Production of edible films causes less waste and pollution, however, their permeability and mechanical properties are generally poorer than synthetic films (Kester and Fennema, 1986). Extensive research is needed on the development of

new materials, methods of films formation, methods to improve film properties and the potential applications.

2. Classification of Edible Films and Coatings

Edible films can be produced from materials with film forming ability. During manufacturing, film materials must be dispersed and dissolved in the solvent such as water, alcohol or mixture of water and alcohol or mixture other solvents. Plasticizer, antimicrobial agent, colors or flavor can be added in this process. Adjusting pH and/or heating the solutions may be done for the specific polymer to facilitate the dispersion. Film solution is then casted and dried at desired temperature and relative humidity to obtain free-standing films. In the food application, film solutions could be applied to food by several methods such as dipping, spraying, brushing and panning followed by a drying step. Kester and Fennema (1986) classified the edible films based on the nature of material as polysaccharide, protein, lipid and composite films.

2.1 Polysaccharides

Polysaccharides used for edible films or coatings include cellulose and derivatives starch and derivatives pectin, seaweed extracts, exudate gums, microbial fermentation gums, chitosan (Krochta and Mulder-Johnson, 1997). Polysaccharides are very generally very hydrophilic resulting in poor water vapor and gas barrier properties. Although coating by polysaccharide polymers may not provide a good water vapor barrier, these coating can act as sacrificing agent retarding moisture loss from food products (Kester and Fennema, 1986).

2.1.1 Cellulose and Derivative

Cellulose is composed of repeating D-glucose units linked through ß-1, 4 glycosidic bonds. In its native state, the hydroxymethyl groups of anhydroglucose residues are alternatively located above and below the plan of the polymer backbone. This results in very tight packing of polymer chains and a highly crystalline structure that resists salvation in aqueous media. Water solubility can be increased by treating

cellulose with alkali to swell the structure, followed by reaction with chloroacetic acid, methyl chloride or propylene oxide to yield carboxy methyl cellulose (CMC), methyl cellulose (MC), hydroxy propyl cellulose (HPMC) or hydroxyl propyl cellulose (HPC). Placement of bulky substituents along the cellulose molecule, in the form of ether linkages at reactive hydroxyls, separates the polymer chains and interferes with formation of the crystalline unit cell, thereby enhancing aqueous solubility (Krumel and Lindsay, 1976). MC, HPMC, HPC and CMC film possess good film-forming characteristic; films are generally odorless and tasteless, flexible and are of moderate strength, transparent, resistance to oil and fats, water-soluble, moderate to moisture and oxygen transmission (Krochta and Mulder-Johnson, 1997). MC is the most resistant to water and it is the lowest hydrophilic cellulose derivatives (Kester and Fennema, 1986); however, the water vapor permeability of cellulose ether film is still relatively high. MC and HPMC have ability to form thermally induced gelatinous coating; they have been used to retard oil absorption in deep frying food product (Kester and Fennema, 1986; Balasubramanium *et al.* 1997). MC could be applied as coating on confectionery products as barrier to lipid migration (Nelson amd Fennema, 1991). A number of groups have investigated composite films composed of MC or HPMC and various kinds of solids, such as beeswax and fatty acids (Debeaufort *et al.* 1993; Greener and Fennema, 1989; Kamper and Fennema, 1984; Kester and Fennema, 1989; Koelsch and Labuza, 1992; Park *et al.* 1994). Many of these have water vapor permeability as low as low density polyethylene (LDPE). These composite films were all polymer-lipid bilayer formed either in one step from aqueous ethanolic solutions of cellulose ether fatty acids.

Cellulose can also be chemically modified to ether, ethyl cellulose (EC), which is biodegradable but not edible. EC films can either be cast from non-aqueous solutions or extruded. Like the other cellulose ethers. EC films are poor moisture barrier, but they have been reported to be good oil and fat barriers (Hanlon, 1992).

2.1.2 Pectin

Pectin is a complex group of structural polysaccharides found in the middle lamella of plant cells. It is composed mainly of (1, 4) α-D-

galactopyranosyluronic acid units with varying degrees of esterification (DE). Chemical de-esterification yields low-methoxyl pectins which when dissolved in aqueous media are capable of forming gels in the presence of calcium ions (Schultz *et al.* 1948). The function of the ionic calcium is to bridge free carboxyl group on adjacent polymer molecules (Morris, 1986). Pectin with DE more than 50% is classified as a high-methoxyl, and lower than 50% is classified as a low methoxyl pectin (deMan, 1990). Pectin may be used to form film alone or blended with other polymers (Coffin and Fishman, 1993). These films or coatings give a glossy, non sticky surface, high water vapor permeability. Although pectinate coatings are poor in moisture barrier, they can reduce the moisture loss from food product by acting as sacrificing (Kester and Fennema, 1986).

2.1.3 Chitin and Chitosan

Chitin is the second most abundant naturally occurring biopolymer (after cellulose) and it found in the exoskeleton of crustaceans, in fungal cell walls and other biological materials (Andrady and Xu, 1997). It is mainly poly (ß-(1-4)-2-acetamide-D-glucose), which is structurally identical to cellulose except that secondary hydroxyl on the second carbon atom of the hexose repeat unit is replaced by an acetamide group. Chitosan is derived from chitin by deacetylation in the presence of alkali. Therefore, chitosan is a copolymer consisting of (ß-(1-4)-2-acetamido-D-glucose and (ß-(1-4)-2-acetamide-D-glucose units with the latter usually exceeding 80%. Chitosans are described in terms of the degree of deacetylation and average molecular weight and their important resides in their antimicrobial properties in conjunction with their cationicity and their-forming properties (Muzzarelli, 1996). Chitosan can form semi-permeable coatings, which can modify the internal atmosphere, thereby delaying ripening and decreasing transpiration rates in fruits and vegetables. Films from aqueous chitosan are clear, tough, flexible and good oxygen barriers (Sandford, 1989; Kalplan *et al.* 1993). Carbon dioxide permeability could be improved by methylation of polymer. Butler and his cooperators (Butler *et al.* 1996) observed that films from chitosan were rather stable and mechanical and barrier properties changed only slightly during storage. Chitosan coatings are usually used on fruit and vegetable products such as strawberries, cucumbers, bell peppers as

antimicrobial coating (El Ghaouth *et al.* 1991a, 1991b), and on apples, pears, peaches and plums as gas barrier (Elson and Hayes, 1985; Davies *et al*, 1989).

2.1.4 Starch

Starch consists of amylose and amylopectin, the ratio of amylose and amylopectin depends on the type and variety of raw material. Amylose is a linear chain of D-glucose residues linked through α-1,4 glycosidic bonds. Amylopectin is a branched molecule consisting of glucose units connected by α-1,4 and α-1,6 linkages (Whistler and Daniel, 1985). High amylose starch as cornstarch is a good source for films formation, free-standing films can be produced from aqueous solution of gelatinized amylose and drying. Normal cornstarch consists of approximately 25% amylose and 75% amylopectin. Mutant varies of corn are produced which contain starch with up to 85% amylose (Whistler and Daniel, 1985). Wolf *et al.* (1951) produced self-supporting films by casting aqueous solutions of gelatinized amylose, followed by solvent evaporation. The films were transparent and had very low permeability to oxygen at low RH (Rankin *et al.* 1958). Mark *et al.* (1966), in fact, reported that films produced from high amylose corn starch (71% amylose) had no detectable oxygen permeability at RH levels less than 100%. This was true for both unplasticized and plasticized (16% glycerol) films. This result is surprising in light of the fact that addition of plasticizers and absorption of water molecules by hydrophilic polymers increase polymer chain mobility and generally lead to increased gas permeability (Banker, 1966). Partial etherification of high-amylose starch with propylene oxide, to yield the hydroxypropylated derivative, improves water solubility. As expected, films produced from hydroxypropylated starch possess virtually no resistance to the passage of water vapor: however, as with the pure amylose films, resistance to oxygen transport is substantial (Jokay *et al.* 1967). Oxygen permeation through plasticized and unplasticized films was not detectable at 25 °C and RH up to 78% (Roth and Mehltretter, 1967). At the high RH, films became distorted due to the moisture absorption and were not tested for oxygen permeability; however, it is likely that oxygen transport increased greatly as the film became hydrate. Jokay *et al.* (1967) applied hydroxylpropylated starch films almond nutmeats, and organoleptic evaluation revealed that the film retarded development of oxidative rancidity during

storage. Starch hydrolysates (dextrin) of low dextrose equivalent (DE) have been suggested for use as protective coatings. Although hydrophilic in nature, starch hydrolyses do provide a limited resistance to transport of water vapor. Allen *et al.* (1963) evaluated the relative barrier properties of edible film materials by coating them onto a cellulose acetate support. Starch films displayed minimal resistance to water transport, while films of low-DE dextrin and corn syrup were approximately 2- and 3-fold more resistant, respectively. Murray and Luft (1973) coated almond nutmeats with a 50% solution of a 10-DE starch hydrolysate. Sensory evaluation indicated that the coated nuts maintained a more desirable texture than uncoated controls during storage. Presumably, this was attributation to a reduction in the rate of moisture absorption by coated almonds. Films of starch hydrolysates may exhibit some resistance to oxygen transmission. Dipping of fresh sliced apples in a 40% solution of a 15-DE hydrolysate prior to dehydration prevented browning of the tissue, probably by retarding the entrance of oxygen (Murray and Luft, 1973).

2.1.5 Seaweed and Gum Polymers

Alginate, carrageenan and agar are seaweed products and have good film forming characteristic. Alginate is the salt of alginic acid, a linear $(1 \rightarrow 4)$ linked polyuronic acid extracted from brown seaweed. Film formations, which may or not involve gelation, can be achieved by evaporation, electrolyte crosslinking, or injection of a water-miscible nonsolvent for alginate (Kelco, 1976). Alginate coating posses good oxygen and lipid barrier but poor water vapor barrier properties (Cottrell and Kovacs, 1980; Conca and Yang, 1993). Additionally, coating with alginate can improve flavor, texture and batter adhesion. Carrageenan is an extract from red seaweed which consists of a family of sulfated polysaccharides of D-galactose and 3, 6-anhydro-D-galactose. Upon cooling a warm aqueous solution of the polymer, gelation occures, presumably by the formation of a double-helix structure to yield a three-dimensional polymer network. In addition, since gelation is salt-specific, interchain salt bridges must be important (Glicksman, 1983). Coating with carrageenan has been used in food to incorporate antimicrobial agents, and reduce moisture loss, oxidation or disintegration (Krochta and Mulder-Johnson, 1997).

2.2 Lipid Films

Lipid compounds utilized as protective coating consist of acetylated monoglycerides, nutural wax, and surfactants. The most effective lipid substances are paraffin wax and beeswax. The primarily function of lipid coating is to block transport of moisture due to their relative low polarity. In contrast, the hydrophobic characteristic of lipid forms thicker and more brittle films. Consequently, they must be associated with film forming agents such as proteins or cellulose derivatives (Polo *et al.* 1992). Generally, water vapor permeability decrease when the concentration of hydrophobicity phase increases. Lipid-based films are often supported on a polymer structure matrix, usually a polysaccharide, to provide mechanical strength.

2.2.1 Waxes and Paraffin

Paraffin wax is derived from distillate fraction of crude petroleum and consists of mixture of solid hydrocarbon resulting from ethylene catalytic polymerization. Paraffin wax is permitted for use on raw fruit and vegetable and cheese. Carnauba wax is exudates from palm tree leaves (*Copoernica cerifera*). Beewax (white wax) is produced from honeybees. Candelilla is obtained from candelilla plant. Mineral oil consists of a mixture of liquid paraffin and naphtheric hydrocarbon (Hernandez, 1994). Waxes are used as barrier films to gas and moisture (skin on fresh fruits) and to improve the surface appearance of various foods (e.g., the sheen on sweet). Applied in a thick layer, they must be removed before consumption (certain cheese); when used in thin layers, they are considered edible. Waxes (notably paraffin, carnauba, candellila and bee wax) are the most efficient edible compounds providing a humidity barrier.

2.2.2 Acetoglyceride

Acetylation of glycerol monosterate by its reaction with acetic anhydride yields 1-stearodiacetin. This acetylated monoglyceride displays the unique characteristic of solidifying from the molten state into a flexible, wax-like solid (Feuge *et al.* 1953). Most lipids in the solid state can be stretched to only about 102% of their original length before fracturing. Acetylated glycerol monostearate, however,

can be stretch up to 800% of its original length (Jackson and Lutton, 1952), water vapor permeability of this film is much less than that of polysaccharide film with the exception of methyl cellulose or ethyl cellulose (Kester and Fennema, 1986). Acetylated monoglyceride coating have been used on poultry and meat cuts to retard the moisture loss during storage (Kester and Fennema, 1986).

2.2.3 Resins and Rosins

2.2.3.1 Shellac Resins

Shellac resins are a secretion by the insect *Laccifer lacca* it is composed of a complex mixture of aliphatic alicyclic hydroxyl acid polymers. This resin is soluble in alcohols and in alkaline solutions. Shellac is not a GRAS substance; it is only permitted as an indirect food additive in food coatings and adhesives. It is mostly used in coating for the pharmaceutical industry, a few work has been reported on foods (Hernandez, 1994). Rosins are obtained from the oleoresins of the pine tree, is a residue left after distillation of volatile from the crude resin. Resin and it derivatives are widely used in coating for citrus and other fruits (Sward, 1972).

2.2.3.2 Surfactants

Coating of foods with surface-active agents (16-18 carbon fatty alcohol) have been used for coating foods to reduce superficial a_w and rate of moisture loss by evaporation. Furthermore, thin film of surface–active agents (lecithin, hydroxylate lecithin or tweens) tend to inhibit undersirable light-induced greening (chlorophyll) synthesis in potato tubes.

2.3 Protein Films

In their native states, proteins generally exist as either fibrous proteins, which are water insoluble and serve as the main structural materials of animal tissues, or globular proteins, which are soluble in water or aqueous solutions of acids bases or salts and function widely in living system (Morrison and Boyd, 1959). The fibrous proteins are fully extended and associated closely with each other in parallel

structures, generally through hydrogen bonding, to form fibers. The globular proteins fold into complicated spherical structures held together by a combination of hydrogen, ionic, hydrophobic and covalent (disulfide) bonds (Bushuk and Wrigley, 1974). The chemical and physical properties of these proteins depend on the relative amounts of the component amino acid residues and their placement along the protein polymer chain. Of the fibrous proteins, collagen has received the most attention in the production of edible films. Several globular proteins, including wheat gluten, corn zein, soy protein, and whey protein, have been investigated for their film properties. Protein films are generally formed from solutions or dispersions of the protein as the solvent/carrier evaporates. The solvent/carrier is generally limited to water, ethanol or ethanol-water mixtures (Kester and Fennema, 1986). Generally, proteins must be denatured by heat, acid, base, and/or solvent in order to form the more extended structures that are required for film formation. Once extended, protein chains can associate through hydrogen, ionic, hydrophobic and covalent bonding. The chain-to-chain interaction that produces a cohesive films are affected by the degree of chain extension and the nature and sequence of amino acid residues. Uniform distribution of polar, hydrophobic, and/or thiol groups along the polymer chain increase the likelihood of the respective interactions. Increased polymer chain-to-chain interaction results in films that are stronger but less flexible and less permeable to gases, vapors and liquids (Kester and Fennema, 1986). Polymers containing groups that can associate through hydrogen or ionic bonding result in films that are excellent oxygen barrier but that are susceptible to moisture (Salame, 1986). Thus, protein films are expected to be good oxygen barriers at low relative humidities. Polymers containing a preponderance of hydrophobic groups are poor oxygen barriers but excellent moisture barriers. The more hydrophobic, water-insoluble proteins. However, the fact that they are not totally hydrophobic and contain predominantly hydrophillic amino acid residues limits their moisture-barrier properties. Creation of protein-versed edible films with low water vapor permeability requires addition of lipid components. This is analogous to the situation with synthetic polymers where moisture- sensitive oxygen-barrier polymers must be either co polymerized with a hydrophobic polymer or sandwiched between hydrophobic polymer layers to limit the ability of water to reduce barrier properties. Various types of protein have been used as edible films.

These include collagen, casein, whey protein, corn zein, wheat gluten, soy protein, mung bean protein, and peanut protein (Gennadois *et al.* 1993).

2.3.1 Collagen Films

Collagen is a fibrous protein generally isolated from hides, tendon, cartilage, bone and connective tissues (Balain and Bowes, 1977). Production of films from animal hides can be accomplished using a dry or wet process with some similarity, including (a) alkaline treatment to dehair and remove collagen from carbohydrates and other proteins, (b) acid swelling and homogenization to form a ~ 4.5% moisture gel (wet process) or ~ 10% moisture gel dough (dry process), (c) extrusion into a tube and (d) neutralization of the extruded tube, washing the tube of salts, treating the tube with plasticizer and cross-linkers and drying to 12-14% moisture, with the order depending on whether the wet or dry process is used (Hood, 1987). Collagen is used to make the most commercially successful edible protein films. Collagen casing have largely replaced natural gut casing for sausage (Hood, 1987). Collagen films are eaten with the meat product after removal of the netting. Besides providing mechanical integrity to meat products, collagen film is generally seen as reducing oxygen and moisture transport (Baker *et al.* 1994).

2.3.2 Gelatin Films

Gelatin, a protein derived from collagen formed by thermally reversible gels when warm aqueous suspensions of the polypeptide are cooled, has good film forming properties. Gelatin films could be formed from gelatin 20-30%, plasticizer (glycerin or sorbitol) 10-30% and water 40-70% followed by drying the gelatin gel (Guilbert, 1986). Gelatin is used to encapsulate low moisture or oil phase food ingredients and pharmaceuticals. Such encapsulation provides protection against oxygen and light, as well as defining ingredient amount or drug dosage (Gennadios *et al.* 1994). In addition, gelatin films have been formed as coatings on meats to reduce oxygen, moisture and oil transport (Gennadios *et al.* 1994). For example, gelatin coating formed on cut poultry before freezing reduced the amount of rancidity

developed during storage (Klose *et al.* 1952). The effect was enhanced by adding an antioxidant to the coating.

2.3.3 Corn Zein Films

Corn zein is prolamin fraction (soluble in 70% ethanol) of corn gluten, making up approximately 70% of the corn gluten. Differential solubility in aqueous ethanol yields two zein fractions. A low content of polar amino acid and high content of non-polar amino acids (leucine, alanine and proline) make corn as in anhydrous alcohol (Gennadios *et al.* 1994). Edible film can be formed by drying aqueous ethanol solution of zein (Gennadios and Weller, 1990). Formation of films is believed to involve development of hydrophobic, hydrogen and limited disulfide bonds between zein chains in the film matrix (Gennadios *et al.* 1994). The resulting films are brittle and therefore require plasticizer addition for increasing flexibility (Park, 1991). Zein films are relatively good water vapor barriers compare to other edible film (Guilbert, 1986). Water vapor barrier properties can be improved by adding fatty acids or by using a cross-linking reagent. But when cross-linking agent was used, the edibility of those films was concern (Alikonis, 1979). Food application of corn zein-acethylated monoglyceride is seen in confectionaries and nut products as oxygen and lipid barrier (Alikonis, 1979; Alikonis and Cosler, 1961) and in intermediate moisture foods to delay the diffusion of antimicrobial chemical (sorbic acid) (Torres, 1987). Zein coating have also been used to coat vitamin-enriched rice, for protecting vitamins from loss (Mackus, 1955). Zein and zein-based coating formulations are markedly commercially for these food-uses related pharmaceutical applications (Andres, 1984). Zein coating have also shown an ability to reduce moisture and firmness loss and delay color change (reduce oxygen and carbon dioxide transmission) in fresh tomatoes (Park *et al.* 1994a). Zein has also been explored as a replacement for collagen in the manufacture of sausage casting (Turbak, 1972) and for the production of water-soluble pouches for dried food (Georgevits, 1967).

2.3.4 Wheat Gluten Films

Wheat gluten is generally term for water-insoluble proteins of wheat flour is composed of a mixture of polypeptide molecules, considered to be globular proteins. Cohesiveness and elasticity of gluten give integrity to wheat dough and facilitate film formation. Wheat gluten contains the prolamine and glutelin fractions of wheat flour proteins, typically referred to as gliadin and glutenin, respectively. While gliadin is soluble in 70% ethanol, glutenin is not (Gennadios and Weller, 1990). Although insoluble in natural water, wheat gluten dissolves in aqueous solutions of high or low pH at low ionic strength (Krull and Inglett, 1971). Edible films can be formed by drying aqueous ethanol solution of wheat gluten (Gennadios and Weller, 1990). Cleavage of native disulfide bonds during heating of film-forming solutions and then formation of new disulfide bonds during film drying are believed to be important to the formation of wheat gluten films structure, along with hydrogen and hydrophobic bonds (Gennadios and Weller, 1990). Addition of plasticizer such as glycerin in gluten films is necessary to improve film flexibility (Gennadios *et al.* 1994). However, increasing film flexibility by increasing sorbitol content reduces film strength, elasticity and water vapor barrier properties (Gontard *et al.* 1992). A review on the field of gluten film was published by Gennadios and Weller (1990). A summary of research results on wheat gluten films formation is presented below.

Gennadios and Weller (1992) confirmed the earlier studies of Wall and Beckwith (1969) on the effect of wheat gluten purity on film's appearance and mechanical properties, i.e., a greater purity gluten results in a stronger and clearer films. However, cost of additional purification steps must be considered. Anker *et al.* (1972) adjusted pH for wheat gluten dispersion in alcohol-water mixture by using volatile alkali such as ammonia, to produce neutral pH films. Herald *et al.* (1995) investigated the effect of plasticizer size of wheat gluten; a film prepared from spray-dried wheat gluten was stronger than films from flash-dried which had larger size particles. When used as a coating on grade A-quality shell eggs, the egg quality was maintained for 30 days. Tensile strength of gluten films can be improved by using a cross-linking agent such as glutaraldehyde, or heat curing at 80 °C (Gennadios and Weller, 1992; Kolster *et al.* 1992).

2.3.5 Soy Protein Films

The protein content of soybeans (38-44%) is much higher than the protein content of cereal grain (8-15%). Most of protein in soybeans is insoluble in water but soluble in dilute neutral salt solutions. Thus, soy protein belongs to the globulin classification (Kinsella, 1979). Soy protein is globular in nature and is further classified into 2S, 7S, 11S and 15S fraction according to relative sedimentation rates (Gennadios *et al.* 1994). The principal components are the 7S (conglycinin) and 11S (glycinin) fractions, both of which have a quaternary (subunit) structure (Kinsella *et al.* 1985). Soy protein is high in asparagine and glutamine residues. Both conglycinin and glycinin are tightly folded proteins. While the extent of disulfide cross-linking of conglycinin is limited due to only two to three cysteine groups per molecule, glycinin contains 20 intramolecular disulfide bonds (Kinsella, 1979). Alkali and heating both cause dissociation and subsequent unfolding of glycinin due to disulfide bond cleavage (Kinsella, 1979). Edible films based on soy protein can be produced in either of two ways: surface film formation on heated soymilk or film formation from solutions of soy protein isolate (SPI) (Gennadios and Weller, 1991). Soymilk is produced by grinding soybeans with water followed by separation of milk from extracted soybeans. To form films from both soymilk and SPI, (a) heating of film solutions to disrupt the protein structure, cleave native disulfide bonds and expose sulfhydryl groups and hydrophobic groups, and then (b) formation of new disulfide, hydrophobic and hydrogen bonds during film drying are believed to be important to the formation of soy protein film structure (Gennadios *et al.* 1994). The use of soy protein in the formation of films or coatings on food products has been investigated (Gennadios *et al.* 1994; Baker *et al.* 1994). Soy protein concentrate and SPI have been used successfully to aid batter adhesion and encase meat fibers to aid flavor retention. Soy protein-based coatings showed limited ability to reduce moisture migration in raisin and dried peas (Bolin, 1976).

2.3.6 Casein Films

Milk proteins are classified into two types: casein and whey protein. Casein consists of three principal components, α, ß, and κ, which together form

colloidal micelles in milk containing large numbers of casein molecules and are stabilized by calcium-phosphate bridge (Kinsella, 1984). The casein molecules possess little defined secondary structure, exhibiting instead an open random-coil structure. Casein, which comprises 80% of milk protein, precipitates when skim milk is acidified to the casein isoelectric pH of approximately 4.6 (Dalgleish, 1989). Acidification of solubilizes the calcium phosphate, thus releasing individual casein molecules, which associate to form insoluble acid casein. The acid casein can be converted to functional soluble caseinates by neutralization through addition of alkali. Sodium and calcium caseinates are most common, but magnesium and potassium caseinates are also available commercially (Kinsella, 1984). Edible films based on various caseinates can be obtained by solubilization in water followed by casting and drying. Caseinates form films from aqueous solution without heat treatment due to their random coil nature. Interactions in the film matrix likely include hydrophobic, ionic, and hydrogen bonding (Avena-Bustillos and Krochta, 1993). Glycerin-plasticized caseinate films are transparent and flexibility, but have poor water barrier properties. Treatment with lactic acid or tannic acid improved water barrier properties (Guilbert, 1986). At comparable test conditions, caseinate films appear to be similar moisture barriers to wheat gluten films and soy protein films and somewhat poorer moisture barriers than corn zein films (Avena-Bustillos and Krochta, 1993). Casein has been investigated in the formation of films and coatings on food products (Gennadios *et al.* 1994). Laminated films that included casein protected dried fruit and vegetables from moisture absorption and oxidation. Caseinate-lipid emulsion coatings were successful in reducing moisture loss from peeled carrot and zucchini (Avena-Bustillos *et al.* 1993).

2.4 Composite Films

Edible films and coatings may be heterogeneous in nature, consisting of a blend of polysaccharides, protein, and/or lipids. This approach enables one to advantageously utilize the distinction functional characteristics of each class of film former (Kester and Fennema, 1986). The combination between polymers to form films could be from proteins and carbohydrates, proteins and lipids, carbohydrates and lipids or synthetic polymers and natural polymers. The main objective to produce

composite films is to improve the permeability or mechanical properties as dictated by the need of a specific application. These heterogeneous films are applied either in the form of an emulsion, suspension, or dispersion of the nonmiscible constituents, or in successive layers (multilayer coating or films), or in the form of a solution in a common solvent. The method of application affects the barrier properties of the films obtained (Guilbert, 1986).

Schultz *et al.* (1948) incorporated lipids into low-methoxy pectinate films to improve resistance to water vapor permeation. Kamper and Fennema (1984) introduced the emulsion films from methyl cellulose and fatty acid to improve water vapor barrier of cellulose films. Recently, many researchers have extensively explored the development of composite films based on the work of Kamper and Fennema (1984). Examples of these studies are using lipid and hydroxypropyl methyl cellulose (Hagenmaier and Shaw, 1990), methyl cellulose (MC) and lipid (Greener and Fennema, 1989b), MC and fatty acid (Sapru and Labuza, 1994), corn zein, MC and fatty acid (Park *et al.* 1996), whey isolate and lipids (McHugh and Krochta, 1994), and casein and lipids (Avena-Bistillos, 1993). Rico-Pena and Torres (1990) used methyl cellulose-palmitic acid films as moisture barrier on sundae ice creams cones. Coated sample could store for 10 weeks without moisture loss at -23 ^{o}C , for 4 weeks at -12 ^{o}C.

Shih (1994) formed films, using soy protein isolate and carbohydrate (sodium alginate and propylene glycol alginate), by deposition method drying at 50 ^{o}C for 15 min. The solubility and emulsifying activity were maintained or improved. Films with alkylation process showed better film-making properties.

Holton *et al.* (1994) combined polyethylene with 6% of corn starch. The corn-starch containing polyethylene package provided protection equal to that made of polyethylene.

3. Factor Affecting Films Formation and Films Properties

3.1 Type of Material

Raw materials used in film solutions are classified, according to their solubility characteristics, into two categories-hydrophilic and hydrophobic.

Hydrophilic materials such as soy protein isolate, whey protein isolate and water soluble fish protein are water soluble where as hydrophobic materials such as corn zein, wax are water-insoluble but they dissolve in non-polar such as alcohol. The difference in soluble properties of these raw materials influence the amount of energy needed to obtain dried films and their use on foods. Carbohydrates such as alginate, carregeenan, pectin, starch, cellulose and cellulose derivatives provide a strong matrix free standing films, but these films are poor water barrier properties because hydrophilic nature of raw materials used (Kester and Fennema, 1986). Proteins provide a good gas barrier but poor water vapor barrier properties, however, some protein films such as corn zein films exhibit better water resistance than protein films because zein contains high amount of hydrophobic side chain amino acid. Lipid films, made from hydrophobic materials such as wax, fatty acid, show excellent water vapor barrier but poor mechanical properties.

3.2 Polymer Chemistry

The regular structure molecule is more diffusible than irregular stereochemical structure where as branched molecule may provide a greater cohesive strength than non-branched molecule. Lower molecular weight fraction show a greater cohesion and a greater change in cohesion with temperature change. In highly polar polymer such as protein and cellulosic, self-adhesion by diffusion is not significant due to the minimal flexibility and fixed order of the macromolecule caused by the internal molecular forces holding the polymer chains. Cellulosics have a rigid ring structure chain back bone whereas proteins tend to form helical chain structure (Banker, 1966).

Kinsellar and Phillips (1989) summarized the desired molecular characteristic of proteins for films formation: 1) high soluble molecules promote rapid diffusion; 2) the large molecules allow more interactions at the interface resulting in strong film; 3) amphiphatic molecules provide unbalanced distribution of charged and apolar residuals for improved interfacial interaction; 4) flexible domains facilitate phase behavior and unfolding at interface; 5) dispersion of charged groups affect protein-protein interaction in the films and charge repulsion between neighboring bubbles; 6) polar residue can provide hydratable or charged residues to keep bubbles

apart, binding and retaining water; 7) retention of structure could be enhance overlap and segmental interactions in film; 8) interactive regions can affect depositions of different functional segments and facilitate secondary interactions in the air, and aqueous phases.

3.3 pH

pH plays an important role in protein films made from water-soluble materials, such as soy protein isolate and whey protein isolate, as solubility of these proteins depend on their isoelectric point (pI). During the dissolution of macromolecular substance, the cohesive forces between the solute macromolecules are neutralized by unions with the solvent molecules (Banker, 1966). The functionality of the polymer is related to solution properties which further influences film characteristics. The charge groups repel each other and produce a stretching of the polymer chain when the functional groups on a linear polymer become ionized during dissolution. The greater the degree dissolution and more extensively the chain is charged, the greater is uncoiling of chain. The interaction between the charged polymer molecules and the molecules of the polar solvent increases with increasing charge on the chain (Banker, 1966). The maximum protein solubility is obtained at pH away from its isoelectric point (pI). But to produce an edible film at extreme pH, the sensory property is also considered along with other films properties. Gennadios *et al.* (1993a) studied the effect of pH on soy protein isolate film and found that highly acidic (pH < 1) or alkaline conditions (pH > 12) inhibit soy protein isolate film formation. Kinsella and Phillip (1989) reported that films formed near the isoelectric point of major proteins are more condensed and stronger.

3.4 Casting Temperature

The interaction forces in protein structure are affected by temperature. Hydrophillic interactions increase, hydrogen bonds and electrostatic interaction decrease when temperature increase (Kinsella and Phillips, 1989) resulting in facilitation of adhesion between polymer films and substrate (Banker, 1966). High temperature (70-100 ^{o}C) affected the forming of rigid structure in protein solutions

because of protein denaturation (Chefel *et al.* 1986). The excessive heat or excessive solvent evaporation rate during process may produce non-cohesive films (Guilbert *et al.* 1986). Water soluble proteins such as soy protein, whey protein need a higher temperature and longer time for films formation than films from alcohol-soluble protein such as corn zein or wheat gluten. The higher drying temperature of water-soluble based-films may limit films use. However, low relative humidity can also be employed for film formation at low temperature as has been shown by McHugh and Krochta (1994) in whey protein film formation (40%RH, 23 °C) and McHugh *et al.* (1996) in fruit puree film formation (40% RH, 24°C).

3.5 Concentration

Concentration of film solutions affect the self adhesion of high polymers and rate of matrix forming in film preparations. At low concentration or high concentration, self-diffusion is promoted. At optimum concentration of film solutions, an intermediate viscosity could be obtained which result in the highest cohesive strength (Banker, 1966; Guilbert, 1986)

3.6 Film Additive

Various materials can be incorporated into edible films to influence mechanical, protective, sensory, or nutritional properties. Plasticizer is a major component of edible films. Generally two types of plasticizers are distinguished. Internal plasticization is a result of modifications to the chemical structure of the polymer, for example, by copolymerization or selected hydrogenation or transesterification in the case of edible fats or similar; external plastification is obtained by adding an agent which modifies the structure and energy within the three-dimensional arrangement of the film polymer (Banker, 1966). It is the second method which, on the basis of the type of material and the technology, is mainly used for edible packaging and coatings. A plasticizer may be defined as a compound, which when added to other materials and under given conditions, modifies certain physical and mechanical properties of the material. The addition of a plasticizer to films produces films, which are less likely to break and is more flexible and stronger. The

reduction of the intermolecular bonds between the polymer chains, and thus the overall cohesion, facilitates elongation of the films and reduces its glass transition temperature. This is manifested by a reduction in the barrier properties to gases, vapors, and film solutes (Banker, 1966; Kumins, 1965). A plasticizing agent must be compatible with the film-forming polymer and be permanently present within the solvent–polymer system and under the conditions used. To be compatible, it must be miscible with the polymer, which implies the use of molecular reactions of similar nature. It is important to remember that the formulation of the whole films system (polymer, solvent, plasticizer, and other additives) has a direct effect on the nature and characteristics of the films produced. As a result, the polymer and the plasticizer must not only be compatible, but must also have similar solubility in the solvent used. A soluble plasticizer will be generally sought for the development of soluble coating and an insoluble plasticizer (or dispersible) for an insoluble coatings or for a slow solubilization. The permanence of a plasticizer is also of prime importance since this influence the physical and mechanical stability of the films. The plasticizer should not be volatile (or only very slightly volatile) and its degree of retention by the films should be high. Other properties, such as its chemical stability, hygroscopicity, color, and flavor and so on, are also more or less important depending on the type of films under consideration. In addition, the concentration of a plasticizer necessarily varies from 10-60 % (dry base) according to the nature and type of films and the method of application.

