Improvement of Biodegradable Rice Starch Film Properties by Using Crosslinking Agent and UV Treatment

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ชื่อวิทยานิพนธ์ การปรับปรุงสมบัติของฟิล์มย่อยสลายจากสตาร์ชข้าวเจ้าโดยสารครอสลิงและยูวีทรีทเมนต์
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บทคัดย่อ

งานวิจัยนี้ศึกษาการปรับปรุงสมบัติของฟิล์มสตาร์ชจากข้าวเจ้าด้วยการใช้สารครอสลิงและการทรีทด้วยรังสียูวี โดยการใช้สารครอสลิงทำปฏิกิริยาครอสลิงระหว่างสตาร์ชข้าวเจ้ากับสารครอสลิงที่แตกต่างกัน 3 ชนิด ที่ระดับความเข้มข้นต่างๆ คือ Sodium trimetaphosphate (ร้อยละ 1, 2 และ 3 โดยน้ำหนักแห้งของสตาร์ช) สารผสมระหว่าง Sodium trimetaphosphate และ Sodium tripolyphosphate (STMP/STPP (99:1) (ที่ระดับร้อยละ 4, 8 และ 12 โดยน้ำหนักแห้งของสตาร์ช) และ Epichlorohydrin (ร้อยละ 0.1, 0.3 และ 0.5 โดยน้ำหนักแห้งของสตาร์ช) ซึ่งทำปฏิกิริยาครอสลิงในสภาวะด่าง (pH 10.5) จากผลการทดลองพบว่าเมื่อความเข้มข้นของสารครอสลิงเพิ่มขึ้นส่งผลให้ค่าความหนืดของครอสลิงสตาร์ชข้าวเจ้าลดลงและอุณหภูมิที่ทำให้แป้งเกิดเจลาติไนต์ (pasting temperature) มีค่าสูงกว่าสตาร์ชข้าวเจ้าที่ไม่ผ่านการครอสลิง นอกจากนี้ระดับของการครอสลิง (degree of cross-linking) มีค่าเพิ่มขึ้นเมื่อความเข้มข้นของสารครอสลิงเพิ่มขึ้น และการครอสลิงสตาร์ชสามารถปรับปรุงสมบัติของฟิล์มสตาร์ชข้าวเจ้าได้ โดยผลการทดลองแสดงให้เห็นว่าสมบัติเชิงกลและสมบัติต้านทานไอน้ำของฟิล์มสตาร์ชข้าวเจ้าสูงขึ้นเมื่อด้วยเกิดปฏิกิริยาครอสลิงระหว่างสตาร์ชข้าวเจ้าและสารครอสลิง นอกจากนี้พบว่าเมื่อปริมาณการเกิดปฏิกิริยา ครอสลิงเพิ่มขึ้นส่งผลต่อการปรับปรุงสมบัติทางความร้อนของฟิล์มสตาร์ชโดยอุณหภูมิการเปลี่ยนสถานะจากของแข็งคล้ายแก้ว (Glass transition temperature, T_g) ของฟิล์มครอสลิงสตาร์ชมีค่าเพิ่มขึ้น และยังชี้ให้เห็นชัดเจนอีกว่าการใช้ STMP และ STMP/STPP ให้ปริมาณการเกิดครอสลิงมากกว่า EPI

ผลของการปรับปรุงสมบัติของฟิล์มสตาร์ชข้าวเจ้าด้วยยูวีทรีทเมนต์ โดย ใช้โซเดียมเบนโซเอท (sodium benzoate) เป็นสารที่ไว้ต่อการกระตุ้นด้วยแสงยูวี (photo-sensitiser) ในการเกิดปฏิกิริยาครอสลิงที่ระดับความเข้มข้นต่างๆ (ร้อยละ 3, 6 และ 9 โดยน้ำหนักแห้งของสตาร์ช) พบว่าการเติมโซเดียมเบนโซเอทและการฉายแสงยูวีสามารถปรับปรุงสมบัติของฟิล์มสตาร์ชข้าวเจ้า

โดยสมบัติเชิงกลและสมบัติต้านทานไอน้ำของฟิล์มสตาร์ชข้าวเจ้ามีค่าสูงขึ้นและที่ความเข้มข้นของโซเดียมเบนโซเอทร้อยละ 6 ให้ฟิล์มมีสมบัติที่ดีที่สุดทั้งสมบัติเชิงกล สมบัติต้านทานไอน้ำและสมบัติทางความร้อน ในขณะที่ความเข้มข้นของโซเดียมเบนโซเอทร้อยละ 9 เป็นปริมาณที่มากเกินไปของสารที่ไว้ต่อการกระตุ้นด้วยแสงยูวีมีผลทำให้ปริมาณการเกิดครอสลิงลดลง นอกจากนี้พบว่าฟิล์มสตาร์ชข้าวเจ้าที่ผ่านการฉายแสงยูวีส่งผลให้อุณหภูมิการเปลี่ยนสถานะจากของแข็งคล้ายแก้ว (Glass transition temperature, T_g) เพิ่มขึ้นเมื่อความเข้มข้นของสารที่ไว้ต่อการกระตุ้นด้วยแสงยูวีเพิ่มขึ้น จากการศึกษาระยะเวลาในการฉายแสงยูวีและความชื้นสัมพัทธ์ก่อนการฉายแสงยูวี พบว่าเวลาระยะเวลาการฉายแสงที่เหมาะสมต่อการปรับปรุงสมบัติของฟิล์มสตาร์ชข้าวเจ้าคือ 10 นาทีและที่ความชื้นสัมพัทธ์ร้อยละ 55 ซึ่งให้ฟิล์มที่มีสมบัติดีที่สุด สำหรับการศึกษาผลการดูดซับความชื้นที่อุณหภูมิคงที่ (Equilibrium Moisture Content) พบว่าฟิล์มจากสตาร์ชข้าวเจ้ามีการดูดซับไอน้ำได้อย่างรวดเร็วในช่วงแรกและค่อยๆ ลดลงเมื่อความเวลาในการดูซับไอน้ำเพิ่มขึ้น ฟิล์มสตาร์ชครอสลิงด้วย STMP ความเข้มข้นร้อยละ 3 มีอัตราการดูดซึมไอน้ำต่ำที่สุดที่ระดับความชื้นสัมพัทธ์ต่ำ นอกจากนี้พบว่าเมื่อระดับความชื้นสัมพัทธ์สูงขึ้นส่งผลให้ฟิล์มสตาร์ชข้าวเจ้ามีค่าการต้านทานแรงดึงลดลง

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ABSTRACT

This research aimed to improve the properties of rice starch film by using cross-linking agent and UV treatment. Rice starch was cross-linked with 3 types of cross-linking agent at various concentration; Sodium trimetaphosphate (1%, 2% and 3% w/w starch), the mixture of Sodium trimetaphosphate and Sodium tripolyphosphate (STMP/STPP (99:1) at 4%, 8% and 12% w/w starch) and Epichlorohydrin (EPI; 0.1%, 0.3% and 0.5% w/w starch). Rice starch was cross-linked under alkaline conditions (pH 10.5). The viscosity of cross-linked rice starch decreased when the contents of cross-linking agent increased concomitant with increasing of pasting temperature. The results showed that the degree of cross-linking increased when concentration of cross-linking agents increase. The cross-linked rice starch provided the improvement of rice starch film properties. The results demonstrated that the mechanical and water barrier properties of rice starch films were enhanced by the cross-linking reaction between rice starch and cross-linking agent. Furthermore, increasing of concentration of cross-linking yielded the enhancement the thermal properties of rice starch films causing the glass transition temperature (T_g) shifted toward higher temperature. The results pointed out that STMP and the mixture of STMP/STPP had a higher cross-link density than EPI.

The effect of UV treatment on the properties of rice starch films was investigated. The results revealed that UV treatment using sodium benzoate at various concentration of sensitiser (3%, 6% and 9% wt.) as photo-sensitiser could improve the rice starch film properties. The rice starch film showed increasing in mechanical and water barrier properties when photo-cross-linking of rice starch was applied. The results demonstrated that the highest mechanical, water barrier and thermal properties were obtained addition 6% of photo-sensitiser was applied. However, addition 9% photo-sensitiser was an excessive amount of photo-sensitiser resulted in lower cross-linking density. Moreover, the glass transition temperature (T_g) shifted toward higher temperature when increasing amount of photo-sensitiser. The effect of irradiation time and the relative humidity (before irradiation) on the properties of rice starch films were also investigated. The results demonstrated that the optimum conditions provided the suitable rice starch film properties were received when 10 minutes of irradiation time and 55% RH was applied. Moisture absorption of rice starch films was more rapidly in the initial stage of moisture absorption and showed decreasing when the absorption time increased. The rice starch film cross-linked with 3% STMP showed the lowest moisture absorption when storage in the low relative humidity. In addition, the lowest TS of rice starch films were observed when storage in higher relative humidity.

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CHAPTER 1

INTRODUCTION

The synthesis of polymer for used as a food packaging was research since 50-60 year ago. Synthetics polymers are durable and resistant to biodegrade well (Mali *et al*., 2002) but in the present found that polymer residues are significant part of the volume of waste, causing environmental problems because it is not biodegradable and difficult to eliminate and recycle (Carvalho *et al*., 2007). The increase awareness of environmental conservation and protection has promoted the presentation of biodegradable polymers produced from renewable sources as an alternative to synthetic polymers for selected industrial applications (Muñoz *et al*., 2003). Since 1970s has been studied about polar polymers or biopolymer such as polysaccharide and protein that is the new alternative for used instead of synthetic polymer in plastic and plastic film manufacturing against environmental problems (Garcia *et al*., 2000). Biopolymer consists of naturally occurring polymers that are found in living organisms. The use of biopolymers will have a less harmful effect on our environment compared to the use of fossil fuel based commodity plastics (Krochta and De Mulder-Johnston, 1997). In the present overview, Clarinval and Halleux (2005) have chosen to classify biopolymers according to their origin into two groups: natural polymers, polymers coming from natural resources and synthetic polymers, polymers synthesized from crude oil.

Starch is important biopolymer that is a new choice for production of biodegradable plastics. Since it is considered to be the one of the most abundant biopolymers, a natural renewable carbohydrate polymer, a major food reserve providing a bulk nutrient and energy source and often at low cost (Zobel and Stephen, 2006). Starches used in industrial applications are usually extracted from cereal seeds (corn, wheat, and rice), tubers (potato), and roots (tapioca) (Chiou *et al*., 2005). Starch has been investigated widely for the potential manufacture of products such as water-soluble pouches for detergents and insecticides, flushable liners and bags, and medical delivery systems and devices. Starch granular organization as well as amylose and amylopectin structure depends on the botanical source. Amylose, the linear D-glucose chain, has on average between 500 and 6,000 glucose units that are distributed among 1 to 20 chains. Each chain has shown an average degree of polymerization (DP) of 500. Amylopectin, the branching polymer of starch, contains short (DP = 20–25) chains linked to the α–D–(1–6) linkages on the mainchain.

Rice is the seed of the monocot plants *Oryza sativa* or *Oryza glaberrima*. As a cereal grain, it is the most important staple food for a large part of the world's human population, especially in East and South Asia, the Middle East, Latin America, and the West Indies. It is the grain with the second-highest worldwide production, after maize (corn) (Cohen *et al.,* 1994). Rice has many important roles in Thai society from food to work. Rice uses over half of the farmable land area and labor force in Thailand. It is one of the main foods and sources of nutrition for most Thai citizens. Chiang Phatthalung rice is native rice that has a high amount of amylose (about 25.36-30.40%). Because of its high amylose that made it’s hard and not favor for Thai consumers. In the other hand, rice starch that has a high amylose which is attractive raw materials for use as barriers in packaging materials. Rice starch have been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renewability, as well as possessing good mechanical properties (Xu *et al.,* 2005) However, wide application of starch film is limited by their mechanical weakness and the fact that they are swollen by water the extent of which depends on the relative humidity (RH) (Seow *et al.*, 1999). Then, starch film need to be improved for benefit of applications.

Chemical modifications such as grafting or crosslinking are able to limit excessive water swelling and macromolecular motion. Classical treatment lead to the modified starches used in food or paper additive applications in which crosslinking occurs in heterogeneous media using dry or semidry blending process. For example, starch networks are usually performed by treating granular starch with the following crosslinking agents in heterogeneous media: sodium trimetaphosphate (STMP) (Woo and Seib, 1997), epichlorohydrin (EPI) (Kuniak and Marchessault, 1973), mixture of sodium trimetaphosphate (STMP) / Sodium tripolyphosphate (STPP). In addition, radiation processing technology is being used to improve the properties of polymer product because of its tendency to undergo the chemical reaction between the polymer molecules under irradiation (Gehring, 2000). The most efficient photo-additive, sodium benzoate, is known to be photolysed by UV irradiation (Ghosh and Gangopadhyay, 2000: Wells, 1972). The sensitized irradiated films are rendered insoluble in the solvents in which they were originally soluble (Deville *et al.,* 2002). In this study was improved the properties of rice starch film by chemical treatment, using crosslinking agents such as STMP, EPI and STMP/STPP mixture, and UV treatment, using sodium benzoate as a photosensitiser. The properties of rice starch film received from chemical treatment and UV treatment were compared on mechanical property, thermal property and structural characteristics.

Review of Literature

1. Biodegradable Polymers

Naturally occurring biodegradable polymers are derived from four broad feedstock areas (Tharanathan 2003). Animal sources provide collagen and gelatin, while marine sources provide chitin which is processed into chitosan. However, the remaining two feedstock areas are the ones receiving the most attention from scientists, and are the sources thought to be the most promising for future development and expansion. Microbial biopolymer feeds tocks are able to produce polylactic acid (PLA) and polyhydroxy alkanoates (PHA). The final category of agricultural feedstocks is the biopolymer source under consideration at the University of Saskatchewan, in Saskatoon, Canada. This variety of polymers falls into the categories of hydrocolloids, and lipids and fats. For many decades, cellulose-based polymers played a key role in a wide range of applications, for example, apparel, food (e.g., for sausages), and nonplastics (e.g., varnishes). In the meantime, these bio-based polymers have lost important markets mainly to polyolefins. Since the 1980s, more and more types of starch polymers have been introduced. Currently, starch polymers are one of the most important groups of commercially available bio-based materials. At the outset, simple products such as pure thermoplastic starch and starch/polyolefin blends were introduced. Due to the incomplete biodegradability of starch/polyolefin blends, these products had a negative impact on the market toward biodegradable polymers, which achieved largely development of copolymers consisting of thermoplastic starch and biodegradable petrochemical copolymers (Patel *et al.*, 2003).

Major progress has been made concerning the production of other types of bio-based polymers at industrial scale, based on biotechnology, contributing to the establishment of a sustainable bio-based economy. Another technological driver is the progress of nanotechnology, which also offers new possibilities for bio-based polymers. With regard to the type of bio-based and/or biodegradable polymers, it is important to describe the biodegradability of these materials. The American Society for Testing of Materials (ASTM 1998) and the International Standards Organization (ISO 2005, 2007) define degradable plastics as those that undergo a significant change in chemical structure under specific environmental conditions. These changes result in a loss of physical and mechanical properties, as measured by standard methods. Bio-based polymers can be, but not necessarily are, biodegradable polymers. For example, starch polymers are generally biodegradable, while crystalline polylactic acid (PLA) is virtually non-biodegradable.

1.1 Formulation of Biodegradable Polymer Films

The formulations of biodegradable polymer films require the use of at least one constituent capable of forming a matrix with adequate continuity and cohesion. Generally, this is composed of polymers or compounds which, under the preparation conditions used form continuous crystalline or amorphous structures (hydrophilic polymer, fats and sugars). In the case of coatings for which the films system can be applied directly to the product, two forces are relevant: that between the molecules of the coating material (cohesion) and that between the coating and the support structure (adhesion). The degree of cohesion produces the barrier and mechanical properties of the film. High structural cohesion is manifested by a reduction in flexibility, porosity, and permeability to gases and solutes (Banker, 1966). The degree of cohesion depends on the chemical structure of the film material, the presence of plasticizing and cross-linking agent, the nature of the solvent used and its dilution, the method of application, the procedure of removal of the solvent, and the final thickness of the film. The highest cohesion is generally obtained for ordered polar polymers with long chains which were precipitated in crystalline form. The preparation of average dilution which constitutes a compromise between salvation and extension of polymer molecules and good initial viscosity is preferable (Baker, 1966).

The cohesion of films generally increases in proportion in their thickness up to a threshold beyond which it remains constant. The speed of evaporation of the solvent and/or excessive temperature may be manifested by inadequate cohesion on account of the premature immobilization of the polymer molecular. Regarding the adhesion of the coatings to the foodstuff, this is generally facilitated by hot application. The formulation of films or coatings base on hydrocolloids requires one of the following mechanisms (Kester and Fennema, 1986):

- Simple coacervation, where a hydrocolloid in a aqueous dispersion is precipitated or undergoes a change of phase by evaporation of the solvent, addition of hydrosoluble nonelectrolyte in which the hydrocolloid is insoluble (e.g., ethanol), addition of an electrolyte which causes “salting out” or bridging or even modification of the pH.
- Complex coacervation, where two hydrocolloid solutions of opposite charge are combined, causing the reaction and precipitation of polymer complexes.
- Gelification or heat coagulation, in which heating the macromolecule causes its denaturation followed by gelification (in the case of certain proteins such as ovalbumin) or precipitation, or cooling of a dispersion of the hydrocolloid causing a sol-gel transition (in the case of gelatin or agar-agar).

Insolubility (if this is sought) and the barrier properties and the retention of solutes can occasionally be increased, thanks to the incorporation of bridging agents, bi-or trivalent ions or by the use of denaturing condition (heat, irradiation, ect.). For insoluble film materials of lipidic nature such as fat or waxes, these can be formed by application as a hot liquid followed by cooling with direct solidification in the form of an emulsion or microemulsion with water, or in solution in an appropriate organic solvent followed by evaporation of the water or the solvent.

1.2 Classification of Biodegradable Polymers

According to their source, biodegradable polymers can be classified into four main classes (Guilbert 2000, Vilpoux and Avérous 2004) (Figure 1):

- Agro-polymers directly extracted or removed from biomass (i.e., polysaccharides, proteins, polypeptides, polynucleotides). They are compostable and renewable polymers and can be processed directly, either plasticized, as fillers or modified by chemical reactions.
- Polymers produced by classical chemical synthesis using renewable bio-based monomers or mixed sources of biomass and petroleum, such as PLA or biopolyester.
- Polymers produced by genetically modified bacteria or by microorganisms through fermentation of agricultural products used as substrate. As examples, one may cite polyhydroxy alkanoates (PHA), bacterial cellulose, xanthan, curdian, and pullan.
- Polymers conventionally obtained from the petrochemical industry by chemical synthesis, such as polycaprolactone (PCL) and polyesteramide (PEA).

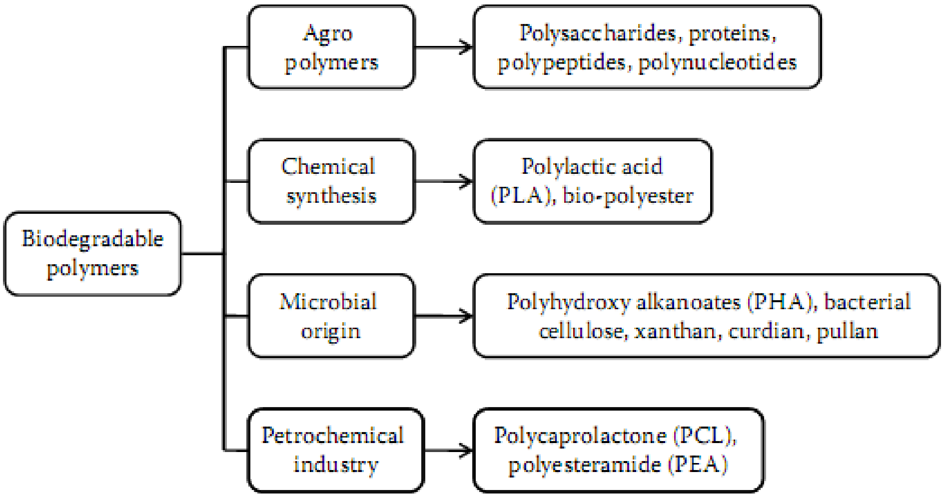


Figure 1. Types of biodegradable polymers and examples of materials in each category.

Source: Souza *et al.* (2010)

Although many types of biodegradable polymers are currently produced at industrial scale (PLA, PHA, PCL, PEA, and others), polymers from agricultural sources are the most studied ones by researchers, especially polysaccharides. This family is represented by different products such as starch and cellulose, both based on glucose units linked in macromolecular chains. Among the films made from polysaccharides, those obtained from starch are the most important (Alves *et al*. 2007, Bertuzzi *et al*. 2007, Famá *et al*. 2006, 2007, Garcia *et al*. 2006, Mali *et al*. 2006, Parra *et al*. 2004, Talja *et al.* 2008, Veiga-Santos *et al.* 2008), because starch is one of the most com-monly used agricultural raw materials, since it is a renewable source, inexpensive (even cheaper than polyethylene), widely available, and relatively easy to handle (Rodríguez *et al.* 2006). Besides, it is found in several forms according to the origin of its raw material.

Nowadays, starch-based polymers are a large part of the bioplastics market, representing from 85% to 90% of the total market. In 2002, about 30,000 metric tons per year were produced, and the market share of these products was about 75%–80% of the bio-based polymers global market (Degli and Bastioli 2002). Moreover, 75% of starch polymers are used for packaging applications including soluble films, films for bags and sacks, and loose fill. Since 1999, in many supermarkets in Scandinavia and in the Mediterranean coast, biodegradable shopping bags for the separate collection of organic waste already exist. Starch has been used as native or slightly modified starch, as well as singly or blended to other materials, which can be conventional synthetic materials or other natural polymers resulting in biodegradable polymers.

1.3 Biodegradation

There are several ways a polymer may degrade in the environment. These include biodegradation, photodegradation, oxidation, and hydrolysis. These processes are often interpreted by the general public as one and the same though they lead to very different end results. It is often conceived that the breakdown of a plastic into small, invisible (to the naked eye) fragments is biodegradation, when in reality these fragments may remain in the environment over significant period of time. Biodegradable polymers when placed in bioactive environments, such as compost, will break down to carbon dioxide and water under the action of bacteria and fungi. There are two major steps in the biodegradation process. The first involves the depolymerization or chain cleavage of the polymer to oligomers, and the second is the resulting mineralization of these oligomers. The depolymerization step normally occurs outside the micro-organism and involves both endo- and exo-enzymes. Endo-enzymes cause random scission on the main chain, while exo-enzymes causes sequential cleavage of the terminal monomer in the polymer main chain. Once depolymerized, sufficiently small-sized oligomeric fragments are formed. These fragments are transported into the cell where they are mineralized. Mineralization is defined as the conversion of the polymer into biomass, minerals, water, CO_2, CH_4, and N_2. The mineralization step usually occurs intracellularly.

Standard test procedures are available to evaluate the biodegradability of plastics. The American Society for Testing and Materials (ASTM), the Ministry of International Trade and Industry (MITI, Japan), 2 and the Organization for Economic Cooperation and Development (OECD) 3 have developed standard test procedures to evaluate the biodegradability of plastics. Most of these test methods measure the percent conversion of the carbon from the designed biodegradable plastic to CO_2 and CH_4 (plus some CO_2) in aerobic and anaerobic environments, respectively. The absence of polymer and residue in the environment indicates complete biodegradation, whereas incomplete biodegradation may leave polymer and/or residue as a result of polymer fragmentation or metabolism in the biodegradation process. Failure in one test does not necessarily exclude biodegradation, it merely indicates that under the environmental conditions and/or the timeframe where the experiment was conducted, no (or incomplete) biodegradation occurred. Usually, more than one test method is needed to fully assess the biodegradability of a given polymer. Table 1 summarizes the various tests under the ASTM standards that may be used to determine biodegradability of a material.

Table 1. Standard methods for estimating biodegradation of plastic materials

Test	Environment	Property measured
ASTMD 5209-92	Aerobic sewage sludge	Carbon-dioxide
ASTMD 5210-92	Anaerobic sewage sludge	Carbon-dioxide/methane
ASTMD 5247-92	Aerobic specific micro-organism	Molecular weight
ASTMD 5271-93	Activated sewage sludge	Oxygen/carbon-dioxide
ASTMD 5338-92	Controlled composting	Carbon-dioxide
ASTMD 5509-94	Simulated compost	Physical properties
ASTMD 5511-94	High solids anaerobic digestion	Carbon-dioxide/Methane
ASTMD 5512-94	Simulated compost using external heated reactor	Physical properties
ASTMD 5525-94	Simulated landfill	Physical properties
ASTMD 5526-94	Accelerated landfill	Carbon-dioxide/Methane
MITI Test	MixedMicrobial	Oxygen

Source: Bhattacharya *et al.* (2005)

2. Cross-linking

Crosslinking means that the polymer molecules are interconnected by some sort of bonding. The bonding can be covalent, ionic, or it can result from intermolecular forces such as hydrogen bonding (Figure 2). With a small degree of crosslinking, a loose network is obtained, such as in vulcanized rubber, in which the crosslinks are formed by sulfur atoms. Highly crosslinked polymers, such as a thermoset plastic, have such a rigid structure that when heated they decompose or burn rather than melt. A crosslinked polymer is one super giant molecule. For example, the polymer in a bowling ball is literally one molecule (Smiley and Jackson, 2002). The crosslink density and crosslink density in homogeneities is inevitable for the assessment of the quality of crosslinked polymers, as well as for the optimization of curing conditions and tailored design of new products with improved mechanical and aging properties. The physical structure of crosslinked polymers, network dynamics and molecular mobility, depend to a large extend on the density of crosslinks. Mechanical and oxidative aging as well as different types and amount of fillers affect the network dynamics, may lead to a change of crosslink density and/or to a degradation of the chemical structure by hydrocarbon chain scissions.

Cross-linking is used in both synthetic polymer chemistry and in the biological sciences. Although the term is used to refer to the "linking of polymer chains" for both sciences, the extent of crosslinking and specificities of the crosslinking agents vary. Crosslinking can be accomplished chemically or by irradiation. Chemical crosslinking with rubber material is called vulcanization. It is accomplished by a heat induced reaction between the polymers and a crosslinking agent. For wire and cable insulations, chemical crosslinking is performed by passing the wire through a long pressurized steam tube, called a continuous vulcanizing (C.V.) machine. Cross-linking is a key technique for modifying the properties of starches and can be achieved by adding intra- and inter-molecular bonds at random locations in the starch granules (Singh *et al.*, 2007). Cross-linking tends to limit the interaction of starch with water and provides a structural integrity of starch-based biodegradable materials during exposure to pressure and moisture (El-Tahlawy *et al.*, 2007). Cross-linking is generally performed by treating starches (semi-dry or slurry) with reagents capable of forming either ether or ester linkages between hydroxyl (–OH) groups on starch molecules. Polyfunctional chemicals such as phosphorus oxychloride ($POCl_3$), sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (EPI), a mixture of adipic and acetic anhydrides, and a mixture of succinic anhydride and vinyl acetate have been commonly used to cross-link starches (Hirsch and Kokini, 2002; Singh *et al.*, 2007). $POCl_3$ is an efficient cross-linking agent in aqueous slurry at pH> 11 in the presence of a neutral salt (Hirsch and Kokini, 2002; Singh *et al.,* 2007). EPI is poorly soluble in water and less uniformly distributed. Additionally, both $POCl_3$ and EPI are toxic and flammable (MSDS, 1996; Woo and Seib, 1997). STMP, on the other hand, is cited as an efficient cross-linking agent and is a solid of low toxicity with no reports about the adverse effects on humans (Woo and Seib, 1997). The phosphorylation reaction produces either monostarch phosphates via substitution or distarch phosphates via cross-linking depending on the reaction conditions including concentration of STMP, amylose content, pH, time and temperature. Singh *et al.* (2007) stated that a combination of substitution and cross-linking can provide stability against acid, thermal, and mechanical degradation of starch and delay retrogradation during storage. Starch phosphates prepared with STMP have been reported to enhance the stability of rice by minimizing granule rupture, solid loss, and water uptake (Rutledge *et al.*, 1972; Rutledge *et al.*, 1974).

Cross-Linking Provides:

1. Higher tensile strength
2. Improved abrasion/cut through
3. Better crush resistance
4. Solder iron resistance
5. Better over load characteristics
6. Resistance to stress cracking
7. Improved fluid resistance
8. Slightly better flame resistance
9. No change of electrical
10. Negligible change in thermal stability
11. Decrease in flexibility
12. Improved high temperature mechanicals

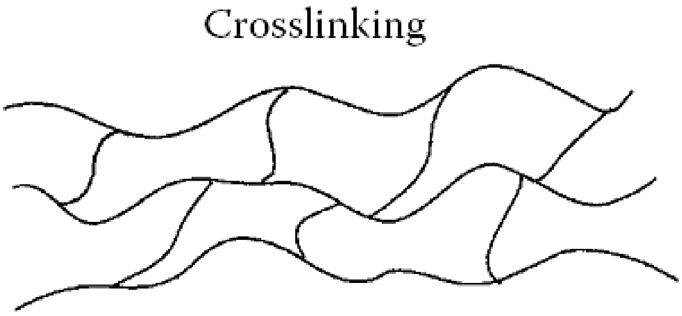


Figure 2. Schematic of cross-linked polymer.
Source: Smiley and Jackson (2002)

3. Starch

Starch is a major storage carbohydrate (polysaccharide) in higher plants and is available in an abundance surpassed only by cellulose as a naturally occurring organic compound. It can be found in fruits, seeds, rhizomes, and tubers. It is composed of a mixture of two polymers: amylose, an essentially linear polysaccharide, and amylopectin, a highly branched polysaccharide. As can be observed in Figure 2-3, amylose (Figure 2) is a linear polymer of anhydroglucose units linked by many units of glucose units bonded by a (1–4) bonds with a molecular weight of (5 × 104 to 2 × 105) kg/mol, whereas amylopectin (Figure 3) is a highly multiple-branched polymer with high molecular weight of (>105) kg/mol, with a (1–4) bonds and branching occurring at a (1–6) bonds every 24–70 glucose units. Simplified three-dimensional models for these two molecules, evidencing the formation of the helical structure, can be found online (Steane, 2008 a, b).

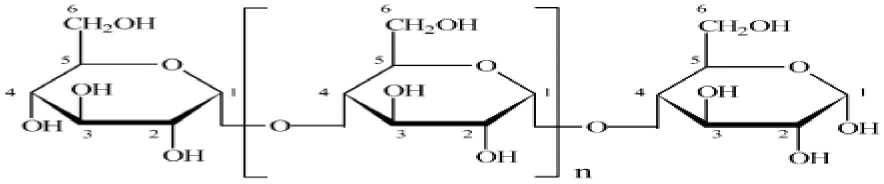


Figure 3. Structural representation of amylose, a linear polymer of glucose molecules existing in starch.

Source : Herrero-Martínez *et al.* (2004)

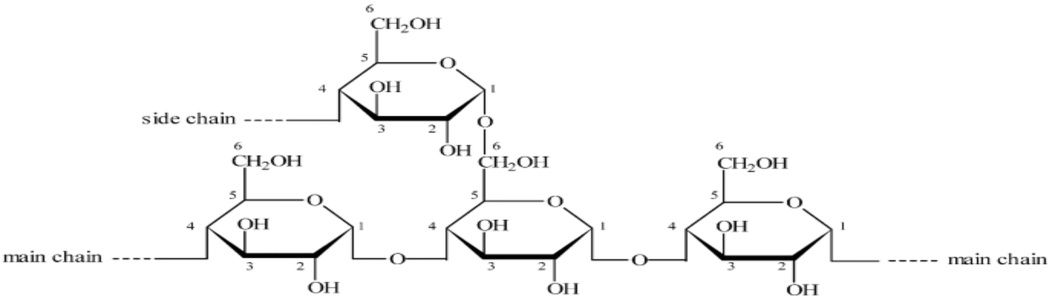


Figure 4. Structural representation of amylopectin, a highly multiple-branched polymer of glucose molecules existing in starch.

