A REVIEW OF WHEAT GLUTEN-BASED BIOPLASTICS PROCESSING AND THEIR APPLICATIONS

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Bioplastics produced from agricultural resources are gaining attraction in recent years because of their sustainability and potential biodegradability. Wheat gluten (a wheat protein) is among the potential feedstocks, which stands out because of its availability, low price, good biodegradability, and good viscoelastic properties. This paper provides state-of-art information on the processing of wheat-based bioplastics with their potential applications. It gives an overview of the structure of wheat gluten, its manufacturing processes (casting, thermoforming, extrusion, compression molding, injection molding), thermal-mechanical properties (tensile strength, elongation at break, young's modulus, water vapor pressure, gas permeability, etc.) of processed plastic films and rigid products, methods to improve the properties, potential applications (packaging, biomedical, adhesives, cosmetics), and limitations and prospects of wheat-based bioplastics.

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DEDICATION

I dedicate this dissertation to my parents Jalal Ahmed and Ferdous Ara Begum, and to my dearest siblings.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

EB	Elongation at break
HMW	High molecular weight
LMW	Low molecular weight
MMT	Montmorillonite
Mpa	Megapascel
MW	Molecular weight
NaOH	Sodium hydroxide
NH_4OH	Ammonium hydroxide
OP	Oxygen permeability
PCL	Polycarprolactone
PHA	Polyhydroxyalkanoates
PE	Polyethylene
PLA	Polylactide
RH	Relative humidity
T_g	Glass transition temperature
T_m	Melting temperature
TS	Tensile strength
WVP	Water vapor pressure
WVTR	Water vapor transmission rate
YM	Young’s modulus

1. INTRODUCTION

Plastics are an essential material for everyday life, often providing performances other materials cannot. However, the increased usages of plastics have become an alarming issue for the environment. Because of their non-degradability, traditional plastics can persevere in the environment for hundreds to thousands of years [1]. Also, plastic accumulated in oceans and cause a large negative impact on marine life [2]. In addition, the incineration of plastics can create toxic and greenhouse gases such as carbon dioxide and methane that result in climate change [3]. Because of all these environmental impacts, the world is looking for alternatives to plastics. In recent years, bioplastics as the alternative to conventional plastics are gaining commercial attraction. Biopolymers, such as proteins, lipids, and polysaccharides, have the potential to be used to manufacture bioplastics [4]. In this case, bioplastics are plastics derived in part or in full form renewable feedstocks. For example, wheat, corn, and soy products are good sources of protein. Therefore, these are ideal feedstock for the production of protein-based bioplastics. Because of its availability, low price, good biodegradability, and good viscoelastic properties, wheat protein has a great potential to be used as a raw material for bioplastics production [5], [6].

The protein portion of the wheat, which is called gluten, can be easily extracted from raw wheat. After washing the starch, granules, and water-soluble portions, approximately 75-85% remaining dry weight is gluten [7]. Proteins are heteropolymers containing up to 20 amino acids joined by peptide bonds (polymerized amino acids). This bond results in a polypeptide chain with a complicated balance of hydrophobic interactions, covalent bonding, hydrogen bonding, and ionic bonding [8]. Several factors, i.e., heat, pressure, irradiation, pH, and solvent, can interrupt the natural configuration of the polypeptide structures, which is called denaturing.

Denaturing results in the change of the chemical structure of the polymer and alters the thermal-mechanical properties of the protein-based plastics. As a result, by regulating the factors affecting denaturing, bioplastics with desired property can be obtained. For example, gluten does not have enough ductility for most industrial applications as the hydrogen bonds make gluten-based plastics relatively weak. Thus, plasticizers or solvents are used to reduce the intermolecular forces and increase chain mobility of the polymer [9] that improves the ductility and reduces the glass transition temperature (T_g) of gluten for the ease of processing [10].

Wheat gluten-based bioplastics are generally manufactured using thermoplastic processing techniques, such as extrusion, injection molding, casting, and compression molding [11], [12]. Different additives are generally added with the raw materials to increase the processing capabilities, namely solvents/plasticizers. In addition, process parameters (i.e., the temperature of processing, the feed rate of raw materials, additives) greatly influence the thermal-mechanical properties of gluten-based bioplastics. Along with thermoplastic processing, there are other compounding processing techniques such as crosslinking, coating, multilayer structure blending, etc. are also used to enhance the final properties of wheat gluten-based bioplastics.

Wheat gluten-based bioplastics have diverse applications, including food packaging, adhesive industries, composite materials, food disposables, and biomedical and pharmaceutical sectors [13]–[16].

The goal of this paper is to review state of the art on the processing of wheat gluten-based bioplastics and their applications. This paper is divided into 13 sections discussing step by step the backgrounds of protein and gluten structure, processing techniques from the wheat grain to gluten-based bioplastics, and the applications of gluten-based bioplastics. Initially, a brief discussion on the definition of bioplastics and how they are different from conventional petrochemical plastics is given in Section 2. The processing methods, the processing parameters, the characteristics and properties of gluten-based bioplastics largely depend on the structure of proteins and gluten. Section 3 and Section 4 of this paper therefore give the necessary background on the structure of proteins and wheat gluten respectively. As gluten is found in wheat grain alongside with other constituents, i.e., carbohydrates, fats, vitamins, minerals, etc., it is important to separate gluten from other constituents in wheat grain. Therefore, the detail on the separation methods or namely milling processes is given in Section 5. Separated gluten can be used for processing of different types of bioplastics. Section 6 provides a brief classification of gluten-based bioplastics. Before going through the detail processing methods, Section 7 describes different additives and their uses in gluten-based bioplastic processing in order to improve the processability of gluten.

The common processing techniques (i.e., casting, thermoforming, extrusion, compression molding, injection molding, etc.) used for gluten-based bioplastics are described in Section 8. The existing studies of different researchers on different processing techniques and their findings are reviewed critically in this section. The most important studies with their summary and findings are illustrated in tables for giving a better understanding to the readers.

As crosslinking is a useful method that is used in thermoset bioplastic processing and largely influences the properties of the final product, Section 9 of this paper provides critical reviews and a concise detail on the crosslinkers of crosslinking methods used by different researchers. Several special processing methods and formulas, i.e., coating, and blending of different polymers, use of nanocomposites, enzymic and chemical treatments are discussed in Section 10. After that, Section 11 provides the applications of gluten-based bioplastics in the packaging, adhesive industry, cosmetics, biomedical industry. This section includes the examples of different gluten-based bioplastics already been used in different sectors and the potential applications that are explored by different researchers in their studies. Lastly, the limitation of this study and the future prospects are suggested in Section 13.

2. BIOPLASTICS

Bioplastics are plastics products produced from renewable sources, e.g., vegetable oil, starch, fiber, etc. [17]. They can be produced from agricultural products or recycled bioplastics. According to the European bioplastic organization, bioplastics are defined as plastics that are either biobased, biodegradable, or both [18]. European bioplastic organization defined biobased as the material or product that is partly or entirely derived from biomass or plants. The agricultural feedstock such as wheat, corn, sugarcane, etc., is an example of a biobased source. According to the European bioplastic organization, biodegradable means the material that can go through a chemical process where microorganisms in the environment convert the material into natural substances such as water, carbon dioxide, compost, etc. The biodegradation does not depend on the resources (e.g., biobased) of the material but rather depends on the material's chemical structure. Thus, fully biobased plastics may be non-biodegradable, and most fully fossil-based plastics can also be biodegradable. Figure 1 shows a graph of the material coordinate system of bioplastics that portrays how the common bioplastics are classified based on biodegradability and bio-based content [18].

Bioplastics made from wheat meet both bio-based and most likely the biodegradability criteria. Wheat is a widely cultivated cereal crop, and it shows very good biodegradability. Domenek et al. showed that wheat gluten products could be fully biodegraded after 36 days in aerophilic fermentation and 50 days in farmland soil [15].

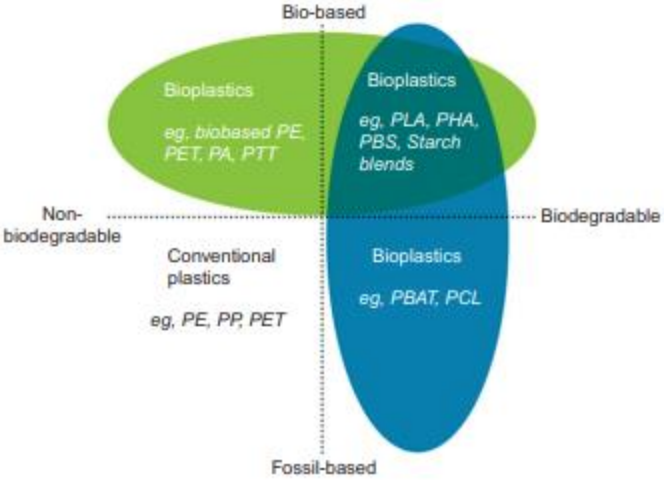

Figure 1: Material classification based on biodegradability and bio-based content [18]

3. PROTEINS

Polysaccharides, proteins, and lipids are widely used for the production of bioplastics. Among these, polysaccharides are abundant and cost-effective [19]. However, when directly used as a polymer, polysaccharides typically have poor water and vapor barrier properties [20]. It is possible to ferment polysaccharides for the production of other monomers, such as lactic acid, or the production of polymers such as PHAs. The resulting polymers can be relatively water stable. In contrast, plastics produced from lipids can show good barrier properties, and they have relatively good elasticity [21]. In addition, protein-based plastics have advantages such as availability and good barrier properties. For example, protein-based plastic films can have better mechanical properties than polysaccharide-based and lipid-based films [22].

Proteins are complex heteropolymers, with amino acids as their monomer unit. Protein contains up to 20 different amino acids, each with particular structures and sequences [23]. Peptide bonds join amino acids. Because of the large variety of structures and sequences, an unlimited number of interactions and chemical reactions can occur between the amino acids and the resulting protein structures [24].

