

Soy protein polymers: Enhancing the water stability property

by

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ABSTRACT

Soy protein based plastics have been processed in the past by researchers for various short-term applications; however a common issue is the high water sensitivity of these plastics. This work concentrates on resolving this water sensitivity issue of soy protein polymers by employing chemical and mechanical interaction at the molecular level during extrusion. The primary chemical interactions employed were anhydride chemistries such as maleic anhydride (MA), phthalic anhydride (PTA), and butylated hydroxyanisole (BHA). These were respectively used in conjunction with glycerol as a plasticizer to produce relatively water stable soy protein based plastics. Formulations with varying additive levels of the chemistries were extruded and injection molded to form the samples for characterization. The additive levels of anhydrides were varied between 3-10% tw/tw (total mass). Results indicated that phthalic anhydride formulations resulted in highest water stability. Plastic formulations with concentration up to 10% phthalic anhydride were observed to have water absorption as low as 21.5% after 24 hrs of exposure to water with respect to 250% for the control formulation. Fourier transform infrared spectroscopy (FTIR) was utilized to characterize and confirm the fundamental mechanisms of water stability achieved by phthalic and maleic anhydride chemistries. In addition, the anhydride formulations were modified by inclusion of cotton fibers and pretreated cotton powder in order to improve mechanical properties. The incorporation of cotton fibers improved the dry strength by 18%, but did not significantly improve

the wet state strength of the plastics. It was also observed that the butylated-hydroxy anisole (BHA) formulation exhibited high extension values in the dry state and had inferior water absorption properties in comparison with anhydride formulations.

CHAPTER 1: INTRODUCTION

Over the past two decades the global economy has been largely influenced by the overall supply and demand of energy, which, to date, is mostly derived from petroleum. The ultimate benefactors of such circumstances have been those countries that hold large reserves of crude oil and ultimately dictate the petroleum prices worldwide. Adding to the complexity of the situation, the war in the Middle East has led to strained diplomatic ties and increased demand and competition for energy among developed nations. Throughout history, after certain events such as the advent of the internal combustion engine; societies have become increasingly reliant on petroleum for its needs of propulsion and energy. Figure 1 shows the energy consumption in the United States over the last 60 years [1], and it can be seen that fossil fuels are the most preferred source of energy, which bolsters the statement.

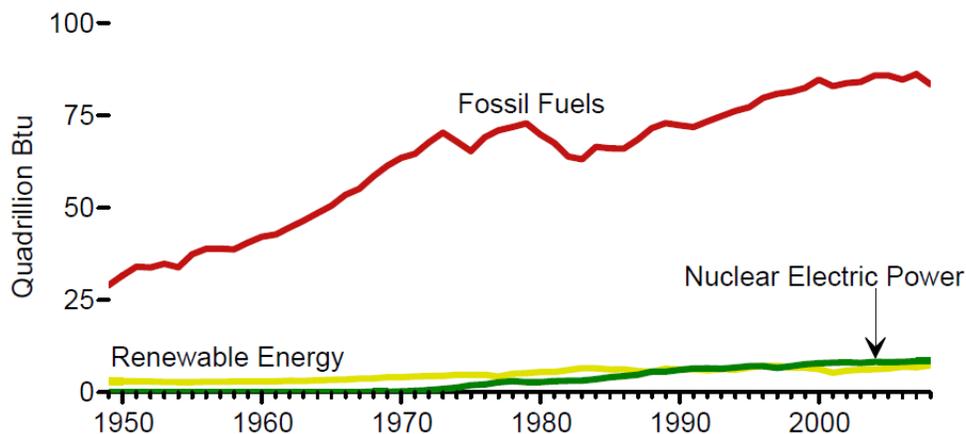


Figure 1 Primary energy consumption (by source), in the United States.[1]

One of the key milestones in the history of materials was the discovery of nylon in 1935 which led us into the new era of petroleum plastics. Since then, many different petroleum based polymers such as polyethyleneteraphthalate (PET), polypropylene (PP), polytetraflouroethlyene (PTFE, Teflon) have been discovered and we have been successful in utilizing these polymers in various applications raging from clothing to building materials. Despite their versatility, petroleum polymers have disadvantages, with disposal being one of the major issues. The commonly used petroleum polymers are innately non-biodegradable due to their chemical makeup and molecular bonding. This property of petroleum polymers, regardless of whether they are disposed in a landfill or in the sea, causes environmental pollution [2] ultimately affecting these environments.

1.1 Abatement of Petroleum Polymer usage

In order to counter the pollution caused by disposal of petroleum plastics, various solutions based on reduction of their use have been proposed and some have been implemented into practice during recent times. Some of the most prominent solutions are recycling of plastics, use of modified/blended petroleum plastics, use of biodegradable plastics produced from fermented sugars and starches, and finally, the use of protein based plastics. The following sections discuss the advantages and disadvantages of each of these solutions in detail respectively; the section on protein plastics is discussed in greater detail as this research concentrates on improving the wet properties of soy protein polymers.

1.1.1 Recycling of petroleum plastics

The process of recovery and recycling of plastics from the waste stream is one of the major post-use processing of plastics besides energy recovery through incineration and landfills. Insight into recycling can be gained by reviewing the statistics published by the Environmental Protection Agency (EPA). As detailed by the EPA, the total amount of municipal solid waste (MSW) generated during 2008 in the United States was 250 million tons and 12% of which (30 mil. tons) comprised of plastics [3] as detailed in Figure 2.

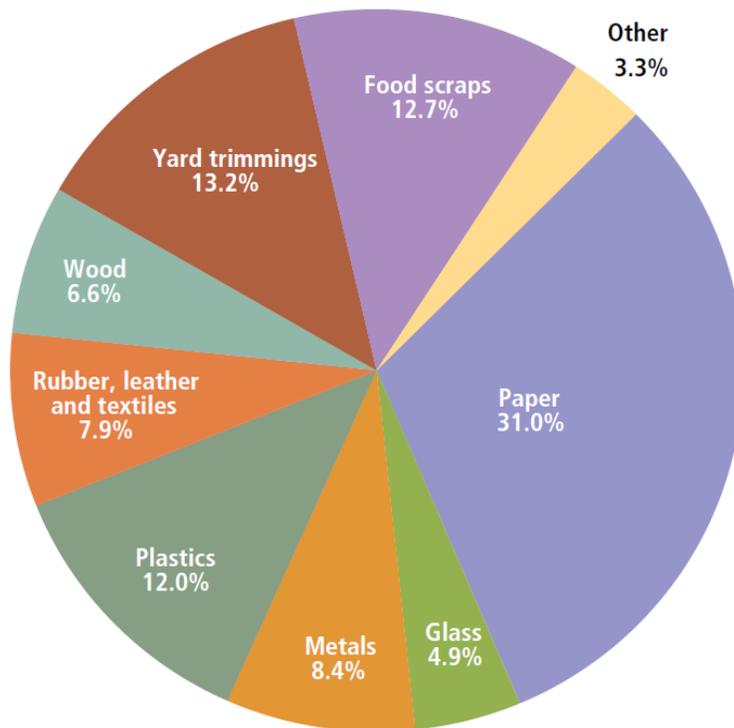


Figure 2 Profile of total MSW generated during 2008 (by Material), 250 Million tons (before recycling).[3]

It should be noted that these numbers only account for plastic wastes generated by the common household (packaging of non durable and durable goods). Plastics used in automobiles for example, are not included in these figures. A detailed analysis of data indicates that only 7.1% (2.12 mil tons) of the total plastic waste generated (30.05 mil tons) is recycled and this is detailed in Table 1 [3].

Table 1 Generation and recovery of materials from MSW, 2008(in million tons and percent generation of each material).[3]

Material	Weight Generated	Weight Recovered	Recovery as Percent of Generation
Paper and paperboard	77.42	42.94	55.5%
Glass	12.15	2.81	23.1%
Metals			
Steel	15.68	5.29	33.7%
Aluminum	3.41	0.72	21.1%
Other nonferrous metalst	1.76	1.21	68.8%
Total metals	20.85	7.22	34.6%
Plastics	30.05	2.12	7.1%
Rubber and leather	7.41	1.06	14.3%
Textiles	12.37	1.89	15.3%
Wood	16.39	1.58	9.6%
Other materials	4.50	1.15	25.6%
Total materials in products	181.14	60.77	33.5%
Other wastes			
Food, other‡	31.79	0.80	2.5%
Yard trimmings	32.90	21.30	64.7%
Miscellaneous inorganic wastes	3.78	Negligible	Negligible
Total other wastes	68.47	22.10	32.3%
Total municipal solid waste	249.61	82.87	33.2%

These low rates of recovery leave a large amount of plastic waste for either landfill or incineration which in turn causes soil pollution and CO₂ emissions adding to the green house effect respectively [2]. Additionally, other nations worldwide dispose of their waste in the ocean, which results not only in marine pollution but also harmful to sea animals. Even though the recycling rate has increased since 1960 as depicted in Figure 3 [3], major issues related to quality and economics of recycled products have impeded recycling rates significantly.

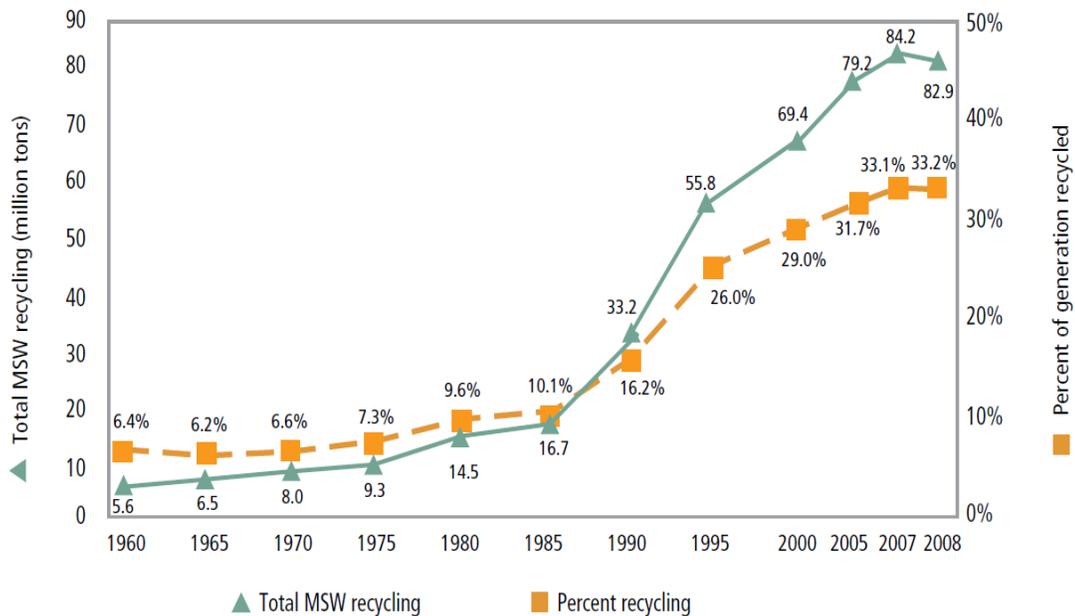


Figure 3 Municipal solid waste recycling rates (1960- 2008). [3]

The heterogeneous mixture of plastics found in MSW and ultimately in the recycling stream has been one of the major issues, because this affects the

reprocessing and the properties of the final recycled products [4]. Concerns regarding lower energy efficiency and cost effectiveness of reprocessing waste stream plastic with respect to economics of using virgin petroleum plastics has also been a factor that has impeded recycling [5].

1.1.2 Modified/Blended petroleum plastics

One of the other approaches to reducing the use of petroleum plastics is to form blends. In order to reduce the overall plastic content in the final product, petroleum plastics have been blended with bio-degradable additives such as starches, proteins, and other plant material and particulates such as fibers, wood chips, and corn cobs. Despite the reduction in the amount of petroleum plastics with this method, upon degradation there remains residue of plastics [2]. An additional problem is that the residue after degradation of such blended plastics can affect the bioactivity of the compost or the degrading environment [6]. Researches and recyclers have expressed their concerns about such blended plastics entering the recycling stream, primarily because, studies indicate that the contamination caused by the bio-components causes problems during processing in terms of change in melt temperature as well as the overall quality of the final recycled product [4].

1.1.3 Bioplastics (starch based and fermented sugar based)

Recently, greater social awareness and extensive public education regarding the adverse effects and pollution caused by petroleum based plastics coupled with higher petroleum prices has led to the increase in the demand for green (environmentally friendly) polymers. Table 2 details the recent trend in consumption of petroleum plastics; it is observed that since 2004 the overall consumption has dropped by 14% (84 K to 72 K million pounds) [7].