Source : Herrero-Martínez *et al.* (2004)

Industrially, starch is used, as ingredient, in the manufacture of paper, textiles, adhesives, and foods. The relative amount of amylose and amylopectin depends upon the starch source, such as amylomaize (rich-amylose starch) or waxy maize (rich-amylopectin starch), and film properties are a function of the amount of each macromolecule present within the starch granule (Tharanathan, 2003). There are many possible sources from which starch can be obtained to make bioplastics, and thus each place can use the most abundant starch source available locally. The main starch sources produced worldwide are corn (64%), sweet potato (13%), and cassava (11%) (FAO, 2004). Many other sources are available like wheat, rice, yam, green banana, potato, peas, beans, sorghum, arrowroot, and lentils, among others (Table 2).

Table 2. Some Typical Amylose–Amylopectin Mass Ratios[a] for Starches

Starch Source	**Amylose**	**Amylopectin**
High-amylose corn	50–85	15–50
Corn	26	74
Waxy corn	1	99
Wheat	25	75
Rice	17	83
Cassava	17	83
Tapioca	17	83
Potato	21	79

[a] Determined by complexation with Bu-1-OH, gel chromatography of enzymatically debranched starch, affinity chromatography on Sepharose-bound concanavalin A, or quantitative precipitation of amylopectin fraction with Con A solution.

Source : Zobel and Stephen (2006)

4. Rice Starch

Rice (*Oryza sativa L.*) in Asia and to some extent in Africa (Wang and J. Wang, 2004; Vandeputte and Delcour,2004), and the drought resistant sorghum (*Sorghum bicolor* Moench) in Africa, are cultivated in very large quantities, which belongs to the *Porceae Gramineae* or grass family has been consumed by humans for at least 5000 years. Rice contains two major sub-species: short grained *japonica* or *sinica* variety and the non-sticky, long grained *indica* variety. *Japonica* are usually cultivated in dry fields, in temperature East Asia, upland areas of southeast Asia and high elevations in South Asia, while *indica* are mainly lowland rice, grow mostly submerged, throughout tropical Asia. Rice is known to come in a variety of colors, including: white, brown, black, purple and red (Kennedy and Burlingame, 2003). Rice starch occurs naturally in rice and is the storage polysaccharide of rice. Starch is polymeric carbohydrate compose of anhydroglucose units. This is not a uniform material and starches contain two types of glucose polymer: a linear chain molecule termed amylose and branched polymer of glucose term amylopectin (Rodriguez *et al.,* 2006). Starch is often used in industrial foods. They have been use to produce biodegradable films to partially or entirely replace plastic polymer because of its low cost and renew ability, and it has good mechanical properties (Xu *et al.,* 2005).

4.1 Chemical Composition

Starch consists of two major types of molecules, primarily linear amylose and highly branched amylopectin. Normal starch consists of about 75% amylopectin and 25% amylose, waxy starches consist of mainly amylopectin and 0–8% amylose, and high-amylose starches consist of 40–70% amylose. A newly developed genetically modified starch has more than 90% amylose content (Sidebottom *et al*., 1998; Case *et al*., 1998). In addition to amylose and amylopectin, most cereal normal starches also contain lipids and phospholipids (Morrison, 1984; Medcalf *et al*., 1968; Lim *et al*., 1994; Kasemsuwan *et al*., 1996), which have profound impacts on the pasting property of the starch (Yoo and Jane, 2002). Most tuber and root starches and some cereal starches consist of phosphate monoester derivatives that are found exclusively on amylopectin molecules (Takeda and Hizukuri, 1982; Lim and Seib, 1993). Sugary-1 starch consists of phytoglycogen that is a water-soluble glucan with a highly branched structure and substantially shorter branch chains. The presence of phytoglycogen is a result of the lack of starch-debranching enzymes. Many starches, such as high-amylose maize starches (Jane and Chen, 1992) and sugary-2 starches, (Perera *et al*., 2001) also contain intermediate components that are branched molecules with smaller molecular weights and longer branch-chain lengths than does amylopectin (Takeda *et al*., 1993). Structures and properties of these components are discussed in the following sections.

4.1.1 Amylose

Amylose is compose of D-glucose molecules, which are linked in an α-1,4 conformation. The glucose monomers therefore form a linear straight chain polymer. Amylose is less predominant (about 20%) and typically constitutes about 20-40% in proportion. Amylose contains α-1,4 bond and is slightly soluble in water. Amylose molecules are arranged in a helical conformation. Amylose forms a blue complex with iodine, which can be read about 650 nm. Amylose is the key component involved in water absorption, swelling and gelation of starch in food processing. It is more susceptible to gelatinization and retrogradation, and hence is most commonly involved in resistant starch formation (Hoseney, 1986).

4.1.2 Amylopectin

Amylopectin is the major component of most starch, consist of a large number of shorter chains that are bound together at their reducing end side by a α-1,6 linkage (Hoseney, 1986). Amylopectin is therefore highly branched as the α-1,4 linear chains are punctuated with the α-1,6 linkages. The α-1,6 constitute about 5% of the structure of amylopectin and gives rise to branching. The amylopectin molecule therefore is much larger than the amylose molecule. Amylopectin has short branched chains and branch linkages and thus cannot form the helical complex with iodine. The branched dextrin of amylopectin, however, gives a purple color with the iodine complex, identifiable at about 550 nm (Bertoft, 2004).

4.1.3 Minor components of Starch

Minor components, such as lipids, phospholipids, and phosphate monoester derivatives, are found in starch and have profound effects on the properties of starch. Lipids and phospholipids are found in cereal starches. Cereal normal starches contain up to 1% lipids, and the level of lipid content is proportional to the amylose content of the normal starch (Morrison, 1993). Starches of different botanical origins consist of different species of lipids. For example, normal maize starch consists of mainly free fatty acids, glycerides, and little phospholipids; normal rice starch contains substantial amount of phospholipids and some free fatty acids; and wheat, barley, rye, and triticale starches consist of exclusively phospholipids (Lim *et al.*, 1994; Kasemsuwan and Jane, 1996; Morrison, 1993). Cereal waxy starches contain few lipids, whereas high-amylose starches contain substantially more lipids. Root and tuber starches contain very little lipids and no detectable phospholipids (McPherson and Jane, 1999).

- Protein: The difference between rice flour and starch is that most of the native proteins and lipids have been removed from starch. The protein content of milled rice in a germplasm collection reportedly range from 4.5 to 15.9% (Kennedy and Burlingame, 2003). Therefore, isolation of starch from rice mainly involves techniques to remove proteins. The majority of rice protein is alkaline soluble, thus alkaline steeping methods are commonly used in industry and research to produce rice starch with good recovery and lower residual protein content. The goal for the protein content of isolated rice starch is generally 0.5% or less (Lumdubwong and Seib, 2000).

- Minor constituents: Beside protein, other minor constituents including lipids, phosphorus and trace elements are commonly found in isolate starch. Non-waxy rice contains 0.3-0.4% bound lipid; waxy rice starch reportedly contains less of this fraction (0.03%). Phosphorus plays an extremely important role in starch functional properties, such as paste clarity, viscosity consistency and paste stability (Jane *et al.*, 1996).

4.2 Structure of Starch Granule

Starch is synthesized in granules inside amyloplasts. Starch granules isolated from different botanical sources and different organs display different shapes and sizes. 55 Diameters of starch granules vary from submicrons to more than 100 µm. Examples of diameters of starch granules are potato starch 15 to 75 µm, wheat A-granules 18 to 33 µm and B-granules 2 to 5 µm, maize starch 5 to 20 µm, rice starch 3 to 8 µm, and amaranth starch 0.5 to 2 µm (Jane, 1994). Starch granules display spherical, oval, disk, polygonal, elongated, kidney, and lobe shapes. Leaf starch has flat-shaped small granules that are submicron in diameter (Zeeman *et al.*, 1998). Wheat, barley, rye, and triticale starches display bimodal granule size distributions: the disk-shaped, large A-granules and the spherical, small B-granules (Jane, 1994). Rice and oats starches are known as compound starches, which are defined as multiple granules synthesized within a single amyloplast (Jane, 1994). Thus, starch granules are tightly packed together and develop into polygonal irregular shapes.

The basic physical structural unit of starch granule. These granules are simple or compound and consist of concentric or eccentric layers of varying density. They are of varying size (2-150 µm), size distribution and shape on the basis of X-ray diffraction experiment, starch granule are said to have a semicrystalline character. About 70% of the mass of a starch granule is regarded as amorphous and 30% as crystalline. The amorphous regions contain the main amount of amylose but also a considerable part of the amylopectin. The crystalline regions consist of primary of amylopectin. Native starches can be divided into type A, B and C. While type A and B are real crystalline modifications, the C-type is a mixed form. The A-type is largely present in cereal starches, and the B-type in potatoes. The C type is not only observed in mixtures of corn and potato starches (Belitz and Grosch, 1992).

4.3 Properties of Rice Starch

Starch is known to go through transformation and gives diverse physical structures and properties (Figure 5). Native granular starch, after being soaked in water, can absorb up to 30%, by weight, of moisture. The water absorbed is present in the amorphous region of the starch granule. This process is reversible, and water can be evaporated on drying at the ambient temperature or at a temperature below that for gelatinization. When starch granules are heated in the presence of water, the granules eventually lose the double-helical crystalline structure and the Maltese cross. This process is known as gelatinization. The gelatinization of starch is an irreversible process, and each starch has its own characteristic gelatinization temperature. When the gelatinized starch is continuously heated in excess water, the starch granules swell, develop viscosity, and become a paste. This is known as pasting. On cooling, viscosity increases as temperature decreases, and starch molecules in the paste develop a network and gel. After an extended storage period or repeated freeze and thaw cycles, starch molecules in the paste or gel crystallize, and the process is known as retrogradation (Jane, 2004).

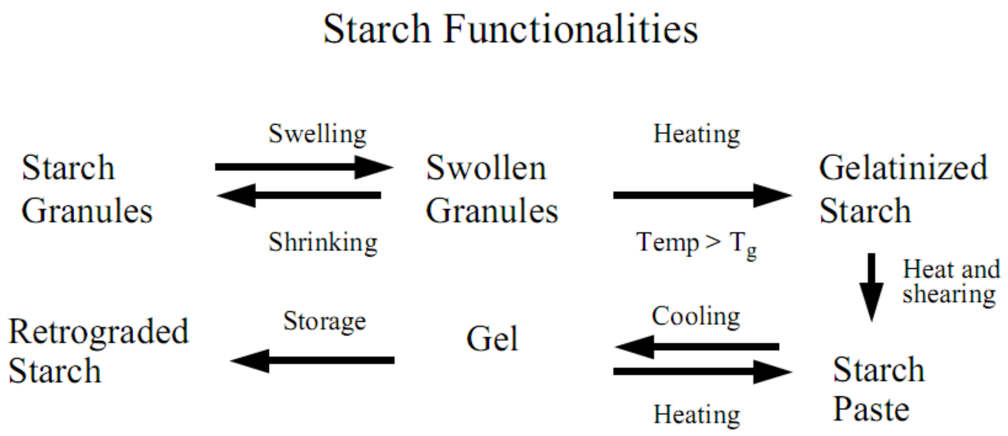


Figure 5. Transformation of starch structures.
Source : Jane (2004)

The versatile physical structures of starch facilitate a wide variety of applications; for example, granular starch is used for facial powder; large A-granules of wheat starch are used in carbonless copy paper, and small particle starch (Jane *et al*., 1992) or small granular amaranth starch (diameter 0.5 to 2 µm, resembling that of a fat micelle) is used for fat substitutes (Hanson *et al*., 1998). Starch pastes are used as sizing agents in paper and textile industries and as thickening agents for soup and canned foods in the food industry. Starch gel is used to make tapioca pudding, desserts, and fillings. Retrograded or crystalline high-amylose maize starch is used as resistant starch that has limited digestibility by humans and is desirable for low-caloric diet foods.

4.3.1 Swelling Power and Solubility

Starch is not water soluble, because granules are too large to form a solution. Starch has a relative high density, about 1.45-1.64 g/cm^3, depending on its source, its prior treatment, and the measurement method. Starch granules absorb some water in suspension at room temperature, but the amount of swelling is limited in intact granules. When heat in water suspension to progressively higher temperature, very little happens, until a critical temperature is reached. At this point the granule begins to swelling rapidly, losing the polarization crosses, a process termed gelatinization.

4.3.2 Gelatinization and Pasting

Starch gelatinization is the collapse (disruption) of the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization. The point of initial gelatinization and range over which it occurs is governed by starch concentration, method of observation, granular type and heterogeneity within the granule population under observation. Pasting is the phenomenon following gelatinization in the dissolution of starch. It involves granule swelling, exudation of molecular components from the granule and eventually, total disruption of the granule (Atwell *et al.*, 1998).

4.3.3 Retrogradation

Under low energy input, as in freezing and chilling, further hydrogen bonding may occur, resulting in further tightening of structure with loss of water-holding capacity, known as retrogradation. Starch retrogradation is a process which occurs when the molecules comprising gelatinized starch begin to reassociate in an ordered structure. In its initial phases, two or more starch chains may form a simple juncture point that may then develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears (Atwell *et al.,* 1998).

4.3.4 Glass-transition Temperature

Glass transition is the kinetic transition of amorphous polymers from a glassy state to a rubbery state. The glass-transition temperature (T_g) is specific to each material and depends on molecular weight of the polymer and presence of plasticizers. T_g of a homologous polymer increases with the increasing number-average molecular weight up to a plateau limit (Billmeyer, 1984) and decreases with an increase of plasticizer concentration. Plasticizers are small chemicals compatible with the polymer and increase flexibility and extensibility. Water is known to be the most powerful plasticizer for starch and many hydrophilic biopolymers (Slade and Levine, 1993; Slade and Levine, 1995). Glass-transition temperature of native wheat starch has been reported to decrease with the increase in moisture content (Zeleznak and Hoseney, 1987). When environmental temperature is above the glass-transition temperature, the polymer has molecular mobility and can go through conformational changes to crystallize and retrograde.

5. Starch Modification

Native starches have many disadvantages for industrial applications such as insolubility in cold water, loss of viscosity, and thickening power after cooking. In addition, retrogradation occurs after loss of ordered structure on starch gelatinization, which results in syneresis or water separation in starchy food systems. However, these shortcomings of native starch could be over- come, for example, by introducing small amounts of ionic or hydrophobic groups onto the molecules. The modifications alter the properties of starch, including solution viscosity, association behavior, and shelf life stability in final products. The functionality of starch can be modified through physical, chemical, and biotechnological means. Another purpose of starch modification is to stabilize starch granules during processing and make starch suitable for many food and industrial applications.

Starch can be physically modified to improve water solubility and to change particle size. The physical modification methods involve the treatment of native starch granules under different temperature/moisture combinations, pressure, shear, and irradiation. Physical modification also includes mechanical attrition to alter the physical size of starch granules. Starch is widely modified by chemical methods. The most common chemical modification processes are acid treatment, cross-linking, oxidation, and substitution, including esterification and etherification. The development of biotechnology provides another means for starch modification during the growth of the plant. Different amylose levels, amylopectin structure, and phosphorus contents from various plant sources can be produced using antisense reduction of enzyme activity of single or multiple enzymes. New starch functionalities can also be identified in naturally occurring mutants, which have been widely employed in food industry because of their natural and specific functional properties.

5.1 Chemical Modification

Chemical modification, which is the most common industrial means of enhancing starch properties, entails reaction or treatment of starch with chemical reagents to introduce new chemical substituent groups, effect molecular scission, or promote oxidation or molecular rearrangements (Wurzburg, 1986b). Chemical modification can be carried out on three starch states: In suspension, where the starch is dispersed in water, the chemical reaction is carried out in water medium until desired properties are achieved. The suspension is then filtered, washed, and air dried. In a paste, where the starch is gelatinized with chemicals in a small amount of water, the paste is stirred, and when the reaction is completed, the starch is air dried. In the solid state, where dry starch is moisturized with chemicals in a water solution, air dried, and finally reacted at a high temperature (i.e., ≥ 100 ºC). The most common chemical modification includes: oxidation, esterification, crosslinking and etherification. The chemical modification of starch results in enhanced molecular stability against mechanical shearing, acidic, and high temperature hydrolysis; obtaining desired viscosity; increasing interaction with ion, electronegative, or electropositive substances; and reducing the retrogradation rate of unmodified starch and limit the excessive water uptake (Fringant *et al*., 1996; Kumar *et al*., 2008).

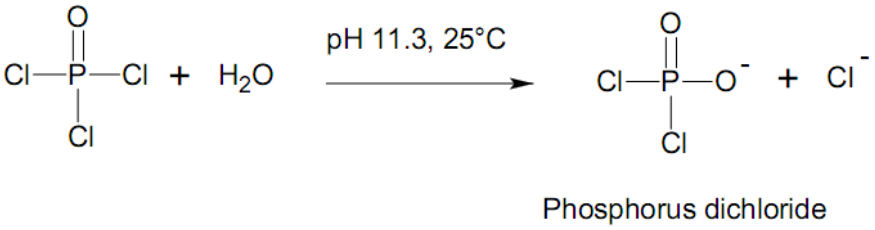
5.1.1 Oxidation Starch

Starch oxidation has been practiced since the early 1800s, and various oxidizing agents have been introduced, for instance, hypochlorite, hydrogen peroxide, periodate, permanganate, dichromate, persulfate, and chlorite (Rutenberg *et al*., 1984). The main uses for oxidized starch are the paper and textile industries. However, the application of oxidized starches in the food industry is increasing because of their low viscosity, high stability, clarity, and binding properties. Oxidized starch for food use is mainly produced by the reaction of starch with sodium hypochlorite. Bleaching agents, such as hydrogen peroxide, peracetic acid, potassium permanganate, and sodium chlorite, are also permitted by the Food and Drug Administration (FDA) in the United States however, their levels used in the reaction are too low to change the behavior of starch therefore, such chemicals are not included in this section. Oxidized starches are used as binding agents in foods, such as batters applied to meats before frying. The tenacious coatings formed on frying may result from the salt bridges between the protein molecules and anionic oxidized starch or the reaction of amino groups with the aldehyde groups on the oxidized starch. An acid-thinned maltodextrin may be oxidized to give a double modification, resulting in low viscosity and nongelling starch which can be used as fillings for chocolate confections (Rutenberg *et al.*, 1984; Wurzburg, 1986).

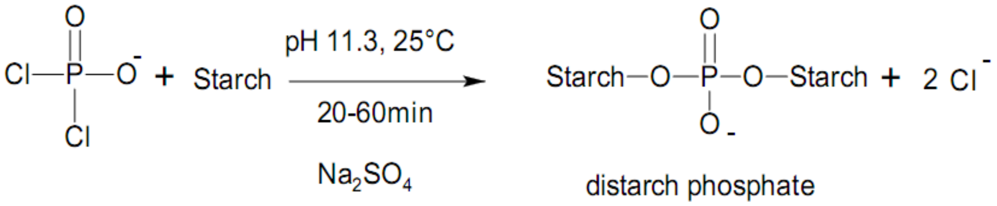
5.1.2 Cross-Linking Starch

Cross-linking reaction is chemically modifying the blended polymer during or after the blending process (Shi *et al.*, 2008). Where the functionality of one of the monomers is greater than 2, then a cross-linked polymer is formed (Figure 4). Starch contains two types of hydroxyls, primary (6-OH) and secondary (2-OH and 3-OH). These hydroxyls are able to react with multifunctional reagents resulting in cross-linked starches. Cross-linking is done to restrict swelling of the starch granule under cooking conditions or to prevent gelatinization of starch. Cross-linking of starch is effected by a low level of reagent. Starch molecules are long chain polymers which occur in close proximity within granules. Starch molecules can be interconnected by reactions with trace amounts of a multifunctional reagent (Seib, 1996). The reagents permitted by FDA for making cross-linking food grade starch are phosphoryl chloride, sodium trimetaphosphate, adipic acetic mixed anhydride, and mixtures of sodium trimetaphosphate and tripolyphosphates. Epichlorohydrin is no longer used by starch manufacturers in the U.S. because chlorohydrins are carcinogens (Seib, 1996).

Cross-linking of starch with phosphorous oxychloride is a rapid reaction which produces a di-starch phosphate. The reaction is especially efficient above pH 11 and in the presence of sodium sulphate (2% based on starch) (Furin, 1973; Wu and Seib, 1990). When phosphoryl chloride is added to a slurry of starch the first chloride ion of phosphoryl chloride reacts with water at 25°C immediately (with half-life of 0.01 sec) (Hudson and Moss, 1962) to form a phosphorous dichloride, which is likely the crosslinking agent. There is no time for phosphoryl chloride to diffuse into starch granules. The remaining two chlorides of phosphorous dichloride react with water almost simultaneously with a half-life of ~4 minutes at 25°C. Practically, phosphoryl chloride can be added as rapidly as possible to a starch slurry in order to generate phosphorous dischloride in situ, which then diffuses into the granule to affect cross-linking6 (Equation 1 and Equation 2).



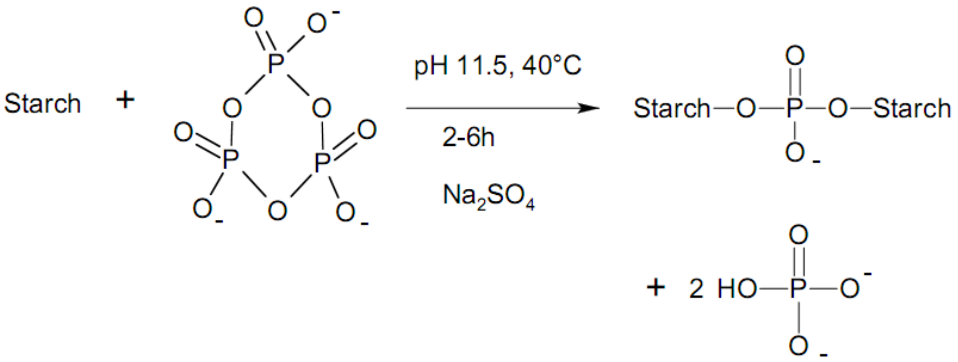
(1)



(2)

Source: Xie *et al*. (2005)

Sodium trimetaphosphate, a nonhazardous solid, is another cross-linking agent. The cross-linking starch with sodium trimetaphosphate proceeds slowly to give distarch phosphate (Equation 3). However, this reaction can be accelerated by increasing the pH and the concentration of sodium sulphate (Seib, 1996).



(3)

Source: Xie *et al*. (2005)

Since low levels of reagents are used in cross-linking, chemical measurement of the extent of cross-linking is difficult. In the case of cross-linking with phosphorylating reagents, 31P-NMR could be used to measure the extent of cross-linking (Kasemsuwan and Jane, 1994). The physical methods related to the change in swelling of starch granules are also used to measure cross-linking. Three popular methods include measurement of fluidity, optical clarity, and pasting curves are available in the literature (Seib, 1996). Cross-linked starches are used in salad dressings to provide thickening with stable viscosity at low pH and high shear during the homogenization process. Cross-linked starches with a slow gelatinization rate are used in canned foods where retort sterilization is applied; such starches provide low initial viscosity, high heat transfer, and rapid temperature increase, which are particularly suitable for quick sterilization.(Rutenberg *et al*., 1984; Evans *et al*., 1969; Rutenberg *et al*., 1975) Cross-linked starches have been applied in soups, gravies, sauces, baby foods, fruit filling, pudding, and deep fried foods. Drum dried cross-linked starches are used to provide a pulpy texture in food systems. Drum dried cross-linked starches containing low amylose content, such as waxy corn, have been used to improve cake volume and crumb softness (Rutenberg *et al*., 1984).

Koo *et al*. (2010) studied the effect of cross-linking on the physicochemical and physiological properties of corn starch. Corn starch was chemically modified by cross-linking with STMP/STPP (99:1. w/w) and the physicochemical and physiological properties (in vitro and in vivo) of the cross-linked corn starch were investigated as a function of the degree of cross-linking. Cross-linking decreased the solubility, swelling factor, and paste clarity of corn starch. While the swelling factor was highly correlated with the degree of crosslinking ($R^2 = 0.878$), the X-ray diffraction patterns did not show any significant alteration in the crystallinity of corn starch. It was shown by SEM measurement that a black zone was observed on the surface of crossed-linked starch granules, which did not occur with native starch. When mice were fed the diets containing the corn starch with low (CLCS-5) and high (CLCS-12) degree of cross-linking (51.3 and 99.1%,respectively), significant effects on the final body weight, weight gain as well as perirenal weight of the mice ($p < 0.05$) were observed. Also, significant decreases in total lipid, triglyceride, and total cholesterol concentrations in serum were detected in CLCS-5 and CLCS-12 groups (p

< 0.05).While total lipid level in the liver decreased with increasing degree of cross-linking, the triglyceride level was not affected by the supplementation with both of CLCS-5 and CLCS-12 corn starch samples.

Luo *et al.* (2009) using waxy potato starch as the raw material, the preparation, and determination of physical and chemical properties of acetylated distarch adipate (ADA) were studied systematically. The experimental variables investigated include temperature, pH, and reaction time, which all have influence on the degree of substitution (DS) of cross-linked waxy potato starch. Results show that the optimal reaction was obtained at a pH range of 9.0–9.5, a temperature of 35 ºC, and a reaction time of 2 h. The structures of ADA starch were characterized by FT-IR, and the results indicate a new absorption peak at 1732 cm^{-1} which is assigned to the C=O stretching vibration. It was found through polarized light microscopy and scanning electron microscopy (SEM) that the starch granule structure did not substantially change after crosslinking. The modified waxy potato starch paste exhibited excellent viscosity stability, acid resistant, salt tolerance properties and good breakdown. The swelling of ADA starch granules was gradual over a wide range of temperatures.

Mirmoghtadaie *et al.* (2009) studied the effect of cross-linking and acetylation on oat starch properties. Starch samples separated from oat were modified with two different levels of POCl3 (0.5 and 1.0 g kg^{-1}) as a cross-linking agent and two different levels of acetic anhydride (6% and 8% (w/w)) for acetylation. Swelling factor, thermal properties and retrogradation measurements were evaluated to characterize the influence of phosphorylation and acetylation on oat starch. Cross-linking decreased the swelling factor and did not improve gelatinization temperature while it increased synaeresis in comparison with native starch. Acetylation increased swelling factor but reduced gelatinization temperature and synaeresis of oat starch.

Ačkar *et al.* (2010) studied the isolation of starch from two wheat varieties and their modification with epichlorohydrin. They were determined the influence of wheat variety and modification with epichlorohydrin on starch properties. Starch was isolated from two wheat varieties: ‘‘Golubica” and ‘‘Srpanjka” and modified with different concentrations of epichlorohydrin (0.1%, 0.3% and 0.5% v/w). Both native and modified starches were characterised. Results showed similar characteristics of native wheat starches. Modification with epichlorohydrin increased temperature of gelatinisation, but gelatinisation and retrogradation enthalpy were affected by the extent of the chemical reaction between starch and epichlorohydrin, due to differences in reactivity between wheat starch varieties. Maximum viscosity decreased as well as breakdown and setback values. Swelling power and solubility were also decreased by modification, while paste clarity and freeze–thaw stability were influenced differently, due to different extent of the chemical reaction between different starch varieties and epichlorohydrin. Colour of starch was not significantly altered by modification. Digestibility of starches can be reduced by proper selection of epichlorohydrin concentration used for modification.

Chung *et al*. (2004) studied the glass transition and enthalpy relaxation of cross-linked corn starches. Thermal transition of cross-linked corn starches was characterized by using a differential scanning calorimeter (DSC) in the presence of excess (67%, based on total weight) or limited (15%) water. Normal corn starch (~24% amylose) was cross-linked by reacting with a mixture (99:1) of sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) in an aqueous alkaline slurry containing sodium sulfate. The amount of the cross-linking agent (STMP/STPP mixture) in the starch slurry (100 g starch, 140 ml water, and 10 g sodium sulfate) was varied (4–12 g), and the level of cross-linking was monitored by the level of incorporated phosphorus, swelling volume, and α-amylase resistance. When analyzed with excess water, the glass transition temperature (T_g' ; 25.6 and 25.7 ºC) of the cross-linked starches was higher than those of native and control starches (26.6 and 26.5 ºC, respectively), and the ice melting enthalpy was also increased by the cross-linking, indicating the increased amount of freezable water. However, in the DSC analysis with limited water, T_g and heat capacity increment ($\triangle C_p$) at T_g decreased by cross-linking. It indicates that the free volume for starch chains was increased by the bulky and ionic phosphate groups. The relaxation enthalpy increased, but the peak temperature of relaxation endotherm decreased as the cross-linking level increased. Retrogradation enthalpy after one week storage at 4ºC was decreased by cross-linking.

5.1.3 Starch Esterification

Starch ester is a group of modified starches in which some hydroxyl groups have been replaced by ester groups. The level of substituents of the hydroxyl groups along the starch chains is often expressed as average degree of substitution (DS). The average degree of substitution is the moles of substituent per mole of D-glucose repeat residue (anhydroglucose unit). The maximum possible DS is 3.0 when all three hydroxyls are substituted on each glucose unit along a starch chain. The DS can be calculated by the following equation:

$$DS = \frac{162\ W}{100\ M - (M - 1)\ W} \tag{4}$$

where W = % by weigh of substituent and M = formula weight of substituent.

The reagents approved by the FDA for preparation of organic and inorganic monoesters of starch intended for food use are acetic anhydride, vinyl acetate, succinic anhydride, 1-octenyl succinic anhydride, and sodium tripolyphosphate. In this section, three types of starch esters are introduced:

- Starch acetates prepared by reacting starch with acetic anhydride
- Starch succinate and starch alkenylsuccinate, which are produced by the reaction of starch with succinic anhydride and alkenyl substituted succinic anhydride, respectively
- Starch phosphate resulting from the reaction of starch with tripolyphosphate and/or trimetaphosphate

5.1.4 Starch Etherification

Unlike ester linkage such as starch acetate, which tends to deacetylate underalkaline condition, ether linkages are more stable even at high pH. Etherification gives starch excellent viscosity stability. Hydroxyalkyl starches, including hydroxyethyl and hydroxypropyl, are mainly produced for industrial applications. Hydroxyethyl starch has not yet been approved as a direct food additive, but can be used as an indirect food additive, such as a sizing agent for paper contacting food, whereas hydroxypropyl starch is primarily used for the food industry. Therefore, the focus of this section will be on hydroxypropyl starch which is made by reacting propylene oxide with starch under alkaline conditions. Hydroxypropyl starches are being widely used in food products where they provide viscosity stability and freeze-thaw stability. These starches are normally combined with cross-linking to provide the desired viscosity, texture, and stability for processing and storage. Hydroxypropyl starches are used as thickeners in fruit pie fillings, puddings, gravies, sauces, and salad dressings. Hydroxypropyl tapioca starch has been successfully used in frozen

6. Photo-Crosslinking Starch

Ultraviolet (UV) irradiation as a physical, cost effective, non-thermal, and environmental friendly technology has received increasing attention during recent years, during which it has been successfully applied for preservation and decontamination of food products (Bintsis *et al.,* 2000; Mertens and Knorr, 1992). UV irradiation has been used in medical and pharmaceutical research to crosslink collagen and gelatin films (Fujimori, 1965; Tomihata *et al.*, 1992; Weadock *et al.*, 1984 ; Bhat and Karim, 2009). Radioactive treatment, in solid or liquid states such as gamma irradiation (Fanta *et al.*, 1974; Kiatkamjornwong and Meechai, 1997; Reyes *et al.*, 1968; Delville *et al.*, 2002) or electron beam (Olivier *et al.*, 2000; Ruckert *et al.*, 1999) have been use to modify starch especially in grafting process. Radiation processing technology is being used to improve the properties of polymer product because of its tendency to undergo the chemical reaction between the polymer molecules under irradiation (Gehring, 2000). Reference (Takakura *et al.*, 1965) used photoadditives such as benzoic acid derivatives for poly (vinyl alcohol) crosslinking. The most efficient photoadditive, sodium benzoate, is known to be photolysed by UV irradiation (Ghosp and Gangopadhay, 2000; Wells, 1972) (Figure. 5). Radiation modification of starchbased plastic sheet was studied (Zhai *et al.*, 2003). The investigation showed that the properties of starch based were improved by radiation induced crosslinking. The sensitized irradiated films are rendered insoluble in the solvents in which they were originally soluble.