The plasticizers that are most often used in the field of edible coatings and films are the following:

- mono-, di-, and oligosaccharides (generally glucose syrups or glucose-fructose honey)

- polyols (principlly glycerol and its derivatives, polyethylene glycols, sorbitol)

- lipids and its derivatives (fatty acids, monoglycerides and their esters, phospholipids and other emulsifiers).

Plasticizing of hydrophillic polymer-based films will generally be achieved by the addition of a compound belonging to one of the first two groups and

that of a wax-or fat-based film by a compound from the third group.The efficiency, stability, compatibility and permanence of a plasticizing agent can be evaluated by various semi-empiric tests. The final method of plasticization consists of adding to the films system relatively inert solids (fillers which reduce the molecular reactions and cohesion of the final films). The size of these particles and their dispersion are of prime importance. Microcrystalline cellulose, various protein isolates, and cocoa have been used as plasticizers, particularly, in fat–based films. Glycerol and polyethylene glycol were found to be the most effective plasticizers for methyl cellulose, MC, (Donhowe and Fennema, 1993) and hydroxypropyl methyl cellulose, HPMC films (Aulton *et al.* 1981). Park *et al.* (1993) also studied the effect of plasticizer on cellulose based films. Three plasticizer-polyethylene glycol (PEG), propylene glycol (PG), glycerin (G)-at 4 level concentrations (0.17, 0.33, 0.50, 0.66 ml/ g of cellulose) were examined. They found decrease in tensile strength (TS) and increase in elongation (E) with plasticizer increase. PEG was found to be most effective to improve flexibility among glycerin and polyethylene glycol. However, PEG did not affect film's permeability properties. Glycerin did not have effect on oxygen permeability (OP) of cellulose films but affected water vapor permeability (WVP), WVP increased with glycerin increase from 0-0.33 G/g but decreased beyond 0.33ml/g cellulose. Water vapor and oxygen permeability of cellulose films increased with PG increase. Chinnan and Park (1995) observed that increasing PEG from 0 to 0.33 ml/g cellulose increased the WVP of hydroxyl propyl cellulose films. Whereas, in MC films WVP decreased when PEG was increased from 0 to 0.11 ml/g cellulose but WVP increased when PEG was increased from 0.11 to 0.33 ml/g cellulose. In whey protein films, Mahmoud and Savello (1992) reported change in water vapor transmission rate for glycerin level of 0.125 to 2%. McHugh and Krochta (1994b) determined the effect of sorbitol and glycerol on whey protein films. Oxygen permeability was affected by glycerol more than that by sorbitol. Films with sorbitol showed lower oxygen permeability than films with glycerol at equal tensile strength. Tensile strength decreased and elongation increased with plasticizer increase. In pectin films, Coffin and Fishman (1993) found glycerin performed better than urea and PEG. In their study, mechanical properties (elongation and tenacity) improved with increasing glycerin (9 to 19% (w/w)).

Guo (1994) investigated the effect of PEG-600 on sucrose permeability of cellulose acetate films which decreased with increasing plasticizer concentration at low concentration (< 30% (w/w)) but increased dramatically when the concentration of plasticizer increased above 30%. The antiplasticizer effect at the low concentration and the formation of plasticizer channels at high concentration were used to explain these finding.

Butler *et al.* (1996) investigated the effect of glycerin on properties of chitosan films at 0.25 and 0.50 ml/g chitosan. As they expected, the barrier properties and elongation at break increased with increasing glycerin but decrease in tensile strength. In order to decrease water vapor permeability on whey protein isolate films by using glycerol or sorbitol as plasticizer, Fairley *et al.* (1996) used sodium dodecyl sulfate (SDS) as plasticizer. SDS could not be used as plasticizer by its own, however, when used as co-plasticizer with sorbitol at mass ratio of SDS to whey protein isolate (WPI) of 1.2 films improved in flexibility and solubility without water vapor permeability change. When SDS was used with glycerol at the same ratio of SDS to WPI, showed less flexibility and solubility with slight increase in water vapor permeability.

Generally, films composed of one substance have either good barrier or good mechanical properties but not both. Indeed, the desirable properties of different materials are combined to form composite films: polysaccharides and proteins establish polymer interactions and create a network responsible for the mechanical properties, but their hydrophilic nature; on the contrary, lipids provide for the film their water vapor barrier property because of their hydrophobic character, but films made from lipids alone are usually too brittle (Kester and Fennema, 1986; and Guilbert, 1986). Many studies have reported the impact of lipid type on WVP of emulsified films. Generally, the WVTR of a film increases as the length of the lipid hydrocarbon chain decreases and the unsaturation degree increases (Koelsch et al, 1992; Kamper et al, 1984; and McHugh et al, 1994). Additionally, a film's water vapor resistance is inversely related to lipid polarity. The barrier efficiency of resulting composite films strongly depends on polarity of films components and distribution of lipid material in the film matrix (Kamper and Fennema, 1984; Debeaufort et al., 1993). The WVP efficiency depends on the polarity of lipids as

observed by Fennema et al. (1994): tristearin and stearic acid were less efficient than stearic alcohol because carbonyl and carboxyl groups are more polar than hydroxyl groups and they can form a water layer around them. Moreover, WVP drops with an increase of hydrophobic compound content. For example, Kamper and Fennema (1984) found that the stearic acid concentration in the emulsified films decreased their WVP until an optimum was reached. Fernandez et al (2007) studied the incorporation of lipids into WPI films. They found that surface tension was significantly decreased by adding unsaturated fatty acid (oleic and linoleic acid), whereas the greatest effect on WVP reduction was achieved with stearic acid. In general, WVP of composite films comprised of biopolymers and lipids strongly depend on the type, structure and quantity of the lipids. For films containing fatty acids or fatty alcohols, the WVP decreased with increased chain-length and degree of saturation of the lipids (Hagenmaier and Shaw, 1990; Kamper and Fennema, 1984a, b; Koelsch and Labuza, 1992; McHugh and Krochta, 1994a). For instance, McHugh and Krochta (1994) showed improved moisture barrier properties when the hydrocarbon chain-length was increased up to 16-18 carbons for fatty alcohols and monoglycerides. For fatty acids, barriers properties were augmented with an increase of chain-length from 12-18 carbon atoms, but from 18-22, barrier properties decreased (Koelsch and Labuza, 1992). This behavior could be explained by the differences in the network tortuosity.

Gontard et al. (1994) found that, for whey protein/lipid composite films, the influence of lipids depended on lipid characteristics and interactions between the lipid and the protein structural matrix. This result agreed with the study conducted by Park et al. (1993) examined the TS properties of fatty acid-laminated MC films, and reported that lamination of fatty acids on the surface of MC films reduced their TS. The trends of the TS properties as a function of the concentration of fatty acids were dependent on the characteristics of the fatty acids. Shellhammer and Krochta (1997) reported decreased in TS with increasing lipid concentration for a variety of WPI/lipid composite films. They suggested that increasing the lipid concentration weakened the TS of the protein phase.

Objectives

1. Investigate the effect of pH, heating temperature, heating time on the properties of edible film from mung bean and red bean proteins.
2. Determine the effect of protein concentration on the properties of edible film from mung bean and red bean proteins.
3. Study the effect of type and concentration of plasticizer on the properties of edible film from mung bean and red bean proteins.
4. Improvement of water barrier property of mung bean and red bean protein films incorporated with lipids.

CHAPTER 2

MATERIAL AND METHODS

1. Preparation of Raw Materials

Mung bean (*Uthong-1* S.) and/or red bean (*Doikham* S.) proteins were prepared by using the classical method of alkaline extraction and acid precipitation from mung and red bean bean flour. Mung bean and/or red bean flour was mixed with distilled water in the ratio of 1:10, stirring and adjusting the final pH to the 9.0 using 1 M NaOH. The suspension was extracted for 1 h using magnetic stirrer. And centrifuged for 30 min at 4 °C, 8000 rpm (Model J2-21M, Beckman Instruments Inc., Palo Alto, USA). The pH of the supernatant was adjusted to 4.5 by 1 M of HCl to allow precipitation and then centrifuge for 30 min at 4 °C, 8000 rpm. The isoelectric form of wet protein concentrate was then freeze-dried for 24 h (Dura-Top/Dura- Dry MP, Model TD97A001, FTS Systems, Inc), ground and placed in plastic box and stored at -20 °C until used. The nitrogen of protein concentrate was analyzed using the Kjeldahl method. A conversion factor of 5.46 was used to calculate the protein content.

2. Raw Materials Analysis

2.1 Protein content (AOAC, 1995)

2.2 Fatty content (AOAC, 1995)

2.3 Moisture content (AOAC, 1995)

2.4 Solubility of protein (Lee *et al*, 1992)

2.5 Dispersion of molecular weight (Laemmli, 1970)

3. Effect of pH, Heating Temperature and Heating Time on the Properties of Edible Protein Film from Mung Bean and/or Red Bean Proteins

3.1 Preparation of Edible Protein Film from Mung Bean and/or Red Bean Proteins

Freeze-dried mung bean and/or red bean proteins (93.52% and 90.26%, respectively) were dissolved in distilled water (3 g/100 ml) to prepare film-solutions. The pH level (8.0, 9.0 and 10.0) was adjusted prior to adding plasticizer (sorbitol) on a protein to sorbitol ratio of 2:1. All components were homogenized (10000 rpm for 2 min) and heated (60, 70 and 80 $^{\circ}$C) for the given time (10, 20 and 30 min). The film-solution was cooled to room temperature, followed by vacuum application to remove any dissolved air before pouring onto leveled non-stick trays to set. Once set, the trays were held overnight at 55 $^{\circ}$C undisturbed, and then cooled to ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccators at 60 % RH for further testing.

3.1.1 Film Testing

3.1.1.1 Conditioning

All films were conditioning prior to permeability and mechanical tests according to Standard method, D618-61 (ASTM, 1993a). Films used for testing water vapor permeability (WVP), tensile strength (TS) and elongation (E) were conditioned at 60% RH and 25$\pm$2 $^{\circ}$C by placing them in a desiccator over a saturated solution of Mg $(NO_3)_2 .6H_2O$ for 48 h or more. For other tests, films were transferred to plastic bags after peeling and placed in desiccator.

3.1.1.2 Moisture and Protein Contents

Moisture content was determined by drying samples under vacuum at 70 $^{\circ}$C (3.4 KPa) for 24 h. Nitrogen content was determined by Kjedahl Method (A.O.A.C., 1995). A protein conversion factor of 6.25 was used to calculated protein content.

3.1.1.3 Film Thickness

Thickness of the films was measured with a micrometer (Gotech Testing Machine, Model GT-313-A, Japan) to the nearest 0.01 mm at five random locations around the film. Precision of the thickness measurements was ±5%. Mean thickness for each sample was calculated and used in water vapor permeability (WVP) and tensile strength TS) calculation.

3.1.1.4 Film Solubility

Method modified from Stuchell and Krochta (1994) was used to measure film solubility. Film pieces 20 mm x 20 mm were dried at 70 °C in a vacuum oven (3.4 kPa) for 24 h, and then weighted to the nearest 0.0001 g for the initial dry weight. Films were immersed into 20 ml of distilled water in 50 ml screw centrifuge tube containing 0.01 % potassium sorbate. The tubes were capped and placed in shaking water bath for 24 h at 25 ± 2 °C. The solution was removed and set aside for later testing of protein solubility as described later. The remaining solution and film piece was pour onto (Whatman #1) qualitative filter paper, rinsed with 10 ml distilled water, and dried at 70 °C in a vacuum oven for 24 h and dried weight of film was determined. Triple measurements were done for each treatment triplicate. Total soluble matter was calculated from the initial gross weight and final dry weight using the following equation:

$$\% \text{ Film solubility (db)} = \frac{(\text{film weight before test} - \text{film weight after test})}{\text{film weight before test}} \times 100$$

3.1.1.5 Protein Solubility

Solution set aside from films solubility was analyzed for protein content by the Lowry method (Lowry *et al.* 1950). A 0.5 ml of test solution was placed in a test tube. A 2.5 ml of the mixture of 0.2-M sodium hydroxide and 4% sodium carbonate was pipetted into each sample and vortexed to mix thoroughly and stand for 10 min. Then 0.25 ml Folin ciocalture & phenol reagent was added into each sample and vortexted to mix thoroughly and stand for 30 min at room

temperature. Absorbance at 750 nm was determined by diode array spectrophotometer (Hewlett Packard Model 6541A, Avondale, PA). A standard curve was developed using bovine serum albumin. The protein solubility (% PS) was calculated as followed:

$$\% \text{ Protein solubility} = \frac{\text{weight of protein in 20 ml solution x 100}}{\text{initial weight of film x (\%protein in film) x (\%dry matter of film)}}$$

3.1.1.6 Film Color

A Hunter Lab (Hunter Associates Laboratory, Inc., Reston, Verginia) was used to determined film L, a and b color value (L = 0 (black) to 100 (white); a = -60 (green) to +60 (red); and b = -60 (blue) to +60 (yellow). Color (means of five measurements at different locations on each specimen) was measured on 10 cm X 10 cm. Prior to color measurement, film specimens were conditioned at 50% RH and 23±2 °C for 3 days.

3.1.1.7 Water Vapor Permeability

The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh *et al.* 1993) was used to determine the WVP of films. The test cups were filled with 20 g of silica gel (desiccant) to produce a 0% RH below the film. A sample of protein film from fish meat was placed in between the cup and the ring cover of each cup coated with silicone sealant (LITHELEN, Leybold System Gmbh, Germany) and held with four screws around the cup's circumference. The air gap was at approximately 1.0 cm between the film surface and desiccant. The water vapor transmission rates (WVTR) of each film was measured at 60± 5 % RH and 25±2° C. After taking initial weight of the test cup, it was placed into an environmental chamber with an air velocity rate of 350 ft/min (Incubator, Model KBF 115). Weight gain measurements were taken by weighing the test cup to the nearest 0.0001g with an electronic scale (Sartorious Corp.) every 3 h for 18 h. A plot of weight gained versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit time (g/h). WVTR was expressed in gram units, per square meter, per day.

Steady state over time (slope) yielded a regression coefficient of 0.99 or greater. Nine samples per treatment were tested. The WVP of film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapor pressure difference across the film.

3.1.1.8 Tensile Strength and Elongation at Break

Tensile strength was performed with an Instron universal testing instrument (LLOYD Instrument, Model LR30K, Hants, England) as per ASTM D882-91 Standard Method (ASTM, 1995). Fifteen samples, 2.54 cm x 10 cm, were cut from each film. Initial grip separation and cross head speed were set at 50 mm and 50 mm/min, respectively. Tensile strength was calculated by dividing the maximum force at break by initial specimen cross-sectional area, and percent elongation at break was calculated as follows;

$$E = 100 \text{ X } (d_{\text{after}} - d_{\text{before}})/d_{\text{before}}$$

Where d was the distance between grips holding the specimen before or after the break of the specimen.

3.1.1.9 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to Laemmli (1970). The freeze dried mung bean and red bean were boiled for 120 s in 10g/100g SDS-8 M Urea and 0.5 M Tris-HCl (pH 6.8) in the presence of 2-mercaptoethanol (2ME) followed by application of 15 μg of proteins on 4g/100g stacking and 12.5g/100g running polyacrylamide gel. The molecular weight standard mixture (stockNo. SDS-7, Sigma Chemical Company, St. Louis, MO) used was made up of bovine serum albumin (66.0 kDa), egg albumin (45.0 kDa), glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle (36.0 kDa), bovine carbonic anhydrase (29.0 kDa), bovine trypsinogen (29.0 kDa), bovine pancrease trypsinogen (24.0 kDa), soybean trypsin inhibitor (20.1 kDa), and bovine milk α-lactalbumin (14.2 kDa). Protein bands were stained with 0.125g/100g of Coomassie blue and destained in 40g/100g methanol and

7g/100g acetic acid solution followed by 5g/100g methanol and 7g/100g acetic acid solution.

3.1.1.10 Protein solubility

Protein solubility of dried protein was determined by the method of Lee *et al.* (1992) with slight modification. The freeze dried mung bean and red bean proteins were diluted to 1% w/v with distilled water and centrifuged for 20 min at 10,000 x *g* in a centrifuge (Model J2-21M, Beckman Instruments Inc., Palo Alto, CA) to sediment insoluble proteins. Protein concentrations in the total solution and supernatant fractions were determined by the Lowry method and Protein solubility was computed as:

$$\text{Protein Solubility} = \text{g } \frac{\text{protein in the supernatant}}{\text{g total protein}} \times 100$$

3.1.2 Statistical Analysis

3.1.2.1 Experimental Design

General Response Surface Methodology (GRSM) was used to determine the optimum combinations of pH, heating temperature and heating time. GRSM are given in terms of coded variable, x_i (Cochran and Cox, 1957; Cox, 1958; Cox, 1958; Mayers, 1971; Thompson, 1982). They are used in specific applications after transformations of the uncoded (actual) variables, ξ_i. In this study a three level, three-factor design was adopted (Box and Behnken, 1960). It fulfills the requirements for multiple factor response surface designs and considers the overall error (variance or sampling error and bias error). Furthermore, the designs and specific for investigation with second order polynomials is ratatable, it provides a reasonably constant variance of response in the three-dimensional space and possesses a high degree of orthogonality (only the constant term β_o and the quadratic estimates β_{ij} and correlated). Selection of levels for independent variables was based on the results from preliminary tests and observations of Cuq et al. (2000). The levels of input variables in coded (x_i) and uncoded (ξ_i) forms are given in Table 3.1.The

experimental design consisted of fifteen experimental points, which included three replications of the center point. The fifteen films were prepared in random order. Each of the thirteen dependent Y variables (responses) was assumed to be affected by the three independent variables. Responses under observation were: tensile strength (Y_1), percentage of elongation (Y_2), water vapor permeability (Y_3), film solubility (Y_4), protein solubility (Y_5), L* value (Y_6), a* value (Y_7) and b* value (Y_8). Each value represented the mean of three determinations. The product thus obtained was analyzed and experimental values were compared with model predictions.

Table 1. Fractional factorial design for selected parameters for sample

Independent variables	Coded	Uncoded	Coded	Uncoded
			1	10.0
pH	x_1	pH	0	9.0
			-1	8.0
			1	80.0
Temperature, °C	x_2	T	0	70.0
			-1	60.0
			1	30.0
Time, min	x_3	t	0	20.0
			-1	10.0

3.1.2.2 Statistical Analysis

The PROC RSREG (response surface regression) procedure of SAS Institute, Inc (1996) was used to determine the effects of independent variable (pH, heating temperature and heating time) on physical, barrier and chemical properties of edible films from mung bean and red bean proteins. Tensile strength (TS), % Elongation (%E), water vapor permeability (WVP), films solubility (FS), protein solubility (PS), L value, a value and b value, were analyzed. RSREG is based on a second order polynomial equation to perform regression:

$$Y = \beta_{ko} + \sum_{i=1}^{3} \beta_{ki} x_i + \sum_{i=1}^{3} \beta_{kii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{kij} x_i x_j \quad \text{.....(1)}$$

Where: β_{ko}, β_{ki}, β_{kij} are constant coefficients and x_i are the coded independent variables. If the lack of fit was not significant, regression coefficients (β_{ko}....β_{k9}) were used to generate contour plot for response variable. If the regression model from RSREG showed a significant lack of fit, simple mathematics transformations were performed on independent and response variable to improve the fit (Box and Draper, 1987) before response surfaces were generated. Response surface and contour plot of responses for these models can also be drawn using the Statistica for Windows Version 5.0 by plotting as a function of two variables, while keeping other variable at the constant value. The optimum conditions of the selected parameters on the properties of edible films from mung bean and red bean proteins can be determined by superimposing the contour plots an acceptably high tensile strength, high elongation at break and low water vapor permeability. After data analysis, the experiments were performed to verify the response models at the optimum conditions using the same film- forming procedure.

4. Effect of Protein Concentration on the Properties of Edible Films from Mung Bean and/or Red Bean

Freeze-dried mung bean and/or red bean proteins were dissolved in distilled water at varying protein concentrations of 1.5, 3.0 and 4.5% w/v to prepare film solutions. The optimum pH (selected from 3.2) was adjusted prior adding plasticizer (sorbitol), the ratio of protein to sorbitol was applied at 2:1. All components were homogenized (10000 rpm for 2 min) and heated at optimum heating temperature on a hot plate with magnetic stirrer at optimum heating time obtained from 3.2. The film-solution was cooled to room temperature, followed by vacuum application to remove any dissolved air before pouring onto leveled non-stick trays to set. Once set, the trays were held overnight at 55 °C undisturbed, and then cooled to ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccators at 60 % RH for further testing.

5. Effect of Type and Concentration of Plasticizer on the Properties of Edible films From Mung Bean and/or Red Bean

Freeze-dried mung bean and/or red bean proteins were dissolved in distilled water at optimum protein concentration selected from 3.3 to prepare film solutions. The optimum pH (selected from 3.2) was adjusted prior adding plasticizer the different type of plasticizer (sorbitol, glycerol and polyethylene glycol) at various concentrations (30, 40, 50 and 60% by weight protein). The film solutions were heated at optimum heating temperature on a hot plate with magnetic stirrer at optimum heating time obtained from 3.2. The film-solution was cooled to room temperature, followed by vacuum application to remove any dissolved air before pouring onto leveled non-stick trays to set. Once set, the trays were held overnight at 55 °C undisturbed, and then cooled to ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccators at 60 % RH for further testing.

6. Effect of Lipid Type and Concentration on the Properties of Edible Film from Mung Bean and Red Bean Proteins

The freeze-dried proteins were dissolved in distilled water to prepare film solutions. The pH was adjusted prior to adding plasticizers (selected from part 5). Three type of lipids which were palm oil, stearic acid, and oleic acid at various concentrations (5, 10,15, 20, 25, wt% of protein) on the film properties were investigated. The solutions were heated on a hot plate with magnetic stirrer at temperature and heating time selected from part 3. The film solution were held overnight at 50 °C for 15 h, and then cooled to ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccators at 60% relative humidity for further testing.

CHAPTER 3

RESULTS AND DISCUSSION

1. Compositional Profile of Mung Bean and Red Bean Proteins

The proximate composition of dried mung bean and red bean proteins were found to be 93.52, 1.37, 0.85 and 4.26 % and 90.26 1.08, 3.99 and 4.67 % of crude protein, crude fat, carbohydrate and ash, respectively (Table 2.).

Table 2. Proximate compositions of dried mung bean and red bean powder

Compositions	Amount (%)	
	Mung bean	Red bean
Protein	93.52	90.26
Fat	1.37	1.08
Carbohydrate	0.85	3.99
Ash	4.26	4.67

2. SDS-PAGE Patterns of Mung Bean and Red Bean Proteins

SDS-PAGE patterns of proteins from mung bean and red bean proteins are illustrated in Figure 1. Proteins from mung bean and red bean, large amounts of proteins had molecular weight (MW) between 24 and 55 kDa with some traces having less than 24 kDa. However, there were small amounts of proteins of MW between 24 and 14.2 kDa, as seen from some bands; however, the amounts were meager.

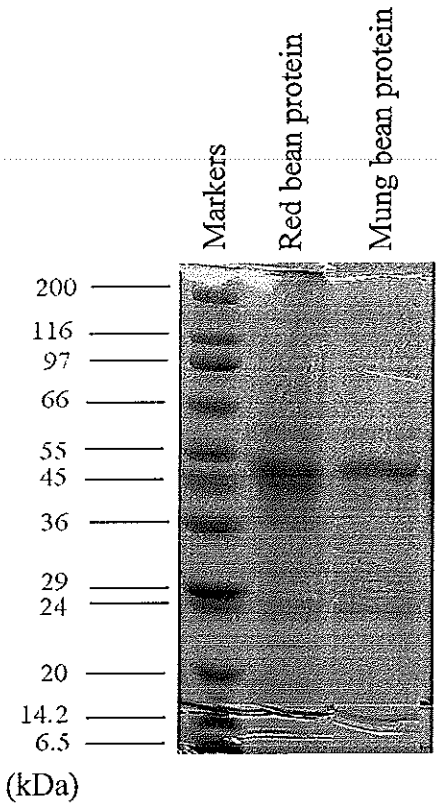


Figure 1. SDS-PAGE patterns of mung bean and red bean proteins

3. The Amino Acid Composition of Mung Bean and Red Bean Proteins

The amino acid composition of mung bean and red bean is shown in Table 3. Mung bean and red bean proteins were rich in essential amino acid such as leucine, isoleucine, lysine, and phenylalanine and also rich in acidic amino acid such as glutamic acid and aspartic acid. However, the sulfur containing amino acid such as methionine and cysteine were also detected in mung bean and red bean proteins (2.75 and 3.62 and 3.08 and 3.59 of mung bean and red bean, respectively follow by Edman degradation (Table 3.).

Table 3. Amino acid content of freeze dried mung bean and red bean proteins

Amino acid	Mung bean proteins (% of protein)	Red bean proteins (% of protein)
Alanine	1.43	2.27
Arginine	8.69	3.24
Glutamic acid	9.45	9.15
Glycine	1.87	2.03
Histidine	3.25	3.07
Isoleucine	5.46	5.55
Leucine	4.70	4.95
Lysine	5.36	5.26
Methionine	2.75	3.08
Phenylalanine	5.98	5.78
Cysteine	3.63	3.59
Appartic acid	6.45	5.69
Tyrosine	1.94	2.57
Proline	6.75	6.62
Serine	1.90	2.03
Threonine	2.71	3.20
Tryptophan	2.11	2.24
Valine	0.90	0.89

4. Protein Solubility of Mung Bean and Red Bean Proteins

Solubility is considered as one of the functional properties of protein, hence the loss of solubility could be taken as a criterion of protein denaturation (Wolf, 1970; Wu and Inglett, 1974). The solubility profiles of dried mung bean and red bean proteins are shown in Table 4. The results showed that the solubility was in the range of 23.57-25.18%.

Table 4. The solubility of freeze dried mung bean and rd bean proteins

Proteins	Solubility (%)
Mung bean	23.57
Red bean	25.18

5. Effect of pH, Heating Temperature and Heating Time on the Properties of Edible Film from Mung Bean Proteins

5.1 Tensile Strength and Elongation at Break

Tensile strength is the maximum tensile stress sustained by the sample during tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile strength at yield or at break, respectively (ASTM, 1991). Elongation at break is an indication of films flexibility and stretch ability (extensibility), which is determined at point when the film breaks under tensile testing and is expressed as the percentage of change of the original length of the specimen between the grips of a film to stretch (extend). The main factors that influenced the film's properties were pH and heating temperature of film-solutions, while heating time had the lowest effect. Contour plots for tensile strength and elongation at break are given in Figure 2. and 3. Depending upon the film conditions, tensile strength showed a high variation between 3.59-6.51 MPa (Figure 2.) and 10.28- 43.74% for elongation at breaks (Figure 2). Comparing at the same heating temperature of film solutions, the results demonstrated that, tensile strength showed increase as pH of film solutions increased from 8-9.5, it would be implied that lower pH of film solutions induced formation of resistant films. Banker (1966) reported that pH plays an important role in protein films made from water-soluble materials. At pH away from the isoelectric point (pH 4.5) promoted denaturation of proteins, unfolds and, solubilize, during the solubilization of proteins, the cohesive forces between the proteins macromolecules are neutralized by unions with the solvent molecules (Banker, 1966). The functionality of the polymers is related to solution properties which further influences film characteristics. The highest tensile strength value was obtained at pH about 9.5 (Figure 2.). The weakest films was observed at lowest pH of film solutions: a very low tensile strength (3.85-4.63 MPa) was observed at pH lower than 8.0, most likely due to less protein-protein interaction. The tensile strength was enhanced as heating temperature of film solutions increased from 60-80 °C. This may be due to higher heating temperature of film solutions induced proteins

denaturation resulted in increase in the number and/or a better localization of bonds between protein chains provided in higher interaction between protein polymers. Hayakawa and Nakai (1985) stated that heating of film solutions induced surface SH group converted to SS bonds thus contributing gel network formation between protein chains resulted in higher interaction between protein polymers. The weakest films demonstrated at lowest heating temperature; a very low tensile strength (3.73-4.06 MPa) was observed at heating temperature of film solutions around 60 °C. The contour plots (Figure 2) indicated an interaction between the effect of pH and heating temperature of film solutions on tensile strength of edible films. It was observed that, lowest tensile strength could be expected with low both pH and heating temperature of film solutions. According to the contour plots, the experimental condition involving higher pH (10.0) and a high heating temperature (75-78 °C) of film solutions, led to higher film formations resulted in a high tensile strength. Heating time also affect the tensile strength. Irrespective of pH and heating temperature of film solution, the result demonstrated that, tensile strength increased as heating time of film solution increased from 10 to 30 min.

Elongation at break value was also most affected by pH and heating temperature of film solutions. All linear, quadratic and interaction terms for pH, heating temperature and heating time were significant. The contour plots of elongation at break (Figure 3.) indicated that edible films from mung bean proteins is an elastic material with elongation at break value between 4.60-43.74 % and showed the most elastic films with a highest elongation at break when higher pH and higher heating temperature of film solutions were employed. An increase in elasticity of heat-induced was suggested to be due to an increased number of intermolecular disulfide (SS bond) bonds (Shimada and Cheftel, 1988). Prolonged heating time, however, resulted in increased in elongation at break.

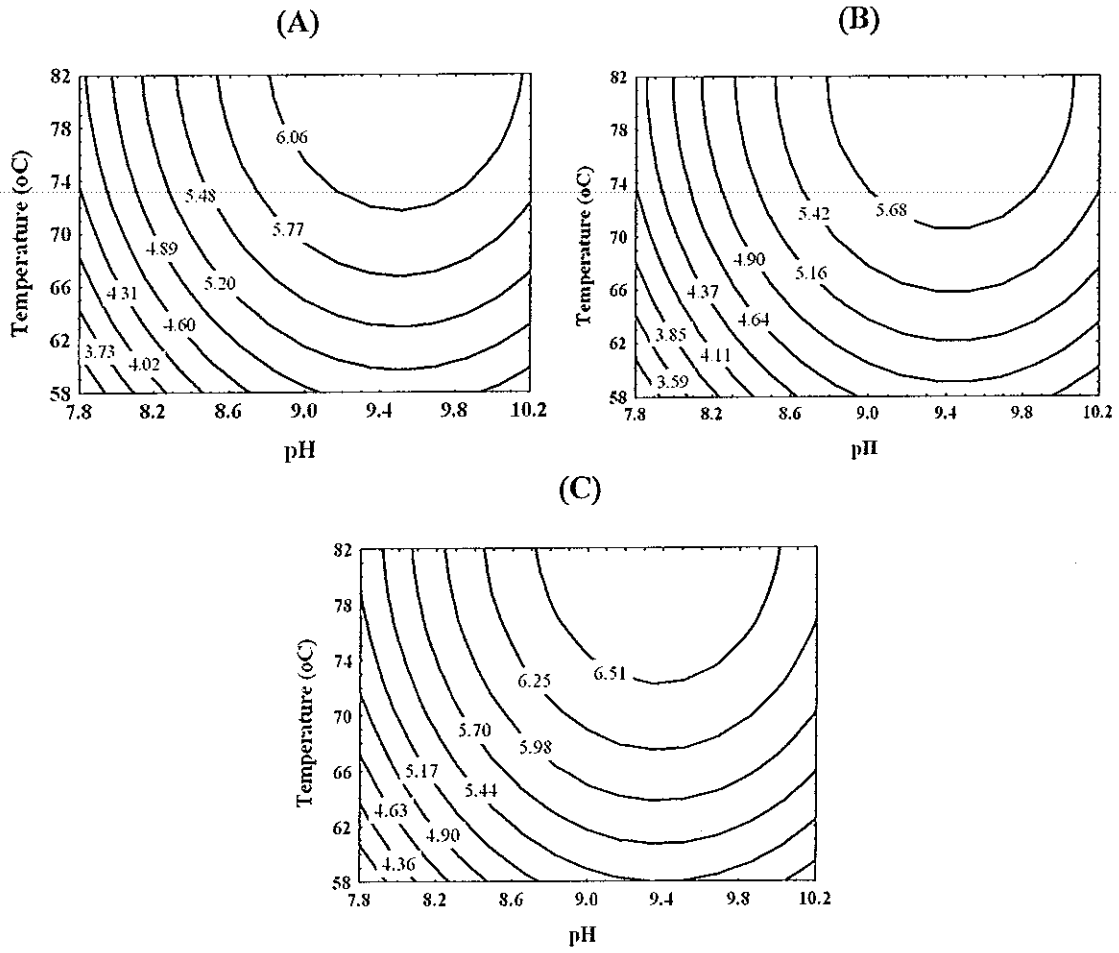


Figure 2. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent tensile strength (kPa) of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

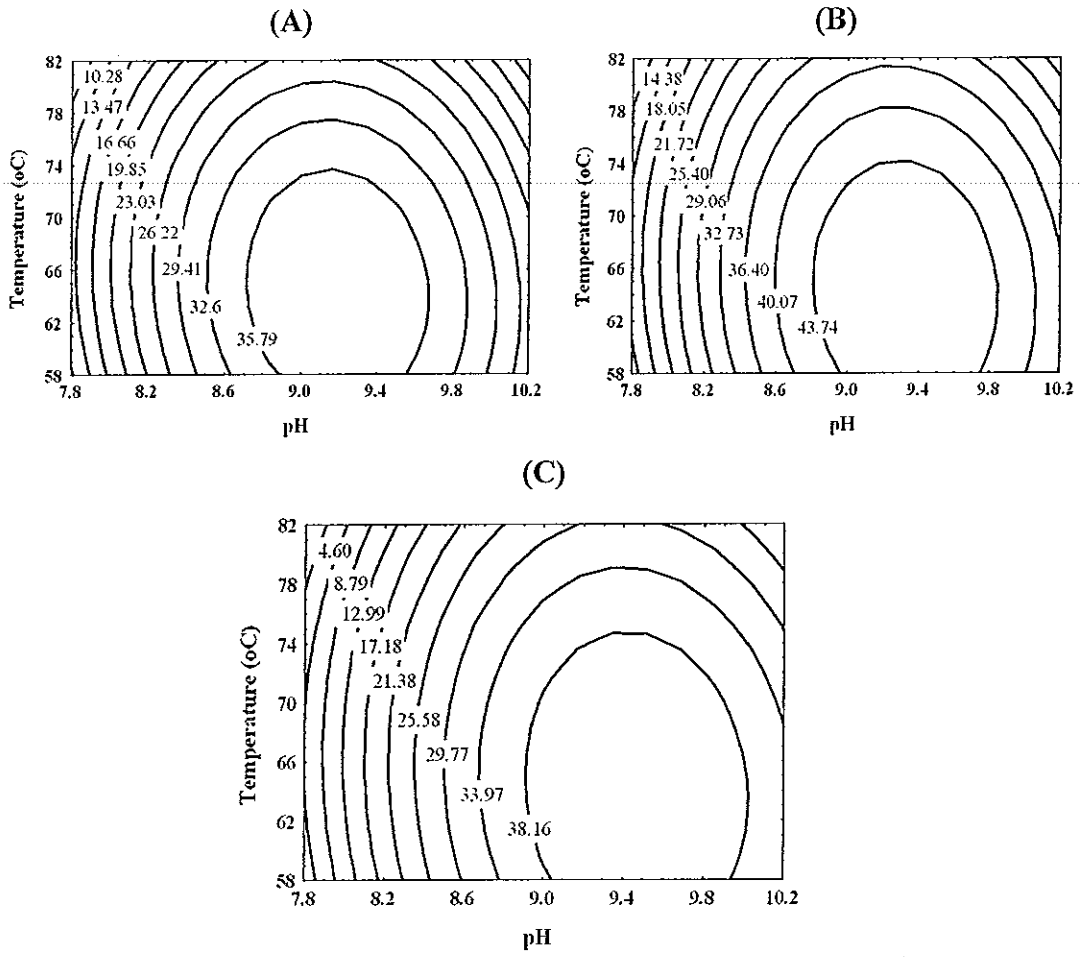


Figure 3. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent elongation at break (%) of films at given heating; (A) = 10 min,(B) = 20 min and (C) = 30 min.