Table 2 Thermoplastics resin sales by major market 2004 -2008

(Millions of pounds, dry weight basis).[7]

Major Market	2004	2005	2006	2007 ¹	2008 ¹
Transportation	4,899	4,647	4,559	3,312	2,751
Packaging	25,952	25,118	26,280	26,527	24,097
Building and Construction	15,676	15,417	15,486	14,289	12,313
Electrical/Electronic	3,096	2,863	2,641	1,980	1,755
Furniture and Furnishings	3,458	3,403	3,332	3,091	2,671
Consumer and Institutional	18,714	17,352	17,823	17,193	15,461
Industrial/Machinery	1,042	998	1,004	943	834
Adhesives/Inks/Coatings	1,196	1,160	1,078	1,069	937
All Other	2,168	2,048	2,031	1,604	1,375
Exports	9,900	9,624	10,244	12,346	11,962
Total	86,101	82,629	84,478	82,354	74,156

Some of the commercially successful examples of green polymers are biopolymers such as polylactic acid (PLA) and poly-hydroxyalkanoates (PHA) which are synthesized by microbial fermentation of sugars from agricultural feed

stocks. The benefits of these bioplastics can be well comprehended by referring to Figure 4, which depicts a cradle to grave model with a recycling sub-process [8]. Under ideal conditions, the cycle is a closed carbon loop with no further generation of carbon emissions due to the carbon sequestration by photosynthesis. Environmentally, such a process would be the ideal situation except that such a closed loop carbon cycle is difficult to achieve realizing the fact that fossil fuels are the source of energy for the harvesting and fermentation (synthesis) steps in the cycle [9].

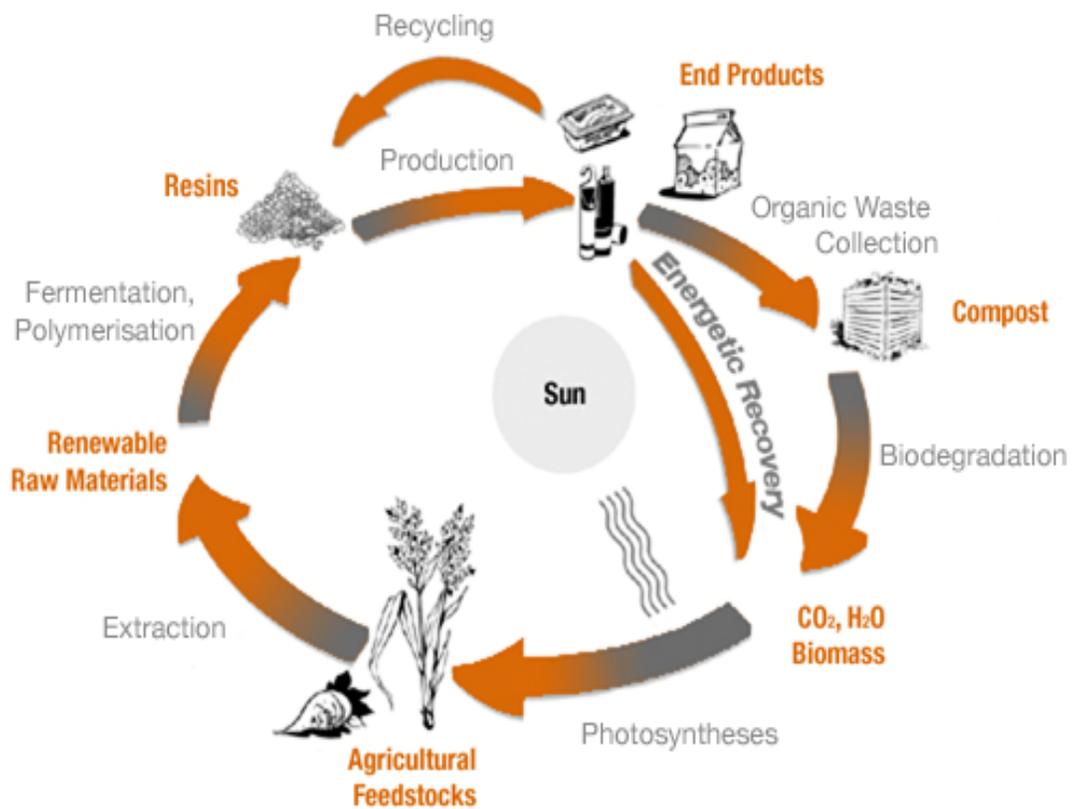


Figure 4 Closed loop life cycle of bioplastics. [8]

With the issues detailed of the previous lifecycle, the issue becomes more complex when considering the recent increase in food prices. According to food economist the increase in food prices are due the use of agricultural feed stocks for biofuels and sugar based plastics causing a shortfall in food supply [10]. However, such an argument can be misleading taking into account the facts and arguments presented by biofuel researchers and economists. Researchers in the field of biofuels argue that the better overall energy efficiency and lower cost of biofuels with respect to petroleum will reduce transportation costs which will ultimately lower food prices. This argument is reinforced by the fact that increase in food prices were in tandem with the increase in crude oil prices that reached \$200 per barrel and not because of biofuels [11]. Though these plastics have matured into commercial products, despite the socio-economic issues discussed earlier, there still exist some processing issues which need to be resolved, thus making it difficult to control the quality of the product made from materials these materials [12].

1.1.4 Protein based plastics

World leading economies, both industrialized and emerging nations have reached a consensus that the development of environmentally friendly materials and sustainable energy sources are essential to reduce their respective contribution on pollution and ultimately the carbon footprint. In light of this, some of the commercially available biopolymers and plastics on the market are polyesters

derived from sugar fermentation or cultured microorganisms which include polymers such as polylactic acid (PLA) and polyhydroxyalkanoate (PHA) polymers. Other classes of bioplastics include starch based thermoplastics and petroleum derived biodegradable polymers such as poly-caprolactone. The other prospective candidates have been protein polymers from plants for the longest time in history [13]. The most suitable and versatile molecules have been the proteins derived from soy and corn due to their higher order of molecular weight and thermoplastic behavior after plasticizing. In the past, a significant amount of research has been completed by researchers on soy protein based plastics for commercial plastic application [14,15]. Plant protein polymers, being derived from agricultural feedstocks, are the only class of biorenewable polymers with which we can overcome the concerns of affecting feedstock. It can be realized from the fact that protein polymers can be sourced from agricultural co-products avoiding the need of designated crops for protein polymer extraction. An attractive proposal is the utilization of co-products generated by the soy and corn based bio-fuels industry to enable a better economic and viable feedstock for plant proteins as a bioplastics. The utilization of the co-products also improves the efficiency of the bio-fuel process, thus reducing their process and raw material cost.

One issue of soy protein based plastics that has restricted their commercialization has been their sensitivity to water due to the hydrophilic nature of the protein molecule [14,16]. Significant amount of research effort that has

focused on addressing this issue involves co-blending soy protein with other biopolymers [17,18]. The water sensitivity of soy-protein plastics is due to the composition of soy protein isolate (SPI), the primary substrate used for this work, contains 90% protein. Soy protein isolate is a mixture of proteins composed of two major sub-fractions of proteins, 11s and 7s each with average molecular weights of 350,000 and 200,000, respectively [19]. Eleven (11)s, the higher molecular weight of the two, is a relatively hydrophobic protein in comparison with 7s due to its functional side groups configuration. However, the 7s protein molecule constitutes approximately (70%) of the total protein content of SPI. Thus, the overall property of SPI is hydrophilic [14,20]. Further details on the individual structures of 11s and 7s soy protein molecules will be discussed in the following section to assist in-depth understanding of the protein globulins.

1.2 Renewable natural polymer- Soy Protein Isolate

Soybeans, one of the major agricultural crops in the U.S., with annual productions of up to 3.19 billion bushels [21], are primarily used in a wide variety of food products either in its native form (dried soy bean snacks) or as a processed product, such a soy milk. Often the food industry uses soy-protein isolate (SPI) as an initial ingredient for many food products. Soy protein isolate contains at least 90% protein [16]. The protein is extracted from crushed or defatted soy meat free of fats that are removed by dissolving in hexane. Following dissolution in caustic

solution at a pH of 9, precipitation of the protein is promoted by acidifying the extract to pH 4-5 modification.

Soy proteins are primarily composed of two protein structures; 7s and 11s also known as β -conglycinin and glycinin globulins. These are main storage proteins in soybeans and have been reported to be key components in determining functional properties of soy products and are distinctly different in their functional properties. The globulin 7s is a trimer (an oligomer with three monomers) formed by any combination of the α , α' and β , sub-units which are helix confirmations of polymerized amino acids as shown in

Figure 5, which are non-covalently linked [22]. Each subunit has one or two *N*-linked (nitrogen) glycosyl groups. The globulin 11s subunits consist of the combination of two polypeptides, *A* and *B*, with acidic and basic isoelectric points, respectively, linked by a disulfide bond. The molecule is formed by six sub-units. The molecular weights of 7s and 11s globulins have been reported to be 150–200 and 300–400 KDa, respectively [23].

The presence of high proportions of glutamic and aspartic acid compared to other proteins make both soy globulins more hydrophilic than globular proteins found in wheat gluten. The sulfhydryl groups and disulfide bonds of 7s globulin are zero and two per molecule, respectively. In contrast, 11s globulin has two sulfhydryl groups and 20 disulfide bonds per molecule.

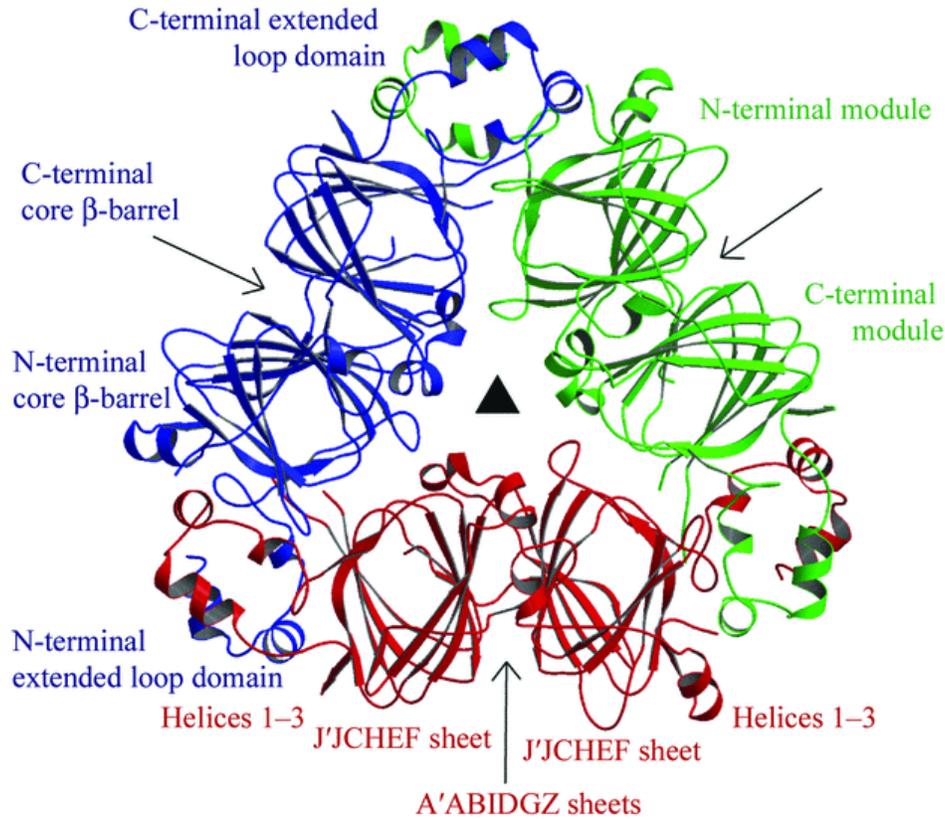


Figure 5 Ribbon diagram of the α_1 trimer as seen along a molecular three-fold axis (black triangle). [24]

1.3 Net Shape forming of plastics

The most common component or systems in the plastics processing industry are extrusion and injection molding, respectively. Primarily utilized for processing thermoplastics, extrusion and injection molding are an integral part of the plastics industry, especially in segments such as bottle manufacturing. They are also used for manufacturing of net resin or composite components, such as short glass reinforced plastics and in processing of thermoset plastics (reactive

extrusion, RTM etc). Both extrusion and injection molding are based on an ancient principle of conveying matter, which is the Archimedes screw (circa 287–212 B.C.) [25]. It was invented as a pump to convey water for irrigation and later employed in ships to pump water fast and efficiently. The simple design has been adopted by the plastics industry for plasticizing, pumping, mixing, metering and shape forming of polymers either into pellets or sample shapes during polymer processing.