Photosensitization is an abnormally high reactivity of a biological substrate to artificial sources or natural sunlight providing, in principle, ineffective doses of UVA, UVB and Vis radiations. Photosensitization requires the presence in the biological medium of certain substances known as photosensitisers which induce the changes in the biological substrate after absorbing appropriate radiation (Beijersbergen van Henegouwen, 1981; Spikes, 1989; Miranda, 1992; Spielmann *et al.*, 1994). The photosensitisers structural requirements to induce photo-toxicity are related with the ability for absorbing those radiation wavelengths which present a better skin penetration (above 310 nm) favoring the subsequent photochemical decomposition to form stable photoproducts, free radicals and/or singlet oxygen (Condorelli *et al.*, 1996a). It is possible to find photosensitisers in the cellular content (e.g. flavins and porphyrins), in foods, cosmetics, some plants or their juices, industrial chemicals (dyes, coal tar, derivatives chlorinated hydrocarbons) and drugs. In addition to so broad distribution, the exogenous photosensitisers may enter into the body through different ways as well: ingestion, inhalation, injection or direct contact with the skin or mucous.

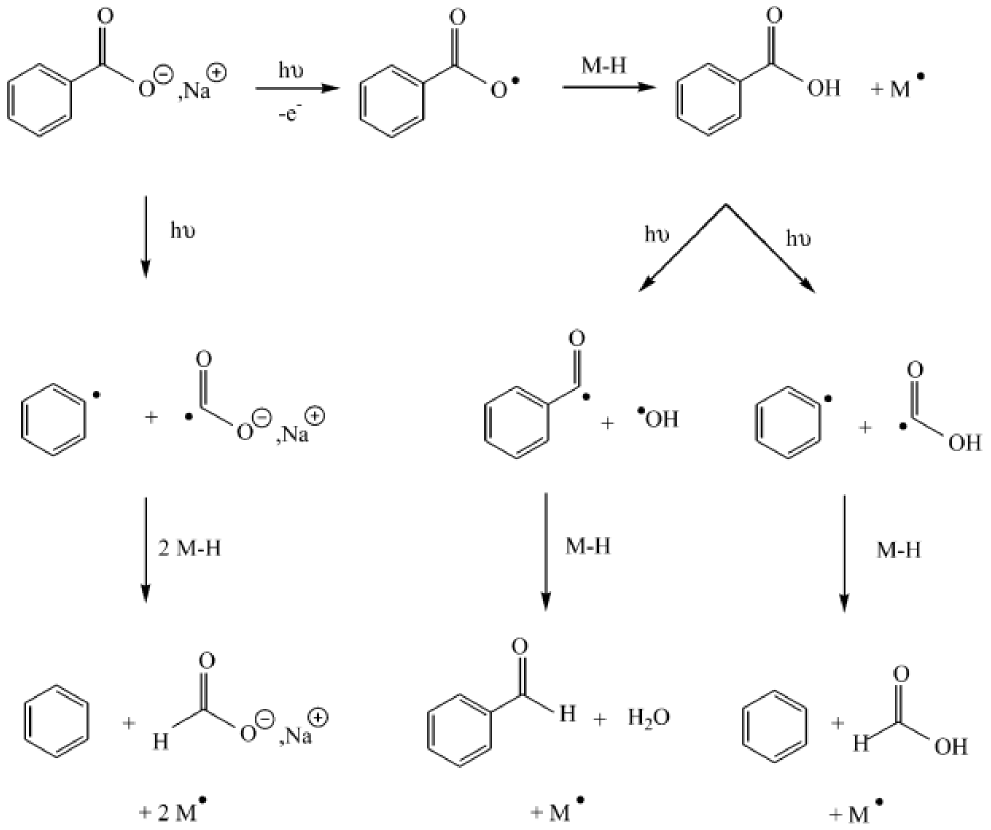


Figure 6. Mechanism involved in sodium benzoate of irradiated rice starch film
Source : Delville *et al.* (2002)

Delville *et al.* (2002) studied a new family of crosslinked starches were synthesized in the solid state by UV irradiation. The crosslinking treatment is novel because starch is treated in the solid state and no crosslinking agent is used. The original photosensitisers used were water soluble and member of the benzoic acid family. They were able to crosslinking starch even at concentrations as low as 0.1%. The crosslinking kinetics was established by the determination of both the swelling degree and the gel fraction in order to characterize the network created. Crosslinking densities can be varied by altering the UV exposure time (1-10 min). Although the final crosslinking density is relatively low, it is still sufficient to render starch nearly insoluble in DMSO. The reactivity is discussed (i) as a function of the plasticisers content (water, glycerol, sorbitol), which can modify both the macromolecular mobility and the material density and (ii) as a function of the presence of a destructuring agent (NaOH) which favours starch accessibilites to the reactants.

Mechanical properties were measured. Due to the relatively low crosslinking rate, only a slight increase is observed for crosslinked samples.

Khan *et al.* (2006) were prepared and characterized ultra violet (UV) radiation cured bio-degradable films of sago starch/PVA blend. Polymer films of sago starch/polyvinyl alcohol (PVA) were prepared by casting and cured under ultra violet (UV) radiation. Different blends were made varying the concentration of sago starch and PVA. Tensile strength (TS) and elongation at break (Eb) of the prepared films were studied. Films made up of sago starch and PVA with a ratio of 1:2 showed the highest TS and Eb. The physico-mechanical properties of prepared films were improved by grafting with acrylic monomers with the aid of UV radiation. A series of formulations was prepared with two monomers 2-ethyl 2-hydroxymethyl 1,3 methacrylate (EHMPTMA) and 2-ethylhexylacrylate (EHA) and a photoinitiator. Monomer concentration, soaking time and radiation dose were optimized in terms of grafting and mechanical properties. The highest TS was at 50% EHMPTMA and 48% EHA and 2% photo initiator at 5 min soaking time and recorded value was 6.58 MPa. The prepared films were further characterized with NMR spectroscopy and scanning electron microscope (SEM).

Lee *et al.* (2005) studied pasting, swelling and solubility properties of UV initiated starch-graft-poly(AA). UV-initiated graft copolymerization of sago starch with acrylic acid (AA) at low level (2.5%, 5%, 7.5% and 10% w/w) was investigated. The UV curing technique and procedure was found to successfully produce starch-graft-poly(acrylic acid) [S-g-poly-(AA)]. The carboxyl group content was found to increase with increasing % of monomer. Rapid visco-analyzer (RVA) pasting profile, swelling and solubility of the samples were studied. UV irradiation of sago starch (control) results in high pasting temperature, high peak viscosity and high setback, besides retarding swelling and solubility as compared to the native untreated starch (p < 0:05). Pasting temperature was decreased after grafting as compared to the control. S-g-poly(AA) showed higher peak viscosity, peak time and setback than native untreated starch (p < 0:05). Breakdown was retarded at 2.5% S-g-poly(AA) but increased at 10%. S-g-poly(AA) exhibited lower swelling power and solubility than the native sample (p < 0:05).

Follain *et al.* (2005) studied the influence of poly vinyl alcohol addition and photocrosslinking on starch based materials mechanical properties. Mechanical properties of starch/PVA blends (maximum 5% wt PVA), with or without crosslinking, have been analyzed in order to study how the increase in the linear to branched chain can improve the material's performances. As a result, elongation is enhanced without significant strength decrease for both casting and extrusion processes. Water sorption and photocrosslinking ability have also been measured showing a fairly good compatibility between the two macromolecular components: PVA and starch. For example, a decrease in water sorption on PVA addition was observed which could not simply be related to additivity between the two polymers. Finally, systems composed of pure glycerol plasticized starch blends were photocrosslinked at very low rates in order to 'lengthen' the macromolecules. The results of the mechanical tests on these materials showed an enhancement of the elongation at break. These results highlighted the fact that a modification of the molecular weight distribution (or linear long chains ratio, i.e. PVA) led to the development of entanglements or long distance interactions (strain at break increase).

Kumar and Singh (2008) prepared biocomposites of cellulose reinforced starch film improved properties by photo-induced crosslinking. In the present study, the composite films have been prepared from the aqueous dispersions of starch with microcrystalline cellulose using glycerol as plasticizer and irradiated under ultraviolet (UV) light using sodium benzoate as photo-sensitizer. Photocrosslinking was characterized by measuring the water absorption under 100% relative humidity, swelling degree and gel fraction in dimethylsulphoxide (DMSO), upon irradiation time. Both, the incorporation of cellulose and photo-irradiation were found to decrease the water absorption, swelling in DMSO and increase the gel fraction. Thermal transitions indicated the anti-plasticization of amylopectin chains at the fiber/matrix interface. With increasing content of cellulose and photo-irradiation time, the tensile modulus and strength were found to improve. It is summarized that the combination of cellulose reinforcement and photo-crosslinking of matrix has improved the physical and mechanical properties.

7 Factor Affecting Edible Films Properties

7.1 Type and Concentration of Plasticizers

A plasticizer is substance that is incorporated into a rigid plastic to increase its flexibility, workability, and distensibility. By reducing the grass transition temperature and increasing chain lubricity, plasticizers also improve processing and extrusion characteristics, reduce the minimum required processing temperature, reduce the plastic's hardness and improve low temperature flexibility. Generally, two types of plasticizers are distinguished. Internal plasticization is a result of modifications to the chemical structure of 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 tree-dimensional arrangement of the film polymer (Banker, 1996). It is the second method which, on the basis of the type of materials and the technology, is mainly used for edible packaging and coatings. A plasticizer may be defined as a compound, which when added to another material and under given conditions, modifies certain physical and mechanical properties of the material. The addition of a plasticizer to a film produces a film which is 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 film and reduces its glass transition temperature. This is manifested by a reduction in the barrier properties to gases, vapors, and film solutes (Banker, 1996; Kumins, 1965).

A plasticizing agent must be compatible with the film-forming polymer and be permeability 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 film system (polymer, solvent, plasticizer, other additives) has a direct effect on the nature and characteristics of the film 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 coating 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 film. The plasticizer should not be volatile (or not only very slightly volatile) and its degree of retention by the film should be high. Other properties, such as its chemical stability, hygroscopicity, color, flavor, and so on, are also more or less important depending on the type of film under consideration. In addition, the concentration of plasticizer necessarily varies from 10-60% (dry basis) according to the nature and type of film 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 (principally glycerol and its derivatives, polyethylene glycols, sorbitol)

-.lipids and its derivatives (fatty acids, monoglycerides and their esters, acetoglycerides, phospholipids, and other emulsifiers). Plasticizing of a hydrophilic polymer-based film will generally be achieved by the addition of a compound belonging to one of the first two groups and that of wax- or fat-based film by a compound from the third group.

The efficiency, stability, compatibility and permeability and permanence of a plasticizing agent can be evaluated by various semi-empire tests. The final method of plasticization consists of adding to the film system relatively inert solids (fillers which reduce the molecular reactions and cohesion of the final film). The size of these particles and their dispersion are of prime importance. Microcrystalline cellulose, various proteic isolates, and cocoa have been used as plasticizers, particularly, in fat-based films (Kester and Fennema, 1986).

7.2 Amylose and Amylopectin Content

Starch consists of two polysaccarides, the essentially linear amylose, and the branched amylopectin (Manners, 1989). Pure amylose films are stronger than amylopectin films (Rindlav-Westling *et al.,* 1998) and they have a higher elasticity (Lourdin *et al*., 1995). The ability of amylose to produce self-supporting films has been known for a long time (Wolff *et al*., 1951) and which is attributed to the ability of its linear chains to interact by hydrogen bonds to a higher extent than the branched amylopectin chains. Amylopectin films, on the other hand, are rather frail due to the higher degree of entanglement caused by the extensive branching and the short average chains length (Rindlav-Westling *et al*., 1998). Lourdin *et al.* (1995) has been found that linear amylose chains confer better mechanical properties compared to branched amylopectin molecules.

7.3 Type and Content of Lipids

A large number of lipidic compounds such as oils and animal and vegetable fats, acetoglycerides, natural waxes, or surfactants have been used to produce edible films or coatings. They are used basically on account of their excellent humidity barrier properties, either as a protective surface layer, or protective barrier between two parts of a heterogeneous foodstuff. They may also fulfill other functions, such as acting as supports for liposoluble additives, protecting the surface from abrasion or scorching of fruits during transport or storage the surface from abrasion or scorching of fruits during transport or storage (Hardenburg, 1967). Edible films are generally prepared using biological polymers such as proteins, polysaccharides, lipids and resins. Natural biopolymers have the advantage over synthetic polymers in that they are biodegradable and renewable, as well as edible. Each group of materials has certain advantages and disadvantages. Protein and polysaccharide films generally provide a good barrier against oxygen at low and intermediate relative humidity, and have good mechanical properties, but their barrier against water vapor is poor due to their hydrophilic nature (Guilbert, 1986; Kester and Fennema, 1986). In contrast, films prepared with lipid materials have good water vapor barrier properties, but are usually opaque and relatively inflexible. Lipid compounds commonly used for the preparation of lipid-based edible films and coatings include neutral lipids, fatty acids, waxes, and resins (Kester and Fennema, 1986; Hernandez, 1994). One way to achieve a better water vapor barrier is to produce a composite film by adding hydrophobic components such as lipid and wax materials. A composite hydrocolloid-lipid film or coating is particularly desirable, since it has acceptable structural integrity imparted by the hydrocolloid materials and good water vapor barrier properties contributed by the lipid materials (Greener and Fennema, 1989b). The efficiency of the lipid materials in composite films and coatings depends on the nature of the lipid used, in particular on its structure, chemical arrangement, hydrophobicity, and physical state (e.g. solid or liquid), and on the lipid interactions with the other components of the film, such as proteins and polysaccharides (Rhim and Shellhammer, 2005).

Objectives

1. To study the effect of cross-linking modification on rice starch film properties.
2. To study the effect of UV treatment on rice starch film properties.
3. To study the effect of relative humidity on rice starch film properties.
4. To compare the property of rice starch films prepared by chemical cross-linking and UV treatment.

CHAPTER 2

MATERIAL AND METHODS

1. Material

1.1 Raw material

Rice starch was extracted from grains of Chiang Phatthalung rice (*Oryza sativa L.*) by using alkaline methods, rice grain purchased from a local grocery.

1.2 Chemicals

Commercial grade sorbitol used as a plasticizer and Sodium benzoate used as photo-sensitizer were purchased from the Vidyasom Co. Ltd. (Thailand). Crosslinking reagent, Epichlorohydrin (EPI) was purchased from MERCK Schuchardt OHG (Hohenbrunn, Germany), Sodium trimetaphosphate (STMP) was obtained from SciencePedia Co., Ltd. (Bangkae, Bangkok) and Sodium tripolyphosphate (STPP) was purchased from SIGMA-ALDRICH Co. Ltd., (St. Louis, MO, USA), Analytical grade (AR) lithium chloride, magnesium nitrate, sodium chloride and potassium nitrate for saturated salt solutions (at 11% RH, 50% RH, 75% RH and 93% RH, respectively)

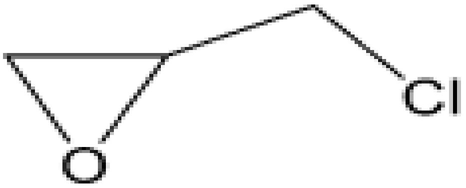


Figure 7. Chemical structure of epichlorohydrin (EPI)

Source : Crini (2005)

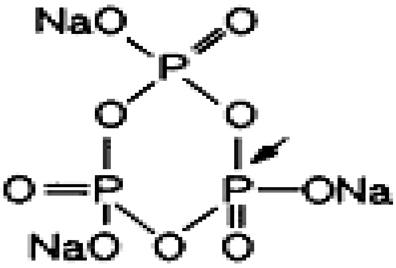


Figure 8. Chemical structure of sodium trimetaphosphate (STMP)

Source : Lim and Seib (1993)

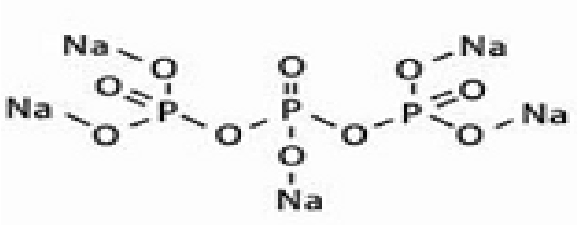


Figure 9. Chemical structure of sodium tripolyphosphate (STPP)

Source : Cho et al. (2010)

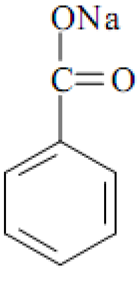


Figure 10. Chemical structure of sodium benzoate

Source : World Health Organization, Geneva (2000)

2. Instruments

Instruments	**Model**	**Company/City/Country**
Magnetic stirrer	RO 10 power	IKA LABORTECHNIK Stanfen, Germany
Stirrer	RW 20n	IKA LABORTECHNIK Stanfen, Germany
Blender	MX-T700GN	National, Japan
Thermogravimetric analyzer	Perkin-Elmer TGA-7	Norwalk, Conn., U.S.A.
Universal testing machine	LR 30K,	Lloyd Instruments Ltd,
Differential scanning calorimeter	Perkin-Elmer DSC-7	Hampshire, UK
Dynamic mechanical thermal analyzer	DMTA-V	USA Rheometric Scientific, US
X-ray diffractometer	X'Pert MPD	Coulter, U.S.
Hunter associates laboratory	Inc., VA	Philips, Netherland,USA
pH meter	pH/Ion 510	Eutech Instruments Pte Ltd., Singapore
Fourier transform infrared spectrometer	Bruker Model Equinox 55	Bruker Co., Ettlingen, Germany
Rapid visco analyser	RVA-Super4	Newport Scientific, Australia

3. Methods

3.1 Preparation of rice starch

Rice starch (Chiang Phatthalung) was prepared by soaked rice grains with water (1:2 w/v) for 6-12 hour then poured water and were wet-milled. The slurry was heated at 55°C and the sheet was milled and sieved through 106 mesh screen. Rice flour was then collected for making rich starch according to the method of Sawai and Morita (1968), rice flour granules were mixed with 5% sodium chloride solution (a ratio of rice starch/NaCl such as 1:4). The suspensions were then continuously stirred. After 2 hours the suspension was centrifuged and the slurry was mixed with 70% ethanol solution (a ratio of rice starch/ethanol such as 1:4). The suspension was then continuously stirred. After 2 hours the suspension were centrifuged and the slurry was mixed with 0.35% sodium hydroxide solution (a ratio of rice starch/NaOH such as 1:5). The suspensions were then continuously stirred for 12 hours. After 10 hours the suspension was washed by successive centrifugations in distilled water for 3 times and adjust pH = 7. The slurry was heated at 55 °C and the sheet was milled and sieved through 106-mesh screen to get a rice starch. The results were test as followed;

- Crystals content of starch granule by X-ray diffractometer
- Gelatinization temperature by Differential Scanning Calorimeter
- Chemical composition of starch;
    - Amylose content (Shanthy *et al.*, 1980)
    - Protein content by AOAC (1999)
    - Lipid content by AOAC (1999)
    - Moisture content by AOAC (1999)
    - Ash content by AOAC (1999)

3.2 Effect of cross-linking agents on the properties of cross-linked rice starch films

3.2.1 Preparation of cross-linked rice starch with epichlorohydrin (EPI)

Cross-linked rice starch with epichlorohydrin (EPI) was prepared using the methodology of Wurtzburg (1960) and Wattanachant *et al.*, 2003. 15 g of sodium chloride (15% based on dry wt. of starch) was added to 250 ml of water when the salt was dissolved, 100 g of rice starch (d.w.b.) (40% rice starch solid in slurry) were added and stirred until the mixture was uniform then a 5% sodium hydroxide solution was added to adjust the slurry to pH 10.5 with vigorous stirring to prevent starch gelatinization. The cross-linking agent 0.1%, 0.3% and 0.5% of EPI was added into the mixture and stirred at room temperature for 5 hours. After that, the 1 M of hydrochloric acid solution was added into slurry to terminated the reaction by adjust the slurry to pH 5.5. Starch slurry was wash with water, then starch was neutralized with 5% NaOH and centrifuged at 8000 rpm for 15 min, thereafter starch was dried at 50 ºC for 24 hour

3.2.2 Preparation of cross-linked rice starch with Sodium trimetaphosphate (STMP)

Cross-linked rice starch with sodium trimetaphosphate (STMP) was prepared according to the method of Woo and Seib (2002). 100 g of rice starch was added in 250 ml of distrilled water and then the cross-linked agents, STMP (1%, 2% and 3% w/w) were added into the slurry. The mixtures were adjusted to pH 10.5 by 5% NaOH. The slurries was stirred continuously, warmed up to 45 ºC and held at this temperature for 2 hour. After that, the 1 M of hydrochloric acid solution was added into slurry to terminated the reaction by adjust the slurry to pH 5.5. Starch slurry was wash with water, then starch was neutralized with 5% NaOH and centrifuged at 8000 rpm for 15 min, thereafter starch was dried at 50 ºC for 24 hour. The resulting cross-linked starches were formed to film by using solution casting method as described in following section.

3.2.3 Preparation of cross-linked rice starch with Sodium trimetaphosphate / Sodium tripolyphosphate (STMP/STPP)

Cross-linked rice starch with mixtures of sodium trimetaphosphate / Sodium tripolyphosphate (STMP/STPP: 99/1 %w/w) were prepared according to the method of Koo *et al.*, 2010. 100 g of rice starch was added in 250 ml of distrilled water and then the cross-linked agents STMP/STPP (4%, 8% and 12% w/w), were added into the slurry. The mixtures were adjusted to pH 10.5 by 5% NaOH. The slurries was stirred continuously, warmed up to 45 ºC and held at this temperature for

2 hour. After that, the 1 M of hydrochloric acid solution was added into slurry to terminated the reaction by adjust the slurry to pH 5.5. Starch slurry was wash with water, then starch was neutralized with 5% NaOH and centrifuged at 8000 rpm for 15 min, thereafter starch was dried at 50 ºC for 24 hour. The resulting cross-linked starches were formed to film by using solution casting method as described in following section.

3.2.4 Preparations of cross-linked rice starch films

Cross-linked rice starch films were prepared by using solution casting. 3% starch solution (w/v) were prepared by adding cross-linked rice starch in distrilled water with vigorous stirring and heating the slurry to 85ºC (gelatinize temperature) and held at this temperature for 10 min. Then the slurry were cooled to 50±5ºC, sorbital (plasticizer) was added as 50% of dry wt. starch stirred for 2 min. The mixtures were cast onto flat, leveled, non-stick trays to set. After that the tray were held overnight at 55ºC for 10-12 h. The resulting films were kept in descicators at 55% RH for further testing. All treatments were made in triplicate.

3.3 Effect of photo-cross linking on the properties of rice starch films

3.3.1 Effect of sodium benzoate (photo-sensitizer) contents on the properties of rice starch films

3% starch solution (w/v) were prepared by adding native rice starch in distrilled water with vigorous stirring and heating the slurry to 85ºC (gelatinize temperature) and held at this temperature for 10 min. Then the slurry were cooled to 50±5ºC, sorbital (plasticizer) was added as 50% of dry wt. starch stirred for 2 min. and then sodium benzoate (3%, 6% and 9% w/w of dry wt.) were added as photo-initiator. Starch was stirred for 2 min. The mixtures were cast onto flat, leveled, non-stick trays to set. After that the tray were held overnight at 55ºC for 10-12 h. The resulting films were kept in descicators at 55% RH for 72 h before irradiation as described in following section. All treatments were made in triplicate.

3.3.2 Effect of irradiation time on the properties of rice starch films

3% starch solution (w/v) were prepared by adding native rice starch in distrilled water with vigorous stirring and heating the slurry to 85ºC (gelatinize temperature) and held at this temperature for 10 min. Then the slurry were cooled to 50±5ºC, sorbital (plasticizer) was added as 50% of dry wt. starch stirred for 2 min. and then sodium benzoate (the optimum content selected from previous study) was added as photo-initiator. Starch stirred for 2 min. The mixtures were cast onto flat, leveled, non-stick trays to set. After that the tray were held overnight at 55ºC for 10-12 h. The resulting films were kept in descicators at 55% RH for 72 h before irradiation as described in following section. All treatments were made in triplicate.

3.3.3 Effect of relative humidity of rice starch film (before irradiation) on the properties of rice starch films

3% starch solution (w/v) were prepared by adding native rice starch in distrilled water with vigorous stirring and heating the slurry to 85ºC (gelatinize temperature) and held at this temperature for 10 min. Then the slurry were cooled to 50±5ºC, sorbital (plastisiser) was added as 50% of dry wt. starch stirred for 2 min. and then sodium benzoate (the optimum content selected from previous study) was added as photo-initiator. Starch stirred for 2 min. The mixtures were cast onto flat, leveled, non-stick trays to set. After that the tray were held overnight at 55ºC for 10-12 h. Storing a film at 0, 55 and 98% RH for 72 hrs before UV treatment for 72 h before irradiation as described in following section. All treatments were made in triplicate.

3.3.4 Ultraviolet irradiation set up

Starch films, which were kept at 55% RH, were irradiated under mercury lamp (400W) supplying radiation longer wavelength than 290 nm for 10, 20 and 30 min. After irradiation, UV treated films were kept at 55% RH in a desiccators for 72 h for further testing.

3.4 Effect of relative humidity on the properties of crosslinked rice starch and photo-crosslinked rice starch films

Effects of relative humidity on the properties of crosslinked rice starch and photo-crosslinked rice starch films (selected films) were determined by placing rice starch films into a controlled humidity environment at a constant temperature until equilibrium. After drying at 75 °C and < 1 mmHg pressure for 24 h (EYELA ^{TM}, Model VOS-300VD, Japan), the films were placed into environments of various relative humidities above salt solutions and constant temperature at 27±2 °C in desiccators. The relative humidities were 11% RH (lithium chloride), 50% RH (magnesium nitrate), 75% RH (sodium chloride) and 90% RH (potassium nitrate). The sorption experiments were carried out by keeping approximately 1,000 mg of films (2 x 2 cm for weighing and 1.5 x 12 cm for Tensile strength) in desiccators, removing at frequent intervals and weighing until they reach constant weight (within ± 5%), after that, the films were tested by tensile strength.

4. Determination of Rice Starch Films Properties

4.1 Conditioning

All films were conditioned prior to subjecting them to permeability and mechanical tests according to a standard method, D 618-61 (ASTM, 1993a). The films were tested for water vapor permeability (WVP), tensile strength (TS), and elongation (E) were conditioned at 60% RH and 27±2°C by placing them in desiccators over a saturated solution of $Mg(NO_3)_2.6H_2O$ for 72 h or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

4.2 Water Vapor Permeability (WVP)

The gravimetric modified cup method based on ASTM E96-92 (McHung *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 was placed in between the cup and the ring cover of each cup coated with silicone sealant (high vacuum grease, Lithelin, Hanau, Germany) and held with four screws around the cup's circumference. The air gap was at approximately 1.5 cm between the film surface and desiccant. The rated water vapor transmission (WVTR) of each film was measured at 60±2% RH and 25±2°C. After taking the initial weight of the test cup, it was placed into a growth chamber with an air velocity rate of 135 m/min (Model KBF115, Contherm Scient, Lower Hutt, New Zealand). Weight gain measurements were taken by weighing the test cup to the nearest 0.0001 g with an electronic scale (Satorious 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). The 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. Six 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.

4.3 Mechanical Properties

Tensile strength (TS) was measured with a LLOYD Instrument (Model LR30K, LLOYD Instruments Ltd., Hampshire, England) as per ASTM D882-91 Standard method (ASTM, 1993b). Ten samples, 1.5 cm x 12 cm, were cut from each film. The initial grip separation and crosshead speed were set at 70 mm and 30 mm/min, respectively. Tensile strength was calculated by dividing the maximum force by the initial specimen cross-sectional area, and the present elongation at break (E) was calculated as follows:

$$E = 100 \times (d_{\mathrm{after}} - d_{\mathrm{before}}) / d_{\mathrm{before}} \tag{5}$$

Where, *d* was the distance between grips holding the specimen before and after the break of the specimen.

4.4 X-ray Diffraction

X-ray patterns of starch powders, starch nanocrystals, starch film and starch film reinforced with starch nanocrystals were analyzed. This was done by using an X-ray diffractometer (Philips X, Pert MPD, Japan) with Cu Kα radiation at a voltage of 40 kV and 30 mA. The samples were scanned between 2θ = 3-40° with a scanning speed of 2°/min. Prior to testing the samples were dried and stored in desecrator. The relative crystallinity index was calculated using equation 1 (Koksel *et al.*, 1993; Muller *et al.,* 2009).

$$\%X_c = \frac{A_c}{A_t} = \frac{A_c}{A_c + A_a} \qquad (6)$$

Where A_c is the crystalline area, A_a is the non-crystalline area and A_t is total area.

4.5 Differential Scanning Calorimeter (DSC)

The thermal properties of rice starch films were analyzed with a Perkin-Elmer DSC-7 (Norwalk, Conn., U.S.A.) equipped with an intra-coolant Thermal Analysis Controller TAC7/DX (Perkin-Elmer). Samples (approx. 20 mg each, db) were weighed into stainless steel pans (Perkin-Elmer) designed to withstand high pressures. The stainless steel pan was sealed with an O-ring, and allowed to reach equilibrium of the moisture overnight. An empty DSC pan was used as a reference pan. The heating rate was programmed by holding the heat at -50°C for 1 min, followed by ramping the temperature rang 0f -50 °C to 180 °C at a rate of 20 °C/min, and holding it at 180 °C for 1 min. The measurements were made at least in duplicate for each treatment.

4.6 Dynamic Mechanical Thermal Analysis (DMTA)

The small deformation analysis of the films was performed in tension in a dynamic mechanical thermal analyzer (Rheometric Scientific, DMTA V). The tested film strips were cut into small strips (20 x 5 mm) and clamped in the instrument with an initial grip separation 5.5 mm. The films were subjected to a sinusoidal strain on top of static deformation. The testing was conducted at a constant frequency of 1 Hz and a strain of 0.02% over a temperature range of 50 to 200 °C, at a heating rate of 5 °C/min. The measurements at each experimental point were done at least in triplicates. Dynamic mechanical spectroscopy was employed within the linear viscoelastic regime to determine T_g. The storage and loss modulus (E' and E'') and loss tangent (tan δ = ΔE'/E'') were measured as a function of temperature at a constant frequency and a selected heating or cooling rate.

4.7 Water Uptake

The kinetics of water absorption was determined for rice starch films addition lipid. The dimensions of specimens were 2 x 2 cm. The films were therefore supposed to be thin enough so that the molecular diffusion was considered to be one-dimensional. Samples were first dried overnight at 100° C. After weighing, they were condition at room temperature in desiccators containing sodium sulfate to ensure a RH ratio of 98%. The conditioning of samples in high moisture atmosphere were preferred to the classical technique of immersion in water because starch is very sensitive to liquid water and can partially dissolve after long time exposure to water. The sample were removed at specific intervals and weighed by using a four-digit balance. The water content or water uptake of the samples was calculated as follows:

$$\text{\% Water uptake} = \frac{M_t - M_0}{M_0} \times 100 \qquad (7)$$

Where M_t is mass of sample at time t; M_0 the mass of sample at t=0.

4.8 Color and transparency

Color of film was determined using a CIE colorimeter (Hunter associates laboratory, Inc., Reston, Virginia, USA), working with D65 (day light). The color parameters were expressed as L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) values. The transparency value of film was calculated by the following equation (Han and Floros, 1997):

$$\text{Transparency value} = -\log T600/x \qquad (8)$$

where T600 is the fractional transmittance at 600 nm and x is the film thickness (mm). The greater value represents the lower transparence of the film.

4.9 Swelling degree and gel fraction measurement

Photo-crosslinked rice starch films was characterized by swelling behavior in dimethylsulphoxide (DMSO) in which neat starch is completely soluble. The method was described by Delville *et al.*, 2002. Starch films were irradiated and condition at 55% RH for 72 h before immersion in DMSO at 25ºC for 24 h (sorption equilibrium). The swollen film was weighted (m_s) and washed with water and ethanol respectively in order to remove the DMSO. Thereafter swollen film was dried at 80ºC for 24 h and reconditioned at 55% RH before weighing (m_d). The swelling Degree (SD) was calculated as in (9).

$$\text{Swelling Degree (SD)} = \frac{m_s - m_d}{m_d} \approx \frac{m_s}{m_d}\ ;\ m_d \ll m_s \qquad (9)$$

The gel fraction (GF) was calculated as in (10).

$$\text{Gel fraction (GF)} = 100\frac{m_d}{m_c} \qquad (10)$$

Where m_c is a corrected weight (i.e. only starch weight content)

4.10 Degree of cross-linking

The degree of cross-linking of modified starches was determined from the viscosity values, according to the procedure of Kaur and Singh (2006) and Koo et al. (2010). Pasting properties of rice starch were measured by using Rapid Visco Analyzer (RVA) by modified AACC method (AACC, 2000). Sample 2.5 g (10 % w/w dry basis) were suspended in 25 ml distilled water and heated from 50 to 95°C, held at 95°C for 3 min, then cooled from 95°C to 50°C and held at 50°C for 6 min. Pasting parameters were automatically computed and reported. The degree of cross-linking was calculated by using the equation below:

$$\text{Degree of cross-linking} = \frac{A-B}{A} \times 100 \qquad (11)$$

Where A is the peak viscosity of the control sample (without crosslink), and B is the peak viscosity of the cross-linked rice starch.