The polypeptide chains allow the protein structures to fold into a secondary, tertiary, and quaternary structure which is called the native conformation [25]. Because of the various chemical structures, amino acids have side groups called as R group. These R groups are the radical groups that distinguish amino acids from each other. These side groups each have different size and physicochemical properties, i.e., polarity, pH, etc., determining the strength and the nature of intra- and inter-molecular interactions in each amino acid. Some of the important amino acids based on their R group are amide (- NH_2), acidic (-COOH), neutral (-OH), basic ((C=O)-NH_2), sulfur-containing (-SH), etc. [8]. These functional side groups promote the primary configuration of the protein structure. The secondary structure refers to the localized folded structure of the polypeptide chain. Secondary structures are bonded by hydrogen bonds (week secondary bonds) between the carbonyl group of one amino acid and the amino group of another. Tertiary structure is a larger three-dimensional polypeptide structure that occurs because of the interactions between the R groups of the amino acids. Hydrogen bonding, ionic bonding, and disulfide bonding are some of the R group interactions that contribute to the folding of the protein, leading to tertiary structure [26]. Quaternary structure only occurs in some of the proteins, consisting of multiples polypeptide chains known as subunits. Quaternary structure occurs because of similar types of interactions that cause the tertiary structure to form.

When external factors such as heat, pressure, pH, solvent are changed, the interactions in the native conformation may be disrupted that can cause the protein to lose its native three-dimensional structure. These disruptions typically do not affect the primary structure of the protein. However, the overall change in the protein configuration is called denaturing. The alterations that occurred because of denaturing can alter the thermal-mechanical properties of the protein and resulting plastics with different properties. These properties include strength, flexibility, oxygen and vapor barrier properties, etc.

4. WHEAT GLUTEN

Wheat is a widely common cereal grain. Like other crops, it consists of protein, carbohydrate, fiber, fat, and other components. The protein portion of the wheat is called gluten. When washed, starch granules and other water-soluble constituents of the wheat are dissolved and can be fractionated. However, gluten is not water-soluble. It consists of approximately 75-85% non-soluble dry weight of wheat [7], separated from the starch by centrifugation and thoroughly washed and dried. Gluten consists of more than hundreds of protein components [27]. These protein components can be separated into two fractions based on the solubility in the alcohol-water solution (e.g., 60% ethanol) [6]. The soluble fraction in alcohol-water solution is called gliadins. They consist of single-chain polypeptides, having a molecular weight ranging from 10kDa to 50kDa. Gliadins are classified based on amino acid sequences and cysteine content. γ-gliadins are high in cysteine. α-, β- and γ-gliadins have sulfur-containing amino acids, and they form intrachain disulfide bridges. In contrast, ω-gliadins lack sulfur-containing amino acids and thus do not form disulfide links. Hydrated gliadins show little elasticity and less cohesiveness and mainly contribute to the viscosity and extensibility of wheat dough [7]. The other fraction of gluten is called glutenin that is insoluble in a higher concentration of alcohol. Glutenins have molecular weights ranging from 50kDa to 300kDa. They are protein aggregates that consist of high-molecular-mass (HMW) and low-molecular-mass (LMW) subunits. They are formed by disulfide links of different polypeptide chains, and when hydrated, they show good cohesiveness and elasticity that contributes greatly to dough strength and elasticity.

Concerning the amino acid composition, the majority of gluten consists of glutamine and proline. The glutamine is mainly responsible for binding with water and comprises approximately 40% gluten [28]. In addition, glutamines form hydrogen bonds and are accountable for the viscous property of gluten. Compared to glutamine, proline includes 30-45% of gluten and is mainly responsible for gluten's elastic nature. Among the amino acids, cysteine plays a vital role in the structure of gluten. They are primarily present in an ionized state, and they can form both intrachain and interchain disulfide bonds. This property makes gluten a great potential in bioplastic applications to allow plastics to be produced with good mechanical properties.

5. GLUTEN MILLING PROCESS

Gluten is the protein portion of wheat that can be used for the processing of bioplastics. The composition of wheat contains only 10-13% proteins. The other constituents of wheat are carbohydrates (69-72%), moisture (10-11%), fat (2-3%), fiber (2-3%), ash (1-2%), etc. For wheat proteins to be used for bioplastic production, the protein portion of wheat must be separated. The separation technique is called the milling of wheat. The milling process is typically completed in one of two techniques: dry milling and wet milling.

5.1. Dry Milling

The structure of wheat grain consists of three parts: the bran, endosperm, and germ. Figure 2 shows the structure of wheat grain. Among the three parts, the endosperm contains most of the starch and protein portions. The objective of the dry milling is to separate the endosperm from the bran and germ. Before starting the milling process, to remove any non-grain part, the grains are cleaned in several steps. First, a vibrating screen is used to remove coarse foreign materials that come from harvesting. Next, the dust particles or other lighter materials are removed by using an aspirator. A disk separator is then used, which separates the materials based on size. A scourer is then used, which breaks the kernel hairs of the wheat grain by throwing the wheat against the drum of the scourer. Any magnetic materials are removed using a magnetic separator. A washer stoner is used at last, separating the heavier and lighter materials from the wheat grains. During the milling, wheat grain is conditioned and tempered to achieve a constant moisture content of approximately 15.5% [29], which is relatively low, and that’s why this is referred to as dry milling. This moisture makes the bran layer flexible. As a result, when undergoing the milling action, bran layers break into large flakes.

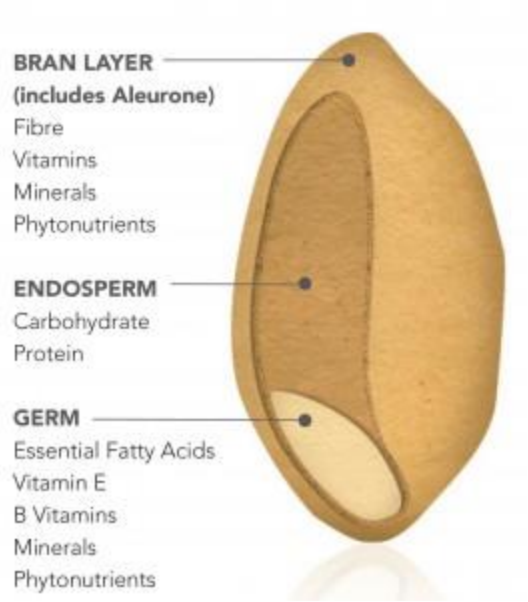

Figure 2: Structure of wheat grain [30]

The main steps of the dry milling process involve grinding on the roller mill, sieving, and purifying [31]. The bran and germs get broken into large flakes because of the continuous grinding of roller mills. The grinded stocks are then sieved, and fine particles are separated. Meanwhile, the large particles are sent to the roller for further grinding. Thus, a continuous reduction process continues where endosperm particles result in fine wheat flour. A process flow diagram of milled wheat from the cleaned wheat is shown in figure 3.

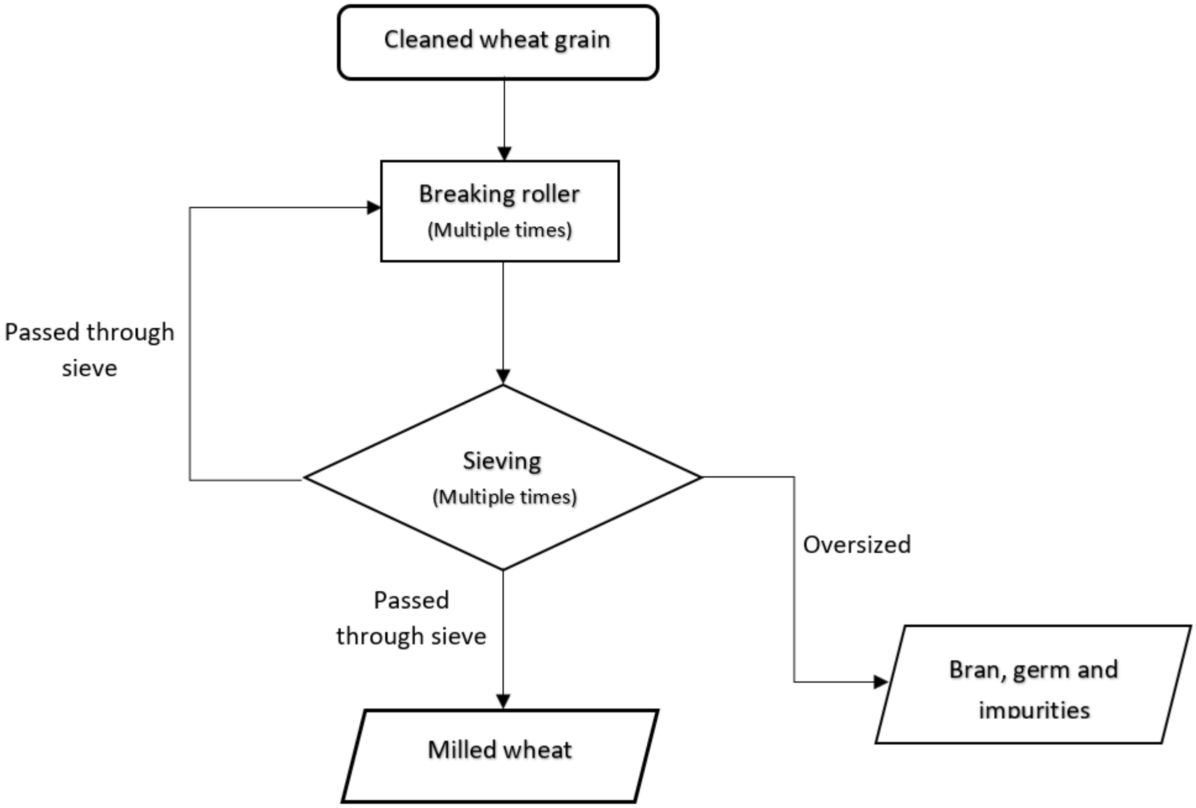

Figure 3: Flow chart of dry milling process from cleaned wheat

5.2. Wet Milling

The wheat flour obtained from dry milling contains protein, starch, fat, fiber, and other constituents. To separate proteins from other constituents, the wet milling process is used. It is a combination of steps where water is used to soften the flour to make it a dough, and several separation techniques based on solubility, sedimentations, filtrations, distillations, centrifugations, etc., are applied to separate the proteins from the other constituents. Wheat flour obtained from the dry milling is used as the starting material in the wet process. Water or a combination of water and other chemicals is added to the flour that provides a dough-like structure. Centrifugation, decantation, hydroclones, etc., are then used to separate the starch and gluten from the wheat flour [31]. The starch and other constituents dissolve in water, where the gluten is not soluble in water. The non-soluble portion is separated and then washed thoroughly and dried. The drying stage involves methods such as freeze-drying which prevents denaturing of the protein structure. The temperature is maintained below 70℃ during this process. If the conditions are not maintained properly, wheat gluten can lose its viscoelastic properties. It is then called nonvital wheat gluten. The wet milling and drying process are controlled in specific conditions to preserve the vitality of the gluten. In this form, the gluten is called vital gluten or unnatured gluten that allows the preservation of the characteristics of natural protein as water absorbability, formation of elastic and extensible mass, etc. When vital gluten is mixed with water, the gliadin and glutenin proteins are hydrated, imparting hydrogen bonds and hydrophilic interactions with water molecules. The dynamic interactions of gliadins and glutenins in the presence of water result in the formation of a viscoelastic gluten network, which is essential for bioplastic production using wheat gluten [32].

