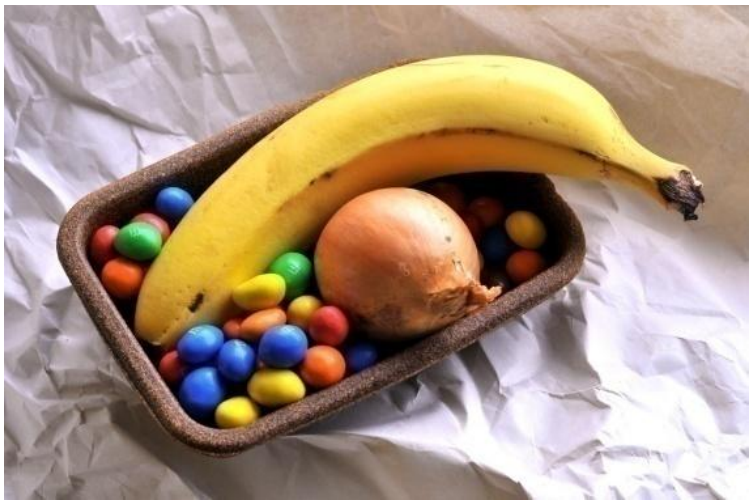
Protein Based Plastics from the Residuals of Industrial Oil Crops



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Cover Photo: Tray from Crambe oilseed residuals,

Summary

The factors that can be utilized in the development of new protein based plastic materials from industrial oilseeds are examined. As this area has not been explored in depth in the literature, experiences from the study of other proteins are used to better understand the possibilities of industrial oilseed protein based material. The review begins with a short introduction of the issued surrounding protein based plastics then follows the conventional production process for oilseeds and the possible alternative processes with a view to maintaining protein quality. A selection of industrial oilseeds is considered for use as protein sources. The factors that go into successful utilization of the oilseed proteins are examined from an industrial perspective, e.g. plasticization and forming useful articles, as well as the inter-protein interactions that shape product properties. After the molecular scale mechanisms are considered, previous work in developing protein based plastics is reviewed. Finally, possible applications are examined.

Preface

Modern life relies on plastic materials for everything from food packaging to car parts and critical medical devices. These products depend on the supply of petroleum which is rapidly being accepted as unsustainable. As an alternative to petroleum plant based resources are rapidly being developed into a new bio-economy. The use of agricultural products such as oils and starches has been extensively studied while uses for proteins in this new bio-economy have been lacking. One promising use for these proteins are in protein based plastics. The development of protein based plastics has a long history from the prehistoric use of natural animal and plant proteins to modern high performance fibres from synthetic spider silk. The understanding of protein behaviour from extraction and purification to chemical reactions is well understood and this knowledge has been applied to the development of protein based plastics. Progress has been made in developing protein based plastics but they have yet to see widespread use. Through the understanding of protein chemical interactions it is hoped that useful plastics can be produced and another product stream from the sustainable bio-economy can be developed.

Contents

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1. Introduction

In our modern society we rely on "plastic" materials for many critical applications, from medical devices like syringes to the packaging that keeps our food fresh. These materials are overwhelmingly produced using petrochemicals that come from fossil oils. In recent years the price stability of fossil oil as well as the security of its supply has come into question. At some point in the future petrochemical feedstock will either become too expensive or simply unavailable. If we are to continue to reap the benefits of the products currently made from fossil oil we will eventually need to find alternate ways of making the products that currently come from petroleum sources. An additional problem with most current petroleum based plastics is that they degrade extremely slowly placing stress on the environment if not disposed of properly.

One proposed alternative to fossil oil for many applications are plant oils (*1*). Plant oils are currently mainly produced for human nutrition from the seeds of many plants such as rapeseed, soy, cottonseed, sunflower and sesame (*2*). Many oil seeds provide both useful oil and high protein residuals for human or animal nutrition. Oil seed development has also been carried out so that the residual meal after oil extraction can be used as animal foodstuff, rapeseed development being one such example (*3*). Although the quality of most current plant oils and residual high protein meal have been developed to meet the needs of human and animal consumption, there is an opportunity to develop industrial oil seed varieties whose oils can replace petrochemicals whose residuals will provide new sources of high protein meal (*4*).

With the possible replacement of some petrochemicals with industrial plant oils it is expected that the supply of protein containing residuals from industrial oil crops will greatly increase. One promising use for this protein rich feedstock is bio-based plastics (*5, 6*). Plant protein based materials are bio-degradable addressing end of life disposal concerns, one of the major issues with petroleum based polymers (*5, 7*). These protein based plastics have also been shown to have attractive gas barrier properties, making them possible replacements for conventional polymers in food packaging applications (*8, 9*). Despite the promise of these materials there are many problems to overcome in utilizing plant proteins as replacements for conventional plastic

materials. Among these hurdles are high cost, poor processability, brittleness, low strength and low stiffness (*5*).

Natural protein based materials are all around us, including the major structures of our bodies (*10*). Although it was not recognised at the time, humans have long made polymers from natural proteins such as glues from the animal protein sources like hides, hooves, blood, milk and egg as well as coatings using milk proteins (*11, 12*). In the modern era casein from milk was used industrially with formaldehyde as a hardening agent from about 1900 (*13*) and later in 1929 with aluminum stearate as a processing aid without a hardening step (*11*). By 1926, 55% of the world production of buttons were made of casein, and the total volume produced had reached 60 000 tons by 1932 (*11*). The 1920's and 30's saw the rise of the "chemurgy", a movement to manufacture industrial products from agricultural feedstock. This trend was subsequently displaced by lower cost alternatives from the petroleum industry (*11, 14*). In 1936 Ford Motor Company utilized over 3 million pounds of oil-free soy meal in soy flour-phenol-formaldehyde-wood flour composites and by 1942, 150 million pounds of this material was being produced (*11*). Plant proteins were also used in the manufacture of commercial fibres during this period using the solution spinning process (*15*). Materials from plant proteins are nothing new.

Plant proteins from oilseeds are an attractive feedstock for industrial production due to their availability and expected low cost. For example rapeseed meal sold for 209 USD/tonne in 2009 (*16*), and industrial oilseed meal that is not appropriate for animal feed can be expected to be less expensive. Despite the attractive price, only limited work has been carried out to date on industrial oilseed residuals used in bio-based plastics (*17-22*). The common amino acid building blocks shared between all proteins allow us to gain insight into industrial oilseed protein based bio-based materials from work with other protein systems. In the native state most plant protein molecules do not exhibit high enough molecular weight or have sufficient intermolecular interaction to form useful bio-based materials. In order to increase the molecular weight, cross links can be formed between molecules using chemical additives or naturally occurring reactions between amino acids (*23, 24*). This cross link network stabilizes the material mechanically as well as binding protein molecules within the network leading to reduced solubility (*25-29*).

Most plant proteins exhibit some ability to cross link using naturally occurring amino-acids such as cysteine, tyrosine and lysine available in the proteins (*28, 29*). These cross links can be

activated in a number of ways including casting from solution, heating, exposure to radiation, changes in pH, introducing denaturing compounds, chemical cross linking and enzymatic action (*5, 25, 30, 31*).

The majority of proteins found in plants are in the glassy state in their pure form at room temperature making materials made from them brittle. In order to decrease the glass transition temperature (Tg) to the desired range and reduce brittleness, plasticizers are used to make the molecules more flexible and thus the material more flexible (*5*). In most cases plasticizers interact by forming hydrogen bonds with the protein molecules, disrupting intermolecular interactions and result in higher protein chain mobility. A high vapour pressure and low diffusion rate are desired so that the plasticizer can remain at sufficient concentration to provide a long lasting effect. Many compounds have been tested as plasticizers such as glycerol, sorbitol, lactic acid, ethylene glycol and fatty acids, with glycerol being the most successful although it suffers from a high diffusion rate (*32-35*). Depending on the application a brittle material may be acceptable and the proteins can be utilized without plasticization (*36*).

Global interest in sustainability, concern over greenhouse gas emissions and the high cost of petroleum is increasing the research related to the use of oilseeds as a source of chemicals for industry (*4, 37-39*). In traditional food targeted oilseed crops (such as rapeseed and soybean) plant breeders have focused on first production of high quality oils for human consumption and secondly on the quality of the residual high protein meal for human or animal consumption (*3*). In the development of oil crops specifically for industrial applications, the desired qualities may preclude the use of the residual meal as human or animal feed due to anti-nutritional factors such as inedible residual oils or glucosinolates. The protein containing residuals of industrial oil production can be made edible by removal of anti-nutritional compounds, but this is likely to be cost prohibitive for food applications (*40-43*). In the development of industrial oilseeds, genetically modified cultivars may be used that create a barrier to their use as a human or animal food source due to legislation in the EU (*44*). The volume of residuals from the use of plant oils to replace petrochemicals is unlikely to find a market of sufficient size to consume it at the required price without the development of new applications.

Current interest in developing industrial oil crops includes plants such as *Crambe abyssinica* (Crambe), *Brassica carinata* (Carinata, Ethiopian Mustard), *Brassica napus* (Rapeseed),

Camelina sativa (Camelina, false flax, gold of pleasure), *Ricinus communis* (castor bean) and others (*4, 38, 39, 45*). An added advantage of some proposed industrial oil crops is that they are being developed to grow under conditions which are marginal for current agricultural crops (*4, 39*). Finding uses for process residuals that do not depend on low levels of anti-nutritional compounds would provide a value added outlet for new industrial oil crops. Proteins from many sources have been investigated as bio-based polymers, indicating the possibility of using protein rich industrial oilseed residuals as a biopolymer feedstock (*5, 25, 30, 31*). However, little direct work has been done in the area of utilizing non-food industrial oil seed crops in bio-based materials production (*17-22*).

2. Industrial Oilseed Production

Oilseeds are one of the major crop groups worldwide with 2.5% of all yearly agricultural production by mass, requiring 16% of cultivated land (*46*). Industrial uses of seed oils can rely on food crop oils as well as dedicated industrial oilseed crops depending on the quality of the oils required. Current oilseeds cultivated specifically for industrial uses include: castor bean for ricinoleic acid used in the production of lubricants, nylons and polyurethane plastics; high erucic acid rapeseed (HEAR) producing erucic acid for the plastic additive erucamide, lubricants and other industrial chemicals; and crops such as camelina for the production of fatty acid methyl esters (biodiesel) (*4*).