5. Experimental Design and Statistical Analysis

A completely randomized experimental design was used to determine the character of composite films. Analysis of variance (ANOVA) was used to compare mean differences of the samples. If the differences in mean existed, multiple comparisons were performed using Duncan's Multiple Range Test (DMRT).

CHAPTER 3

RESULTS AND DISCUSSION

1. Characteristic of Rice Starch

1.1 Compositional of Rice Starch

The proximate compositions of rice starch were shown in Table 3. The amylose, moisture, protein, fat and ash content were 30.40± 0.01%, 11.23 ± 0.05%, 0.56 ± 0.02%, 0.51 ± 0.01% and 0.21 ± 0.07%, respectively. While the peak viscosity and pH value were 338.60 RVU and 6.5-7.0, and rice starch color was white, respectively.

Table 3. The proximate compositions of rice starch.

Compositions	Amount (%)
Amylose	30.40 ± 0.01
Moisture	11.23 ± 0.05
Protein	0.56 ± 0.02
Fat	0.51 ± 0.01
Ash	0.21 ± 0.07
pH	6.5-7.0
Peak viscosity, RVU	338.60 ± 0.42
Color	White

1.2 Rice Starch Crystallinity

The X-ray diffractograms of rice starch powder was depicted in Figure 11. Actually, rice starch has the A-type XRD pattern (Bao and Bergman, 2004) with strong reflection at 15.29°, 17.29° and 22.73° because It is seen that high amylopectin starch (Liu *et al*., 2009). Gallant *et al*. (1997) described that the crystalline structures of native granular starches give x-ray patterns that fit the three main types A, B, and C and amylose gives characteristic V-patterns but the V-pattern is not routinely found in common starches, it has been detected together with A, B and C structures in starches. The % crystallinity values of rice starch were estimated as 23.19%.

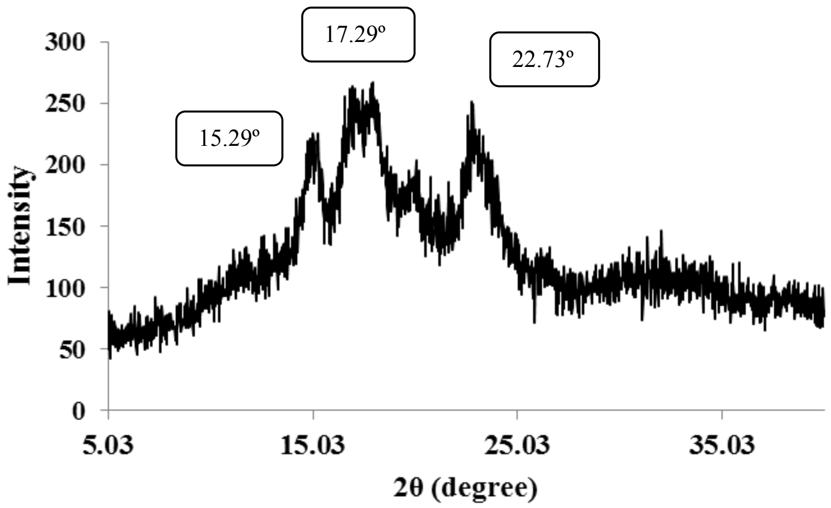


Figure 11. XRD diffractograms of rice starch.

2. Effect of crosslinking agents on the properties of crosslinked rice starch powder.

2.1 Viscosity and pasting temperature of crosslinked rice starch.

The viscosity and pasting temperature of untreated rice starch (control) and crosslinked rice starch with EPI, STMP and STMP/STPP were demonstrated in Table 4 and Figure 12 (A-C). The peak viscosity of untreated rice starch (338.60 RVU) showed higher peak viscosity than modified rice starch. The reduction in peak viscosity would be resulted from the presence of the crosslinking agent which may reduce interactions of starch molecules with water molecules resulting in lower peak viscosity values. However, the results demonstrated that the initial pasting temperature of modified rice starch presented higher than untreated rice starch. These results could be explained by the crosslinking of molecular chains makes the starch granules more ordered and consequently more energy would be required for swelling. Hirsch and Kokini (2002) reported that the higher levels of crosslinking generally lead to reduced granule swelling solubility, paste clarity and paste peak viscosity (Kaur *et al.*, 2006; Wongsagonsup *et al.*, 2005). According to the effect of type and content of crosslinking agents on viscosity and pasting temperature, found that rice starch modified with 8%STMP/STPP yielded the lowest viscosity than 0.5%EPI and 3%STMP due to a higher density of crosslinks was occurred. Moreover the viscosity peaks of rice starch crosslinked with EPI (Figure 12(A)) and STMP (Figure 12(B)) were slightly decreased while the viscosity peaks of STMP/STPP (Figure 12(C)) were dramatically decreased when the concentration of crosslinking agents increased. Application of STMP/STPP provided a greater reduction in viscosity. It due to the high concentration of crosslinking agent could provide the high crosslinking density. Addition, the results showed that the crosslinked rice starch had higher pasting temperature than untreated rice starch. These results are in agreement with the study of Xiao *et al*. (2011) reported that pasting properties of untreated potato starch and crosslinked starch with epichlorohydrin (EPI) found that crosslinking treatment decreased peak, setback and final viscosities of potato starch. Moreover, Lim and Seib (1993) investigated the preparation of starch phosphates and showed that a mixture of phosphate salts (sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP)) gave better results than using STMP alone to prepare distarch phosphate (crosslinked starch). Since the formation of crosslink between starches molecules strengthen the swollen granules against breakage under conditions of high temperature and shear. In addition, after starch granule rupture, the covalent crosslinks may provide sufficient granule integrity to keep the swollen granules intact and minimize or prevent loss in viscosity (Jyothi *et al*., 2006; Lim and Seib, 1993). The amylose does not leach out from the granule because the phosphate groups have strengthened the granule and prevented it from swelling (Muhammad *et al*., 2000).

Table 4. Peak viscosity and pasting temperature of crosslinked rice starch powder.

		Viscosity		
Crosslinking agent	Peak Viscosity (RVU)	Viscosity@ 95ºC (RVU)	Viscosity@ 50ºC (RVU)	Pasting temperature (ºC)
Rice starch	338.60±0.42^{a}	316.80±3.25^{a}	346.50±4.24^{a}	84.03±0.03^{f}
0.1%EPI	188.80±1.41^{b}	176.55±0.77^{b}	182.90±2.12^{b}	92.30±0.42^{e}
0.3%EPI	158.10±1.97^{c}	149.10±1.83^{c}	155.55±2.82^{c}	93.10±0.60^{bc}
0.5%EPI	156.50±1.41^{cd}	148.05±0.63^{d}	150.70±2.82^{d}	93.83±0.71^{b}
1%STMP	156.10±0.28^{d}	148.60±042^{d}	151.25±2.12^{d}	94.30±0.03^{b}
2%STMP	154.55±1.06^{e}	146.00±1.83^{e}	148.80±4.14^{e}	94.38±0.00^{b}
3%STMP	153.15±1.06^{e}	142.65±0.63^{g}	145.60±7.07^{f}	94.70±0.64^{b}
4%STMP/STPP	153.65±0.18^{e}	144.05±0.64^{f}	146.55±7.78^{f}	92.75±0.07^{d}
8%STMP/STPP	125.15±1.40^{g}	118.35±1.27^{i}	120.55±9.09^{h}	95.08±0.03^{a}
12%STMP/STPP	146.80±0.67^{f}	135.40±0.78^{h}	138.65±6.06^{g}	94.30±0.00^{b}

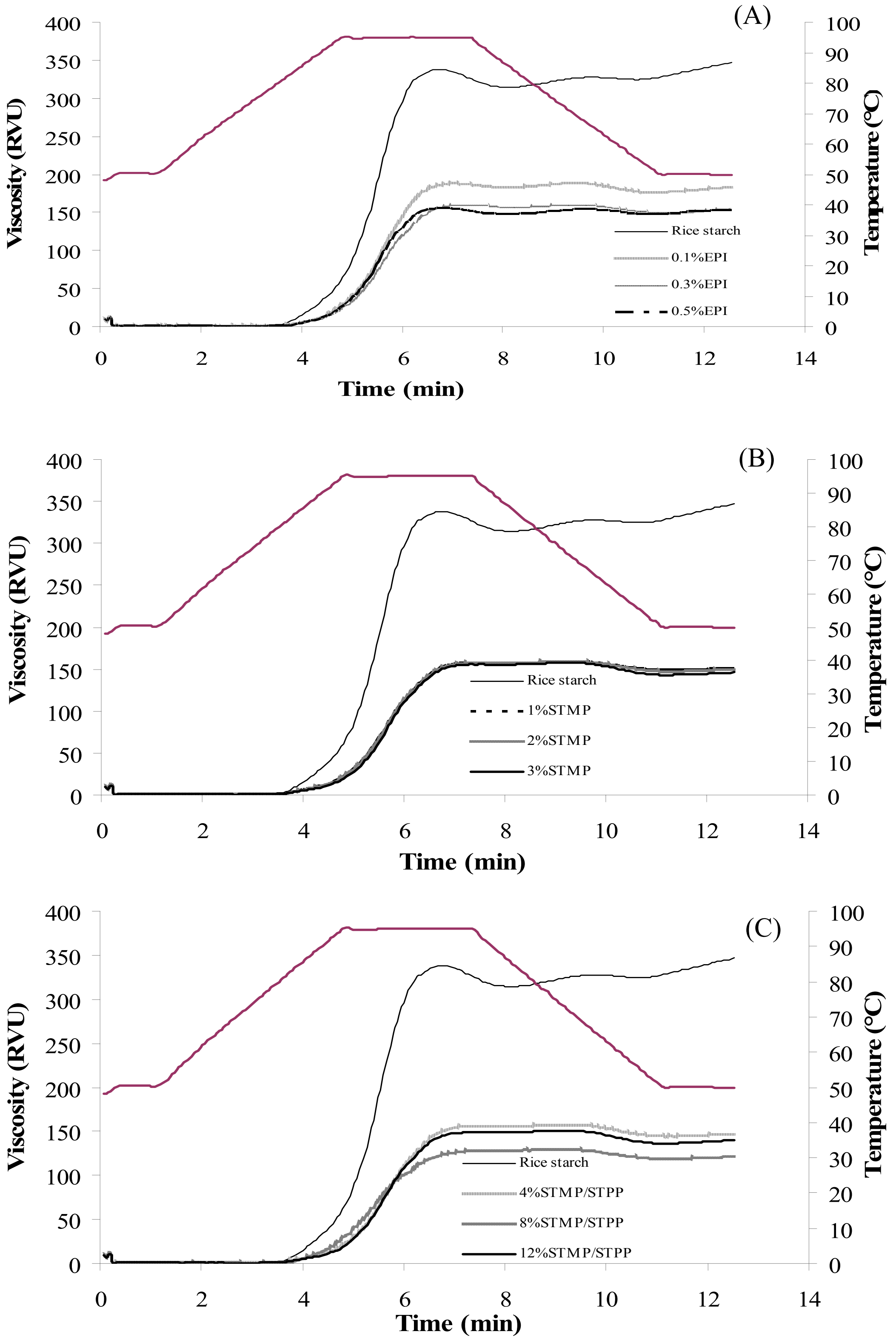


Figure 12.Viscosity profile of untreated rice starch comparison with EPI (A), STMP (B) and STMP/STPP (C) crosslinked rice starch.

2.2 Degree of crosslinking of crosslinked rice starch.

Degree of crosslinking of crosslinked rice starch showed in Table 5. Degree of crosslinking was calculated from maximum viscosity values, as described by Kaur *et al.* (2006); Koo *et al.* (2010) shown in Eq. (11). Then, the degree of cross linking is therefore directly proportional to the number of covalent bonds of formation of cross inking which are produced during reaction with cross linking agent. The higher levels of crosslinking generally lead to reduced granule swelling (Hirsch and Kokini, 2002) solubility, paste clarity and paste peak viscosity (Kaur *et al.*, 2006; Wongsagonsup *et al.*, 2005). According to the results found that the degree of crosslinking increased with increase of crosslinking agents (EPI, STMP and STMP/STPP) concentration. The results demonstrated that the highest degree of crosslinking was found when 0.5% EPI, 3% STMP and 8% STMP/STPP were used. However, using of 12% STMP/STPP yielded the decreasing degree of crosslinking from 63.04±0.42 (8% STMP/STPP) to 56.65±1.12 (12% STMP/STPP) due to it was an excessive amount as described above. Hirsch and Kokini (2002) also observed that the degree crosslinking agent of waxy maize starch increased when the concentration of phosphorous oxychloride (POCl3), sodium trimetaphosphate (STMP) and epichlorohydrin (EPI) increase. Moreover, Bajpai and Bhanu (2007) studied the impact of varying crosslink density on the percent dissolution of starch microspheres that crosslinked with EPI and the result indicated that the extent of dissolution gradually decreased with increased concentrations of crosslinking agent. It may be explained by the fact that when increasing the concentration of crosslinker the crosslink density of starch microspheres also increases which keeps the microspheres compact and resistant to dissolution. Ayoub and Rizvi (2007) reported that an increase in the EPI concentration from 0 to 0.5% was accompanied by an increase in the degree of crosslinking of extruded wheat starch due to the concentration of EPI was increased, the relative self-diffusion coefficient (D) decreased rapidly, reaching a negligible value at EPI concentration higher than 0.5% in untreated starch, confirmed good crosslinking.

Table 5. Degree of crosslinking of crosslinked rice starch.

Crosslinking agent	Degree of crosslinking (%)
rice starch	0.00
0.1%EPI	44.24±1.14^{i}
0.3%EPI	53.31±1.97^{h}
0.5%EPI	53.78±1.44^{g}
1%STMP	53.90±0.28^{f}
2%STMP	54.36±1.06^{e}
3%STMP	54.77±1.06^{c}
4%STMP/STPP	54.62±1.41^{d}
8%STMP/STPP	63.04±0.42^{a}
12%STMP/STPP	56.65±1.12^{b}

3. Effect of crosslinking agents on the properties of crosslinked rice starch film

The thickness of resulted rice starch films were measured with a precision digital micrometer and the average thickness of rice starch films was approximate 0.13±0.01 mm. Mean thickness values of sample were calculated and used to determine in tensile strength (TS), water vapor permeability (WVP) and transparency value of rice starch films. In XRD, DSC, FT-IR and DMTA analysis; the crosslinked rice starch films were selected 0.5%EPI, 3% STMP and 8% STMP/STPP crosslinked rice starch films that provided a high crosslinking density, good mechanical and barrier properties.

3.1 Water vapor permeability (WVP)

Starch is the biopolymer that sensitive to moisture, which affects the mechanical properties of thermoplastic starch materials, any improvement in decreasing moisture sensitivity and enhancing water resistance of thermoplastic starch material is seriously important (Zhou *et al*., 2008). Water vapor permeability (WVP) results can be useful to understand possible mass transfer mechanism and solute and polymer interactions in biodegradable films. According to the thermodynamic of irreversible process, water chemical potential difference is the driving force of water transfer though a films. When the process occurs at constant temperature and pressures the water chemical potential difference results proportional to water vapor concentration difference between the two faces (Morillon *et al*., 2000; Bertuzzi *et al*., 2007). WVP values of untreated rice starch and crosslinked rice starch films are shown in Figure 12. The crosslinked rice starch films showed superior water barrier properties than untreated rice starch films. The WVP of untreated rice starch film was 6.19 g.mm/m^2.day.KPa, whereas rice starch films crosslinked with 0.1%, 0.3% and 0.5% EPI were 3.02, 2.44 and 1.89 g.mm/m^2.day.KPa, respectively, rice starch films crosslinked with 1%, 2% and 3% STMP were 4.08, 2.19 and 2.28 g.mm/m^2.day.KPa, respectively and rice starch films crosslinked with 4%, 8% and 12% STMP/STPP were 2.72, 2.48 and 3.84 g.mm/m^2.day.KPa, respectively. This results could be explained the decreasing of WVP value by a hydrophilic group (OH$^-$) of rice starch decreased when crosslinking reaction occurred between rice starch and crosslinking agent molecules and crosslinking agent limits water absorption by restricting the mobility of starch chains in the amorphous region (Gunaratne and Corke, 2007). Addition, Muñoz *et al*., (2003) reported the WVP of glutenin-rich film values decreased by around 30% when formaldehyde (FA), glutaraldehyde (GTA) or glyoxal (GLY), used as crosslinking agents. Theoretically, maximum WVP can only be as high as would be achieved by the untreated rice starch.

Figure 13 depicted that WVP of rice starch crosslinked with EPI (Figure 13(A)) and STMP (Figure 13(B)) films were slightly decreased when the concentration of EPI were increased; because EPI and STMP were slow acting crosslinking agent and it's gradually penetrated into starch granule and slowly induced the crosslinking reaction within starch granule. The results demonstrated that the WVP of rice starch film that prepared from STMP/STPP (Figure 13(C)) showed dramatically decreased when the concentration of STMP/STPP increased from 4% and 8% STMP/STPP. However, when STMP/STPP at 12% was applied, the WVP value of resulted films showed increasing due to at 12% STMP/STPP was an excess concentration of STMP/STPP. According to this results could be described by to high content of cross linking agent provided an increasing the bulky molecules and free volume in the films resulting to increase the hydrophilic of starch film. Chung *et al*. (2004), reported that the crosslinked corn starches that crosslinked with STMP/STPP had an excess concentrate measured at 10% concentration of the mixture of STMP/STPP.

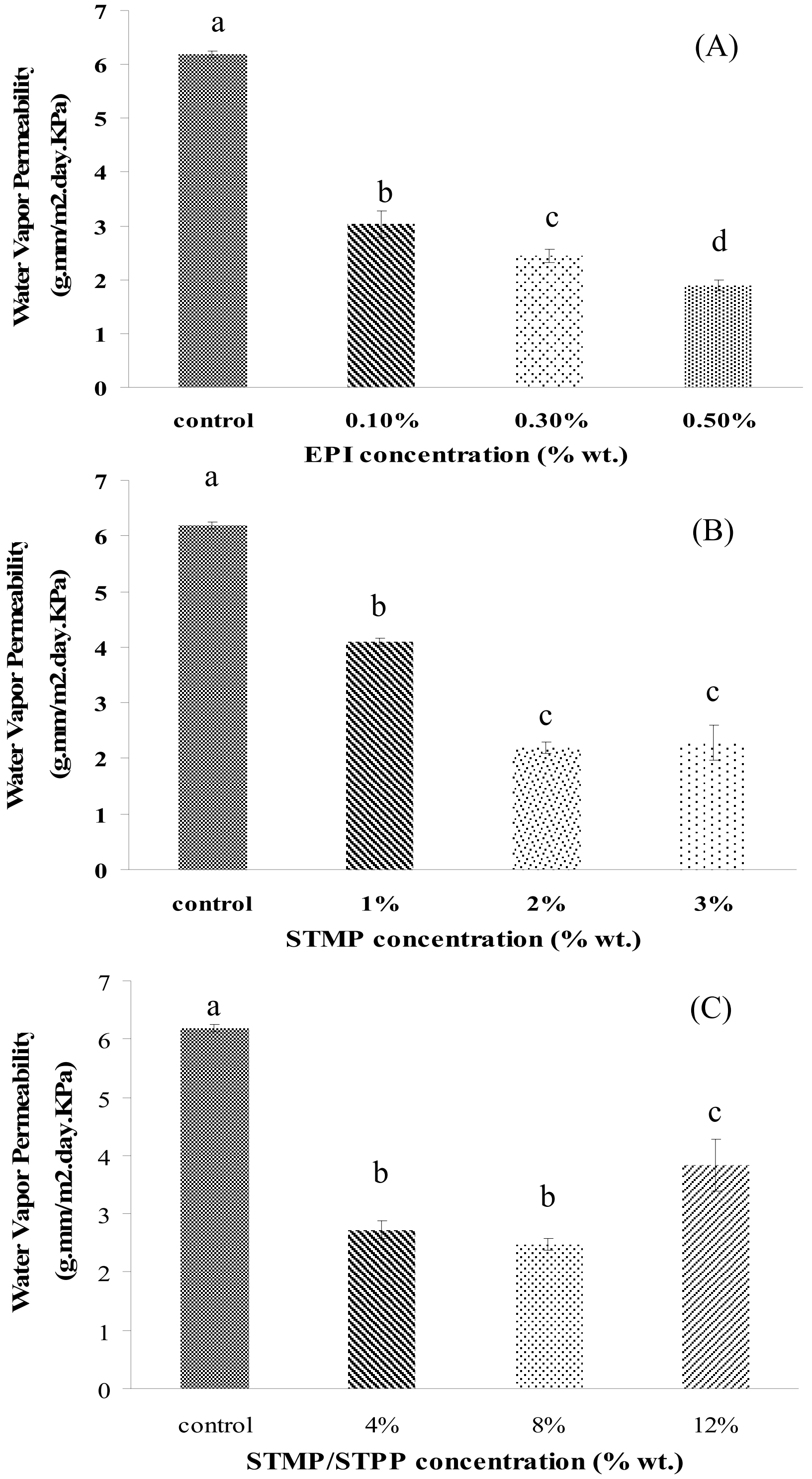


Figure 13. Effect of crosslinking agent on Water vapor permeability (WVP) of untreated rice starch comparison with EPI (A), STMP (B) and STMP/STPP (C) crosslinked rice starch films. Mean values with different letter are significantly different ($p<0.05$).

3.2 Tensile strength (TS) and Elongation at break (E)

Tensile strength is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile at yield or at break, respectively (ASTM, D882-91). The effect crosslinking agents' concentration on tensile strength (TS) and elongation at break (E) of rice starch films were presented in Figure 14 (A-C) and 15(A-C).The results showed that concentration of crosslinking agents affected the TS of rice starch films. The TS of crosslinked rice starch films showed higher than untreated rice starch film. The TS of untreated rice starch film was 5.01 MPa, whereas rice starch films crosslinked with 0.1%, 0.3% and 0.5% EPI were, 4.70, 5.42, 7.99 MPa, respectively. The TS of rice starch films crosslinked with 1%, 2% and 3% STMP were 6.30, 7.23, 8.23 MPa, respectively and the TS of rice starch film crosslinked with 4%, 8% and 12% STMP/STPP were 5.84, 7.57 and 4.36 MPa, respectively. The increase in TS is due to the increase of crosslinking density in starch films that causing by the crosslinking reaction that reacted between hydroxyl groups and crosslinking agent resulting in crosslinked starches. Similar results were reported by Kim and Lee (2002) reported that the TS of the films prepared with crosslinked corn starch were higher than that prepared with untreated corn starch. Moreover, Khan *et al*., (2006) reported that crosslinked sago starch/PVA blend films had TS higher than untreated sago starch/PVA blend films

Results pointed out that, rice starch films modified with 0.1% EPI provided relatively low improvement in TS. Besides, using EPI above 0.1% showed significantly increase in TS of the films. Addition, the TS was also slightly increased when the concentration of STMP/STPP agents were increased. However, the TS of the films showed inversely trend when 12% STMP/STPP was applied. It could be explained by the bulky molecules of STMP/STPP was coagulate and limited the crosslinking reaction in starch granules and the free volume was increased resulting in decreased the TS value of film.

According to the Figure 14(B) and 14(C), can be revealed that STMP and the mixture of STMP/STPP seem to fasting acting result in increased TS values and EPI (Figure 14(A)) was the lowest action. The optimum concentration of crosslinking agent is an important factor for improved the mechanical properties of crosslinked starch film. At low concentrations of crosslinking agent, there is not enough crosslinking between the starch molecules to improve the TS of the films. At high concentrations, there is excess crosslinking that limits the mobility of the starch molecules, leading to lower TS (Yang *et al*., 1996, 1997).

Elongation at break (E) is an indication of the films flexibility and stretchability (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. Figure 15(A-C) showed the effect of type and concentration of crosslinking agents on E of rice starch films. Found that E of untreated rice starch film showed higher than modified rice starch films. Results demonstrated that when the concentration of crosslinking agents increase resulted in decreasing of E from 116% to about 58% and E were dramatically decreased when using 0.5%EPI, 3%STMP and 8%STMP/STPP and the lowest E was occurred when using 0.5%EPI. By the reason of the crosslinking reaction limited chain movement and strengthen starch chain. The crosslinked starch film has more rigidity than uncrosslinking film. Besides, Yin *et al*. (2005) also reported that starch–polyethylene films crosslinked with boric acid (0.5–2.5%) had TS ranging from 8.9 to 10.1 MPa and the breaking elongation decreased from 60% to 8% after crosslinking. Comparison between types of crosslinking agents on E revealed that type of crosslinking agents did not significant effect on the E of resulted films.

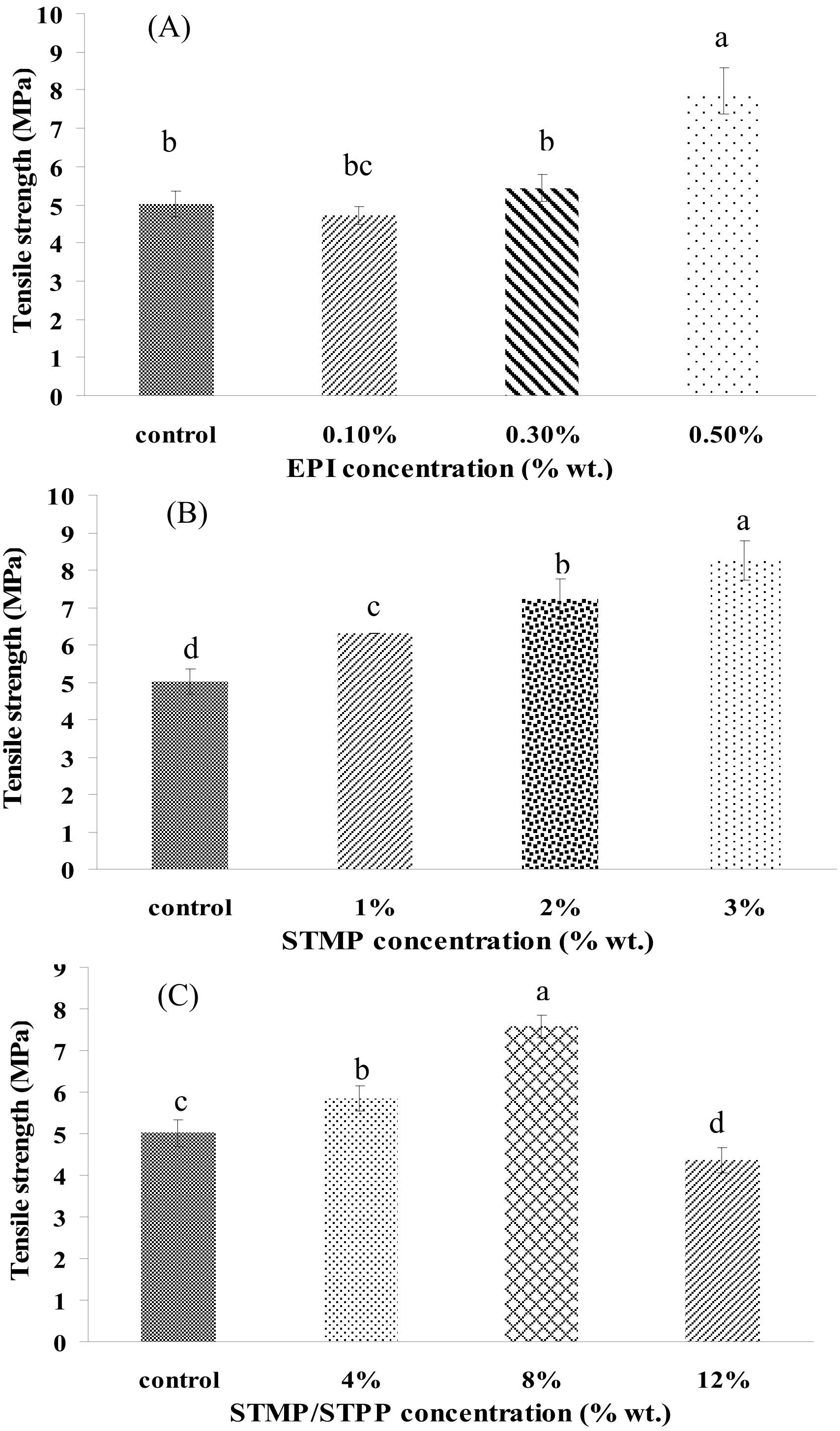


Figure 14. Effect of crosslinking agent on Tensile strength of untreated rice starch comparison with EPI (A), STMP (B) and STMP/STPP (C) crosslinked rice starch films. Mean values with different letter are significantly different ($p<0.05$).

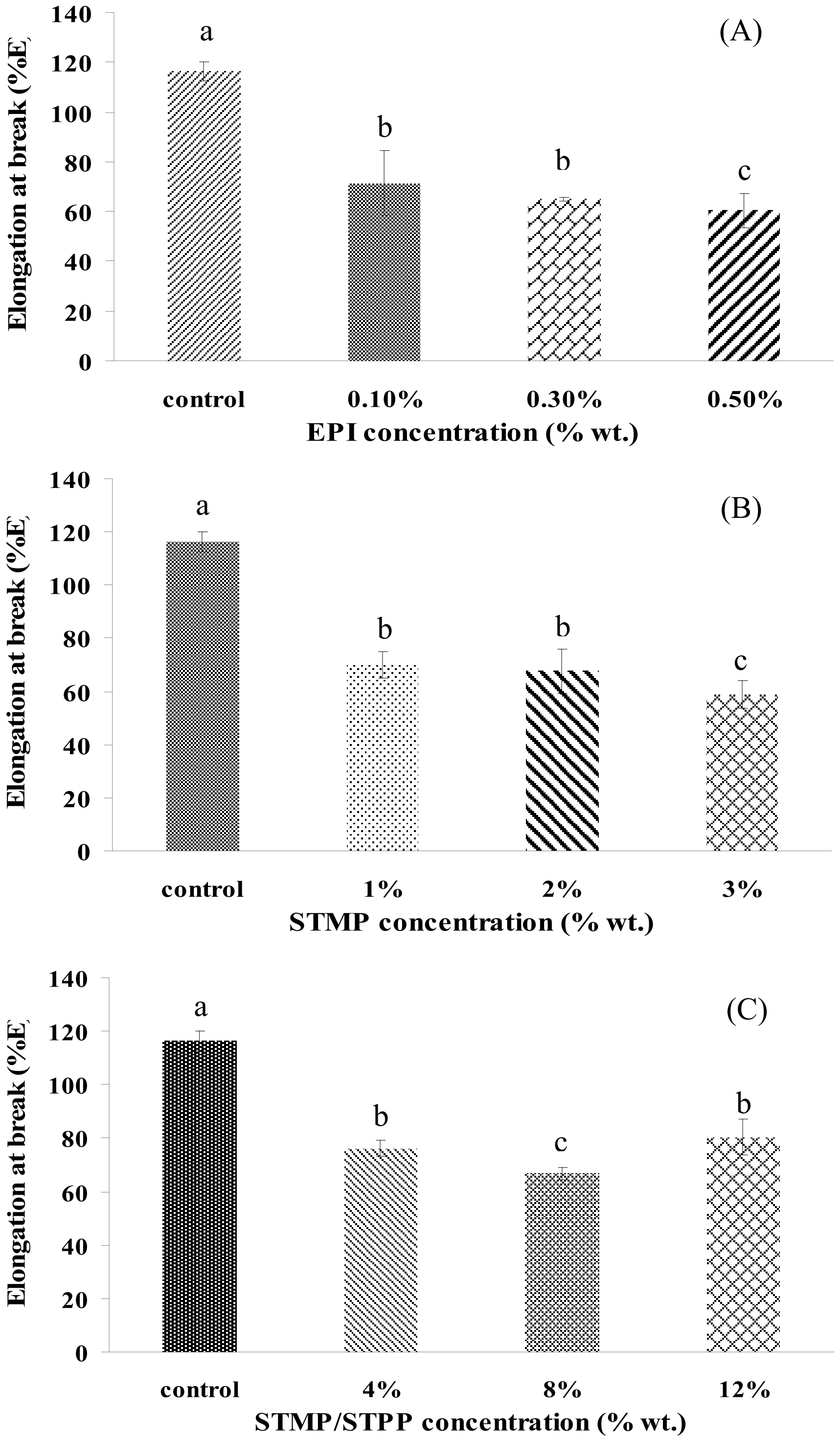


Figure 15. Effect of crosslinking agent on Elongation at break of untreated rice starch comparison with EPI (A), STMP (B) and STMP/STPP (C) crosslinked rice starch films. Mean values with different letter are significantly different ($p<0.05$).