5.2 Water Vapor Permeability

Water vapor permeability is an important property that greatly utility in food systems (Granasambandam *et al.* 1997). Since a main function of an edible films or coatings are often to impede moisture transfer between food and the surrounding atmosphere, or between two components of a heterogeneous food products, water vapor permeability should be low as possible. However, hydrophillic (edible or nonedible) materials, such as protein films, derivative from this ideal behavior due to interactions of permeating water molecules with polar groups in the films structure (Hagenmaier and Shaw, 1990). Deviation from the ideal behavior can also be induced by temperature effects on materials (Myers *et al.* 1962). The main factor influencing water vapor permeability of edible film produced from mung bean proteins were pH and heating temperature of film solutions. The contour plots (Figure 4.) were characteristics of the effect of these variables and showed that water vapor permeability value demonstrated the highest at pH of film solutions around 9.0 (4.24-15.55 g.mm/m^2.day.KPa) and tend to decreased when pH of film solutions were reached to 9.8-10.0 (4.25-4.65 gram.mm/m^2.day.KPa). At higher pH protein denatures, unfolds and solubilizes, facilitating favorable molecule orientation pronounced higher the formation of intermolecular disulfide bond by thiol-disulfide interchange and thiol oxidation reactions. The function of disulfide bonds on protein insolubilization during drying of soymilk was studied by Fukushima and Van Burea (1970). Thiol-disulfide interchanged by thiol oxidation has also been implicated in whey protein gelation studies (Donovan and Mulvihill, 1987; Shimada and Cheftel, 1989). The highest water vapor permeability came at lowest pH. The water vapor permeability of edible films was also affected by heating temperature of films solutions. Basically, proteins must be denatured (by heating) in order to form the more extended structures that are required for film formations. Once extended, protein chains can associate through hydrogen, ionic, hydrophobic and covalent bonding. The chain-to-chain interaction that produces cohesive films is affected by the degree of chain extension and the nature and sequence of amino acid residues. Uniform distribution of polar, hydrophobic, and/or thiol groups

along the polymer chain increase the likelihood of the respective interactions. (Kester and Fennema, 1986). The results showed that increasing of heating temperature of film solutions (60-80 °C) resulted in lower water vapor permeability (Figure 4) most likely result from increasing of heating temperature of film solutions promoted greater cross-link between protein-protein chains resulting in a tight and compact protein network and structure. Shimada and Matsushita (1980) have reported that the first step of ovalbumin aggregation involved the formation of SS bonds and the exposure of hydrophobic groups, and that, during further heating, ovalbumin was then polymerized intermolecular sulfhydryl/disulfide (SH/SS) exchange to form a higher protein net work structure. The highest water vapor permeability of edible films was found at lowest heating temperature of film solutions. The effect of heating time of film solutions on water vapor permeability of edible films showed similar trend with heating temperature.

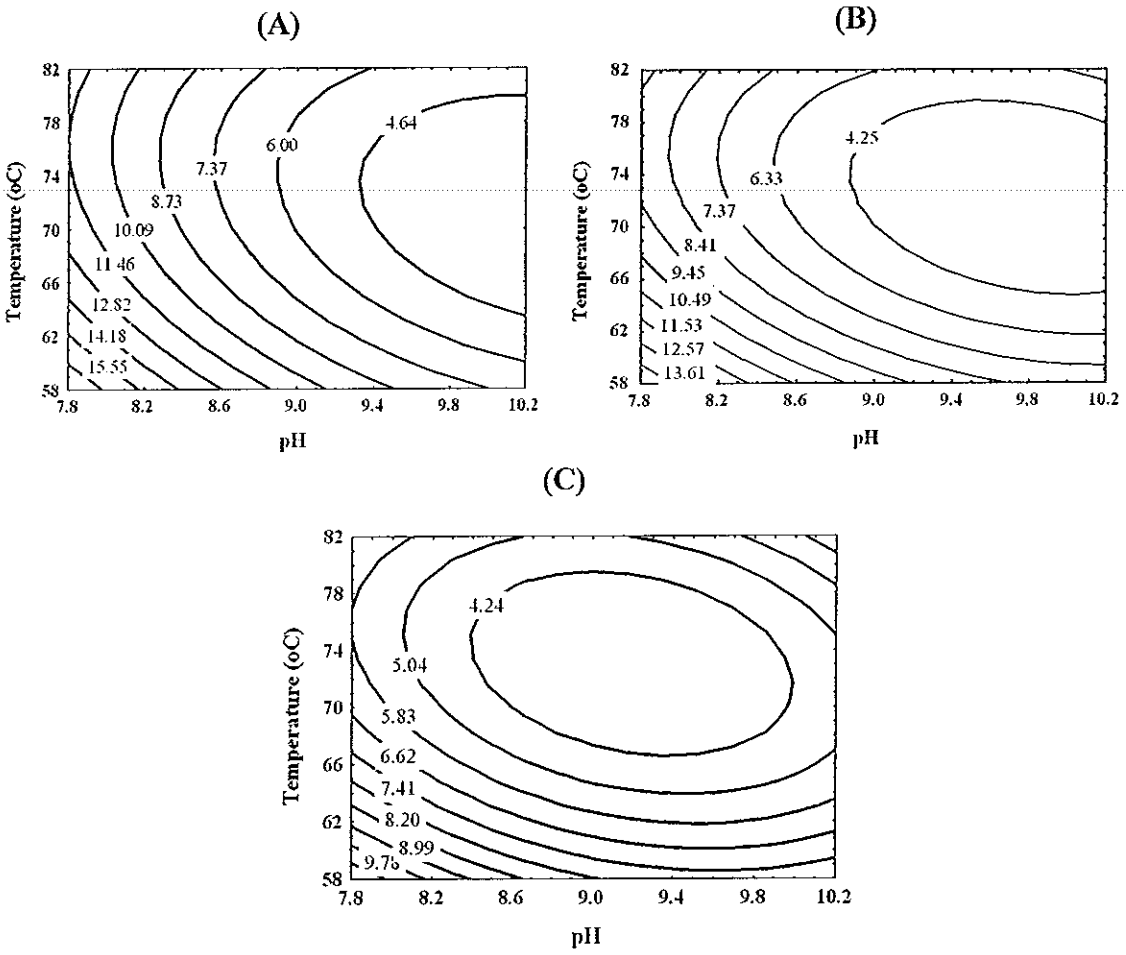


Figure 4. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent water vapor permeability (g.mm/m^2.day.kPa) of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

5.3 Films Solubility and Proteins Solubility

Water resistance is an important property of edible films for applications as food protection where water activity is high, or when the film must be in contact with water during processing of the coated food e.g. to avoid exudation of fresh or frozen products (Gontard *et al.* 1992). Generally, higher solubility indicates lower water resistance. However, a high solubility may have an advantage for some applications. High solubility is advantageous in case that the films are to be consumed with a product and may also be important factor that determines biodegradability of films when used as packaging wrap. Edible film pieces produced from mung bean proteins maintain their integrity (i.e., did not dissolve or break apart) even after 24 hr of incubation with gentle motion. This indicates that the protein polymer network remained intact and those only monomers, small peptides and non-protein material were soluble (Stuchell and Krochta, 1994). The pH and heating temperature of film solutions were the main effect on films and proteins solubility, while heating time showed less effect. The contour plots of films and proteins solubility showed decreased when pH of film solutions were increased (Figure 5. and 6.). It was observed that both films and proteins solubility showed lower solubility values when pH of film solutions were higher than 9.0. Decreasing of films and proteins solubility as a result of higher pH was the probable reason for this. Decreased soluble matters may be due to decreased protein solubility. Lower pH of film solutions (pH < 9.0), dispersion in water might result in loosening the film structure, causing dissolution of the non-protein materials (Gnanasambandam *et al.* 1997). It was observed that both films solubility and proteins solubility demonstrated lowest at pH around 9.4-10.0, most likely due to better films formation. The contour plots of the effect of heating temperature of film solutions on films solubility and proteins solubility are shown in Figure 5 and Figure 6. Increasing in heating temperature of film solutions from 60 to 80 ^{o}C resulted in decrease in films solubility and proteins solubility. Roy *et al.* (1999) reported that wheat gluten films solubility and proteins solubility decreased ($p < 0.05$) as heating temperature of film solutions increased. This was attributed to more pronounced heat-induced protein denaturation at higher temperatures. Heat induced

protein denaturation (unfolds), resulted in exposing previously "buried" groups such as hydrophobic and sulfhydryl (SH) groups which producing a strong films resulted in lower both films solubility and proteins solubility (Fukushima and Van Buren, 1970; Farnum *et al.* 1976; Schofield *et al.* 1983 and Mine *et al.* 1990). Heating time of film solutions from this studied seemed to be less effect on films solubility and proteins solubility of edible films.

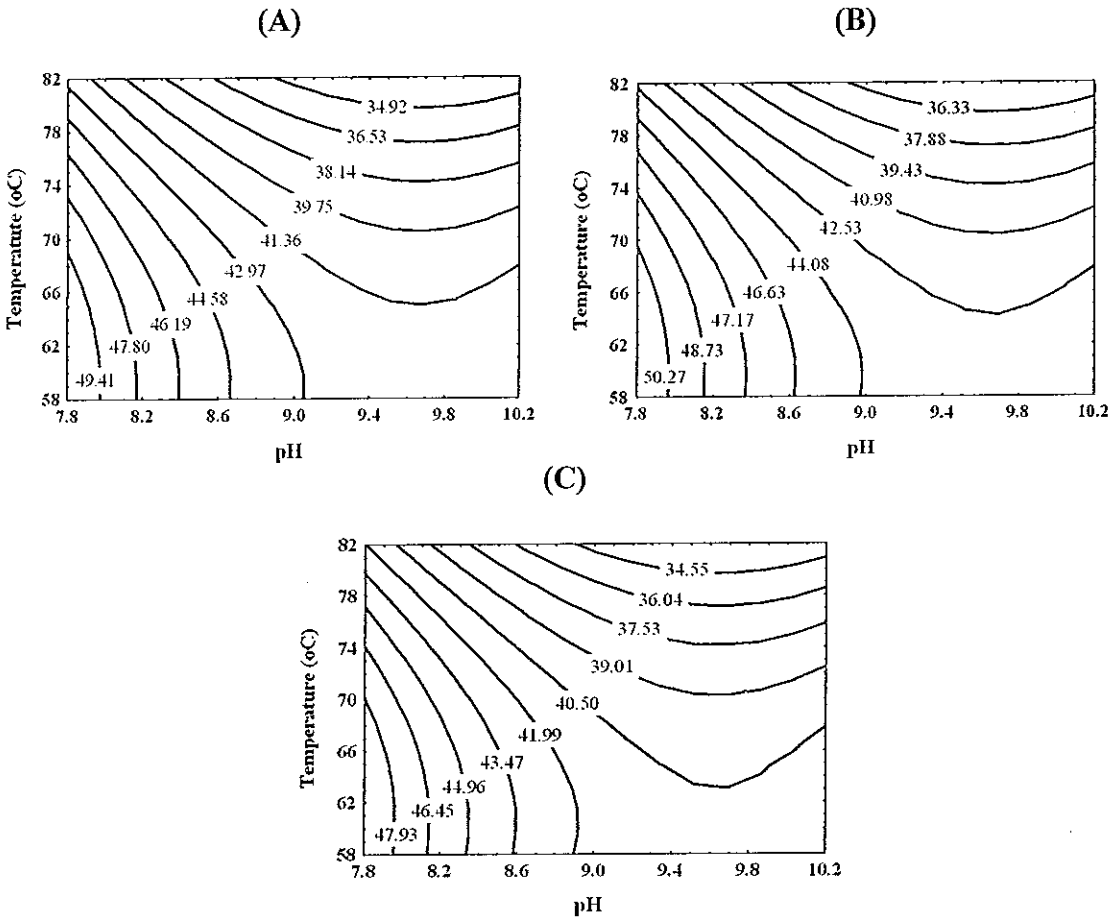


Figure 5. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent film solubility (%) of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

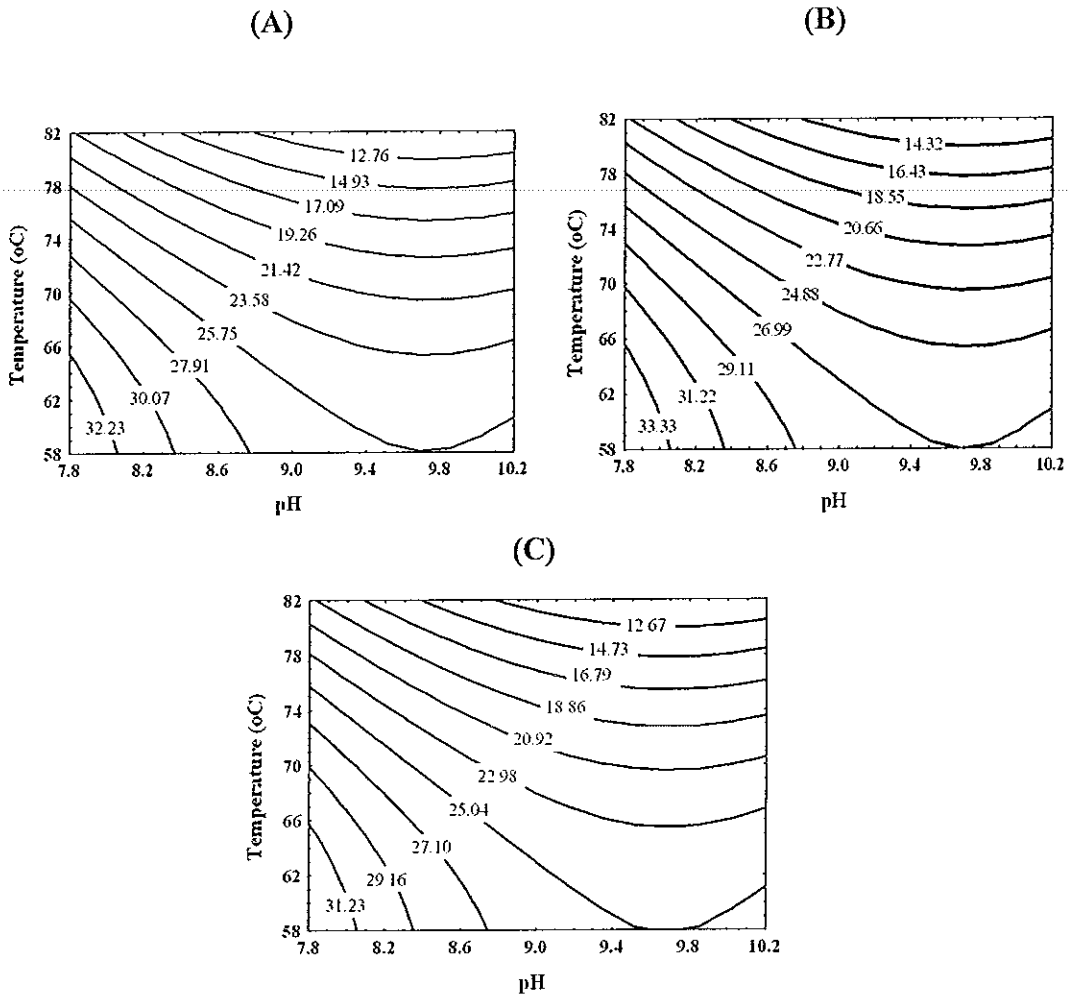


Figure 6. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent protein solubility (%) of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

5.4 Films Color

The results of the measurements performed on the films color were expressed in accordance with the Hunter system, and the rectangular coordinates (L*, a* and b*) were defined. The film colors were the most affected by pH of film solutions, while heating temperature and heating time were little affected. Films formed at lower both pH and heating temperature were lighter yellow than films formed at higher pH and heating temperature. Instrumental color parameters L* and a* little increased with increasing in pH and heating temperature of film solutions (Figure 7. and 8.), however, value b* dramatically increased with increasing in pH and heating temperature of film solutions (Figure 9.), and this made films appeared more yellowish. At alkali pH, proteins were observed to from complexes with polyphenolic compounds. Such complexes might have contributed to discoloration of films prepared at higher pH (Grananasambandam *et al.,* 1997). The value an increased as pH of film solutions increased from 9.0-10.0, which concomitant with change in heating temperature (Figure 9) which produced a greenish yellow films.

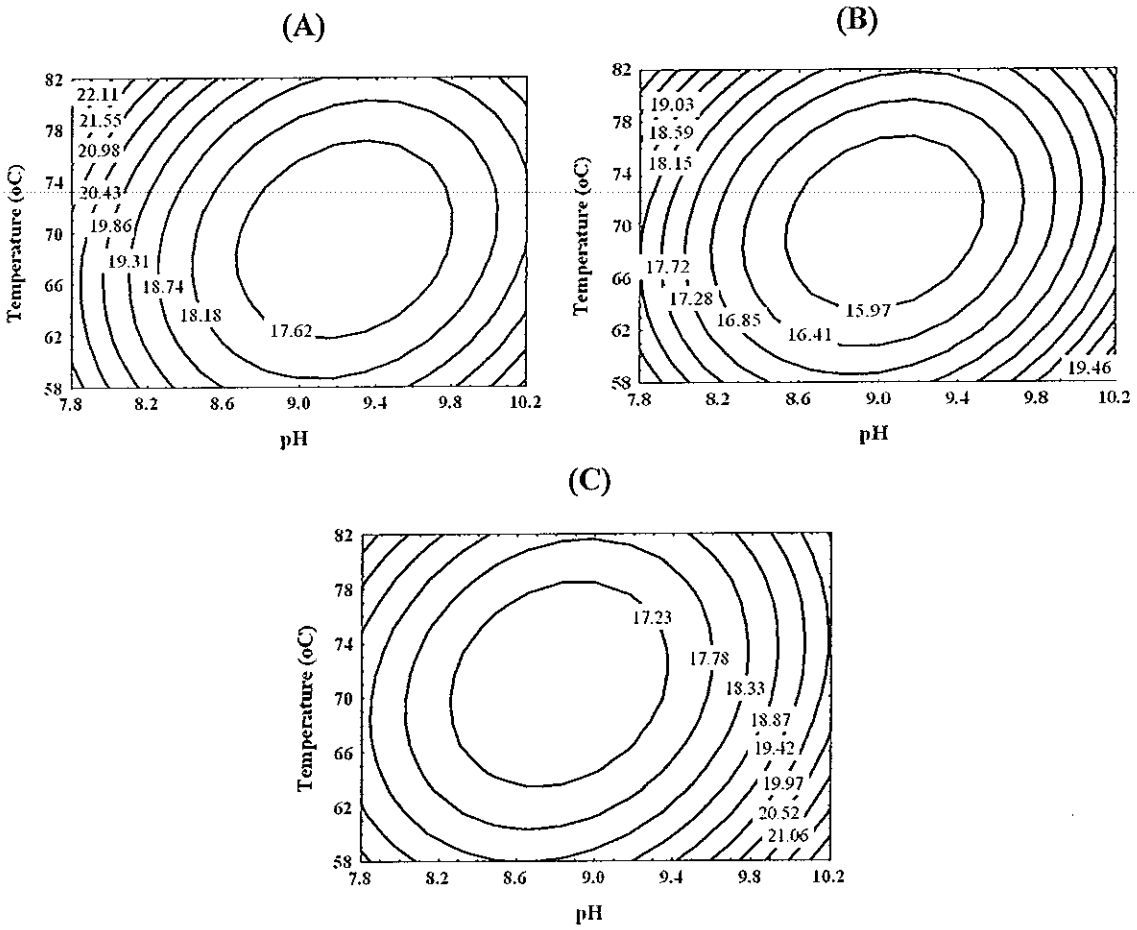


Figure 7. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent L* value of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

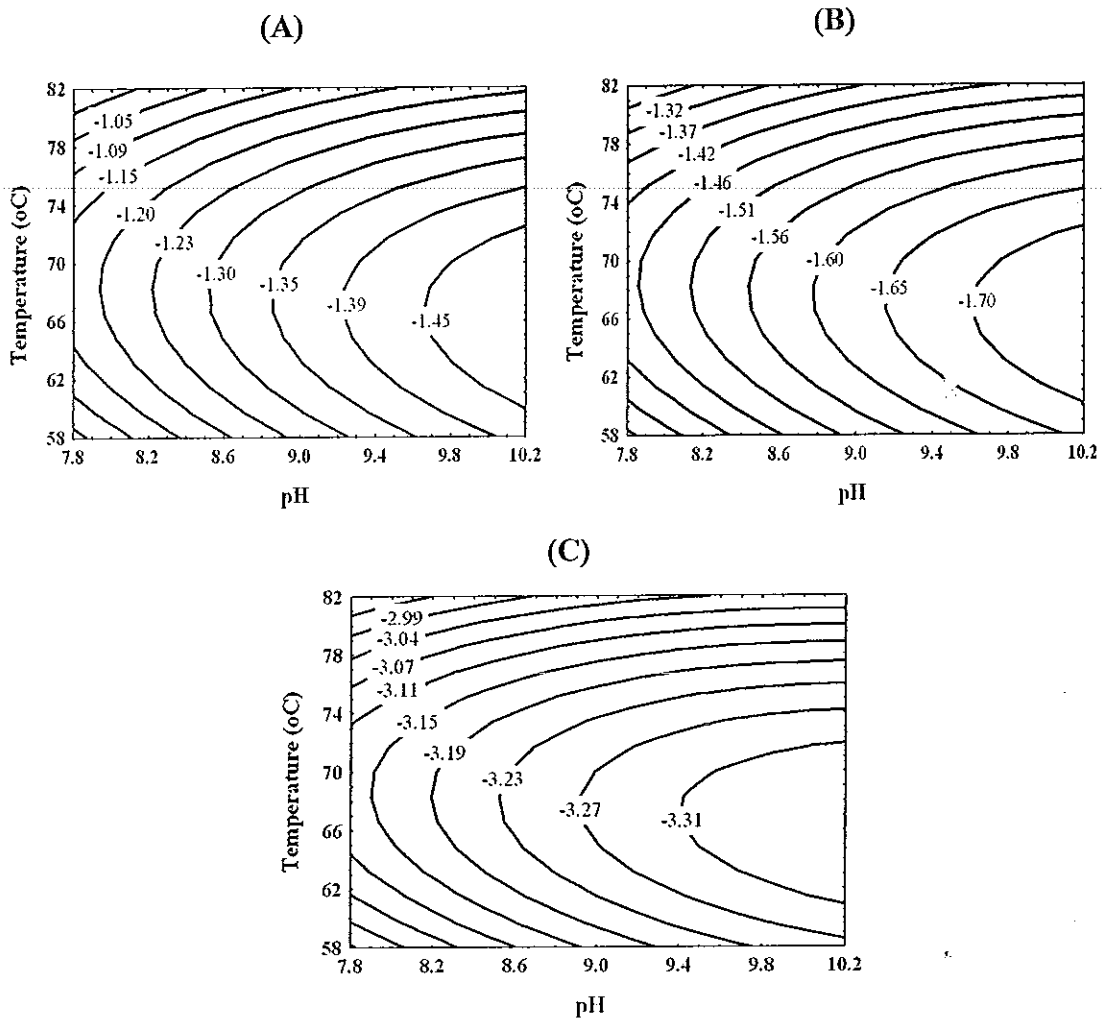


Figure 8. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent a* value of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

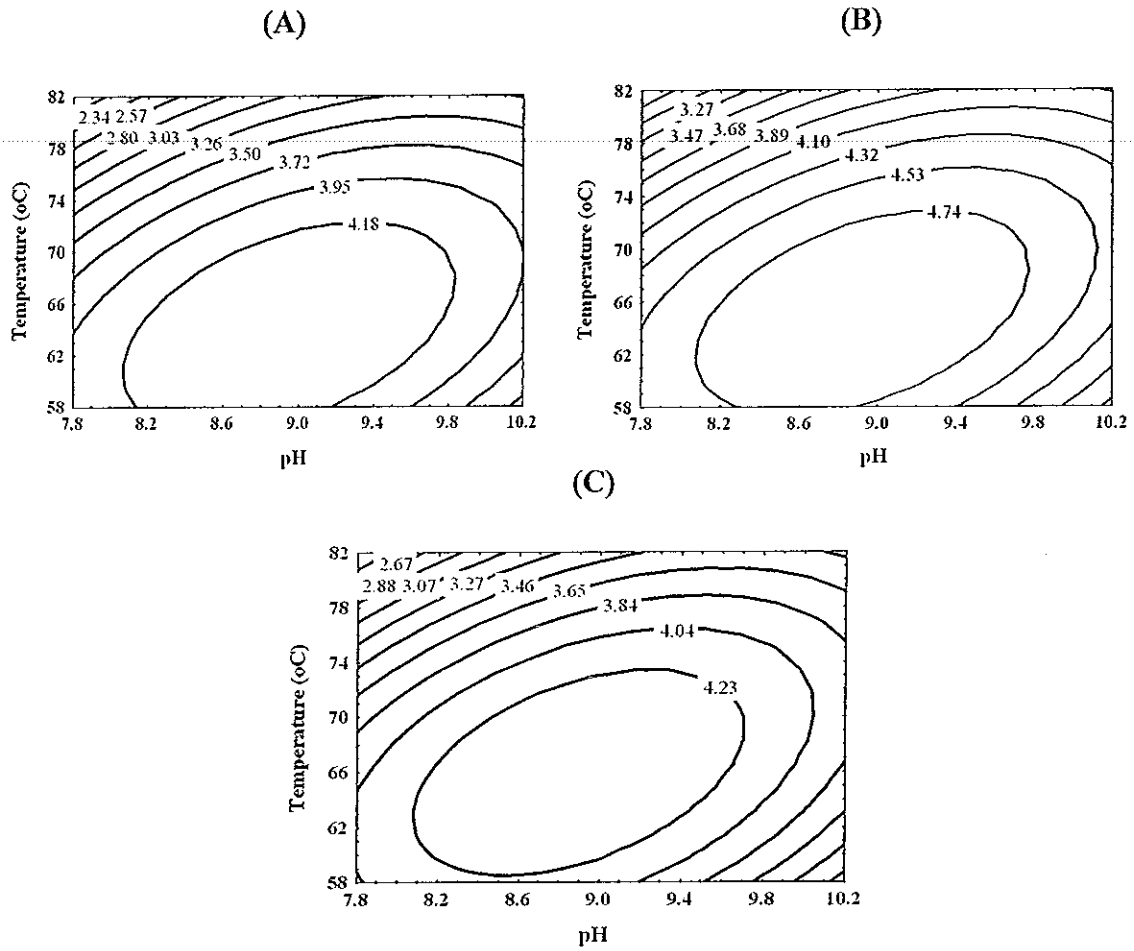


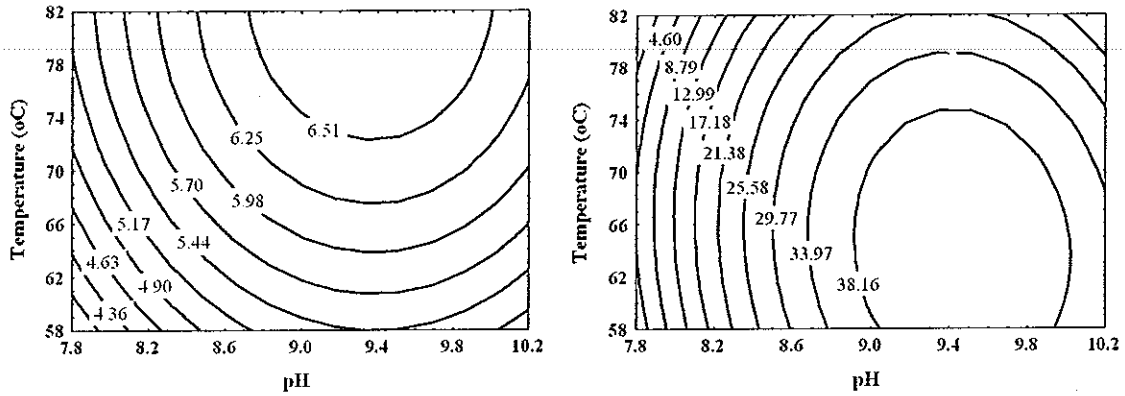
Figure 9. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent b* value of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

5.5 Localization of Optimum Conditions

To determine the optimum conditions of the effect of pH, heating temperature and heating time on the properties of edible film from mung bean proteins, the graphical method used in RSM was employed. The variable tensile strength and water vapor permeability were considered the most important of the 8 responses followed by elongation at break. The contour plots in Figure 11. were obtained from the predictive model of tensile strength, elongation at break and water vapor permeability at 30 min of heating time (Figure 10.). Plot of Figure 11. were superimposed over those of Figure 10(A), 10(B) and 10(C) to locate regions the highest of tensile strength, elongation at break and lowest water vapor permeability. The shaded area in Figure 11. satisfies following highest tensile strength and elongation at break and lowest water vapor permeability. As shown, the optimum condition for edible film from mung bean proteins at shaded area: pH of film solutions of 9.40 and heating temperature of 73.5 °C for 30 min of heating time. At this condition demonstrated 6.45 MPa, 39.10% and 10.57 g.mm/m^2.day.kPa of tensile strength, elongation at break and water vapor permeability, respectively.

(A) Tensile strength
(heating time = 30 min)

(B) Elongation at break
(heating time = 30 min)



(C) Water vapor permeability
(heating time = 30 min)

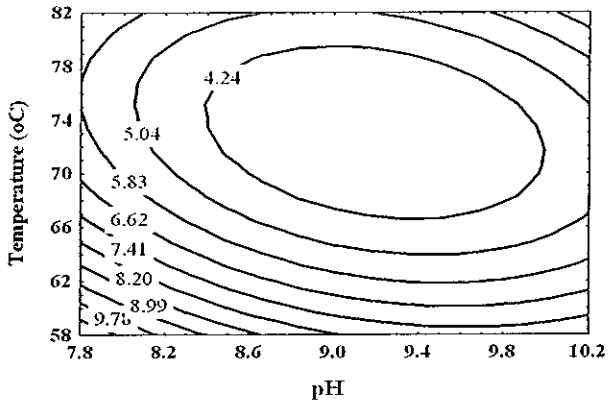


Figure 10. Contour plots showing response behavior of pH and heating temperature of film solutions heated for 20 min on the; (A) tensile strength (MPa), (B) elongation at break (%) and (C) water vapor permeability (g.mm/m^2.day.kPa).