2.1 Preparation of Seeds for Processing

The various stages of processing between harvest and the preparation of biomaterials from industrial oilseed proteins can affect protein quality through biological action or other environmental variables. Proper handling of oilseeds is important to maintain the quality of both the extracted oil and residual meal. Deterioration of oilseeds in storage results in the release of carbon dioxide and heat generation by oxidation reactions increasing deterioration rates and possibly charring seeds. Damage can be in the form of mechanical injuries to the seeds during harvest or from freezing and also contributes to deterioration (*47*). High temperatures during storage may result in damage to the proteins and affect their use as bio-materials.

High moisture content during storage (above 14-15%) has a negative effect on both oil and the residual protein meal. Free fatty acid generation may be accelerated by microbial growth and enzymatic activity supported by high moisture content. High moisture content also supports insects and mites that damage seeds thus accelerating decomposition reactions. Sprouting can also occur during storage of high moisture seeds with the accompanying increase in enzyme activity and breakdown of both proteins and oils. Air dryers are used to ensure low moisture content in seeds before entering storage and ventilation is used to cool the seeds in storage preventing heat damage from oxidation reactions. Drying must be carried out at a temperature low enough to prevent damage that can affect protein functionality.

Individual operations are used for the preparation of oilseeds for processing which vary depending on the physical characteristics of the seeds. Most oilseeds go through the process of cleaning, drying, dehulling, size reduction, cooking and tempering prior to oil extraction (*47, 48*). Some of these processes can affect the protein quality in the residual meal (*40, 49-54*).

In usual industrial practice oilseeds are tempered to denature proteins, help release oils from the cells, inactivate enzymes and improve palatability if they are to be used as animal feed. Some industrial oilseeds contain glucosinolates, these compounds are hydrolyzed by enzymes releasing isothiocyanates and nitriles as a predator defence mechanism (*55*)(Table 1). For example, rapeseed is heated with steam during processing in order to inactivate the enzyme myrosinase, preventing the production of isothiocyanates and nitriles making the resulting meal more palatable to animals (*41*). Following tempering the cooked seeds are immediately pressed to separate oil. Tempering may be done to simply improve flaking and extraction performance rather than to deactivate enzymes (*48, 56*). Depending on the temperature, cooking/tempering may impact the behaviour of proteins in the residual meal (*40, 49-54*).

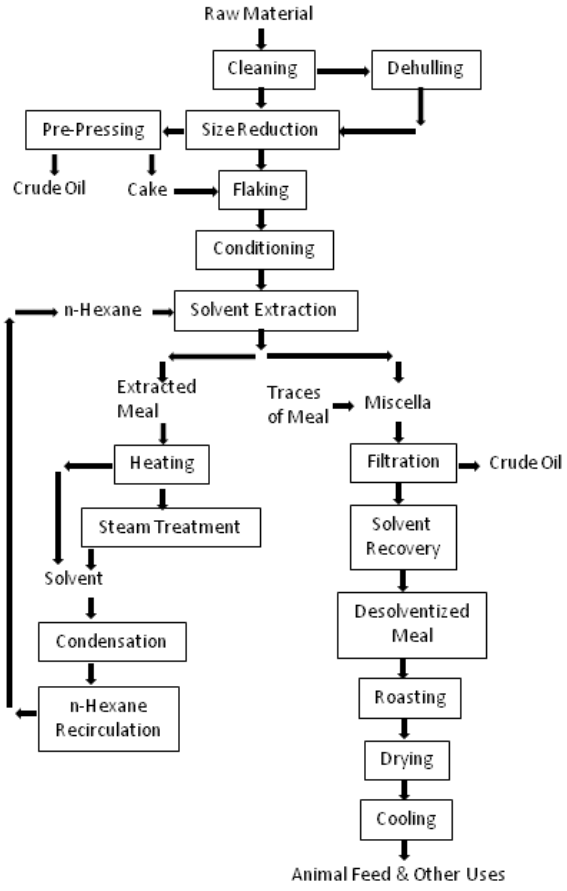

Figure 1 – Solvent oil extraction schematic (redrawn from [47]).

2.2 Oil Extraction Techniques

Oil extraction techniques are important in our context as they may affect the quality of the oil and proteins in the residual meal and will be designed based on economic considerations of the oil and meal value. The most common techniques for oil extraction are solvent extraction, mechanical extraction via a screw press and their use in combination.

Solvent extraction

Solvent extraction is the most common commercial method for the treatment of oilseeds. In solvent extraction oils are preferentially dissolved by a liquid solvent. The effectiveness depends on the oilseed preparation, temperature and equipment design. It is usual for the oil in residual meal to be less than 1% from commercial solvent extraction. Hexane is most widely used for commercial oil solvent extraction. The use of alcohols and water as solvents for oil extraction are hampered by low solubility and high energy requirements for solvent recovery and meal drying (*47*).

Since oil solubility and diffusivity increase and oil solution viscosity decreases with increasing extraction temperature, extraction with a hot solvent is attractive from a processing point of view. The energy required for solvent recovery decreases with higher extraction temperatures. Despite the advantages of hot solvent extraction, elevated temperatures may cause degradation of protein quality in the residual meal (*20*). Although it is not known to be in commercial use, solvent extraction of oils can be combined with the removal of unwanted compounds from the meal. The use of a hexane-ethyl alcohol-water ternary solvent has been shown to remove oil as well as polyphenols while maintaining protein quality (*40*).

There are 3 major steps in solvent extraction (Figure 1): oil extraction, solvent removal/recovery and meal toasting.

Following oil extraction the remaining solvent in the meal is removed by a desolventizer-toaster. The meal is heated to evaporate solvent without unduly damaging the value of the meal. The maximum allowable temperature depends on the type of proteins present and their intended end use. If only nutritional uses are intended the allowable temperature may be higher than if the proteins will be required to have certain functionality (*20*).

Mechanical oil extraction

Modern mechanical oil extraction of oilseeds relies on the continuous screw press. Screw press units are popular as they are less expensive than the complex solvent extraction systems and are available with capacities from 100 tonnes/day down to laboratory sizes. Although often referred to as “cold pressing” the mechanical action of the screw press or external heating can cause a

considerable increase in temperature which can reach in excess of 70^{o}C (*20*). Each mechanical processing step has the potential to degrade protein quality if the processing temperature is too high.

Aqueous extraction

Traditional aqueous extraction or water floatation involves heating oil containing material, grinding with or without water, and boiling with water to liberate the oil. The oil is then allowed to float to the surface where it is collected and further heated to remove moisture (*48, 49*). This process can be used to extract proteins in the residual liquid as well as oil in the flotation layer. Oil yields of 50% are generally found in traditional non-commercial aqueous processing (*48*). To improve protein and oil extraction at milder conditions enzymes and surfactants can be added to the extraction medium (*49*). Recent work aimed at producing highly functional proteins utilizes aqueous extraction of cold pressed meal in conjunction with residual oil recovery, ultrafiltration, dialysis, and salting in/out techniques (*51-54*).

Supercritical fluid extraction

Rising costs of meeting environmental and safety standards using the solvent extraction method have focused attention on supercritical fluid extraction technologies as an alternative (*49, 57*). The most attractive supercritical solvent is CO_2, which makes an acceptable oil solvent as a liquid above the critical pressure. When the pressure is reduced to below critical, the CO_2 returns to its gaseous state and is easily removed from the oil and then recycled into the process. Supercritical CO_2 extraction of oil has been shown to maintain the quality of the residual proteins (*58*).

2.3 Protein Extraction and Purification

Depending on the application, the proteins from industrial oilseeds may need to be extracted from the residual meal and further purified. Applications such as fibres and thin films certainly require all large size residual material, such as hull fragments, to be removed as they are generally larger than the desired thickness of the final product. If the produced meal cannot provide adequate properties as a bio-based plastic, protein extraction can be used to increase protein concentration and thus improve performance. Compounds in the residual meal that

interact strongly with proteins, such as phytates, may need to be separated in order to increase the reactivity of the proteins in the final material (*40, 59*). Processes such as film casting, the wet spinning of fibres and the formulating of adhesives require protein solutions which may be formed concurrently with protein extraction (*15, 60*).

Proteins may be fractionated using a modification of the traditional Osborne procedure where extractions are performed serially in solutions as follows: 5% NaCl (globulins and albumins) followed by dialysis to precipitate globulins, aqueous ethanol (70%) solutions (prolamins), and 0.1M NaOH (glutelins). The replacement of the traditional initial step of deionised water while substituting dialysis of the salt extracted proteins is required due to the presence of salts in the seeds that cause the coextraction of globulins and albumins (*20*). From initial extractions and determination of the desired proteins available an extraction procedure can be designed.

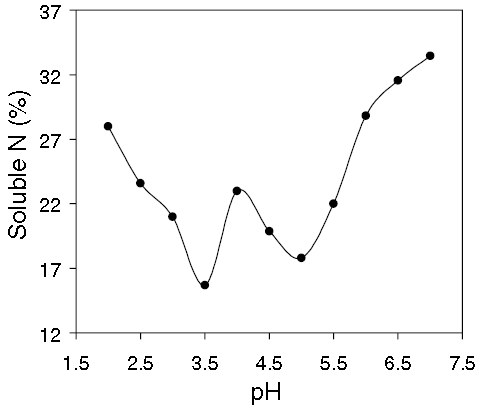

Figure 2 – Effect of pH on the solubility of Carinata proteins [62].

High or low pH extraction with isoelectric precipitation

It is well known that the aqueous solubility of seed proteins varies with the pH of solution (*61*)(Figure 2). This effect can be used to extract proteins into solution at their most soluble pH followed by shifting the pH to their lowest solubility (the isoelectric point) thereby causing them to precipitate. The precipitated proteins can then be removed by centrifugation. In current industrial practice it is common to extract oilseed proteins with high pH aqueous solutions followed by isoelectric precipitation (*5, 40, 62, 63*). This may be the most efficient method in terms of cost and protein recovery rate but can have unintended negative consequences. At high pH proteins are soluble, but can become denatured and react with each other and non-protein components of the meal (*40*). Changes in conformation and other reactions during extraction can have an impact on protein functionality (*50, 64, 65*).

