NOVEL SUPERABSORBENT MATERIALS OBTAINED FROM PLANT PROTEINS

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Introductory Paper at the Faculty of Landscape Architecture, Horticulture and Crop Production Science 2017:2
Swedish University of Agricultural Sciences
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Cover photo: “Biological resources for greener diapers”,

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Summary

This work reviews the potential of plant protein-based materials as superabsorbent polymers (SAP). The review also discusses important topics of relevance for the current state of petroleum-based SAP and explains the background of the high water uptake of such materials. As diapers represent one of the most significant example of SAP applications, their industrial assemblying is highlighted. The research in absorbent materials has shown that treated and functionalized proteins may play a role in daily-care applications where super absorbency is required. Consequently, a description of proteins and the interactions that can take place within these natural polymers are reviewed. Protein sidestream candidates from biorefinery processes are listed and a further description of protein extraction mechanisms is given. This review also summarizes the results that have been obtained so far in the area of protein-based SAP materials. Finally, the challenges that protein-based SAPs face, as a possible candidate to replace petroleum-based ones, are discussed.

Preface

Super absorbent polymers (SAPs) have had an important role in the development of the daily-care product industry, such as diapers. Petroleum-based SAP materials have been utilized in these products practically since the first disposable item containing absorbent polymers appeared on the market. In order to adapt this industry into the *green* and sustainable era, these products have to be re-invented. Therefore, scientists have put an effort into producing new eco-friendly alternatives to petroleum-based SAP materials. Previous work has shown that bio-based materials such as proteins have the ability to absorb water in a similar range as synthetic SAP. In addition, the agricultural industry has proven to be able to provide an important protein concentrated feedstock from sidestreams related to the biorefinery field, for instance when producing bioethanol from wheat or starch from tubers such as potatoes. Consequently, once we understand the transformation processes of protein structures and interactions neccesary for promoting water uptake in protein based SAPs, a suitable alternative for the unsustainable petroleum-based SAP may be developed.

Contents

List of Abbreviations

DMAP: 4-dimethylaminopyridine
AUL: Absorbency under load
AAc: Acrylic Acid
ACN: Acrylonitrile
AAm: Acryl Amide
APS: Ammonium Persulfate
CMC: Carboxymethyl Cellulose
CRC: Centrifuge Retention Capacity
C-L: Crosslinks
DSC: Differential Scanning Calorimetry.
DVS: Divinylsulphone
EDTAD: Ethylenediaminetetraacetic Dianhydride
EGDGE: Ethylene glycol diglycidyl ether
EDGMA: ethylene glycol dimethacrylate
FT-IR/IR: Fourier Transformed Infrared Spectroscopy
FSC: Free Swelling Capacity
GA: Glutaraldehyde
HEC: Hydroxyethyl Cellulose
IPN: Interpenetrating Networks
LiCl: Lithium Chrolide
MBA: methylene bis-acrylamide
NFC: Nanofibril Cellulose
NMR: Nuclear Magnetic Resonance,
PAAc: Poly(Acrylic Acid)
KPS: potassium persulfate
PAas: Polyaspactic Acid
PEG: Poly(ethylene Glycol)
PVA: Poly(vinyl Alcohol)
PIP: Protein Isolectric Precipitation
PMM: Protein Micellar Mass
Semi-IPNs: Semi Interpenetrating Networks
SA: Succinic Anhydride
SAP: Super Absorbent Polymer(s)
TGA: Thermal Gravimetric Analysis
TNBS: Trinitrobenzenesulfonic Acid

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1. Introduction

Super absorbent polymer (SAP) materials have played an important role in the personal and hygienic care industry since their first synthesis, during the 1970's [1]. SAPs consist of slightly crosslinked hydrophilic polymer chains that form a stable 3D network structure. These materials have the potential to absorb and retain: water, saline solution and body fluids [2]. The range of absorption varies with the solvent used, but can be up to 1000 times their dry weight for pure water [3]. At the moment, the main SAPs used at the industrial scale are partially neutralized polyacrylates, such as Sodium neutralized Polyacrylic Acid (PAAc) [4-6]. Among the many applications where these materials are implemented, their use in daily-care products is extensive and has helped manufacturers to produce more efficient and less expensive products while keeping important liquid absorbency properties [7,8].

Most of the commercially available daily-care products (*i.e.* diapers) have an absorption core which contains partially or fully synthetic and neutralized polyacrylic acid (PAAc) [9]. The inexpensive petroleum-based PAAc fulfills the requirements for super absorbents, *i.e.* high absorption rate, equilibrium absorption and retention of the liquid within the network [10]. In addition, the techniques used for polymerizing acrylic acid are based on solution and suspension polymerization, processes that are convenient at the industrial scale. In order to produce crosslinks and avoid the SAP dissolving in polar media, a low concentration of crosslink agents are typically added. The most common among these agents are ethylene glycol dimethylacrylate (EDGMA) and methylene bis-acrylamide (MBA) [11].

The aim of recent researches are focusing on producing PAAc materials that are more absorbent compared to today's commercially available SAP [12,13] as well as making PAAc more capable of keeping their mechanical properties even after being submitted to severe mechanical stress conditions [14]. Nonetheless, sustainability concerns regarding the use of petroleum-based materials for fabricating disposable daily-care products are rising, resulting in a need for new material alternatives. The global market have already pointed out that relying on petroleum-based SAPs is not sustaible and represent a problem when it comes to meeting the demand that daily-care products will signify in the future [15,16]. Moreover, there are still toxicity and carcinogenicity fears concerning synthetic SAP, especially for PAAc [17,18]. Although the industry employs rigorous safety testing procedures [19], the proven risk of inhalation irritation,

monomer residual traces, and rash after skin contact is imminent and keeps the use of such materials under discussion [20].

Currently, the problems of waste management have raised a new drawback to petroleum-based SAP. According to the literature, by the late 20th century the global SAP production amounted 1 million tons per year [16,21]. However, an enormous increase in capacity has recently been noted for the SAP industry in order to adapt the manufacturing of these polymers to the increasing global demand. In 2014 the industry had a capacity of *ca.* 3 million tons of SAP [22], meaning a 3 times increase within 10 years. Since SAPs are petroleum-based, the production of these products represents an obvious environmental problem and a non-sustainable solution. Additionally, taking into account that just some countries in the world have a good and established waste management policy, the disposal of diapers has become a global concern since large amounts are disposed in landfills. Moreover, polyacrylate polymers essentially only undergo a low degree of degradation below 150 ºC, where the governing degradation mechanism is intramolecular anhydride cyclization [23]. Just over 210 ºC more efficient mechanisms take place, with decarboxylation and actual chain scission of the PAAc macromolecule [24].