6. GLUTEN-BASED BIOPLASTICS

Based on the chemical structure, plastics and bioplastics from wheat gluten can be classified into two broad categories: thermoplastic and thermoset.

6.1. Thermoplastics

A thermoplastic is a form of polymer that becomes pliable when heated. Typically, thermoplastic materials can be thermally cycled many times with little degradation, allowing them to be reprocessed and recycled. The cycle begins with heating, where the materials are plasticized. Plasticized materials can then be shaped depending on the processing techniques. After cooling, the materials become rigid. However, the processing temperature, ambient atmosphere, geometry of the product can affect the properties of finished products [33]. The heat can be provided by radiation, internally generated through fiction, or a range of other processes. Wheat gluten or its fractions, along with some other additives, can be used for the production of thermoplastic. Figure 4 shows the structure of the thermoplastic polymers.

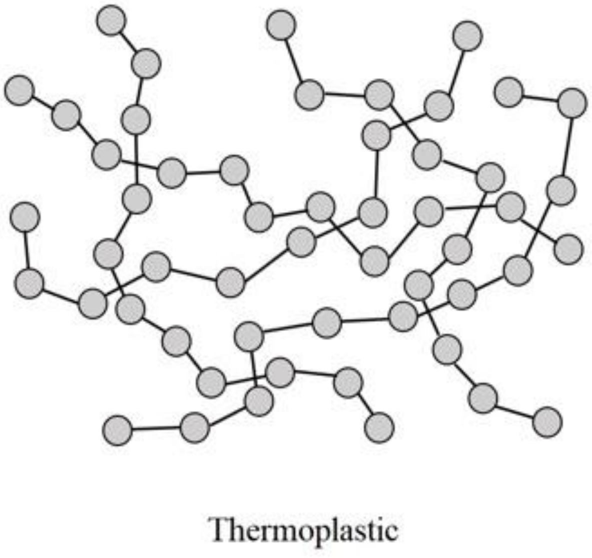

Figure 4: Structure of thermoplastic polymers

Thermoplastic materials are broadly classified into two classes: amorphous and semi-crystalline thermoplastics. In semi-crystalline material, there are regions where the molecules fold onto each other forming a regional crystalline structure surrounded by amorphous regions. Crystalline material has both glass transition temperature (T_g) and melting temperature (T_m). T_g is the temperature where the materials start to change from a hard and rigid state to a more flexible or rubbery state. Whereas, melting temperature (T_m) of a material is the temperature where the material turns to liquid form. The semi-crystalline thermoplastics are shaped at a temperature over the melting temperature. Semi-crystalline polymers have a highly ordered molecular structure that results in a well-defined melting point. They remain solid below that melting point and only transform to low viscous liquid after the melting point. Semi-crystalline thermoplastics show a very good fatigue resistance and resistance to shear cracking.

There is no crystalline structure of the molecules in an amorphous material, and the entire structure is random. Amorphous thermoplastics are shaped at a temperature that is well above the T_g. This causes them to have a range of temperatures at which they will soften and have no well-defined melting point. They are generally easy to transform when heated as the molecules become mobile as heat is increased. The amorphous thermoplastics show poor stress cracking and fatigue resistance. Wheat gluten is an example of amorphous materials.

6.2. Thermoset

Thermosets are polymers that cure irreversibly and become hard and stiff permanently after curing. Curing can be promoted by applying heat or irradiation or by the addition of a catalyst. Curing causes a chemical reaction in the polymer that results in crosslinking. Crosslinking is the process of chemically joining two or more molecules by covalent bonds. The covalent bonds form between the polymer chains of the molecules. Crosslinkers or crosslinking agents have two or more reactive ends which can chemically attach with two or more specific functional groups, i.e., amines, suflydryls, etc., of proteins.

Crosslinking is a chemical process of forming covalent bonds to join two or more polymers. Crosslinkers or crosslinking agents generally have two or more reactive ends that chemically react with particular functional groups. Crosslinking of these functional groups (i.e., primary amines, sulfhydryls, etc.) can also occur in protein molecules. Two groups in a protein can create intramolecular crosslinks that can cause the stability of the tertiary and quaternary structure of the protein [8].

Crosslinked polymers are generally processed and shaped before crosslinking occurs. Once crosslinking happens in the polymer, the material cannot be reshaped through heating because the polymer network becomes chemically rigid. Thus, crosslinked polymers are thermosets. In thermoset processing of wheat gluten, wheat gluten or its fractions are mixed in a liquid solution, and then the liquid is crosslinked in the molding process. The mold is given to the desired shape before the crosslink occurs. Figure 5 shows the structure of thermoset polymers.

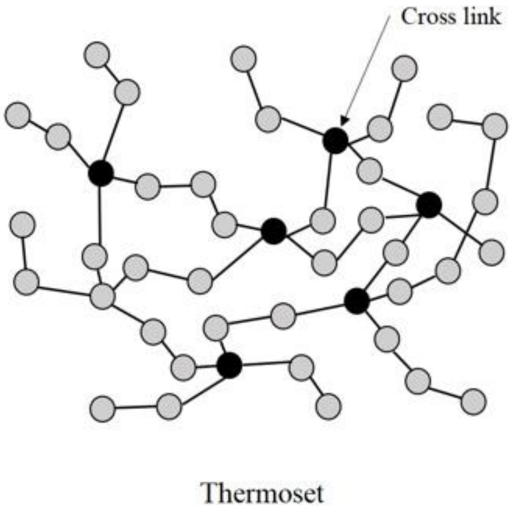

Figure 5: Structure of thermoset polymers

7. COMPOUNDING OF WHEAT GLUTEN BIOPLASTICS

The compounding of wheat gluten involves processing wheat gluten as raw material and adding different additives, i.e., plasticizers, fillers, stabilizers, pigments, etc., to achieve specific properties, performance, and appearance. Among these additives, plasticizers are a major component of the wheat gluten compounding process. Without a plasticizer, wheat gluten films and bioplastics are typically brittle. To enhance the mechanical properties, plasticizers are added to the plastic. Plasticizers are substances that can increase the flexibility and the workability of a material [34]. They can initiate physical reactions between the polymer chain, causing flexibility, elasticity, extensibility, and other mechanical property to improve [22], [35]. Plasticizers can reduce the intermolecular forces and reduce the glass transition temperature (T_g) of wheat gluten, which results in improved mobility in the polymer chain [10], [36]. The most common plasticizers used for protein processing include monosaccharides, oligosaccharides, lipids, polyols, water, alcohol, and other derivatives [37]. Glycerol, sorbitol, polyethylene glycol, sucrose are examples of those plasticizers. Among these, glycerol is widely used in the processing of bioplastics. The structure of glycerol can easily enter and position itself inside the 3-dimensional polymer network [38]. It has a low molecular weight and easily interacts with polymer chains [4].

Fillers are added to plastics to increase mechanical properties. They occupy the space in plastics to replace the expensive resins or polymers and make the plastics cost-efficient. In addition, they give the plastics better hardness, stiffness, strength, and workability. The most common examples of fillers that are used are calcium carbonate, clay, silica, wood flour, sawdust, gypsum, cotton fibers, etc. Aside from the previous uses, different filler materials can be used to give different special properties to the plastics, i.e., quartz and mica improve hardness, barium salts make the plastic impervious to x-rays, clay improves electrical properties and processability.

To improve the thermal stability of the plastics during processing and retard the degradation in their lifespan, stabilizers can be added to the wheat gluten bioplastics. The most common stabilizers that are used in plastic processing are mixed metal salts (barium-zinc, cadmium-zinc, etc.), organotins (dibultin maleate, n-alkyltin mercaptides, etc.), lead (lead sulfates, lead phthalate, etc.), and epoxidized vegetable oils (epoxidized soybean oil, epoxidized linseed oil, etc.).

Catalysts are another additive that is mainly used in the compounding of thermosetting plastics. Catalysts speed up the crosslinking rate of thermoset plastics. Examples of catalysts used are hydrogen peroxide, zinc oxide, benzoyl peroxide, etc.

Pigments are added to plastics to achieve the desired color and appearance. Some of the examples of pigments are zinc oxide, carbon black, chromium trioxide, etc. Lubricants prevent the plastic material from sticking to the mold, which gives a glossy finish to the plastics. Waxes, oil, soaps are used as lubricants.

The compounding of plastics with additives usually takes place in an extruder machine. The wheat gluten is fed into the extruder, where it is melted/plasticized and moved forward through the action of the screw. Additives are added along the length of the barrel and mixed with wheat gluten. Wheat gluten and all the additives get thoroughly mixed when they reach the end of the extruder. The extrudate comes typically in strands, and they are cooled and then cut into pellets by a cutter.

8. GLUTEN-BASED BIOPLASTICS PROCESSING

Bioplastic processing of wheat gluten typically involves mixing wheat gluten or its various fractions with a solvent/plasticizer and applying heat and pressure to shape the mixture to desired products [8]. Bioplastics can be processed by common plastic processing techniques such as casting, extrusion, thermoforming, injection molding, compression molding, etc. Depending on the final product, the processing of wheat-gluten bioplastics can be a single technique or a combination of several techniques. In this section, five of the most popular techniques: casting, extrusion, thermoforming, injection molding are discussed.

8.1. Casting

Casting is a common processing technique for wheat-gluten-based bioplastics. In casting, wheat gluten is dissolved in a solvent to make a solution. Plasticizers and other materials can be added to the solution that helps in the functional properties of the casted product. This solution is then poured into a mold that contains a cavity of the shape of the desired product. Typically, heat is applied to alter intermolecular interactions, including any cross-linked disulfide bonds [39]. When the liquid mass dries (evaporation of the solvent), it solidifies and takes the shape of the desired product.