3.3 X-ray Diffraction analysis

The XRD graphs of the individual components; rice starch powder, rice starch film and crosslinked rice starch films were selected 0.5%EPI, 3% STMP and 8% STMP/STPP crosslinked rice starch films for XRD analysis, the result was showed in Figure 16. The results pointed out that the gelatinized untreated rice starch and crosslinked rice films had difference structure from the untreated rice starch powder. The characteristic diffraction peaks of untreated rice starch powder was observed at 15.29°, 17.29°, 22.73° and weak diffraction peaks at 19.95° and rice starch films and crosslinked rice starch films with 0.5%EPI, 3% STMP and 8% STMP/STPP were observed at about 17.08° and 19.58°. According to the XRD pattern described by Zobel, (1964) and Kim and Lee, (2002), the untreated rice starch and the crosslinked rice starches belong to the A type and this result was in agreement with Xiao *et al.*, (2011) reported that A-type crystallinity is characterized by a well-defined peak at about 16.9-17.5 ° ($2\ \theta$). The crystallinity values of rice starch film and crosslinked rice starch films were displayed in Table 6. The reduction of % crystallinity of rice starch and crosslinked rice starch films due to starch was gelatinized for forming film, after gelatinization the crystalline of starch granules were destroyed (Zhang *et al.*, 2007) and their participation in crosslinking reaction (Das *et al.*, 2010). The results suggest that the crosslinking reaction decreased the crystallinity of rice starch films because the increase in density fluctuation as an increase of lattice defects introduced by crosslinking within the crystalline phase or to the change in local density in the vicinity of the crosslinks in the amorphous phase (Chen and Yeh, 1991).

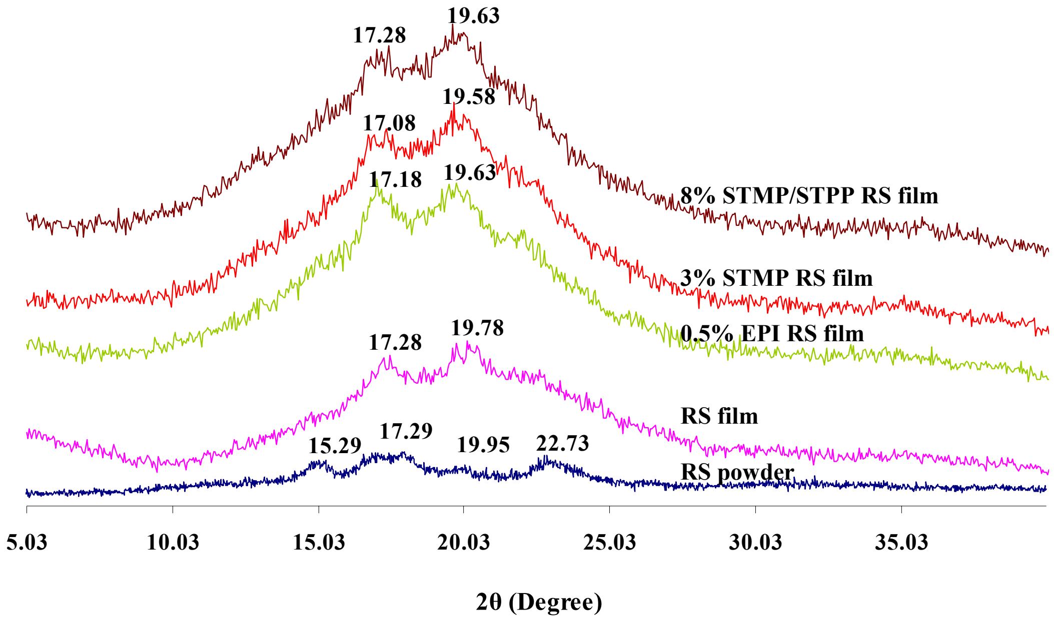


Figure 16. XRD patterns of rice starch powder, rice starch films (control) and crosslinked rice starch films (0.5% EPI, 3% STMP and 8% STMP/STPP)

Table 6. Relative crystallinity of crosslinked rice starch films

Type of starch	%Crystallinity (Area(peak)/Area(total)x100)
rice starch powder	23.19
rice starch film	16.10
0.5%EPI rice starch film	15.67
3%STMP rice starch film	15.13
8%STMP/STPP rice starch film	14.79

3.4 FT-IR analysis

FT-IR spectroscopy was used to verify the change in the chemical structure of starch molecules resulting from crosslinking reaction with EPI, STMP and STMP/STPP. FT-IR spectra of all films were depicted in Figure 17. The broad band of rice starch film at 3332.96 cm^{-1} was the O-H stretching. The peak at 2931.77 cm^{-1}

corresponded to the C-H stretching vibration (Sung *et al.*, 2006). Another characteristic absorption band occurred at 1018.05 cm^{-1} and about 990 cm^{-1} in crosslinked films, which attributed to C–O stretching. One characteristic absorption band occurred at 1647.19 cm^{-1} which was presumable originates from tightly bound water present in the starch molecules. The 0.5% EPI rice starch film demonstrated a weak intensity peak that should correspond to the ether stretch band at 1207.42 cm^{-1}. In STMP found the peak at 2850.75 and 1415.37 cm^{-1} correspond to the CH stretch attached to O and OH bending band and the peak at 1012.79 and 1261.38 cm^{-1}that correspond to phosphate stretching (P-O-C) and vibration (P=O) (Singh and Nath, 2012), respectively in 3% STMP rice starch film. And in STMP/STPP found the peak at 1035.75 correspond to phosphate stretching (P-O-C).

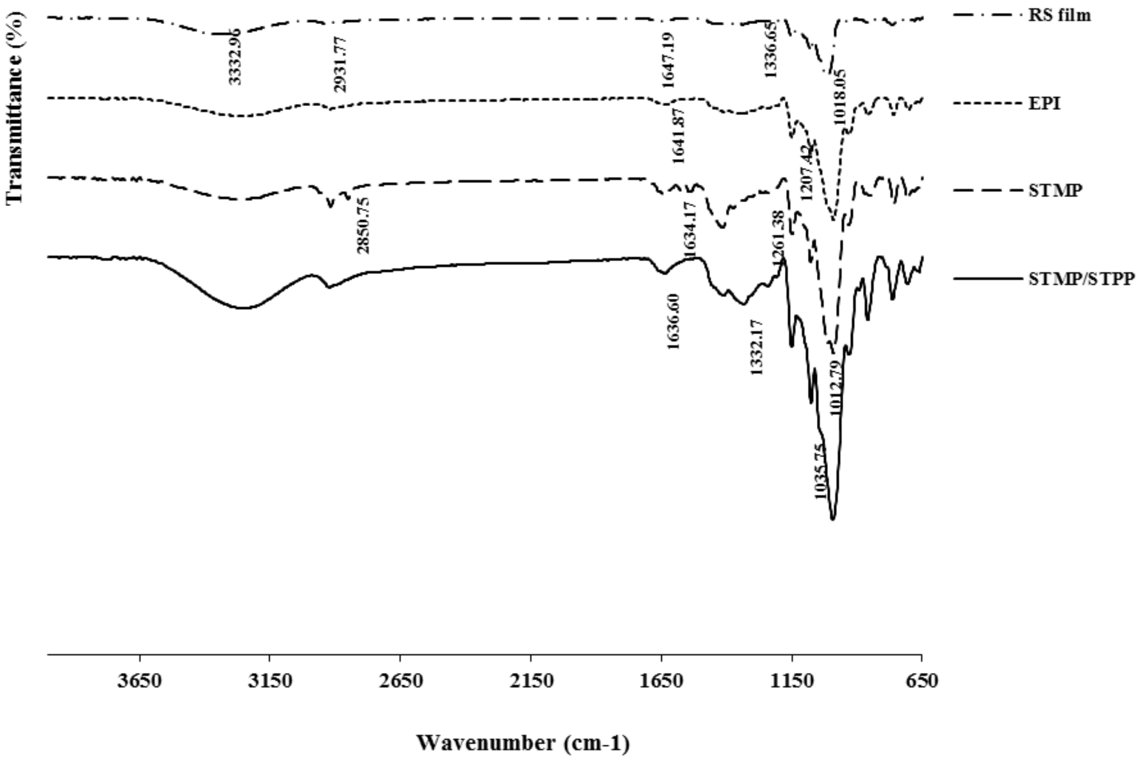


Figure 17. FTIR spectra of rice starch, 0.5% EPI, 3% STMP and 8% STMP/STPP of rice starch films.

3.5 Differential scanning calorimeter (DSC)

Differential scanning calorimeter (DSC) measurements were performed for untreated rice starch films (control) and crosslinked rice starch films. All the samples were heated from -50 to 180 °C with a heating rate of 20 °C/min. The DSC traces are shown in Figure 18. The crosslinking effect of chemical crosslinking are reflected in mechanical properties of the films, increasing film rigidity to a different extent as a consequence of the molecular mobility being reduced by covalent bonds (Muñoz *et al.*, 2003). The values obtained from DSC curve depicted in Table 7. The results showed the glass transition temperature (T_g) of untreated rice starch film was 25.60 ºC and crosslinked rice starch films with EPI, STMP and STMP/STPP were 35.59 ºC, 29.45 ºC and 31.67 ºC, respectively. The modified starches with STMP/STPP and EPI present an increase in the glass transition temperature. These results could be explained that the introduction of phosphate groups (STMP/STPP) and glycerol bond (EPI) into starch tightened the molecular organization in the starch molecules, thus gelatinization is carry out at a higher temperature. The increasing of T_g due to its occurred crosslinking which resulted in decreased chain mobility in the film matrix. Ačkar *et al.* (2010) reported that the glass transition temperature of wheat starch film was increased by modification in 0.1 and 0.3% (v/w) of EPI. On the other hand Chung *et al.* (2004) reported that the crosslinked starches with STMP/STPP displayed decreases in T_g. Figure 18 depicted the decreasing of endothermic heat flow when rice starch film was crosslinked, which is in accordance with research of Carmona-Garcia *et al.* (2009) and Jyothi *et al.* (2006). On the basis of DSC studies, Woo and Seib (2002) and Deetae *et al.* (2008) also reported that the crosslinked starches showed higher gelatinization temperatures and lower gelatinization enthalpies than their untreated counterparts. The decrease of heat flow of crosslinked film due to the increased of free volume in starch chain when the presence of bulky groups of crosslinking agents. The increasing of T_g and the heat capacity change (△Cp) demonstrated the crosslinked starches were resistant to a higher temperature than the untreated starch film. Crosslinking bonds can affect other bonds, such as hydrogen bonding, in the granule by acting as bridges between molecules.

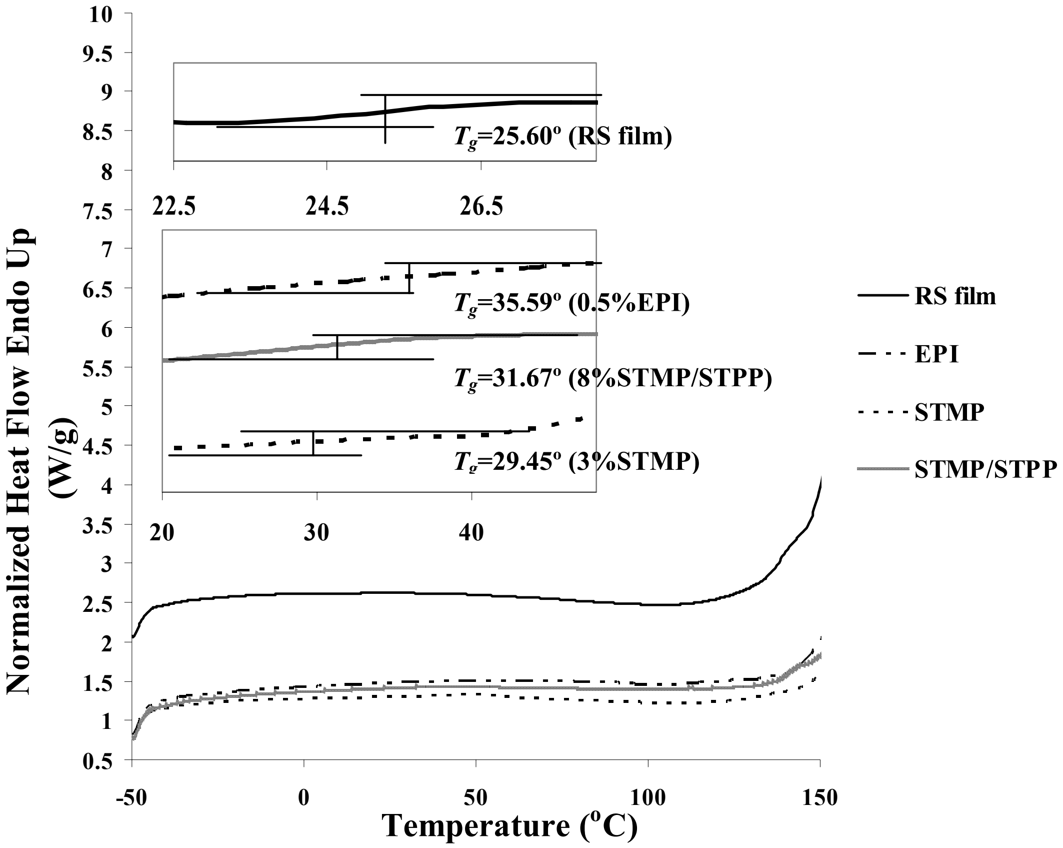


Figure 18. DSC curves of rice starch films (control) and crosslinked rice starch films.

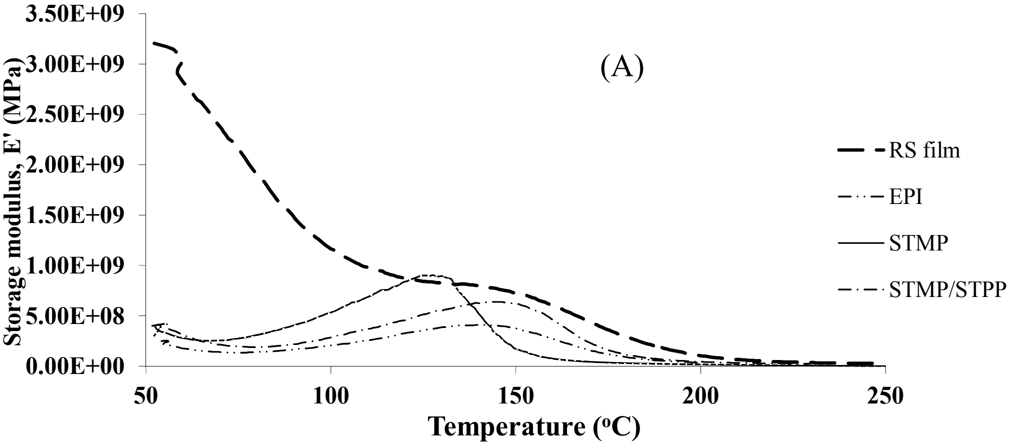
Table 7. Glass transition temperatures and specific heat change values of rice starch film and crosslinked rice starch films.

FILM	Transition temperature (°C)			△Cp
	*T*g onset	*T*g midpoint	*T*g endpoint	(J/g* °C)
rice starch	23.33	25.60	27.00	0.001195
0.5%EPI	23.00	35.59	47.66	0.098895
3%STMP	21.04	29.45	37.33	0.074048
8%STMP/STPP	24.004	31.67	35.67	0.841237

3.6 DMTA

The thermo-mechanical behavior of rice starch film and crosslinked rice starch films was studied using a dynamic mechanical thermal analyzer (DMTA). Figure 19(A) shows the storage modulus (E′) as a function of the temperature of rice starch film and crosslinked rice starch films. From the results found that storage modulus (E′) of crosslinked rice starch films was lower than untreated rice starch films. For example, the storage modulus of untreated rice starch films, crosslinked rice starch films with 0.5% EPI, crosslinked rice starch films with 3% STMP and crosslinked rice starch films with 8% STMP/STPP presented about 3.02 X 10^9 MPa, 2.21 X 10^8 MPa, 3.01 X 10^8 MPa and 4.00 X 10^8 MPa, respectively. The storage modulus (E′) of chemical crosslinking was decreased for 2 times of untreated rice starch films. The decrease of the storage modulus (E′) due to structural symmetry and H-bonding were decreased while some covalent bond was formed between the starch molecules (Ray *et al.*, 2009).

Figure 19(B) shows the tan δ curve of untreated rice starch film and crosslinked rice starch films as a function of temperature. Usually the position of the tan δ peak is used as the definition of transition temperature. According to the result, transition temperature of crosslinked rice starch film were slightly increased and found that crosslinked rice starch films with 8% STMP/STPP showed the highest transition temperature. The transition temperature increase indicates that structural changes in the amorphous phase in starch. Normally, an increase in crosslinking density reduces the chain mobility and consequently increases the T_g. This effect can be understood in terms of decreasing free volume (Chung *et al.*, 2004; Montserrat, 1995). However, crosslinked rice starch films with 3% STMP demonstrated that the transition temperature was decreased due to the bulky molecule of crosslinking agent that remained in starch molecule cause to the increased of free volume.



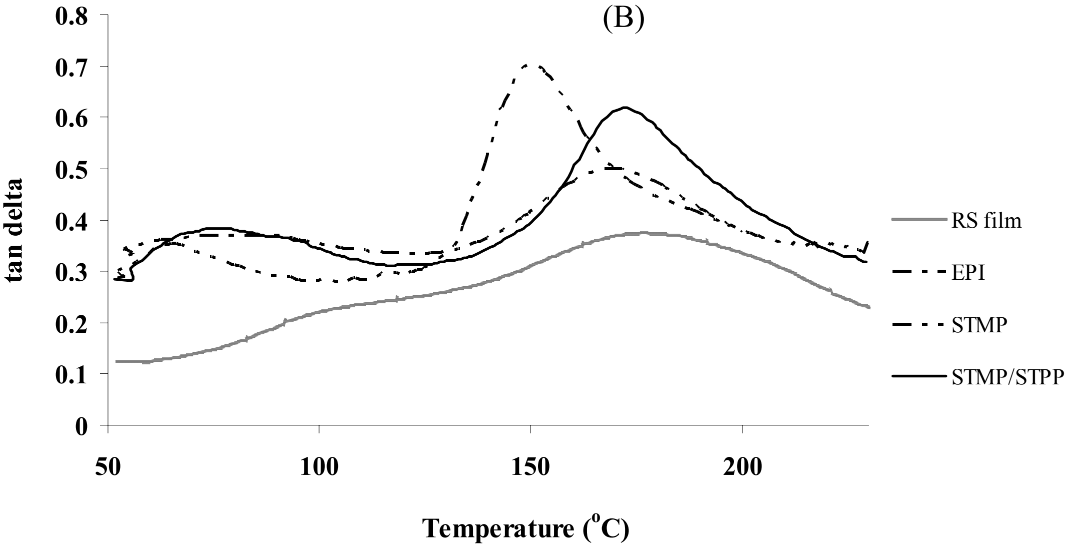


Figure 19. The dynamic mechanical behaviors of both (A) storage modulus (*E′*) and (B) loss factor (tan δ) as a function of temperature for rice starch film and crosslinked rice starch films.

3.7 Color and transparency

Figure 20-22 (A-C) shows the results from the effect of content of crosslinking agent on color (L*, a*, b*) and Figure 23 (A-C) depicted the transparency of untreated rice starch and crosslinked rice films. The measurements performed on the rice starch film's color were expressed in accordance with CIELAB system. The results showed the L* value slightly decreased when addition of EPI (Figure 20(A)), STMP (Figure 20(B)) and STMP/STPP (Figure 20(C)) as the crosslinking agent but the a* (Figure 21 (A-C)) and the b*(Figure 22 (A-C)) value were increased. This indicated the decreasing lightness (L*) and increasing yellowness (b*) and greenness (a*) of crosslinked rice film. From the results pointed that the additions of crosslinking agent change the color of rice starch film. Kim and Lee (2002) reported that LLDPE films containing the crosslinked starch were whiter than the LLDPE films containing the untreated starch. The transparency may be affected by various factors including film thickness (Bangyekan *et al.*, 2006) and it is also of importance in some instances, when used as packaging materials (Phattaraporn *et al.*, 2010). The results showed that the transparency (Figure 23(A-C)) was slightly increased in the crosslinked films and crosslinked films were off-white in color but highly translucent. Lim and Seib (1993) reported that increasing of light transmittance by crosslinking with STMP and STPP. The results suggest that the translucency slightly decreased with increasing crosslinking agent contents. It's due to the degree of crosslinking agent dispersion in the matrix starch (Kampeerapappun *et al.*, 2004).

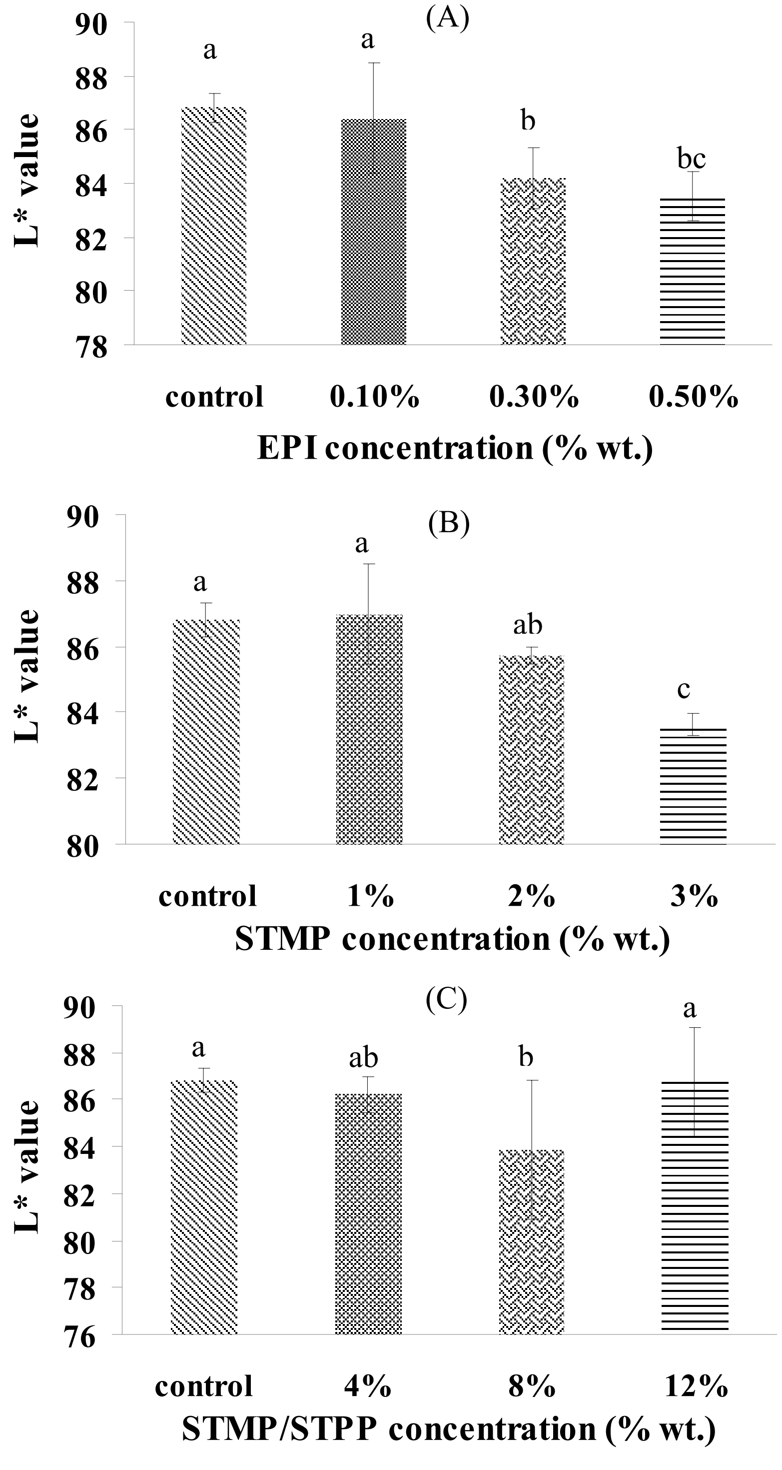


Figure 20. Effect of type of crosslinking agent on L* value of untreated rice starch comparison with EPI (A), STMP (B) and STMP/STPP (C) crosslinked rice starch films. Mean values with different letter are significantly different ($p<0.05$).

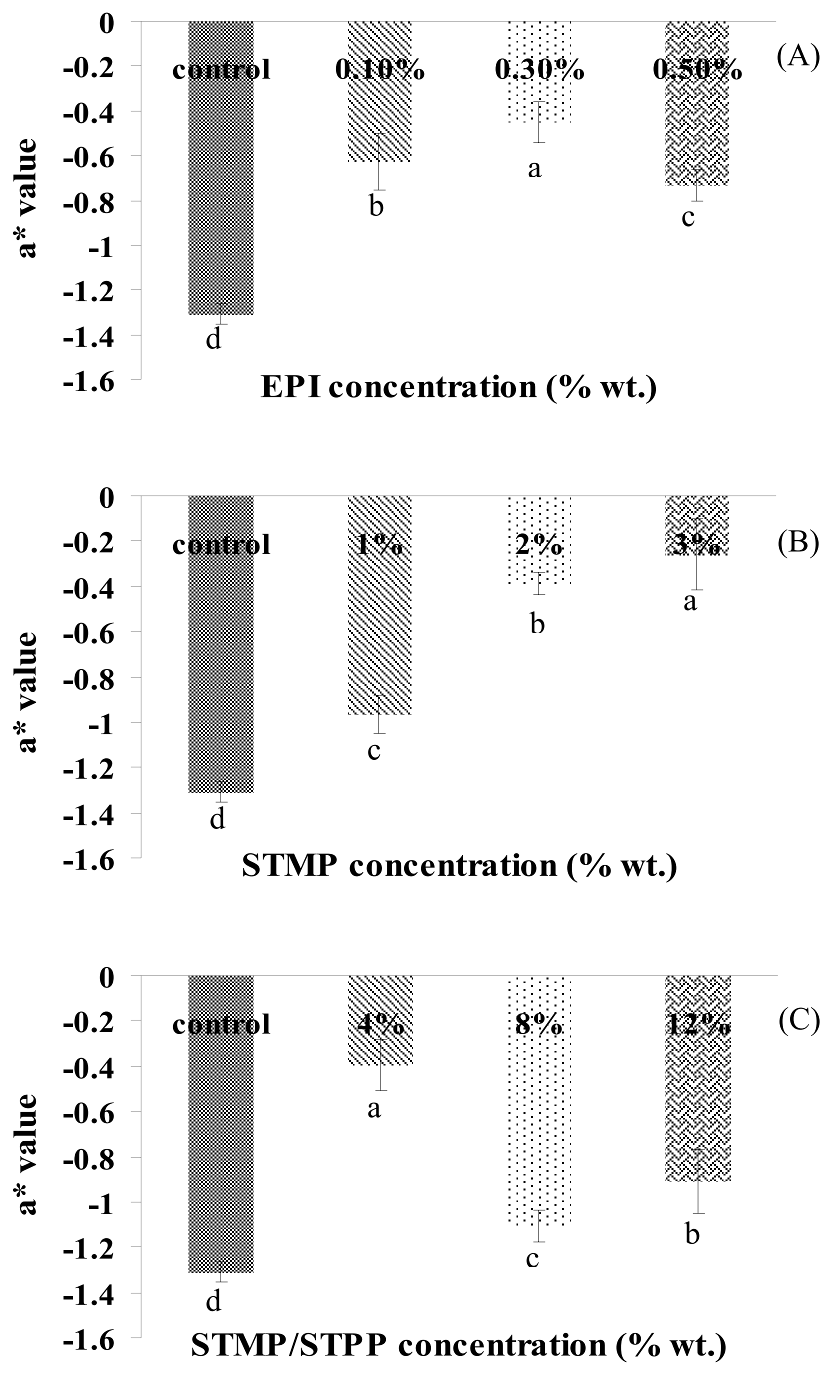


Figure 21. Effect of type of crosslinking agent on a* value of untreated rice starch comparison with EPI (A), STMP (B) and STMP/STPP (C) crosslinked rice starch films. Mean values with different letter are significantly different ($p<0.05$).

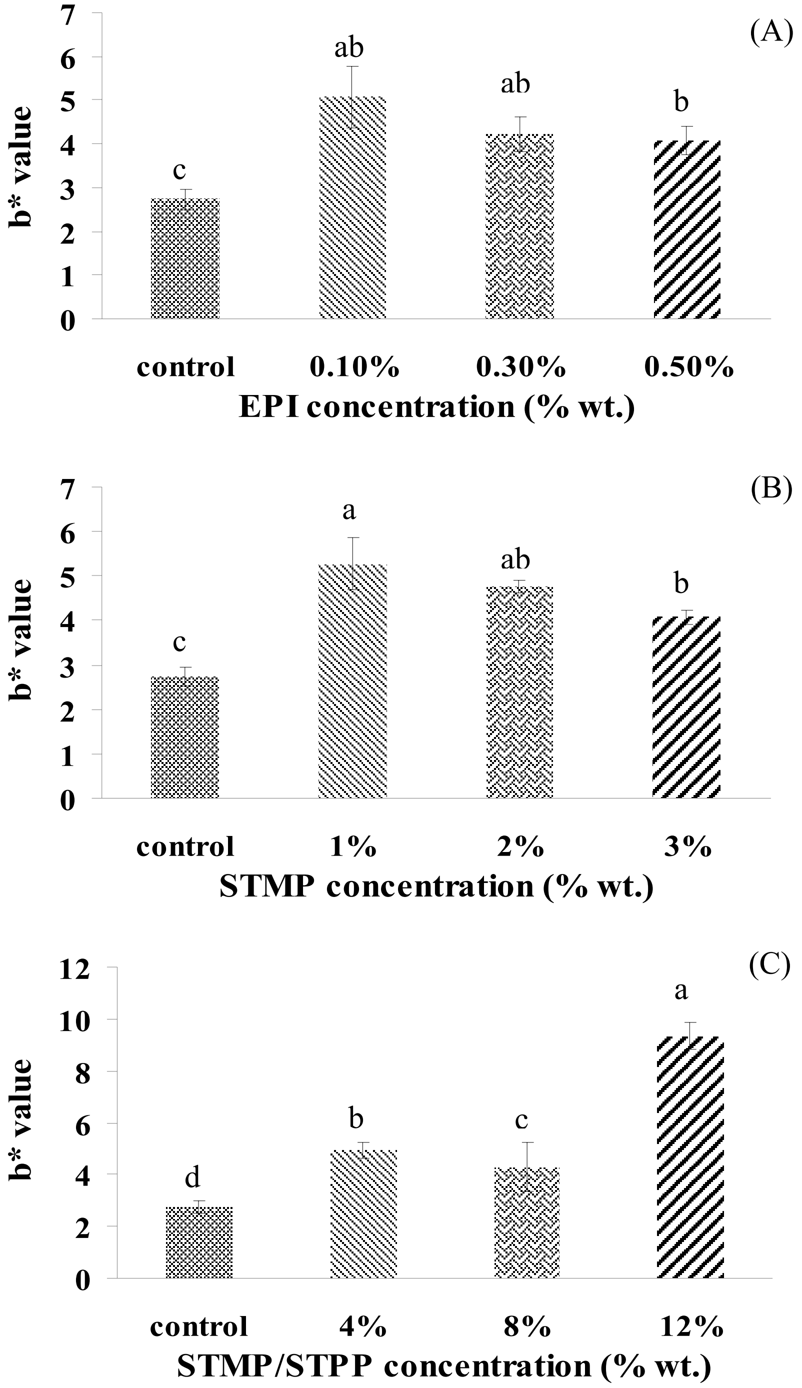


Figure 22. Effect of type of crosslinking agent on b* value of untreated rice starch comparison with EPI (A), STMP (B) and STMP/STPP (C) crosslinked rice starch films. Mean values with different letter are significantly different ($p<0.05$).