Consequently, despite the broadly well-known benefits of synthetic SAP, the non-biodegradability and lack of sustainability, together with the massive production of these materials might be a dead-end for its utilization [5]. Still, to my knowledge, a feasible alternative that could properly and efficiently replace petroleum-based SAP is lacking.

In order to overcome the addressed downsides of synthetic SAP, a variety of “green” alternatives have been evaluated. Bio-superabsorbent gels have been obtained by using graft co-polymerasation of cellulose, gelatine, and other bio-based materials [16,21,25,26]. Natural polymers have also shown their ability to perform as SAP in medical, biological and pharmaceutical applications due to their biocompatibility and degradability. Polysaccharides (*e.g.* cellulose [27], hyaluronic acid [28] and chitosan [29]) have been studied and have been reported as alternatives to synthetic SAPs. Likewise, other studies have shown the ability of protein-based materials, *e.g.* fish protein isolates, to accomplish superabsorbent behavior with water uptakes of *ca.* 500 g/g [17,18,30,31]. Proteins are an extremely versatile material due to their high molecular weight and the composition of different amino acids held together by peptide bonds [32], therefore their properties can be fine-tuned in order to produce superabsorbent materials [15,17,33]. Even though the equilibrium swelling plateau of naturally-based

superabsorbent materials is comparable to that of petroleum-based PAAc, the absorbency rate is lower than the latter and thus is unattractive as a commercial alternative to synthetic SAP.

Recently, the agriculture industry have come out with new responsibilities in terms of producing sustainable alternatives to the non-viable petroleum market. The manufacture of cereals, seeds, and plants for different purposes, produces a number of sidestreams which can be used as an inexpensive and a reliable feedstock source. By 2014 approximately 1000, 729, 381 and 23 million tons of corn, wheat, potatoes, and oat, respectively, were produced worldwide [38]. For instance wheat was shown to have potential as raw material for: bioethanol fuel production after wet processing, gluten biobased plastics, foams [34-36], refinery aid [37], etc. Likewise, residuals from de-oiling procedures of rapeseed and *Carinata* seeds have been used for producing protein-rich bioplastics with interesting mechanical properties [39,40]. Thus, several crops consumed worldwide can boost the biopolymer industry in a green and novel way in order to replace petrochemical products in the market.

The idea of turning protein-rich sidestreams or cereals into a suitable bio-superabsorbent material has become an interesting topic due to a lack of research using polypeptide structures for high water-uptake [16]. Previous work has shown that foams based on 100% proteins have the ability to absorb water in a similar range as synthetic SAP [36]. The main downside of such foams are the observed sponge effect; upon mechanical compression the material releases nearly all the absorbed water.

Consequently, an important focus given in this paper is to understand the underlying transformation processes that a protein is submitted to when applying different thermal and chemical treatments for producing different structures, *e.g.* foams. Similarly, the interactions that can happen between the protein chain and the water molecules after the absorption process will be discussed. This understanding of the mechanisms that bind water molecules to different protein structures and the development of novel protein-based superabsorbent alternatives for producing sustainable daily-care products.

2. SAP state-of-the-art

Since the beginning of the 1970's, SAPs have received increasing scientific attention, and one main interest has been to understand how swelling properties of highly hydrophilic polymers can be controlled. The research has been focusing on how to prevent partial or complete dissolution of the material when placed in aqueous solutions [10,41]. Early findings allowed

researchers to develop macromolecules slightly cross-linked and neutralized, thereby being able to take up more than 500 g of water per gram of dry matter (g/g) [7,42]. A polymer can be considered absorbent when a high liquid uptake is obtained even when counter ions are present. However, in order for an absorbing polymer to be entitled as superabsorbent, the high absorbance must also occur under pressure (*ca.* 2.10 kPa) [7,42-45]. Sodium-Polyacrylic acid and Polyacrylamide are among the most preferred polymers and among those mostly implemented for applications in personal-care products matrixes for producing SAP at industrial scale [43,46,47]. The Global Superabsorbent Polymers Industry has accounted the synthetic SAP production to reach over 2 million (by 2014) and the estimated increase is 7% per year [22]. Unlike other highly absorbent structures such as cellulose fibre pads [48-50], sponges[51,52] and biofoams, also encountered in daily-care products[53], SAP retains a significant amount of water within the gel structure upon mechanical compression [3,36,54].

As illustrated in figure 2.1, Polyacrylic acid possesses a carboxyl group as the building block unit, which gives rise to the strong water affinity [16,21]. For success of the SAP, intermolecular bridges or crosslinks must be present or formed for the creation of a network that prevents the polymer from dissolving and/or releasing molecules into the liquid. According to the literature, 0.0008 mol of crosslinks per mol of PAAc are required to accomplish this goal [42,43,55]. Related to mechanical rubber theories, a lower crosslink concentration attributes more flexibility of the polymer chains between the junctions [56]. A slightly crosslinked network will permit large expansions of the molecules when in contact with water and will hinder the dissolution of the polymer [3]. After the synthesis of the SAP, the functional groups are neutralized with monovalent cations (*e.g.* Na^+) stabilizing the ionic forces presented by the carboxyl acid groups. The stabilization also provides a high osmotic pressure that develops in the macromolecule upon contact with water. This pressure is acting towards the absorption of the liquid for balancing the chemical potential between the polymer surface and the liquid [8,42,55-57]. This will be further discussed in section 2.1.3.

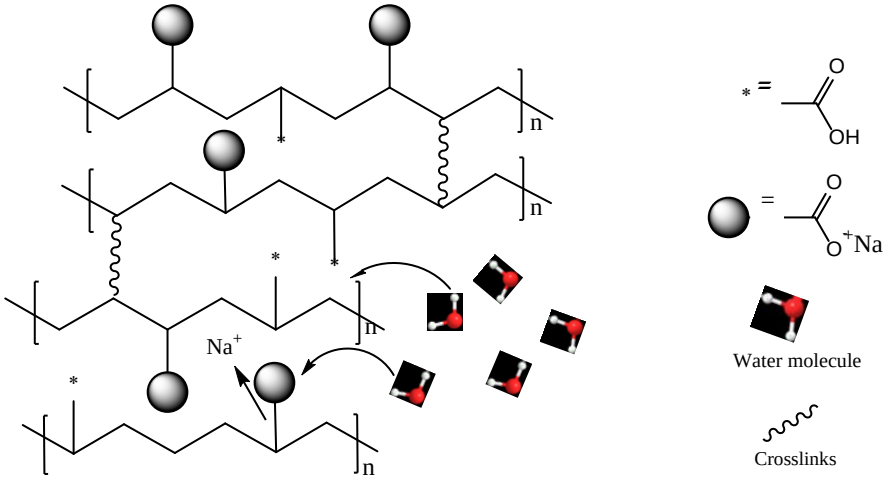

Figure 2.1: Scheme of a Sodium-polyacrylate network. The development of an osmotic pressure is partially illustrated with the releasing of Na^+ ions when the SAP is exposed to water.