1.3.1 Extrusion

Extrusion can be described as a process where plastics (resins), usually in the form of beads or pellets, are continuously fed through a heated chamber and conveyed by a feed screw. The feed screw is driven by a drive/motor and speed and torque control for quality control reasons. As the plastic is conveyed it is sheared, melted, compressed and forced through a die that has a predefined profile. The cooling of the melt results in hardening of that plastic into a continually drawn piece whose cross section matches the die pattern [26]. It is important to note that a phenomenon of “die swell” occurs due to relaxation of stresses in the sheared molten material, immediately after exiting the die. As a result the extrudate has larger dimensions than the profile on the die. Die swell also depends on other factors such as speed of the screw, drawing rate and material properties.

In an extruder, most of the heating is produced by friction between the inner barrel wall, the material, and the screw surface. Thus, as the screw rotates, it both shears and keeps the material at the proper processing temperatures inside the barrel. A simple schematic of an extrusion process is shown in Figure 5. In some cases the die at the end/exit can be designed such that the extruded shape of the material is a tube, a film and as complex as a reinforced window treatment frame.

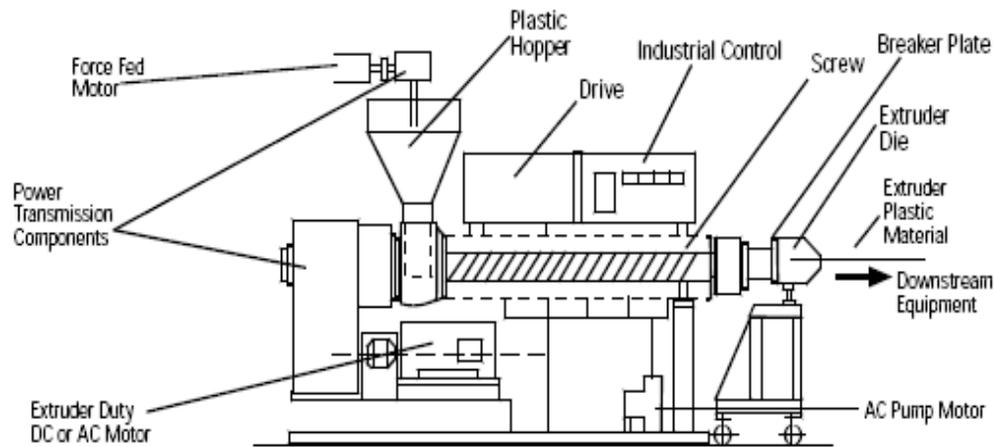


Figure 6 Drawing of extrusion process. [26]

1.3.2 Injection molding

Injection molding, which uses extrusion to melt and pressurize plastics, in contrast is a batch process. That is to say, multiple or single components are manufactured in repetitive steps. In this case a plunger or reciprocating screw injects the molten material into a mold cavity. A clamp force keeps the mold

closed so as not to leak and because of the pressure on injection molding (35-70 MPa) the clamp force is typically high (50.0-60000 KN). Once filled with a preset amount of material, also called the shot size, the screw translates forward creating injection pressures between 0.03-140 MPa and displaces the material from the barrel into the mold. The material is rapidly cooled to solidify the melt inside the mold. Once the solid part is formed it is ejected and the process is typically repeated. Figure 7 shows a schematic of a typical injection molding machine and cycle.

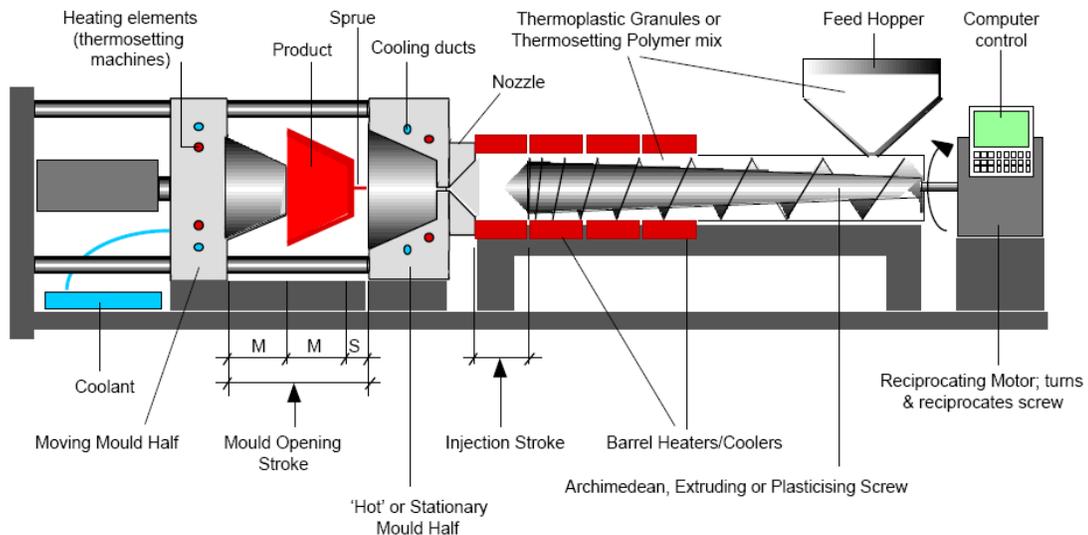


Figure 7 Drawing of injection molding process [27]

1.4 Objective

The research in this thesis is focused on resolving the water sensitivity issue of soy protein polymers by utilizing chemical modifications coupled with

various mechanical additives such as cotton to enhance the properties of the plastics. Preliminary trials with particulate and fiber additives indicated improved mechanical properties as well as enhancing the water stability of the plastic composite and chemical modifications, which can lead to water stable soy protein plastics.

1.5 Literature review and proposed methods

1.5.1 Previous research

Earlier research has utilized polar plasticizers such as glycerol and water as a solvent to produce SPI thermoplastics. Because of the water stability issues of soy protein based plastics, researchers have employed various approaches to address these issues. Some of these issues are discussed here. The prominent methods include blending with other biodegradable polyesters, heat treatment, and compounding with chemical components that improve the surface of the final component hydrophobic properties of the resultant plastics. The following sections discuss these approaches in detail after which the chemistries and additive employed for this research work will be discussed.

1.5.1.1 Polyester blends –PCL and Biomax

Poly-caprolactone is a petroleum based biodegradable polymer, that is relatively resistant to water was used by Rui et.al (2006) to make water-resistant

composite plastics. In this case, samples were prepared from soy protein isolate (SPI) or soy dreg (SD), poly(ϵ -caprolactone) (PCL) and toluene- 2,4-diisocyanate (TDI) as the compatibilizer, by blending and one-step reactive extrusion (REX) followed by compression-molding. The results indicated that SPI and SD series exhibited high water resistance and good tensile strength (14.8 MPa for SPI35 and 16.3 MPa for SD35). This paper provides a convenient way to prepare new soy protein plastics with good biodegradability and water resistivity [28]. Other similar approaches by Mungara et.al. (2002) and Graiver et.al. (2004) involved the use of two component polyester blends and biodegradable co-polyesters such as PCL-Biomax and Easter bio copolyester, in conjunction with SPI [18,19]. The results of these works indicated dry strengths of 27-33 MPa for these blends along with very low water absorption values. However, compatibilizer such as di-Isocyanate compound does not lend the final blend to be environmentally “green”.

1.5.1.2 Heat treatment

Extensive studies carried out on the level of cross-linking in soy protein by Clau'dia et.al. 2003 indicate that heat treatment at 80°C for 24 hours reduces the number of free amines and carboxyl groups which were measured in an untreated and heat treated soy protein samples, respectively. It was also observed that other than improved water stability, the mechanical properties were superior in comparison with a glyoxyl cross-linked sample group, which exhibited lower

count of free amine and carboxyl reactive side group than the heat treated specimens [29]. Further expansion of the previous work was done by Baboi et.al. (2007) by increasing the thermo-cycling temperatures to 100 and 120°C. The results of this work indicate improved water stability coupled with degradation in mechanical properties with increase in thermo-cycling temperature].

1.5.1.3 Salts of fatty acid-Zinc stearate

Previous research by Baboi et.al. (2007) utilized such fatty acid to improve the surface properties of SPI based plastics. Zinc stearate is a salt of a stearic acid $Zn(C_{18}H_{35}O_2)_2$, a zinc soap that repels water and is insoluble in polar solvents. The results of the study indicate that the use of fatty acid salts did improve the water absorption properties, but not significantly, with respect to polyester blends.

1.5.2 Proposed chemical reactants

The chemical reactants that will be employed in this research are anhydrides, particularly those with bi-functional side groups. It is theorized that anhydride functional molecules, which have high affinity for hydroxyl groups, would react with the hydroxyl sites on the protein molecule. The decrease/conversion of the hydroxyl reactive sites on the protein molecule will reduce the protein's affinity for water leading to reduction in the hydrophilic characteristics of the protein. In addition, the anticipated synergetic effects of this

chemistry will allow linking and attachment of the plasticizer molecules to the protein molecule to occur. Other chemical additives that will be employed are potassium phosphate (dibasic), a general anticoagulant [30]. It was conceptualized that the use of anticoagulants simultaneously with anhydrides will increase reactive efficiency with protein side groups. The effect of food antioxidants such as butylated hydroxy anisole (BHA) [31] will also be investigated in order to observe their respective effects on the overall hydrophilicity of soy plastics. As previously mentioned, particulate and fiber forms of cotton will be used to enhance the mechanical properties. An effect that was observed is that the addition of cotton fibers improved the overall water stability of the composite plastic.

1.5.3 Processing techniques

Historically, soy protein polymers have been utilized to develop various biodegradable applications. Some of the prominent examples have been soy protein based foam sheets for packaging [32], protein based extruded films, soy protein–polyester blends and natural fiber based composites [33]. Because of the compatibility of soy protein with plasticizers such as glycerol and water, the majority of the soy based plastic applications were developed using traditional thermoplastic processing techniques such as extrusion and injection molding. The majority of the work published in this field report the use of co-rotating twin screw extruders for compounding and plasticization. This is because twin screw

extruders achieve a higher level of mixing and plasticizing which results in a homogeneous extrudate in comparison with single screw extruders. In addition, net shape forming of the plastic pellets obtained from compounding has been done with different processes such as compression molding, injection molding, or extrusion, depending on the final product. In order to obtain ASTM D638-08 type 1 plastic samples [34] using the soy based resins, previous research has cited injection molding because the samples processed with compression molding typically had weld lines at the pellet/pellet interfaces, which primarily occur due to insufficient fusion at the pellet boundaries. However, samples processed with injection molding were found to be more consistent and homogeneous [35] which resulted in consistent test results.

1.5.4 Testing methods

In addition to the common tensile strength and water absorption tests, researchers have frequently employed Fourier transform infrared spectroscopy (FTIR) as a tool to investigate the changes and improvement of the protein polymers at the molecular level. FTIR is a technique which employs a high intensity infrared beam which when made incident / passed through a test sample is partly absorbed by the sample yielding an absorption spectrum. Each type of bonding in a molecule, upon absorption of the IR energy, vibrates at a certain resonant frequency depending on the bond geometry and atomic masses involved. These vibrating frequencies of the atoms correspond to the frequency of the

absorbed radiation of a particular corresponding to a wave number. In IR active organic compounds, the molecules vibrate with six basic modes of vibration, which are symmetrical and antisymmetrical stretching, scissoring, rocking, wagging, and twisting. Depending on whether the molecules are linear or non linear, the molecules have $3N-5$ or $3N-6$ modes of vibration, where N is the number of atoms in the molecule/side group and each set of vibration modes are distinct for a particular bond/molecule type. This spectrum of absorption obtained from the analysis can be interpreted to investigate the type of atomic bonding/side groups present in the molecules.

CHAPTER 2: RESEARCH QUESTIONS

2.1 The Primary objectives/research questions

1) Do Anhydrides improve the water stability of the soy protein molecule and soy protein based plastics without using blends?

2) Does the use of glycerol as the plasticizer along with anhydrides make it possible to process protein based plastics with conventional thermoplastic processing equipment?

3) Does the use of environmentally friendly additives and reactants help improve the water stability of protein based plastics?

The following chapter discusses in detail how the above stated research questions/objectives were achieved by this research work and explains the test results obtained.

CHAPTER 3: IMPROVEMENT OF WATER STABILITY OF SOY PROTEIN BASED PLASTICS

3.1 Synopsis-Processing and evaluation.

The raw polymer, soy protein isolate (SPI), was compounded with plasticizers and solvents such as glycerol and water; along with preservative salts and sulfide link cleavage agent, potassium sorbate and sodium sulfite, respectively, to form the base formulation. Anhydride chemical reactants, phthalic (PA) and maleic anhydride (MA) were included in concentrations varying from 0-10% (w/w). All formulations were compounded with a reactive extrusion process followed by injection molding to obtain ASTM dog-bone samples [34]. Composite formulations were processed following a similar method with an additional fiber /particulate mixing step prior to extrusion processing. Following ASTM standards, the samples were tested for water absorption and tensile strength properties following a one day open air conditioning step. Based on the results observed, formulations with the best water absorption properties were further analyzed employing Fourier transform infrared spectroscopy (FTIR) technique. Formulations PA10% and composite formulations of PA10% were observed to have the lowest water absorption values of 19.0% and 16.8% after 24 hrs respectively .The wet state tensile strength was observed to be above 1MPa with in the first 6 hrs of exposure to water for C_fPA10% formulation (cotton fiber and phthalic anhydride).