Extraction with aqueous salt solution

Where the target proteins present in the oilseed residuals are albumins and globulins they can be removed with an adequate strength of aqueous salt solution (*53, 66, 67*). This procedure does less damage to the proteins compared to extraction at alkaline pH where denaturation and reactions with non protein components can occur (*40, 59, 64, 65, 68*). To remove the proteins from solution, dialysis can be used or additional water added to reduce the salt content, thus precipitating globulins. The remaining albumins and globulins can be removed by ultrafiltration or simply drying the solution (*51, 66, 67*).

When the above procedure is carried out on rapeseed residuals, a protein isolate is produced with improved functional properties compared to the conventional procedure of high pH extraction followed by isoelectric precipitation (*50, 66*). This highlights the importance of protein conformation on function and the importance of selecting an extraction method that maintains functionality, not simply the highest yield.

3. Industrial Oil Crops

For the purposes of this discussion industrial oil crops are defined as crops that are grown for oil that serves as an industrial feedstock and are not primarily intended for human or animal consumption. These crops are developed with their primary industrial use in mind and their residuals are a secondary consideration. The residuals usually have little value due to their high levels of anti-nutritional components (*38, 50, 62, 69*) or possibly due to regulatory issues in the case of genetically modified crops (*44*).

3.1 *Crambe Abyssinica* (Crambe)

The annual plant Crambe (*Crambe Abyssinica* Hochst. ex R.E. Fries) from the family Cruciferae is one possible industrial oil crop. Crambe requires 90 to 100 days to mature and can tolerate temperatures down to -4C. It is relatively drought tolerant, but best yields are found with moist conditions during pod set and filling with a following dry period towards maturity (*70*). Well drained soils with a pH of 6-7 are suitable; it will not tolerate waterlogged or heavy clay soils. Crambe produces single seeds with individual pods with an oil content up to 38% (60% when dehulled) and yields of up to 1 t/ha (*4*). The oil contains 50 to 60% erucic acid (*71*), a long chain fatty acid used in the manufacture of lubricants, the production of erucamide for the plastic industry (*45*) and possibly brassylic acid for Nylon 1313 production (*72*). Crambe does not cross with any other currently used oil crops. This fact makes Crambe of interest for genetic modification as it is unlikely that the modified genetic material will enter into the food chain (*4*). Crambe is mainly of interest due to its oil quality and not for agronomic aspects. The specifics of the Crambe storage proteins have been little studied outside the overall amino acid composition (*71*) (see Table 1). It is suggested from nitrogen solubility studies that the major protein families are water or salt soluble (albumins and globulins respectively) with a small amount of alcohol soluble prolamins (*73*).

3.2 *Brassica Carinata* (Ethiopian Mustard)

Carinata (*Brassica Carinata* A. Braun) is an annual plant of the family Cruciferae. Maturity requires 101 to 119 days (*74*). Advantages of Carinata are its heat and drought tolerance as well

as resistance to many pests and diseases (*4, 74*). Seed yields of 3t/ha with an oil content of 42% have been obtained with 40% erucic acid content (*74*). Residual meal of Carinata has a high protein content and well balanced amino acid profile for feed (Table 1). However, Carinata meal contains high levels of glucosinolates and phytates making it unsuitable as feed without further processing (*62, 74*). The low crossability of Carinata with other brassicas make it attractive for genetic modification (*4, 39*). The drought tolerance of Carinata makes it a candidate for cultivation in marginal areas while its resistance to pests and diseases may reduce off farm inputs to cultivation.

3.3 *Camelina Sativa* (False Flax, Gold of Pleasure)

Camelina (*Camelina Sativa* (L.) Crantz) is from the family Cruciferae and can be grown as an annual or biannual winter crop (*75*). Summer varieties of Camelina take 120 days to mature. Seed yields of 2.6 t/ha and 3.3 t/ha have been achieved with summer and winter varieties respectively. Oil production is 42% and 45% of seed weight for summer and winter varieties respectively. The oil contains 90% unsaturated fatty acids with only 3% erucic acid, making it of interest for human nutrition (*75*). Camelina possesses some unique agronomic traits that make it attractive, such as compatibility with low tillage systems, double cropping, winter surface seeding and use on marginal lands (*76*). It does not cross with other oilseeds in cultivation making it attractive for genetic modification (*4*). These traits do not strictly fit into the definition of an industrial oil crop as Camelina is a possible food crop. However, Camelina does not currently have a well defined end use and has been proposed as an industrial oil crop, therefore it is included here.

3.4 *Brassica Napus* (Rapeseed)

Rapeseed (*Brassica Napus* L.) is grown commercially as winter and spring varieties. Worldwide 58 million tonnes of rapeseed was produced in 2008 utilizing 30.8 million hectares (*46*). There are many varieties available including double “zero” varieties designed for human oil consumption (low erucic acid) with animal consumption of the meal (low glucosinolates). Single “zero” varieties contain either low erucic acid or glucosinolate levels. High erucic acid rapeseed (HEAR) varieties are grown for the production of industrial oils. Spring varieties of rapeseed

mature in 74 to 140 days (*77*) and typical yields are in the range of 2 to 3.5 t/Ha in northwest Europe (*78*), with a worldwide average for all rapeseed varieties of 1.88 t/Ha in 2008 (*46*).

Commercial rapeseed varieties produce seeds with an oil content of about 45% of seed weight (*79*). Rapeseed development has been focused mainly on oil quality, especially on increasing or decreasing erucic acid content depending on the intended use. This has resulted in a range of oil quality from near zero erucic acid for human consumption to industrial oil varieties up to 55% of the oil containing erucic acid. The theoretical maximum for erucic acid content from conventional breeding is believed to be <66% (*80*). With the development of many different varieties of rapeseed the total protein content varies, although the protein content is considered to be about 25% for dehulled seed (*79*). The concentrations of various amino acids in rapeseed protein can be found in Table 2.

The proteins of rapeseed fall into 3 main groups in mature seeds. The storage protein groups cruciferin (12S globulin) and napin (1.7S albumin) representing 40-50% and approximately 20% of the total protein, respectively. Oil body membranes, oleosin, a 19 kDa hydrophobic peptide represents a further 20% of the total protein, with the remainder of the protein content uncategorized (*79*). The structure of rapeseed storage proteins has been studied more extensively than the other industrial oils. Rapeseed cruciferin has been found to consist of 6 subunits each of which contain 2 disulphide linked precursors (*81*) while the napins consist of 2 precursors linked by disulphide bonds (*82*). The oleosins do not generally form oligomers but are known to form dimers and trimers under certain conditions (*83*).

Table 1. Components of industrial oilseed cakes, values adjusted to wt% of fat free dry matter where necessary. Columns do not add up to 100% due to differences in data sources.

	Carinata[a]	**Crambe**[b] **(with hulls)**	**Crambe (dehulled)**	**Camelina**	**Rapeseed**
Proteins	42.7	31.1	49.3[c]	43.6[e]	42.5[h]
Ash	5.7	7.4	9.1[c]	5.1[f]	7.8[h]
Glucosinolates	5.6	4.5-7	8.6[c]	0.44-0.7[g]	1.5[i]
Phytic acid	3.4	NR	2.7[d]	3.2-4.5[g]	1.2[h]
Fibre	34.9	22.1	18.8[c]	14.5[e]	46.8[h]
Soluble sugars	6.2	NR	14[c]	NR	6.6[j]
Polyphenols	0.3	NR	0.5[d]	0.15-0.36[g]	1.44[h]

a-(*62*). b- (*71*) , remaining amount is "Nitrogen free extract". c- (*43*), sugars are a sum of sucrose and dextrose. d –[Matthäus (*84*) '97] , from whole seed data adjusted for 35% oil[a] and 6.5% moisture (assumed). e - (*85*). f - (*86*), from seed data adjusted for 31% oil and 6.8% moisture. g - (*87*), from seed adjusted to 43.3% oil, 6.5% moisture (assumed), glucosinolates calculated as glucocamelinin (M_w 201.35g/mol). h - (*88*). i - (*89*), "double zero" rapeseed meal, calculated as progoitrin (Mw388.4g/mol). j - (*90*).

Although rapeseed is grown as a food crop, many varieties such as HEAR have significant anti-nutritional content placing them under our definition of an industrial crop. The extensive knowledge obtained from rapeseed breeding and genetic manipulation makes rapeseed attractive as a possible platform for future industrial crops. Agronomists are already familiar with growing rapeseed on a commercial scale which would ease the introduction of industrial rapeseed varieties compared to novel industrial oilseed crops. In the case of genetic manipulation of rapeseed there are regulatory issues to overcome as the modified material could easily transfer to food crops (*44*).

Table 2. Amino acid content of 4 industrial oilseeds, expressed as g/100g protein.

Amino Acid	**Carinata** (*62*)	**Crambe** (*71*)	**Camelina** (*75*)	**Rapeseed*** **(*75*)**
Alanine	4.6	3.8-4.2	4.2	4.6
Arginine	8.4	5.7-7.3	7.4	5.9
Asparagine + aspartic acid	8.8	6.0-7.6	8.1	7.5
Cystine	2.0	2.6-2.8	1.9	2.5
Glutamine + glutamic acid	20.7	14.2-17.0	14.3	17.3
Glycine	5.5	4.7-5.3	5.3	5.2
Histidine	2.7	2.2-2.7	2.5	2.8
Hydroxyproline	NR	0.6-0.9	NR	NR
Isoleucine	NR	3.7-4.1	3.5	3.9
Leucine	NR	5.9-6.8	5.7	7.1
Lysine	5.9	4.9-5.7	4.6	5.5
Methionine	1.8	1.6-1.9	1.7	2
Phenylalanine	4.4	3.4-4.0	3.8	4
Proline	6.9	5.5-6.2	4.5	6
Serine	4.9	3.5-4.1	4.7	4.4
Threonine	4.6	3.1-4.6	3.9	4.4
Tryptophan	.6	1.0-2.0	X	1.3
Tyrosine	2.8	2.5-3.0	X	3
Valine	4.6	4.5-5.6	4.8	5.2

NR - not reported, X – not determined, * - commercial meal

4. Bio-based Plastics From Plant Proteins

Proteins from natural plant sources are an abundant material that has been proposed as a replacement for petrochemical based polymers (*5, 6, 91, 92*). Plant based protein based plastics have the advantages of biodegradabity (*5, 7*), renewability, low O_2 and CO_2 permeability ((*17, 26, 93*) and (*94, 95*) respectively) and possibly low cost. However, applications for plant protein based plastics are limited by the disadvantages of materials developed to date; they degrade too easily for some applications (*96*), have low mechanical properties (strength and stiffness), high water permeability and they are susceptible to dissolution and swelling in water and other solvents (*5, 92*).