In order to synthetize a SAP based on Polyacrylic acid (or other hydrophilic acrylate macromolecules), free-radical polymerization of the sodium-acrylic acid monomer is extensively implemented [16,43,58]. To obtain the final products with different qualities, either suspension or bulk polymerization systems are used. By optimization of the kinetic reaction parameters, *e.g.* temperature, time, monomer, crosslinker concentration, and the addition of chain-transfer agents, production of very specific quality SAP materials are possible [3,10,43,45,59-62].

As a consequence of the polymerization procedure, the synthetized and dried polymer keeps a rounded-shape and a porous structure [63]. Particle size distribution may vary depending on the processing parameters and techniques applied, but the range oscillates from 100 to 1000 μm [3,44] whilst the reported pore size varies from 0.5 to 50 μm [42,64]. As regards to the drying step, the simplest mechanisms used are forced air ovens or hot air dryers [59]. However, since the polymerization process involves an exothermic reaction, this heat can be used also for drying the material [10,43]. A second superficial polymerization step is applied for promoting gel strength and a dry-feeling of the particles after swelling. This last procedure involves spraying of the crosslinkers onto the material to promote a higher crosslink density on the surface [65,66]. Thereafter, unreacted monomers are extracted using liquid chromatography techniques. Crosslink density, obtained from the amount of extractable materials, is together with the network stability important parameters of the SAP [43]. Additionally, gravimetric measurements of the extracted fractions are used for characterization of the SAP product [44].

Traditional tests for measuring the superabsorbent capacity of a material include: i) free swelling capacity (FSC; both in pure and salt solutions), ii) centrifuge retention capacity (CRC), and, iii) absorbency under load (AUL). Free swelling capacity relates to the liquid absorption

of the dry powder, contained in a plastic-fabric bag with dimensions of 40x30 mm^2 [67,68]. Centrifuge retention capacity measures the binding strength of the water molecules in the swollen gel. The gel is submitted to centrifugal forces of *ca.* 250x g and the liquid retention is determined [10,42-44]. Lastly, the AUL test involves the compression of a dry powder in a cylindrical chamber with a piston of internal diameter of 25.4 cm (weighing *ca.* 4.4 g). This experiment replicates the absorption under compression forces, similar to the potential compression occurring in a diaper when worn [10,43,45].

2.1. Physics behind absorption

Many different factors are involved in the physics behind the absorption of a liquid in a hydrophilic polymer network. If we consider a SAP particle, the first phenomena which is involved is actually a diffusion of the liquid molecules through the material. Diffusion in gels contains strong dependences on polymer concentration, type and nature of the polymer, swelling media, etc. [69,70]. Hedenqvist *et al.* described swelling kinetics of a solid material as in two stages [71]. First, there is a time-dependent diffusion of the molecules through the material, giving rise to a gradual increase in the total mass. At this stage, not all the material is participating in the absorption. Once the solvent has penetrated all the gel, a rapid expansion of the matrix occurs until saturation is achieved [70-72].

However, as explained earlier in section 2, it is common to include open-cell pores in SAP materials. When porosity is present in the system, different absorption mechanisms from diffusion are involved in the transport of the liquid within the material [73-76]. Darcy's law illustrates the influence in the volumetric flow of a liquid (Q) as proportional to the difference in the pressure generated by the presence of a porous medium [77]. The equation for a steady-state systems is shown in Equation 1.

$$Q = -\frac{K_d\, A\, \Delta P}{\mu\, L} \quad \textbf{(Equation 1)}$$

Where "A" represents the cross-sectional area, "L" the travel distance of the liquid in the sample, "μ" the viscosity of the liquid, "Kd" is a geometry factor from the porous media and "ΔP" is the pressure difference developed.

Although different physical forces can contribute to the volumetric flow of the liquid within a porous material, the most prominent is a capillary effect. This comes from a competition between cohesive and adhesives forces through a capillary of "R" radius, with a surface tension (σ). Hence, the liquid is driven to move towards areas with less pressure. The mechanism for

describing the capillarity pressure (cp) is described by Laplace's Law and is shown in Equation 2 [78,79].

$$cp = \rho\, g\, h = {}^{2\,\sigma}/_{R} \quad \textbf{(Equation 2)}$$

The capillary force is the main phenomena responsible for the fast initial absorption among all the commercial available SAP [7]. Sannino *et al.* and Kabiri *et al.* have shown that the porosity of SAP provides an important potential absorption capacity to the material, even at different SAP composition and under different solvents [60,80-82].

SAPs are polymeric materials which contain charged groups along the main chain [83]. Consequently, the description of their performance is usually compared to polyelectrolytes [84]. This fact leads to significant considerations regarding the behaviour of these materials compared to uncharged polymers, especially when they are placed in contact with water. The coexistence of counter ions as charge balancing groups in SAP gives rise to an important contribution of osmotic pressure for the liquid absorption [83,85]. In fact, non-neutralized polyacrylates show a distinctly poor absorbent behaviour due to the lack of counter ions and the low ionization of the carboxyl groups in neutral pH solutions [86,87]. The critical contribution in the super absorption by the osmotic pressure is challenged when the material is exposed to saline solution, where the liquid uptake can be reduced by approximately 1 order of magnitude by the presence of 0.9%wt. NaCl [62,88-90]. The difference of the total ionic species (C_i) in solution and gel will provide an osmotic contribution (Πion) due to the ion/counter ion interactions in the polyelectrolyte gel (Van't Hoffs law, described in Equation 3) [86,87]. Nonetheless, in SAP we ought to consider the entropic deformation of the network and the free energy of mixing that are developed when the material is placed in a solvent. Consequently, the osmotic pressure contribution (Π) for SAP includes rubber elasticity and Flory-Huggins mixing theories as well; marked in blue and red, respectively (Equation 4) [3,43].

$$\Delta\Pi ion = RT\,(\sum C_{gel} - \sum C_{solution}) \quad \textbf{(Equation 3)}$$

$$\upsilon_a \Pi = \mathrm{kT}\left[-\boldsymbol{Ln(1-\phi) - \phi - \chi\phi^2} + \frac{\phi}{Nb}\left(\frac{1}{2} - \left(\frac{\phi_o}{\phi}\right)^{2/3}\right)\right] \quad \textbf{(Equation 4)}$$

Where υ_a represents molecular volume, Nb the chain length, ϕ_o and ϕ the polymer volume fraction in dry and swollen state respectively.