3.2 Materials:

Soy protein isolate (SPI, ~90% protein) was obtained from Solae Company, St. Louis, MO. Plasticizer, salts, i.e. glycerol (Gly), sodium sulfite, sorbic acid (potassium salt), and potassium phosphate dibasic (DPP) were obtained from Fisher Scientific (Pittsburgh, PA). Maleic anhydride (MA), Phthalic anhydride (PA), and Butylated hydroxy anisole (BHA) were procured from Sigma Aldrich and Acros Organics. Cotton fiber (C_f) was prepared by cutting mercerized cotton yarn (from local sources) hank to 19 to 25 mm lengths followed by fluffing to individualize the fibers. Cotton powder (C_p) was acquired from Northern Technologies Inc, MN.

3.3 Preparation and processing:

3.3.1 Base formulation

All formulations were developed from a base formulation (BF) as the platform, each of which started out with two major portions, a solid and a liquid fraction, respectively. The solid fraction constituted of 1 kg of SPI, which was constant for all formulations. The liquid fraction for the base formulation constituted a solution mixture of 80 parts of water, 30 parts glycerol, and 0.5 parts of both sodium sulfite and sorbic acid (K-salt). The latter mentioned proportions/ratio of the components remained constant for all the formulations.

3.3.2 Anhydride formulations and salt additives

For maleic (MA) and phthalic anhydride (PA) formulations, the measurements were based on the final percentage (%) concentration in the molded plastic as detailed in Table 3 and 4. The respective mass of anhydride reactants was combined with the liquid fraction by reducing equivalent mass of glycerol such that the total mass of the liquid fraction remained the same for all formulations. It is important to note that, for phthalic anhydride (PA)

Table 3 Formulation matrix for Plastics with chemical additives only

	Solid Fraction	Liquid Fraction				
Component	SPI	Base Solution	MA	PA	BHA	DPP
Formulation						
Control	100 P	B.S	-	-	-	-
MA. 3%	100 P	BS -(4.5P Gly)	4.5 P	-	-	-
MA. 5%	100 P	BS -(7.5P Gly)	7.5 P		-	-
PA. 3.5%	100 P	BS -(5.3P Gly)	-	5.3 P	-	-
PA. 5%	100 P	BS -(7.5P Gly)	-	7.5 P	-	-
PA. 10%	100 P	BS -(15P Gly)	-	15 P	-	-
BA. 0.5%	100 P	BS	-	-	0.7 P	
D+PA. 5%	100 P	BS -(7.5P Gly)	-	7.5 P	-	1. P
D+PA. 10%	100 P	BS -(15P Gly)	-	15 P	-	1 .P

Table 4 Formulation matrix for plastics composites with chemical and particulate additives.

Component Formulation	Solid Fraction			Liquid Fraction		
	SPI	C _f	C _p	Base Solution	MA	PA
Control	100 P		-	B.S	-	-
C _f	100 P	2 P	-	B.S	-	-
C _f MA3 %	100 P	2 P	-	BS -(4.5P Gly)	4.5 P	
C _f PA5%	100 P	2 P	-	BS -(7.5P Gly)	-	7.5 P
C _f PA10%	100 P	2 P	-	BS -(15P Gly)	-	15 P
C _p	100 P	-	20 P	BS	-	-
C _p PA10%	100 P	-	20 P	BS	-	15 P

formulations, 1.0 parts of sodium sulfite was used in place of 0.5 parts. This change in formulation was done to improve the reactivity between the anhydrides and the hydroxyl sites on the protein. These formulations are summarized in Table 3 and 4. The addition of the antioxidant additive and anticoagulant salts was done similarly, except the amount of sodium sulfite added remained at 0.5 parts.

3.3.3 Composite formulations

Composite formulations with non-reactive fillers such as cotton fibers and cotton powder, were added in parts to the total mass of SPI in the solids fraction

prior to mixing with the liquid fraction. The various ratios/proportions of the non-reactive fillers added are detailed in Table 4.

3.3.4 Mixing and compounding

Once the solid and liquid fractions were prepared, both were mixed together in a high speed mixer (Henschel Mixers American, Inc., Houston, TX) to produce a moist “dough-like” resin mixture. This dough was further extruded on a compounding twin-screw extruder (Liestriz Micro18, L/D ratio 30, American Liestriz Corp., Somerville, NJ), in order to obtain a plastic extrudate, which was then pelletized with a pellet mill (Scheer Bay Inc. WI). The temperature profile during extrusion followed a gradation of 95-110°C from the hopper to the die for all formulations except for phthalic anhydride formulations, which ranged between 95-120°C. The higher extrusion temperatures for phthalic anhydride formulation were based on material behavior during trials. It was observed that phthalic anhydride formulations plasticized better with marginally higher temperatures, where as other formulation processed well at 110°C. The pellets were conditioned to a final moisture level of 10-15% depending on the various formulations and was further injection molded using a 22 S Boy machine (20 ton clamping force) into ASTM 638-08 sample type 1 standard tensile test samples [34]. The injection molding was completed at temperatures between 120 to 135°C for all formulations except maleic anhydride formulations which were completed at 110 to 120°C. Samples were randomly selected for testing from each batch.

3.4 Testing and evaluation:

3.4.1 Water absorption testing

All sample formulations were tested for their water absorption in accordance with the ASTM D570-98 standard [34]. The samples were conditioned initially for one day and exposed to a 100% moisture environment by immersing them in distilled water. The absorption of the samples were measured every 2 hrs for the first 8 hrs, and after which the final reading was taken after 24 hrs for duplicate sets. The water absorption value (WAV) was determined by calculating the difference in weight between the initial dry and wet samples and expressed as percentage over the dry weight of the sample. Each data point collected was the average water absorption of two samples at each time interval.

3.4.2 Wet state tensile strength testing

Samples were tested for tensile strength in their wet state following ASTM D638-08 standard [34] and tests were conducted utilizing the duplicate sample set from the water absorption test using a 4500 series Instron testing frame. The injection molded samples were of standard geometry-sample type-1, with a cross section of 12.7mm x 3.2mm (length 65mm) and the samples were loaded at a constant grip displacement rate of 5mm per min as stipulated by standard ASTM D 638-08.

3.4.3 Fourier transform infrared spectroscopy (FTIR)

Both phthalic and maleic anhydride formulations were characterized using FTIR technique. The characterizations were done with mid-IR wave numbers (500- 4000 cm^{-1}), cesium iodide being used as background material.

3.5 Results and observations

Soy plastics compounded with maleic anhydride were observed to have a better water resistance in comparison with the control formulation. The water resistance of the plastics improved with increasing concentration of maleic anhydride seen in Figure 8. In reference to the maleic anhydride formulations, MA 3% and MA 5%, the water absorption after 24 hrs dropped from 70% to 27%, respectively. While the Maleic anhydride formulations showed significant improvement in water absorption properties, the wet samples of MA 5% formulation lost their structural integrity (strength) after 24 hrs of exposure to water, which made it difficult to test their wet state strength. As a result of similar sample behavior observed with MA 10% formulations, both water absorption and wet state tensile strength were not recorded for the same. With phthalic anhydride formulations, PA 3.5%, 5%, and 10%, the overall water absorption property of the plastics improved with the increase of phthalic anhydride concentration, which was similar to the trend observed with MA 3% and 5%. Formulation PA 10% had water absorption of 19% after 24 hrs, which was

observed to be the lowest among the anhydride plastic formulations, as seen in Figure 8.

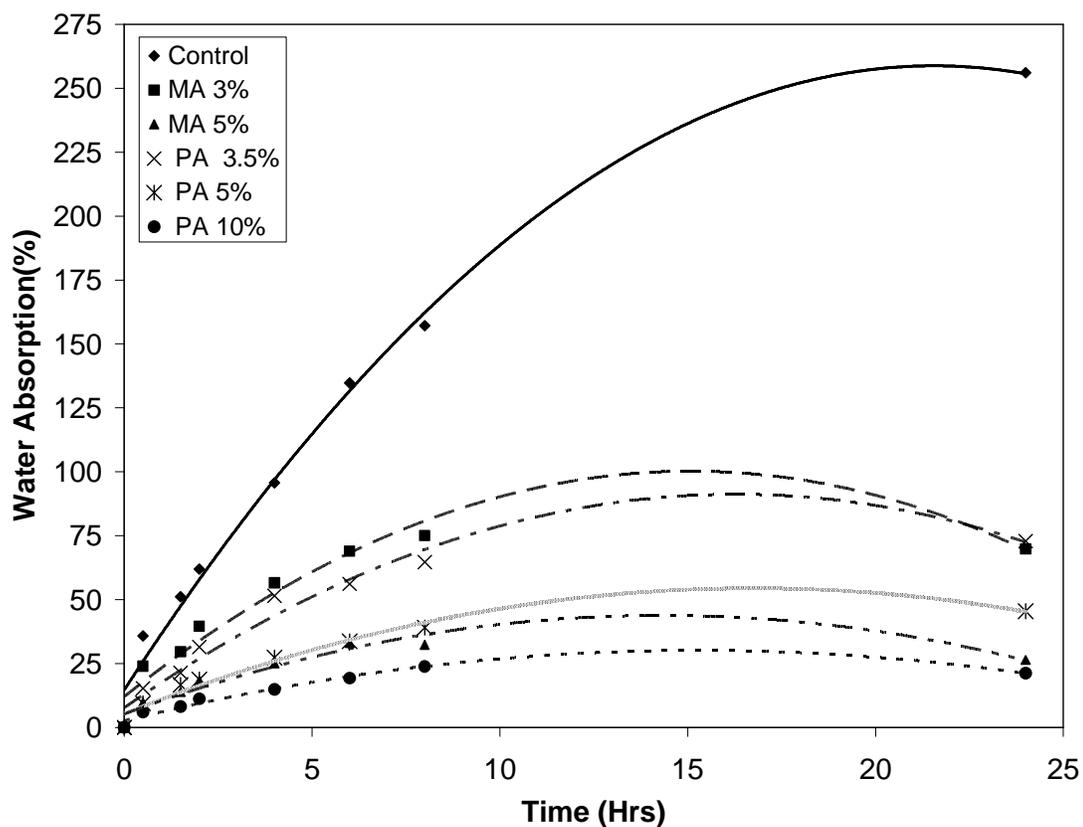


Figure 8 Water absorption of maleic and phthalic anhydride formulations over 24hrs. (Polynomial fit trend lines, degree-2)

These results support the theory that anhydrides, as a functional chemistry/reactant, have an affinity for hydroxyl groups on the protein (which are responsible for the hydrophilic nature), consequently making the protein less hydrophilic. In addition, water absorption was inversely proportional to anhydride concentration in the plastic. This further reinforces the theory of using anhydrides

as reactants to promote water stability or hydrophobicity of soy protein based plastics. Formulation BA 0.5 exhibited improved water stability, but were not as significant as anhydride formulations. Formulations based on dibasic potassium salt along with phthalic anhydride D+PA 5% and D+PA 10% were observed to have higher water absorption values in comparison with the respective phthalic anhydride formulations (i.e. having the same respective concentration), as seen in Figure 9.