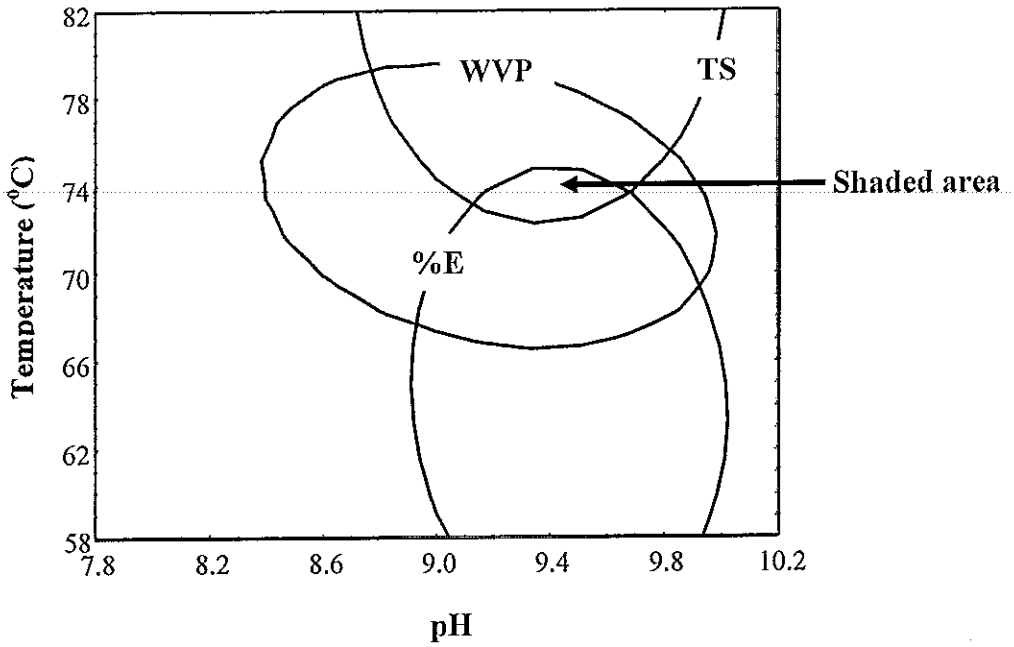


Figure 11. Optimum film solutions condition as a function of the independent variables after superimposition of contour plots over those of 10(A), 10(B) and 10 (C). Shaded area indicates regions the highest of tensile strength, elongation at break and lowest water vapor permeability.

Validation tests are performed to determine the adequacy of the SOP model (Floros and Chinnan, 1988; Mudahar et al, 1990). This is performed because a fractional factorial design was used as the experimental design. A model is deemed adequate if the predicted values (of the model) are close to the experimental values observed during the validation tests. Table 5 shows the predicted and observed values for the responses at optimum condition for the effect of some process parameters on the properties of edible film from mung bean proteins. The experimental values were averages of three replicates and were very close to the predicted values indicating that the SOP models generated were acceptable. The high CV values for some models were due to their lesser reproducibility (Montgomery, 1984) that may have contributed to the statistical insignificance of some of these models. Despite the lesser effect of these responses to the optimum conditions, predictions were within fairly acceptable limits.

Table 5. Predicted and observed values for the independent variables after superimposition conditions of edible film from mung bean proteins.

Response variable	Predicted value	Actual value ± SD
Tensile strength (MPa)	6.45	6.28± 0.43 (6.84%)
Elongation at break (%)	39.10	35.74 ± 5.07 (14.18%)
Water vapor permeability ($g.mm/m^2.day.KPa$)	10.57	11.43 ± 1.07 (9.3%)

Numbers in parentheses are coefficients of variation (CV)

6. Effect of Protein Concentrations on the Properties of Edible Films from Mung Bean Proteins

The effect of protein concentrations on the tensile strength and elongation at break of edible films from mung bean proteins are presented in Figure 4.12. Edible films with containing protein concentrations 1.5, 3.0 and 4.5% at fixed pH of 9.40, heating temperature of 73.5 and 30 min heating time were investigated. When 4.5% of protein concentration was used, the formation of films was inhibited due to the high viscosity of proteins. Hence, only the effect of proteins concentration at 1.5% and 3.0% w/w were compared. Different in the proteins concentration influenced the tensile strength (Figure 12.). Increasing of protein concentration at 3.0% w/w showed significantly ($p < 0.05$) higher tensile strength than using 1.5% w/w, (Figure 12.), this implied that, higher protein content (3.0%) induced favorable structure regarding the ability of the films to form. Comparing of the elongation at break of edible films from mung bean proteins at various content of proteins is given in Figure 12. It was observed that, the proteins concentration was significantly ($p < 0.05$) effect on elongation at break. Increasing the concentration of proteins of film solutions from1.5 to 3.0 % w/w provided higher elongation at break. Increasing in elongation at break of edible films formed at higher (3.0% w/w) indicated difference protein net work was formed. At the lower protein concentration is probably less protein-protein interaction, while higher proteins concentration (3.0% w/w) induced favorable structure regarding the ability of the films to form and stretch.

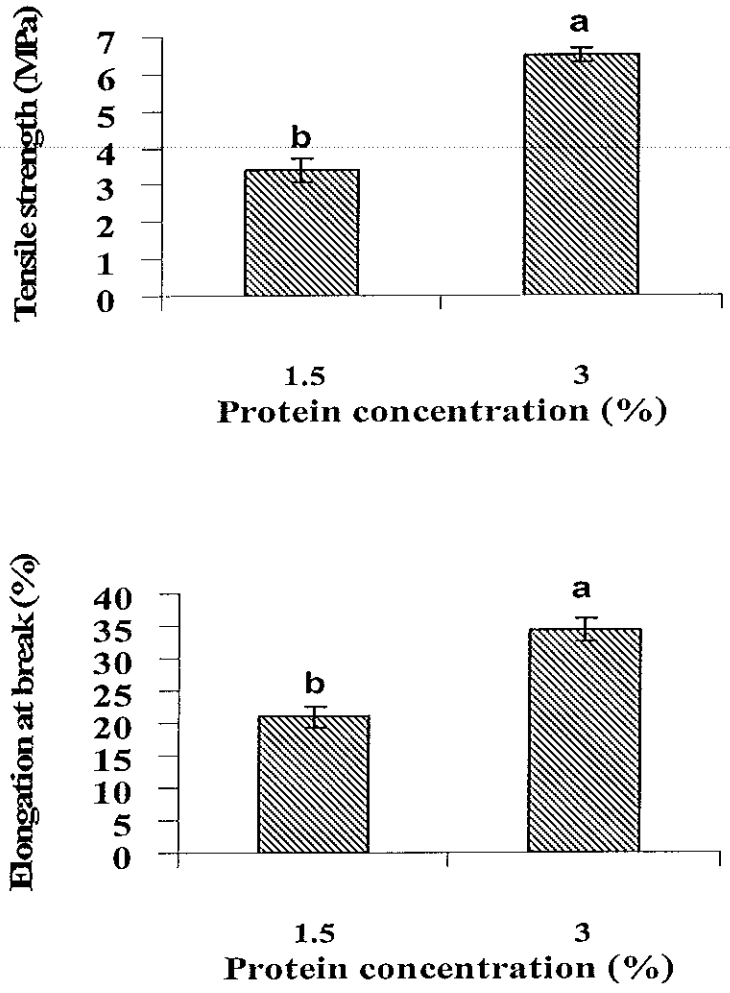


Figure 12. Effect of protein concentration on the tensile strength and elongation at break of edible films from mung bean proteins film. Standard error bars are shown. a-b, tensile strength and elongation at break value means with different letters represent significantly different value at $p < 0.05$ using Duncan's Multiple Range Test.

Water vapor permeability of edible films from mung bean proteins at various concentrations 1.5 and 3.0w/w were investigated (Figure 13.). The protein concentrations had significantly ($p < 0.05$) effect on water vapor permeability (Figure 13.). This result might be due to the fact that, higher protein concentration (3.0 %), a more aggregated structure with a denser protein structure network formed (McHugh and Krochta, 1994). As a result, decrease in water vapor permeability concomitantly with decrease in film and protein solubility obtained (Figure 14.). However, Anker *et al.* (2000) reported that varying of whey protein isolated (WPI) concentration over and under the critical gel concentration (Cg) elucidated the influence of the polymer network on the film properties. The strain at break showed a maximum at the Cg and this implied that the most favorable structure regarding the ability of the film to stretch was formed at this concentration. Reduced mechanical properties for films formed below and above Cg indicated that different protein network was formed. However effect of varying the concentration of WPI influencing the barrier property was studied by McHugh *et al.* (1994). Miller and Krochta (1997) further confirmed that the permeability was highly affected by how closely packed the polymer chains were. Although this showed how large an effect of the protein concentration was on the barrier properties, several other factors were known to affect the permeability: the microstructure, the plasticizer, the density, the orientation, cross-linking, and the molecular weight of the polymer chains, the nature of the permeant etc (McHugh *et al.,* 1994).

The color of edible films from mung bean proteins was affected by protein concentrations, higher protein concentration showed significantly ($p \leq 0.05$) higher in b* , but lower in a* (Figure 15), hence, the films color was darker and more yellow than that found at lower content of protein. However, the lightness (L*) of films was not significantly ($p \leq 0.05$) different (Figure 15.). It was well known that protein could undergo browning reactions during processing, causing yellowing and loss of nutritional value of product (Coultate, 1988). Yellowing was attributed to the reaction of protein lysine group with reducing sugars such as lactose and glucose. Labuza and Saltmarch (1981) found that the rate of browning pigment formation in whey powder increased as

storage temperature and water activity increased from 25-45 °C and 0.33 to 0.65, respectively. Heat curing of whey protein isolated film at 60, 70 and 80 °C for up to 48 h qualitatively increased film yellowing (Miller *et al.* 1997).

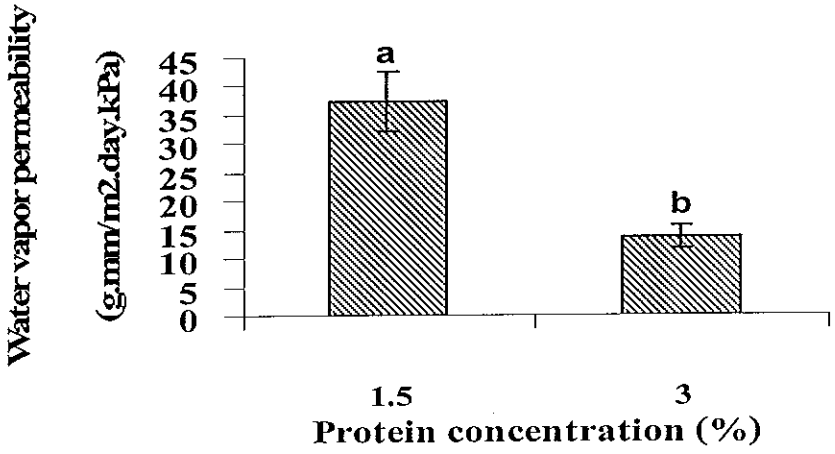


Figure 13. Effect of protein concentration on the water vapor permeability of edible films from mung bean protein film. Standard error bars are shown. a-b, water vapor permeability value means with different letters represent significantly different value at $p < 0.05$ using Duncan's Multiple Range Test.

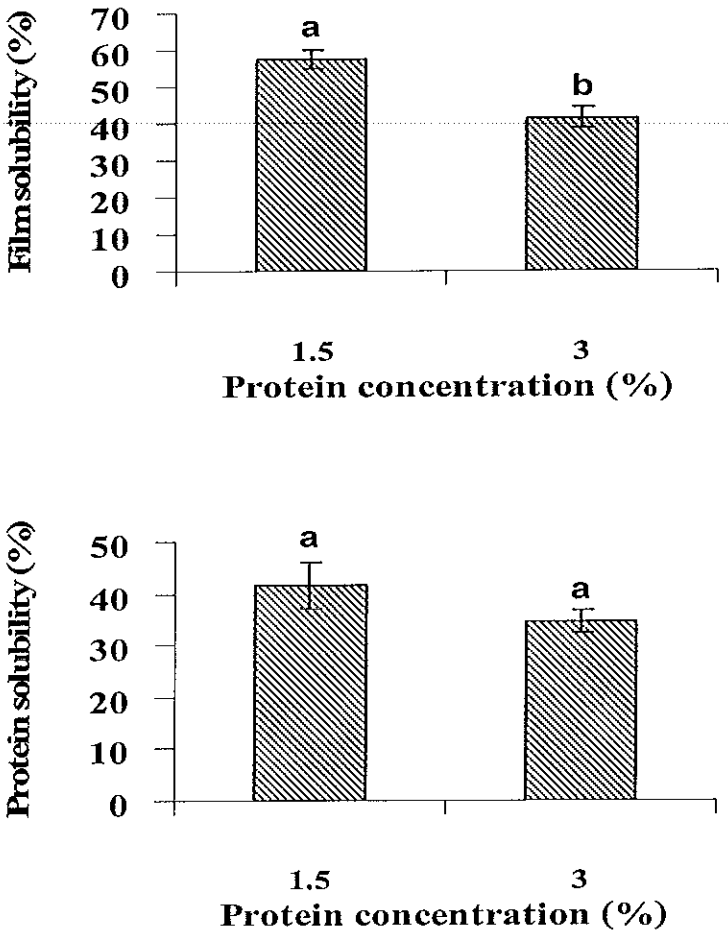


Figure 14. Effect of protein concentration on the film and protein solubility of edible films from mung bean protein film. Standard error bars are shown. a-b, film and protein solubility value means with different letters represent significantly different value at $p < 0.05$ using Duncan's Multiple Range Test.

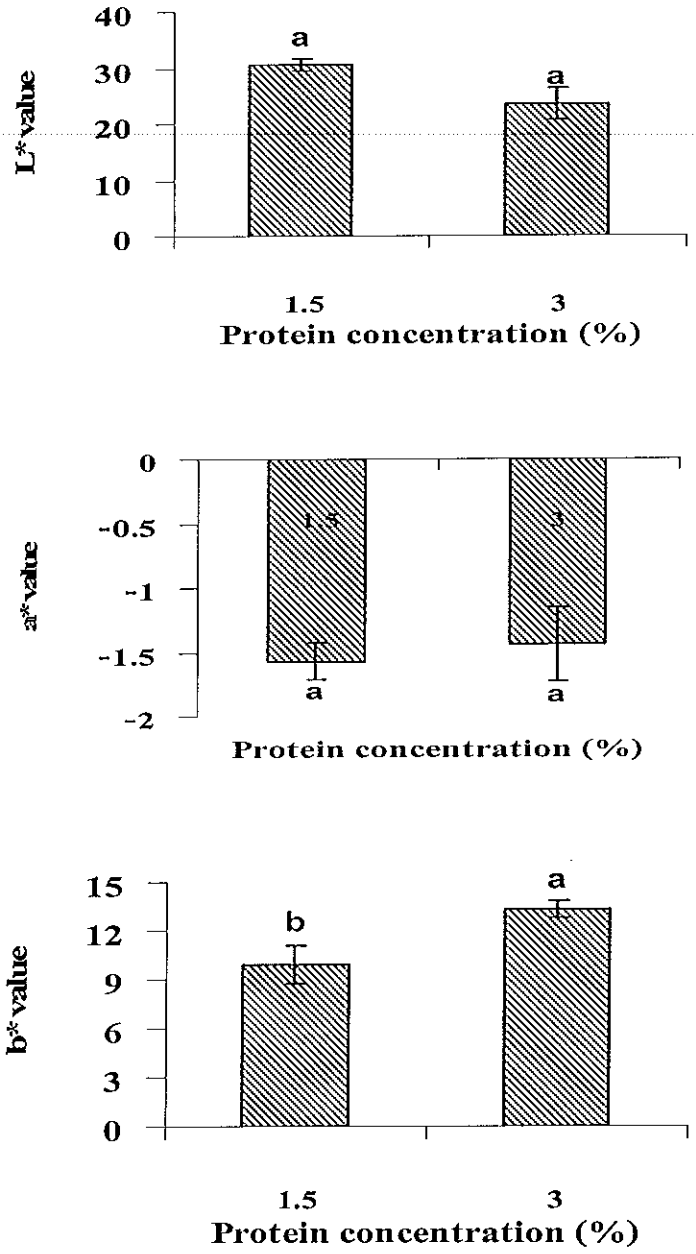


Figure 15. Effect of protein concentration on the L*, a* and b* values of edible films from mung bean protein film. Standard error bars are shown. a-b, L*, a* and b* value means with different letters represent significantly different value at $p < 0.05$ using Duncan's Multiple Range Test.

7. Effect of Type and Concentration of Plasticizer on the Properties of Edible Film from Mung Bean Protein

The plasticizers which were selected in this study had been used to plasticize in protein and polysaccharides film based on compatibility (no phase separation). Our work found that the edible film from mung bean proteins was compatible with all plasticizers in this study. The plasticizers represent different chemical compositions, sizes and shapes, thus providing the opportunity to explore the effects of these factors on film mechanical properties. Preliminary work demonstrated that the edible film from mung bean proteins formed without plasticizer were relatively brittle and broken easily when peeled off.

7.1 Effect of Type and Concentration of Plasticizer on Tensile Strength and Elongation at Break of Edible Film from Mung Bean Protein

Preliminary work demonstrated that edible film from mung bean proteins formed without plasticizer as relatively brittle and broke easily when peeled off. Hence desirable mechanical properties of edible films were improved by using four types of plasticizer (sorbitol, glycerol and polyethylene glycol) at different concentrations (30, 40, 50 and 60%). The mechanical properties of films plasticized by sorbitol, glycerol, polyethylene glycol, at different concentration were assessed by measuring their tensile strength and elongation at break. The results are shown in Figure 16. It was observed that an increase in the content of these plasticizers resulted in decrease in mechanical resistance (decrease in tensile strength) and tend to increase in extensibility (increase in percentage of elongation). Tensile strength decreased from 7.23 to 2.40, 3.75 to 1.25 and 5.07 to 2.39 MPa when the sorbitol, glycerol and polyethylene glycol concentration increased from 30 to 60 % w/w., while, elongation at break increased from 6.73 to 27.66, 15.59 to 21.25 and 9.16 to 24.81%. Sorbitol, glycerol and polyethylene glycol are low molecular weight hydrophilic molecules that could easily fit into protein chains and establish hydrogen bonding with reactive groups of proteins. Bringing together plasticizers and proteins induced formation protein-plasticizer interactions to the

detriment of protein-protein interactions. As a consequence, the density of intermolecular interaction in material decreased and the free volume between polymer chains increased (Cuq *et al.,* 1997). The changes in mechanical properties as affected by hydrophilic plasticizers were observed for various hydrocolloid-based films (Park and Chinnan, 1990; Gontard *et al.*, 1993). The mechanical properties of sorbitol, glycerol and polyethylene glycol plasticized films at an equal concentration were compared (Figure 16.). The sorbitol plasticized films had significantly ($p \leq 0.05$) higher tensile strength and lower elongation at break than polyethylene glycol and glycerol and plasticized films at all concentrations. This could be attributed to the ring molecular conformation of sorbitol molecules, which may sterically hinder insertion between the protein chains resulted in less effective in disrupting the protein-protein interruptions. McHugh and Krochta (1994) studied whey protein isolated/sorbitol (1:1) and whey protein isolated/glycerol (2:3) films and presented similar tensile strength values. They concluded that a higher amount of sorbitol than glycerol was needed to obtain similar tensile strength properties. The glycerol and polyethylene glycol plasticized films were more stretchable than the sorbitol plasticized films (Figure 16), suggesting that glycerol and polyethylene glycol could be a more effective plasticizer in edible films than sorbitol and sucrose. The effectiveness of glycerol and polyethylene glycol in the edible film from mung bean proteins are most likely due to its small size and configuration which allows it to be more readily inserted between the polymer chains, and consequently exert more influence on the mechanical properties than the larger molecule. Donhowe and Fennema (1993) found that plasticizer with low molecular weights such as glycerol was more effective than those with high molecular weights in methylcellulose-based films. Similarly, McHugh and Krochta (1994) suggested that smaller size plasticizer was more effective than larger size plasticizer in whey protein films. In addition, at an equal percentage concentration, the total number of glycerol molecules in the film-solution was greater than that of the higher molecular weight polyethylene glycol, therefore glycerol had more functional groups (-OH) than polyethylene glycol, which should promote the plasticizer-polymers interactions in the films (Donhowe and Fennema, 1993; McHugh

and Krochta, 1994). Gennadios *et al.* (1993) reported that, the polar group (-OH) along plasticizer chains are believed to developed polymer-plasticizer hydrogen bonds replacing the polymer-polymer interaction in the biopolymer films. Molecular size, configuration and total number of functional hydroxide groups of the plasticizer as well as its compatibility with the polymer could affect the interactions between the plasticizer and the polymer (Yang and Paulson, 2000)

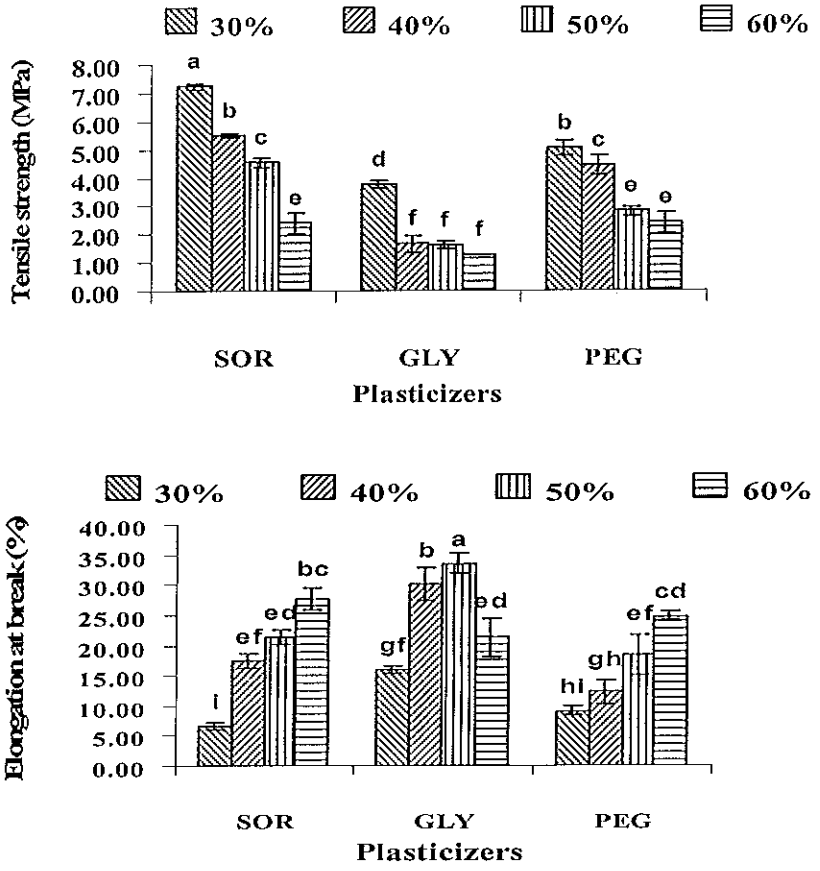


Figure 16. Effect of plasticizer type and concentration on the tensile strength and elongation at break of edible films from mung bean protein film. Standard error bars are shown. a-i; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test, where, SOR = sorbitol, GLY = glycerol and PEG = poly ethylene glycol.

7.2 Effect of Type and Concentration of Plasticizer on Water Vapor Permeability of Edible Film from Mung Bean Protein

Water vapor permeability of edible film from mung bean proteins with different type and concentration of plasticizer were examined (Figure 17.). The water vapor permeability increased with increasing of plasticizer concentration. The water vapor permeability increased from 44.38 to 68.48, 125.16 to 238.20 and 78.38-204.18 g.mm/m^2.d.kPa respectively, when the concentration of sorbitol, glycerol and polyethylene glycol increased from 30 to 60 % w/w (Figure 17.). This tendency could be explained by structural modifications of the protein network. The incorporation of plasticizers modified the molecular organization of the protein network, with an increase in free volume. The network becomes less dense and as a consequence more permeable (Ashley, 1985). Permeability increased with plasticizer content could be related to hydrophillicity of plasticizer molecules. Introducing hydrophillic plasticizers, favorable to adsorption and desorption of water molecules, was reported to enhance the water vapor permeability of hydrocolloid-based films (Gontard *et al.*, 1993; McHugh *et al.* 1994). Comparing of the successive values of the water vapor permeability for each plasticized film was shown in Figure 17. Films plasticized with sorbitol had lower water vapor permeability than those with polyethylene glycol and glycerol at each plasticizer concentration, respectively due to the fact that sorbitol had ability to bind less water than sucrose, polyethylene glycol and glycerol and, thereby, provided a lower water vapor permeability (McHugh *et al.*, 1994). Chick and Ustanol (1998) reported that casein-based films plasticized with glycerol had higher water vapor permeability values than films plasticized with sorbitol when the same amounts of plasticizers were used. The high hydrophillicity of glycerol and polyethylene glycol molecules, which is favorable to the adsorption of water molecules, could also be contribute to the increase in the films water vapor permeability (Gennadios *et al.*, 1993b). The increase in water vapor permeability with increasing hydrophillicity plasticizer concentration was also common in edible films (McHugh *et al.*, 1994; Cuq *et al.*, 1997). Sorbal *et al.* (2001) reported that hydrophilicity of the plasticizers will increase the water content of the films,

consequently increasing the mobility of the molecules. In addition, increasing water content could also affect permeate solubility in the film.

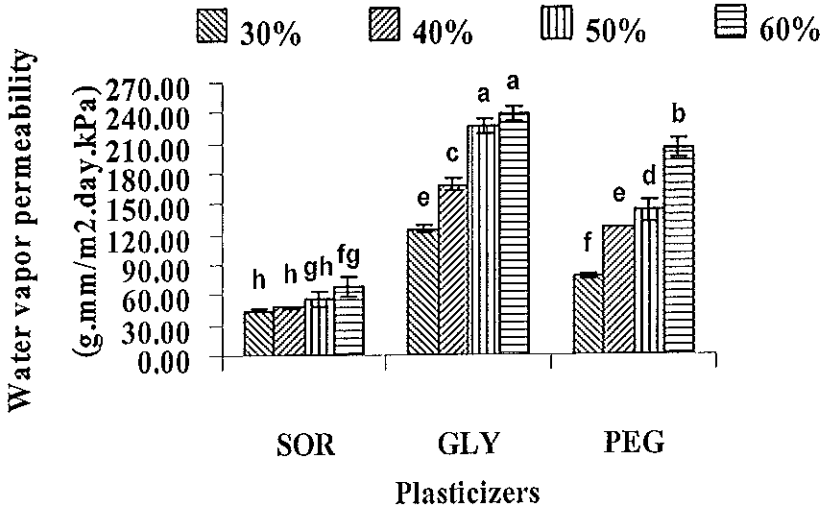


Figure 17. Effect of plasticizer type and concentration on the water vapor permeability of edible films from mung bean protein film. Standard error bars are shown. a-h; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan' Multiple Range Test, where, SOR = sorbitol, GLY = glycerol and PEG = poly ethylene glycol.

7.3 Effect of Type and Concentration of Plasticizer on Film and Protein Solubility of Edible Film from Mung Bean Protein

From visual observations and irrespective of plasticizer type and content, the edible films from mung bean proteins clearly did not lose integrity after a 24 h immersion in water. Irrespective of the type, an increase in plasticizer content leads to an increase in films and proteins solubilities (Figure 18.). It could be hastily concluded that hydrophilic plasticizers enhanced films solubility in water. Low molecular weight protein chains (i.e. monomers and small peptides) formed during storage of film solutions and entrapped in the network (Cuq *et al.*, 1995) could then constitute the protein-based

materials that solubilize in water. The dry matter solubilized in water was likely to be composed mainly of the plasticizer. The protein network was then not likely to solubilize or disperse in water. High interaction density and more certainly, the presence of intermolecular covalent bonds or "physical knots" (i.e. chain entailments) are responsible for partial insolubility of these films. This water solubility behavior could not be generalized, and understanding the films solubility remains a complex subject. Plasticizer solubilization in water was already observed for films based on wheat gluten or treated soy proteins or transglutaminase catalytic cross-linking whey protein (Gontard *et al.*, 1992; Stuchell and Krochta, 1994). Stuchell and Krochta (1994) pointed out that increase in the content of protein solubilized in water was obtained when the hydrophilic content of treated whey protein-and soy protein-based films increased. A decrease in the polymer network interaction density due to the presence of plasticizer was thus associated with this increase in solubility property. The lowest films and proteins solubility of edible film from mung bean proteins plasticized by 30% w/w of these plasticizers were noticed, while increasing the amount of plasticizer content showed higher films solubility and proteins solubility (Figure 18.). It could be explained that, at higher content of plasticizer, more molecules of plasticizer were untrapped in the protein cross linked network and able to escape into solution, while, lower content of plasticizer gave lowered plasticizer molecules untrapped in the crosslinked network and less ability to escape into solution. The film and protein solubilities were higher for the sorbitol followed by polyethylene glycol plasticized film comparing with those plasticized with glycerol. The sorbitol and polyethylene glycol had a ring and height molecular weight, which may sterically hinder insertion between the protein chains (Yang and Paulson, 2000) thus, facilitated its escape into solution, while glycerol have a small molecules, which promote the insertion between protein-protein chains.

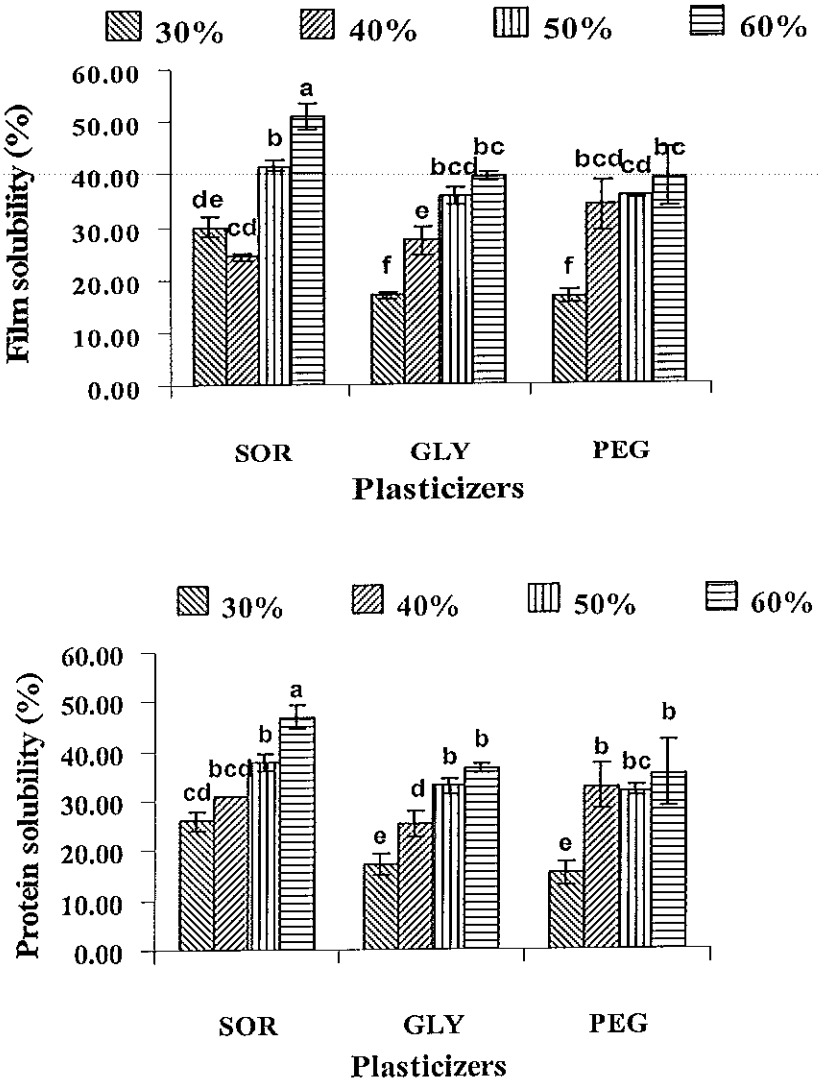


Figure 18. Effect of plasticizer type and concentration on the film and protein solubility of edible films from mung bean protein film. Standard error bars are shown. a-f; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test, where, SOR = sorbitol, GLY = glycerol and PEG = poly ethylene glycol.

7.4 Effect of Type and Concentration of Plasticizer on Film Color of Edible Film from Mung Bean Protein

The results of the measurements performed on the films color were expressed in accordance with the CIELAB system, and the rectangular coordinates (L*, a* and b*) were defined. The color of films was more affected by the nature of the plasticizer rather than by concentration. L* and a* values of edible film from mung bean proteins plasticized by sorbitol, glycerol and polyethylene glycol seemed not significantly different ($p > 0.05$) (Figure 19.). In contrast, increased yellowness (b*) occurred when higher plasticizer concentration involving with sucrose and sorbitol were used (Figure 19.). This was somewhat expected since color change mainly depend on the type of plasticizer.