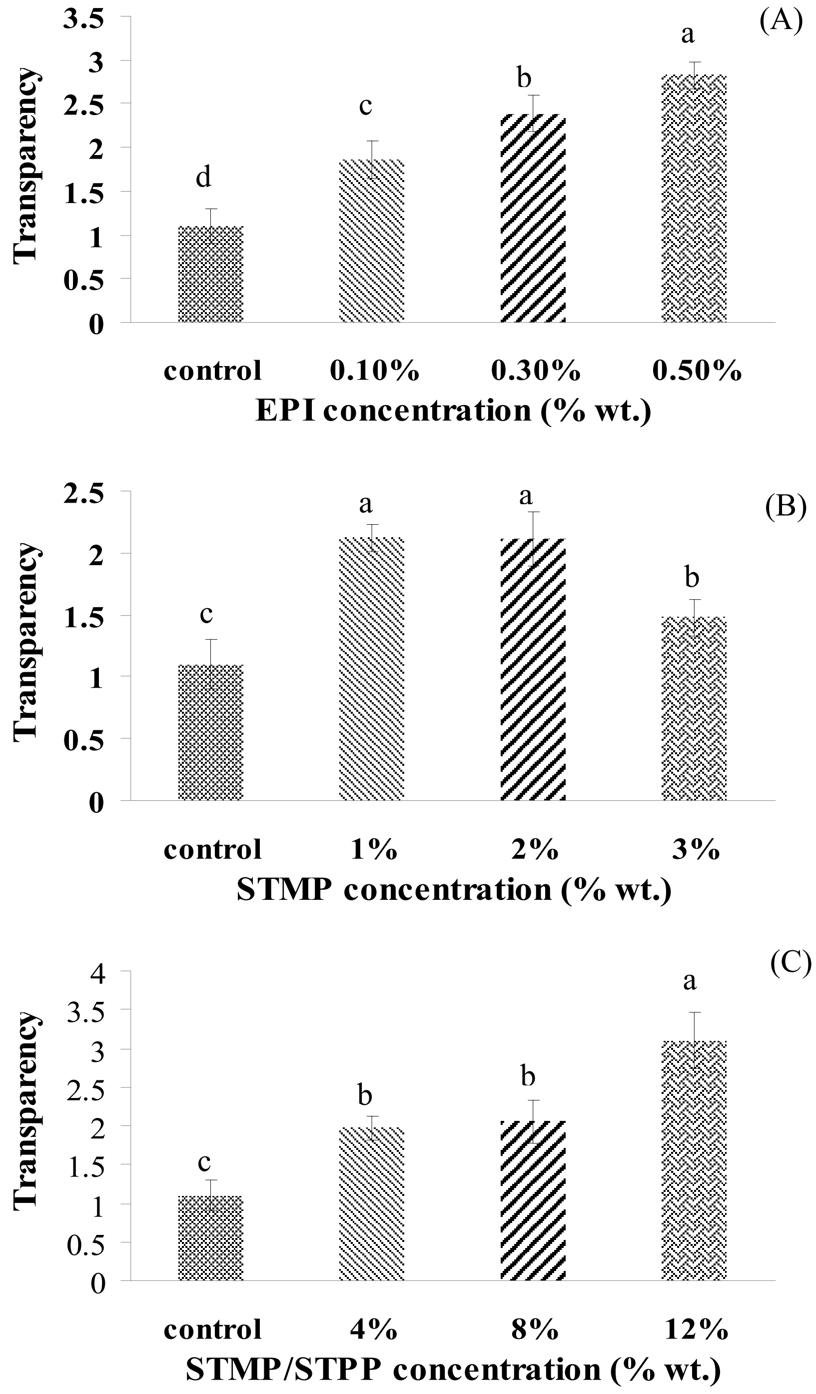


Figure 23. Effect of crosslinking agent on transparency of untreated rice starch comparison with EPI (A), STMP (B) and STMP/STPP (C) crosslinked rice starch films. Mean values with different letter are significantly different ($p<0.05$).

3.8 Water Uptake

The water uptake of untreated rice starch film (control) and crosslinked rice starch films (EPI, STMP and STMP/STPP) during conditioning in 98% RH as a function of time were shown in figure 24 (A-C). The results revealed that the crosslinking agent concentration had a great effect on the water uptake values of crosslinked rice starch film compared with untreated rice starch film. The water uptake curve demonstrated two state of water absorption in all type of crosslinking agents. From the results found that in $t < 2$ days, the absorption kinetics was dramatically increased, while after t > 2 days the absorption process was slightly increased, reaching to plateau, corresponding to the water absorption at equilibrium. The water uptake at equilibrium was significantly decreased with increasing the content of all type of crosslinking agents (EPI, STMP and STMP/STPP). The crosslinking agents react with the -OH groups present in starch and make ether linkages with the available hydroxyl groups. This helps to increase the mechanical properties of the films as well as reduce their water absorption behavior (Das *et al*., 2010). Rutledge *et al*. (1972, 1974) reported a decrease in both solid loss and water uptake of parboiled rice after crosslinking with STMP. Due to crosslinking reinforces the structure of starch granules and limits water absorption of starch thereby restricting the mobility of the starch chain in the amorphous region (Gunaratne and Corke, 2007; Manoi and Rizvi, 2010). According to the results of Das *et al*. (2010) reported the moisture absorption was considerably decreased in the crosslinked starch/poly(vinyl alcohol) (PVA) blend films.

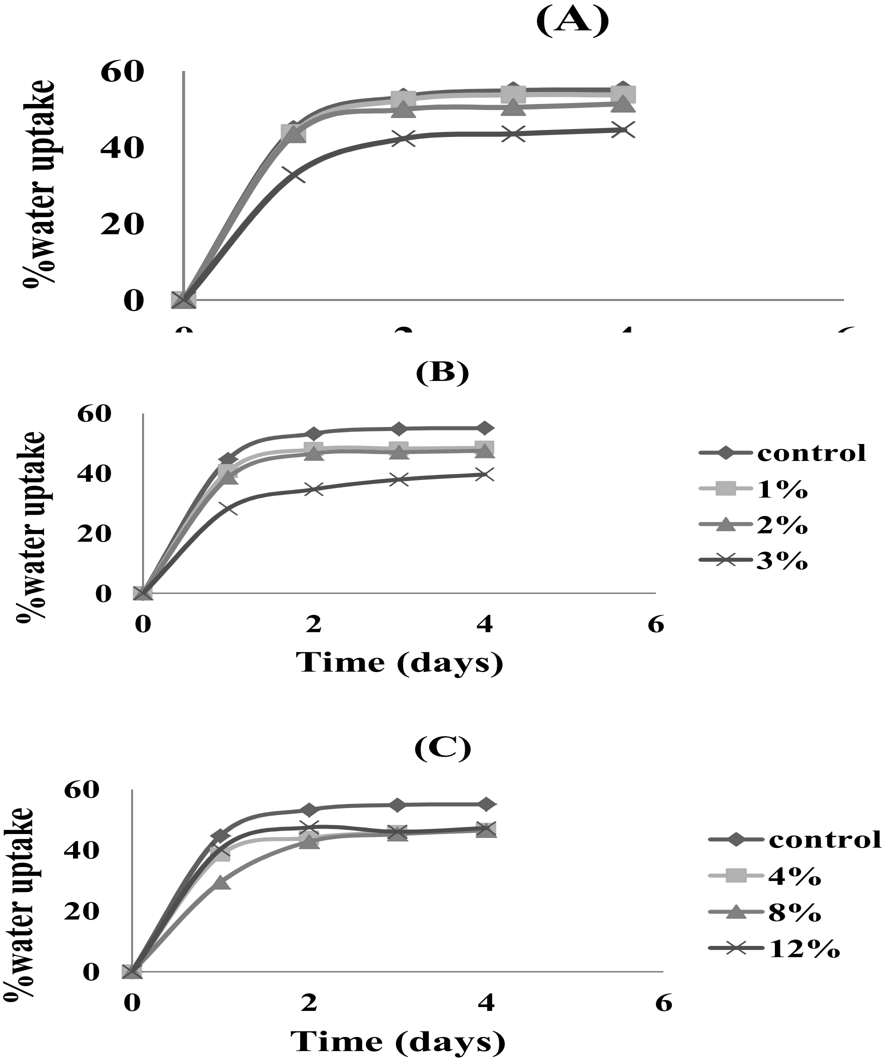


Figure 24. Effect of types and concentration of crosslinking agent on water uptake of rice starch films: (A) Epichlorohydrin (EPI), (B) Sodium trimetaphosphate (STMP) and (C) Sodium trimetaphosphate/ Sodium tripolyphosphate (STMP/STPP).

4. The effect of photocrosslinking on the properties of the UV treated rice starch films.

4.1 The effect of sodium benzoate (sensitizer) contents on the properties of rice starch films.

4.1.1 Water vapor permeability (WVP)

In the formation of biodegradable films, polysaccharides were generally used for their ability to establish polymer interactions and create a continuous network responsible for the mechanical properties of the films (Krochta, 1992; Callegarin *et al*., 1997). Biodegradable films produced from polysaccharides, however, are highly sensitive to water and provide limited resistance to moisture transmission due to the substantial inherent hydrophilicity of the film-forming substances and to the considerable amount of hydrophilic plasticizers incorporated into the films (Guilbert, 1986; Krochta, 1992; Gennadios *et al*., 1994b; Guilbert *et al*., 1996; Callegarin *et al*., 1997). Permeability is influenced by the hydrophobic or hydrophilic nature of the material, by the presence of voids or cracks, and by the stearic hindrance and tortuosity in the structure (Ghanbarzadeh *et al*., 2006; Wang *et al*., 2005). Water vapor permeability (WVP) results can be useful to understand possible mass transfer mechanism and solute and polymer interactions in biodegradable films. According to the thermodynamic of irreversible process, water chemical potential difference is the driving force of water transfer though a films. When the process occurs at constant temperature and pressure, the water chemical potential difference results proportional to water vapor concentration difference between the two faces (Morillon *et al.,* 2000; Bertuzzi *et al.,* 2007). WVP values of untreated rice starch films and UV treated rice starch films demonstrated in Figure 25. The UV treated rice starch films showed better water barrier properties than untreated rice starch films. The resulted showed that WVP values decreased when the amount of sodium benzoate increased. However, WVP value was slowly increased when the amount of sensitizer was excessive. Since the over amount of photochemical filter which limits UV penetration into the exposed film, so leading to a lower crosslinking density, characterized by crosslinking gradient (Decker and Moussa, 1990; Delville *et al.,* 2002). The WVP of untreated rice starch film was 6.19 g.mm/m^2.day.KPa, whereas the UV treated rice starch films contained sodium benzoate had WVP between 5.34 to 4.12 g.mm/m^2.day.KPa and found that the lowest WVP was observed when 6% sodium benzoate was used (4.12 g.mm/m^2.day.KPa.). Regarding to these results could be explained by a hydrophilic group (OH$^-$) of rice starch decreased when crosslinking reaction occurred between rice starch and sodium benzoate molecules and using 6% sodium benzoate provided a highest crosslinking density. Sabato *et al.* (2001) reported that crosslinked soy and whey protein based films with gamma-irradiation showed decreased WVP in the present of carboxymethylcellulose (CMC). Crosslinking has been shown to increase resistance to water vapor and gas transport (Ghanbarzadeh *et al.,* 2006).

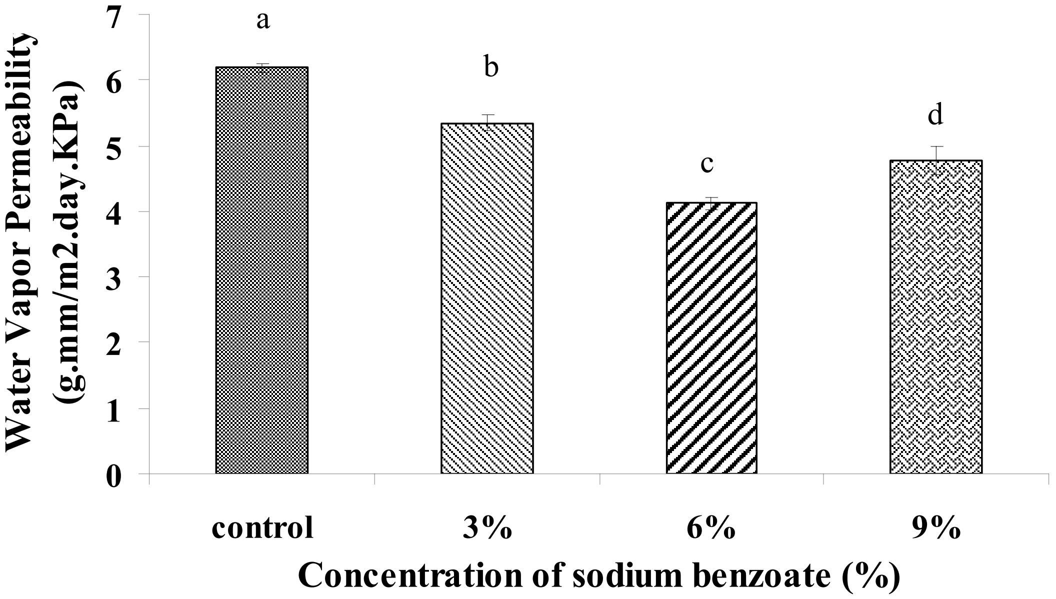


Figure 25. Effect of content of sodium benzoate on Water vapor permeability (WVP) of UV treated rice starch films at various content of sensitizer; 3% , 6% and 9%. Mean values with different letter are significantly different ($p<0.05$).

4.1.2 Tensile strength (TS) and Elongation at break (E)

The effect of content of sensitizer on tensile strength (TS) and elongation at break (E) of rice starch films depicted in Figure 26 (A) and (B). The TS of rice starch film was affected by content of sensitizer. The results demonstrated that the TS of rice starch films were enhanced as addition of sensitizer. Furthermore, increasing of sensitizer content from 3 to 6% resulted in an increase of TS (Figure 26(A)), indicated that a high interaction occurs between starch molecule and the performances (e.g., mechanical properties). In addition, the increase in TS may due to the increase of crosslinking density. Besides, at lower sensitizer content interaction is very poor so that TS will not occur properly. The result showed that rice starch film contained 6% of sodium benzoate had the highest TS but too high sensitizer yielded lower TS. Khan *et al*. (2006) also observed similar results for sago starch/PVA blend film crosslinked with UV irradiation, the TS values increased with UV radiation doses up to a certain limit and then decreased. The crosslinking reaction occurs through a radical mechanism, the sensitizer (sodium benzoate) are excited or decompose to produce radicals upon irradiation with UV light and this radical reacted with starch molecule as crosslinking reaction to increase the TS of UV treated rice starch films but the maximum sensitizer inhibited UV penetrated and decreased hydrogen abstraction (Delville *et al.,* 2002), its mean when the sensitizer accessed the TS value was decreased and the E was increased in 9% sensitizers. In generally, food packaging requires high stress with deformation according to the intended application to protect the stuff while in used (Gontard *et al.,* 1992). From the results demonstrated that the amount of sensitizer that made rice starch presented a good mechanical properties were in rang 0.1-6% by used sorbital as plasticizer. Regarding the elongation at break (E) is an indication of the 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. Figure 26(B) showed the effect of sensitizer content on at break of UV treated rice starch films, it was indicated that E valued showed decrease from 116% to 67% when amount of sensitizer increase from 3-6 wt% and E valued were slowly increased when sensitizer increased up to 9 %. The tendency increase of TS was relatively low compared to UV-cured synthetic thermosets. Moreover, Delville *et al. (*2002) reported that UV treated rich starch film is to be related to the low crosslinking density that achieved by the photo-crosslinking treatment.

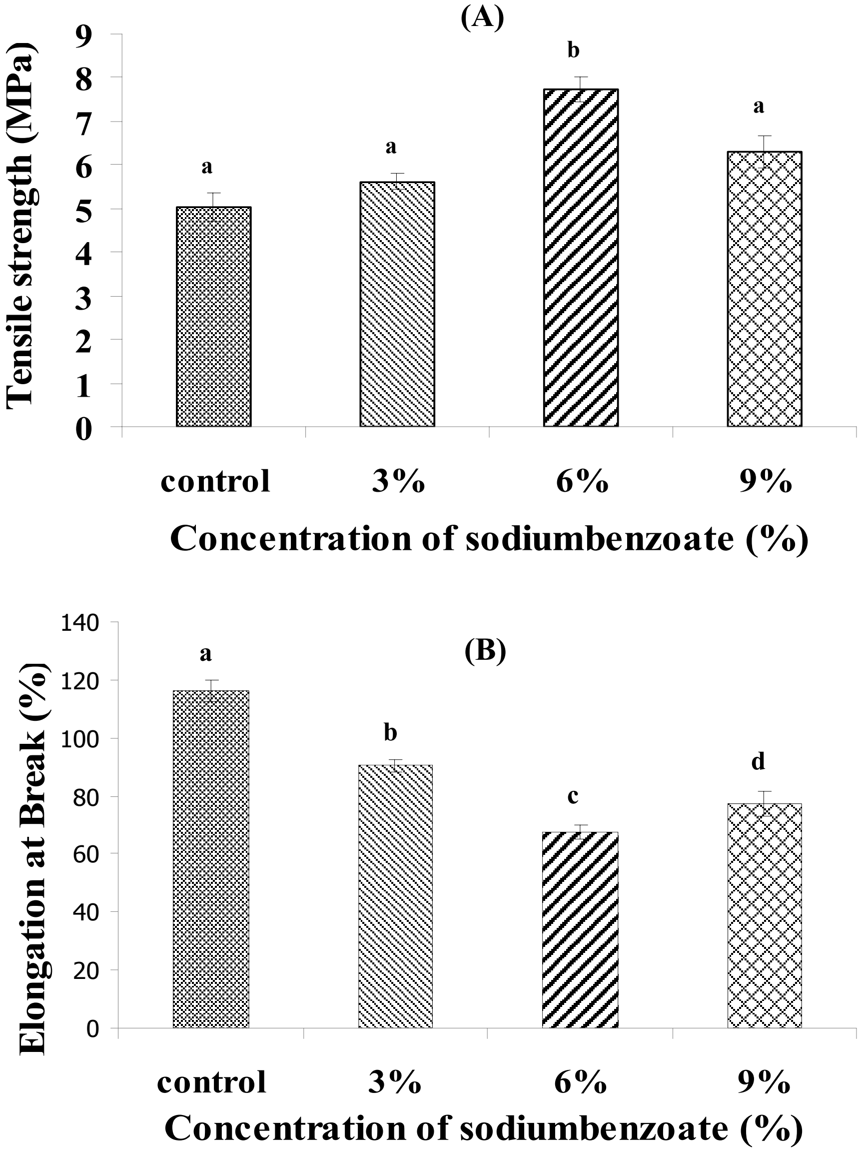


Figure 26. Effect of content of sodium benzoate on Tensile strength (A) and Elongation at break (B) of UV treated rice starch films at various content of sensitizer; 3% , 6% and 9%. Mean values with different letter are significantly different ($p<0.05$).

4.1.3 X-ray Diffraction analysis

The XRD graphs of the individual components; rice starch powder, rice starch film and UV treated rich starch film contained sensitizer were showed in Figure 27. Different types of crystallinity have been revealed by X-ray diffraction depending on the amylose content of starch (Van Soest *et al.*, 1996a,b), the starch origin (Forssell et al., 1999), the transformation process used (Miles *et al.*, 1985; Rindlav-Westing *et al.*, 1998; Van Soest *et al.*, 1996a,b) (thermo molding, extrusion or casting), and the additives. It is well known that rice starch has the A-type XRD pattern (Bao and Bergman, 2004) with strong reflection at 15.29°, 17.29° and 22.73° due to high amylopectin content (Liu *et al.*, 2009) and % crystallinity values were estimated as 23.19%. The characteristic diffraction peaks of rice starch film presented at 17.5° and 20.5° and UV treated rice starch films were observed at about 16° and 20.5°. The gelatinized rice starch films had difference structure due to the present of plasticizer; it increased the free volume, and crosslink reaction in rice starch molecule. Ummi-Shafiqah *et al.* (2012) reported the XRD pattern of sago and mung bean starch blend films and UV treated starch blend films could be assigned to a B-type. Starch films normally show a B-type x-ray diffraction, regardless of the original polymorphs of starch from which the films are made (Bader and Göritz, 1994; Rindlav *et al.*, 1997; Rindlav-Westling *et al.*, 1998; Mali *et al.*, 2002; Myllärinen *et al.*, 2002a). The crystallinity values of untreated rice starch film (control), 3% sensitizer, 6% sensitizer and 9% sensitizer were estimated (Table 8) as 16.10%, 15.95%, 14.97% and 14.79%, respectively. Thus, it can be concluded that UV treatment of starch has been reported not to alter the crystalline structure of starch or its gelatinization enthalpy (Mestres and Rouau, 1997).

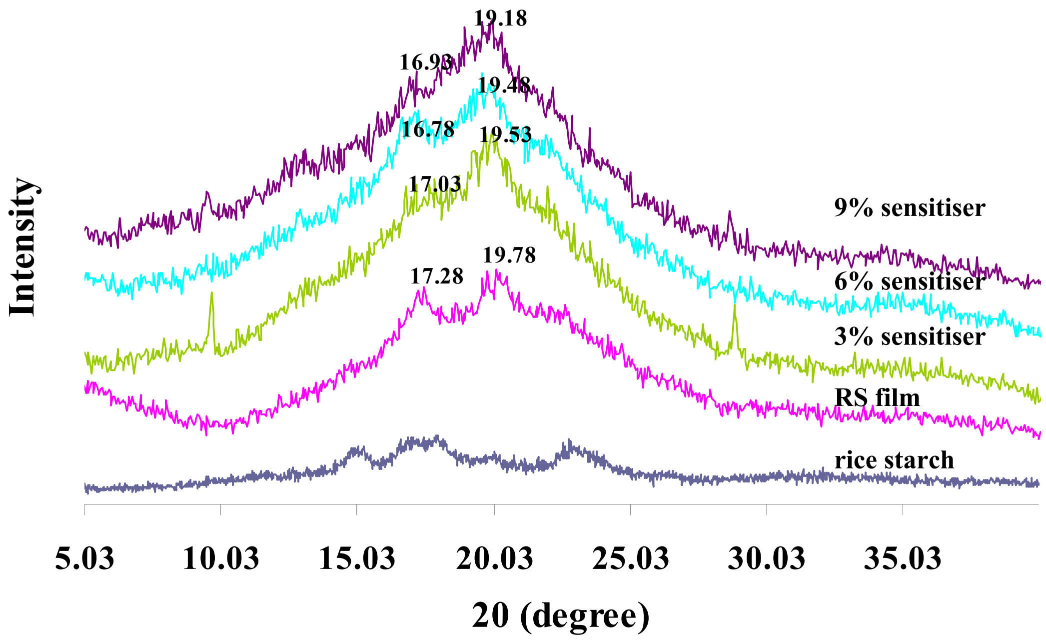


Figure 27. XRD patterns of rice starch powder, rice starch film (control) and UV treated rice starch films at various contents of sensitizer.

Table 8. Relative crystallinity of rice starch powder, rice starch film and UV treated rice starch films at various contents of sensitizer.

Type of starch	**%Crystallinity = (A(peak)/A(total)x100)**
rice starch powder	23.19
rice starch film	16.10
3% sensitizer	15.95
6% sensitizer	14.97
9% sensitizer	14.79

4.1.4 Differential scanning calorimeter (DSC)

DSC was used to examine the transition temperatures, such as glass transition temperature (T_g) and specific heat change values (△Cp), of the films. The crosslinking effect of UV treatment is reflected in resistant to high temperature, low pH, and high shear, and improve viscosity and textural properties of the untreated starch. The sensitizer bind neighboring anhydroglucose units (AGU) in the amorphous regions of the rice starch amylopectin. In this experiment, DSC measurements were performed for untreated rice starch films (control) and UV treated rice starch films. All the samples were heated from -50 to 180 °C with a heating rate of 20 °C/min. The DSC traces are shown in Figure 28. For the DSC scan, it was observed the wide endothermic peaks around 25 ºC to 140 ºC and there were no exothermic peak was observed during heating. The values obtained from DSC curve depicted in Table 9. The results showed the glass transition temperature of rice starch film was 25.60 ºC and UV treated rice starch films contained 3%, 6% and 9% of sensitizer were 29.37 ºC, 27.67 and 30.33 ºC, respectively. The T_g is a very important physical parameter, which serves to explain the physical and chemical behavior of material system, and it is defined as the temperature at which the material changes from the glassy state to the rubbery state for a given heating rate (Perdomo *et al.*, 2009). This transition was associated with molecular segmental motion of amorphous structure. From the result, UV treated rice starch film pointed out a slightly increased T_g due to its occurred crosslinking between polymer chain which resulted in decreased chain mobility in amorphous region of resulted film matrix. The results is similar to the studied of Bhat and Karim (2009) who investigated commercially procured fish gelatin treated with UV light to improve the properties of fish gelatin and reported that enthalpy (DH) revealed a significant increase after UV treatments. This increase might be attributed to the UV-induced crosslinking of fish gelatin.

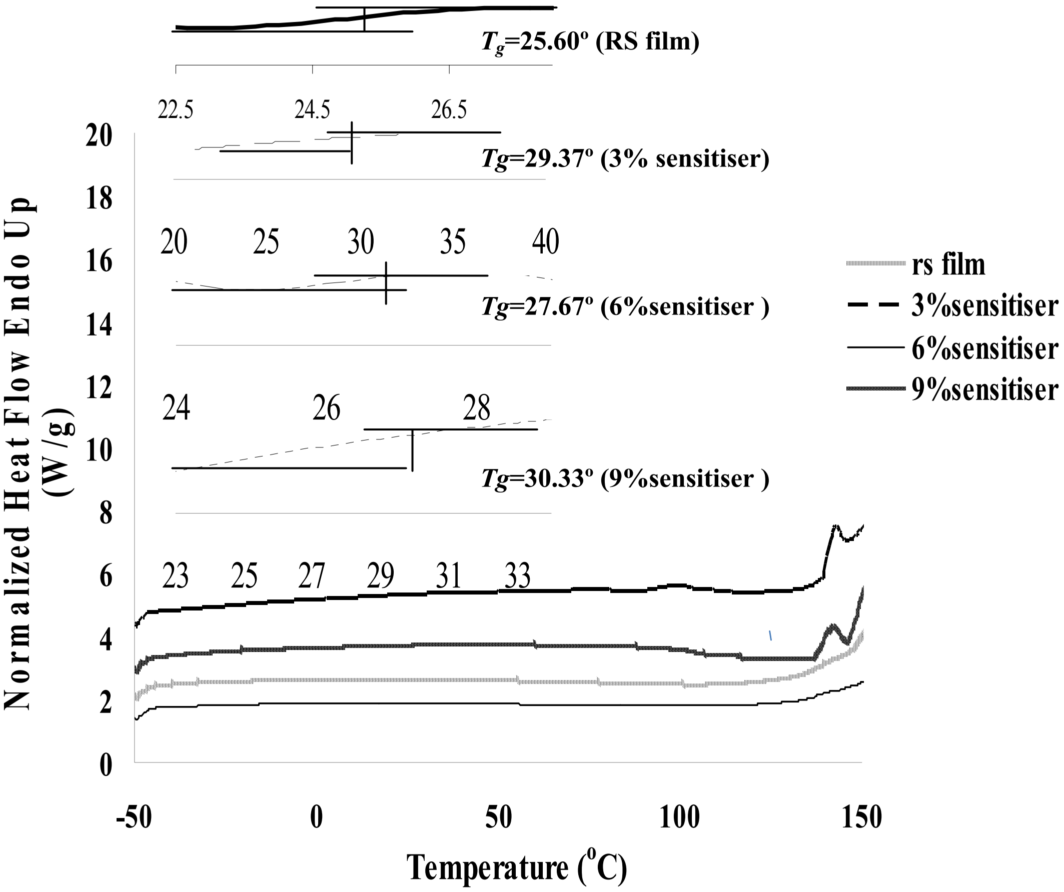


Figure 28. DSC curves of rice starch films (control) and UV treated rice starch films contained various contents of sensitizer.

Table 9. Glass transition temperatures and specific heat change values of rice starch film and UV treated rice starch films contained various contents of sensitizer.

FILM	Transition temperature (ºC)			△Cp
	T_g onset	T_g midpoint	T_g endpoint	(J/g* ºC)
rice starch	23.33	25.60	27.00	0.001195
3% sensitizer	23.67	29.37	37	0.173673
6% sensitizer	24	27.67	28.67	0.00055
9% sensitizer	23.45	30.33	33.67	0.070471

4.1.5 FT-IR analysis

FTIR spectra of all films are presented in Figure 29. The broad band of rice starch film at 3332.96 cm^{-1} was the O-H stretching. The peak at 2931.26 cm^{-1} corresponded to the C-H stretching, while the band at 1338.58 cm^{-1} was the O-H of water. The UV treated rice starch films spectrum demonstrated a weak intensity peak that should correspond to the benzoate anion and benzoic acid. The peak at 1550.34, 1553.88 and 1550.02 cm^{-1} (3%. 6% and 9% sensitizer, respectively) corresponded to the CO_2^- asymmetric (ν_{as}) and the peak at 1374.18 and 1380.73 cm^{-1} (6% and 9% sensitizer) corresponded to the CO_2^- symmetric (ν_s) stretching vibrations of carboxylate group (Tejedor *et al.,* 1990). Additionally, the 6% sensitizer showed the highest absorption peak at 1553.88 cm^{-1} confirmed 6% sensitizer film had a higher decomposed of free radicals of sodium benzoate than 3% and 9% sensitizer film. As UV light provides lower energy level than other source of ionizing radiation, it is impossible for direct cleavage of C-C or C-H bond of starch molecules to occur for the formation of free radical. Hence, for crosslinking propose, there need for sensitizer (photo initiator) that can absorb a low-energy photon (UV light) and become activated, leading to the formation of free radicals (Bhat and Karim, 2009) and also leads to macro radical combination by hydrogen abstraction (Delville *et al*., 2002). The films that had a high free radical could possess a high crosslinking of rice starch molecules.

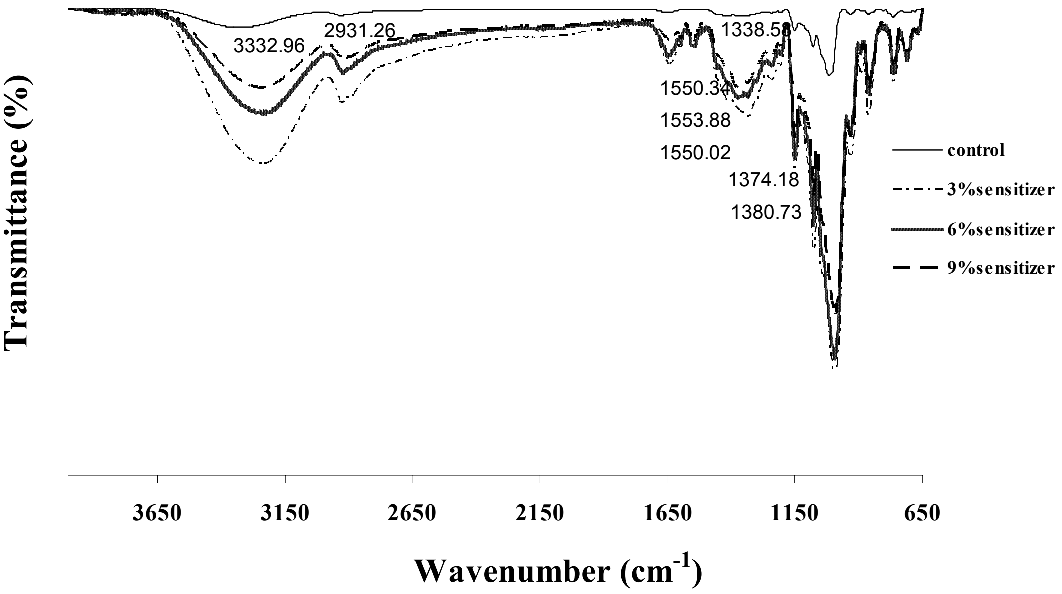


Figure 29. Attenuated total reflection (ATR) spectra of rice starch film and UV treated rice starch films and UV treated rice starch films contained various contents of sensitizer; 3% , 6% and 9% .