Several factors can affect the properties of the casted product. The drying temperature is one of the important factors that affect the mechanical properties of casted products. As the temperature increases, more crosslinking reactions can occur in the protein network, which causes intermolecular covalent bonds to form. As a result, the mechanical properties, i.e., tensile strength, elongation at break, young’s modulus, etc., improve the casted product. A similar result was found in several researchers' studies [40]–[43]. The authors of these studies suggested that the tensile strength (TS) and young’s modulus increase with the increase of drying temperature.

Casted products can also be influenced by other materials and methods. Lee et al. investigated the effect of gamma-irradiation on the properties of gluten films [44]. Gamma-radiation treatment of the solution caused alteration in gluten molecule structure. The TS of gluten films was increased significantly by the gamma-radiation. Increased TS was assumed to be related to the increased crosslinking of polypeptide chains. Balaguer et al. [45] used cinnamaldehyde as a cross-linking agent. Cross-linked casted films showed increased TS and lower elongation at break (EB). Also, the films showed better transparency which makes them well-suited for food packaging applications.

Although casting is a common technique for processing protein-based bioplastics, there are several limitations. For example, the solvents used can be expensive, and the costs of the evaporation of the solvent can be significant [8]. For these reasons, casting has limited applications in gluten bioplastic processing.

Table 1 gives a summary of significant studies where the casting method was used for the manufacturing of wheat gluten-based bioplastics.

Table 1: Wheat gluten bioplastics processing using casting.

Protein	Plasticizer	Variables/ Parameters	Properties	Findings	Reference
Wheat gluten	20% glycerol	Different drying temperatures, 1.5% sodium dodecyl sulfate	TS: 3.3-8.2 MPa EB: 109-328% YM: 40-110 MPa	Drying temperature affected the mechanical and physical properties of gluten films	[43]
Wheat gluten	20-60% glycerol	Glycerol content varied	TS: 0.6 MPa EB: 732%	Cast films showed lower stress value compared to thermo-pressed films	[46]
Wheat gluten	30% glycerol	Irradiation applied to the film-forming solution	TS: 2.68-3.99 MPa EB: 108-282%	With increased irradiation TS increased, EB decreased, water vapor permeability decreased	[44]
Wheat gliadin-rich fraction	25% glycerol	Cinnamaldehyde added	TS: 1.6-9.9 MPa EB: 144-319% YB: 2.1-112.1MPa	Cross-linked films showed higher TS, lower EB, improved transparency, and other mechanical properties	[45]
Wheat gluten	-	No plasticizer used	EB: 70-200%	Gliadin free gluten could be used for tissue engineering purposes	[47]
Wheat gluten	3-7% glycerol	Glycerol concentration varied	EB: 30-250%	With the increase of glycerol concentration, TS decreased, EB increased, WVTR increased	[48]

8.2. Thermoforming

The thermoforming process involves heating plastic sheets and forming the sheets into final products through pressure and/or vacuum. The process starts by securing a plastic sheet above a mold. Then heat is applied to the plastic sheet until it becomes malleable and reaches its “rubbery” state. The heat can be supplied by three different methods: conduction, convection, and infrared radiation. Conduction heating is contact heating where plastic sheets and heated plates are in direct contact. This method is limited because most plastics have low thermal conductivity. In convection heating, heated fluid is used for heating. Convection heating can be used for preheating the plastic sheet. The third method: Infrared radiation is the most efficient method for heating the plastic sheets. The infrared energy can penetrate the plastic and bulk heat the plastic without limitations related to conduction.

The sheet is forced into a mold by pressure or vacuum to ensure that the plastic sheet conforms to the shape of that mold. The heat is discontinued, and the material is allowed to cool. After cooling, the molded part is removed from the mold.

The properties of wheat gluten-based bioplastics made by thermoforming depend on several factors, i.e., molding temperature, reducing reagents, plasticizer content, etc. Sun et al. [49] investigated the influence of molding temperature for wheat gluten bioplastics. The result showed that the molding temperature has a significant effect on the properties of wheat gluten plastics. In more detail, the TS, YM both increased with the increase of molding temperature. The authors suggested that this increase is related to the molecular mobility and the crosslinking density of the gluten network. The molecular mobility was too low at low temperatures, which caused inadequate protein unfolding and an inhomogeneous network. Also, crosslinking did not occur at a low temperature. However, at higher molding temperatures, plasticized proteins tended to develop a homogenous network between the crosslinking sites, which resulted in high extensibility and high strength.

Song et al. [50] used sodium bisulfite, sodium sulfite orthioglycolic acid, along with glycerol in their study. Sodium bisulfite, sodium sulfite and orthioglycolic acid work as reducing agents. Reducing agents can cleave the covalent disulfide linkages between cysteine residues and cause denaturation to occur. The authors found that with the use of these reducing agents, the young's modulus and flexibility increased significantly. Because reducing agents can break the disulfide bonds into thiol groups [51], [52], this causes the reduction of molecular protein weight

[53] as a result of the localized depolymerization. Localized depolymerization provides more molecular freedom and increases the flexibility of the resulting plastics.

Glycerol content is also an important factor that significantly affects the mechanical properties of gluten-based bioplastics. Sun et al. [54] investigated the role of glycerol content in the mechanical properties of wheat gliadin bioplastics. Sun's studies showed that TS and YM were generally inversely proportional to glycerol content in the samples. The authors suggested that gliadins produce strong hydrogen bonds and hydrophobic reactions [55]. It is usually believed that glycerol reduced the intermolecular bonds in gliadin bioplastics. As a result, the flexibility and extensibility generally increased with glycerol content [10], which leads to the decrease in YM, TS, and stiffness of gliadin-based bioplastics.

Hernandez-Munoz et al. [56] investigated the change of glycerol content on both gliadin and glutenin fractions. Increased Gliadin fractions showed better mechanical properties compared to glutenin fractions. The authors suggested that the high crosslinking rate of gliadin was the main reason for the improved mechanical properties. It was also seen that heat treatment of protein could modify the disulfide gluten structure by the unfolding of gliadin and glutenin structure through disulfide/sulphydryl interchange reactions. The disulfide/sulphydryl interchange reactions usually occur at 55-75℃ for glutenins and higher than 70℃ for the gliadins [57]. Lavelli et al. showed that crosslinking occurs more comprehensively in the gliadin-rich fraction than glutenin-rich fraction [58]. Thus, because of the high rate of crosslinking, gliadin-based bioplastics show better mechanical properties than glutenin-based bioplastics.

Table 2 shows a summary of significant studies where wheat gluten-based bioplastics were manufactured with the thermoforming method.

Table 2: Wheat gluten-based bioplastics were obtained by the thermoforming method.

Protein	Plasticizer	Variables/ Parameters	Properties	Findings	Reference
Gluten electrophoresis	30% glycerol	-	TS: 0.49-3.41 MPa WVP: 1.028-8.7 g/msPa YM: 8.15-28.74 MPa	It could be used in eggshell coating and degradable packing material	[59]
Gluten	33.6% glycerol	Temperature: 65-95℃, heat treatment time: 2-24 h	TS: 3.8-17 MPa EB: 5-170% WVP: 1.54-1.93 g/msPa	Significant increase in TS and WVP and decrease in EB with the rise of temperature and exposure time	[42]
Gluten	11-66% glycerol	Glycerol content varied	TS: 1-34.3 MPa EB: 5-374%	Glutenin fraction showed higher TS and lower EB and water vapor permeability compared to the gliadin fraction	[60]
Gluten	40% glycerol	Temperature effect 80-135℃	TS: 0.26-2.04 MPa EB: 236-468%	With the increase of temperature, the films showed increased TS and decreased EB	[61]
Gluten	20-60% glycerol	Glycerol content varied	TS: 1.65-4.15 MPa EB: 144-200%	Glycerol amount did not show a significant effect on TS	[46]
Gliadin- and glutenin-rich fraction	33% glycerol	Temperature: 40-115℃	TS: 0.61-14 MPa EB: 5-380%	Higher temperature facilitates cross-linking of polymer chains	[56]
Gluten	35% glycerol	Molding temperature 65, 85, 105, 125℃	TS: 0.52–6.69 MPa EB: 173–288% YM: 1.2–36 MPa	Increased temperature improved crosslinking density and increased TS, YM, and relaxation time	[49]
Glutenin rich fraction	40% glycerol	Sodium bisulfite, sodium sulfite orthioglycolic acid as reducing reagents	TS: 1.54–1.8 MPa EB: 88–133% Weight loss: 30.8–35.7% Water uptake: 105–116%	Reducing agents showed reduced Young's modulus in the plasticized dough	[50]
Gluten	36% glycerol	Mixing temperature varied	TS: 0.3-1 MPa YM: 1.3-10.2 MPa EB: 30-127%	SME values decreased with the increase of mixing temperature	[62]

8.3. Extrusion

Extrusion is a widely popular manufacturing technique for plastic processing. In extrusion, typically, plastic pellets or powder feedstock are added to the hopper, and this material is pushed forward by a screw where it is heated through friction and conduction. This melted plastic is conveyed to a die. After passing through the die of the desired shape, it takes the shape of that die. The key parameters include screw speed, temperature settings at the various zones, feed rate, the screw design, length-to-diameter ratio, die size, material compensation, and moisture. Specific Mechanical Energy input is a measure of extrusion condition, which directly impacts the final extrudate properties [11]. Specific mechanical energy is calculated using torque, screw speed, and mass flow rate. Redl et al. [63] investigated different extrusion processing conditions on plasticized wheat gluten. The authors found both smooth surface extrudate and disrupted extrudate depending upon different processing conditions. When a twin-screw extruder is used, because of a high screw speed, the specific mechanical energy input becomes too high for the process. This excessive specific mechanical energy leads to viscous heat dissipation, which results in higher temperature and hence leads to excessive crosslinking [64]. Pommet et al. [23] also found in their study that high specific mechanical energy and high temperature can result in excessive crosslinking. Verbeek et al. [11] indicated that the control of protein/protein interactions during the extrusion process could lead to successful processing. The high crosslinking rate inside the extruder barrel can increase the viscosity and reduce the chain mobility, leading to an increase in residence time, torque, and pressure in the metering zone. This can cause protein degradation and can even lead to sessing the screw in the barrel.