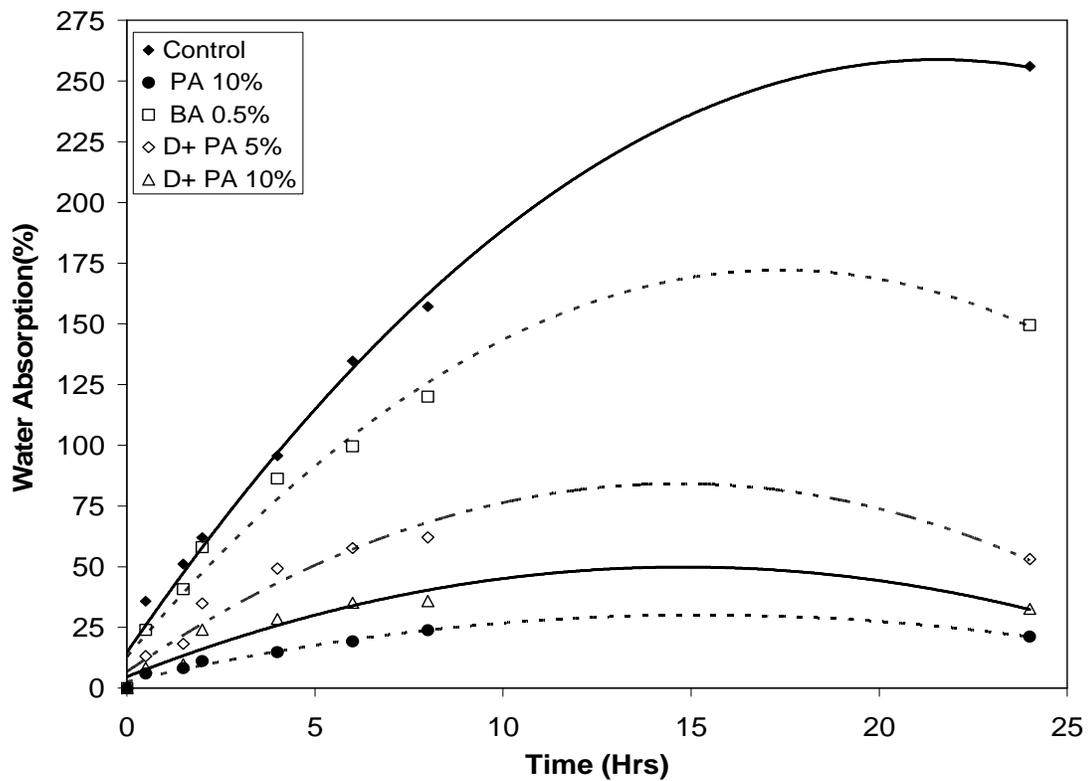


Figure 9 Water absorption antioxidant additives and anhydride formulations with salts over 24hrs. (Polynomial fit trend lines, degree-2)

FTIR characterization was conducted on the formulations that performed the best in the water absorption test. The control formulation, PA 10% and MA 5%, were characterized using the FTIR technique, because they resulted in the lowest water absorption, as well as representing the untreated sample (control formulation). It can be seen in Figure 10 that the FTIR spectrums for all three formulations exhibit common peaks at wave numbers of 1672, 1545 and 1254 cm^{-1} , corresponding to amide I (C=O), amide II (N-H), and amide III (C-N and N-H) bond vibrations respectively, existing in the soy protein molecule[36].

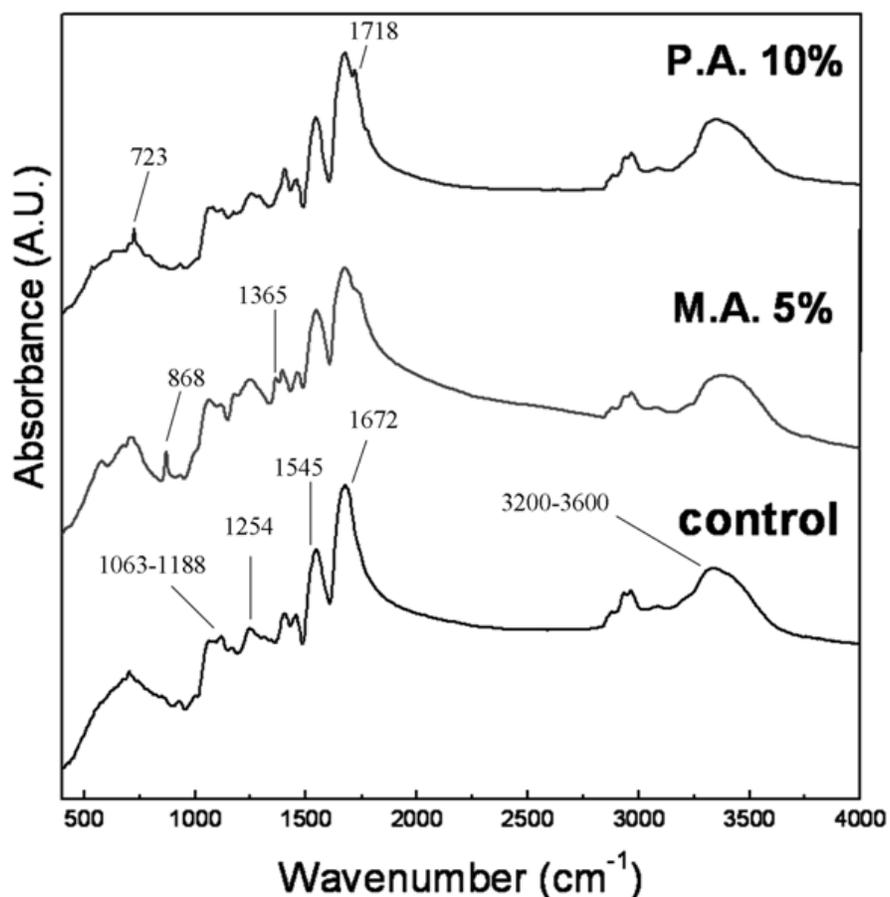


Figure 10 FTIR absorbance spectrum for control, MA 5% and PA 10%.

The broad peak from 3200-3600 cm^{-1} corresponds to the free and bounded -OH and -NH groups [36]. Peaks at 1063 and 1118 cm^{-1} correspond to the vibrations of C-C and C-OH bonds present in the glycerol molecule [37]. The spectrum of MA.5% exhibits a distinct peak at 868 cm^{-1} , being attributed to the C-H bonds situated on the opened ring of maleic anhydride molecule (derived from aromatic source). The spectrum of PA 10% shows a sharp peak at 723 cm^{-1} , being attributed to the C-H bonds on the aromatic ring of phthalic anhydride molecule [38]. A twin peak at 1718 cm^{-1} exhibited by both PA 10% and MA 5% spectrums suggests the formation of new saturated carboxylic acid side groups and reformation of amide I bonds [38]. In addition, slight shifts in position, as well as change in the shape of the amide III peaks suggest breakage and reformation of linkages and side groups [36]. Thus, based on the results of the FTIR, it is believed that the theorized chemical reactions with anhydride molecules did improve water stability of soy protein based plastics.

Composite plastic formulations using cotton fiber (C_f) and cotton powder (C_p) in combination with anhydrides did exhibit marginally better water resistance properties with respect to anhydride plastics formulations (PA 10 %). In Figure 11 it is seen that the formulations C_f and C_p had lowered water absorption in comparison with control samples. Cotton fiber and powder with phthalic anhydride, formulations C_f PA10% and C_p PA10% were observed to have 19% and 16.8% water absorption value after 24 hrs, as seen in Figure 11. However, the wet strength of all formulations dropped below 1 MPa within the first 5 hrs of

submersion in water except for formulations PA10% and Cf PA10. It is believed that the addition of particulates and fibers, which are a denser network of cellulose, might have created a mechanical barrier and formed a composite structure that reduces the rate of water diffusion through the plastic matrix.

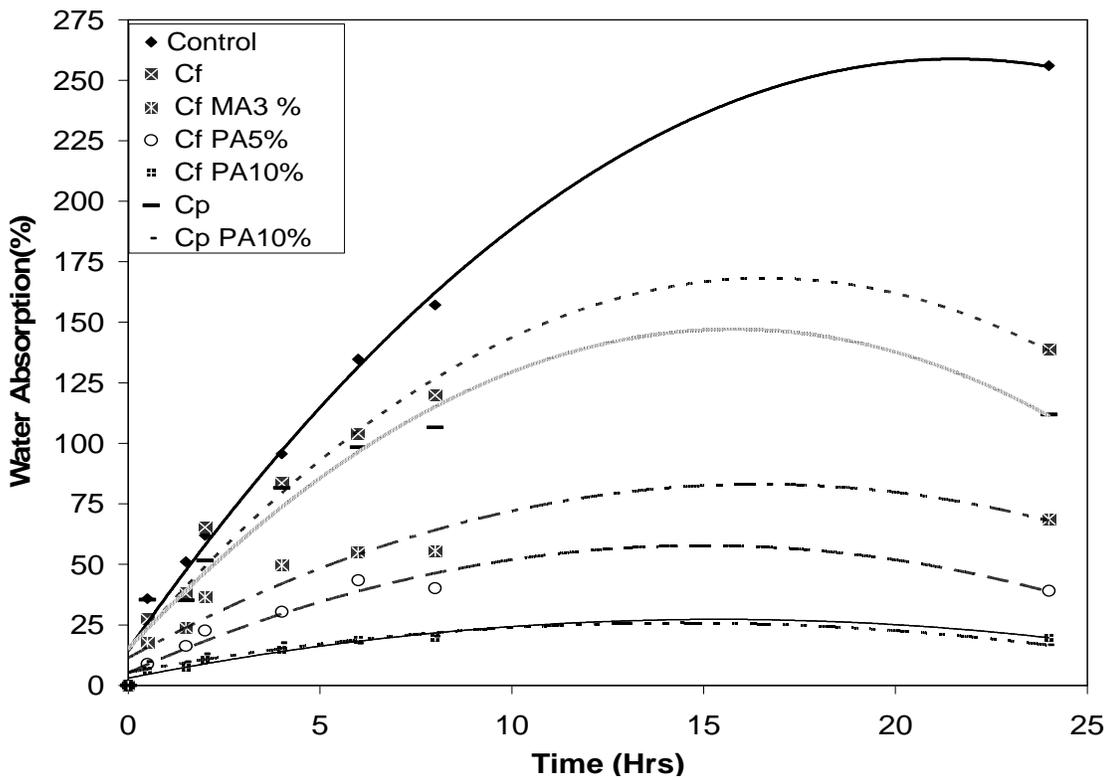


Figure 11 Water absorption of cotton fiber and cotton powder plastic formulations over 24hrs. (Polynomial fit trend lines, degree-2)

The objective of improving wet state strength with addition of particulates was partially achieved as seen in Figure 12, that the strength was maintained for the initial 1-2 hrs of the test.

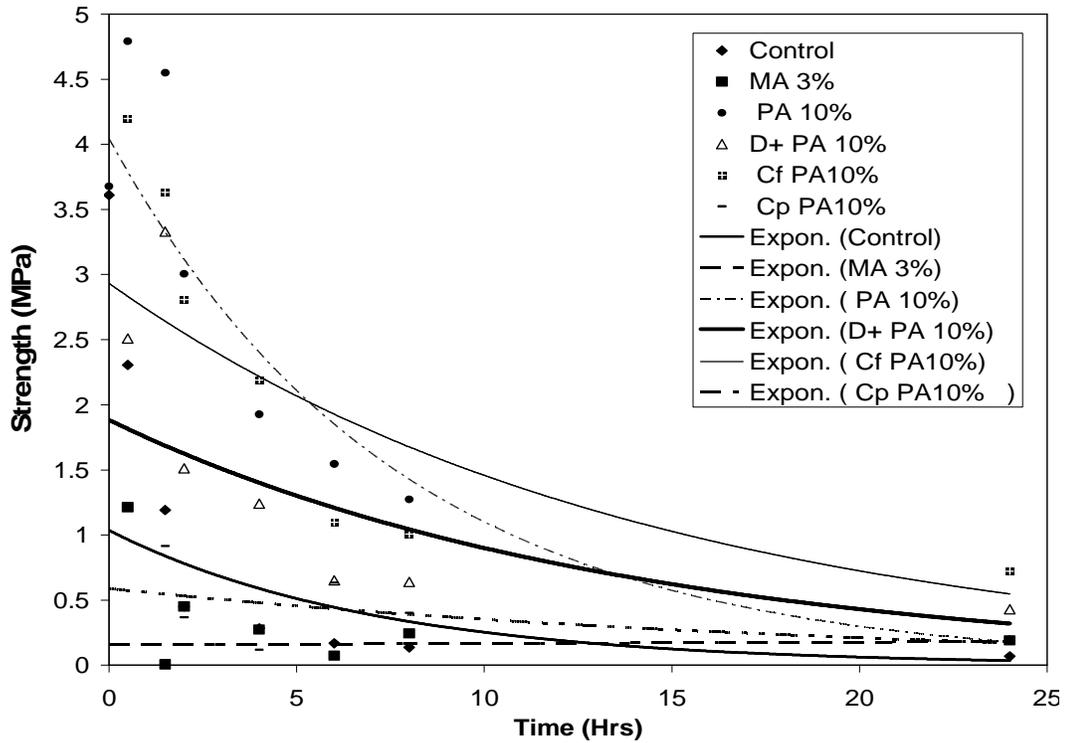


Figure 12 Wet state tensile strength at intermittent intervals over 24 hrs
(exponential trend lines).

It was observed that wet strengths after 24 hrs were only marginally better than the formulations without the particulate additives. It is believed that while the cotton reinforces the composite, the matrix (protein polymer) was weakened by the water, preventing load transmission to the fibers, resulting in a reduction of strength of the composite.