2.2 Chemistry behind absorption

The chemistry behind the absorption can be studied from the function that carboxylic acid moeities provide within the material. Among other hydrophilic functional groups (*e.g.* aldehydes, ketones, ether and esters) carboxyl group units can form hydrogen bonds with water

through both the carbonyl (C=O) and hydroxyl (-OH) groups. The strong interaction that the carboxyl group can form gives rise to their high boiling temperatures compared with other functional groups [91]. Even in the gas phase, carboxylic acid molecules are always present in a dimeric form [92]. The cooperative chemical potential between the -C=O and –OH creates a resonance and open structure which has a distinct behaviour as compared to similar chemical compounds such as alcohols and esters [93]. In addition, the carbonyl group in carboxylic acids has a more basic nature than in esters. However, the charged state of the carboxyl groups depends on the pH of the system, making them weak polyelectrolytes [3,94,95]. A 3D representation of the group is shown in figure 2.2.

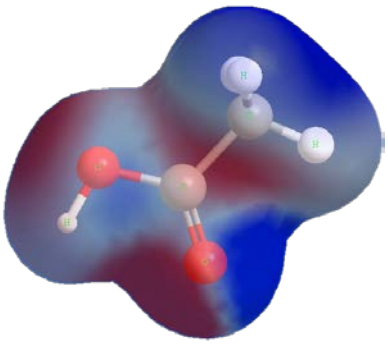

Figure 2.2: Carboxylic acid 3D representation (example of Formic acid). The red color illustrates electronegativity clouds. Drawn in ChemBioDraw ®

Carboxylic acid units can be neutralized under basic conditions, *e.g.* by using sodium hydroxide (NaOH) [92]. The neutralization process is necessary in polyacrylic acid to fully (or partially) dissassociate the ions thus enabling the fixation of more charges onto the polymer backbone. The superabsorbency of the material is strongly influenced by the degree of neutralization of the acrylic acid [3,7]. If the neutralization is not carried out, the polymer does not behave as a polyelectrolyte in solution [86,87]. In addition, the charges produced by carboxyl dissociation promote electroestatic repulsion within the polymer chains which favors the rapid expansion of the network when the material is placed in water. As expected, a decrease in pH or increase in the counter ion content in solution will favour the shrinkage of the chains, *i.e.* less network expansion occurs during swelling at such conditions [96].

3. Structure of modern disposable diapers

A disposable diaper can be divided in three main parts: the top sheet, core or fluff pulp and back sheet (Fig. 3.1, a) [97,98]. The first layer consists of non-woven polypropylene fibres which allow the transport of the body fluids to the fluff pulp [3,98,99]. The function of the top sheet is to allow the flow of the liquid through the sheet while keeping a dry feeling [100]. The core

comprises SAP particles embedded within a fluff of non-woven cellulose fibres. The addition of the SAP is achieved by either layering or blending the dry particles in the fibres; both techniques showing similar performance during the utilization of the product [97,101]. The purpose of the fibres is to permit an even spread and transport of the liquid through the whole pad as well as to act as a fluid reservoir [7,102]. Lastly, the back sheet is a combination of cloth fibres and hydrophobic plastic films, often polyethylene (Fig. 3.1, b). This sheet provides flexibility and micro porosity which provides for breathability in the product. The porosity in the back sheet is achieved during the processing of the material by the inclusion of calcium carbonate particles and the subsequent stretching of the film for producing micro-voids [103].

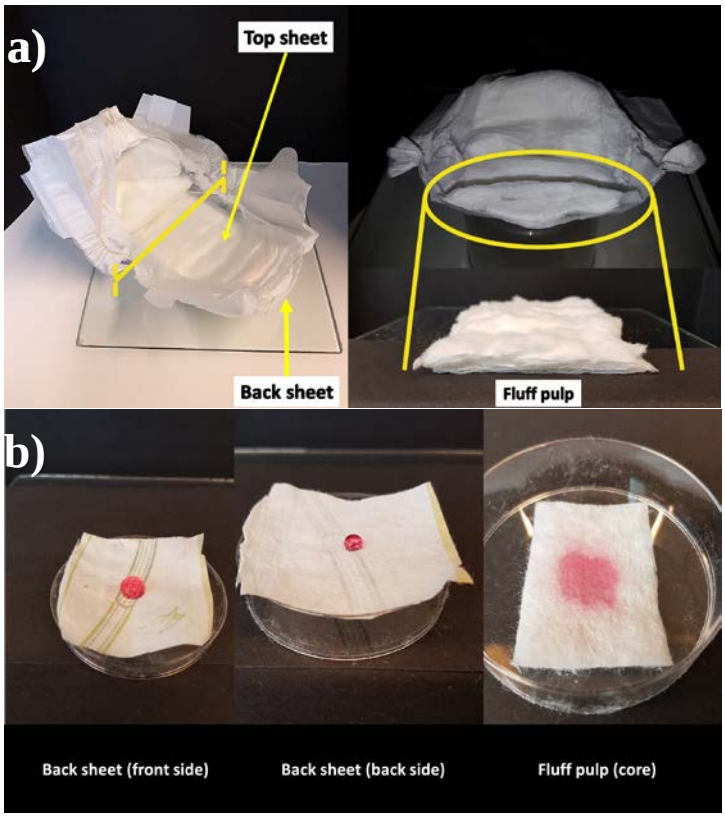

Figure 3.1: Disassembly of a disposable diaper (a) and evaluation of the main parts using dyed water (b). Dyed water was used for illustrating the functionality of the sheets regarding to the liquid transportation.

The disposable diaper manufacturers are aiming towards the fabrication of thinner products by reducing the thickness of the fluff pulp. This trend has given rise to an increase in SAP content in the fluff pulp layer in modern diapers, which accounts for 10 g per item [7,16]. In considering a SAP suitable for diapers the free swelling, absorbency under load (AUL) and residual monomer (RM) in saline solution are required to be 50g/g, 30 g/g and 4-5 ppm, respectively [16,104].

4. Proteins as raw materials

Proteins are known for being one of the main nutrients in the human diet. The physical and chemical tasks that a protein carries on within living organisms are broad, including: transportation, catalysis and structural tissues [105]. Protein composition, molecular weight, protein content, etc., are important parameters that determine the nutrionional quality of a cultivar and can vary within the same crop [106,107]. In addition, the functionality and application of proteins are not only dependent on the parameters mentioned before, but also on the inter and intramolecular interactions that they form [108].