The window of gluten processing using the extrusion process is very narrow [9]. In more detail, the extrusion of gluten has a relatively narrow temperature range. In addition, the extrusion of gluten has a relatively low-temperature limit. For glycerol plasticized gluten, the low-temperature limit is approximately 90℃, which is determined by the denaturation of the gluten structure. The upper temperature limit is determined by the increased viscosity due to extensive protein aggregation [65], [66]. This aggregation is mainly the thiol-disulfide exchange reactions that restructure the disulfide bonds. Sulfhydryl of the cysteine amino acid can form new disulfide cross-links during oxidation that can also lead to aggregation. However, the upper temperature limit can be increased by delaying or limiting the disulfide reactions [67], which can be done by adding radical scavengers that impede the crosslinking reactions.

Ullsten et al. used salicylic acid as a radical scavenger in their study [67]. Salicylic acid successfully improved the temperature range of the process. It did not impact the ductility of the plastic film, but it did affect the cross-linking rate. Later the same authors in another study [68] added sodium hydroxide with salicylic acid to get an increased alkalic condition. Wheat gluten shows nonhomogeneity at the pH value close to its isoelectric point, pH 7.5. Previous studies showed that homogenous gluten films could be found at pH 2-4 and pH 9-13. The increased pH value with the addition of sodium hydroxide produced good homogenous films. The addition of sodium hydroxide also showed an improved gas barrier and improved mechanical properties such as TS and EB. However, not many studies were conducted using NaOH as a radical scavenger because of its toxic properties. Instead of NaOH, NH_4OH (Ammonium hydroxide) was used as a radical scavenger by Ullsten et al. [69]. Although NH_4OH resulted in better gas permeability and WVP, the mechanical properties were compromised. Later, urea was used instead of NH_4OH by Ture et al. [70]. The produced film showed a slightly better result.

A summary of important literature where wheat gluten-based bioplastic was manufactured using the casting method is given in table 3.

Table 3: Summary of wheat gluten bioplastics processing using casting.

Protein	**Plasticizer**	**Variables/ Parameters**	**Properties**	**Findings**	**Reference**
Gluten	30% glycerol	1-2% salicylic acid addition	TS: 0.9-3MPa EB: 47.3–159% YM: 3.2–36.7 MPa Moisture content: 9.4-17.6%	Salicylic acid improved the working temperature for the extrusion. It did not impact the ductility but affect the cross-linking rate	[67]
Gluten	30% glycerol	3-5% sodium hydroxide or 1% salicylic acid	TS: 1.4–2.4 MPa EB: 79–143% YM: 10–95 MPa	NaOH addition improved the oxygen barrier and showed better mechanical properties compared to the mixture of both NaOH and salicylic acid	[68]
Gluten	30% glycerol	10-20% urea addition	TS: 2.2.–3.6 MPa EB: 30–87% YM: 62–73 MPa WVTR: 32–57 gmm/m2day OP: 0.16–0.32 cm3mm/m2 day atm	Urea can be used for solvent-free extrusion processes.	[70]
Gluten	30% glycerol	0-50% kraft lignin (KF) was used	TS: 1.1-2.6 MPa YM: 4-107 MPa EB: 3-109%	Increased range of processing temperature, improved processability	[64]
Gluten	Glycerol	Glyoxal and xantham gum were added as additives	TS: 0.6-1.4 MPa YM: 3-4 MPa	Glyoxal and xanthan gum improved the water uptake capacity, but the YM decreased	[71]

8.4. Compression Molding

Compression molding involves the molding of molten plastic through compression and typically heat. The compression molding arrangement includes two metal mold plates where the lower plate is static, and the upper plate is movable. The gluten, along with plasticizers and other additives, is placed into the lower mold cavity. The upper plate is moved to the lower plate, and heat and pressure are applied. The polymer placed in the mold cavity becomes soft and because of the application of heat and pressure, the materials take the shape of the mold. Molding temperature, molding time, moisture content, applied pressure, plasticizer content are parameters controlled during the manufacturing process to produce the desired property from the molded films.

The molding temperature is one of the most critical parameters in determining the properties of the final product. Both glutenin and gliadin fractions of gluten get unfolded on heating at approximately 75℃, which accelerates the sulfhydryl-disulfide interchange promoting denaturing of the proteins, and upon cooling, they retain the denatured configuration [72]. For gliadins, the crosslinking reactions occur at approximately 90℃, whereas for glutenin, the crosslinking reactions generally occur at 60-70℃. Thus, the amount of sulfhydryl-disulfide interchanges is generally proportional to the temperature of both gliadin and gluten fractions, which facilitates the formation of intermolecular covalent bonding. This results in the increase of YM and TS as the processing temperature increases. Zubeldia et al. [73] investigated the YM and YS for gluten film compression molded at 80℃ and 100℃. With the increase of the temperature from 80℃ to 100℃, they found the increase of YM and TS as high as 50% and 7.5%, respectively. Gallestedt et al. [74] in their study, found that the resultant product was dough-like when the molding temperature was below 90℃. The authors studied color change as a function of temperature. The films produced at higher temperatures (130 ℃) were darker in color, likely caused by the crosslinking density, extensive aggregation, temperature effects of the pigments.

Moisture content is also an important parameter for the properties of the compression-molded gluten plastic. The water can plasticize gluten that results in decreased intermolecular forces and increased chain mobility. Molecular mobility is generally proportional to moisture content. However, excessive moisture can lead to porosity. Jansens et al. [75] investigated the effect of molding condition and moisture content (without any additional plasticizer) in the mechanical properties of gluten powder. The authors observed that the flexural modulus of gluten bioplastics does not depend on the primary interactions (e.g., hydrogen bonds, crosslinking) but rather relies on the hydrogen bonds and thermal interactions. The primary bonds in the protein network can cause delocalization of local strain in the outside area of largest stress, which can bring toughness. Thus, toughness was found to be generally proportional to the moisture content in selected conditions. However, excessive moisture and higher processing temperature can lead to a loss of toughness.

A summary of these studies is illustrated in table 4.

Table 4: Summary of wheat gluten-based bioplastic processing using compression molding

Protein	**Plasticizer**	**Variables/ Parameters**	**Properties**	**Findings**	**Reference**
Gluten	25-40% glycerol	Glycerol content, molding temperature	TS: 1.2-17.2 MPa YM: 0.1-363 MPa	Higher glycerol content resulted in higher OP and WVP, but lower YM	[74]
Gluten	-	No plasticizer, moisture content, molding temperature, molding time	TS: 32.6-47.6 MPa T_g: 65-76.7 ℃	Moisture content, molding temperature and molding time affect the mechanical properties	[75]
Gluten	16% glycerol	Alkoxysilicone added	EB: 42-160%	High elongation, low TS	[76]
Gliadin and glutenin	10-40% glycerol	Glycerol content	YM: 30-500 MPa TS: 1-15 MPa EB: 10-400%	10% glycerol showed the best results in gliadin and glutenin products	[77]
Gluten	15-25% glycerol	Glycerol content, pressing temperature, mixing time	TS: 1.86-2 MPa EB: 37-57% YM: 15-22 MPa	Glycerol content has a huge effect on mechanical properties and WVP	[73]

8.5. Injection Molding

Injection molding is one of the most popular thermoplastic processing techniques that is specially used for complex-shaped products with high dimensional accuracy. The injection molding machine includes a screw in a barrel where plastics are fed in pellet form through a hopper. The material plasticizes inside the barrel as heat is provided by the heating units and shear heating. The screw rotation pushes forward the plasticized material. The material is thus injected into the mold cavity under high pressure. The mold cavity represents the shape of the desired product. Once the injected material enters the mold cavity, it begins to cool and solidifies to take the shape of the mold. After a specific cooling time, the mold is opened. The ejector pin pushed the product out of the mold. A schematic diagram of the injection molding process is shown in figure 6.

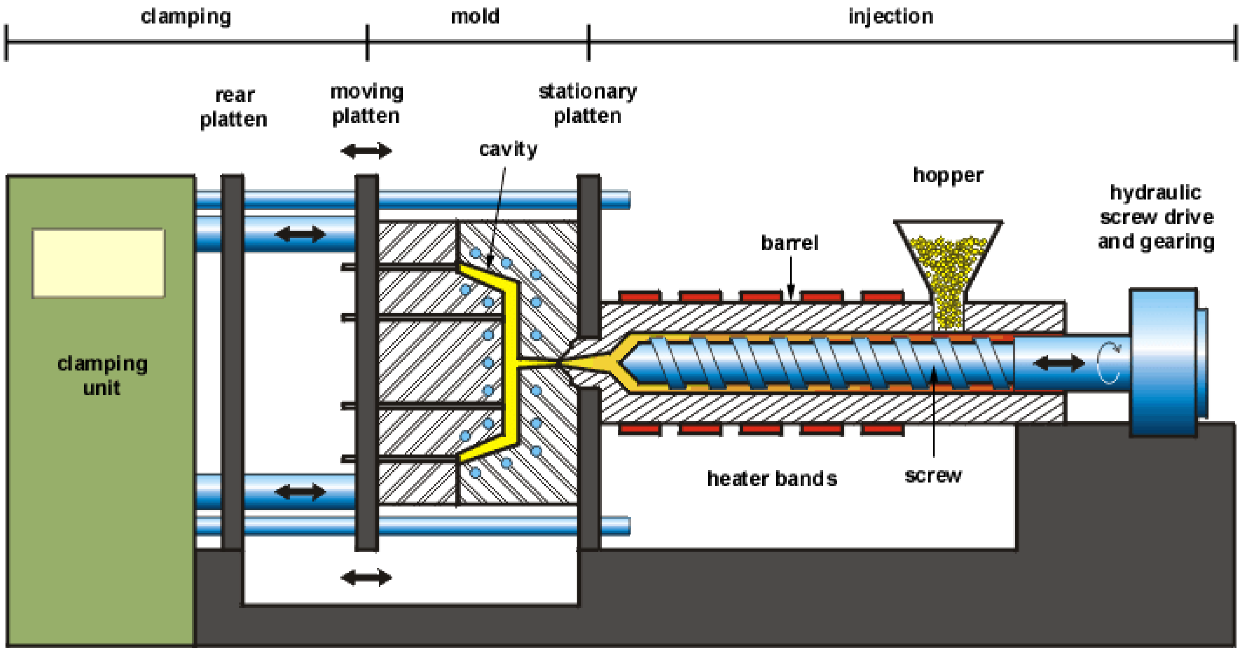

Figure 6: Injection molding process [78].