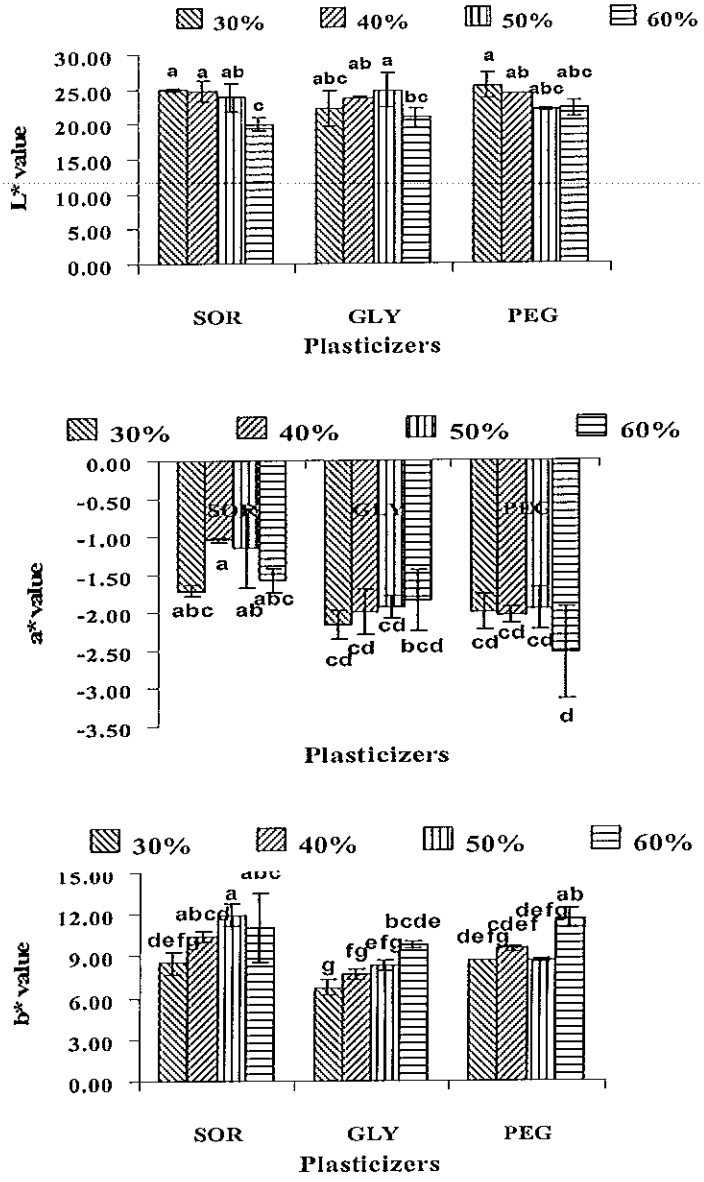


Figure .19 Effect of plasticizer type and concentration on the L*, a* and b* of edible films from mung bean protein film. Standard error bars are shown. a-g; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test, where, SOR = sorbitol, GLY = glycerol and PEG = poly ethylene glycol.

8. Effect of Lipid Type and Concentration on the Properties of Edible Film from Mung Bean Proteins.

8.1 Effect of Type and Concentration of Lipid on Tensile Strength and Elongation at Break of Edible Film from Mung Bean Protein

Gernerally, protein films (hydrophilic films) have good mechanical properties and are excellent gas, aroma, and lipid barriers but inefficient against water transfer. Several research groups have attempted to improve the water vapor barrier properties of protein film by adding lipid as an emulsion (Koelsch and Labuza, 1992; Gontard et al., 1994; and Quezada et al., 2000). Hence, in this study the mechanical properties of edible films were improved by using three types of lipid (oleic acid, stearic acid and palm oil) at different concentrations (5, 10, 15, 20, and 25%). The mechanical properties of films were assessed by measuring their tensile strength and elongation at break. The results are shown in Figure 20. It was observed that the TS of protein film from mung bean proteins decreased with the addition of lipid. Previously work demonstrated that the maximum occurred when no lipid was added (6.45 MPa). The decreased of TS of films as addition of lipids (Figure 20.). These results agreed with the study conducted by Gontard et al, (1994) in which they found that, for whey protein/lipid composite films, the influence of lipids depended on lipid characteristics and interactions between the lipid and the protein structural matrix. Similarly, Shaw et al, (2002), who found that increasing soya oil concentration led to decreased in TS. Weller et al. (1998) stated that the decrease in Young's modulus of protein film accompanying the increase in lipid concentration was related to the weakening effect of lipid on protein network, due to the lack of structural integrity of the lipid. The interactions between nonpolar lipid molecules and between the polar polymer and nonpolar lipid molecules are believed to be much lower than those between the polar polymer molecules. Typically, strength reduction of edible composite films with lipid incorporation has also been reported (Debeaufort and Voilley, 1995; Shellhammer and Krochta, 1997; Yang and Paulson, 2000; Bertan et al., 2005). The addition of palm oil resulted in a greater reduction in TS

of the mung bean protein films than the addition of oleic acid and stearic acid (Figure 20.). The differences in mechanical properties between these films could be related to their physical state, structure and chemical nature of the lipids. No significant difference ($p > 0.05$) in TS occurred between the oleic acid and stearic acid-incorporated films.

The elongation at break of mung bean protein films with the addition of lipids decreased as the concentration of lipids increased (Figure 20.). This is in agreement with the findings of Javanmard and Golestan, (2007) that %E decreased as olive oil concentration increased; thus, the flexibility of whey protein emulsion films was reduced. This may be attributed to noncontinuous film matrix formation probably because of the presence of lipid globules. The lower continuity and cohesiveness of protein network in the presence of lipid globules might result in the decrease in EAB (Anker et al., 2002; and Peroval et al., 2002). Lower water content of film containing lipids may also cause the decreased elongation (Gallo et al., 2000). Oleic acid added into the film resulted in higher %E than stearic acid and palm oil-incorporated films. The effect of lipid type is due to their chemical structure. The low molecular weight and liquid phase possibly exhibited more plasticizing effect than high molecular weight and solid lipids.

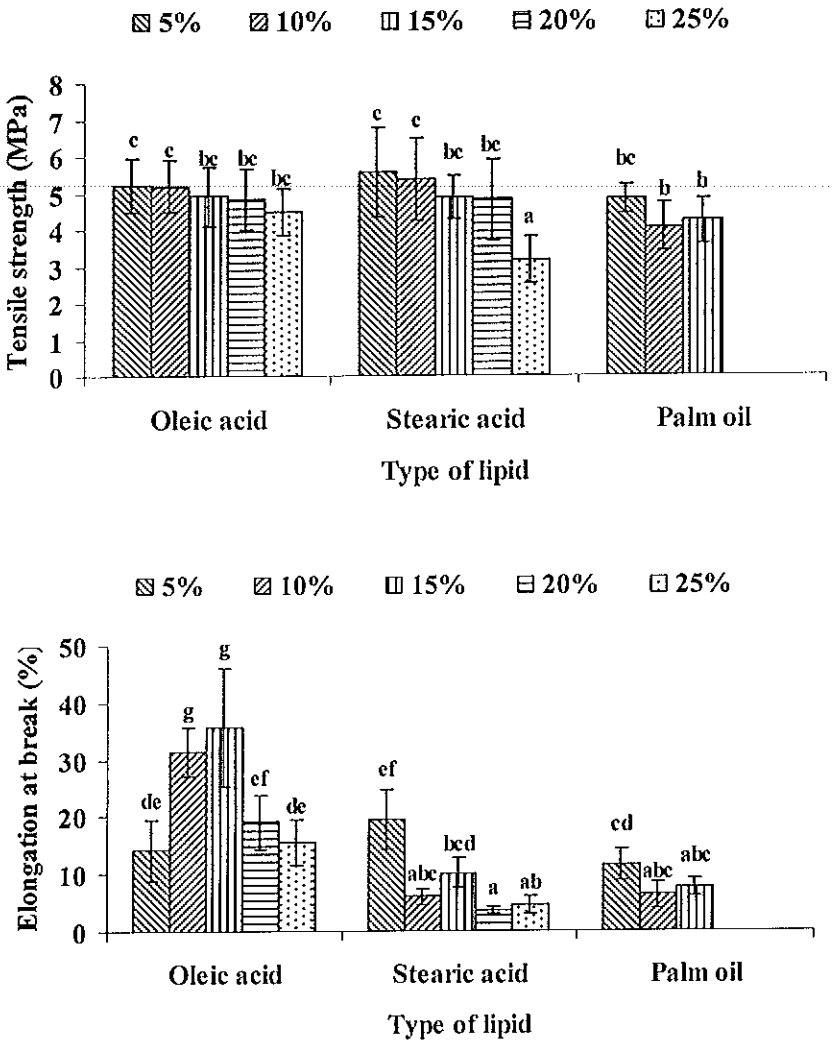


Figure 20. Effect of lipid type and concentration on the tensile strength and elongation at break of edible films from mung bean protein film. Standard error bars are shown. a-f; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test.

8.2 Effect of Type and Concentration of Lipid on Water Vapor Permeability of Edible Film from Mung Bean Protein

The edible film from mung bean protein with the addition of lipids were malleable and visually homogeneous. The WVP of mung bean protein film without the addition of lipids were more water vapor permeable (10.57 g.mm/m^2.day.kPa) than incorporated films with lipids, resulting from the greater hydrophilic property of the films without the addition of lipids (Figure 21.). The addition and increasing concentration of lipids tended to decrease WVP (Figure 21.), due to the film's contained higher hydrophobicity (Morillon et al., 2002; Vergano and Weller, 1994 and Shellhammer and Krochta, 1997). Gontard et al, (1994) observed that when lipids were used in composite film formulations, large amount of lipids could promote a protective effect on WVP. Comparing with the same concentration of lipids, the result showed that the addition of oleic acid and stearic acid resulted in a greater reduction in WVP than the addition of palm oil (Figure 21.). In general, WVP of emulsion films comprised of biopolymer and lipids strongly depend on the type, structure and quantity of lipids. For film containing fatty acid, the WVP decreased with increased chain length and degree of saturation of the lipids (Hagenmaier and Shaw, 1990; Kamper and Fennema, 1984a, b; Koelsch and Labuza, 1992; McHugh and Krochta, 1994). Moreover, the increase of solid fat content especially between 0% and 30% allows for the improvement of the barrier efficiency (Kamper and Fennema, 1984a; Morrilon et al., 2002). This is because the CH_2 group of the liquid aliphatic chains have a greater effect than when they are crystallized (Morrilon et al. 2002). So solid structure of fats is denser and limits the diffusion of water. Moreover, the solubility of water in solid lipids is also reduced (Kamper and Fennema, 1984a; Callegarin and Quezada, 1997). Beside, for solid fat contents higher than a critical value that depends of the lipid nature, permeability could increase due to structure defects within the films (Morillon et al, 2002). However, WVP of film corporated with oleic acid was not different from the stearic acid incorporated film, but both films demonstrated lower WVP than palm oil incorporated film. Morillon et al, (2002) reported that fats with

short chain length are more efficient to reduce moisture transfers through films than long chain.

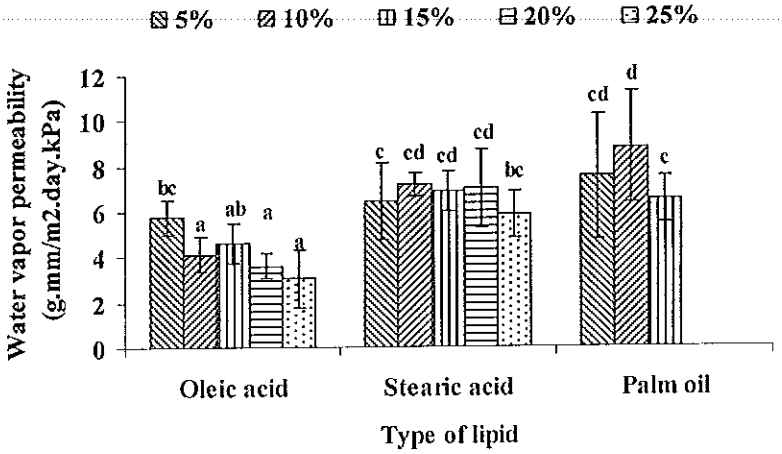


Figure 21. Effect of lipid type and concentration on the water vapor permeability of edible films from mung bean protein film. Standard error bars are shown. a-d; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test.

8.3 Effect of Type and Concentration of Lipid on Film Solubility of Edible Film from Mung Bean Protein

The water resistance of edible films is an important property in food applications especially when the water activity is high or when the film has to be in contact with water. Conversely, edible films with higher water solubility may be required, for instance, to contain portions that will be dissolved in water or hot food (Guilbert and Biquet, 1989). The effect of lipid types and concentrations on film solubility is shown in Figure 22. Film solubility markedly decreased when the lipid were incorporated. The loss in solubility was pronounced when greater level of lipids were incorporated. Addition of lipids thus increased hydrophobicity of the mung bean proteins composite film. Similar results have been reported by Kim and Ustanol, (2001) on lipid-

whey protein emulsion films. All lipids no significant difference ($p>0.05$) effect film solubility (Figure 22.).

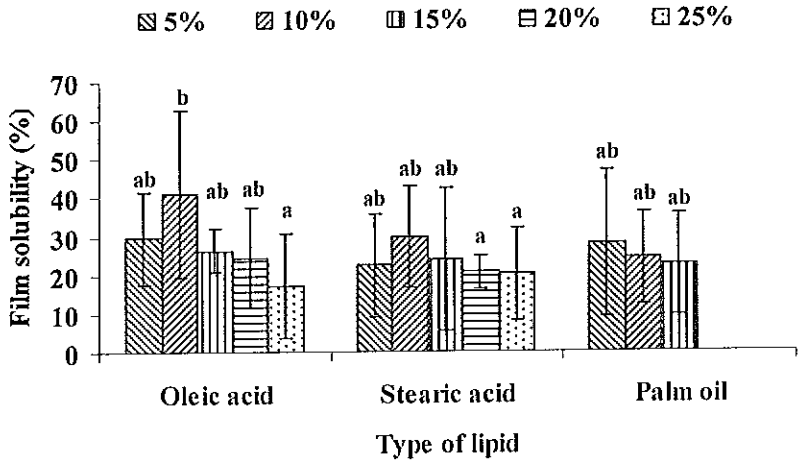


Figure 22. Effect of lipid type and concentration on the film solubility of edible films from mung bean protein film. Standard error bars are shown. a-b; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test.

8.4 Effect of Type and Concentration of Lipid on Film Color of Edible Film from Mung Bean Protein

The color of edible film from mung bean proteins with the addition of lipids was affected by the lipid type and concentration. The film became lighter yellow as evidenced by the increased L* and b* values when the concentration of lipid increased (Figure 23.). The decrease in a* value was noticeable as the lipids was incorporated into the films. Comparing with the same amount of lipids, the results pointed out that composite films with palm oil showed higher b* values but lower L* and a* values than oleic acid and stearic acid. Yang and Paulson (2000) have reported that the differences in opacity of film were determined by the optical properties of lipids incorporated.

Furthermore, addition of lipids generally causes the films to lose or reduce their transparency (Yang and Paulson, 2000; Shaw et al., 2002; Pommet et al., 2003; Bertan et al., 2005). However, when lipids are added, the films lose transparency. The degree of opacity depends on the lipid content and particle size. In general, films with small particle size and low lipid content were translucent; however, as particle size and lipid content increased, the films became more opaque (Prez-Gago and Krochta, 2001).

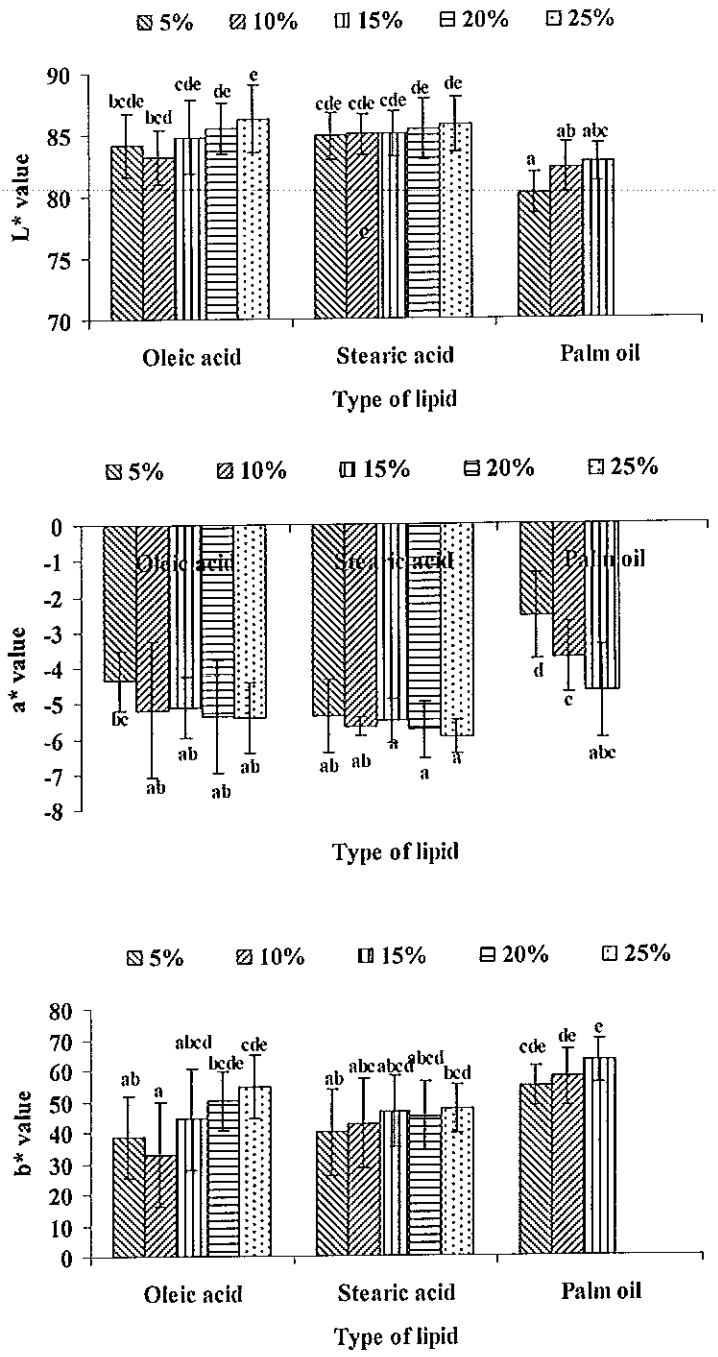


Figure 23. Effect of lipid type and concentration on the L*, a* and b* of edible films from mung bean protein film. Standard error bars are shown. a-e; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test.

8.5 Microstructue

The relationship between surface characteristics and moisture barrier properties of film from mung bean proteins with the addition of lipids, SEM was used to determine the surface morphology of the film with 5, 10, 15, 20 and 25% of oleic acid. It was observed that the films with 5% of oleic acid had continuous surface without porous and/or hole of air bubbles (Figure 24.). The micrographs also showed increased surface irregularity and oil droplets were more intense on the surface when the addition of lipids increased (Figure 24.).

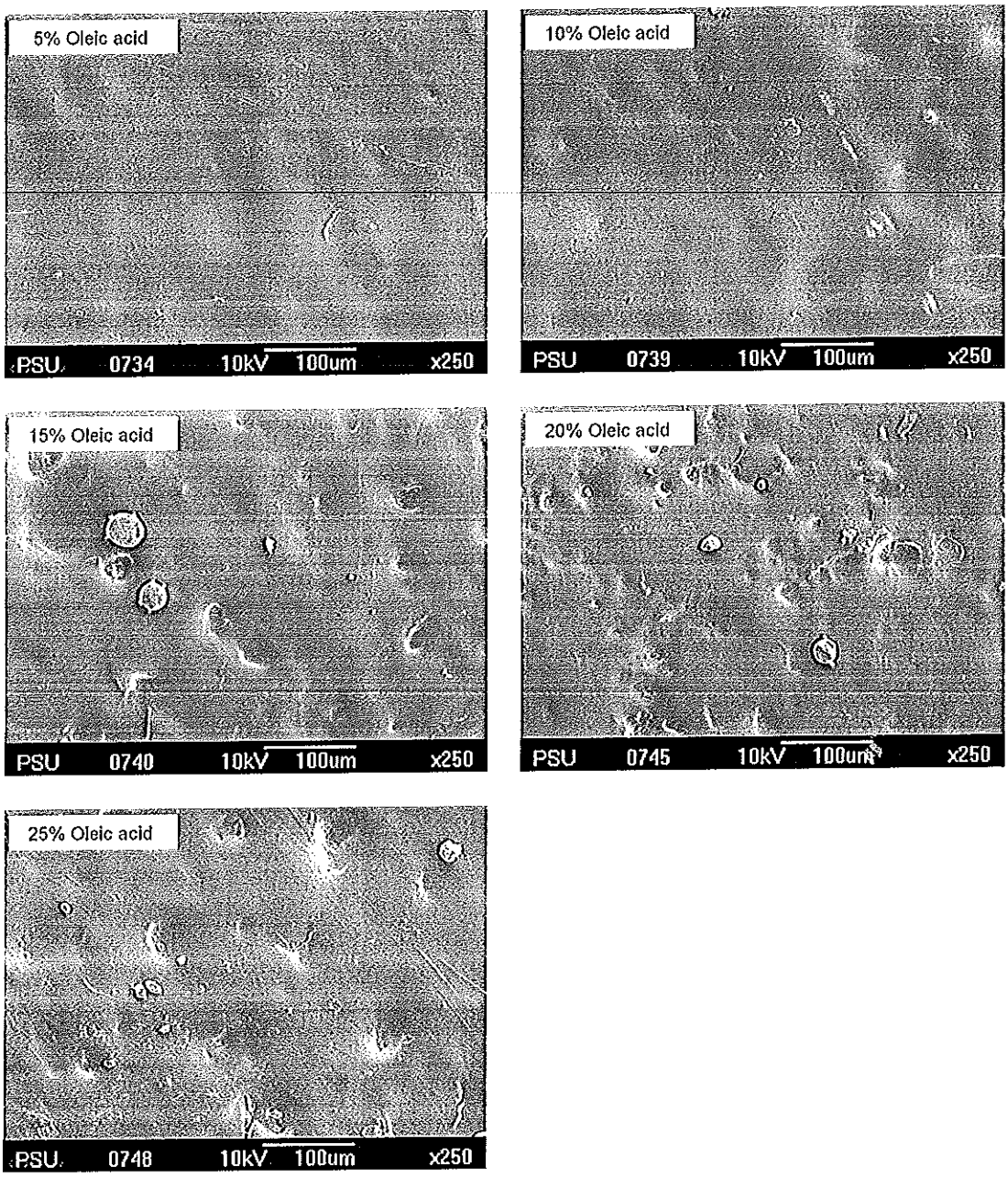


Figure 24. SEM of mung bean protein/lipid composite films containing various concentrations of oleic acid.

Comparing with the same concentration of lipids, the results showed that the surface irregularity with the addition of lipids. However, oleic acid appeared to be well incorporated and embedded in the composite films resulting in a relatively smooth and continuous surface more than composite films with stearic acid and palm oil, respectively (Figure 25.). The film with palm oil had more irregular surface than stearic acid, which may have resulted from oil droplets of palm oil in the film.

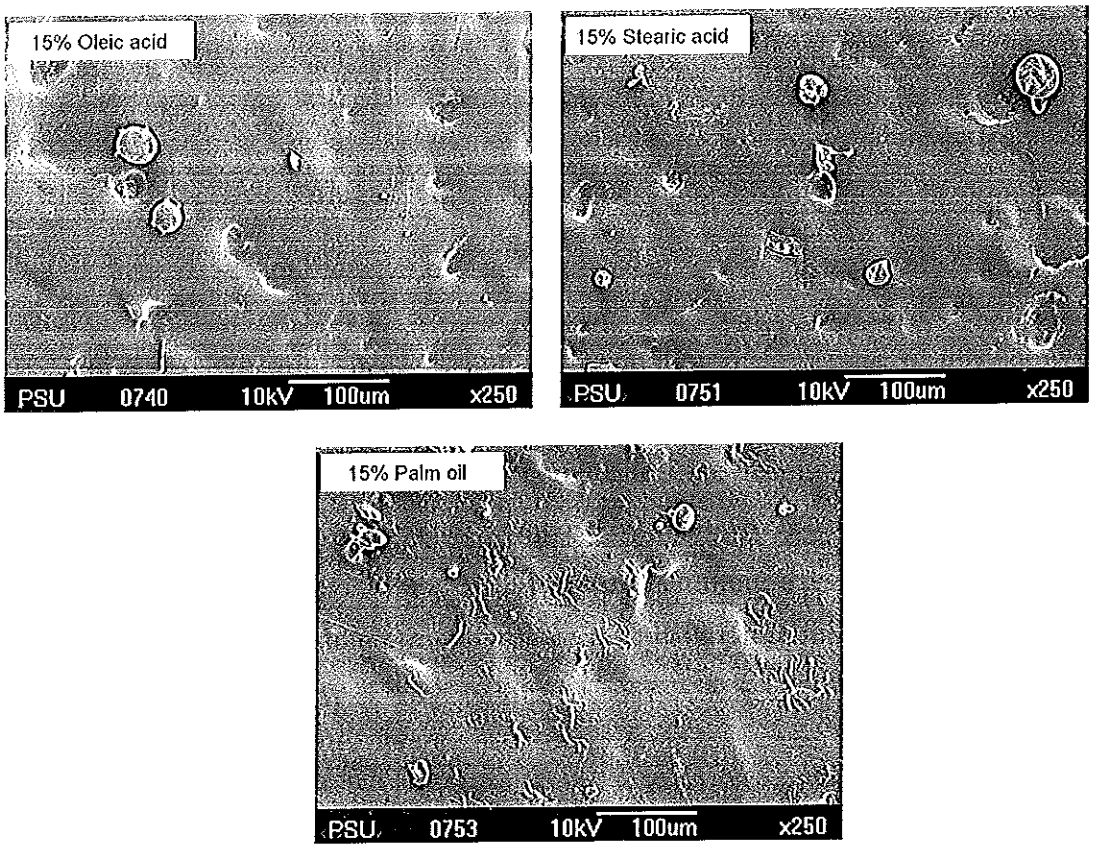


Figure 25. SEM of mung bean proteins/lipid composite films containing 15% oleic acid, 15% stearic acid and 15% palm oil.

9. Effect of pH, Heating Temperature and Heating Time on the Properties of Edible Film from Red Bean Proteins

9.1 Tensile Strength and Elongation at Break

An edible film must withstand the normal stress encountered during its application, subsequent shipping and handling of the food, to maintain its integrity and also barrier properties. High tensile strength is generally required but deformation values must be adjusted according to the intended application of the films. That is whether it is undeformable material to provide structural integrity or reinforce structure of the food (Gontard *et al.* 1992). Tensile strength is an important mechanical property that expresses the maximum stress developed in films during tensile testing (Gennadios *et al.* 1993), while elongation at break is an indication of films flexibility and stretchability (extensibility). High tensile strength is generally required but deformation values must be adjusted according to the intended application of the films. That is whether it is undeformable material to provide structural integrity or reinforce structure of the food (Gontard *et al.* 1992). If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile strength at yield or at break, respectively (ASTM, 1991). The main factors that influenced the film properties were pH of film solutions, while heating temperature and heating time had the low effect. The contour plots for tensile strength and elongation at break are given in Figure 26. and 27. Depending upon the film conditions, tensile strength showed a high variation between 2.64-9.11 MPa (Figure 26.) and 1.88-7.50 % for elongation at breaks (Figure 27.). Comparing at the same heating temperature of film solutions, the results demonstrated that, tensile strength showed increase as pH of film solutions increased, it would be implied that higher pH of film solutions induced formation of resistant films. Banker (1966) reported that pH plays an important role in protein films made from water-soluble materials. At alkaline condition, pH away from the isoelectric point promoted denaturation of proteins, unfolds and, solubilize, during the solubilization of proteins, the cohesive forces between the proteins macromolecules are neutralized by unions with the solvent molecules (Banker,

1966). The functionality of the polymers is related to solution properties which further influences film characteristics. The charge groups repel each other and produce a stretching of the polymer chain when the functional groups on a linear polymer become ionized during dissolution facilitating favorable molecule orientation and fine-stranded network (Banker, 1966). The resultant interaction between polymers may have been responsible for this result. Anker *et al.* (2000) reported that, when the pH of the film solutions from ß-lactoglobulin is increased above 8, SH/S-S interchange reactions or thiol/thiol (SH/SH) oxidations can occur upon heating and intermolecular disulfide (S-S) bonds can be formed. The highest tensile strength value was obtained at pH about 9.8-10.0 (Figure 26.). The weakest films showed at lowest pH of film solutions: a very low tensile strength (2.64 MPa) was observed at pH lower than 9.0, most likely due to less protein-protein interaction. The tensile strength was enhanced as heating temperature of film solutions increased from 60-80 °C. The results demonstrated that, tensile strength increased from 2.64-3.67 MPa almost to 7.91-9.11MPa when heating temperature of film solutions increased from 60 to 80 °C. This may be due to higher heating temperature of film solutions induced proteins denaturation resulted in increase in the number and/or a better localization of bonds between protein chains provided in higher interaction between protein polymers. The weakest films demonstrated at lowest heating temperature; a very low tensile strength (2.64-3.67 MPa) was observed at heating temperature of film solutions around 60 °C. The contour plots (Figure 26) indicated an interaction between the effect of pH and heating temperature of film solutions on tensile strength of edible films. It was observed that, lowest tensile strength could be expected with low pH and relative low heating temperature of film solutions. According to the contour plots, the experimental condition involving higher both pH (9.8-10.0) and heating temperature and of film solutions, led to higher film formations resulted in a high tensile strength. Heating time seemed to be no effect on tensile strength.

Elongation at break value was most affected by pH and heating temperature of film solutions. All linear, quadratic and interaction terms for pH, heating temperature and heating time were significant. The contour plots of elongation at break

(Figure 27.) indicated that edible films from red bean proteins is an elastic material with elongation at break value between 1.88-9.02% and showed the most elastic films with a highest elongation at break when higher both pH and heating temperature of film solutions were employed. An increase in elasticity of heat-induced was suggested to be due to an increased number of intermolecular disulfide (SS bond) bonds (Shimada and Cheftel, 1988). Prolonged heating time, however, resulted in no increased in elongation at break.

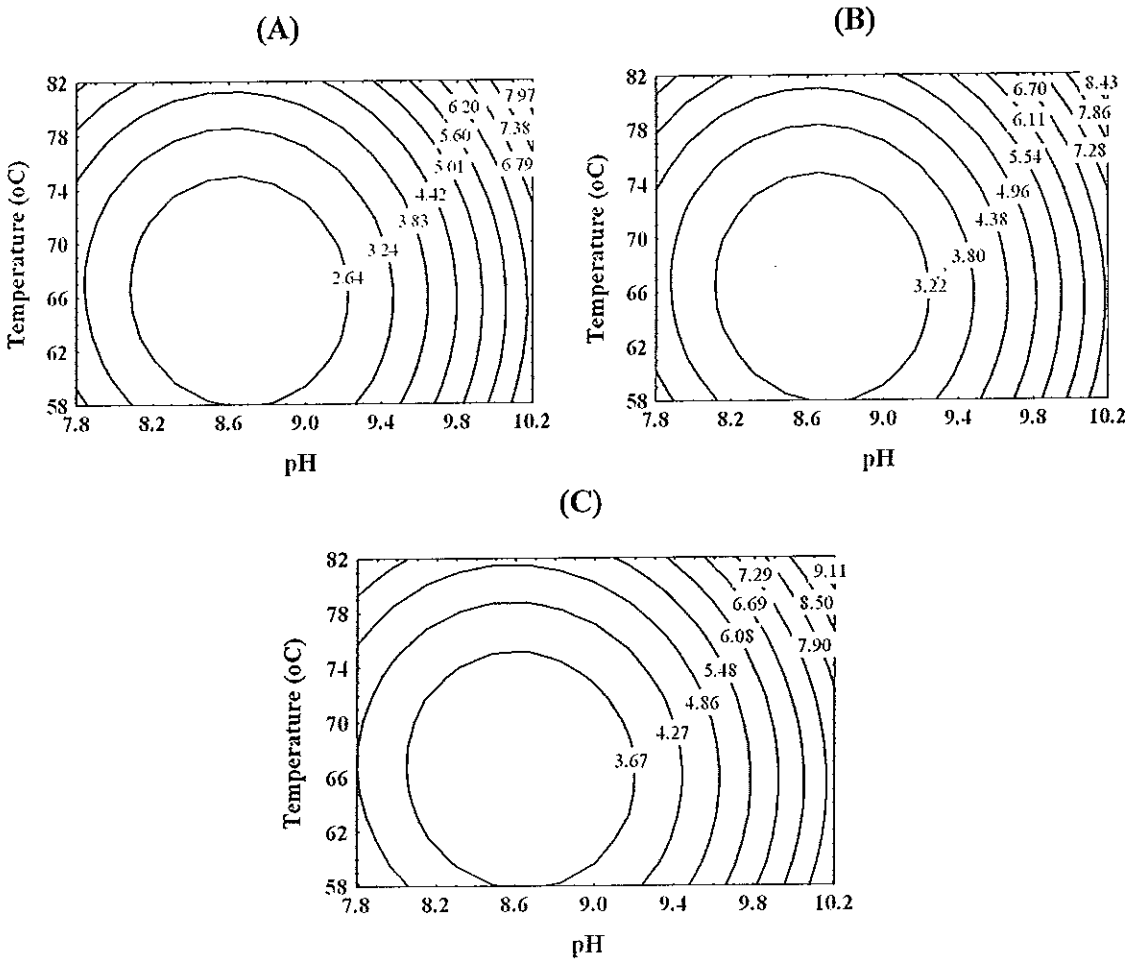


Figure 26. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent tensile strength (kPa) of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

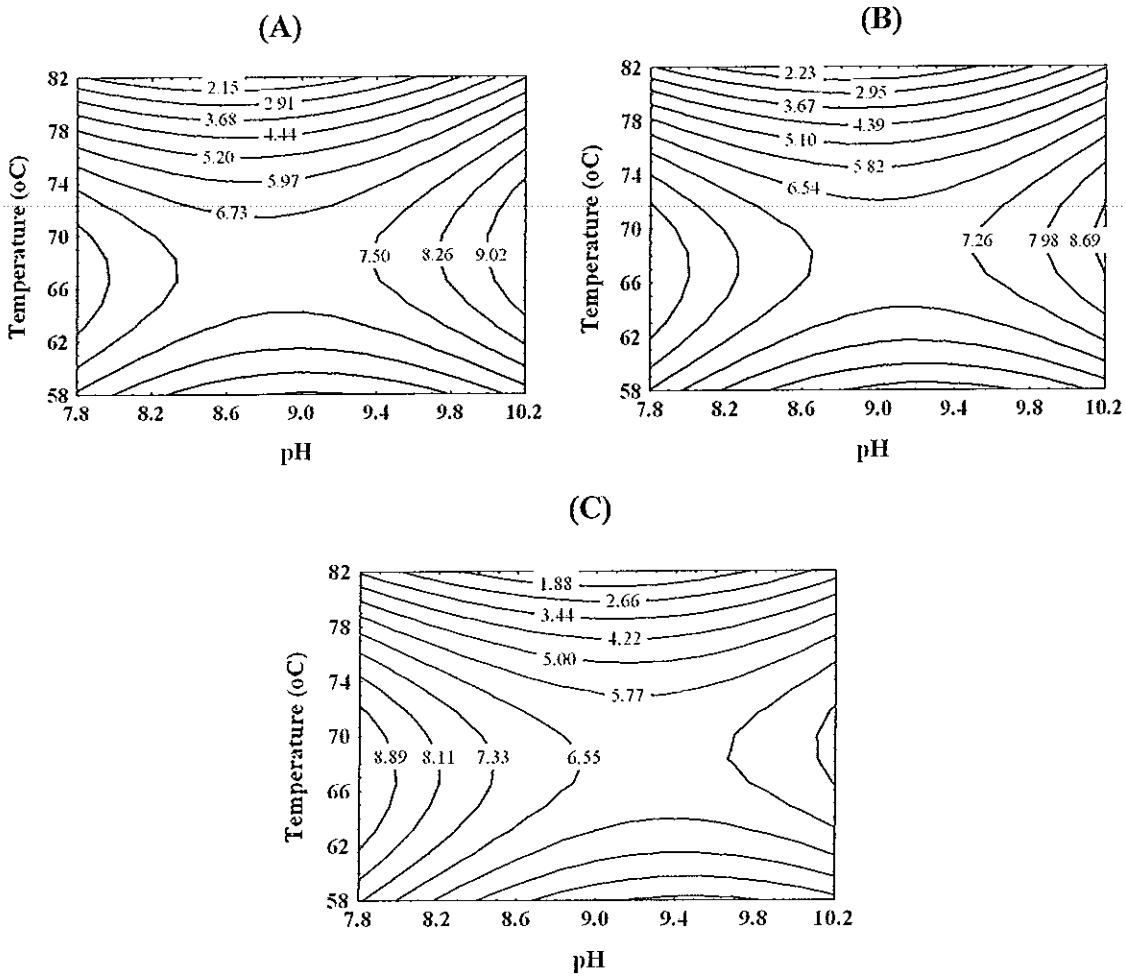


Figure 27. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent elongation at break (%) of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

9.2 Water Vapor Permeability

The main factor influencing water vapor permeability of edible film from red bean proteins was pH of film solution, followed by heating temperature and heating time of film solution. The contour plots (Figure 28.) were characteristics of the effect of these variables and showed that water vapor permeability value demonstrated the highest at pH of film solutions around 8.0 (30.93-36.48 g.mm/m^2.day.KPa) and tend to decreased when pH of film solutions were reached to 10.0 (23.80-27.98 g.mm/m^2.day.KPa). At higher pH protein denatures, unfolds and solubilizes, facilitating favorable molecule orientation pronounced higher the formation of intermolecular disulfide bond by thiol-disulfide interchange and thiol oxidation reactions. The function of disulfide bonds on protein insolubilization during drying of soymilk was studied by Fukushima and Van Burea (1970). Thiol-disulfide interchanged by thiol oxidation has also been implicated in whey protein gelation studies (Donovan and Mulvihill, 1987; Shimada and Cheftel, 1989). The highest water vapor permeability came at lowest pH; very high water vapor permeability. The water vapor permeability of edible films was also affected by heating temperature of films solutions. Basically, proteins must be denatured (by heating) in order to form the more extended structures that are required for film formations. Once extended, protein chains can associate through hydrogen, ionic, hydrophobic and covalent bonding. The chain-to-chain interaction that produces cohesive films is affected by the degree of chain extension and the nature and sequence of amino acid residues. Uniform distribution of polar, hydrophobic, and/or thiol groups along the polymer chain increase the likelihood of the respective interactions. (Kester and Fennema, 1986). The results showed that increasing of heating temperature of film solutions (60-80 °C) resulted in lower water vapor permeability (Figure 28.) most likely result from increasing of heating temperature of film solutions promoted greater cross-link between protein-protein chains resulting in a tight and compact protein network and structure. The highest water vapor permeability of edible films was found at lowest heating temperature of film solutions. Heating temperature and heating time at the range of this studied seemed to be not effect on the water vapor permeability of edible film from red bean proteins.