The building blocks of proteins consist of amino acid residues, and differ from each other depending on the functional group "R" attached to the α-carbon (See figure 4.1, a). Approximately 100-500 amino acid building blocks are held together by peptide bonds in a protein (see figure 4.1, b). Each individual polypeptide can interact in different manner with other chains, giving rise to a macromolecule complex [105,107]. There are at least 20 different functional groups that can be linked in the α-carbon, forming a highly heterogeneous chain and structure [109]. The heterogeneity of proteins is one feature that makes these natural molecules unique, compared to other type of biomolecules such as polysaccharides (based on single or few combinations of saccharides) [107,109]. The functionality and intramolecular structure of proteins are dependent on the amino acid composition, leading to different physical and chemical properties for each individual type of proteins [105].

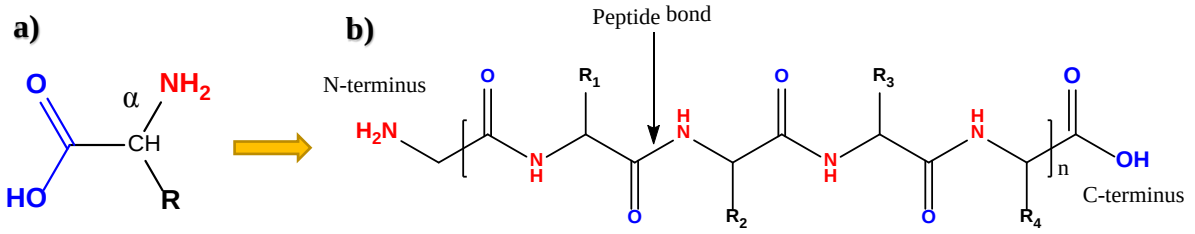

Figure 4.1: Representation of an amino acid monomer (a) and an illustration of the assembly of the amino acids into a polypeptide (b).

Regarding the amino acid chemistry, the double bond of the carbonyl group (C=O) has a resonance structure, whereas the –C-N bond has physical features that resemble a double bond. Therefore, the energy required for changing the conformational state of the proteins is higher than that for other natural macromolecules, *e.g.* polysaccharides [105]. This is expressed in the high rigidity of polypeptide chains and it correlates with the glass transition temperature (Tg) of proteins, typically being above room temperature [110]. Nonetheless, brittleness in protein-based materials can be reduced with the addition of plasticizers (*e.g.* glycerol), increasing their

processability and still keeping good mechanical properties [107,110-112]. Proteins are arranged in a hierarchical manner, forming inter and intramolecular interactions, complexes, hydrophobic regions, etc., which give rise to attractive structural features [105,107]. Protein chemistry and physical properties can be fine tuned through different processes, *i.e.* the use of additives, thermal treatment, and purification [108,113]. Consequently, proteins are a good alternative for producing inexpensive and smart materials with the potential to replace petroleum-based ones [16].

4.1. Interactions in Proteins

The complexity of protein structures can be partially explained by the interactions that may occur within and/or between single and multiple protein chains. Interactions in proteins can lead to the formation of several types of covalent, ionic or hydrogen bonds [108]. The most common covalent intra- or intermolecular link between chains (excluding main chain bonds) ocurring in proteins is the formation of disulfide bridges [105]. The bonds are formed between cysteine moieties (a type of amino acid residue) and are one of the main sources of stabilization of the protein structure in the solid state [107,109].

Some amino acid groups can be readily ionized in aqueous solution giving a charge distribution onto the protein chain. This fact brings about the possibility of forming ionic interactions between the chains which stabilize the 3D structure of the protein [114]. These bonds can be easily disrupted by shifts in pH through neutralization of the ionic link. The process gives rise to an unfolding of the protein, which thereby looses its native macromolecular structure, this process is known as denaturation [105,114-116]. Denaturation can also be achieved by disruption of hydrogen bonds when heat treatments are applied to the protein [107]. Similarly, hydrogen bonds are affected by the presence of compounds with higher tendency to form these H-bridges than the protein itself, *e.g.* urea [117]. Hydrogen bonds in proteins, along with cystine bonds, have the responsibility to keep the folded structure stable [105].

Stabilization of the folded-chains in proteins is also based on weak van der Waals' interactions occurring at the atomic level. Hydrophobic interactions are another type of specific interactions present in polypeptides, especially in those being water soluble [108,114]. The sum of

all the interactions that can take place in proteins leads to the formation of their corresponding macromolecular structures. A summary of such hierarchical structures is given in section 4.2.

4.2. Structures in proteins

As previously described, different amino acid residues are linked together by peptide bonds, forming the polypeptide (protein) chain. The sequence of each specific amino acid within the protein chain is what forms the basic structure of the macromolecule, known as ***Primary structure*** [105,118]. The basic information of the protein is encoded in this structure [119].

Polypeptides show different types of chain folding depending on the intra and inter hydrogen bonds combined with other interactions. The folding of the amino acid chain leads to the formation of α-helices or β-sheets, determining the ***Secondary structure*** of the protein [118,119]. A combination of different secondary structures are formed that minimize the conformational energy of the protein. Hence, a 3D structure is formed as a consequence of the secondary arrangement, known as ***Tertiary structure*** [105,119]. Many of the physical and chemical properties of proteins in their native state come from this structure [120]. Finally, complexes are formed in the protein due to the presence of many different polypeptide chains interacting with each other [118]. These complexes are once more estabilized by non-covalent bonds and give rise to the proteins larger scale macromolecular 3D structure; the ***Quaternary structure*** [119].

4.3. Protein sidestream candidates

The focus of this work is to review the feasibility of plant protein sidestreams from the agricultural and food industries to perform as superabsorbent polymers (SAP). There have not been reports in the literature regarding protein-based superabsorbent materials where the sidestream candidates selected in the current review paper have been used. The amino acid composition of the principal protein candidates chosen can be found in Table 4.1. Although soy protein is not studied within this literature review, it has been the protein on which the major work has been done regarding modified protein for addressing superabsorbent functionality [16].

Table 4.1: Amino acid composition of different proteins. All values are expressed in g amino acid/ 16 g Nitrogen.