Dry state tensile strength for phthalic anhydride formulations were compared and it was observed that PA 3.5% and PA 5% performed better in terms of dry strength with respect to control and PA10% formulations as detailed in Figure 13. Formulation PA 10% was observed to have the best performance in

terms of wet state strength and water absorption properties. The error bars in Figure 13 are standard deviations of the respective formulation data sets used as the positive and negative limits.

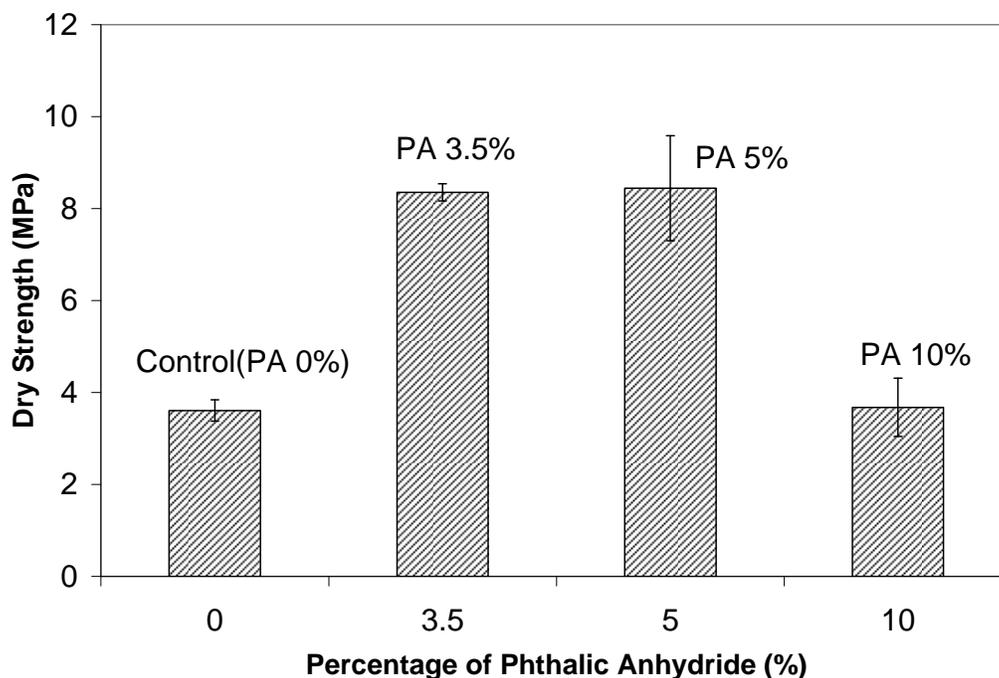


Figure 13 Dry strength of Phthalic anhydride formulations vs. PA concentration.

It was also observed that the plastic samples formed with phthalic anhydride formulations became brittle, (even though maleic anhydride formulations remained flexible enough, low yield of quality samples at injection molding did not allow a full scale dry strength to be conducted for maleic anhydride formulations). The differences in brittleness of the samples were believed to be the result of anhydride reactivity and the molecular properties of the anhydride

chemistry. Such a hypothesis is consistent with the observations as phthalic anhydride has a benzene ring, which is a very stiff molecule, might have caused the brittleness. Another explanation was that the higher order of reactivity for phthalic anhydride based formulation might have caused faster diffusion of the free water molecules resulting in faster drying and brittleness of the sample. Similarly, maleic anhydride formulation was flexible due to relatively less stiff molecule with respect to phthalic anhydride. It was also theorized that phthalic anhydride may have promoted a certain amount of crosslinking.

CHAPTER 4: STATISTICAL ANALYSIS AND VALIDATION

Formulations with significant improvement in water absorption properties with respect to control, in this case phthalic anhydride formulations; were tested statistically to validate the data set.

4.1 Dry strength data

Statistical validation for dry strength data was conducted using two primary tests - one way ANOVA and means with Tukey-Kramer HSD test. The dry strength data test was conducted using the of phthalic anhydride data set as detailed in Figure 13 and one-way ANOVA yielded the results as seen in Figure 14, and analysis summary with the following results.

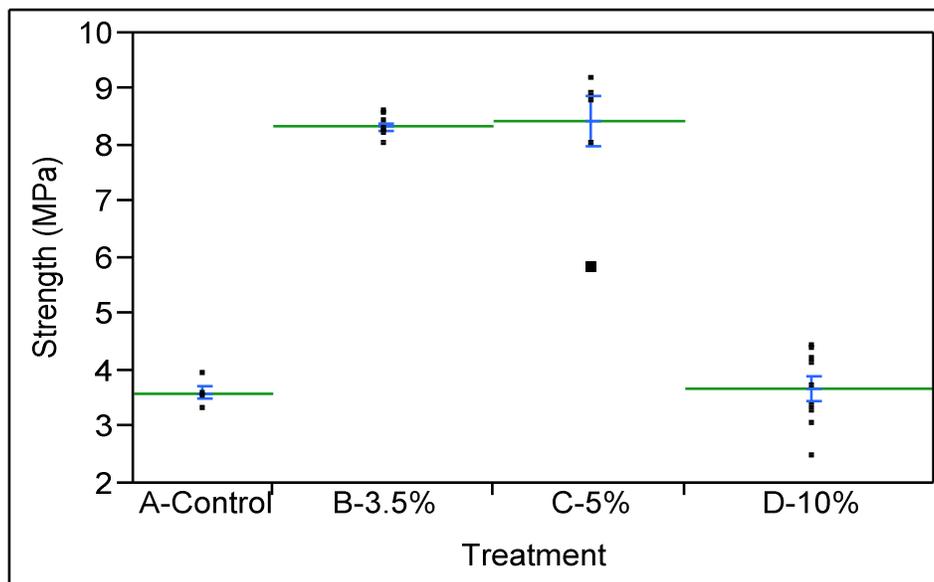


Figure 14 One-way analysis of strength (MPa) by treatment (phthalic anhydride formulations)

4.1.1 Dry strength analysis –Summary

Comparisons for all pairs using Tukey-Kramer HSD

Table 5 Comparison of means: dry strength for phthalic anhydride formulations.

Abs(Dif)-LSD	C-5%	B-3.5%	D-10%	A-Control
C-5%	-1.01258	-0.89331	3.808339	3.721714
B-3.5%	-0.89331	-0.94718	3.755398	3.663871
D-10%	3.808339	3.755398	-0.89301	-0.98869
A-Control	3.721714	3.663871	-0.98869	-1.1981

*Positive values show pairs of means that are significantly different.

Table 6 Comparison of means: Result of significance test for phthalic anhydride formulations

Level			Mean
C-5%	A		8.4403400
B-3.5%	A		8.3532250
D-10%		B	3.6773333
A-Control		B	3.6094020

*Levels not connected by same letter are significantly different.

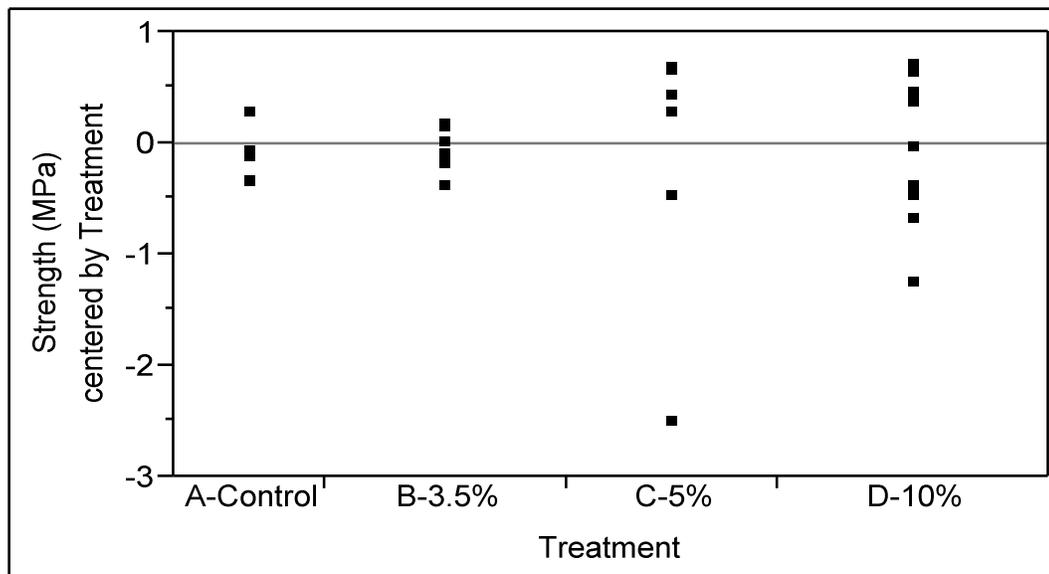


Figure 15 Plot of Residuals vs. Treatment for phthalic anhydride formulations

From the analysis summary and the comparisons for all pairs using Tukey-Kramer HSD as shown in Tables 5 and 6, it can be seen that the control and PA10% formulations are not statistically different from each other and similarly PA3.5% is not statistically different from PA5%. This can be seen from the negative values of Abs (Dif)-LSD (least square difference) for the corresponding combination/comparison of formulations and is further affirmed by assigning alphabetical categorization (this analysis is highlighted in yellow). However, the means of PA3.5% and PA5% are statistically different from the means of control and PA10% in term of dry strength. It is important to note that a single outlier data point in the PA5% data set, seen in the plot of residual Figure 15; did not make a difference in the outcome of the statistical analysis, despite excluding the data point, thus data point was not excluded from the final analysis.

4.2 Wet state data

The water absorption data of PA and control formulations were analyzed and fitted to polynomial form curves by JMP 8.0 statistical analytical software and these are as seen in Figure 16. Following ANOVA, the difference in the value of intercepts of the fitted polynomial equation for different PA formulations suggests that the data sets are different from each other. It is understood from the negative values of parameter estimates terms of ‘Hours steeping*Treatment [B-3.5%]’ and ‘Hours Steeping*Treatment[C-5%]’ as shown in Table 7 in the analysis summary below, indicates that the fitted water absorption curves of PA

formulation are statistically different from control.

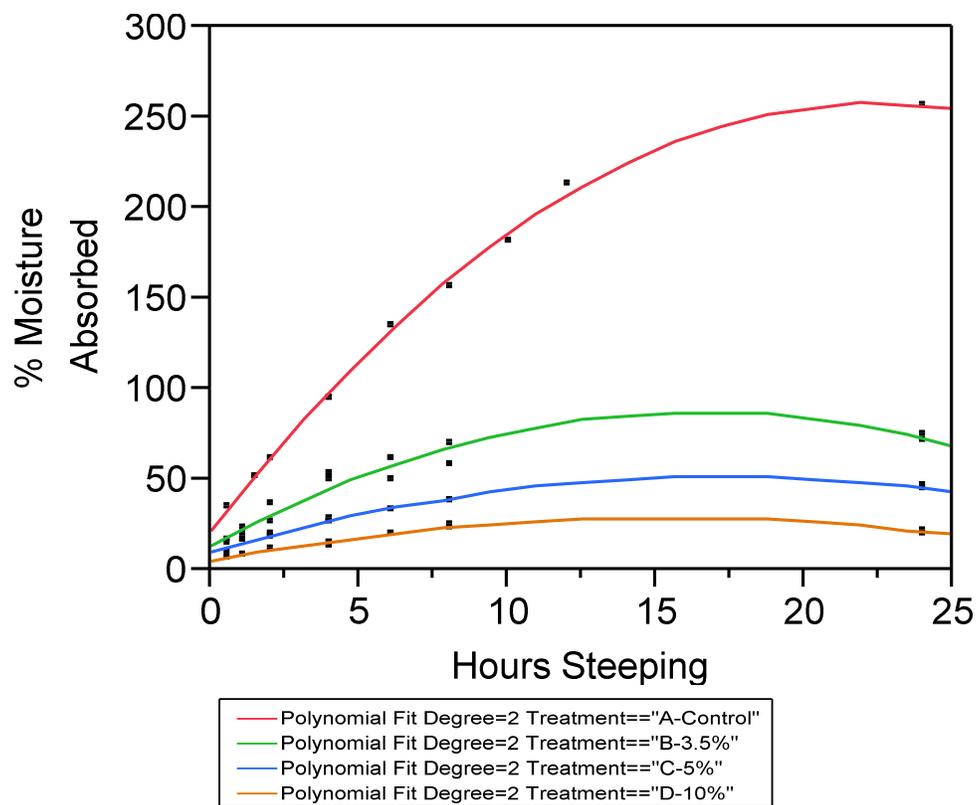


Figure 16 Bivariate fit of % moisture absorbed by hours steeping
for phthalic anhydride formulations