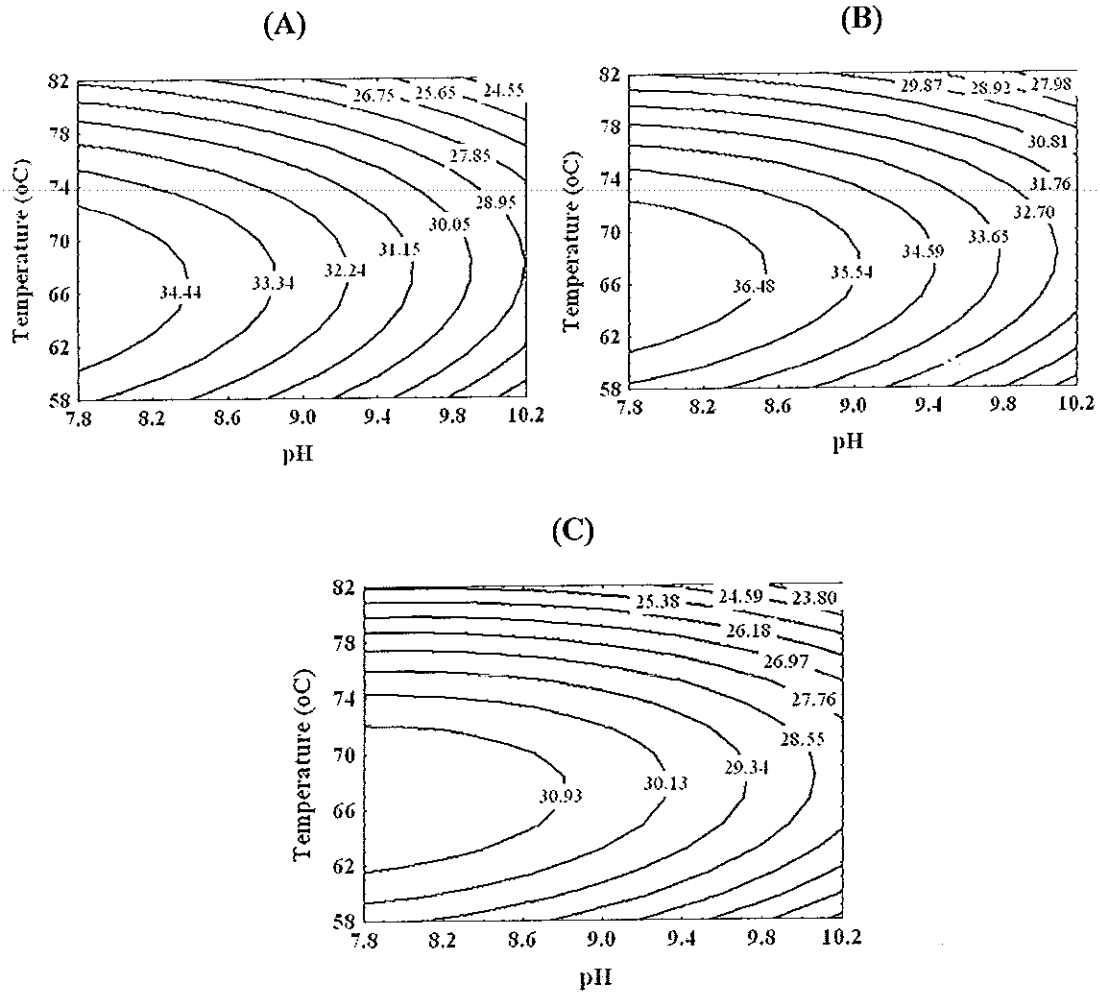


Figure 28. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent water vapor permeability (g.mm/m^2.day.kPa) of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

9.3 Films Solubility and Proteins Solubility

The edible from red bean proteins maintained their integrity (i.e., did not dissolve or break apart) even after 24 hr of incubation with gentle motion. This indicates that the protein polymer network remained intact and those only monomers, small peptides and non-protein material were soluble (Stuchell and Krochta, 1994). The pH and heating temperature of film solutions were the main effect on films and proteins solubility, while heating time demonstrated less effect. The contour plots of films and proteins solubility showed decreased when pH of film solutions were increased (Figure 29. and 30.). It was observed that both films and proteins solubility showed lower solubility values when pH of film solutions higher than 9.0. Decreasing of films and proteins solubility as a result of higher pH was the probable reason for this. Decreased soluble matters may be due to decreased protein solubility. Lower pH of film solutions (pH < 9.0.0), dispersion in water might result in loosening the film structure, causing dissolution of the non-protein materials (Gnanasambandam *et al.* 1997). It was observed that both films solubility and proteins solubility demonstrated lowest at pH around 9.8-10.0, most likely due to better films formation. The contour plots of the effect of heating temperature of film solutions on films solubility and proteins solubility are shown in Figure 29. and 30. Increasing in heating temperature of film solutions from 60 to 80 °C resulted in decrease in films solubility and proteins solubility. Roy *et al.* (1999) reported that wheat gluten films solubility and proteins solubility decreased ($p < 0.05$) as heating temperature of film solutions increased. This was attributed to more pronounced heat-induced protein denaturation at higher temperatures. Heat induced protein denaturation (unfolds), resulted in exposing previously "buried" groups such as hydrophobic and sulfhydryl (SH) groups which producing a strong films resulted in lower both films solubility and proteins solubility (Schofield *et al.* 1983 and Mine *et al.* 1990). Heating time of film solutions on film and protein solubility showed similar trend with heating temperature of film solution.

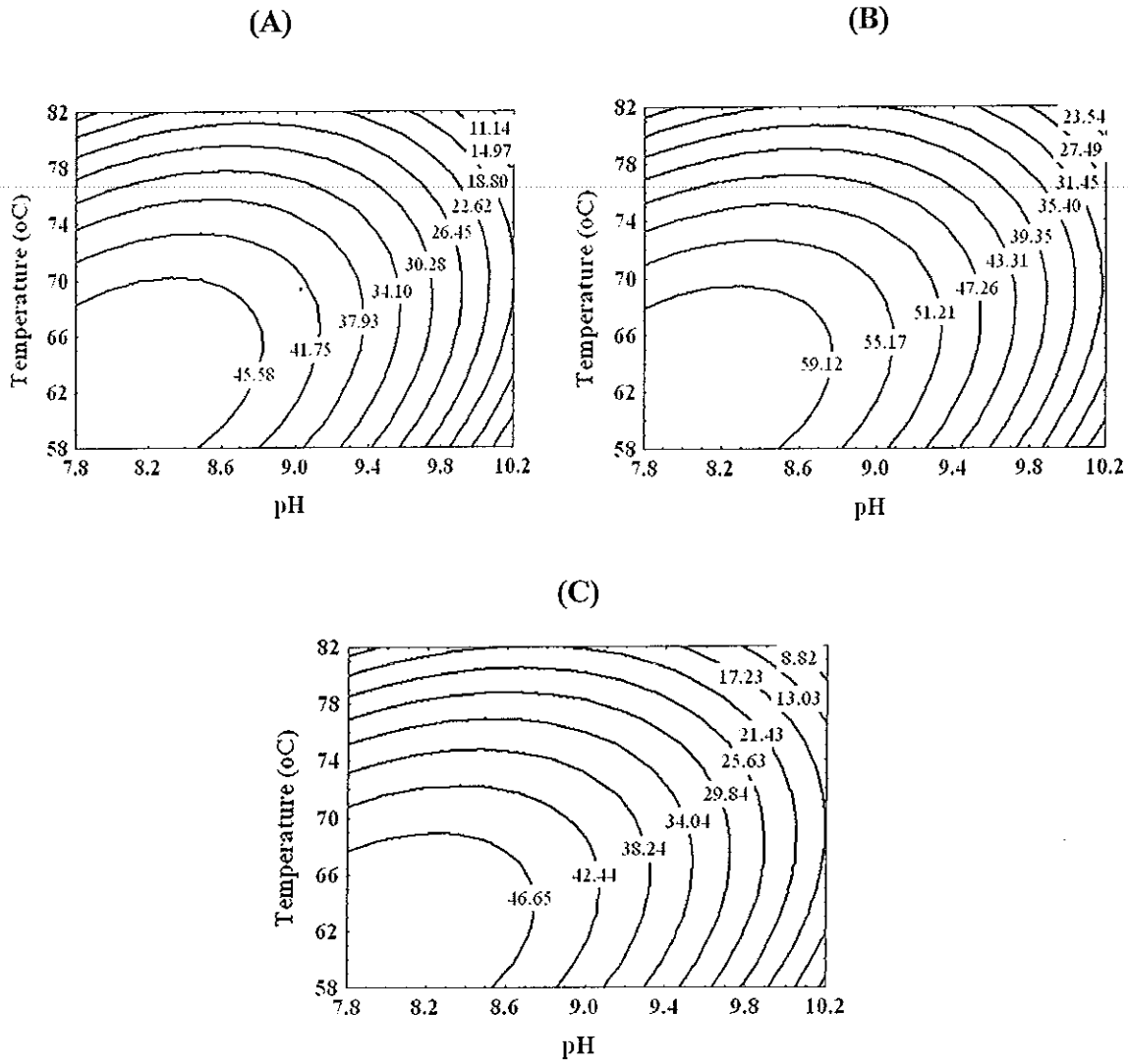


Figure 29. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent film solubility (%) of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

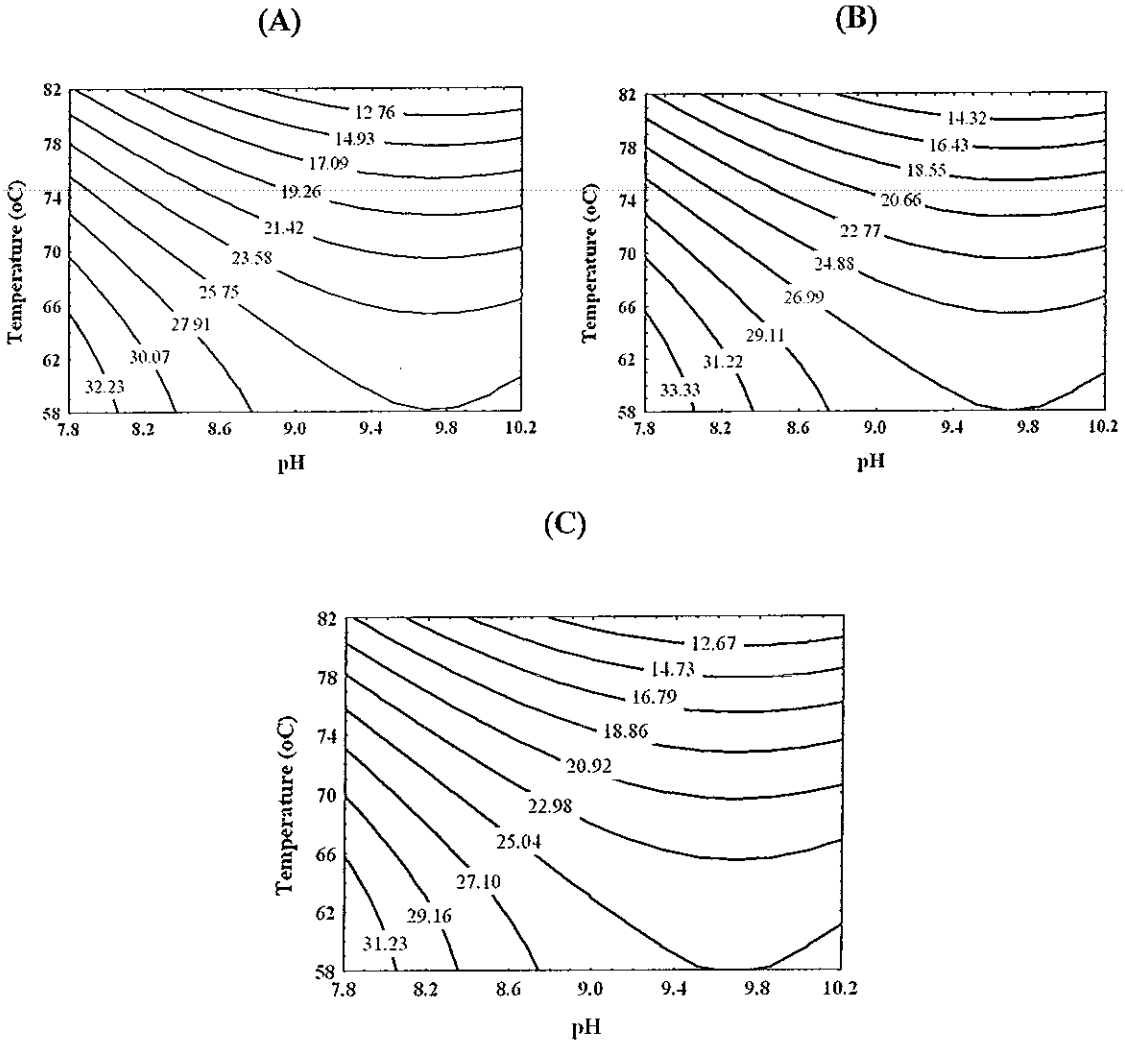


Figure 30. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent protein solubility (%) of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

9.4 Films Color

The results of the measurements performed on the films were expressed in accordance with the Hunter system, and the rectangular coordinates (L*, a* and b*) were defined. The film colors were the most affected by pH of film solutions, while heating temperature and heating time were little affected. Films formed at lower both pH and heating temperature were lighter yellow than films formed at higher pH and heating temperature. Instrumental color parameters L* showed decrease as pH of film solutions increased (Figure 31.), however, value b* dramatically increased with increasing in pH and heating temperature of film solutions (Figure 33.), and this made films appeared more yellowish. The value a* increased as pH of film solutions increased from 8.0-10.0 concomitant change in heating temperature (Figure 32.) which produced a reddish yellow films.

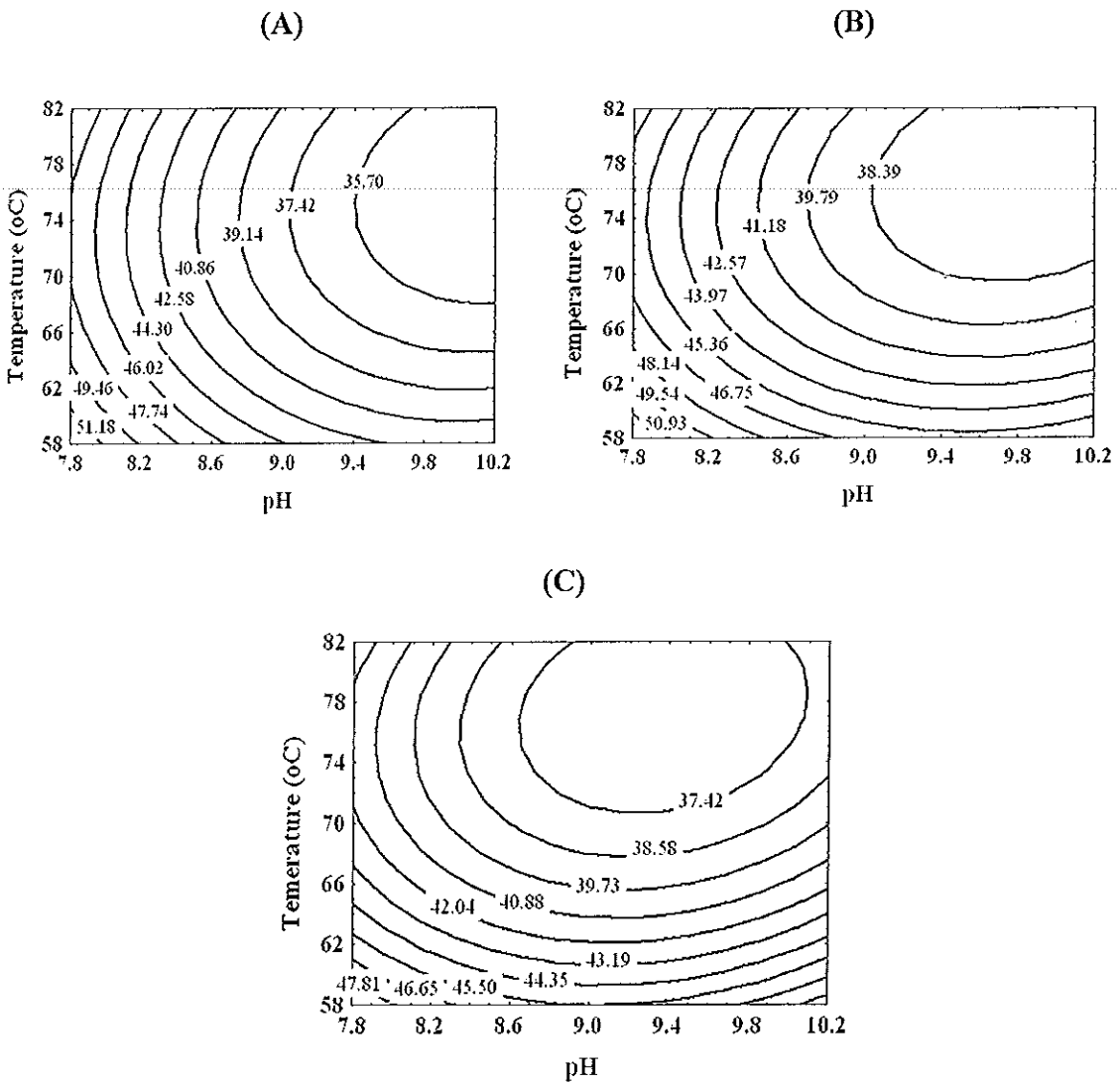


Figure 31. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent L* value of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

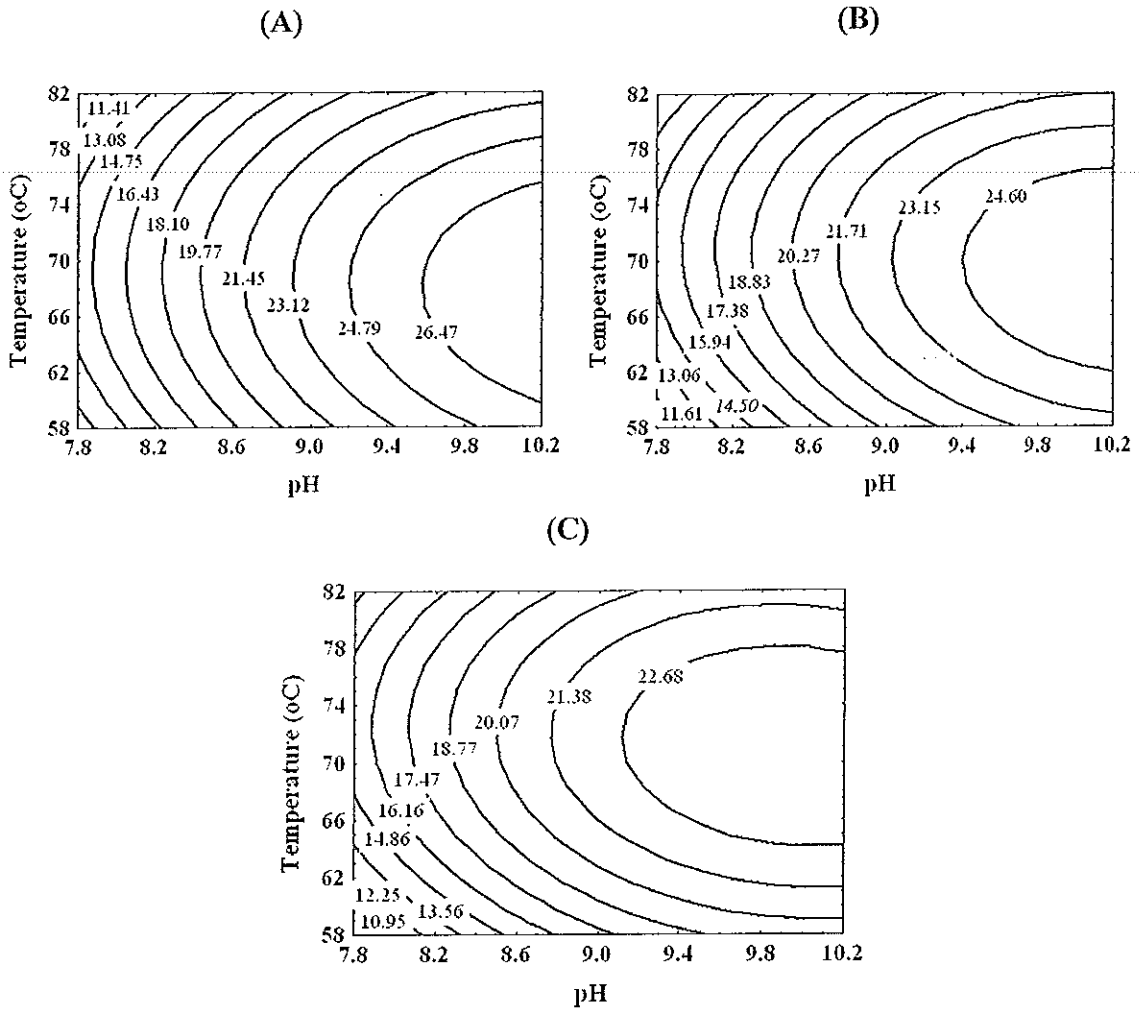


Figure 32. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent a* value of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

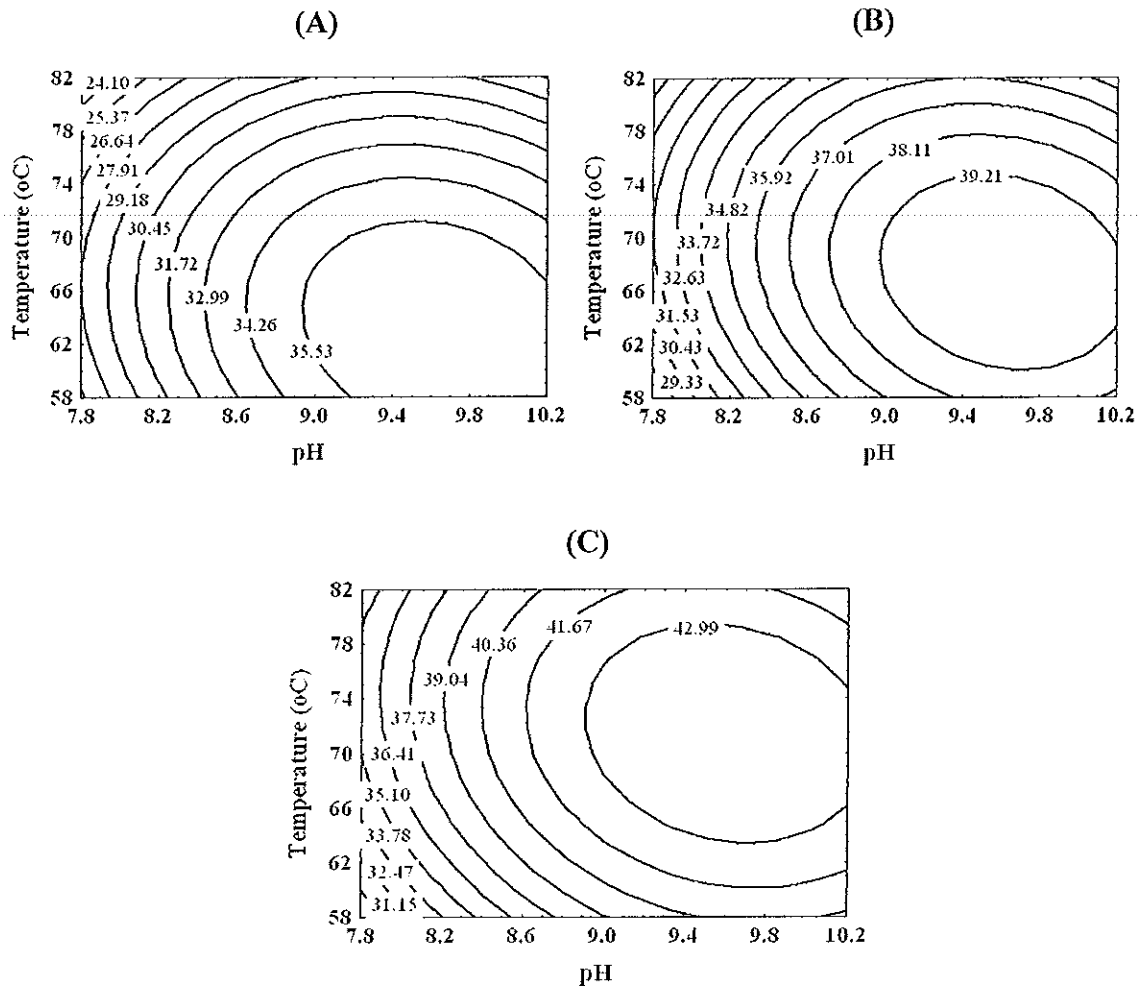
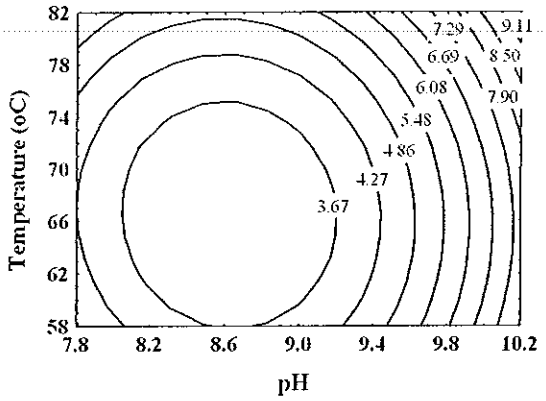


Figure 33. Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent b* value of films at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

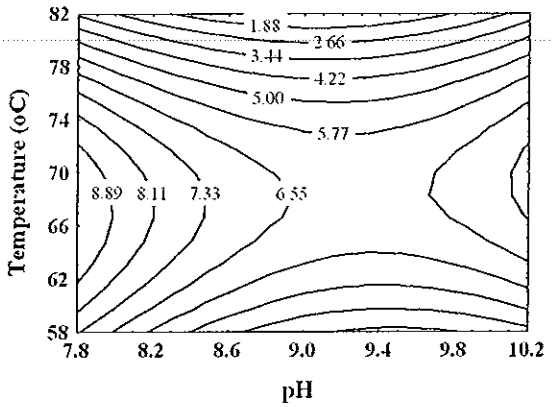
9.5 Localization of Optimum Conditions

To determine the optimum conditions the effect of pH, heating temperature and heating time on the properties of edible film from red bean proteins, the graphical method used in RSM was employed. The variable tensile strength and water vapor permeability were considered the most important of the 8 responses followed by elongation at break. The contour plots in Figure 35. were obtained from the predictive model of tensile strength, elongation at break and water vapor permeability at 30 min of heating time. Plot of Figure 35. were superimposed over those of Figure 34.(A), 34.(B) and 34.(C) to locate regions the highest of tensile strength, elongation at break and lowest water vapor permeability. The shaded area in Figure 35. satisfies following highest tensile strength and elongation at break and lowest water vapor permeability. As shown, the optimum condition for edible film red bean proteins shaded area: pH of film solutions of 10.0 and heating temperature of 81.5 ^{o}C for 30 min of heating time. At these condition demonstrated 8.48 MPa, 2.80 % and 23.64 g.mm/m^2.day.kPa. of tensile strength, elongation at break and water vapor permeability, respectively.

(A) Tensile strength
(heating time = 30 min)



(B) Elongation at break
(heating time = 30 min)



(C) Water vapor permeability
(heating time = 20 min)

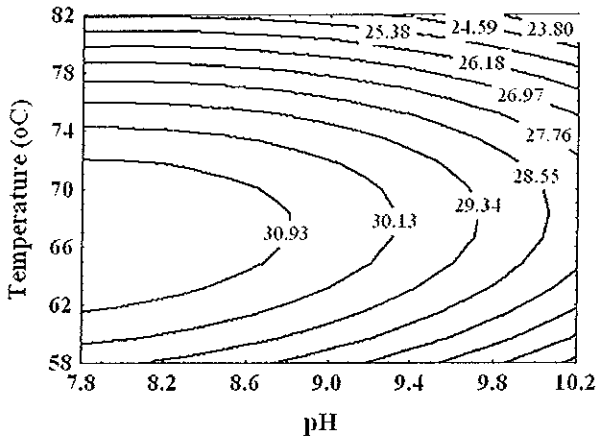


Figure 34. Contour plots showing response behavior of pH and heating temperature of film solutions heated for 30 min on the ; tensile strength (MPa), (B) elongation at break (%) and (C) water vapor permeability (g.mm/m^2.day.KPa).

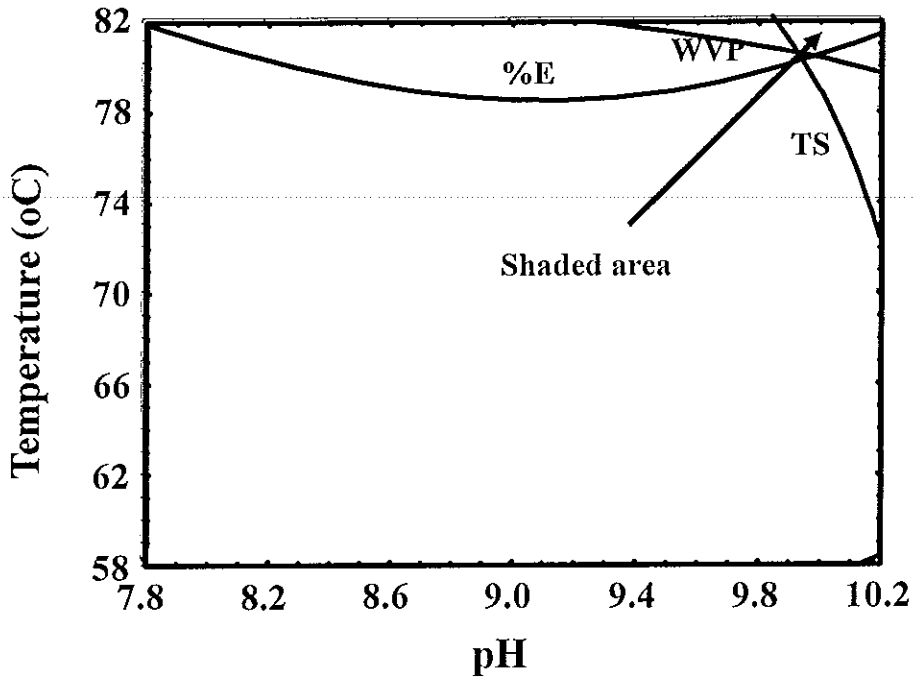


Figure 35. Optimum film solutions condition as a function of the independent variables after superimposition of contour plots over those of 34.(A), 34.(B) and 34.(C). Shaded area indicates regions the highest of tensile strength, Elongation at break and lowest water vapor permeability.