4.1.5 Color

Color of the packaging is an important factor in terms of general appearance and consumer acceptance (Srinivasa *et al.*, 2007). The results of the measurements performed on the rice starch film's color were expressed in accordance with CIELAB system and the rectangular coordinates (L*, a* and b*). Figure 30 showed the effect of sensitizer using sodium benzoate from 3%-9% of UV on color of rice starch films. The results demonstrated that content of sensitizer showed less affected of rice starch film color. However, it was found that a* value trend to decreased when amount of sodium benzoate increased, while the L* and b* value were increased. Addition of sodium benzoate into the rice starch film yielded increase both lightness and yellowish was due to the oxidation reaction that induced by UV light (Hamaguchp *et al.*, 2003). However, too high content of sensitizer (9%) provided decrease of b* value due to the excessive amount of sodium benzoate limited UV penetration into the films (Delville *et al.,* 2002).

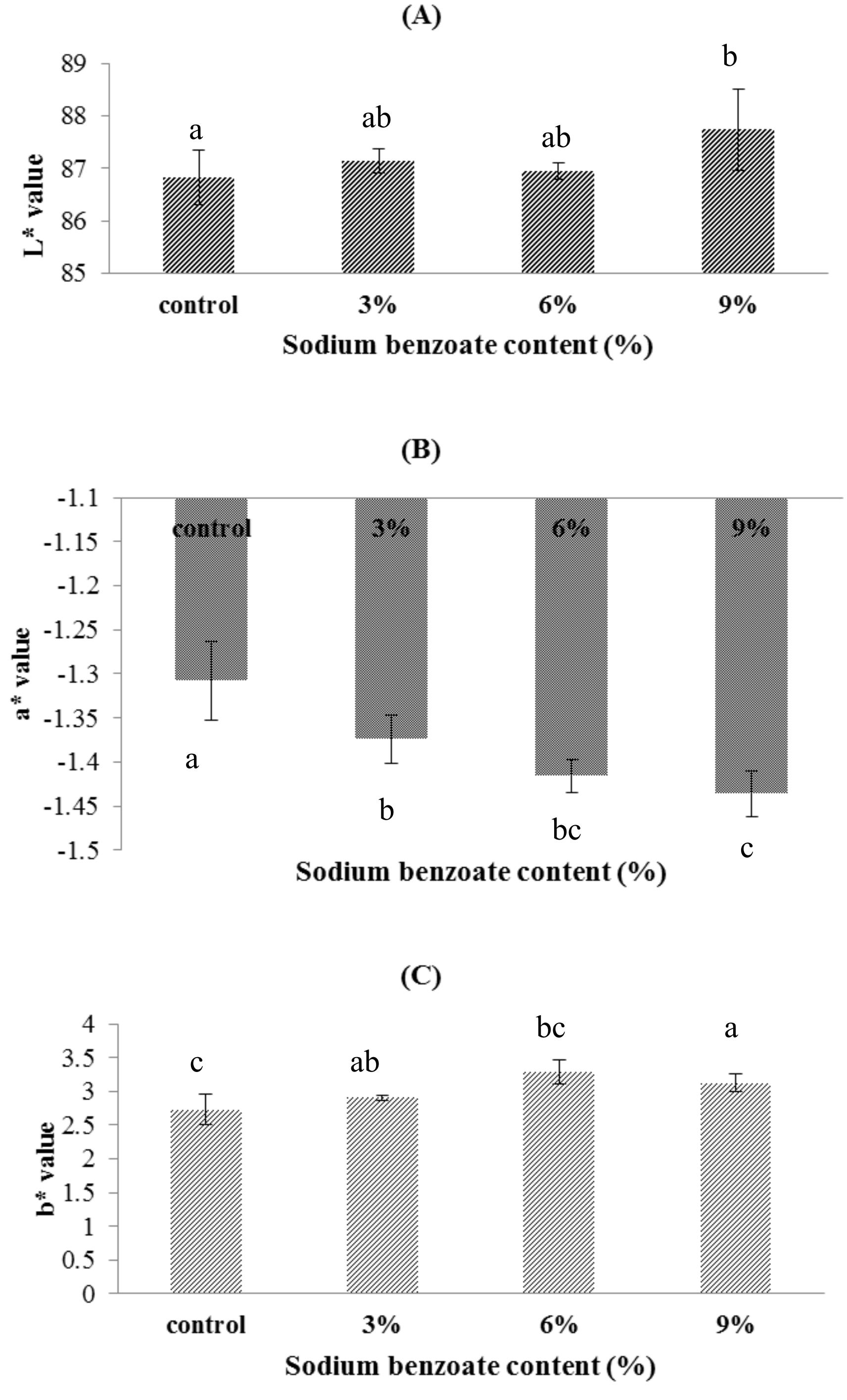


Figure 30. Effect of amount of sensitizer on L* (A); a* (B) and b* (C) of rice starch film and UV treated rice starch films contained various contents of sensitizer; 3% (B), 6% (C) and 9% (D). Mean values with different letter are significantly different ($p<0.05$).

4.2 Effect of irradiation time on the properties of UV treated rice starch films

According to the previous study addition of 6% sodium benzoate into rice starch films provided a good mechanical and barrier properties of resulted films. Hence, the rice starch films with 6% sodium benzoate were selected for further investigating on the effect of irradiation time on the properties of UV treated rice starch films.

4.2.1 Mechanical properties

The formation of starch macromolecular network in rice starch film could change the mechanical properties of the film and the irradiation times have an importance effect on crosslinking reaction that occurred while UV light penetrated into rice starch film. It is worthy to examine the influence of the irradiation time for photocrosslinking treatment on mechanical properties. It’s the important criterion for many practical applications of rice starch film (Zhou *et al*., 2008). Figure 31 (A-C) showed the effect of the irradiation time on mechanical properties of UV treated rice starch film. The results demonstrated that when the irradiation time increased the TS of 3%sensitizer (Figure 31 (A)) and 6% sensitizer (Figure 31 (B)) were slightly decreased but 9% sensitizer (Figure 31 (C)) showed the extremely decreased. It due to the macromolecular chain scission may occur. Moreover, rice starch film that contained 6% sensitizer still has higher TS value than 3% and 9% sensitizer rice starch films because 6% sensitizer could provide the highest amounts of active radicals to occurred photo-crosslinking within rice starch molecules. When the exposure is prolonged the UV irradiation dose was increased and it induced starch molecule chain slit and the mechanical properties were decreased. Figure 32(A-C) showed the effect of irradiation time on E of UV treated rice starch films, it was indicated that E value increased when irradiation time was increased from 10 to 30 min and 6% sensitizer gave starch film with lowest E value. Khan *et al*. (2006) reported that TS values increased with UV radiation doses up to a certain limit and then decreased in (UV) radiation cured bio-degradable films of sago starch/PVA blend.

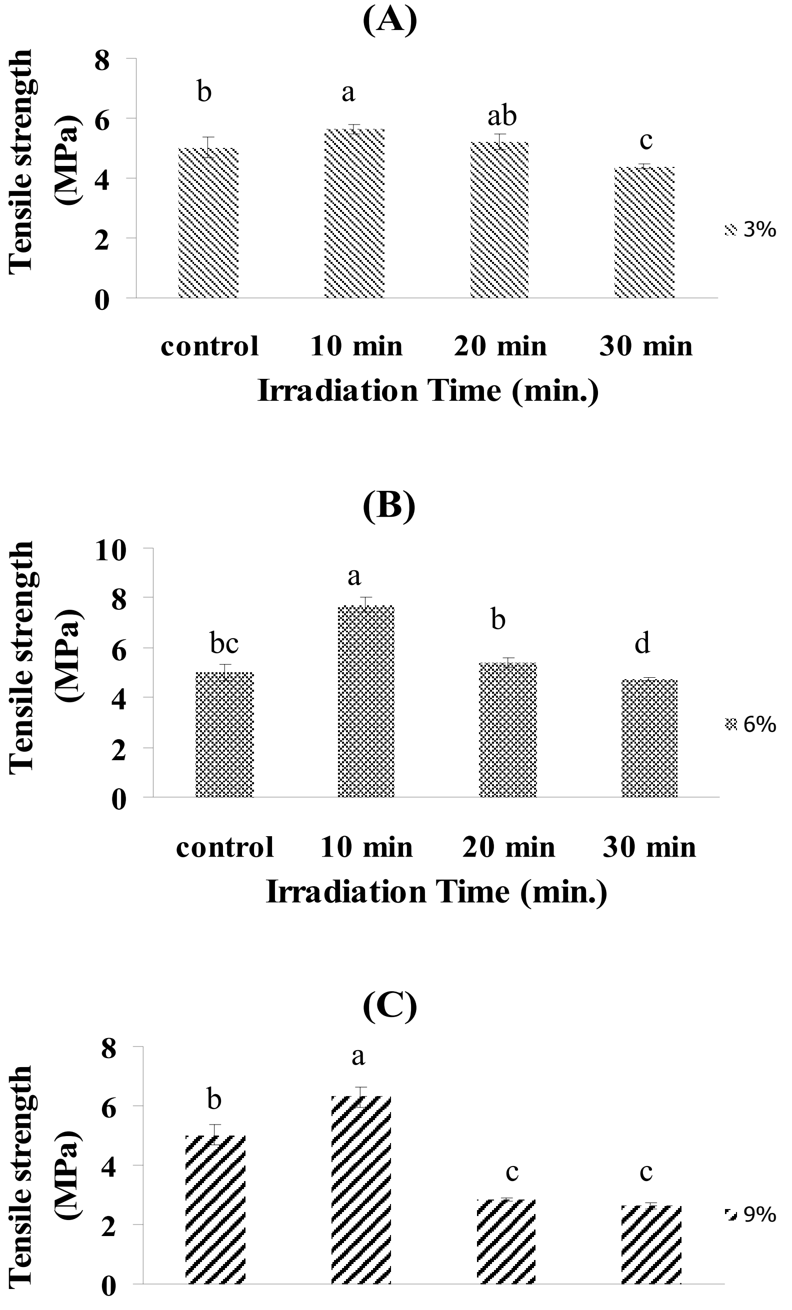


Figure 31. Effect of the irradiation time on Tensile strength of UV treated rice starch films contained various concentration of sensitizer; 3% (A), 6% (B) and 9% (C) and various irradiation time from 10 – 30 min. Mean values with different letter are significantly different ($p<0.05$).

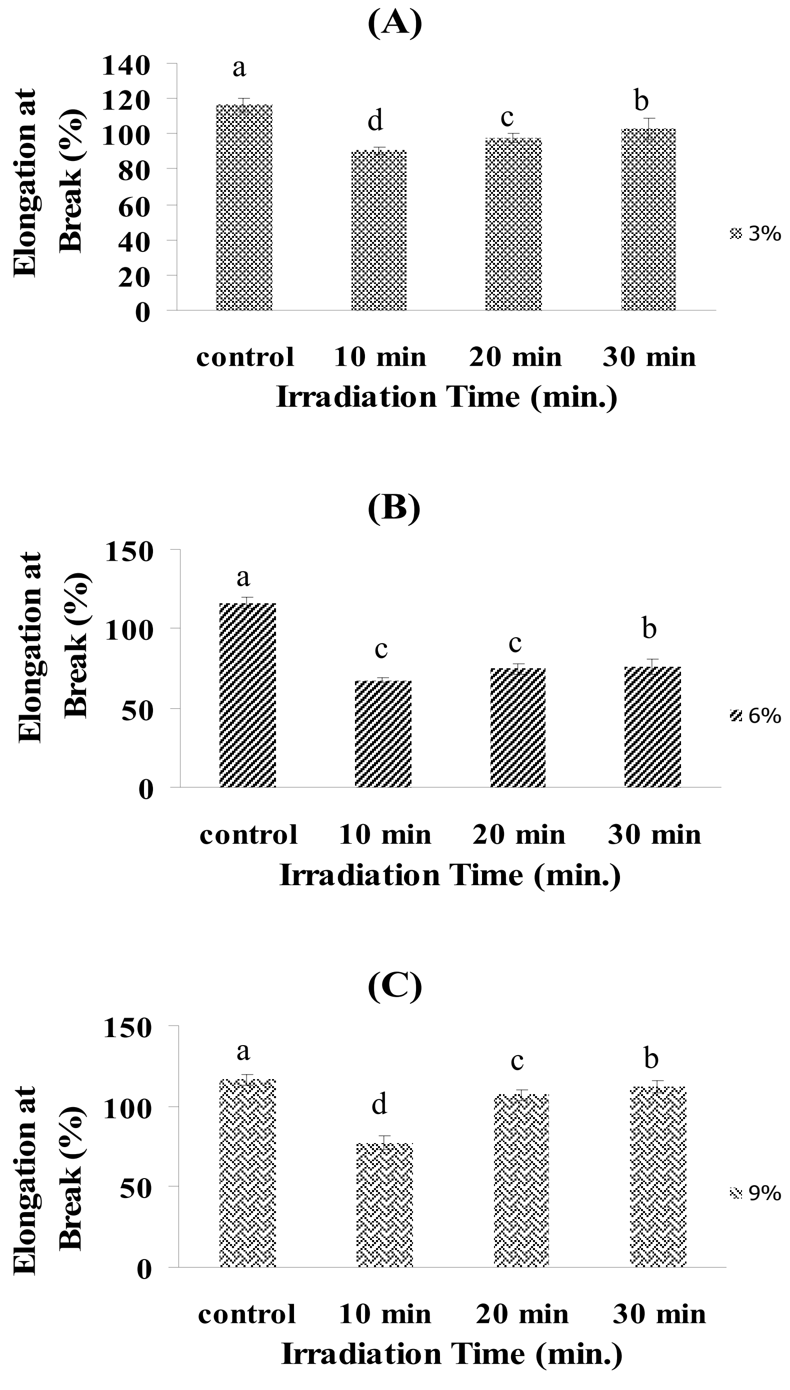


Figure 32. Effect of the irradiation time on Elongation at break of UV treated rice starch films contained various concentration of sensitizer; 3% (A), 6% (B) and 9% (C) and various irradiation time from 10 – 30 min. Mean values with different letter are significantly different ($p<0.05$).

4.2.2 Water vapor permeability (WVP)

Effect of the irradiation time on water vapor permeability (WVP) of UV treated rice starch films contained various contents of sensitizer; 3%, 6% and 9% and various irradiation times from 10 to 30 min was shown in Figure 33(A-C). Results depicted that WVP value increased when increased both irradiation time and sensitizer concentration. In 6%sensitizer (figure 33(B)) the WVP value were dramatically increased when the irradiation time increased. Kumar and Singh (2008) reported that biocomposites of cellulose reinforced starch films was found that water absorption decreased when increasing time of UV irradiation, it is the effect of photo-irradiation. The plasticized starch (NeatSt) reaches its plateau level after 15 min of irradiation whereas composites reach after 20 min. From the results 6% sensitizer still had the lower WVP value than 3% and 9% sensitizer and when the irradiation time increased WPV value of all UV treated rice starch films were reaches its plateau after 20 min. The increasing in WVP value in UV treated rice starch films due to an excess UV light can cause starch molecule chain slit and limit crosslinking reaction and water resistance of UV treated rice starch films were decreased.

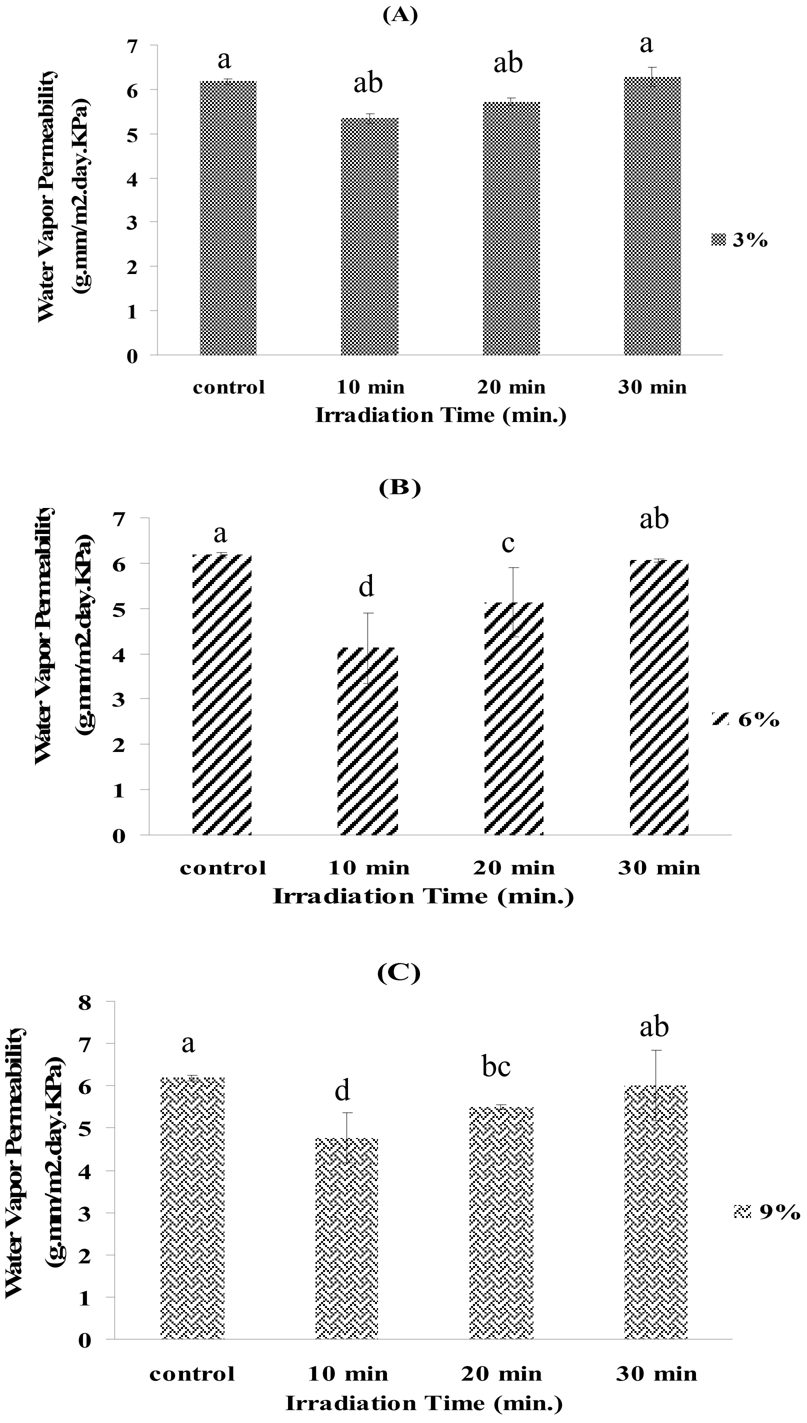


Figure 33. Effect of the irradiation time on Water vapor permeability (WVP) of UV treated rice starch films contained various content of sensitizer; 3% (A), 6% (B) and 9% (C) and various irradiation time from 10 – 30 min. Mean values with different letter are significantly different ($p<0.05$).

4.2.3 Color

Color value was recorded as L* (lightness, 0=black, 100=white), a* (a*=greenness, +a* = redness), and b* (-b* =blueness, +b* =yellowness). In figure 34-36 showed the effect of irradiation time (10 min, 20 min and 30 min) on color (L*, a*, b*) of UV treated rice starch films used sodium benzoate (3%, 6% and 9% wt.) as sensitizer. The results depicted that L* value increased when irradiation time increased, whereas b* value was slightly increased when irradiation time increased since UV treated rice starch films had a lower moisture content. As described by Gontard *et al*. (1993) and McHugh *et al*. (1994), the photocrosslinking had lower moisture contents than the untreated rice starch films. Rhim *et al*. (2000) reported soy protein isolate (SPI) films modified with UV irradiation had slightly lower L* values (decreased lightness) and notably greater b* values (increased yellowness) than untreated rice starch films. From the results found that when the irradiation time was increased induced the photo-oxidation in rice starch films yielded the degradation of polymer and resulted in polymers brittleness, with loss of resistance and changes of color (Berre and Lala, 1989).

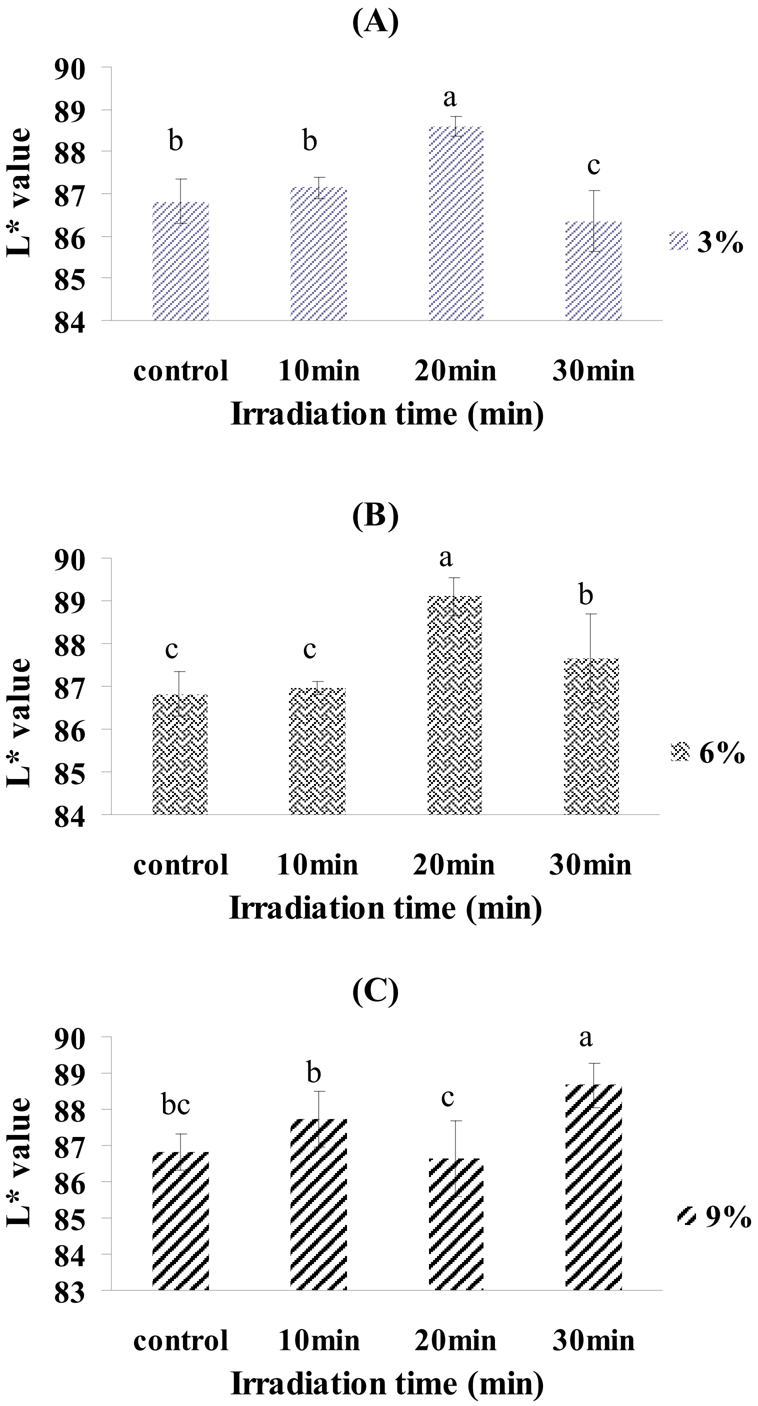


Figure 34. Effect of irradiation time on L* value of rice starch film and UV treated rice starch films contained various content of sensitizer; 3% (A), 6% (B) and 9% (C) with various irradiation time from 10 – 30 min. Mean values with different letter are significantly different ($p<0.05$).

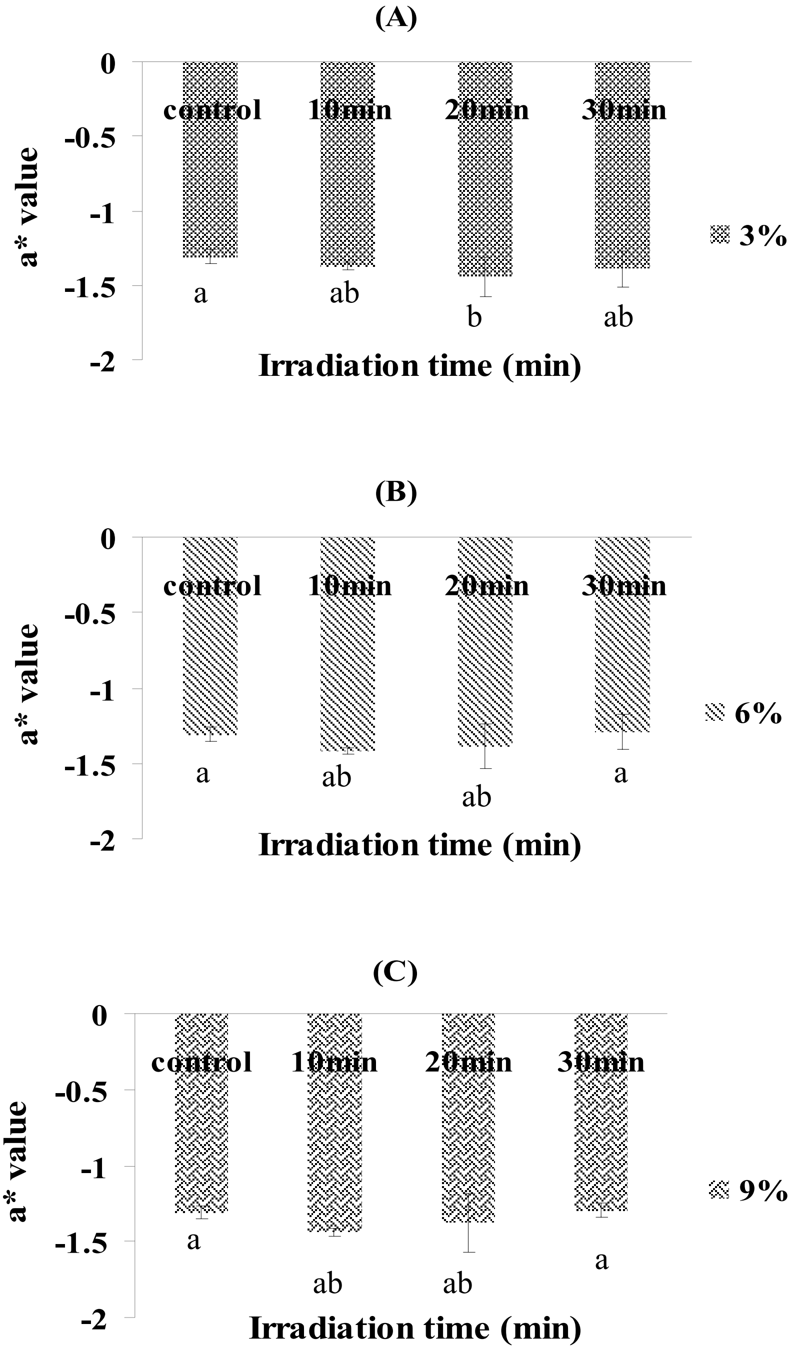
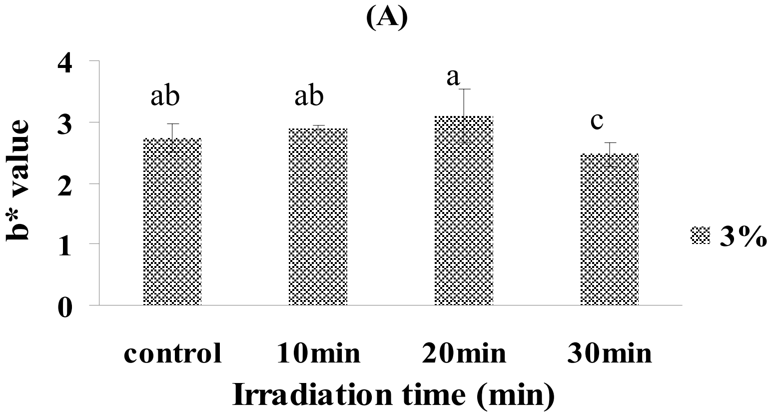
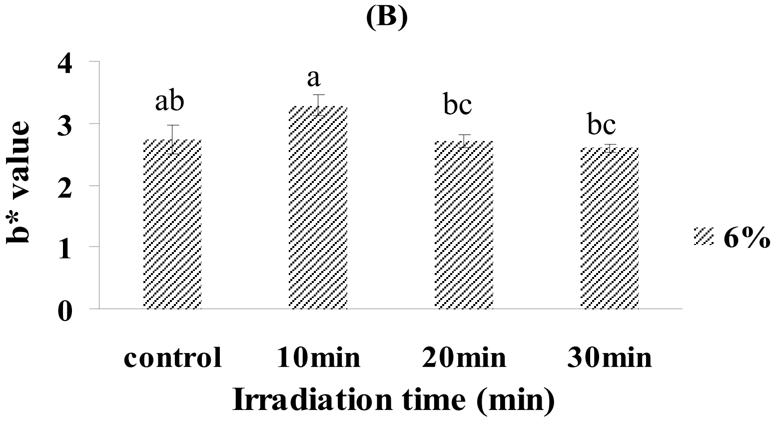


Figure 35. Effect of irradiation time on a* value of rice starch film and UV treated rice starch films contained various content of sensitizer; 3% (A), 6% (B) and 9% (C) with various irradiation time from 10 – 30 min. Mean values with different letter are significantly different ($p<0.05$).





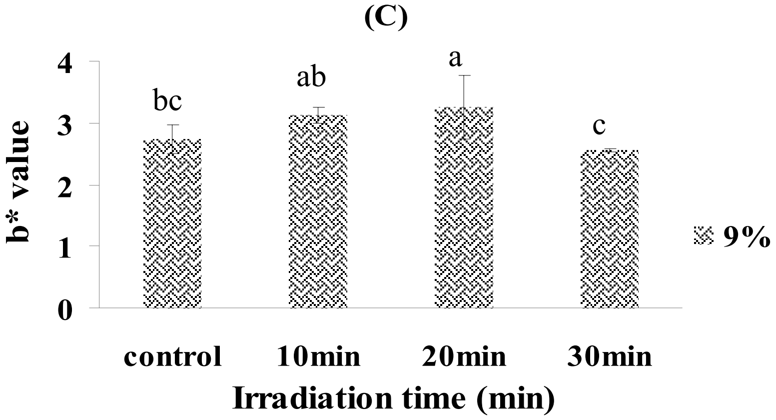


Figure 36. Effect of irradiation time on b* value of rice starch film and UV treated rice starch films contained various content of sensitizer; 3% (A), 6% (B) and 9% (C) with various irradiation time from 10 – 30 min. Mean values with different letter are significantly different ($p<0.05$).

4.2.4 Transparency

Figure 37 shows the transparency value of rice starch film and UV treated rice starch film contained various contents of sodium benzoate (3-9%), and irradiation time from 10 to 30 min at a wavelength of 600 nm. The transparency may be affected by various factors including film thickness (Bangyekan *et al.*, 2006) and it is also of importance in some instances, when used as packaging materials (Phattaraporn *et al.*, 2010). The results showed that addition of sodium benzoate and increasing of irradiation time affected the decreasing transparent (increasing transparency value) of resulted films. The transparency value of rice starch film was 1.09 and those of UV treated rice starch film were 1.87 - 3.2. Rice starch film without UV treated showed the highest transparent (lowest transparency value). However, when the irradiation time increased the transparency of UV treated films were slightly decreased. The presents of sodium benzoate and the films exposed with UV light decreased the transparency. However, it can be said that rice starch film exhibits higher transparent than the UV treated rice starch film.