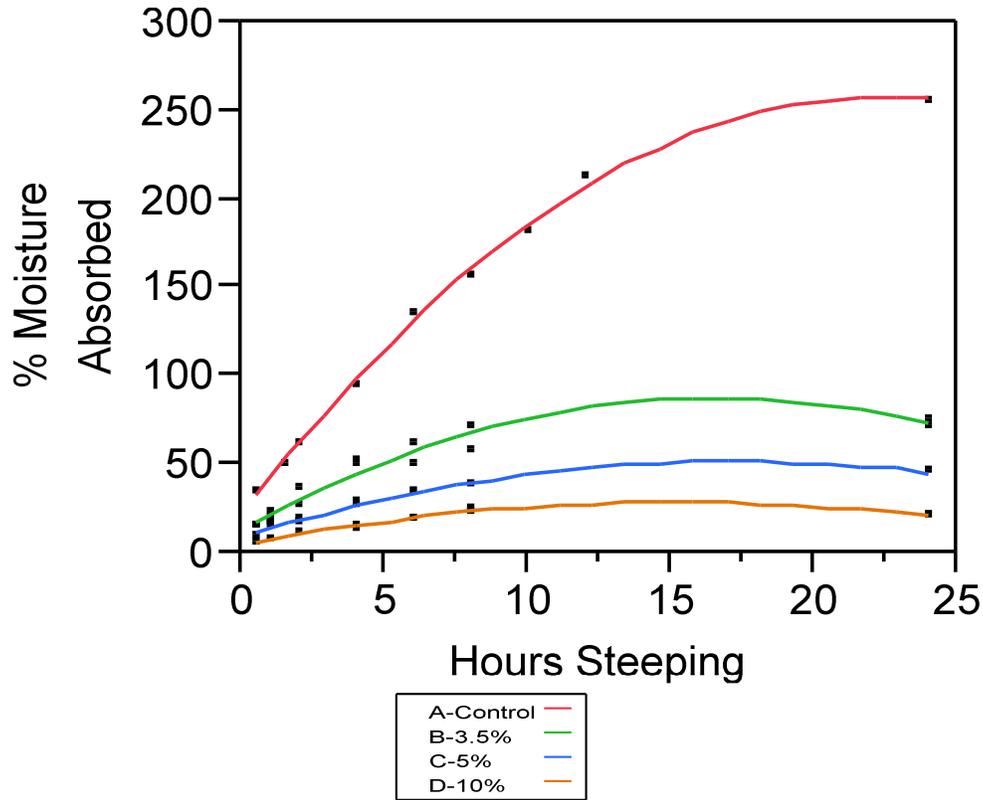


Figure 17 Response moisture absorbed regression plot for phthalic anhydride formulations

Table 7 Parameter estimates for polynomial fit of water absorption data for phthalic anhydride formulations

Term	Estimate	Std Error	Prob> t
Intercept	12.497291	0.961205	<.0001*
Treatment[A-Control]	9.6252708	1.914542	<.0001*
Treatment[B-3.5%]	1.1475404	1.572844	0.4700
Treatment[C-5%]	-3.058163	1.572844	0.0591
Hours Steeping	9.4777571	0.262927	<.0001*
Hours Steeping*Hours Steeping	-0.244759	0.010259	<.0001*
Hours Steeping*Treatment[A-Control]	11.549786	0.462944	<.0001*
Hours Steeping*Treatment[B-3.5%]	-0.592346	0.452861	0.1985
Hours Steeping*Treatment[C-5%]	-4.5867	0.452861	<.0001*
Hours Steeping*Hours Steeping*Treatment[A-Control]	-0.224223	0.018337	<.0001*
Hours Steeping*Hours Steeping*Treatment[B-3.5%]	-0.023027	0.017577	0.1978
Hours Steeping*Hours Steeping*Treatment[C-5%]	0.103427	0.017577	<.0001*

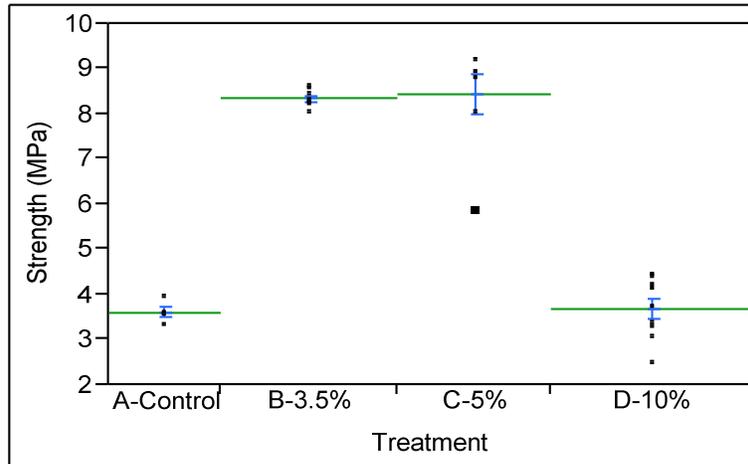
From the above analysis, in particular from different values of intercepts the polynomial fits as depicted in Figure 16 it can be seen that each of the treatments are different statistically. To support this, the negative values of the triple products 'Hours Steeping*Hours Steeping*Treatment [A-Control]' and 'Hours Steeping*Hours Steeping*Treatment [B-3.5%]' in the parameter estimate terms above in the analysis summary shows the difference in treatments.

CHAPTER 5: CONCLUSION

Water absorption property of soy protein based plastics can be improved by reacting anhydrides with the pendant hydrophilic groups of the protein. The water absorption test and FTIR studies were in good agreement with the theory that the anhydrides react and bond to the hydroxyl reactive sites on the protein molecule making the protein polymer less hydrophilic. In addition, the water absorption properties were observed to improve with the inclusion of non reactive additives such as cotton powder and fiber. The water absorption properties were observed to improve as a function of increase in additive percentages and this was found to be true for anhydride chemistries and cotton fiber as the additive. The mechanical properties were increased by using fillers such as cotton fibers and powder. The wet state mechanical properties were observed to the highest with formulations containing both anhydride chemistries and cotton fiber additives. The use of anhydrides in conjunction with glycerol as the plasticizer made processing possible with conventional thermoplastic processing equipment, even though water absorption properties of soy protein plastics were similar to some commercial polyamides, the wet state mechanical strengths were low.

APPENDIX A: STATISTICAL ANALYSIS OF DRY STRENGTH RESULTS FOR PHTHALIC ANHYDRIDE DATA SET

Oneway Analysis of Strength (MPa) By Treatment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
A-Control	5	3.60940	0.23090	0.10326	3.3227	3.8961
B-3.5%	8	8.35323	0.18575	0.06567	8.1979	8.5085
C-5%	7	8.44034	1.13970	0.43077	7.3863	9.4944
D-10%	9	3.67733	0.67168	0.22389	3.1610	4.1936

Oneway Anova

Summary of Fit

Rsquare	0.932108
Adj Rsquare	0.92396
Root Mean Square Error	0.688697
Mean of Response	6.105213
Observations (or Sum Wgts)	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	3	162.79495	54.2650	114.4098	<.0001*
Error	25	11.85759	0.4743		
C. Total	28	174.65254			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
A-Control	5	3.60940	0.30799	2.9751	4.2437
B-3.5%	8	8.35323	0.24349	7.8517	8.8547

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C-5%	7	8.44034	0.26030	7.9042	8.9764
D-10%	9	3.67733	0.22957	3.2045	4.1501

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
2.75064	0.05

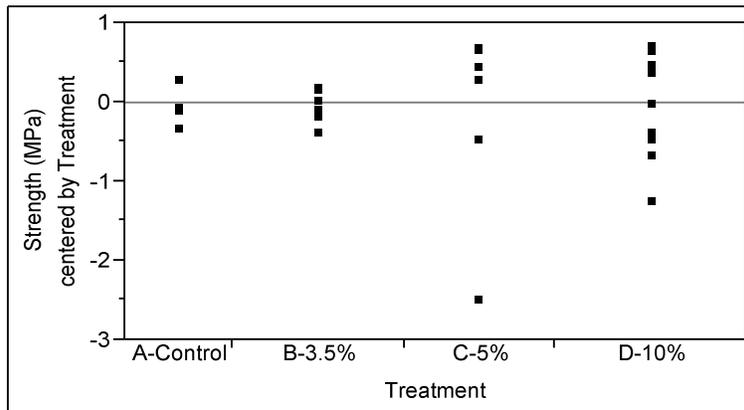
Abs(Dif)-LSD	C-5%	B-3.5%	D-10%	A-Control
C-5%	-1.01258	-0.89331	3.808339	3.721714
B-3.5%	-0.89331	-0.94718	3.755398	3.663871
D-10%	3.808339	3.755398	-0.89301	-0.98869
A-Control	3.721714	3.663871	-0.98869	-1.1981

Positive values show pairs of means that are significantly different.

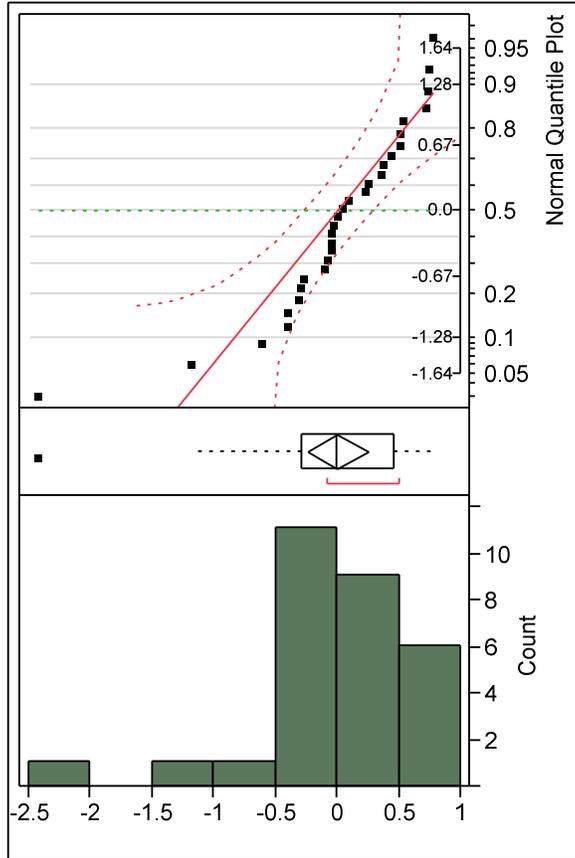
Level		Mean
C-5%	A	8.4403400
B-3.5%	A	8.3532250
D-10%	B	3.6773333
A-Control	B	3.6094020

Levels not connected by same letter are significantly different.

Plot of Residuals vs Treatment

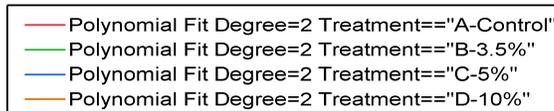
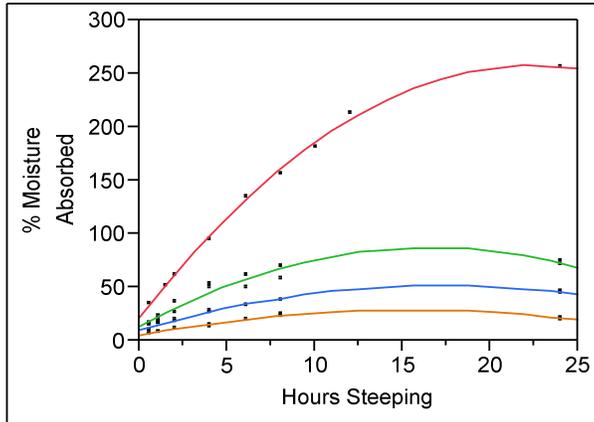


Distribution of Residuals



APPENDIX B: STATISTICAL ANALYSIS OF FOR WATER ABSORPTION RESULTS FOR PHTHALIC ANHYDRIDE DATA SET.