Amino Acid	Molecular weight (g/mol)	Wheat Gluten[a] [121]	Potato protein[b] [122,123]	Oat Protein[c] [124]	Rapeseed Protein[d] [125]	Soy Protein[e] [126]	Zein Protein[f] [135]
Alanine	89.09	2.40	4.00	5.00	4.60	4.40	8.30
Arginine	174.2	2.40	4.98	6.90	5.90	8.00	1.80
Aspartic Acid	133.16	2.90	13.12	8.90	7.50	12.0	4.50
Cysteine	121.16	2.10	0.59	1.60	2.50	1.26	0.80
Glutamic Acid	147.13	37.3	17.6	23.9	17.3	17.7	21.4
Glycine	75.07	3.10	3.52	4.90	5.20	4.40	0.70
Histidine	155.16	2.20	1.76	2.20	2.50	2.70	1.10
Isoleucine	121.18	4.00	3.84	3.90	3.50	4.80	6.20
Leucine	131.17	6.80	6.24	7.40	5.70	7.80	19.3
Lysine	146.19	1.20	5.28	4.20	4.60	6.80	NR
Methionine	149.21	1.80	1.12	2.50	1.70	0.76	2.00
Phenylalanine	165.19	4.90	3.36	5.30	3.80	5.20	6.80
Proline	115.13	13.7	3.84	4.70	4.50	5.10	9.00
Serine	105.09	5.20	3.52	4.20	4.70	5.20	5.70
Threonine	119.12	2.50	3.84	3.30	3.90	4.00	2.70
Tryptophan	119.12	1.00	1.65	NR	NR	NR	NR
Tyrosine	181.19	3.80	2.72	3.10	NR	4.00	5.10
Valine	117.15	4.10	5.76	5.30	4.80	4.80	3.10

[a] Based on gluten fraction. [b] Based on FAO data, 1972. [c] Based mean value of 289 *A. Sativa* cultivars. [d] Based on commercial meal. [e] Based on ground defatted soybean. [f] Based on commercial zein Pomes (1971). NR; not reported.

4.3.1. Oat

The high nutritional value of oat (*Avena sativa*) regarding protein and soluble fiber content make it an important cereal seed for human consumption [127,128]. The worldwide oat production for 2014 was accounted to be 23 million of tons [38]. About 12 to 24 wt% of the oat is composed of proteins, which are mainly located in the groat [129]. The major protein group in oats is avelin (globulin-like protein) representing up to 80% of the total protein content. The second largest group belongs to a prolamine-like protein, avenin. The molecular weight of oat proteins is reported to be from 52 to 70 kDa [128,130]. Oat proteins possess a ridgid structure which gives rise to a high glass transition temperature and good heat-resistance properties [128]. Furthermore, in order to obtain unfolding (denaturation) of the protein, temperatures of *ca*. 110 ºC are needed [131]. The most representative application of oat proteins is in films [132,133]. Avenin-based films with high oxygen permeability have been produced. However, the mechanical properties of

such films did not show better performance than other protein-based films reported in the literature [134].

4.3.2. Maize

Maize (*Zea mays*) represents the fourth largest and most important food crop, after wheat and rice. The total production by 2014 was more than 1000 million tons [38]. The biorefining of maize produces mainly starch, oil and ethanol, which generates a protein rich meal as a by-product [135,136]. The total protein content in corn is about 12%, where the largest group is represented by zein [107]. This prolamine accounts for *ca.* 45% of the total protein content in corn. Zein has been shown to be an important protein candidate for different material applications [135]. Primary applications for zein-based materials have been based on textiles [137] and coatings in cosmetic products [138]. Barrier and tensile properties of zein-based materials are attractive and have been intensively studied for the production of biodegradable films [107]. Zein mixed with 40% glycerol have shown to give films with 5 MPa tensile strength and the highest elongation at break among the protein candidates studied [134]. Recent publications have shown opportunities to produce biocompatible zein foams with interesting mechanical properties [139-140]. Also, hybrid zein-based foams having a bimodal nanometric porous structure have been recently produced [141].

4.3.3. Rapeseed

Rapeseed (*Brassica Napus*) is considered as the third largest seed produced for oil extraction due to its high oil content (*ca.* 45 wt%) and agronomic characteristics [125,142]. FAO's data records a worldwide rapeseed production of 74 million tons in 2014. The oil extraction process leaves a meal that contains *ca.* 38 wt% of rapeseed proteins, composed mainly by globulins (25-65%) and albumins [143]. The former represented by cruciferin (300 kDa) and the later by napin (up to 14 kDa) [144]. Rapeseed proteins signicant uses are in animal feeding and fertilizers, whilst food application is rare due to the high phenolic and glucosinolate content [142,144]. The study of rapeseed-based materials has focused on the purification/extraction of the proteins from the residual cake after the oil has been removed [142,144]. Hu *et al.* and Gryglewicz *et al.* have utilized the oil from rapeseed to produce polyols which were further used for the production of rigid polyurethanes [145,146]. A recent study made by Johansson *et al*. used hot-pressed rapeseed sidestream protein concentrate, mixed with glycerol at different compositions. The results showed that a material with a stiffness of *ca.* 925 MPa is possible to produce by pressing the samples at 180 ºC, although the strain at break showed a brittle behavior [147].

4.3.4. Wheat

Wheat (*Triticum aestivum*) is accounted as one of the largest and most important cultivated crops [148]. The worldwide production of wheat was 729 million tons in 2014, according to FAO [38]. The total protein content of wheat varies with the genotype and cultivation conditions, but a typical range goes from 11 up to 20wt%. Wheat proteins can be divided in non-gluten and gluten proteins [149]. The latter group is the major component, and accounts 75-85% of the total protein content in wheat [150]. Gluten proteins are composed of gliadins (30-60 kDa) and glutenins (30-3000 kDa) [148,150]. The gluten proteins are resposible for the mechanical integrity in breadmaking [121,149,150]. Wheat gluten is also acknowledged as showing similar viscoelastic properties as those encountered in synthetic plastics. The important functional and structural features of wheat gluten proteins have made them an interesting feedstock to be used for productions of functional bio-based materials [148,151]. Wheat gluten based materials have shown good mechanical properties and are a suitable alternative for some applications where petroleum-based plastics are used. These materials have been successfully produced by using common plastic processing techniques such as extrusion [122,152], compression moulding[153] and foaming [35]. The protein can be obtained as a concentrate after starch extraction from wheat [154].

4.3.5. Potato

Potato protein concentrate comes as a by-product of the starch extraction from potato tubers [123]. After starch has been removed, the suspension sidestream product (potato fruit water) contains *ca.* 5% of solids [155]. The dry particles have shown to contain at least 35 wt% of potato protein, representing 20-60% of the total protein present in potatoes [155,156]. The major potato protein fraction is patatin (a glycoprotein), followed by protease inhibitors and a complex of proteins [157]. Patatin accounts for approximately 40% of the total potato protein content and its molecular weight is reported to be 40 kDa [157,158]. Newson *et al.* have produced bio-based materials from potato protein concentrate and interesting mechanical properties when mixed with 15, 20 and 25 wt% glycerol were reported, utilizing compression moulding techniques [111].