Validation tests are performed to determine the adequacy of the SOP model (Flores and Chinnan, 1988; Mudahar et al, 1990). This is performed because a fractional factorial design was used as the experimental design. A model is deemed adequate if the predicted values (of the model) are close to the experimental values observed during the validation tests. Table 6. shows the predicted and observed values for the responses at optimum condition for the effect of pH, heating temperature and heating time on the properties of edible film red bean proteins. The experimental values were averages of three replicates and were very close to the predicted values indicating that the SOP models generated were acceptable. The high CV values for some models were due to their lesser reproducibility (Montgomery, 1984) that may have contributed to

the statistical insignificance of some of these models. Despite the lesser effect of these responses to the optimum conditions, predictions were within fairly acceptable limits.

Table 6. Predicted and observed values for the independent variables after superimposition conditions of edible film from red bean proteins.

Response variable	Predicted value	Actual value ± SD
Tensile strength (MPa)	8.48	8.02± 0.49 (6.00%)
Elongation at break (%)	2.80	2.95 ± 0.56 (18.88%)
Water vapor permeability ($g.mm/m^2.day.KPa$)	23.64	20.61 ± 3.60 (17.51%)

Numbers in parentheses are coefficients of variation (CV)

10. Effect of Protein Concentrations on the Properties of Edible Films from Red Bean Proteins

The effect of protein concentrations on the tensile strength and elongation at break of edible films from red bean proteins are presented in Figure 36. Edible films with containing protein concentrations 1.5, 3.0 and 4.5% (pH 10.0 and heating temperature of 81.5 for 30 min of heating time) were investigated. Different in the proteins concentration of this study (1.5, 3.0 and 4.5% w/w) was significantly effect on the tensile strength (Figure 36.). The highest tensile strength about 6.45 MPa when 4.5% w/w of protein concentration was used, and tend to decreased when 1.5% w/w of protein concentration of film solutions was used. However, the results showed that the tensile strength was not significant different ($p>0.05$) when 3.0 and 4.5% of protein concentration was used. Comparing of the elongation at break of edible films at various content of proteins is given in Figure 36. It was observed that, the proteins concentration was significantly ($p < 0.05$) effect on elongation at break. Increasing the concentration of proteins of film solutions from 1.5 to 3.0 % w/w provided an increase in elongation at break from 12.24 to 30.08%. Increasing in elongation at break of edible films formed at lowest (1.5% w/w) and highest protein concentration (4.5%) indicated difference protein net work was formed.

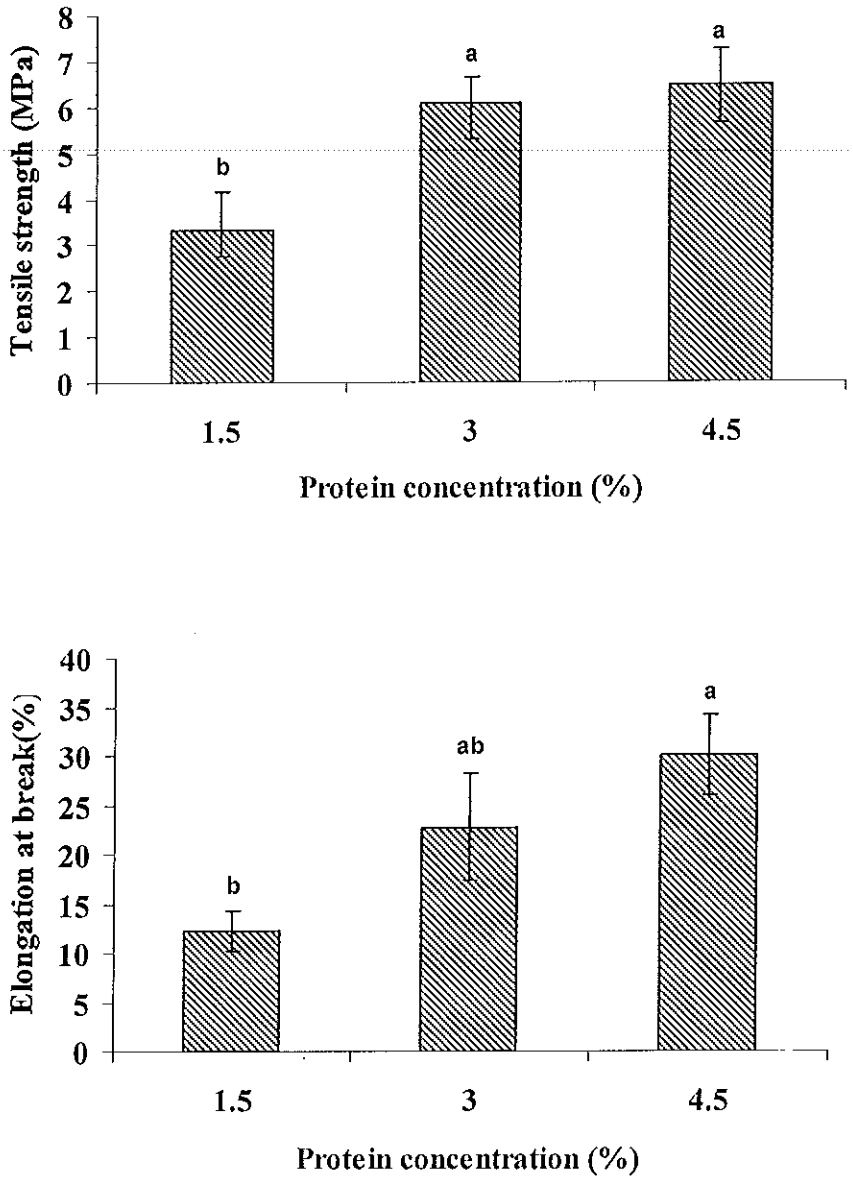


Figure 36. Effect of protein concentration on the tensile strength and elongation at break of edible films from red bean protein film. Standard error bars are shown. a-b, tensile strength and elongation at break value means with different letters represent significantly different value at $p < 0.05$ using Duncan's Multiple Range Test.

Water vapor permeability of edible films from red bean proteins at various concentrations 1.5, 3.0 and 4.5% w/w was investigated (Figure 37.). The protein concentrations were not significantly ($p > 0.05$) effect on water vapor permeability (Figure 37.). Films solubility and proteins solubility of edible films from red bean proteins was not affected by protein concentration (Figure 38.). However, 4.5% w/w of protein concentration demonstrated little lower proteins solubility than 1.5 and 3.5% w/w of protein. It could be the result from more aggregated structure was formed, with a denser protein structure, provided a decreased in proteins solubility when 4.5% w/w of protein concentration were used.

The color of edible films from red bean proteins was affected by protein concentration, higher protein concentration showed significantly higher in b*, but lower in L* (Figure 39.), hence, the films color was more yellow than lower of protein concentration. However, a* value of films was not significant different (Figure 39.).

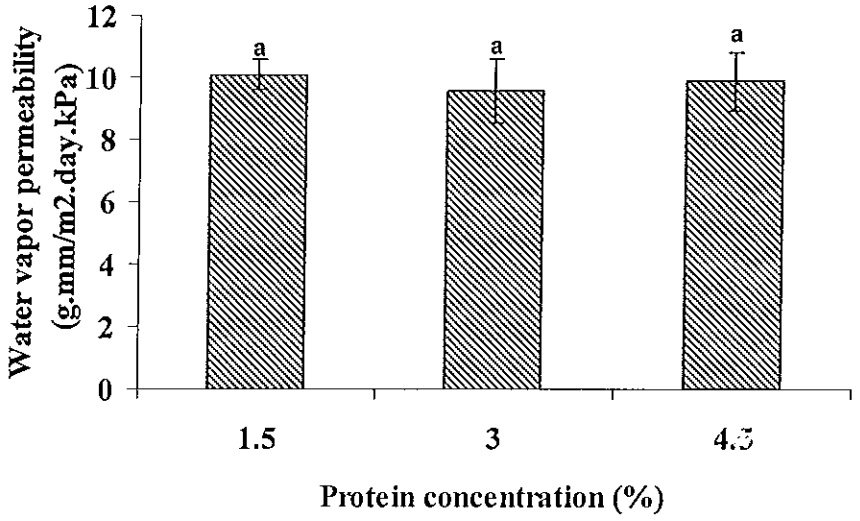


Figure 37. Effect of protein concentration on water vapor permeability of edible films from red bean protein film. Standard error bars are shown. a, water vapor permeability value means with different letters represent significantly different value at $p < 0.05$ using Duncan's Multiple Range Test.

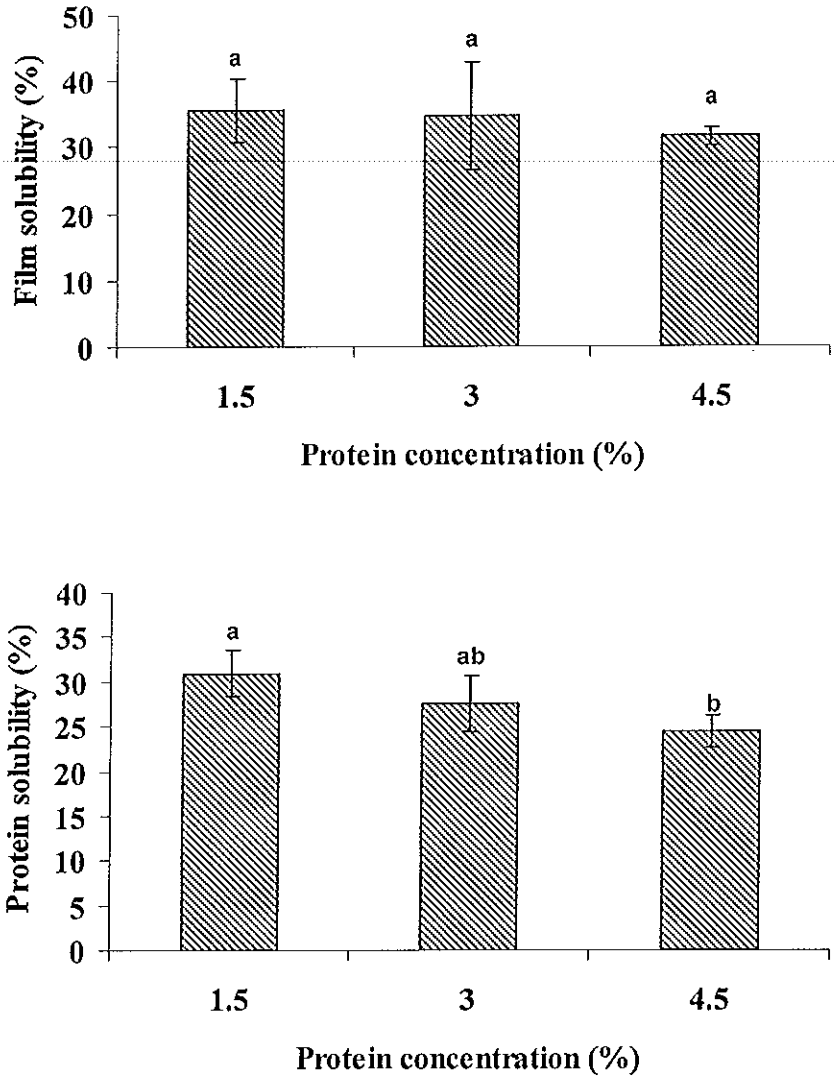


Figure 38. Effect of protein concentration on the film and protein solubility of edible films from red bean protein film. Standard error bars are shown. a-b, film and protein solubility value means with different letters represent significantly different value at $p < 0.05$ using Duncan's Multiple Range Test.

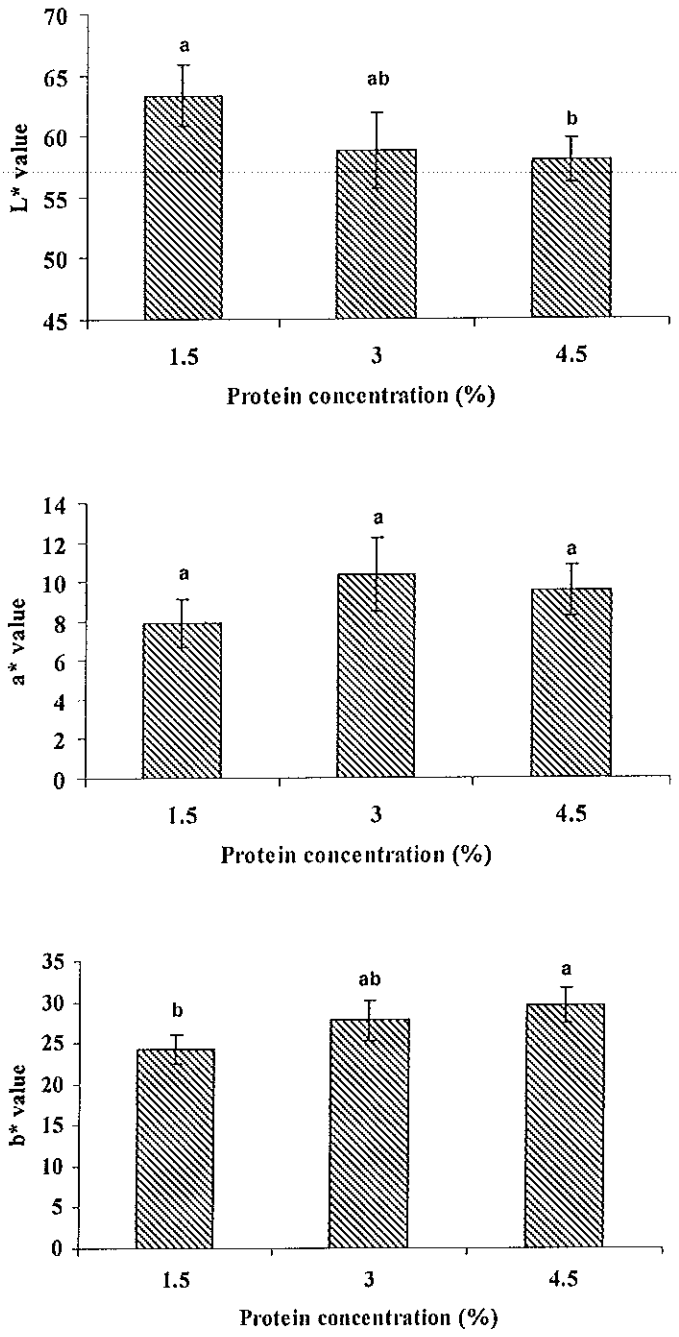


Figure 39. Effect of protein concentration on the L*, a* and b* values of edible films from red bean protein film. Standard error bars are shown. a-b, L*, a* and b* value means with different letters represent significantly different value at $p < 0.05$ using Duncan's Multiple Range Test.

11. Effect of Type and Concentration of Plasticizer on the Properties of Edible Film from Red Bean Proteins

11.1 Effect of Type and Concentration of Plasticizer on Tensile Strength and Elongation at Break of Edible Film from Red Bean Proteins

The mechanical properties of edible films plasticized by sorbitol, glycerol, polyethylene glycol or sucrose were assessed by measuring their tensile strength and elongation at break for four types of plasticizer (sorbitol, glycerol, and polyethylene glycol sucrose) at different concentrations (30, 40, 50 and 60%). The results were shown in Figure 40. It was observed that an increase in content of these plasticizers resulted in decrease in tensile strength and increase in elongation at break. Tensile strength decreased dramatically from 8.61 to 4.78, 6.74 to 1.26 and 4.60 to 3.05 MPa when the sorbitol, glycerol and polyethylene glycol concentration increased from 30 to 60 % w/w, while, elongation at break increased from 2.22 to 14.55, 15.93 to 44.04 and 6.38 to 25.51%, respectively (Figure 40.). Gontard *et al.* (1993) observed a linear reduction of the mechanical resistance (puncture force) in gluten film, from 1.9 N to 0.3 N when glycerol increased from 19 to 49 %. Cuq *et al.* (1997) also observed a linear reduction of the puncture force of edible films based on myofibrillar proteins of Atlantic sardine from 5.1 to 2.6 N, when glycerol increased from 0 to 40 g of glycerol/100 g of protein. The changes in mechanical properties as affected by hydrophilic plasticizers were previous observed for various hydrocolloid-based films (Park and Chinnan, 1990; Gontard *et al.*, 1993). The mechanical property changes characterize decrease in density and reversibility of intermolecular molecular interaction occurring in the edible films from red bean proteins network. The mechanical properties of sorbitol, glycerol and polyethylene glycol plasticized films at an equal concentration were compared (Figure 40.). The sorbitol plasticized films had significantly ($p \leq 0.05$) higher tensile strength and lower elongation at break than polyethylene glycol and glycerol plasticized films. This could be attributed to the ring molecular conformation of sorbitol molecules, which may

sterically hinder insertion between the proteins chains resulted in less effect in disrupting the protein-protein interruptions.

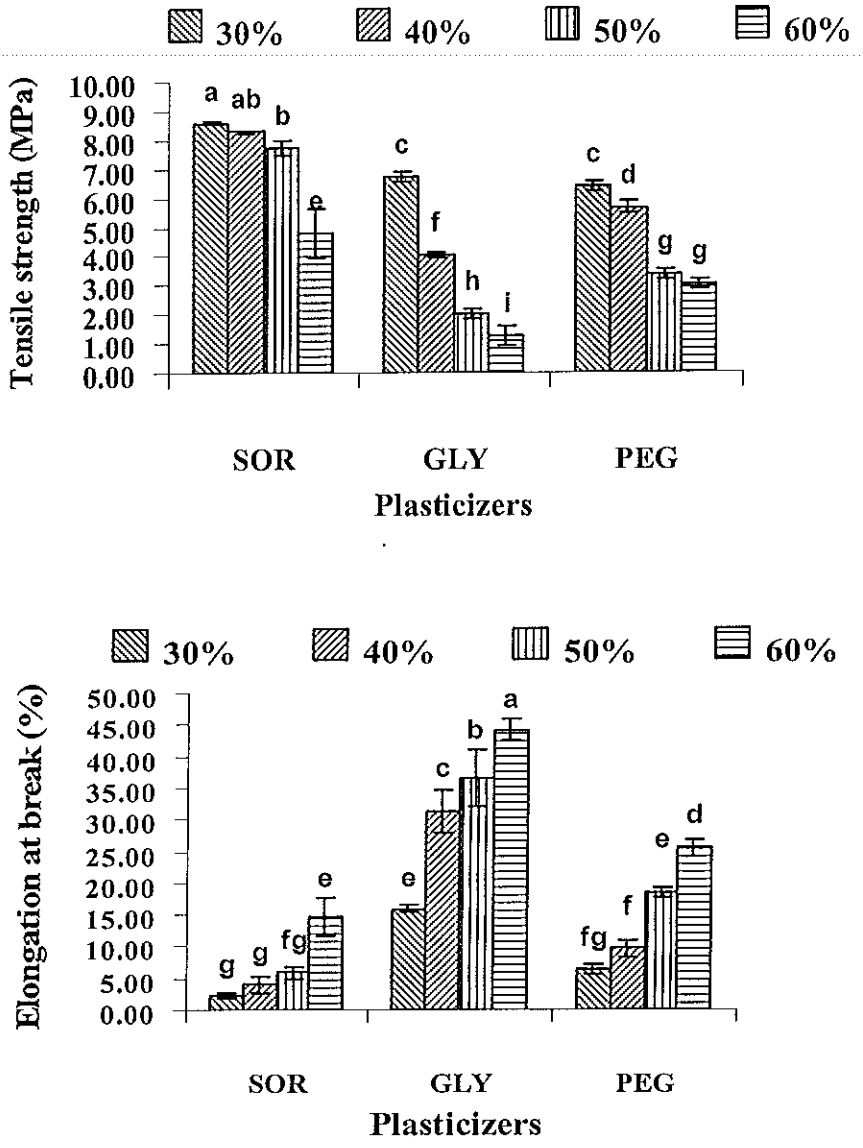


Figure 40. Effect of plasticizer type and concentration on the tensile strength and elongation at break of edible films from red bean protein film. Standard error bars are shown. a-i; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test, where, SOR = sorbitol, GLY = glycerol and PEG = poly ethylene glycol.

11.2 Effect of Type and Concentration of Plasticizer on Water Vapor Permeability of Edible Film from Red Bean Proteins

Water vapor permeability of edible films from red bean proteins with different type and concentration of plasticizer were examined (Figure 41.). The results demonstrated that water vapor permeability increased with increase of plasticizer concentration. The water vapor permeability of the film increased from 13.70 to 48.54, 62.65 to 162.53 and 30.69 to 82.54 g.mm/m^2.d.kPa, respectively, when the sorbitol, glycerol and polyethylene glycol concentration increased from 30 to 60 %w/w (Figure 41). An increase in plasticizer content enhanced the mobility of the polymer matrix thereby facilitating the water vapor or gas diffusion and permeation. Plasticization of the polymer leads to widening of the interchain hydrogen bonds, thus, facilitating permeation (Park *et al.*, 1994). The incorporation of plasticizers modifies the molecular organization of the protein network resulted in increase in free volume of protein network. Permeability increased with plasticizer content could be related to hydrophilicity of plasticizer molecules. Introducing hydrophilic plasticizers, favorable to adsorption and desorption of water molecules, was known to enhance the water vapor permeability of hydrocolloid-based films (Gontard *et al.*, 1993; McHugh *et al.*, 1994). Comparison of the successive values of the water vapor permeability for each plasticized edible films were shown in Figure 41. Films plasticized with sorbitol and had the lower water vapor permeability than polyethylene glycol and glycerol plasticized films due to the fact that sorbitol had ability to bind less water than polyethylene glycol and glycerol, thereby, provided lower water vapor permeability (McHugh *et al.*, 1994a). Chick and Ustanol (1998) showed that casein-based films plasticized with glycerol had higher water vapor permeability values than films plasticized with sorbitol when the same amounts of plasticizer were used. The high hydrophilicity of polyethylene glycol and glycerol molecules, which is favorable to the adsorption of water molecules, could also be contributed to the increase in the film water vapor permeability (Gennadios *et al.*, 1993). The increase in water vapor permeability with increasing hydrophilicity plasticizer concentration is also common in edible films (McHugh *et al.*, 1994a; Cuq *et al.*, 1997).

Sorbal *et al.* (2001) reported that hydrophilicity of the plasticizers will increase the water content of the films, consequently increasing the mobility of the molecules. In addition, increasing water content could also affect permeant solubility in the films. Effect of edible films plasticized by polyethylene and glycol glycerol on water vapor permeability (Figure 41.). Water vapor permeability of edible films plasticized by glycerol was significantly ($p \leq 0.05$) higher than those of edible films plasticized by polyethylene glycol (Figure 41). It could be explained by the effect of the small size of glycerol which allows it to be more readily inserted between the polymer chains, and consequently exert more influence on oxygen permeability properties than the larger polyethylene glycol molecule. Moreover, comparing at an equal percentage concentration, the total number of glycerol molecules in the film solutions is greater than that of the higher molecular weight polyethylene glycol and therefore glycerol has more hydrophilic group than polyethylene glycol which should promote the solubility and diffusivity of water through film structure resulted in higher water vapor permeability.

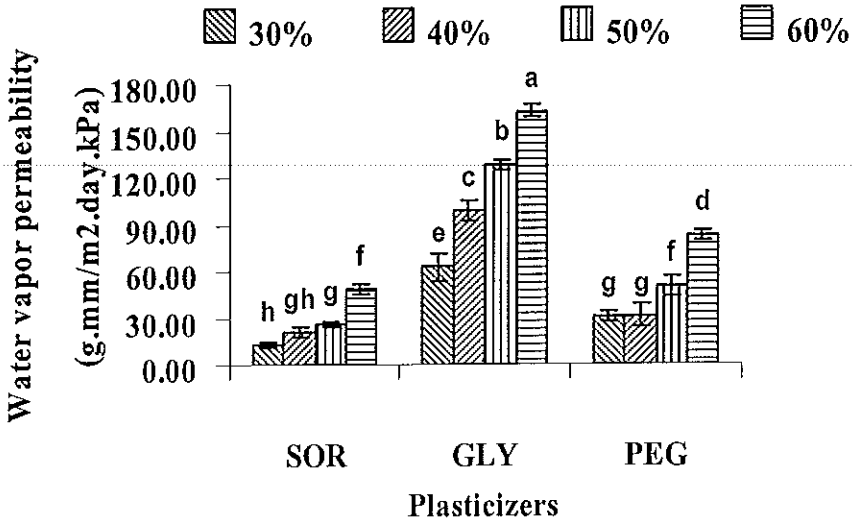


Figure 41. Effect of plasticizer type and concentration on the water vapor permeability of edible films from red bean protein film. Standard error bars are shown. a-h; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan' Multiple Range Test, where, SOR = sorbitol, GLY = glycerol and PEG = poly ethylene glycol.

11.3 Effect of Type and Concentration of Plasticizer on Film and Protein Solubility of Edible Film from Red Bean Proteins

Irrespective of type, an increase in plasticizer content led to an increase in film solubility and protein solubility (Figure 42.). It could be hastily concluded that hydrophilic plasticizers enhance films solubility in water. Low molecular weight protein chains (i.e. monomers and small peptides) formed during storage of film- solutions and entrapped in the network (Cuq *et al.*, 1995) could thus constitute the protein-based materials that solubilize in water. The dry matter solubilized in water was likely to be constituted mainly by the plasticizer. Plasticizer solubilization in water was already observed for film based on wheat gluten or treated soy proteins or produced by transglutaminase catalytic cross-linking of whey protein (Gontard *et al.,* 1992; Stuchell and Krochta, 1994). Stuchell and Krochta (1994) had pointed out increase in the content

of protein solubilized in water when the hydrophilic content increased for treated whey protein-and soy protein-based films. A decrease in the polymer network interaction density due to the presence of plasticizer was thus associated with this increase in solubility properties. The lowest film solubility and protein solubility of edible films plasticized by 30% w/w of these plasticizer were noticed, while increasing the amount of plasticizer resulted in higher film solubility and protein solubility (Figure 42.). It could be explained by the fact that at the higher content of plasticizer, there were more molecules of plasticizer untrapped in the cross linked network and able to escape into solution, while, lower content of plasticizer provided lowered plasticizer molecules untrapped in the cross linked network resulted in lesser ability to escape into solution. The film solubility and protein solubility of films plasticized by polyethylene glycol was trend higher than those of glycerol and significantly sorbitol.

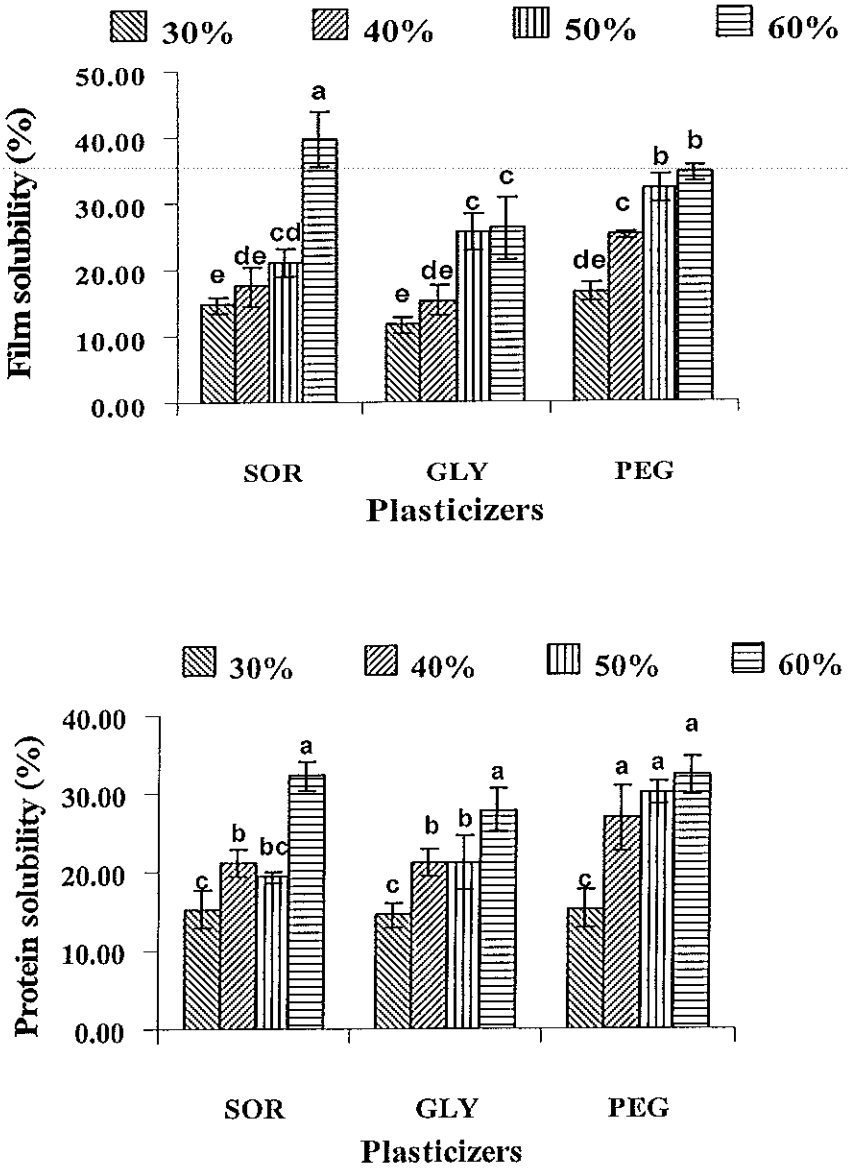


Figure 42. Effect of plasticizer type and concentration on the film and protein solubility of edible films from red bean protein film. Standard error bars are shown. a-e; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test, where, SOR = sorbitol, GLY = glycerol and PEG = poly ethylene glycol.

11.4 Effect of Type and Concentration of Plasticizer on Color of Edible Film from Red Bean Proteins

The results of the measurements performed on the films color were expressed in accordance with the CIELAB system, and the rectangular coordinates (L*, a* and b*) were defined. The color of films was more affected by the content of the plasticizer than by its type. L* value of edible film from red bean proteins plasticized by sorbitol, glycerol and polyethylene glycol sucrose were not significantly different ($p > 0.05$) (Figure 43.). It was observed that a*and b* values of the film showed significantly ($p \leq 0.05$) higher but lower in L* value when higher plasticizer were used (Figure 43.); hence, the films color was more yellow than lower plasticized films.

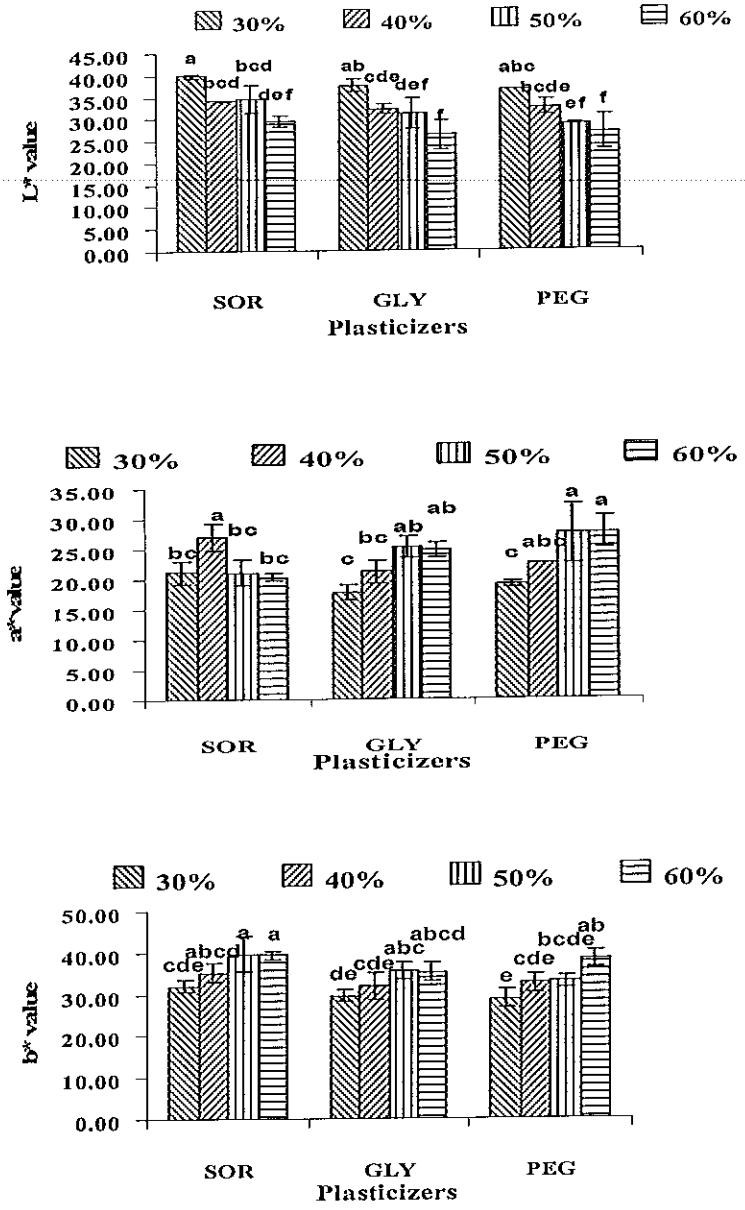


Figure 43. Effect of plasticizer type and concentration on the L*, a* and b* of edible films from red bean protein film. Standard error bars are shown. a-f; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test, where, SOR = sorbitol, GLY = glycerol and PEG = poly ethylene glycol

12. Effect of Lipid Type and Concentration on the Properties of Edible Film from Red Bean Proteins

12.1 Effect of Type and Concentration of Lipid on Tensile Strength and Elongation at Break of Edible Film from Red Bean Protein

The TS of edible film from red bean proteins with different types and concentration of lipids shown in Figure 44. It was observed that the TS of protein film from red bean proteins decreased with the addition of lipid. Previously work demonstrated that the maximum occurred when no lipid was added (8.48 MPa). The trend of TS of films decreased as concentration of lipids increased (Figure 44). These results agreed with the study conducted by Gontard et al, (1994) in which they found that, for whey protein/lipid composite films, the influence of lipids depended on lipid characteristics and interactions between the lipid and the protein structural matrix. Similarly, Shaw et al, (2002), who found that increasing soya oil concentration led to decreases in TS. Weller et al. (1998) stated that the decrease in Young's modulus of protein film accompanying the increase in lipid concentration was related to the weakening effect of lipid on protein network, due to the lack of structural integrity of the lipid. The interactions between nonpolar lipid molecules and between the polar polymer and nonpolar lipid molecules are believed to be much lower than those between the polar polymer molecules. Typically, strength reduction of edible composite films with lipid incorporation has also been reported (Debeaufort and Voilley, 1995; Shellhammer and Krochta, 1997; Yang and Paulson, 2000; Bertan et al., 2005). The addition of palm oil in a greater reduction in TS of the red bean proteins films than the addition of oleic acid and stearic acid (Figure 44.). The differences in mechanical properties between these films could be related to their physical state, structure and chemical nature of the lipids. No significant difference ($p > 0.05$) in TS occurred between the oleic acid and stearic acid-incorporated films.