Bivariate Fit of % Moisture Absorbed By Hours Steeping



Polynomial Fit Degree=2 Treatment=="A-Control"

% Moisture Absorbed = 22.122562 + 21.027543*Hours Steeping - 0.4689824*Hours Steeping^2

Summary of Fit

RSquare	0.997849
RSquare Adj	0.997132
Root Mean Square Error	4.108539
Mean of Response	131.8844
Observations (or Sum Wgts)	9

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	46979.679	23489.8	1391.571
Error	6	101.281	16.9	Prob > F
C. Total	8	47080.959		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	22.122562	2.821531	7.84	0.0002*
Hours Steeping	21.027543	0.649306	32.38	<.0001*
Hours Steeping^2	-0.468982	0.025898	-18.11	<.0001*

Polynomial Fit Degree=2 Treatment=="B-3.5%"

% Moisture Absorbed = 13.644831 + 8.8854111*Hours Steeping - 0.2677859*Hours Steeping^2

Summary of Fit

RSquare	0.947917
RSquare Adj	0.938447
Root Mean Square Error	5.369162
Mean of Response	44.72661
Observations (or Sum Wgts)	14

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	5771.3725	2885.69	100.1005
Error	11	317.1069	28.83	Prob > F
C. Total	13	6088.4794		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	13.644831	2.772429	4.92	0.0005*
Hours Steeping	8.8854111	0.821105	10.82	<.0001*
Hours Steeping^2	-0.267786	0.031782	-8.43	<.0001*

Polynomial Fit Degree=2 Treatment=="C-5%"

% Moisture Absorbed = 9.4391284 + 4.891057*Hours Steeping - 0.141332*Hours Steeping^2

Summary of Fit

RSquare	0.985865
RSquare Adj	0.983295
Root Mean Square Error	1.617826
Mean of Response	27.15332
Observations (or Sum Wgts)	14

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	2008.1117	1004.06	383.6137
Error	11	28.7910	2.62	Prob > F
C. Total	13	2036.9027		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	9.4391284	0.835383	11.30	<.0001*
Hours Steeping	4.891057	0.247414	19.77	<.0001*
Hours Steeping^2	-0.141332	0.009577	-14.76	<.0001*

Polynomial Fit Degree=2 Treatment=="D-10%"

% Moisture Absorbed = 4.7826421 + 3.1070172*Hours Steeping - 0.1009356*Hours Steeping^2

Summary of Fit

RSquare	0.988814
RSquare Adj	0.98678
Root Mean Square Error	0.753012
Mean of Response	14.92435
Observations (or Sum Wgts)	14

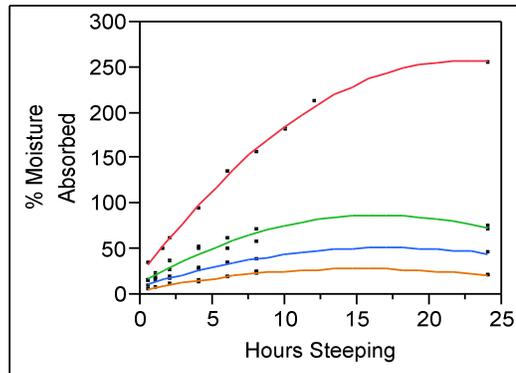
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	551.35864	275.679	486.1832
Error	11	6.23730	0.567	Prob > F
C. Total	13	557.59595		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	4.7826421	0.388827	12.30	<.0001*
Hours Steeping	3.1070172	0.115158	26.98	<.0001*
Hours Steeping^2	-0.100936	0.004457	-22.64	<.0001*

Response % Moisture Absorbed Regression Plot



A-Control	—
B-3.5%	—
C-5%	—
D-10%	—

Summary of Fit

RSquare	0.996775
RSquare Adj	0.995866
Root Mean Square Error	3.409699
Mean of Response	47.10235
Observations (or Sum Wgts)	51

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	11	140149.00	12740.8	1095.886
Error	39	453.42	11.6	Prob > F
C. Total	50	140602.42		<.0001*

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	18	256.90938	14.2727	1.5253
Pure Error	21	196.50634	9.3574	Prob > F

Source	DF	Sum of Squares	Mean Square	F Ratio
Total Error	39	453.41572		0.1760
				Max RSq
				0.9986

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	12.497291	0.961205	13.00	<.0001*
Treatment[A-Control]	9.6252708	1.914542	5.03	<.0001*
Treatment[B-3.5%]	1.1475404	1.572844	0.73	0.4700
Treatment[C-5%]	-3.058163	1.572844	-1.94	0.0591
Hours Steeping	9.4777571	0.262927	36.05	<.0001*
Hours Steeping*Hours Steeping	-0.244759	0.010259	-23.86	<.0001*
Hours Steeping*Treatment[A-Control]	11.549786	0.462944	24.95	<.0001*
Hours Steeping*Treatment[B-3.5%]	-0.592346	0.452861	-1.31	0.1985
Hours Steeping*Treatment[C-5%]	-4.5867	0.452861	-10.13	<.0001*
Hours Steeping*Hours Steeping*Treatment[A-Control]	-0.224223	0.018337	-12.23	<.0001*
Hours Steeping*Hours Steeping*Treatment[B-3.5%]	-0.023027	0.017577	-1.31	0.1978
Hours Steeping*Hours Steeping*Treatment[C-5%]	0.103427	0.017577	5.88	<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	3	3	441.259	12.6514	<.0001*
Hours Steeping	1	1	15106.840	1299.396	<.0001*
Hours Steeping*Hours Steeping	1	1	6617.175	569.1683	<.0001*
Hours Steeping*Treatment	3	3	7985.062	228.9418	<.0001*
Hours Steeping*Hours Steeping*Treatment	3	3	2170.895	62.2423	<.0001*

REFERENCES

- 1 Annual Energy Review 2008, published by Energy Information Administration. sourced from
(<http://www.eia.doe.gov/emeu/aer/pdf/perspectives.pdf>)
- 2 Brian P. MOONEY Pg 219, “The second green revolution? Production of plant-based biodegradable plastics”; Biochemistry Journal. (2009) 418
- 3 Municipal Solid Waste Generation, Recycling, and Disposal in the United States: Facts and Figures for 2008. Published by the Environmental Pollution Agency (EPA). Sourced from
(<http://www.epa.gov/waste/nonhaz/municipal/pubs/msw2008rpt.pdf>)
- 4 Achyut K. Panda , R.K. Singh , D.K. Mishra, Pg-233, “Thermolysis of waste plastics to liquid fuel:A suitable method for plastic waste management and manufacture of value added products—A world prospective”, Renewable and Sustainable Energy Reviews Vol. 14 (2010)
- 5 Terrance L. Pohlen, M. Theodore Farris, Pg 35 “Reverse Logistics in Plastics Recycling”; International Journal of Physical Distribution & Logistics Management; Vol. 22; Issue 7
- 6 Kenneth E. Johnson, Anthony L. Pometto III,* AND Ziviko L. Nokolov, Pg 1155, “Degradation of Degradable Starch-Polyethylene Plastics in a Compost Environment”; Applied and Environmental microbiology Vol. 59; No. 4 (1993).
- 7 Total Sales & Captive Use by Major Market, 2004-2008, Published by American chemistry council. Sourced from
(http://www.americanchemistry.com/s_acc/sec_policyissues.asp?CID=996&DID=7515)
- 8 Idealized closed loop life cycle of BPDs, Bioplastics at a glance, Published by European bioplastics. Sourced from
(<http://www.european-bioplastics.org/index.php?id=182>)
- 9 Tad W. Patzek, Pg 255,“A First-Law Thermodynamic Analysis of the Corn-Ethanol Cycle”; Natural Resources Research; Vol. 15, No. 4 (2006).
- 10 Prof. Dr.-Ing. Igor Čatić, M. Sc.-Ing. Maja Rujnić-Sokele, Pg 701, “Agriculture products – food for living being or for machinery”; Kunststoffe 61(11) (2008).

- 11 Bruce Dale, Pg 3885, "Biofuels: Thinking Clearly about the Issues" Journal of Agricultural and Food Chemistry; 56 (2008).
- 12 V. P. Martino, A. Jimenez, R. A. Ruseckaite, Pg 2010, "Processing and Characterization of Poly(lactic acid) Films Plasticized with Commercial Adipates"; Journal of Applied Polymer Science 112(4) (2009).
- 13 John W. Lawton¹, Pg 1, "Zein: A History of Processing and Use", Cereal chemistry; Vol. 79; No. 1 (2002).
- 14 S. N. Swain, S. M. Biswal, P. K. Nanda and P. L. Nayak ,Pg 35, "Biodegradable Soy-Based Plastics: Opportunities and Challenges "; Journal of Polymers and Environment.; Vol. 12 (2004).
- 15 J. Zhang, P. Mungara and J. Jane, Pg 2569 "Mechanical and thermal properties of extruded soy protein sheets"; Polymer. Vol. 42, (2001).
- 16 P. Lodha and A.N.Netravali., Pg 49 "Thermal and mechanical properties of environment-friendly 'green' plastics from stearic acid modifiedsoy protein isolate"; Industrial Crops and Products. 21; Issue 1; 49 (2005).
- 17 J. Zhang, L. Jiang, L. Zhu, J. Jane, and P. Mungara, Pg 1551, "Morphology and Properties of Soy Protein and Polylactide Blends" Biomacromolecules; 7(5) (2006).
- 18 P. Mungara, T. Chang, J. Zhu and J. Jane, Pg 10, "Processing and physical properties of plastics made from soy protein polyester blends"; Journal. of Polymers. and the Environment; 31 (2002).
- 19 D. Graiver, L. H. Waikul, C. Berger and R. Narayan, Pg 3231, "Biodegradable soy protein-polyester blends by reactive extrusion process"; Journal. of Applied Polymer. Science; 92 (2004).
- 20 X. Zhao., Q. Ao , F.Chen. ,W. Xue ,L. Li and J. Liu, Pg 116, "Effect of reverse micelle on conformation of soy globulins: A Raman study"; Food Chemistry; 176 (2009).
- 21 National agricultural statistics services 2006 field crop production report from USDA. Sourced from (<http://www.usda.gov/wps/portal/usdahome>)

- 22 Dora Y. M. Lui, Edward T. White, James D. Litster, J. Agric. , Pg 2467, “Dissolution Behavior of Soy Proteins and Effect of Initial Concentration”; Food Chemistry; 55 (2007).
- 23 A. Morales and J. L. Kokinia, Pg 315, “State diagrams of soy globulins”; Journal of Rheology; Vol. 43 ; Issue 2(1999).
- 24 P. J. Kraulis, Pg 946,” Program to produce both detailed and schematic plots of protein structures”; Journal of Applied Crystallography; .24 (1991).
- 25 Chris Rorres, Pg 72, “The Turn Of The screw: Optimal Design of an Archimedes Screw”, Journal of hydraulic engineering Jan (2007).
- 26 Extrusion Process for Reliance electric, Rockwell automation. Sourced from (<http://www.reliance.com/prodserv/standriv/appnotes/d7741.pdf>).
- 27 Introduction to thermo forming :Injection molding from C R Clark catalogs chapter 5
- 28 Rui Deng a, Yun Chen a, Pu Chen a, Lina Zhang, Bing Liao, Pg 2189 “Properties and biodegradability of water resistant soy protein/poly(ϵ -caprolactone)/toluene-2,4- diisocyanate composites”; Journal of Polymer Degradation and Stability ; 91 (2006).
- 29 Cla´udia M. Vaz, Patrick F. N. M. van Doeveren, G. Yilmaz, Leontine A. de Graaf, Rui L. Reis, Ant´onio M. Cunha, Pg 604, “Processing and Characterization of Biodegradable Soy Plastics: Effects of Crosslinking with Glyoxal and Thermal Treatment”; Journal of Applied Polymer Science; Vol. 97 (2005).
- 30 G. Fuller Gerald. “Compositions and methods for joining non-conjoined lumens” U.S. Patent Application 20090162438 (2009).
- 31 M. J. Mulky,. Pg 134 “Butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) as foods antioxidants.” ; Journal of Oil Technologist’. Association of India; 8; Iss:4 (1976).
- 32 Mungara, P.; Zhang, J.; Jane, J., Pg. 148, “Extrusion processing of soy protein -based foam.” Polymer Preprints, 39(2) (American Chemical Society, Division of Polymer Chemistry) (1998).
- 33 Mohanty, A. K.; Tummala, P.; Liu, W.; Misra, M.; Mulukutla, P. V.; Drzal, L. T , Pg 279, “Injection molded biocomposites from soy protein based

bioplastic and short industrial Hemp fiber.”; *Journal of Polymers and the Environment*,13(3) (2005).

34 American Society for Testing and Materials Vol 08.01 Plastics(I)

35 Huang, H. C.; Chang, T. C.; Jane, J. Pg 1101, “Mechanical and physical properties of protein -starch based plastics produced by extrusion and injection molding.” *Journal of the American Oil Chemists' Society* (76) (1999).

36 Guang-Heng, Wang; An-ning, Zhou; Xiao-Bing, Hu. Pg 3134 “Effect of coal filler on the properties of soy protein plastics”. *Journal of Applied Polymer Science*, 102(4) (2006) .

37 Chen, P.; Tian, H. F.; Zhang, L. N.; Chang, P. R., Pg 9389 “Structure and Properties of Soy Protein Plastics with ϵ -caprolactone/Glycerol as Binary Plasticizers”. *Industrial and Engineering . Chemistry . Research.*; 47 (2008).

38 R.Kumar ; L.Zhang,. , Pg 485 , ‘ Soy protein films with the hydrophobic surface created through non - covalent interactions.’ *Industrial Crops and Products*;29(2-3) (2009).