Familiar natural animal protein based materials such as silk, horn, sinew and feathers have been used as traditional materials in their natural state, but the proteins from plant sources require modification to make useful bio-based plastics. As plant proteins usually are below their Tg at the required use conditions, the material made from these proteins is normally brittle. In this case the glass transition occurs at the temperature where coordinated molecular motion can occur in a polymeric chain (*97*). Plasticizers disrupt interactions between segments of the protein chain allowing easier segmental motion lowering the glass transition temperature. Thus, the addition of plasticizers in the material is required to increase flexibility. Protein based plastics do not only need to be flexible in their final use, but also during forming when they are forced into the required shape. In the case of oilseed proteins, the proteins do not generally react strongly with each other making the formation of useful plastics difficult. Special procedures or additives are needed to form a cohesive solid from the oilseed proteins. Such procedures increase the molecular weight of the proteins and form a continuous network through protein-protein interactions (*65*).

The development of protein based bio-based materials specifically from oil seed residuals is still in its early stages with no specific information on these interactions. The general literature about protein behaviour related to food quality (*23, 64, 65*) and to bio-based plastics (*5*) can provide information on protein-protein and protein-additive interactions that can be applied to the development of oilseed protein based bio-materials.

The protein source has a large impact on the behaviour of the bio-material during forming as well as on the final properties. Some plant proteins, such as wheat gluten, contain adequate numbers of reactive residues (cystine) to form intermolecular covalent bonds leading to network formation through simple heating or solution casting (*98*). For other plant proteins such as most oilseed proteins a low number of these reaction sites are present (Table 2). Depending on the path of the protein from seed to biopolymer feedstock, heat or chemical processing can modify the presence of reactive sites (*64, 65*). Reactive sites can also be affected by other components of the seed, such as phenolics, that interact strongly with proteins reducing protein reactivity (*99*).

4.1 Plasticizers

Macromolecules such as synthetic polymers and large proteins exhibit a phenomenon known as a glass transition (*100, 101*). Recent simulations have demonstrated that below the Tg the elements of these large molecules are basically "jammed" and unable to move (*102*). When the material crosses the glass transition the lack of mobility at the molecular level translates to changes in material properties, the material becomes brittle and cracks are formed when stress is applied rather than the material flowing into a new shape. The transition can be probed with differential scanning calorimetry (*101*) and by monitoring the development of the materials mechanical properties with changes in temperature using dynamic mechanical thermal analysis (*35*).

In their natural state proteins are generally below their glass transition at the expected use temperatures for protein based plastics making them brittle and prone to breakage. It is also necessary to have the protein based plastic in a highly flexible state during initial forming. The required flexibility for forming can be achieved by heating above the Tg, but in most cases the Tg of the unplasticized protein is too high to avoid degradation during processing (*103*). As with synthetic polymers (*97*), the glass transition of proteins can be lowered by the introduction of plasticizers (*32, 33*) allowing the protein based plastic to meet requirements for flexibility in use and during forming. Compounds that make good plasticizers are those that not only interact with the protein to lower its Tg but also resist evaporation and migration to the surface ensuring a long lasting plasticizing effect.

Many compounds have been investigated for use as protein plasticizers such as water, glycerol, sorbitol, ethylene glycol and fatty acids (*5*). Plasticizers have been shown to decrease the Tg of proteins according to their molar proportions rather than their percentage by weight. Thus, smaller molecules tend to be favoured as more plasticization is accomplished with low mass addition levels (*35*). The downside of using lower molecular weight plasticizers is that they have high migration rates, possibly resulting in their loss to the environment over time (*104*).

Glycerol has been the plasticizer of choice for many studies as it is easy to mix with the target proteins and has a high vapour pressure and thus resists evaporation. Glycerol's hydrophilicity promotes water uptake by the bio-material increasing water vapour permeability and decreasing O_2 and CO_2 permeability. The rather weak interaction between glycerol and proteins results in high plasticizer migration rates when in contact with a substrate that absorbs glycerol, such as in protein bio-polymer coated paper and composites (*104, 105*). It has also been observed that with immersion in water of wheat gluten based plastics all glycerol is lost after 15 minutes, being replaced with water (*106*). The possibility of plasticizer migration and its consequences must be considered in selecting a protein-plasticizer system for a certain application.

It has been proposed that branched thiol terminated polymers can act as an internal plasticizer as well as a cross linking agent in wheat gluten (*107*). It is unclear whether the improvement in elongation of the protein based plastics incorporating the branched thiol terminated polymer additive was due to a plasticizing effect or some other origin as the Tg of the protein based plastic was not probed. Various reactive groups (ethyl vinyl sulphone, acrylic acid, butadiene sulphone, maleimide) have been attached to ovalbumin as a model system showing that direct attachment of a plasticizer to a protein could reduce the Tg without the migration issues associated with other plasticizers while also increasing the thermal degradation temperature (*34*). Stearic acid has been suggested as an internal plasticizer in soy protein based plastics, exhibiting plasticization while covalently attached to the proteins (*108*).

4.2 Forming

Protein based biomaterials must be formed into the desired shape in order to make useful products. Some shapes are simple, such as a fibre or film, while others can be quite complex, such as injection moulded consumer products. Each conversion process in the production of

protein based biomaterials has specific needs in order to make the material compatible with its use. The properties of the protein based plastic required to successfully use a certain process may affect the final performance of the formed product; thus, the material, process and final end-use must be considered if the final product is to be successful.

Film casting

Solution cast films are generally made in the laboratory as a convenient way to examine the properties of proteins with various casting conditions, solvents and additives (*5, 25*). The protein in question is dissolved or dispersed in a solvent and poured into a container in a thin layer, usually with a release layer on the bottom such as Teflon^{TM}. The solvent is subsequently removed by evaporation leaving a protein film behind. This film is removed and examined for the effect of the casting conditions (solvent, additives, temperature etc.) on the properties of the resulting film.

Some of the advantages of film casting are that it is easy to perform in the laboratory and it makes the distribution of additives throughout the protein mass straightforward. It is unlikely that this method will be successful in manufacturing commercial protein based bio-plastics as the removal of the solvent is costly and it is limited to monolayer films without multiple processing steps. Solvent casting is currently used for the production of commercial specialty polymer films and was used in the past for the production of gelatine protein films for the photographic industry (*109*). Casting of polyvinylidene fluoride dispersions onto polypropylene as a barrier layer is common for commercial high barrier food packaging applications. A similar process can be envisioned for protein based barrier layers (*110*).

Compression moulding

In compression moulding the protein material is first mixed with additives, charged into the moulding apparatus, compressed to shape, heated to complete the necessary reactions, cooled if necessary and the resulting part removed (*26, 111*). For simple shapes such as sheets or films this is relatively easy as the material does not need to flow appreciably. For even slightly more complex shapes such as a shallow dish (see cover), the material needs to flow and fill the mould entirely, requiring a certain flexibility before the reactions are complete. As the amount of flow

during compression is low, there is no shear mixing of the additives and protein once in the press making the original mixing procedure critical.

Extrusion

In extrusion the protein material and additives are first mixed and charged into the extruder feed throat. There are many types of extruder with the most common being single screw, counter-rotating twin screw, and co-rotating twin screw (*30, 112*). All extruder types serve to heat, mix and pump the proteins and additives simultaneously. At the exit of the extruder the stream of material can be shaped by an exit die. The exit die can form the material into an infinite number of shapes such as thin films, tubes, large rods or almost any perimeter shape given the proper die (*113*). For the successful extrusion of protein based biopolymers the material must be fluid enough to be pumped by the screw(s) and cross linking reactions controlled to maintain the necessary rheological properties (*31, 114*). Extrusion is a common process in the plastics industry and its use would allow protein based materials to be replacements for petroleum based plastics within the same processing infrastructure (*31*).

Thermoforming

In the industrial process of plastic thermoforming a preformed sheet of material is heated and formed over a shaped tool, usually by vacuum, where it is cooled and later trimmed. The material must be strong enough to support itself during initial heating, flexible enough when heated to conform to the tool and have flow properties during forming that allow it to maintain an acceptable wall thickness in all areas of the part (*115*). Soy protein isolate based plastic has been shown to have thermoforming ability at least equivalent to polypropylene or polyvinylcholoride in a demonstration package (*116*).

Wet spun fibres

In the wet spinning of protein fibres the protein is first dissolved in a “spinning dope” with additives and usually allowed to age for a certain time to obtain the properties required for successful spinning. This dope is pumped out of small openings into a bath that causes the proteins in the dope to coagulate and form a solid strand of fibre. The fibre is then washed to remove the coagulation solution and remaining solution from the spinning dope. This washed

fibre is usually further processed to improve its properties by stretching, heat setting or chemical treatment (*117-121*). Wet spinning has been used to make commercial fibres from proteins in the past (*15, 119*) and the wet spinning technique was used to make approximately 20% of all commercial fibres in 2000, making it a familiar commercial process (*122*). An advantage of the wet spinning technique is that the initial solution and coagulation bath provide an opportunity to chemically modify the protein fibre. The main disadvantage is that large amounts of solutions are produced from the coagulation bath and subsequent washing that must be regenerated or disposed of.

Melt spun fibres

Melt spinning is related to the extrusion process where fibre forming material is forced at high pressures through small holes in a die to form fibres without added solvent (*123*). In order to accomplish this, a protein based plastic must have flow properties that allow fibre formation at acceptable pressures and speeds. Immediately after exiting the die opening the fibre must have properties that allow it to maintain high quality under the severe change in boundary conditions such as avoiding "melt fracture" (*113*) and withstand the tension applied to carry the fibre through the process. Once formed, these fibres can be stretched to reduce their diameter, heated, molecularly oriented or chemically treated to improve their properties. The melt spinning method is attractive as it is the main process used for the production of synthetic polymer fibres (*122*) and is efficient with no waste products unless produced using subsequent chemical treatments.