5. Protein extraction mechanisms

Although the major raw sidestream candidates selected within this review are protein concentrates (see section 4.3), it is important to get familiar with protein extraction methods

and their consecuences for the protein structure. A purer protein fraction, as well as the removal of secondary components coming with the protein concentrate (*e.g.* starch, ash, etc.), have been shown to contribute towards improved mechanical performance of the material [159,160]. The functionalization of proteins for addressing specific behaviors are typically carried out in aqueous suspension. As consequence, the pH solubility profile and the isoelectric point of the protein must be taken into consideration when performing wet chemistry [161,162]. Thus, when different extraction/purification routes are applied to the same protein, denaturation of the protein structure might be one reason explaining differences in properties [151,159-161,163-165]. The extraction procedure can also be used to fine tune properties while fractionating the proteins available in the raw sidestream [113].

5.1. Alkali extraction and Protein Isoelectric Precipitation (PIP)

As described in section 4, proteins are structured from amino acid sequences which comprise their primary structure. Some amino acids have ionizable, charged side-groups and free residues, giving rise to a polymer containing an average electric charge on its surface (either positive or negative), also known as polyelectrolytes [105]. Therefore, the behaviour of proteins in solution are pH dependent since the presence of more or less ionic groups in solution leads to changes in the net charge and thus to different behaviours with changes in pH. Under acidic conditions, an amino acid residue can be protonated increassing the overall charge of the macromolecule to positive. A similar phenomenon is observed under basic conditions but obtaining an average negative charge (see equations 5 and 6, respectively) [105,166]. For charged molecules, there would be a point where the average net charge between all the ionizable groups is zero, known as isoelectric point (IP). Hence, at the IP the electrostatic repulsion is eliminated, favoring the aggregation and precipitation of the proteins [166,167]. The described behaviour allows purification and concentration of proteins by precipitation. Knowing the IP of a specific fraction allows a separation of the fraction from the other proteins in the system [168,169]. Nonetheless, treating the sample at high or low pHs can give rise to denaturation, chemical reactions and structural damage to the macromolecule [31,105,163,165,166,170].

$$^{+}NH_3\text{-}CH_2\text{-}COO^{-} + H_3O^{+} \rightleftharpoons {}^{+}NH_3\text{-}CH_2\text{-}COOH + H_2O \quad \textbf{(Equation 5)}$$

$$^{+}NH_3\text{-}CH_2\text{-}COO^{-} + OH^{-} \rightleftharpoons NH_2\text{-}CH_2\text{-}COO^{-} + H_2O \quad \textbf{(Equation 6)}$$

5.2. Protein Micellar Mass (PMM)

The ionic strength of proteins (treated as polyelectrolytes in solution) is another feature that can be used for its extraction/purification. When ions are introduced into the system by the

addition of salts, the solubility of protein increases since the activity of the polyelectrolyte is affected. The effect is evident for salt-soluble protein such as globulins, where a solvation complex is formed between the salt ions and the protein [105]. Hence, the salt-soluble protein fraction can be extracted from the material, decanted and precipitated by diluting the saline solution below the solubility range of the protein. The dilution process initiates the formation of protein aggregates, also known as micelles [171]. The purification/extraction process is known as protein micellar mass (PMM).

Although it is an expensive method since it uses large amounts of salt, PMM does not produce damage in the protein structure due to the nature of the mechanism itself, [172,309]. Consequently, a fairly pure extraction of the salt-soluble fraction is possible once the maximum solubility in the aqueous solution is known [173,174]. Moreover, the extraction of specific fractions under PMM gives higher and purer yields than alkali extractions [166]. Other functional properties related to the structure of the protein, such as emulsification, can be kept by using PMM instead of PIP [175].

5.3. Other extraction procedures

Depending on the application, more specific extraction procedures might be useful for addressing certain functional properties in proteins. Hernández-Muñoz *et al.* have shown that gliadins, the alcohol-soluble fraction of wheat gluten proteins (See section 4.3.4), are suitable for producing films with an enhanced elongation at break and integrity in water [176]. Wheat gluten proteins purified with ethanol and mixed with natural fibers have shown to be suitable for producing high-performance bio-based composites [177]. Foams based on a gliadin-rich fraction having low compression modulus and low density have been developed by dispersing gluten proteins in a 70% v/v ethanol solution [178]. Also, 45% w/w ethanol solution has been used for extracting the alcohol-soluble fraction, *i.e.* avenin, of the oat proteins [127]. When a purification procedure is applied to protein, a disruption of some interactions between the different protein fractions and/or non-protein components can occur, which leads to a modification of the protein properties [179].

6. The role of natural-based materials as SAP

Petroleum-based SAP displays absorption properties which suit them for many different applications. However, despite these materials fulfilling all the requirements needed to be

considered a superabsorbent product, *i.e.* high rate and maximum water uptake, absorption in pure water of *ca.* 1200 g/g, high retention of the liquid under load (AUL) and tolerable gel strength; there are still some important downsides that synthetic SAP is facing. According to the new era of green chemistry and its 12 principles [180], these oil-based materials lack sustainability and the degradation time in open air systems is long. Thus, during recent years several studies have addressed the problems of synthetic SAP by encouraging the research/production of natural-based super absorbents, *e.g.* polysaccharides and hydrogels [1,181-183].

Some authors have concluded that despite the emerging use of polysaccharides in the super absorbent industry, little strong research has been carried out applying other bio-based alternatives, such as proteins [21,184]. The main focus in all the studies is in the increase of carboxylic acid units, trying to mimic syntethic SAP [3]. The high affinity that both carbonyl and hydroxyl sub-units have for hydrogen bonding with water molecules renders carboxyl groups of extra interest as the solution to bio-based SAP [185].

Natural materials such as homogenous poly(amino acids), *i.e.* biodegradable polypeptides formed by the covalent union of a single amino acid monomer, have been studied as candidates for producing super absorbent properties. Zhao *et al.* used condensation and further polymerization of Aspartic Acid in N,N-dimethylformamide (DMF) as solvent. Subsequent hydrolysis of the polyaspactic acid (PAsp) resulted in a carboxyl pendant group on each monomer unit and water absorbency of 400 g of water / g of dry resin [62,184]. The reaction mechanism is illustrated in figure 6.1.