Because of gluten's high melt viscosity, injection molding can be challenging. Cho et al. investigated the potential use of injection molding of wheat gluten bioplastics [79]. The researchers fed wheat gluten powder into the injection molding screw at different temperatures ranging from 110 to 200 ℃ with varying combinations of glycerol content. Plasticized gluten was found to be brittle and foamy below the temperatures of 140℃. Mechanical properties, i.e., YM, EB, TS, increased as the temperature increased. The best result was obtained at 180-200 ℃ for the 20% glycerol and 170-190 ℃ for the 30% glycerol. 5% MMT clay was also used in the experiments, which produced improved thermal stability and WVP. The authors also concluded that the stiffness and strength were higher in the circumferential direction than in the radial direction when molding circular components, suggesting shear thinning.

As with the polymer processing techniques, plasticizer plays an important role in the injection molding of gluten-based bioplastics. Alonso-gonzalez et al. [80] investigated the effect of sucrose and trehalose in gluten-based bioplastics in the presence or absence of water. The result showed that sugar behaves as a plasticizer and increases the viscosity of the blend and better processability.

In addition, from using the gluten alone, gluten was mixed with polycaprolactone (PCL) to produce a blend for the injection molding was studied by John et al. [81]. The addition of PCL improved the TS of the final product. However, when the gluten content was increased to more than 60%, the viscosity of the blend increased significantly, and without the plasticizers, the mold filling was insufficient, and jetting was observed even at maximum injection pressure. When the plasticizer is added, the mold filling was complete. However, the TS value decreased sharply.

8.6. Blow Film Extrusion Molding

Blow film extrusion molding is an extrusion process to produce film products. In blow film extrusion molding, the plasticized polymer exits the extruder and is forced into an annular die to produce a tube. Air pressure is applied to the inside of the tube, which creates a thin tubular bubble. This tube is then passed through several nip rolls and guide rolls to finally be taken to the winder. Figure 7 shows a schematic diagram of a blown film production machine.

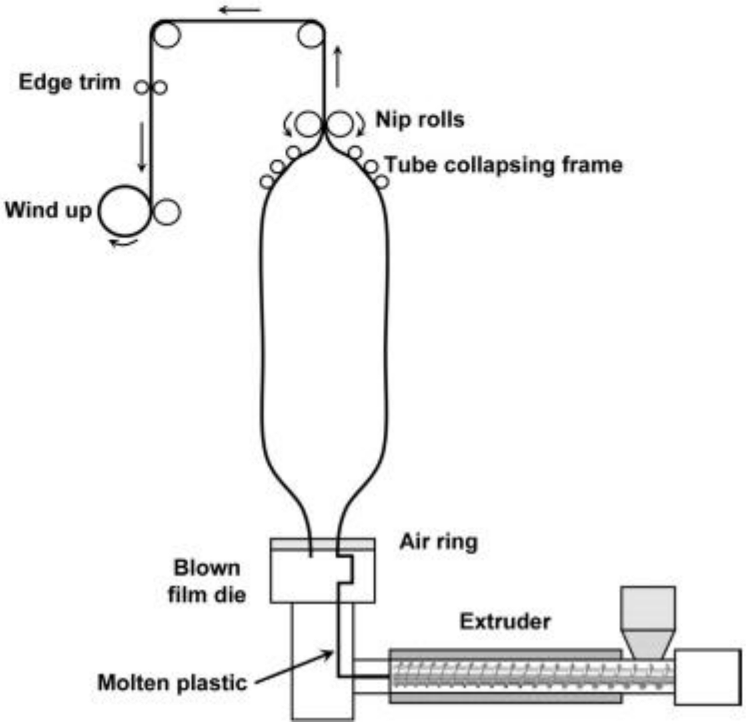

Figure 7: Schematic diagram of blow film extrusion [82]

This process is widely used in packaging applications of conventional plastics. However, the studies where this process is used for processing gluten-based bioplastics are rare. However, there are some studies where the application of other bioplastic materials such as pea protein [83], corn zein [84], soy protein [85], sodium caseinate [86], [87], Yucca schidigera [88] are explored.

9. CROSSLINKING

Crosslinking is a chemical process of forming covalent bonds to join two or more polymers. Crosslinkers or crosslinking agents generally have two or more reactive ends that can chemically react with particular functional groups. Crosslinking of these functional groups (i.e., primary amines, sulfhydryls, etc.) can also occur in protein molecules. Two groups in a protein can create intramolecular crosslinks that can cause the stability of the tertiary and quaternary structure of the protein [8].

Crosslinked polymers are generally processed and shaped before crosslinking occurs. Once crosslinking happens in the polymer, the material cannot be reshaped through heating because the polymer network becomes chemically rigid. Thus, crosslinked polymers are thermosets.

During the processing of proteins, it is possible to produce new covalent bonds, which can also be new cross-links bonds [89]. Disulfide bonds can be broken by the applied heat that can lead to new disulfide bonds with different configurations. The formation of these bonds can be increased by increasing temperature or by alkaline conditions. Several aldehyde products, i.e., glutaraldehyde, glyoxal, formaldehyde, etc., are used to regulate alkaline conditions. The cross-linking formation rate can be increased by employing heat, pressure, and shear or adding chemistry such as cysteine and sulfite ions, or ϵ-NH_2 groups of lysine [90].

Cysteine plays a significant role in the crosslinking of gluten. Cysteine is one of the most reactive amino acids. It can form both inter- and intra-molecular disulfide bonds. Disulfide crosslinking can cleave and reform disulfide bonds that create a new three-dimensional structure. At elevated temperatures and/or pressures, shear or the addition of reducing agents can unfold these native structures. Hernandez et al. [91] used cysteine for polymerized gliadin processing.

The researchers found that gliadin films crosslinked and with the addition of cysteine, while the EB was reduced, but their TS was similar to regular gliadin films and similar TS to regular gliadin films. The authors suggested that cysteine addition can result in intermolecular crosslinking of gliadins and form a high molecular weight protein.

Several aldehyde cross-linkers have been widely used by researchers to improve wheat gluten bioplastics properties. For example, Hernandez et al. [92] applied glutaraldehyde, glyoxal, and formaldehyde to investigate the effects of aldehyde cross-linkers on wheat glutenin-rich films. Formaldehyde-modified films showed the highest TS and lowest EB compared to the others. The researchers suggested that the numerous reaction site for the aldehyde to promote crosslinking resulted in an increase in strength and stiffness. In more detail, formaldehyde can react with amines, sulphydryl, phenolic, imidazolyl, indolyl, and guanidinyl groups. A similar reason was suggested by Sun et al. [54]. The authors indicated that the possible reaction capability of formaldehyde with amine groups of lysine and side chains of cysteine, tyrosine, histidine, tryptophan, and arginine promoted significant crosslinked structure in gluten.

Tropini et al. [93] used 1-Ethyl-3-(3-dimethaylaminopropyl) carbodiimide and N-hydrooxysuccinimide as cross-linking agents to improve water sensitivity and increase the TS of the wheat gluten films. Because these crosslinkers show innate toxicity, the application of these cross-linkers was excluded from food packaging applications. Cinnamaldehyde as a cross-linker was applied to improve the properties of gluten films later by the researchers. In more detail, Balaguer et al. [45] used cinnamaldehyde as a crosslinking agent to wheat gluten, which significantly improved TS and YM of the modified films. Also, the modified films showed higher transparency, making them suitable for use in the packaging industry. Epichlorohydrin is another cross-linker that was applied to wheat gliadin films by song et al. [94], which showed significant improvements in TS and EB properties.

Transglutaminase can be used for the enzymic treatment of wheat gluten-based bioplastics. Transglutaminases are a family of enzymes that can catalyze the formation of an isopeptide bond between γ- carboxamide group of glutamine and ε- amino group of lysine. It can initiate reactions between γ-carboxyamide group and ϵ -amino groups from lysine residue. ϵ(γ-glutaminyl) lysine is formed from the reactions, which results in different inter- and intra-molecular isopeptide bonds [100]. These reactions can cause changes in physicochemical properties and the rheological behavior of gluten [99]. In more detail, maximum resistance to extension (R_{max}) and maximum extensibility are the two rheological properties that were investigated by the researchers. The maximum resistance to extension estimates the strength of gluten, whereas maximum extensibility estimates the deformation value of gluten before it is ruptured. The author found that both of the rheological measurements increased with the increase of transglutaminase in the mixture. Also, after the enzymic treatment of transglutaminase, the altered gluten showed a lower variation of storage modulus with temperature, which indicated that the altered gluten is less sensitive to thermal processing than the unmodified gluten.

Some significant studies of using cross-linkers in wheat gluten processing are listed in table 5.

Table 5: Wheat gluten processing using different cross-linker

Protein	Processing	Cross-linker	Improvements	Reference
Wheat gluten	Casting	Ultraviolet Irradiation	Higher WVP, lower solubility	[95]
Wheat gluten		Transglutaminase	Improved physicochemical properties and rheological behavior, less sensitivity to thermal change	[96]
Wheat gluten gliadin-rich fraction	Dispersing	Cysteine	Improved mechanical properties and WVP	[97]
Wheat gluten gliadin-rich fraction	Thermoforming	Formaldehyde	Improved water barrier, increased mechanical properties	[98]
Wheat gluten gliadin-rich fraction	Thermoforming	Glutaraldehyde, glyoxal, formaldehyde	Higher TS, better WVP	[92]
Wheat gluten gliadin-rich fraction	Thermoforming	Cysteine	Ethanol soluble films, improved TS	[91]
Wheat gluten	Casting	1-Ethyl-3-(3-dimethaylaminopropyl) carbodiimide, N-hydrooxysuccinimide	Better mechanical properties, lower swelling	[93]
Wheat gluten gliadin-rich fraction	-	Epichlorohydrin	Improves TS and EB, reduction weight loss in water	[94]
Wheat gluten gliadin-rich fraction	Casting	Cinnamaldehyde	Higher TS, lower EB, improved transparency, and other mechanical properties	[45]
Wheat gluten	Thermomoulding	L-cysteine, glutaraldehyde and formaldehyde	Much higher T_g for L-cysteine crosslinked gluten compared to aldehyde crosslinked gluten	[54]
Wheat gluten fiber	Spinning	glutaraldehyde	excellent waterstability, improved mechanical properties	[99]

10. SOME SPECIAL BIOPLASTIC FORMULATIONS USING WHEAT GLUTEN

There are several compounding processing methods that can be used to improve the properties of wheat gluten-made bioplastics that involve several chemical, physical or enzymic treatments, producing multilayer structures such as laminating with other plastics to form multi-layer films, blending of other polymers, and/or addition of micro- or nanoparticles. This section details these methods and their potential applications in the processing of bioplastics made from wheat gluten.