Injection moulding

Injection moulding is perhaps the most versatile of the processes used to form plastics. Generally, the feed material is mixed, heated and pumped with a single screw extruder at the end of which is placed in a reservoir. When the mould is empty the reservoir of material is forced to fill the mould through a small opening called a gate. Depending on the mould anything from very simple to extremely complex 3 dimensional shapes can be formed (*124*). In order for the material to proceed through an injection moulding machine it must be capable of being severely sheared and deformed at high rates as it is injected. Injection speeds are generally quite high which can result in flow induced local heating due to severe shearing. For successful moulding in existing machines protein based plastics must be able to withstand the initial feed device (screw

extrusion). The proteins must also resist cross linking while residing in the reservoir waiting to be injected and flow at high shear rates during injection without degradation. Several authors have successfully demonstrated protein based plastic injection moulding from plant sources such as wheat (*125*), soy (*126*), sunflower (*36, 127*) and rapeseed (*18*).

4.3 Mechanisms of Network Formation

Thermoplastic synthetic polymers usually have very high molecular weights and can rely on entanglement or crystal forming to provide acceptable properties (*97*). For plant proteins there is a need to form a cross linked network in order to improve their strength, elongation to break and resistance to water and other solvents. This cross linked network prevents dissolution by anchoring the individual proteins to each other, while the connections oppose the swelling of the network that is necessary for solvent uptake (*106, 128*). Although increased cross linking improves some properties, too high a cross link density can result in a loss of flexibility as the molecules freedom of motion is restricted (*121*). Cross links can form in protein systems through the interaction of certain amino acids with each other, reactions of amino acids with naturally occurring substances in plant systems and by the addition of synthetic chemicals.

4.3.1 Cross linking Between Amino Acids

Disulphide bonding

Disulphide bonding in plant protein systems occurs through cystine bond formation between 2 cysteine amino acid residues, known as the disulphide interchange reaction. These bonds are common in proteins and can exist as inter or intra molecular bonds. The native structure of many proteins are stabilized by intra molecular cystine bonds and proteins can also be composed of multiple subunits connected through intermolecular cystine bonds, for example the subunits of rapeseed napin and cruciferin (*82, 129*). In oilseed proteins, the cysteine residues are involved in intra and inter molecular bonding in the native state making it difficult to form additional intermolecular bonds during processing. Intra molecular cystine bonds are reported to be responsible for the properties of wheat dough and wheat gluten based plastics (*26, 130*). There are indications that intra molecular cystine bonds can be converted to inter molecular bonds given the proper processing conditions in wheat gluten proteins (*131*). If a protein system is low

in available cysteine, disulphide bond functionality can be added by the use of 2-iminothiolane (Traut's reagent) to modify lysine and improve disulphide cross linking (*132*).

Tyrosine bonding

Evidence has been found for dityrosine bond formation in wheat gluten during bread making promoted by ascorbic acid and $KBrO_3$ and it is expected that similar conditions could exist in other protein systems (*133*). The formation of dityrosine bonds has also been proposed from the use of glucose oxidase in wheat flour where they cross link the globulins and albumins (*134*). In the formation of caseinate films γ-irradiation has been shown to promote dityrosine bonds (*135*). Tyrosine cross links involving 2 to 4 tyrosine residues have been identified in native structural proteins such as insect resilin and plant cell walls and are believed to be essential in obtaining their high resilience and elasticity (*29, 136-138*). Protein based biomaterials containing recombinant pro-resilin sequences demonstrate the possibilities of dityrosine bonds outside natural systems (*139*).

Isopeptide bonds

Isopeptide bonds are defined as any amide bond between a carboxyl group and amino group of the residues of 2 separate proteins or peptides. The main groups usually involved in isopeptide formation are lysine, asparagine, and glutamine (*29, 98*). Isopeptides have been proposed as a mechanism for network formation in protein based materials (*26, 35, 93, 140*). Transglutaminazes have been shown to support isopeptide reactions between free amine groups such as in lysine and glutamine in regenerated protein fibres (*15*). Recent work has provided strong evidence for the presence of isopeptide type bonding in heated wheat gluten systems (*141*).

Lysinoalanine

Lysinoalanine (LAL) forms cross links between protein molecules by a two step process (*65*). The first step is the elimination of the H_2S of cysteine through hydroxide-ion catalysis and its reaction with serine yielding dehydroalanine as an intermediate. Next, the dehydroalanine double bond reacts with the available $-NH_2$ group of lysine forming LAL. As this reaction makes the nutritionally important amino acid lysine unavailable in food sources for non ruminants it has

been studied in terms of food processing conditions (*65, 142*). In aqueous solution the formation of LAL is favored by high temperatures, elevated pH and long reaction times, while the presence of ascorbic, malic and citric acids, glucose, sodium sulphite and ammonia retards its formation (*65*). The presence of LAL has been qualitatively observed in wheat gluten films cast under alkali conditions and correlated to improved properties (*143*), while other work has shown that it is absent in heated wheat gluten systems (*141*).

4.3.2 Chemical Cross Linkers and Cross Link Promoters

In addition to the naturally occurring and enzyme catalyzed cross links found in proteins, synthetic chemicals can be used to form cross links. These synthetic cross linkers have 2 or more functional groups that interact with protein residues to form cross links. These functional groups can be the same (homofunctional cross linkers) or different (heterofunctional cross linkers) (*144, 145*). Tables of protein based bio-polymer systems and the various cross linkers used can be found in recent reviews (*5, 25*).

Aldehydes

Aldehydes have a long history as general cross linkers in protein containing systems (*146*). Among the aldehydes used in the development of protein based bio-plastics are formaldehyde (*24, 119, 147-150*), glutaraldehyde (*24, 147, 148, 151-153*), glyceraldehydes (*149*), glyoxal (*24, 148, 154, 155*), and paraformaldehyde (*150*). It has been shown using cotton seed protein that despite the fact that all the above cross linkers have aldehyde reactive groups, each of them reacts differently with proteins (*24*). Aldehydes exist in many oligomeric forms in solution making an exact description of their behavior difficult (*24, 146*). The aldehyde cross linking of protein based biomaterials has been demonstrated in solution (*24, 147, 148, 151, 152*), during thermal processing (*150, 151, 154, 155*), through the exposure to vapours (*156*) and post processing of fibres (*118, 119, 153*).

Isocyanates

Using genetically engineered proteins, synthetic protein films have been formed using diisocyanates (*157*). Diisocyanates have also been used in improving soy protein in blends with polycaprolactone, a synthetic aliphatic polyester (*158*) and in improving the bond strength of rice

bran based adhesives where it may have interacted positively with the protein fraction (*159*). Although not specifically used for cross linking, proteins have been included in isocyanate based polyurethane foams, coatings and adhesives with good results (*160, 161*). Lack of use of isocyanates as cross linkers in protein based plastics is surprising as they are an easily available and well known cross linker. Isocyanates are used in large quantities as a chemical intermediate and for the manufacture of coatings and cross linked synthetic resins, such as polyurethanes.

Multifuncitonal carboxylic acids

The reaction of multifunctional carboxylic acids (malic, citric, or butanetetracarboxylic acid) is expected with the reactive amino groups of proteins. Successful reaction conditions have been demonstrated applying multifunctional carboxylic acids to proteins in the presence of either phosphate (NaH_2PO_2.H2O) (*162-164*) or NaOH (*121*). Other studies have found that multifunctional carboxylic acids do not produce cross links in plant proteins without the proper conditions (*165*). It appears that the reaction of carboxyl groups with amine side groups can be performed in solution (*163, 166, 167*) or after fibres have been formed by wet spinning (*121*). It has also been shown that multifunctional carboxylic acids can be used to graft other molecules to the surfaces of protein fibres such as attaching chitosan and cyclodextrin molecules to keratin protein fibres (*164, 168*).

Oxidation

The introduction of oxygen and potassium iodate (a fast acting oxidant) into solutions of wheat proteins has been shown to result in the formation of cross links, as examined through SDS solubility and molecular weight changes although the specific mechanism was not investigated (*169*). Formation of dityrosine cross links through oxidation is expected for proteins in the presence of peroxides. However, it has been shown that this reaction is limited in collagen and soy protein isolate without the presence of peroxidases (*136*).

Grafting and cross linking with synthetic polymers

Larger molecules have been used to graft plant proteins (namely wheat gluten) to make them reactive for cross linking. Molecules with reactive epoxy groups such as poly(ethylene oxide) diglycidyl ether (PEODGE) have been used to react with residues containing free amine groups

and when combined with ethyl diamine form cross linked structures (*170*). Glycidyl methacrylate and 2-acryloyloxyethyltrimethylammonium chloride (AETAC) combined with ethylene diamine have also been shown to form cross linked structures in wheat gluten (*171*). Thiol terminated star branched molecules have been used to react with cystine residues of wheat gluten forming a cross linked network (*107*). It has been proposed that the synthetic polymers with longer connections between cross link sites not only serve to form a cross linked network, but also to reduce the Tg of the composite material (*107, 170, 171*).

Ammonia as a cross link promoter

The addition of ammonia to protein systems such as wheat gluten (*172, 173*) and oilseed protein or residual meal lignocellulosic composites (*174*) has been shown to modify the final properties. In the case of oilseed proteins it is described as an "end stage maillard reaction" and was shown to require temperatures in excess of 175^{o}C and the presence of carbohydrates for the mechanism to be activated (*174*). The addition of ammonia to glycerol plasticized wheat gluten changes their behaviour markedly. When these materials are heated to 120^{o}C during extrusion their solubility to low levels in the presence of both sodium dodecyl sulphate (SDS) and the reducing agent dithiothreitol (DTT) demonstrating the presence of non-cysteine cross links. The treatment with ammonia simultaneously improves their mechanical performance (strength and stiffness) and oxygen barrier (*172, 173*). In both the protein-lignocellulose and wheat gluten systems above the specific mechanism of action for ammonia is not understood. Ullsten *et al.* proposes isopeptide bonds (*173*) as the cross link mechanism, while Feretti proposes the formation of Maillard type polymers in conjunction with lignocellulosic or glucosidic components (*174, 175*). The appearance of hexagonally arranged structures and increased β-sheet content in extruded ammonia containing wheat gluten has been reported by Kuktaite *et al.* as an explanation for improved properties in the presence of ammonia (*172*).

Irradiation

Irradiation in the form of ultraviolet (UV), gamma and visible light has been used to modify the properties of protein based biomaterials. Soy protein isolate films cast from solution then irradiated with UV showed an increase in stiffness and decrease in elongation (*176*). Cast films of wheat gluten, corn zein, egg albumin and sodium caseinate irradiated with UV in the solid

state showed various responses in terms of tensile strength, total solubility and water vapour permeability (*177*). Differences in response did not correlate to the expected cross linking behaviour of phenylalanine and tyrosine present in the protein systems (*177*). Gamma irradiation has been applied to cast spy protein isolate films with no apparent change in tensile strength or elongation to break (*178*).