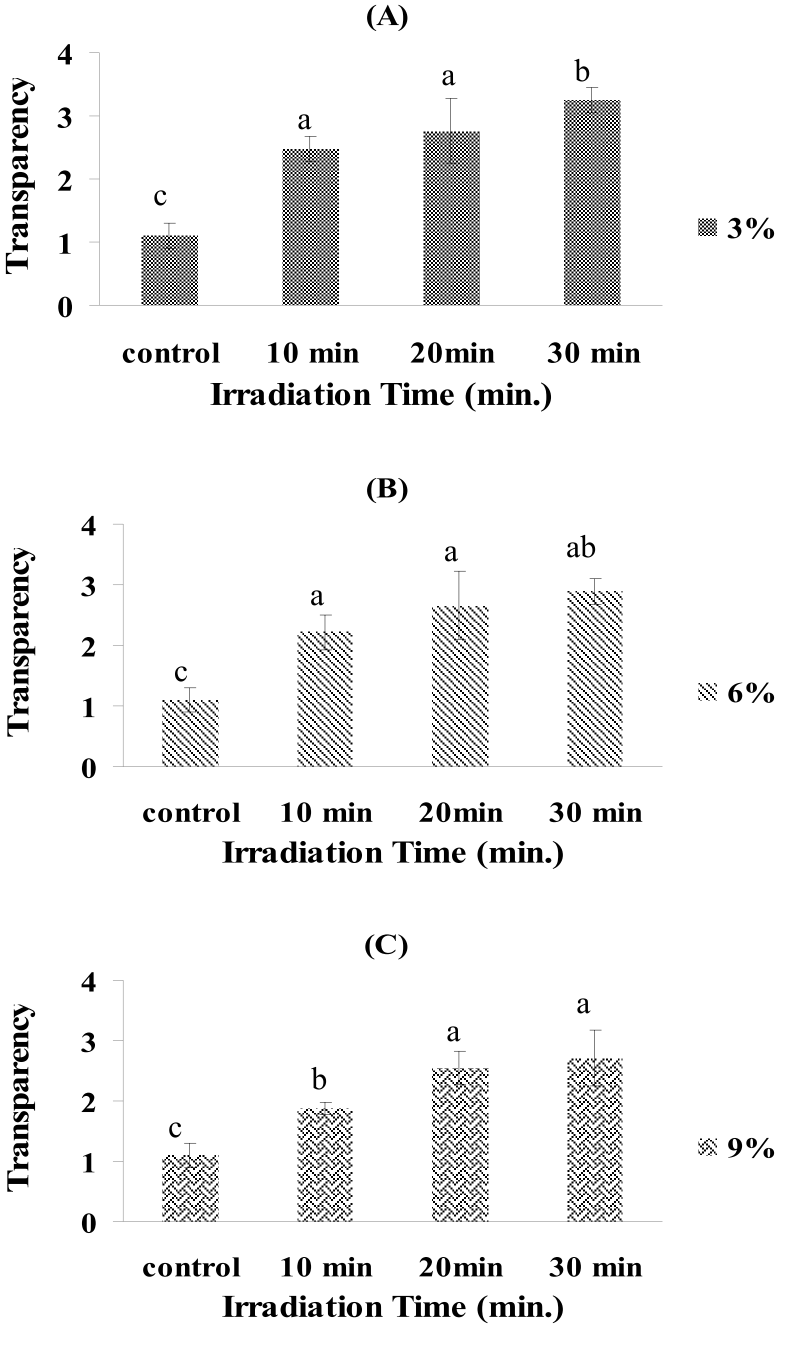


Figure 37. Effect of the irradiation time on transparency of rice starch film and UV treated rice starch films contained various content of sensitizer; 3% (A), 6% (B) and 9% (C). Mean values with different letter are significantly different ($p<0.05$).

4.2.5 Swelling degree and gel fraction

The degree of crosslinking of the starch macromolecular network formed by crosslinking due to UV irradiation can be characterized by the swelling degree (SD) and gel fraction (GF) in Figure 38. GF is directly related to the mass of starch macromolecules involved in the crosslink network and the SD is related to the density of the newly created network (Zhou *et al*., 2008). The results showed that when the irradiation time increased the GF decreased and SD increased due to the crosslink density was decreased from chain scission in starch molecule chain. Rice starch films contained 6% photo-sensitizer demonstrated the lowest of SD and the highest of GF in 10 minutes of irradiation time. Previous work of Delville *et al.* (2002) has demonstrated a network structure formation occurring during UV irradiation of wheat starch films used sodium benzoate as sensitizer, the Gel Fraction (GF) and Swelling Degree (SD) were measured in DMSO. They reported that crosslinking densities can be varied by altering the UV exposure time: 1-10 min and the final crosslinking is relatively low compared with synthetic thermosets. The irradiation time showed better crosslinking density of rice starch films and using 10 min provided the lowest SD. The SD decrease and GF increased with irradiation, which is characteristic of network formation (Delville *et al*., 2002).

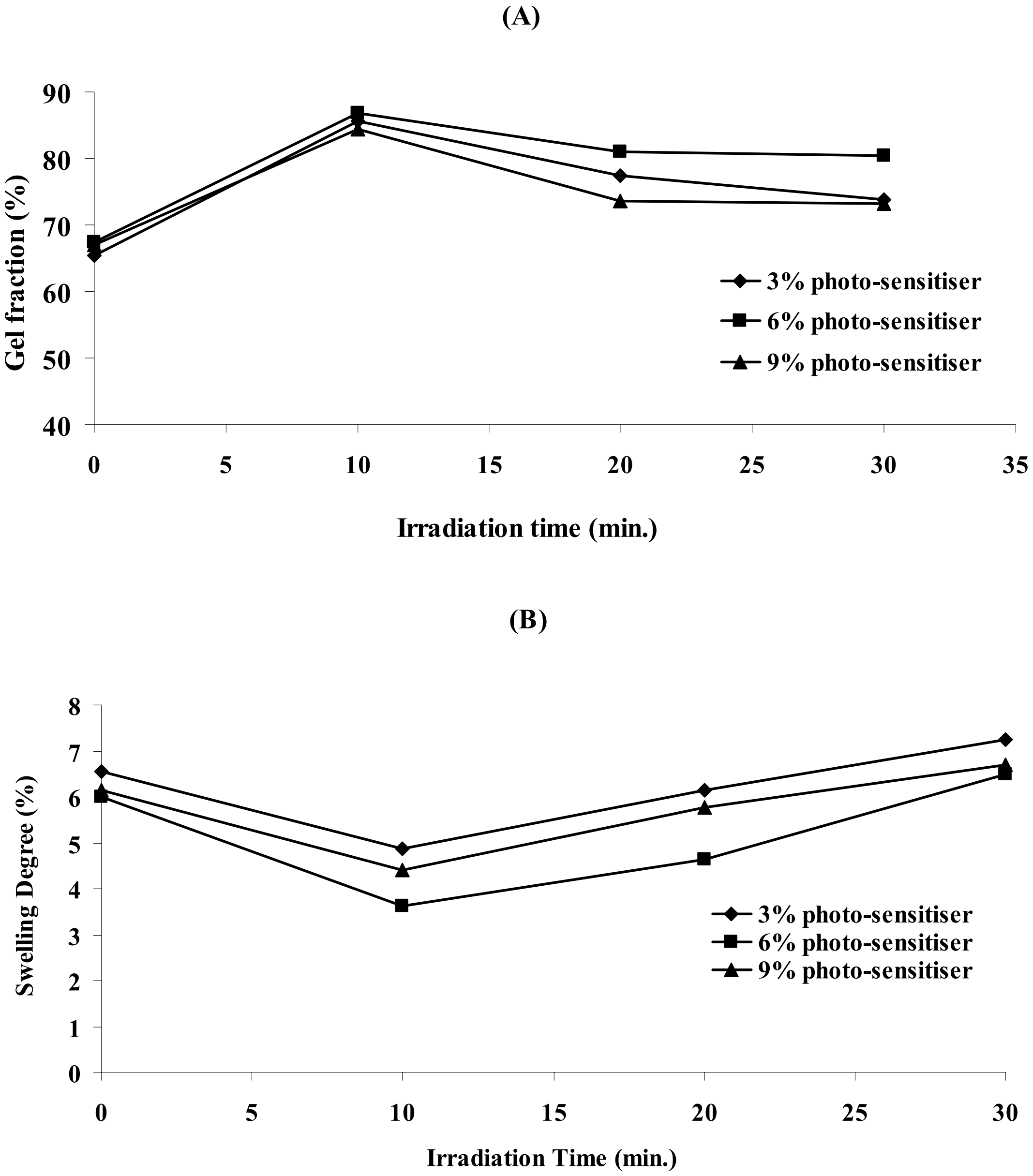


Figure 38. Effect of the irradiation time on Gel fraction (GF): A and Swelling degree (SD): B of rice starch film and UV treated rice starch films contained various concentration of sensitizer; 3% (B), 6% (C) and 9% (D) sensitizer. Mean values with different letter are significantly different ($p<0.05$).

4.3 Effect of relative humidity (before irradiation) on rice starch film properties

According to the previous study addition of 6% sodium benzoate into rice starch films and 10 min. of irradiation time provided a good mechanical and barrier properties of resulted films. Hence, the rice starch films with 6% sodium benzoate were selected for investigating the effect of relative humidity of rice starch film (before irradiation) on the properties of rice starch films.

4.3.1 Mechanical properties

Tensile strength (TS) and Elongation at break (E) of the UV treated rice starch film using 6% sensitizer and kept the films in various relative humidity from 0 %RH to 98 %RH before irradiated with UV light for 10 min were shown in Figure 39 (A-B). Basically, starch is hygroscopic and will gain or lose water to achieve equilibrium with the ambient air (Christensen *et al.*, 1982). Water is also a plasticizer for starches (Hoseney, 1994). Water reacts differently depending on the level of water content in the system. It is successively adsorbed on primary sites (monomolecular layer of water molecules bound via strong H-bonds which is comparable to BET layer (Brunauer *et al.*, 1938)). Followed by multilayers and finally condensed into a liquid phase. The first steps of water adsorption can easily be explained by stoichiometric ratios of water molecules bound to polysaccharide hydroxyls (OHs). It was recently found (Fringant *et al.*, 1996; Joly *et al.*, 1996) that water molecules were doubly bound to sorption sites in polysaccharides: (i) the sorption energy determined using microcalorimetry shows a decrease in function of equilibrium relative humidity (ERH); (ii) moreover, the water content at 80% ERH was assigned to the complete saturation of OHs assuming a double bridging mechanism (Fringant *et al.*, 1996). From the results found that TS trend to decrease when the relative humidity increased. Rice starch film that kept at 0% RH became a brittle films resulting in high TS value and low E value because the films had a low free volume and limited molecular mobility. Lawton (1996) reported TS of the PVA/starch films decreased as relative humidity increases, which is consistent for polymers that contain increasing amounts of plasticizer. Where with water is an excellent plasticizer but it has some disadvantages since water content varies with humidity. At low humidity there are problems with brittleness and at high humidity with softness (Bangyekan *et al.*, 2006).

Tongdeesoontorn *et al*. (2011) prepared the cassava starch (5%w/v) based films and kept films in varied relative humidity (34 and 54%RH) on the mechanical properties of the films reported the TS and E of cassava starch based films were inversely related. At the lower % RH, cassava starch based films had significantly higher TS but lower E than untreated rice starch films. Figure 39(B) showed effect of relative humidity on E of rice starch films. The results demonstrated that when relative humidity increase provided an increasing of elongation at break. Since water is a plasticizer, changing the water content in the film will change the properties of the films. Increasing plasticizer levels in synthetic plastic has been shown to increase %E (Sears and Touchette, 1989). Lawton and Fanta (1994) reported Glycerol-plasticized films prepared from starch-poly(vinyl alcohol) mixtures were increased in %E when poly(ethylene-co-acrylic acid) (EAA), PVA and glycerol were increased together. Equilibration of starch film samples at relative humidities lower than 50% severely reduced %E and increased ultimate TS (UTS), indicating the importance of the plasticizing effect of water.

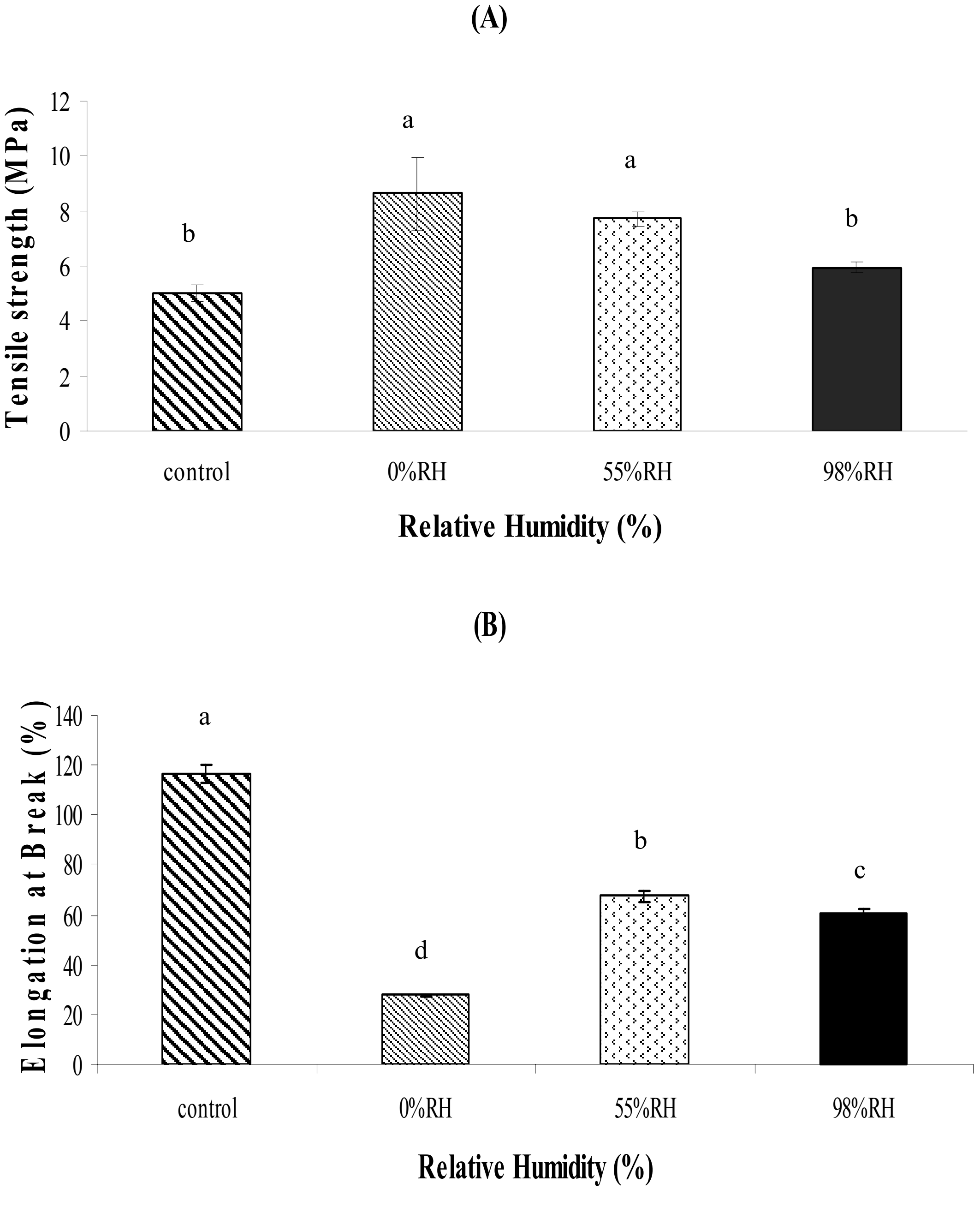


Figure 39. Effect of the relative humidity of rice starch films on tensile strength (A) and Elongation at break (B) of UV treated rice starch films. Mean values with different letter are significantly different ($p<0.05$).

4.3.2 Color

The results of the measurements performed on the 6% sensitizer UV treated rice starch film's color were expressed in accordance with CIELAB system and the rectangular coordinates (L*, a* and b*) were calculated**.** Figure 40 showed the effect of relative humidity on rice starch film (before irradiation) on color (L*, a*, b*) of rice starch films. The L* value and a* value of UV treated rice starch films were not significantly different ($p<0.05$) when relative humidity increased. The b* value of the rice starch films increased with increased of relative humidity. This indicated that the increasing yellowness (b*) of film, which could be attributed to the increasing of plasticizers (water) within rice starch films and the photo-oxidation in rice starch films.

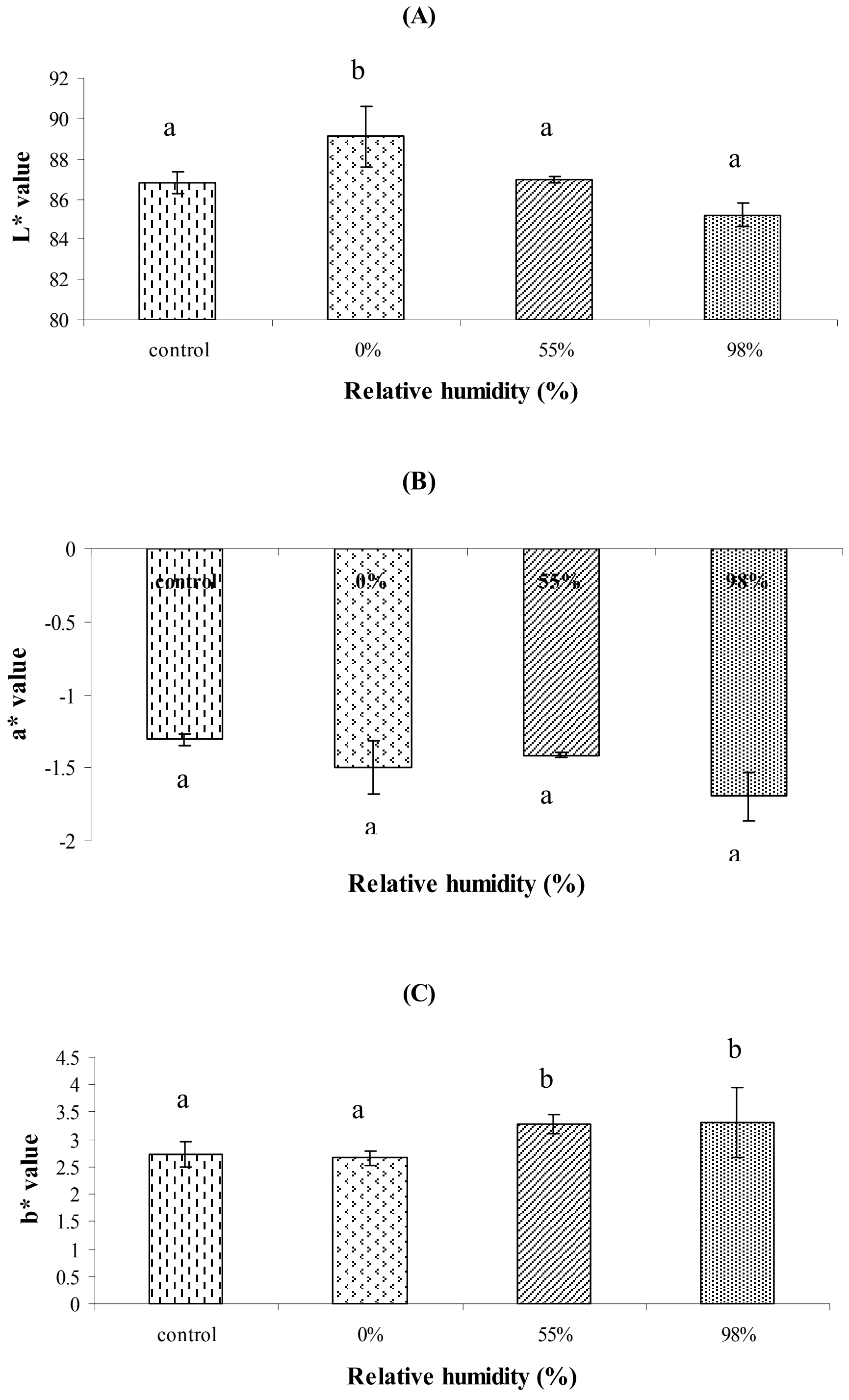


Figure 40. Effect of relative humidity on L* (A); a* (B) and b* (C) of UV treated rice starch films; 6% sensitizer. Mean values with different letter are significantly different ($p<0.05$).

5. Effect of Relative Humidity on Properties of UV treated and Crosslinked Rice Starch films.

The moisture content in starch films can also affect significantly the physical and barrier properties of starch-based films; due to their inherent hydrophilic nature, starch films tend to absorb large quantities of water at elevated relative humidity (RH) conditions. The moisture content can affect significantly the physical and barrier properties of starch films (Mali *et al*., 2005). Therefore, there is a need to address the sorption influence of starch present in the films in order to make it suitable for food packaging. Moisture sorption curves of rice starch film, crosslinked rice starch (0.5%EPI, 3% STMP and 8%STMP/STPP) and UV treated rice starch films contained 6% sensitizer were shown in Figure 41. As time increased, the sorption was more rapid at the initial stages and lower amounts of water were absorbed. Then, the moisture content of the films reached a plateau indicating that they became equilibrated with surrounding RH. The films that stored at 90%RH needed more time to reach the equilibrium and untreated film showed a trend to adsorb more water than crosslinked rice starch films and UV treated rice starch films. Chang *et al*. (2000) who prepared crosslinked tapioca film reported the modification of starch by crosslinking did not appear to affect the sorption properties of the films. Most crosslinking reactions produce a relatively low degree of crosslinking sufficient to strengthen the outer envelope of the starch granules. This amount of crosslinking, although probably having a substantial effect on certain properties (for example, granule swelling, paste viscosity, and retrogradation), may actually contribute little to water sorption properties, as has been previously reported for potato starches (Chilton and Collison, 1974). However, the results demonstrated that using 3% STMP crosslinked rice starch film showed lower moisture absorption than other films. Due to 3% STMP rice starch film has a high degree of crosslinking. The crosslinks appear to be much more effective in preventing the water absorption of films (Hirsch and Kokini, 2002). Figure 42 showed the TS of rice starch films at different relative humidities. The results depicted the TS of untreated rice starch film (control), crosslinked rice starch films (0.5%EPI, 3%STMP and 8%STMP/STPP) and UV treated rice starch films contained 6% sensitizer were decreased as the relative humidity increased. The rice starch films were strongly sensitive to the moisture content and being in the rubbery state at room temperature. From the results found that rice starch films treated with photo-crosslinking has a lower TS value than rice starch that treated with chemical crosslinking due to the crosslinked rice starch films had a higher crosslinking density and lower granule swelling. Mali *et al*. (2005) reported a decrease in the tensile stress was evidenced when RH increased and the tensile stress-strain curves of cassava starch film show the more brittle character of the starch film stored at low relative humidity.

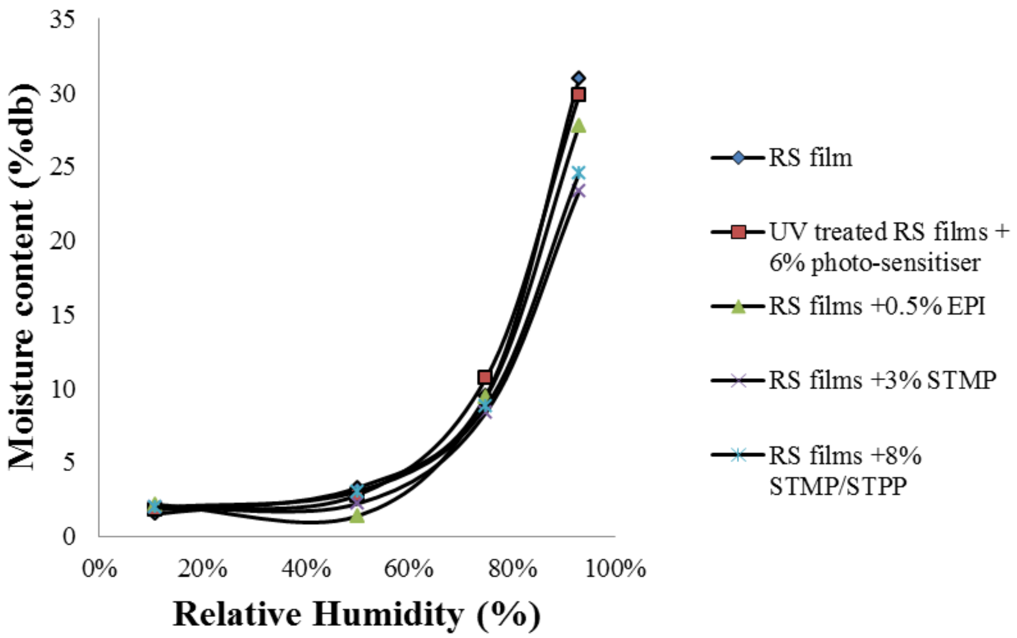


Figure 41. Moisture sorption isotherm curves of rice starch film (control), UV treated rice starch films +6% sensitizer, rice starch films +0.5% EPI, rice starch films +3% STMP, rice starch films +8% STMP/STPP.

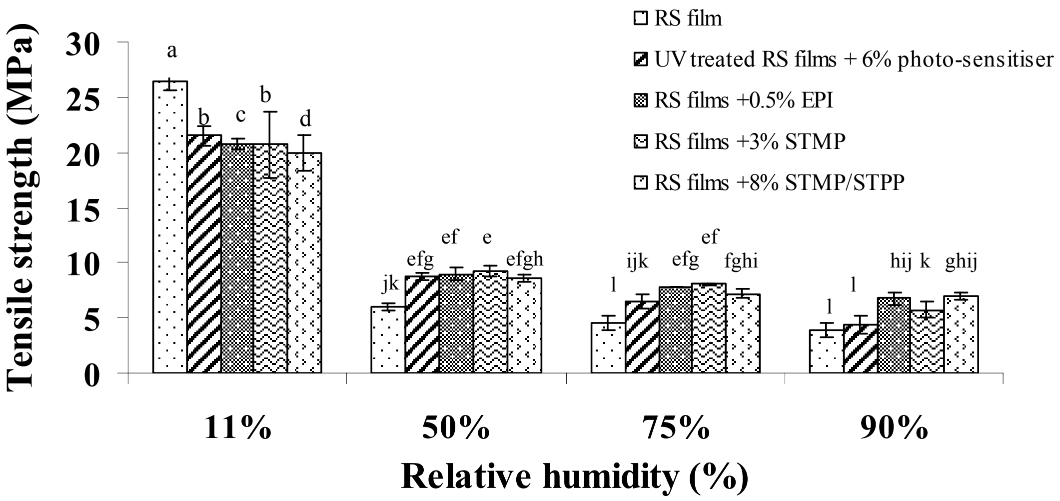


Figure 42. Tensile strength of the rice starch film (control), UV treated RS films +6% sensitizer, rice starch films +0.5% EPI, rice starch films +3% STMP, rice starch films +8% STMP/STPP. Mean values with different letter are significantly different ($p<0.05$).

CHAPTER 4

CONCLUSION

1. The viscosity of cross-linked rice starch decreased when the contents of cross-linking agent increased concomitant with increasing of pasting temperature. Addition, the degree of cross-linking increased when concentration of cross-linking agents increase.

2. The cross-linked rice starch showed the improvement of rice starch film properties. The mechanical and water barrier properties of rice starch films were enhanced by the cross-linking reaction between rice starch and cross-linking agent. Furthermore, increasing of concentration of cross-linking yielded the enhancement the thermal properties of rice starch films causing the glass transition temperature (T_g) shifted toward higher temperature. STMP and the mixture of STMP/STPP showed a higher cross-link density than EPI.

3. UV treatment using sodium benzoateas photo-sensitiser could improve the rice starch film properties. The increasing in mechanical and water barrier properties was obtained when photo-cross-linking was applied. The highest mechanical, water barrier and thermal propertieswere obtained when 6% of photo-sensitiser was used. However, too excessive amount of photo-sensitiser (9%) resulted in lower cross-linking density. The glass transition temperature (T_g) shifted toward higher temperature when increasing amount of photo-sensitiser.

4. The optimum conditions provided the suitable rice starch film properties were received when 10 minutes of irradiation time and 55%RH was applied. Moisture absorption of rice starch films was more rapidly in the initial stage of moisture absorption and showed decreasingwhen the absorption time increased.

5. The rice starch film cross-linked with 3% STMP showed the lowest moisture absorption when storage in the low relative humidity. In addition, the lowest TS of rice starch films were observed when storage in higher relative humidity.

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APPENDIX

ANALYTICAL METHODS

1. Moisture content (AOAC, 1999)

Method

1. Dry the empty dish and lid in the oven at 105°C for 30 min and transfer to desiccator to cool (30 min). Weigh the empty dish and lid.
2. Weigh about 5 g of sample to the dish. Spread the sample with spatula.
3. Place the dish with sample in the oven. Dry for 16 h or overnight at 105°C.
4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried content.

Calculation

$$\% \text{ Moisture} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where W_1 = weight (g) of sample before drying

W_2 = weight (g) of sample after drying

2. Fat (AOAC, 1999)

Reagent

- Petroleum ether

Method

1. Place the bottle and lid in the incubator at 105°C overnight to ensure that weight of bottle is stable.
2. Weigh about 3-5 g of sample to paper filter and wrap.
3. Take the sample into extraction thimble and transfer into soxhlet.
4. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle.
5. Connect the soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 14 h (heat rate of 150 drop/min).
7. Evaporate the solvent by using the vacuum condenser.
8. Incubate the bottle at 80-90°C until solvent is completely evaporate and bottle is completely dry.
9. After drying, transfer the bottle with partially covered lid to the desiccator to cool. Reweigh the bottle and its dried content.

Calculation

$$\%\ \text{Fat content} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

3. Ash (AOAC, 1999)

Method

1. The crucible and lid is firstly placed in the furnace at 550°C overnight to ensure that impurities on the surface of crucible is burned off. The crucible is then cool in the desiccator (30 min).
2. Weigh the crucible and lid to 3 decimal places.
3. Weigh about 5 g sample into the crucible. Heat over low Bunsen flame with lid haft covered. When fumes are no longer produced, place crucible and lid in furnace.
4. Heat at 550°C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
5. Weigh the ash with crucible and lid when the sample turns to gray. If not, return the crucible and lid to the furnace for the further ashing.

Calculation

$$\%\ \text{Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

4. Protein (AOAC, 1999)

Reagents

- Kjedahl catalyst: Mix 9 part of potassium sulphate (K_2SO_4) anhydrous, nitrogen free with 1 part of copper sulphate ($CuSO_4$)
- Sulfuric acid (H_2SO_4)
- 40% NaOH solution (w/v)
- 0.2 N HCl solution
- 4% H_3BO_3
- Indicator solution: Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol)

Method

1. Place sample (0.5-1.0 g) in digestion flask.
2. Add 5 g Kjeldahl catalyst, and 200 ml of conc. H_2SO_4.
3. Prepare a tube containing the above chemical except sample as blank. Place flasks in inclined position and heat gently unit frothing ceases. Boil briskly until solution clears.
4. Cool and add 60 ml distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser, and with tip of condenser immersed in standard acid and 5-7 indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 is distilled.

6. Remove receiver, wash tip of condenser, and titrate excess standard acid distilled with standard NaOH solution.

Calculation

$$\% \text{ Protein} = \frac{(A-B) \times N \times 1.4007 \times 5.95}{W}$$

Where:

A = volume (ml) of 0.2 N HCl used sample titration

B = volume (ml) of 0.2 N HCl used in blank titration

N = Normality of HCl

W = weight (g) of sample

14.007 = atomic weight of nitrogen

5.95 = the protein-nitrogen conversion factor for starch and its by-products

5. Amylose Content

Reagents

- 95% Ethanol
- 1N HCl
- 1N NaOH
- 0.2% Iodine solution
- Standard amylose

Instruments

- Water bath
- Spectrophotometer

Method

1. Weigh 100 mg rice starch into 100 ml volumetric flask.
2. Add 1 ml 95% ethanol and 50 ml distilled water.
3. Heat the sample for 20 minutes in boiling water bath, cool it in room temperature and make up the volume to 100 ml.
4. Pipette out 5 ml starch solution adds it into 50 ml distilled water and neutralized with 1N HCl.
5. Add 2ml Iodine solution and make up the volume to 100 ml
6. Stand for 20 minutes and determine the % Transmittance at 630 nm using a Spectrophotometer and calculate the equivalent of amylose content.

Calculation

$$AE = \frac{R \ x \ A \ x \ 20}{A \ x \ R}$$

Where:

AE = Amount of soluble amylose
R = Sample absorbance
A = Standard amylose absorbance
r = Sample weight (db)
a = Standard amylose weight

6. Specific heat change values (△Cp)

Calculation

$$Cp = \frac{E \text{ x } H \text{ x } 60}{Hr \text{ x } M}$$

△Cp = Cp (literature value) – Cp (measured value)

Where:

Cp = Specific Heat Capacity (J/g/°C)

E = Calibration Constant (dimensionless)

H = Heat Flow (mW)

60 = conversion constant (min → sec)

Hr = Heating Rate (°C/min)

M = Sample Mass (mg)

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