10.1. Coatings

A coating is the application of a thin film onto a surface (substrate). Coatings can be both hydrophobic and hydrophilic. Hydrophobic molecules are nonpolar. As the water molecule is polar, they don't dissolve in water. Oils, fats, alkanes are examples of hydrophobic molecules. A hydrophobic surface tends to repel the water. Thus water-repelling and water-proof coatings are typically produced with a hydrophobic surface. In contrast, hydrophilic molecules are polar. They attract water and readily dissolve in water. They have a wide variety of applications in the agricultural and biomedical fields.

Hydrophilic coatings are generally cross-linked polymer coating. Wheat gluten is used in hydrophilic coatings.

As in all coating applications, compatibility between wheat gluten coating and the substrate is critical. This is mainly dependent on the adhesiveness and hydrophobicity of the coating and substrate surface. For this, several physical, chemical, or enzymic treatments can be used to improve the adherence between wheat gluten and substrate. As the typical preparation of the coating, a solution is often prepared. The coating solution is then spread over a substrate. When the materials are dry or crosslink, they form a hard/durable coating. It is important to note that cleaning and pretreatment of the surface are often completed prior to the application of the coating to promote better adhesion.

One of the most common coating applications of wheat gluten is wheat gluten as a coating on paper. Paper is made from cellulose, and coating it with wheat gluten makes the finished product more water-resistant and retains paper’s natural biodegradability and recyclable properties [100]. Because of the hydrophilic property of gluten, it exhibits good compatibility with paper.

One of the important properties of coated paper is WVP. Guillaume et al. [101] found that gluten-coated papers show a significant decrease in WVP. The WVP reduction was as high as 45% for gluten-coated paper with surface treatment and 56% for gluten-coated paper without any treatment compared to the WVP of regular uncoated paper. Wheat gluten can impregnate the porous channels of the paper that can reduce the transmission of water vapor. More studies were conducted in [102]–[104], that validated the potential application of wheat gluten for the coating on paper. Chalier et al. [104] used 2-helptanone and 2-nonanone in their study. They found the potential application of wheat gluten for antimicrobial uses. Overall, from all these studies mentioned earlier, the final product shows good resistance to oxygen, carbon dioxide, and water. Some other researchers added a mixture of chitosan and gluten for the coating on paper [105]–[107]. The results found from these studies showed good antimicrobial properties to the finished products.

10.2. Blending of Different Polymers and Multilayer Structures

Multilayer structures of polymers can demonstrate significant improvements in thermos-mechanical properties compared to single-layer structures. Acting as a hybrid, these materials allow the properties of each material used in the structure to be realized. Wheat gluten was used with other polymers as a multilayer structure by many researchers [36], [64], [95], [98]. To further promote environmental degradation, some of the studies included PLA and gluten. These studies showed that when mixed with PLA, the plastic film exhibits improved mechanical properties, improved gas barrier, and low glycerol loss.

In addition, blending different types of polymers can introduce improved finished products with enhanced properties. The properties of blended polymers can be incorporated into the final product [108]. Blending proteins with a polar material such as different fatty acids, esters, oils can improve the WVP of the final product [109].

To prevent potential contamination, food packaging products are often subjected to different food stabilization techniques [110]. Some of the techniques include UV light, irradiation, ozone, cold plasma. These treatments can be applied after the packaging of the food or before the packing of foods. These methods disinfect and sterilize the packaging materials. High-pressure treatment is another stabilization technique where both food and packaging materials are exposed to hydrostatic pressures above 150 MPa, allowing the disruption of microorganisms and enzymes. The organoleptic and nutritional properties of food are kept in this treatment process.

The use of nanocomposites and bioplastics can also enhance the performance of the materials, and particularly for packaging materials [110]. Wheat gluten with nanocomposites can produce environmentally friendly packaging [111]. However, the migration of nano clay from the packaging materials to food could be an issue as this cannot be prevented by regular stabilization techniques. Silvestre et al. [112] also indicated that the nano clay could negatively impact environmental and human health hazards. Thus, more studies are needed before the widespread use of these materials. Table 6 shows the summary of some significant studies using multilayer structures and polymer blending.

Table 6: Wheat gluten bioplastics processing using multilayer structures, polymer blending, and nanoparticles

Coating layers	**Plasticizer**	**Processing**	**Improvements**	**Reference**
Gluten, PLA	0-30% glycerol	Compression molding	Better strength, better water vapor transmission rate, better oxygen permeability, lower glycerol loss	[105]
Gluten, Paper	-	Coating	Improved WVP, and O_2, CO_2 transfer property	[101]
Gluten, Paper	20% glycerol	Coating	Improved aroma barrier properties,	[104]
Gluten, chitosan	30% glycerol	Coating	Good oxygen barrier properties on chitosan-coated paper, increased TS and toughness	[106]
Gluten, fish scale	30% glycerol	Compression molding	Storage modulus was reduced, can control the degradation rate of gluten-based bioplastics	[111]
Gliadin, chitosan	20% glycerol	Casting	It could be used for antimicrobial packaging, good WVP and mechanical properties	[107],[113]
Gluten	15% glycerol	Montmorillonite	Strong intermolecular interactions, improve mechanical properties	[114]
Gluten	37.5% glycerol	Montmorillonite	No or very less migration of nanocomposite to food	[115]
Gluten	15% glycerol	Compression molding	Good antimicrobial properties, improved TS, WVP	[116]

11. APPLICATIONS

Wheat gluten-based bioplastics have diverse applications, including food packaging, adhesive industries, composite applications, and biomedical and pharmaceutical sectors. In this section, the application of gluten-based bioplastics in the packaging, adhesive industry, cosmetics, and biomedical sectors are discussed.

11.1. Packaging

Wheat gluten has a potential widespread application in the packaging industry [117]. Wheat gluten exhibits some important properties such as viscoelasticity, glossiness, transparency, strength, and good gas barrier property when properly processes and/or compounded. The viscoelasticity is the property for which the materials show both viscous deformation and elastic deformations. Packaging materials go through a lot of stress. Due to the elastic property, they can quickly return to their original state when the stress is removed, while due to the viscous property, they behave like fluid and can be processed easily. As a result of these simultaneous properties, wheat gluten has great potential in packaging industries.

However, one of the disadvantages of conventional plastics is the potential risk of contamination to food. According to Lau et al. [118], there are five different ways conventional plastics can contaminate the food: gradual degradation of the plastic food container, benzene and other volatiles involved in the plastic structure, environmental contamination, i.e., naphthalene vapor in the air can be absorbed by packaging material and migrate into food, contamination from various chemical agents used in the plastic processing, other contaminants specific to certain monomers. As gluten-based bioplastics are biodegradable, environmentally friendly, and potentially have no chemical volatiles, they can be less harmful in reference to contaminating the food. With the use of the proper additives, the impact of contamination can be reduced greatly.

Fernandez-Saiz et al. found excellent antimicrobial properties in gliadin-chitosonium acetate films [113]. The film showed good potential in food packaging and can be used for extended shelf-life in food products. Li et al. also suggested a great potential of gliadin-chitosan films in food packaging and product shelf life [107]. Ture et al. used potassium sorbate with wheat gluten to make compression molded films which showed good antimicrobial properties and the potential to be used as edible packaging [116]. There are other studies where several blends, i.e., wheat gluten-ZnO [119], nisil poly-wheat gluten-ZrO_2 [120], [121], were used to produce antimicrobial films. The application of edible packaging using gluten-sorbic acid was suggested by Redl et al. [122]. In addition, wheat gluten with nisin was also formulated to made edible packaging [123], [124]. Several researchers used various additives and chemical agents to improve the mechanical properties and gas barrier properties of gluten-based bioplastics [13], [102], [106], [109], [110], [113].

Coatings to a food product is another potential application of gluten-based bioplastics. Tanada-Palmu et al. showed that wheat gluten coating on Sharon fruit and cherry tomatoes could reduce moisture loss [125].