Film forming solutions of proteins have been subjected to γ-irradiation before casting by various authors in order to modify their behaviour. A wide range of doses, from 0 to 50 kGy, have been shown to increase the strength and reduce elongation at break of soy protein isolate films cast from irradiated solutions (*179*). The combination of heating protein solutions (90^{o}C) with γ-irradiation before casting improved the puncture strength and elongation of soy protein isolate-whey films, but not soy protein isolate films. It was proposed that the heat activated disulphide cross linking and the irradiation produced hydroxyl radicals that induced dityrosine bonds during irradiation when whey was present (*180*).

Visible light has been used to produce protein based fibres from analogs to insect resilin protein sequences, forming a highly resilient protein fibres cross linked by dityrosine (*139*). Using a catalyst system consisting of tris(2,20-bipyridyl)dichlororuthenium(II)hexahydrate ($[RuII(bpy)]_3Cl_2$) and ammonium persulphate the presence of visible light forms Ru^{3+} and persulphate radicals that initiate dityrosine bonds (*181*). This system may be applicable to other systems amenable to tyrosine cross link formation, making rapid on demand cross linking possible.

Possible reactions with other constituents of industrial oilseeds

Naturally occurring compounds in oilseed meal such as tannins and other phenolics may play a role in cross linking reactions. Phenolic compounds are known to associate strongly with oilseed proteins. These compounds are readily oxidized to quinones during alkaline protein extraction and target the amine group of lysine, the indole of tryptophan and the sulphur bridges of methioniene (*40, 99, 182*). Removal of the phenolics has been suggested to improve oilseed protein activity (*40*). These compounds are also implicated in the poor organoleptics of some oilseed protein products (*40, 182, 183*), and may affect the organoleptics of oilseed protein based materials. In contrast, polyphenolic aldehydes such as gossypol, which naturally occurs in cotton

seed, have been shown to result in improved properties of cotton seed protein films (*147*). By adding condensed tannins to a phenolic/tannin free system, such as gelatine, the reactions between phenolics and proteins can be used as an advantage, resulting in cross linking (*184*). It was found that the tannins caffeic acid and tannic acid formed covalent cross links in gelatine involving amino acids with amino containing side groups (mainly lysine) at pH > 9 in the presence of oxygen.

4.4 Plant Protein Based Plastics

The development of plastics from proteins has been of two streams, finding a use for residual proteins from existing products (wheat gluten, zein, whey, feathers and oilseed residuals) and highly purified proteins, such as those from insect silk, or specifically engineered recombinant proteins. All proteins are based on the same amino acids and much can be learned from protein materials previously studied. Although our focus is on industrial oilseed proteins, insights from other protein systems can inform us of possible routes to improve the performance of protein based plastics from industrial oilseeds.

4.4.1 Rapeseed, Carinata and Crambe

Although rapeseed is one of the most common oilseed crops (*46*) little has been done to study its use in protein based bio-plastics. Residual rapeseed meal from oil extraction and hot compression moulding was used by Baganz *et al.* to manufacture bio-based plastics (*18*). Various treatments of the meal are described including pulverization, hydrothermal treatment, alkaline treatment and the addition of ground wheat straw filler and the binding agents cellulose acetate and partly acetelated starch. Pulverization to uniformly small particle size (99.9% < 70µm) reduced the need for binding agents. Hydrothermal treatment (moist environment,130^{o}C, 2.5 bar, 2h followed by drying) increased strength, while alkaline treatment (2g NaOH/100g meal 20% protein in aqueous solution, 2h followed by drying) lead to 15% higher strength (no specific mechanical properties were reported for these treatments). The addition of lignocellulosic fillers (ground straw) decreased water absorption while increasing flexural modulus, failure stress and failure strain. It was stated that prototype injection moulded parts were produced within normal parameters, but the formulae and conditions were not stated. Despite the small amount of information in Baganz et al. (*18*) it demonstrates possibilities for

oilseed residuals as useful materials and provides hope that practical compounds can be generated.

Wasche et al. (*17*) prepared films from the residuals of cold hexane defatted rapeseed meal and the protein rich fraction (PRF) that remains from aqueous oil extraction. Isolates were prepared from cold hexane defatted rapeseed meal by alkaline extraction followed by acid precipitation and ultrafiltration. Alkaline solutions (pH 12) of the isolates and PRF (32% oil) were prepared and cast into films. The PRF was further treated with supercritical CO_2 to reduce the oil content and films of this material were cast in the same way. No mechanical property data was given, but the PRF (32% oil) material returned a water vapour permeability of 1370 $g/m^2 \cdot d$ and when deoiled with supercritical CO_2 a water vapour permeability of 2230 $g/m^2 \cdot d$. The residual oil had a positive effect on water vapour permeability, although its effect on other properties was not noted. Films cast from the redissolved ultrafiltrated rapeseed isolate returned an O_2 permeability of 85 $cm^3/m^2 \cdot h \cdot bar$.

Canola protein isolate has been used in a composite with a biodegradeable co-polyester (35% and 40% w/w, respectively) (*20*). Polyvinylpyrrolidone was used as a compatibilizer (2%), zinc sulphate as an ionic cross linker (1%) and water (7%) was included as a processing aid. The composites were plasticized with glycerol, sorbitol, polyethylene glycol or polyvinylalcohol (15%) and compounded in a co-rotating twin screw extruder, pelletized and injection moulded. Glycerol produced the highest values for toughness, tensile elongation and strength. Contrary to most studies, increases in both tensile elongation and modulus were found with the use of glycerol as a plasticizer. This effect was attributed to improved mixing producing a superior microstructure. Polyvinylalcohol produced the highest modulus, with this behaviour explained by the high molecular weight of the polyvinylalcohol reducing its plasticizing effectiveness. All plasticizers exhibited the same water absorption behaviour, with slightly higher water absorption for unplasticized specimens.

Cold pressed rapeseed meal and solvent extracted flour from commercial production was used by Bettini to manufacture bio-plastics through compression moulding at 140 to 180^{o}C for 5 to 30 min. (*22*) Glycerol was used as a plasticizer (10, 20%wt), resulting in brittle materials with a maximum elongation at break of only 1.5%. Beyond simple plasticizer addition a standard pressing protocol was used (140^{o}C, 10% glycerol, 10 min) with the addition of additives; glyoxal

(40% aq) was applied to increase cross linking (1, 3, 5% wt), urea (8M) as a denaturing agent (1, 3, 5% wt) and NaOH used to control pH of the pressing environment (1, 3, 5% wt).

Despite the high degree of uncertainty in the data in Bettini, some general trends can be observed although generally they are not statistically significant (*22*). The presence of oil and the native protein conformation in the cold pressed rapeseed cake generally had a positive effect on all tensile properties of the unplasticized meal at the conditions studied. With the addition of glycerol the solvent extracted and roasted commercial flour was superior. Higher temperatures generally increased the modulus and strength while decreasing the elongation to break, until the onset of thermal degradation. The addition of glycerol had a marked effect; decreasing modulus and maximum stress while increasing strain at break, as expected from a plasticizer. Glyoxal additions reduced modulus with increasing levels with little effect on other properties. Urea addition had a minor effect with a drop in modulus with increasing urea. The addition of NaOH showed no statistically significant effect on the properties in this case.

Three oilseed meals from the brassica family; rapeseed, crambe and carinata were used by Johansson to investigate the production of materials from whole oilseed residuals (*19*). As the residuals contained some plant oils a siccative, manganese III acetylacetonate, was used to try and cross link oils into the overall matrix. All three meals were processed by compression moulding of as received, ball milled, and pre-extruded material. All testable specimens were produced with 15% glycerol and pressed at 100^{o}C for 12 minutes. The ease of processing the meals was in the order carinata, rapeseed, crambe regardless of method. Crambe produced materials with the highest modulus (350 MPa) was more than twice the stiffness of rapeseed or carinata. Strain at break for carinata was the highest (up to 2%), 4 times the level for the other 2 meals. Carinata showed reduced strain at break with ball milling, while the other meals were unaffected. The maximum stress values were similar for crambe and carinata (approx. 1.4MPa) while rapeseed meal only reached a maximum stress of 0.5 MPa. There was no statistically significant effect of ball milling on maximum stress. In the cases when a siccative was used the meal was heat treated for 0, 3, or 6 hours at 60^{o}C in order to examine the effect of reaction time on the cross linking reaction. No conclusions could be drawn from the heat treatment or siccative addition.

4.4.2 Soybean

Soybean (soy) is perhaps the most studied of the plant proteins for protein based plastics applications, but the chemistry of soy residuals is quite different from other oilseeds. The level of phenolics and other anti-nutritional components are low, reducing the number of complicating factors in protein behaviour. Also, raw material availability is high as commercial soy protein concentrates and isolates are easily available. Soy protein based materials have been made using all of the common plastic material forming techniques; film casting, wet and dry fibre spinning, extrusion, compression moulding, thermoforming and injection moulding.

Film casting has been used by many authors to form materials from soy. Park *et al*. have reviewed many of the pertinent factors in forming soy films (*185*). Some of the main lessons in the literature are that the heating of the film forming solution and increased initial protein concentration leads to increased properties of materials made from the flour, concentrate and isolate of soy (*185*). The pH of the casting solution plays a significant role in determining water vapour permeability (WVP), with higher pH leading to lower WVP of the flour, concentrate and isolate films (*185*). The incorporation of SDS in soy protein isolate (SPI) casting solution (up to 40% based on SPI content) resulted in a reduction in tensile strength and increase in elongation to break, similar to the behaviour of a plasticizer. With increasing SDS levels the WVP of the films decreased, but their solubility increased (*186*). Soy protein films have been modified with additions of glutaraldehyde to the casting solution (pH 2 to 10) resulting in increased tensile strength and elongation to break, with decreased solubility (*152*). Heating films after casting (as opposed to heating the casting solution) has been shown to increase tensile strength, decrease elongation at break and decrease WVP (*187*).