The elongation at break (%E) of edible film from red bean proteins decreased as addition of lipids (Figure 44.). This is in agreement with the findings of Javanmard and Golestan, (2007) who found that %E decreased as olive oil concentration increased; thus, the flexibility of whey protein emulsion films was reduced. This may be attributed to noncontinuous film matrix formation probably

because of the presence of lipid globules. The lower continuity and cohesiveness of protein network in the presence of lipid globules might result in the decrease in EAB (Anker et al., 2002; and Peroval et al., 2002). Lower water content of film containing lipids may also cause the decreased elongation (Gallo et al., 2000). Oleic acid added into the film resulted in higher %E than stearic acid and palm oil incorporated films (Figure 44.). The effect of lipid type is due to their chemical structure. The low molecular weight and liquid phase possibly exhibited more plasticizing effect than high molecular weight and solid lipids.

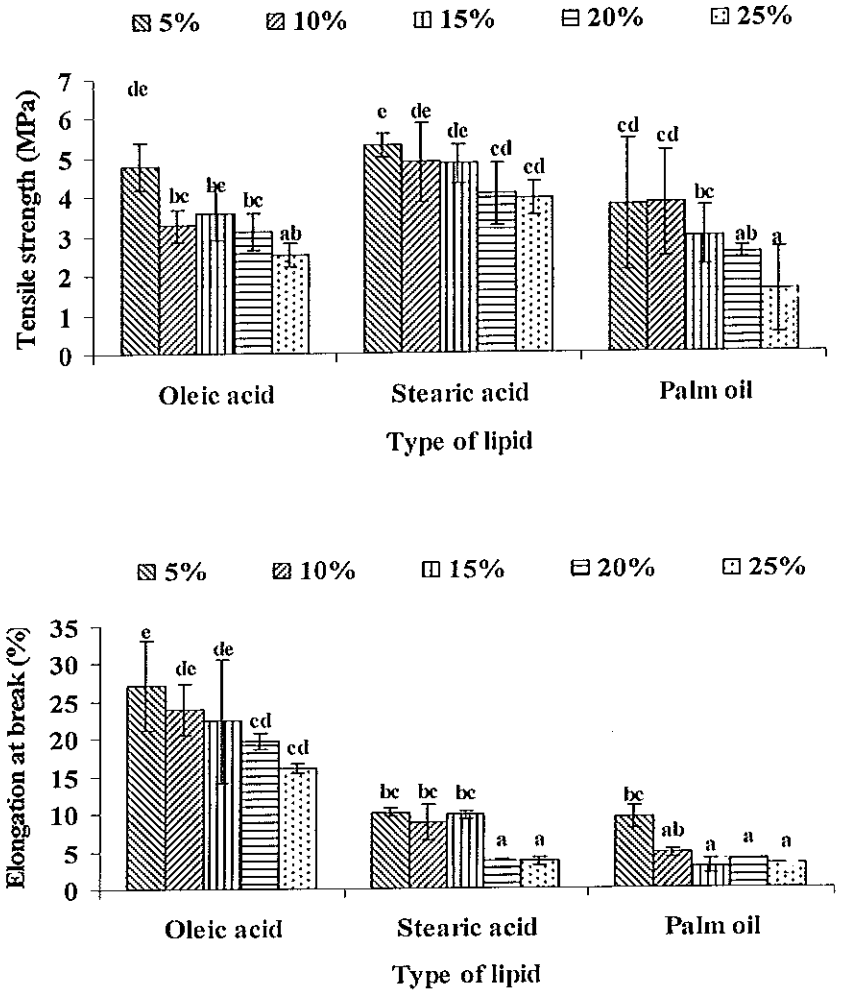


Figure 44. Effect of lipid type and concentration on the tensile strength and elongation at break of edible films from red bean protein film. Standard error bars are shown. a-e; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test.

12.2 Effect of Type and Concentration of Lipid on Water Vapor Permeability of Edible Film from Red Bean Protein

In general, the WVP of edible film without the addition of lipids were more water vapor permeable than incorporated films with lipids. Figure 45. shows WVP of edible film from red bean proteins as affected by different types and concentration of lipids added. The result demonstrated the addition and increasing of concentration of lipids tended to decrease WVP (Figure 45.), due to the film's higher hydrophobicity (Morillon et al., 2002; Vergano and Weller, 1994 and Shellhammer and Krochta, 1997). Gontard et al, (1994) observed that when lipids were used in composite film formulations, large amount of lipids could promote a protective effect on WVP. Comparing with the same concentration of lipids, the result showed that the addition of oleic acid and stearic acid resulted in a greater reduction in WVP than the addition of palm oil (Figure 45.). In general, WVP of emulsion films comprised of biopolymer and lipids strongly depend on the type, structure and quantity of lipids. For film containing fatty acid, the WVP decreased with increased chain length and degree of saturation of the lipids (Hagenmaier and Shaw, 1990;. Kamper and Fennema, 1984a, b; Koelsch and Labuza, 1992; McHugh and Krochta, 1994). Moreover, the increase of solid fat content especially between 0% and 30% allows for the improvement of the barrier efficiency (Kamper and Fennema, 1984a; Morrilon et al., 2002). This is because the CH_2 group of the liquid aliphatic chains have a greater effect than when they are crystallized (Morrilon et al. 2002). So solid structure of fats is denser and limits the diffusion of water. Moreover, the solubility of water in solid lipids is also reduced (Kamper and Fennema, 1984a; Callegarin and Quezada, 1997). However, for solid fat contents higher than a critical value that depends on the lipid nature, permeability could increase due to structure defects within the films (Morillon et al, 2002). However, WVP of film with oleic acid was not different from the film with stearic acid but both films demonstrated lower WVP than palm oil incorporated film. Morillon et al, (2002) reported that fats with short chain length are more efficient to reduce moisture transfers through films than long chain.

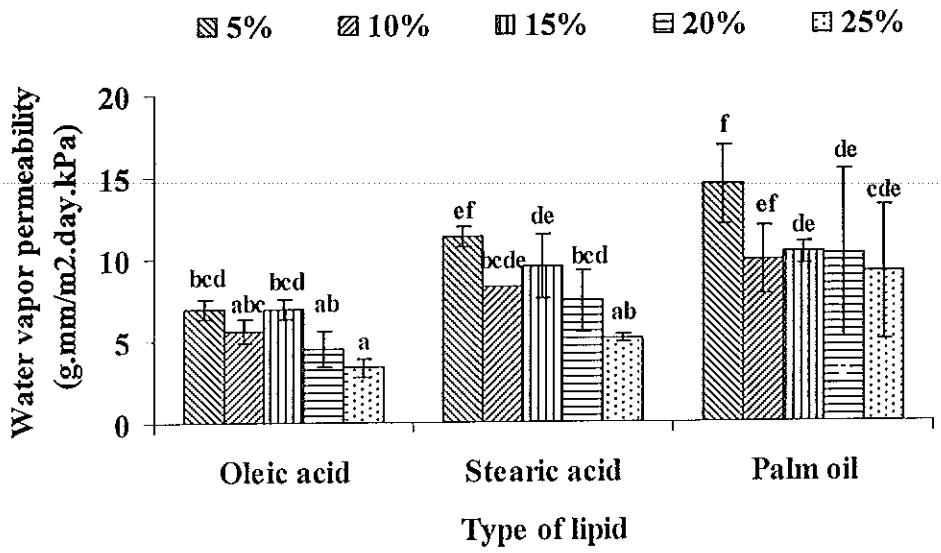


Figure 45. Effect of lipid type and concentration on the water vapor permeability of edible films from red bean protein film. Standard error bars are shown. a-f; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test.

12.3 Effect of Type and Concentration of Lipid Film Solubility of Edible Film from Red Bean Protein

Film solubility of edible film from red bean proteins decreased with the addition of lipids. Addition of lipids thus increased hydrophobicity of the red bean proteins with the addition of lipids. Similar results were reported by Kim and Ustanol, (2001) on lipid-whey protein emulsion films. All lipids no significant difference ($p>0.05$) effect film solubility (Figure 46.).

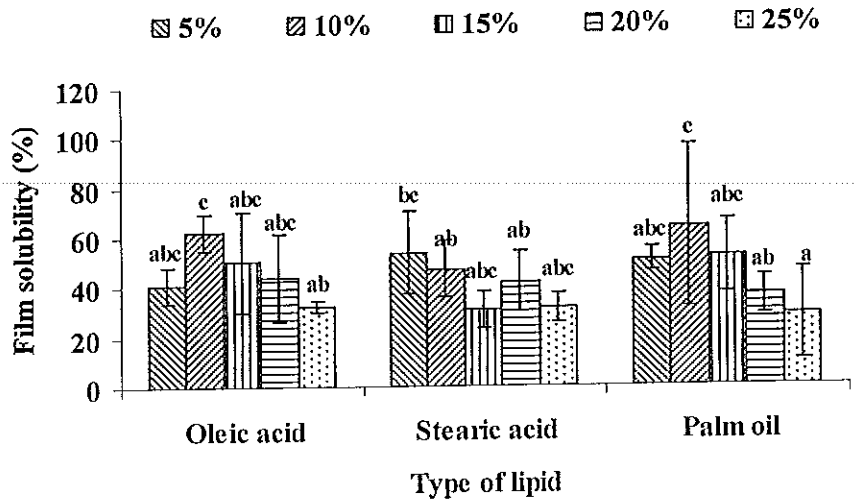


Figure 46. Effect of lipid type and concentration on the film solubility of edible films from red bean protein film. Standard error bars are shown. a-c; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test.

12.4 Effect of Type and Concentration of Lipid on Film Color of Edible Film from Red Bean Protein

The color of edible film from red bean proteins incorporated with lipids was affected by the lipid type and concentration. The film became lighter yellow as evidenced by the increased L* and b* values when the concentration of lipid increased (Figure 47.). The decrease in a* value was noticeable as the lipids was incorporated into the films. Comparing with the same amount of lipids, the results pointed out that composite films with stearic acid showed higher L* and b* values but lower a* values than oleic acid and palm oil. Yang and Paulson (2000) reported that the differences in opacity of film were determined by the optical properties of lipids incorporated. Furthermore, addition of lipids generally causes the films to lose or reduce their transparency (Yang and Paulson, 2000; Shaw et al., 2002; Pommet et al., 2003; Bertan et al., 2005). However, when lipids are added, the films lose transparency. The degree of opacity depends on the lipid content and particle size. In general, films with small particle size and low lipid content were translucent;

however, as particle size and lipid content increased, the films became more opaque (Prez-Gago and Krochta, 2001).

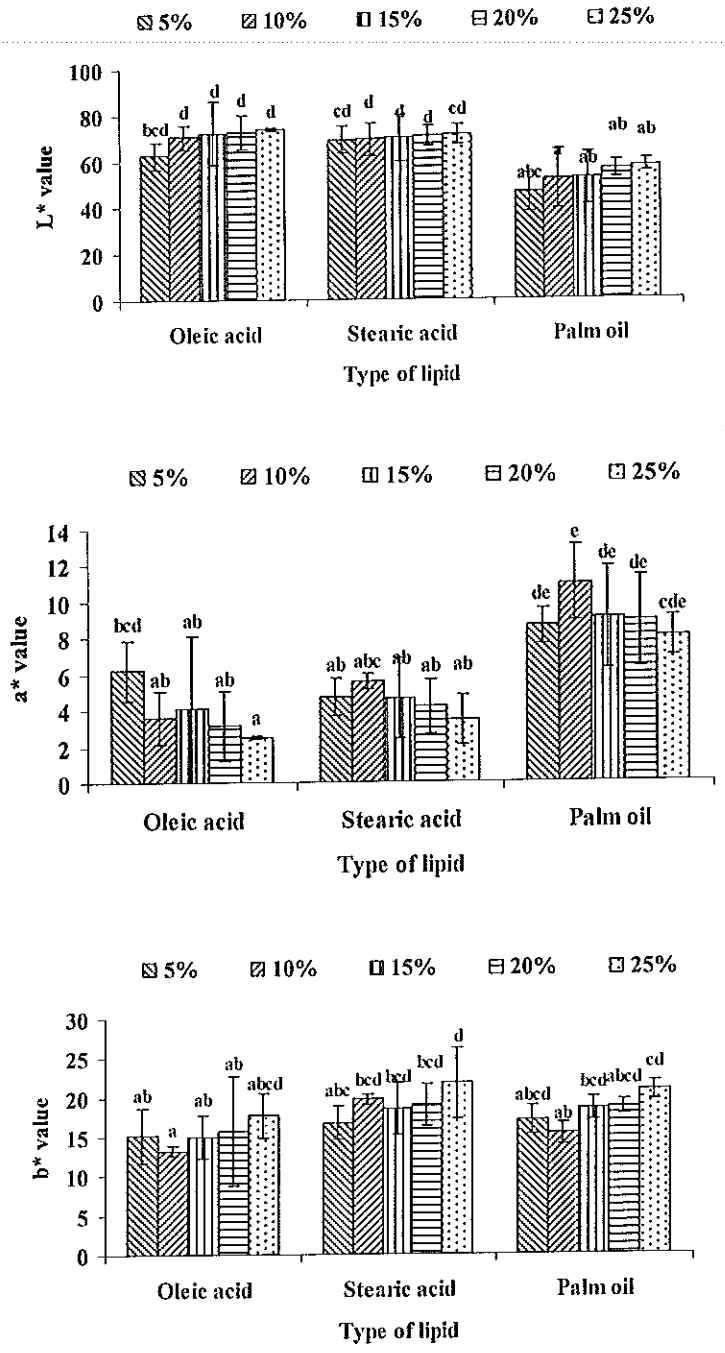


Figure 47. Effect of lipid type and concentration on the L*, a* and b* of edible films from red bean protein film. Standard error bars are shown. a-e; means with different letters represent significantly different value at $p \leq 0.05$ using Duncan's Multiple Range Test.

12.5 Microstructure

The surface of edible film from red bean proteins with addition of lipids. SEM was used to determine the surface morphology of the films containing various concentration of oleic acid. The results showed that the films with 5% of oleic acid had continuous surface and slightly of air bubbles (Figure 48.). The micrographs also showed increased surface irregularity, large air bubbles and oil droplets were more intense on the surface when the addition of lipids increased (Figure 48.).

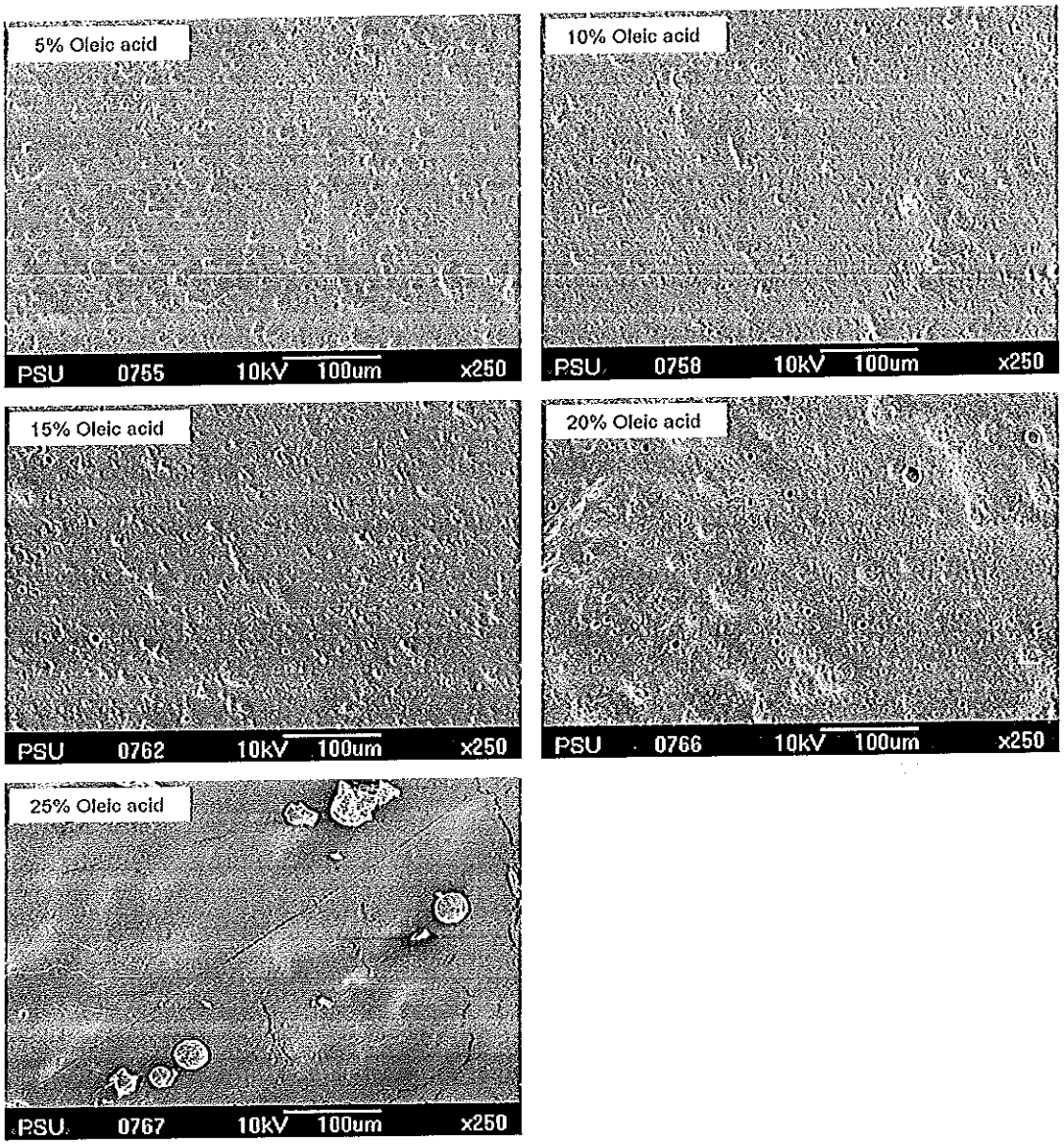


Figure 48. SEM of red bean protein/lipid composite films containing various concentrations of oleic acid.

Comparing with the same concentration of lipids, the results showed that the surface irregularity with the addition of lipids. However, oleic acid appeared to be well incorporated and embedded in the composite films resulting in a relatively smooth and continuous surface more than composite films with stearic acid and palm oil, respectively (Figure 49.). The film with palm oil had more irregular surface than stearic acid, which may have resulted from oil droplets of palm oil in the film.

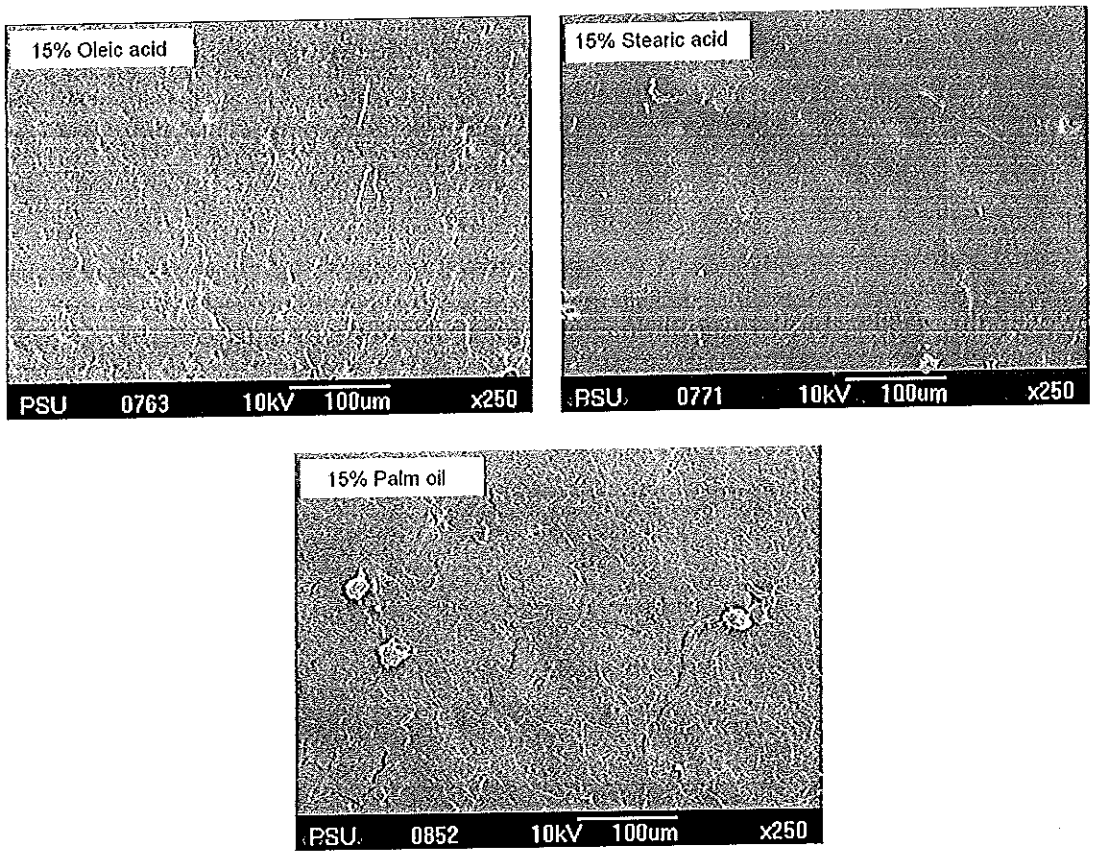


Figure 49. SEM of red bean protein/lipid composite films containing 15% oleic acid, 15% stearic acid and 15% palm oil.

13. Comparing the Properties of Edible Film from Mung Bean and Red Bean Proteins with Edible Film that Produced from other Protein Sources, Polysaccharide and Synthetic Polymer.

The mechanical properties (tensile strength and elongation at break) of mung bean and red bean proteins and of various films were compared (Table 7.). Edible film from mung bean and red bean proteins had mechanical properties better than those other protein sources. Tensile strength of edible films from mung bean and red bean proteins were above (films were more mechanically resistant) but elongation was slightly below (film were less deformable) than casein, soy protein isolate, wheat gluten, peanut proteins and the water-soluble fish proteins reported by Iwata *et al.* (2000) (Table 7.). Edible films from mung bean and red bean proteins had substantially lower both tensile strength and elongation at break than synthetic polymer (high density polyethylene, polyvinyl chloride, cellulose acetate and polyester) (Table 7.). However, edible films from mung bean and red bean proteins had higher tensile strength (6.28-8.02 MPa) than low density polyethylene films, meanwhile elongation at break of edible films from mung bean proteins had almost similar to polyvinylidine chloride and cellulose acetate films (Table 7.).

Water vapor permeability of edible films from mung bean and red bean proteins and various films were presented in Table 8. Water vapor permeability of edible films from mung bean and red bean proteins were characterized by relative low water vapor barrier properties than other protein sources (soy protein isolate, whey protein isolate, milk proteins and wheat gluten) especially the edible films from mung bean proteins. Water vapor permeability determined in this study of edible films from mung bean and red bean proteins were higher than lower density polyethylene and high density polyethylene, however edible films from mung bean proteins had almost similar water vapor permeability to cellophane (Table 8.). Resistance of protein-based edible films to water vapor transmission is limited due to the inherent hydrophilicity of proteins. Transmission of water vapor through protein-based edible film is also facilitated by the presence of, a hydrophilic plasticizer, which favors adsorption of water molecules (Cuq *et al.*, 1995).

Table 7. Tensile strength (TS) and elongation at break (E) of various films

Film Type (polymer: plasticizer)	Test condition	Tensile strength (MPa)	Elongation at break (%)	Reference
Mung bean proteins	25 °C, 50% RH	6.28	35.74	Current study
Red bean proteins	25 °C, 50% RH	8.02	2.95	Current study
Casein: Glycerol (49: 1)	25 °C, 50% RH	4.1	38.00	Motoki *et al.* (1987)
Soy protein isolate: Glycerol (5:3)	25 °C, 50% RH	3.6	139.00	Gennadiose *et al.* (1993)
Wheat gluten: Glycerol (12: 4.4)	25 °C, 50% RH	4.4	170.00	Gennadiose *et al.* (1993)
Milk protein: Glycerol (25:7.5)	25 °C, 65% RH	8.6	22.10	Maynes and Krochta (1994)
Wheat gluten: Glycerol (3: 1)	23 °C, 55% RH	2.12	-	Herald *et al.* (1995)
Peanut protein: Glycerol (1: 2)	25 °C, 50% RH	4.35	105.00	Jangchud and Chinnan (1999)
Water-soluble fish proteins: Glycerol (2:1)	23 °C, 50% RH	3.0-5.5	40-70	Iwata *et al.* (1999)
Water-soluble fish proteins: Glycerol (2:1)		1.84	48.72	Kerdsup *et al.* (2002)
Low density polyethylene	38 °C, 90% RH	7.60-17.30	500	Briston (1988)
High density polyethylene	38 °C, 90% RH	17.3-34.6	300	Briston (1988)
Polyvinylidine chloride	38 °C, 90% RH	48.4-138.0	20-40	Briston (1988)
Cellulose acetate	38 °C, 90% RH	48.5-82.7	15-45	Briston (1988)
Polyester	38 °C, 90% RH	178.0	70.0-100.0	Briston (1988)

Table 8. Water vapor permeability (WVP)

Film Type (polymer: plasticizer)	Test condition	Water vapor permeability ($gram.mm/m^2.day.kPa$)	Reference
Mung bean proteins	25 °C, 50% RH	11.43	Current study
Red bean proteins	25 °C, 50% RH	20.61	Current study
Soy protein isolate: Glycerol (5:3)	25 °C, 50/0% RH	284	Gennadiose *et al.* (1993)
Whey protein isolate: Glycerol (1.6:1)	25 °C, 0/65% RH	119.8	McHugh *et al.* (1994)
Whey protein isolate: Sorbitol (1.6:1)	25 °C, 0/79% RH	62.0	McHugh *et al.* (1994)
Whey protein isolate: Glycerol (2.3:1)	25 °C, 50/0% RH	-	McHugh and Krochta (1994)
Whey protein isolate: Glycerol (3.5:1)	25 °C, 70/0% RH	-	McHugh and Krochta (1994)
Non fat dried milk: Glycerol (4:1)	25 °C, 0/65% RH	70.3	Maynes and Krochta (1994)
Wheat gluten: glycerol (3:1)	23 °C, 55/0% RH	66.37	Herald *et al.* (1995)
Water soluble fish proteins: Glycerol (2:1)	30 °C, 100/0% RH	10.08	Iwata *et al.* (1999)
Peanut protein: Glycerol (3:5)	37.8 °C, 55/0% RH	10.35	Jangchud and Chinnan (1999)
Amylose: Glycerol (7:3)	20 °C, 50/0% RH	-	Forssell *et al.* (2002)
Amylopectin: Glycerol (7:3)	20 °C, 50/0% RH	-	Forssell *et al.* (2002)
Low density polyethylene	38°C 90/0% RH	0.079	Smith (1986)
High density polyethylene	38°C 90/0% RH	0.02	Smith (1986)
Cellophane	38°C 90/0% RH	7.27	Taylor (1986)

CHAPTER 4

CONCLUSIONS

1. Compositional Profile of Mung Bean and Red Bean Proteins

- The proximate composition of dried mung bean and red bean proteins were found to be 93.52, 1.37, 0.85 and 4.26 % and 90.26 1.08, 3.99 and 4.67 % of crude protein, crude fat, carbohydrate and ash, respectively

- Proteins from mung bean and red bean, large amounts of proteins had molecular weight (MW) between 24 and 55 kDa with some traces having less than 24 kDa. However, there were small amounts of proteins of MW between 24 and 14.2 kDa.

- Mung bean and red bean proteins were rich in essential amino acid such as leucine, isoleucine, lysine, and phenylalanine and also acidic amino acid such as glutamic acid and aspartic acid. The sulfur containing amino acids such as methionine and cysteine were also detected in mung bean and red bean proteins.

2. Effect of pH, Heating Temperature, Heating Time, Protein Concentration, Plasticizer and Lipids on the Properties of Edible Films from Mung Bean Proteins

- The pH and heating temperature of film-solutions had the greatest impact on the physico-chemical and permeability properties of edible films from mung bean proteins.

- The films produced at pH ~9.4 at 73.5 °C exhibited the highest tensile strength and elongation at break, while water vapor permeability were at there lowest.

- There was a direct correlation between the film solubility and protein solubility and heating temperature, which reversed with change in pH.

- Color of films turned darker and more yellow with increase in the pH.

- Increasing the protein concentration provided the films with a higher tensile strength and elongation at break, but lowered of water vapor permeability, film solubility and protein solubility and showed a darker and more yellowish film.

- Sorbitol resulted in greatest mechanical resistance, but poorest in film flexibility.

- Glycerol and polyethylene glycol provided the film with flexible structure, however, the mechanical strength was low, but their water vapor permeability was high.

- Increase in plasticizers concentration resulted in decrease of tensile strength with concomitant increase of elongation at break and water vapor permeability.

- Increasing plasticizer concentration resulted in higher solubility.

- Sorbitol plasticized films showed higher solubility than glycerol and polyethylene glycol plasticized films.

- The change in color of edible film depended on the plasticizer type.

- Mung bean protein film with lipids improved the WVP more than mung bean protein film without lipids but film's mechanical properties are lowered.

- Oleic acid incorporated films provided the films with smoother surface and higher TS and %E but lower WVP than stearic acid and palm oil, respectively.

- Addition of lipids significantly increased film yellowness for incorporated films

3. Effect of pH, Heating Temperature, Heating Time, Protein Concentration, Plasticizer and Lipids on the Properties of Edible Films from Red Bean Proteins

- The pH and temperature of film-solutions had the greatest impact on the physico-chemical and permeability properties of edible films from red bean proteins.

- The films produced at pH ~10.0 at 81.5 ^{o}C exhibited high tensile strength and elongation at break, while water vapor permeability were at their lowest.

- Increasing heating temperature of film solutions from 60 to 80 ^{o}C, resulted in increase in tensile strength but decrease in water vapor permeability, film solubility and protein solubility.

- Positive correlation was observed between both film solubility and protein solubility with change in pH of film-solutions.

- Excessive pH and heating temperature of film-solutions resulted in darker and more yellowish color of edible films.

- Increasing the protein concentration provided the films with higher both tensile strength and elongation at break, but lower water vapor permeability.

- The effects of type and concentration of these plasticizers on physico-chemical properties of the films showed a similar trend to edible films produced from mung bean proteins.

- Red bean protein film with lipids improved the WVP more than red bean protein film without lipids but film's mechanical properties are lowered.

- Oleic acid incorporated films provided the films with smoother surface and higher TS and %E but lower WVP than stearic acid and palm oil, respectively.

- Addition of lipids significantly increased film yellowness for incorporated films.

4. Comparing the Properties of Edible Film from Mung Bean and Red Bean Proteins with Edible Film Produced from other Protein Sources, Polysaccharide and Synthetic polymer.

- Edible film from mung bean and red bean proteins had mechanical properties better than those other protein sources.

- Edible films from mung bean and red bean proteins had substantially lower both tensile strength and elongation at break than high density polyethylene, polyvinyl chloride, cellulose acetate and polyester. However, edible films from mung bean and red bean proteins had higher tensile strength than low density polyethylene films.

- Water vapor permeability of edible films from mung bean and red bean proteins were characterized by relative low water vapor barrier properties than other protein sources, especially the edible films from mung bean proteins.

- Water vapor permeability determined in this study of edible films from mung bean and red bean proteins were higher than lower density polyethylene and high density polyethylene, however edible films from mung bean proteins had almost similar water vapor permeability to cellophane.

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