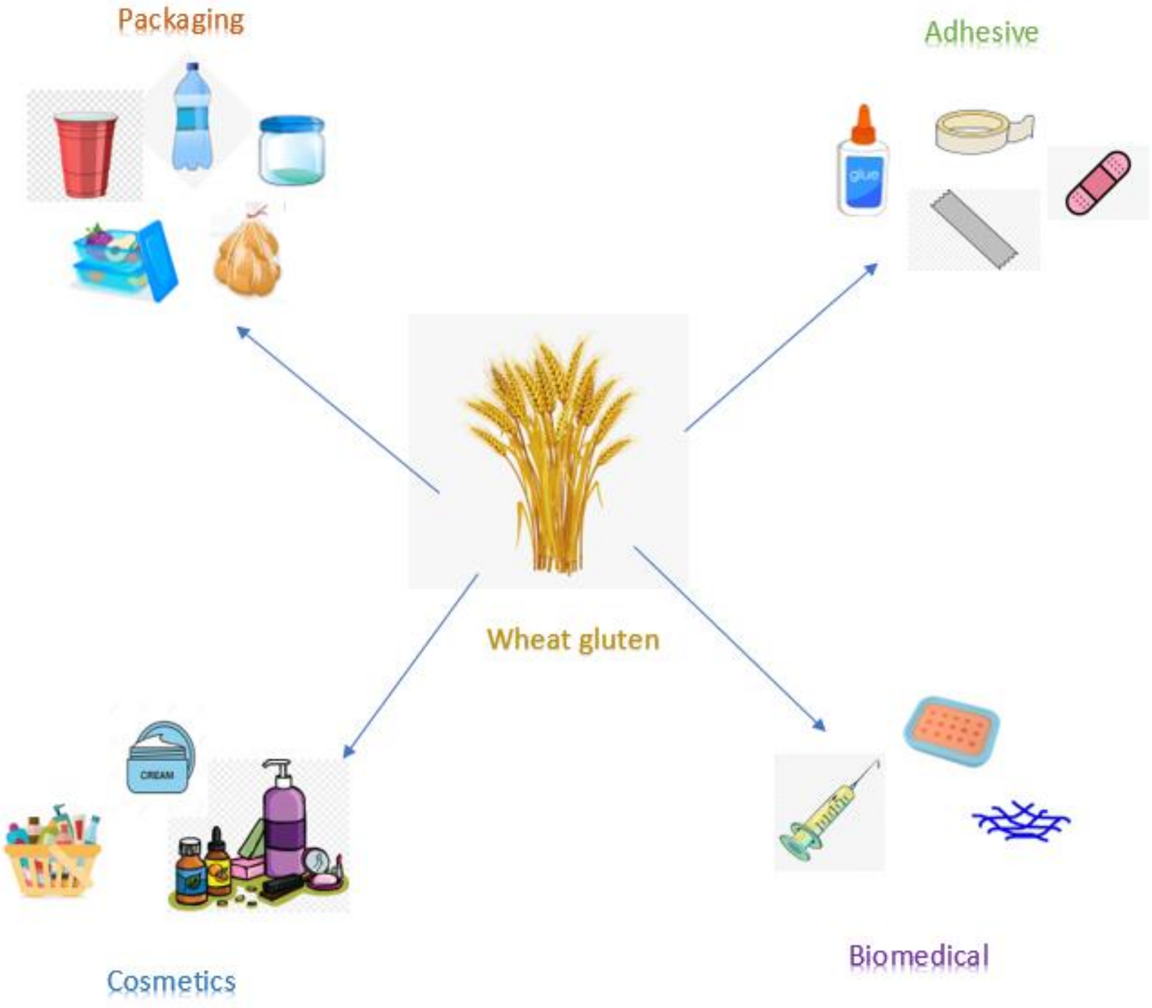

Figure 8: Application of wheat gluten-based bioplastics

Hernandez-Munoz [98] explored nonfood packaging applications for gluten-made bioplastics. These include the packaging of fresh flowers and agricultural mulch. One of the other important applications of bioplastics is coating [59]. Gluten can be used to coat paper and improve the property of paper. Sun et al. [72] showed that cysteine-cross-linked gliadin films present the advantage that they are ethanol-soluble for application as a coating for food or surfaces.

Other potential packaging applications involve the use of nanoclay with wheat gluten [126], [127], [128]. Gluten-made bioplastics using nanoclay show improved mechanical properties. Mauricio-Iglesias et al. [115] investigated the potential of migration of nanoclay from packaging materials to food. Experimental results showed that the migration rate of nanoclay on aluminum or silicon had a very low migration rate. For aluminum, the migration rate was as low as 8.6 mg of aluminum/kg, which was significantly less than the 60 mg of aluminum/kg limit proposed by the European Food Safety Authority.

11.2. Adhesive Industry

Wheat gluten-made bioplastics have good potentials in the adhesive industry. Thermoplasticity and good film-forming ability make wheat gluten a potential material for adhesive production [129]. The properties of adhesive can be adjusted by using plasticizers and crosslinkers. Xi et al. [126] used glutaraldehyde mixed with polyethyleneimine as a crosslinking agent to modify regular gluten-based adhesive. A significant improvement in bonding performance was found in that study. The glutaraldehyde-modified plywood panels showed significantly better bonding performance compared to traditional gluten adhesives. In addition, the modified material showed a higher storage modulus which represents higher elastic deformation of the adhesives. Sartori et al. [130] showed that the adhesive properties depend on the glycerol concentration and the relative humidity (RH). The authors suggested that the adhesives obtained from the study can be used in the food and pharmaceutical industry. Oh et al. [131] showed in their study that wheat gluten-based adhesives containing cellulose nanofibrils and glutaraldehyde show a higher modulus of elasticity, modulus of rupture, and water resistance than that of zein-based adhesives. This suggests the great potential of wheat gluten in manufacturing engineering wood [131]. The adhesive properties of gluten with using other additives, make the wheat gluten effective in producing medical bandages and adhesive tapes [129].

11.3. Cosmetics

With the application of enzymic and chemical hydrolysis of wheat gluten, polypeptide, oligopeptide, and other peptide-containing structures can be produced [132]. These materials are used in different hair- and skin-care products. Some of the commercial examples are moisturizing products from Rachel Perry, Croda, and MGP, Polytriticum (a biodegradable resin), Foam Pro L (a hair product) [129].

Wheat gluten is an allergen declared by the US Food and Drug Administration (FDA). Also, there could be impurities and contamination of bacteria during the processing, which can present a health concern. Therefore, Belsito et al. [133] conducted a thorough assessment of most of the popular commercially used cosmetics products made from wheat gluten and their derivatives. The researchers did not find any major concerning issues regarding this.

11.4. Biomedical Applications

Proteins are major constituents of the human body. Thus, biodegradable plastics produced from protein can be suitable for biomedical applications. The plastics made from wheat gluten have excellent biodegradability, and they have a wide range of applications in the biomedical field. Some of these applications are scaffolds in tissue regeneration, vascular grafting, suture equipment, drug delivery equipment, implants, artificially made skin, etc. [134]. In addition, Reddy et al. showed that wheat gluten fiber shows better mechanical properties compared to soy protein and zein and has great potential in biomedical applications [135].

Bioplastics made from wheat gluten are very flexible, have good elasticity, and are water-soluble. In addition, they can be compounded with nanofibers for the enhancement of mechanical properties. Nanofibers have wide use applications in anti-cancer and drug delivery products. Reddy et al. [47] explored the application of both gliadin and glutenin films as a substrate in tissue engineering. The authors found gliadin to be cytotoxic, which hampers cell growth. They found that gliadin-free wheat gluten films can be an excellent substrate (better than PLA) in tissue engineering. Lubasova et al. [136] used a blend of soy flour, gluten, and PVA to be electrospun mats which can be used in scaffolds in tissue engineering, wound healing, or making biosensors. Reddy and yang [135] also found from their research; wheat gluten can be used in the oral administration (capsules, tables, or hydrogels) of drugs. The potential application of wheat gluten fibers in loading and releasing three popular drugs: metformin, diclofenac, and 5-fluorouracil were explored by Xu et al. [137]. The use of gluten bioplastics for the tissue scaffolds and hemostatic products was also showed by Woerdaman et al. [138]. Aziz et al. [134] showed that azathioprine containing gluten/PVA fibers made from electrospinning could be used in drug delivery systems.

12. LIMITATIONS AND FUTURE PROSPECTS

The authors of this paper hope to deliver a basic and comprehensive understanding of the wheat gluten-based bioplastic processing techniques and potential applications with its state-of-the-art research to both general and in-depth readers. Readers can find some basic concepts about wheat gluten and why it has the potential to be an important bioplastic feedstock. The summary includes most of the existing techniques available. Some less-used processing techniques are not discussed in detail. However, references are given to the readers so that they can explore the relevant topics in more detail. Also, among the numerous applications, the readers are provided with information about packaging, cosmetics, adhesives, and biomedical applications.

The key findings of this paper and future research prospects are summarized below.

- When compared with conventional plastics, unless in some special cases, i.e., biobased PET and biobased PE, bioplastics generally show inferior properties. For that, it is unlikely for bioplastics to replace conventional plastics fully in the near future. Thus, it is essential to find applications where bioplastics can replace traditional petrochemical plastics.
- Another possible issue of some bioplastics is their higher cost compared to conventional plastics. Fortunately, wheat is an amply available crop and has a low cost of the material. However, the processing cost of wheat gluten-based bioplastics remains relatively high. Because of this increased cost, bioplastics made from wheat gluten and other plant proteins cannot compete fully against conventional plastics in some applications. Yet, there are scopes of exploring cost-effective and better additives to reduce costs and enhance performance.

- The extrusion process is one of the most cost-effective and mass-production processes for plastics. However, obtaining gluten-based bioplastics from the extrusion process can be challenging, mainly because of high protein aggregation. Some researchers already used different additives in their studies which can retard crosslinking and improve the scope of wheat gluten-based bioplastics processing. However, there is potential to improve further and make the process more suitable for mass production with protein-based plastic. Injection molding, another mass production process can also be challenging and has limited application in gluten-based bioplastic processing because of the relatively high melt viscosity of gluten.
- One of the most important characteristics of wheat gluten is that it can be modified by physical, chemical, enzymic, and other treatments. It is possible to apply these techniques to blend with other materials, produce multilayer structures and coatings, enhance properties through nanoparticles, etc. Applying these processes can ensure more applications for gluten-based bioplastics.

13. CONCLUSION

The increased awareness of the problems of acquiring conventional plastic materials and their environmental impacts led to the search for biobased and biodegradable polymers from renewable sources to replace synthetic polymers to process plastics. Protein-based polymers have gained significant attraction in recent years because of their improved physiochemical properties, which make them possible for adoption into various applications. Thus, several review articles have been published on the processing and applications of general protein-based bioplastics. However, review papers based on wheat gluten-based bioplastics are less common. As wheat gluten is a widely available crop and has some unique properties over other protein-based bioplastics, there is a growing interest in general information (such as a review article) about the processing of gluten-based bioplastics that might help connect the existing gaps in current research.

This paper provides a brief understanding of bioplastics and why wheat gluten is considered a feedstock for bioplastic production. As a protein, wheat gluten has a special structure that plays a vital role in the physiochemical properties of the bioplastic made from them. The general structure of protein and wheat gluten and its constituents are discussed briefly to provide a general understanding. This paper detailing the wheat milling process, separations of gluten from wheat grain, and use of gluten mixed with other additives in conventional plastic processing techniques. In addition, the classification of bioplastics and the typical processing techniques of wheat gluten are discussed. Conventional plastic processing techniques can be used for the processing of bioplastics made from wheat gluten. However, the processing parameters largely depend on the structure of wheat gluten and the additives used during the processing, which primarily influence the properties of the bioplastics made from them. Based on the studies conducted by other researchers, this paper provides a thorough discussion on the parameters and the importance of the structure in the processing techniques. From the overall discussion, it can be seen that mass production techniques such as extrusion and injection molding have very limited uses in the processing of bioplastics made from gluten because of the extensive aggregation of the protein. However, there are studies that suggest these bioplastics can be processed. The paper also provides concise detail on applying wheat-based bioplastics in the packaging, adhesive industries, cosmetics, and biomedical sectors. Some of the applications have great potential for wheat-based gluten bioplastics, where they can fully replace conventional plastics, such as tissue scaffolds, drug delivery products, artificially made skin, cosmetic products, plant pots, etc. There are some other applications where gluten-based bioplastics can not compete because of the cost-effectiveness and inferior qualities compared to other conventional plastics. However, more studies can be conducted to make gluten-based bioplastics more cost-efficient and have better quality.

The authors of this paper hope that the concerned people will come forward to find out the existing gaps in this research field and work together to guide the path to use wheat gluten-based bioplastics in more applications.

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