Wet spinning of fibres has been used to make useful materials from soy proteins for more than 70 years (*119*). The properties of the fibres can be improved using aldehyde cross linking (*118, 119*). More recent work has focused on environmentally friendly methods using alkali catalyzed multifunctional carboxylic acid cross linkers where post spinning treatment increased fibre strength while decreasing elongation to break (*121*). Huang *et al.* have examined wet spinning parameters for soy, finding that a combination of post treatment with 25% glutaraldehyde and stretching to 170% of the original length was the most promising (*118*).

Wet spinning has also been attempted in films instead of fibres with disappointing strength and stiffness (*188*). Soy films were formed by coagulation of an alkaline solution in an acetate buffer bath. A similar spinning solution was treated to precipitate proteins without spinning for comparison and the spun film was stretched to induce molecular changes. No significant difference was found between the proteins in the coagulated film, post stretched film, and precipitated proteins. No effect of stretching was found, contrary to previous work on wet spun soy fibres (*118*) and was attributed to the low take up speed during coagulation and differences in die design (*188*).

Work has also been done by Huang *et al.* on the extrusion of soy fibres comparing it to wet spinning (*118*). Glycerol, sorbitol and various metallic salts were examined as plasticizing agents; with glycerol as the only substantial success. Post production modification of the fibres was performed with acetic anhydride, acetaldehyde, glutaraldehyde, and stretching. The best performance was obtained with extruded fibres post treated with a combination of glutaraldehyde, acetic aldehyde and stretching to 150% of their original length.

Soy protein based biomaterials have been successfully extruded by various investigators. Pol *et al.* processed corn zein and soy protein isolate (*189*). Soy isolate, glycerol and water were compounded using a twin screw extruder to form pellets that were formed into films using single screw extrusion and chill-roll casting. Successful films were produced, but the process window was small for both compounding (no more than 30% glycerol and 10% water, max temperature 110^{o}C) and single screw extrusion to feed the chill roll (maximum temperature 140^{o}C, minimum temperature 135^{o}C) (*189*).

Zhang *et al*. produced extruded sheets through twin screw compounding followed by single screw extrusion (*190*). Glycerol, water and methyl glucoside were used as plasticizers while $ZnSO_4$, epiclorhydrin and glutaric dialdehyde (glutaraldehyde) were used to enhance cross linking. A wider processing window was found compared to Pol *et al.* (*189*) and $ZnSO_4$ was found to increase Young's modulus, the strength and elongation to break were not significantly affected at levels below 2% while water uptake was reduced by 30%. Epiclorhydrin and glutaric dialdehyde were successful in enhancing properties and were not found to reduce water uptake of the extruded films, but increased processing difficulties during extrusion (*190*).

Foams of soy protein isolate plasticized with glycerol have been formed by extrusion with chemical blowing agents (*191*). Many packaging and product protection applications utilize foamed materials making foaming of interest. Water and glycerol were found to be necessary for acceptable processability. The salts $CaCl_2$ and $ZnSO_4$ were used as ionic cross linkers and increased all of tensile strength, elongation at break and Young's modulus. The density of foam sheets was reduced to the range of 0.4 to 0.6 g/cm^3 with lower glycerol levels giving the lowest densities with all other conditions constant (*191*). Similar results were found for compression moulded foam materials (*191, 192*) with densities ranging from 0.14 to 1.04 g/cm^3. Polymeric materials are not always easily foamed as the stability of foams depends on many factors (*193*) and a demonstration of foamability in protein based biopolymers is significant.

Soy flour bio-polymers have also been produced through reactive extrusion (*194*). Glycidyl methacrylate, maleic anhydride, styrene and a polymerization initiator (Lupresol) were added to soy flour followed by glycerol addition and reaction in a co-rotating twin screw extruder at a maximum temperature of 135^{o}C. The reacted extrudate was then ground and used for compression moulding. All samples with additives showed increased strength and stiffness, but a small increase in elongation at break. For the modified materials the impact strength was diminished. Differential scanning calorimitery results indicate changes in the T_g of the soy protein, indicating interaction with the reactants, decreasing with maleic anhydride and glycidyl methacrylate, and increasing with styrene. The formation of *in situ* linkages with the proteins as well as styrene polymerization is proposed for the changes in properties.

Instrumented single screw extrusion was used by Ralston and Osswald to examine the viscosity of soy protein based bio-plastics (*195*). Soy protein isolate was extruded with various additives; cornstarch, soy oil, titanate and sodium sulphite with glycerol as a plasticizer. Viscosity measurements are critical to the proper process design in the industrial use of all polymers (*113*). Viscosity decreased with increasing shear rate in all cases, so called "shear thinning behaviour", and additives could be used to modify the specifics of viscosity change with shear rate. Over the shear rate range of the study (100-800/s) all of the viscosity vs. shear rate lines intersect at some point; although there are measureable differences in behaviour the compounds are quite similar. When the compounds were re-run through the screw viscometer the behaviour of the compounds with different additives was more similar. At high shear rates and the highest levels of SPI die

wall stick-slip was observed similar to petroleum based thermoplastics at the limit of their processability (*113*). These observations hold the promise that protein based materials may be used in standard polymer processing equipment using standard methods within certain limits.

Pateau *et al.* (*111*) examined the effect of isoelectric precipitation procedure, water content and pressing temperature on both soy concentrate and isolate based bio-plastics formed by compression moulding. Soy protein isolate and concentrate were prepared by dissolution in water followed by isoelectric precipitation using acetic, propionic, hydrochloric and sulphuric acids. The use of hydrochloric and sulphuric acids for isoelectric precipitation reduced the strength and elongation to break. Utilizing soy concentrate as a feedstock, hydrochloric acid precipitation resulted in decreased tensile strength, while untreated concentrate and propionic acid treated concentrate were of equal strength. Both hydrochloric and propionic acid precipitation reduced water uptake.

Cross linking soy proteins in solution was examined by Paeteau *et al.* (*196*) who produced materials of soy protein isolate modified with formaldehyde, glyoxal and adipic/acetic anhydride. The chemical modification of proteins was carried out in solution followed by isoelectric precipitation, centrifugation, drying at 50^{o}C and grinding. The resulting powders were compression moulded at 160^{o}C. It was found that treatment with 5% formaldehyde gave an increase in all of tensile strength, strain at break and a small increase in Young's modulus. Glyoxal treatment up to 5% did not affect tensile strength but decreased elongation to break and increased Young's modulus. Adipic/acetic anhydride treatment decreased tensile strength and elongation to break while increasing Young's modulus. All cross linking treatments decreased water uptake, and DSC showed changes from all treatments indicating successful protein modification.

The problem of increasing strength, stiffness and elongation to break in soy protein bio-plastics have been approached with the concept of molecular orientation (*118, 188, 197*). Kurose *et al.* (*197*) found that by stretching soy isolate based plastics with water as a removable plasticizer allowed the manipulation of the strength, stiffness and elongation at break. Specimens were manufactured by compression moulding followed by stretching up to 2.5 times the original length while still containing high water content (25%). The stretched samples were held for 15 hours in their extended state while drying. This treatment was found to universally increase the

tensile strength. Specimens containing low glycerol (0, 10%) showed increased strain at break with increasing drawing while those with higher glycerol (20, 30%) showing decreased strain at break with increased drawing. Only small changes were seen in Young's modulus. Wide angle X-ray and DSC measurements did not indicate the existence of new crystalline phases and attenuated total reflectance Fourier transform infrared spectroscopy was somewhat inconclusive regarding structural changes from drawing. Microwave molecular orientation analysis revealed increasing molecular orientation with increased drawing. Other attempts at inducing orientation in protein based bio-polymers have been unsuccessful (*188*) perhaps due to their lack of structural pinning provided by the initial heat treatment at 140^{o}C in the case of Kurose *et al.* (*197*).

The findings of Kurose *et al.* are significant as they demonstrate that the molecular orientation of protein based bio-plastics can be directly controlled (*197*). This is the basis for many successful products and processes in the use of petroleum based polymers, from high barrier biaxially oriented polypropylene packaging films and stretch blow moulded polyethylene terephthalate beverage bottles to textile fibres of polypropylene, nylon, and polyester (*198-200*). The control of both molecular orientation and protein-protein interactions with respect to conformation (denaturation) and bonding (cross linking) opens up new opportunities for microstructural control that can lead the way to tailored properties.

In order to provide cost effective soy based bio-polymers Cao *et al.* investigated a process of utilizing protein rich water extract from the whole soy bean to form edible films (*201*). The film properties varied with ratio of beans to extraction water, pH, plasticizer composition and concentration. The extracts contained proteins, lipids, carbohydrates and ash, forming a lipid-protein emulsion film. Testing revealed high elongation to break of 217 to 270% and strength of 1.6 to 2.3 MPa, comparing favourably to some published results on soy protein isolate films (*202*). The strongest films of Cao *et al.* were obtained at a pH of 10, it was proposed that below this pH the proteins have not unfolded adequately to increase their interaction. The successful use of a whole bean extract for film casting indicates a possible low cost route to formation of bio-polymers from oilseeds.

4.4.3 Sunflower

The protein rich residual material from sunflower oil extraction has been investigated as a possible source for protein based plastic by Rigal and co-workers (*36, 127, 149, 203, 204*). Sunflower protein isolates were extracted and assessed for their behaviour with various plasticizers (*203*). Glycerol and triethylene glycol were found to be the most successful in terms of processability, plasticizer retention and strain at break retention during aging. Water vapour permeability of all samples was over 800 times lower than soy (*203*). Sunflower protein isolate based thermomolded plastics have been examined regarding their response to various aldehydes, tannins, fatty alcohols and fatty acids (*149*). The proteins behaved similarly to other oilseed protein isolates with the significant finding that natural tannins can be used to replace the more toxic aldehydes in improving mechanical properties and reducing protein solubility. Octonal was efficient in increasing tensile elongation, decanol at increasing hydrophobicity and octanoic acid at increasing strength and reducing water uptake.

Using extrusion viscometry up to 120^{o}C investigated the effects of glycerol, water and a sodium sulphite reducing agent on viscosity (*127*). Water was required for effective plasticization. For all formulations a decrease in viscosity with increasing shear rate, so called "shear thinning" behaviour was observed. The effect of sodium sulphite reduced viscosity up to additions of 3%, increasing the viscosity at higher loadings. This behaviour was attributed to reducing viscosity by initially breaking interchain disulphide bonds then at higher concentrations increasing viscosity as intrachain disulphide bond breakage allowed the molecules to extend thus increasing viscosity.

Successful injection mouldings were made using 100 parts sunflower protein isolate, 18 parts water, 4-22 parts glycerol and 4 parts sodium sulphite. However, the parts suffered from poor water resistance. A die temperature of at least 145^{o}C and the presence of water was needed to create a consistent film, significantly above the thermal denaturation temperature of sunflower proteins (*204*). Increasing the die temperature to 160^{o}C resulted in increased tensile strength, Young's modulus, elongation at break and increased swelling in water. This behaviour was attributed to increased cross linking rather than thermal denaturation.

Sunflower protein based plastics have been produced from whole sunflower oil cake (*36, 205*). Due to the structure of the sunflower hull, techniques were developed to fibreize the hull during mixing of the cake in a co-rotating twin screw extruder with 20-30% water and 0-5% sodium sulphite (*36*). Injection mouldings could be made with the unmodified sunflower oil cake, but with very poor mechanical properties. Fibreized material had much improved flexural strength and flexural modulus with a reduced amount of water required for plasticization. The incorporation of sodium sulphite further improved flexural strength and reduced the water required for plasticization. Heat treatment at 200^{o}C under nitrogen was used to improve the water uptake and flexural modulus with some reduction of strength.

4.4.4 Cottonseed

For cottonseed significant findings have been reported regarding protein-cross linker interactions, including the action of natural compounds present in the seed that are informative for other proteins (*24, 147, 206, 207*). Cottonseed residuals of glandless or glanded flour (*147*) and delipidated flours (*24, 147, 206, 207*) were extracted at 40^{o}C and pH 10 in a solution of triethylammonium or ammonium for 1 hour followed by centrifugation. The supernatant was blended with glycerol for plasticization and formaldehyde, glutaraldehyde and gossypol (in the case of glandless flour) as cross linkers. It should be noted that this method using triethylammonium or ammonium forms films with neutral final pH, which may be useful in food contact applications. Films were then cast from the solutions first with evaporation for 24 hours at room temperature and final evaporation at 60^{o}C.

Reactive lysine (*24, 206, 207*) and overall amino acid analysis (*24*) of protein hydrosylates after casting were performed via high performance liquid chromatography techniques to elucidate the specific reactions occurring with the different cross linking agents. Reactive lysine analysis measures the amount of lysine that has formed bonds that are stable under acid hydrolysis (*147, 206, 207*). By coupling reactive lysine analysis with overall amino acid analysis, other non acid liable reactions such as lysine-tyrosine linkages and glyoxal-arginine reactions can be examined (*24*).

It was found that films from glanded flours naturally containing the dialdehyde gossypol have similar behaviour to glandless flours where gossypol is added, with decreases in solubility and

increases in puncture strength compared to gossypol free films. The activity of naturally occurring gossypol had a positive effect, although the gossypol in the film forming solution appeared to inhibit formaldehyde and glutaraldehyde action (*147*). Formaldehyde added to the film forming solution increased the puncture resistance up to a formaldehyde/reactive lysine ratio of 8. Further increases in formaldehyde level produced no change in puncture resistance (*206, 207*). Comparison of the acid laible amino acid profile, with and without formaldehyde treatment, showed changes in lysine and tyrosine (*24*). The balance of acid resistant bonds formed per mol of formaldehyde present indicated lysine-tyrosine linkages as the reaction responsible for increased puncture resistance and decreased solubility.

Glutaraldehyde added to the film forming solution increased the puncture resistance up to a glutaraldehyde/reactive lysine ratio of 4. Further increases in glutaraldehyde level produced a reduction in the puncture resistance, which was attributed to polymers of glutaraldehyde forcing the proteins apart thus having a plasticizing effect (*206, 207*). It was demonstrated that glutaraldehyde only reacted with lysine while forming polyglutaraldehyde with 8 glutaraldehyde molecules forming bridges between 2 lysine groups (*24*).

Glyoxal added to the film forming solution increased the puncture resistance up to a glyoxal /reactive lysine ratio of 8 (as with formaldehyde) (*206, 207*). Further increases in glyoxal level produced a reduction in the puncture resistance as with glutaraldehyde (*206, 207*). Amino acid analysis demonstrated that glyoxal reacted with both arginine and lysine leading to acid resistant compounds (*24*). Reactions between glyoxal and arginine are considered unlikely to form cross links, while the reduction in reactive lysine with glyoxal addition correlated with increases in puncture resistance.

Marquié demonstrates that the specifics of amino acid cross linking in protein based polymers can be examined in detail beyond the empirical examination of final physical properties to elucidate specific protein-cross linker interactions (*24*). With an increased understanding of these interactions it is hoped that routes to specific outcomes through cross linking can be obtained.

4.5 Applications

In 2009 the total worldwide market for plastics was 230 Mtonne, 45 Mtonne of that in Europe (Figure 3 (*208*)). Compared to the total worldwide production of oilseeds of approximately 159 Mtonne (*46*), with only a portion being useable for making protein based plastics, the use of oilseed protein based plastics as a general petrochemical polymer replacement is unlikely due to the volume required. Niche markets, such as specialty packaging, are proposed targets for protein based plastics and demonstration packages produced (*8*). Although being sustainably sourced and biodegradable are becoming valuable on their own in the marketplace (*209*), protein based plastics must bring specific price/performance advantages to applications in order to compete with alternatives.

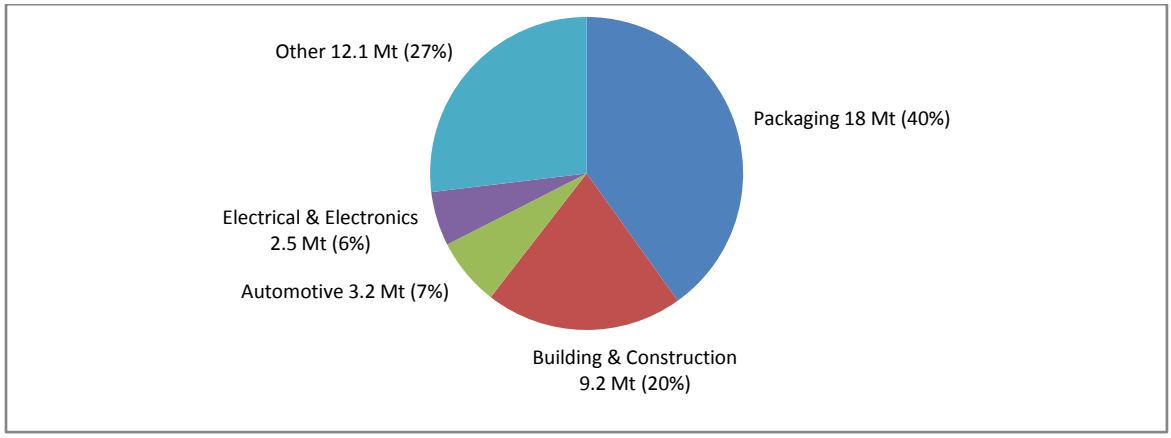

Figure 3. European plastics consumption by use, total 45 Mtonne (*208*).

One often cited advantage of protein based plastics is their low permeability to certain gases (*5*, *128*). A low oxygen permeability is an advantage in many packaging applications where oxidation leads to product damage. Low permeability to hydrocarbons can be an advantage in containing desirable food volatiles (*30*, *128*). A property often cited as a problem for protein based biopolymers is their sensitivity to water, which depending on the application can be an advantage. For example, packaging could be developed where the barrier properties of protein based plastics could be combined with their dissolvability in water to make single dose packages for user convenience (*210*) or reducing exposure to concentrated chemicals (*211*). Agricultural seed tapes could be manufactured of protein based plastics taking advantage of both their dissolvability and in soil biodegradeability (*212*).

The use of dissolvable films for laundry collection in hospitals reduces worker exposure to possibly infectious agents and in the hotel industry reduces workload as dissolvable laundry bags can be directly loaded into washing machines (*213*). A similar approach can be used in the manufacture of single dose detergent packs for home dishwashers using dissolvable films (*214*). Finding applications for protein based plastics may be less about improving their properties rather than finding applications where their existing properties are an advantage.

In many applications a single polymer material cannot meet all the physical property requirements, which has lead to the development of multilayer products. Depending on the use and materials involved, these layers can be extruded together to form multilayer films (*215*), laminated together from preformed films (*216*) or co-injection moulded to encapsulate one material in another (*217*). In this way the best properties of each material can be utilized, for example in a multilayer film with a water resistant poly lacticacid outer surfaces and a protein based core (*9, 216*). Some multilayer structures may take advantage of the poor water resistance of protein based plastics, allowing the dissolvable layer to be removed by washing during recycling operations(*217*). In order to accomplish the commercial application of these multilayer structures, methods for the economical conversion of protein based plastics into final products must be developed. The most common method used in industry is multilayer film extrusion, but film casting (*109*) or substrate coating (*218*) are alternative commercial methods to form films for later lamination. Film casting or substrate coatings are attractive if delivering the proteins in solution is the most convenient. For example a fibreboard substrate coated with wheat gluten demonstrates competitive oxygen permeability to commercial coated fibreboard barriers (*218*).

If multilayer films of protein based plastic are proven feasible there are a myriad of applications possible. In food packaging there are many opportunities for protein based layers in multilayer products. Layers of polyvinyl alcohol (PVA), ethelene vinyl alcohol copolymer (EVOH), nylon and polyvinylidene cholride (PVDC) are commonly used in multilayer packages as oxygen and aroma barriers and their functionality in terms of oxygen barrier can be approached by protein based plastics (*128*). In laminated “stand up” pouches protein based plastic layers could be used to protect the contents from oxygen or contain volatiles, as in packaging for household liquid soaps. Using edible proteins, dissolvable oxygen barrier packaging for convenience foods has been demonstrated for olive oils using zein and soy proteins (*8*). These packages were shown to

be superior to commercial non-dissolvable polymer pouches of polyethylene with a nylon barrier layer. This superior performance points to applications in multilayer pouch or bottle packaging for similar plant oils or other non-food oxygen sensitive products.

Specialised micro scale packaging in the form of core/shell microspheres have been constructed with protein shells containing hydrophobic liquids (*219, 220*) or gases (*221*). Such structures could be used for the formation of stable colloids, for instance allowing the use of aqueous application of pesticides that are not water soluble while providing controlled pesticide release.

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