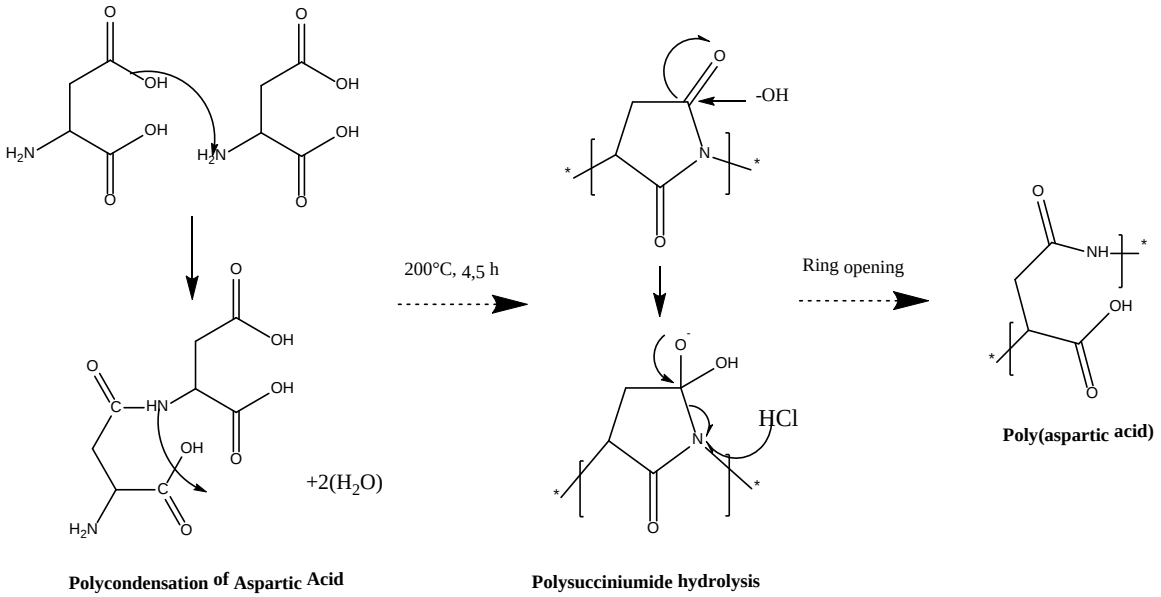

Figure 6.1: Polycondensation reaction and ring opening polymerization of polysuccinimide from condensation of aspartic acid monomer. Inspired by Zhao *et al.* [62,184].

Related work has been carried out using interpenetrating polymer networks (IPN) to form hydrogels with water uptake [186-188]. By the synthesis of semi-interpenetrating networks (semi-IPN) features similar to traditional IPNs have been obtained [62]. In semi-IPNs, a pre-formed polymer network (A) is mixed with a non-crosslinked polymer (B), followed by a subsequent reticulation of "B" [189]. The induced structural features allow for an increase in water retention of the liquid on mechanical stress, which is lacking in IPN systems [186,187]. Further details are listed in sections 6.1.2 and 6.1.4.

Modyfing the reaction mechanisms in order to produce different network architectures, either by favouring IPN or semi-IPN, has generated knowledge that has led to patented bio-based products as an alternative to petroleum-based superabsorbent materials. For instance, Haar *et al.* and Sikes *et al.* have polymerised a random co-poly(amino acid) absorbent material based on thermal polymerization of Succinimide in the presense of α-Aspartate by using Phosphoric Acid media, which give rise to the formation of semi-IPN-like structures [57,190].

The extent of obtaining carboxyl-like structures in natural materials has given rise to an increase in studies where functionalization, crosslinking reactions and grafting steps are considered for tailoring super absorbent properties. For example, the latter concept was investigated by Samaha *et al.*, where acetate groups were graft-polymerized onto starch polymeric chains [191]. Similarly, related works have included the use of silk sericin protein[192] and gelatin[5,90,193] as suporting polymeric backbone for the grafting of synthetic acrylic acid (AA) and acrylamide (AAm), which increases water superabsorbent properties in the material. Furthermore, as previously mentioned, functionalization of polysaccharides and proteins by different reaction mechanisms have been used in many studies to tailor water uptake as well as retention [16,21,64,194-196]. As an illustrative example, figure 6.2 shows a proposed functionalization mechanism using Succinic Anhydride (SA) which interacts and condenses on the hydroxyl groups of cotton cellulose in Lithium Chrloride (LiCl) / N-methyl-2-pyrrolidinone (NMP) solutions. No crosslinks (C-L) were detected and a pure water absorption of 400 g/g was reported [54]. The crosslinking process is a step that is often studied in the development of SAP materials. Several studies concerning different types of crosslink[11,195,197], crosslinking agent[1,16,21,198,199], crosslinking conditions[200], and spacing between junction points (C-L density)[82] have stressed the importance of considering these factors, since they can dramatically affect water absorption.

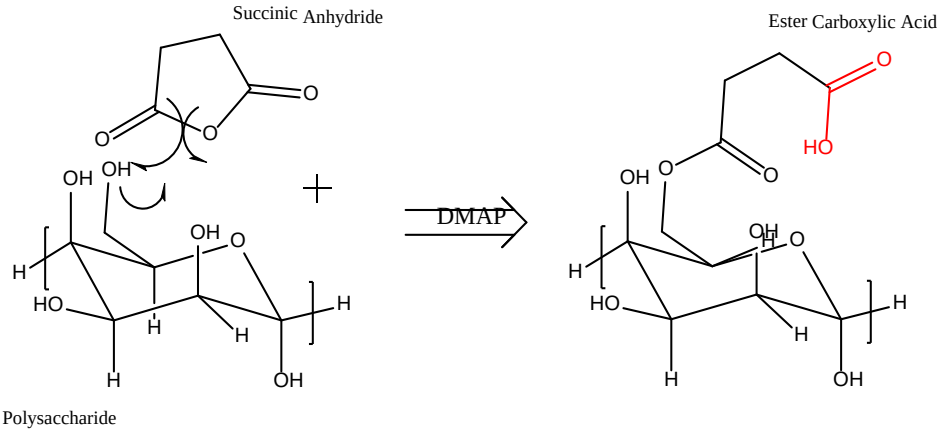

Figure 6.2: Carboxylation reaction of hydroxyl groups contained in cotton cellulose by using Succinic Anhydride. Inspired by Yoshimura *et al.* [196].

In general aspects, the well-known absorbency property of natural material such as polysaccharides has allowed tuning it into different applications. For example, although cellulose is not able to dissolve in water due to its crystallinity and the strong intermolecular hydrogen-bonds it forms, it is possible for water to penetrate inside the structure rather efficiently causing a high degree of swelling [201,202]. In that sense, if the cellulose is prepared from solution and crosslinked with epichlorohydrin the swelling degree can reach *ca.* 1000 g/g after 7 days of immersion in water [27,181,194]. This swelling behaviour has been used to conduct important research in polyelectrolyte absorption onto cellulose fibres in order to tailor surface specificity. Therefore, it is possible to address different affinities on polysaccharide fibres and they thus serve as sensors [96]. The behaviour makes the self-assembly of different Layer-by-layer (LbL) compositions onto cellulose a possible process as well.

New ideas have emerged concerning the use of nanomaterials as a next step in combining superabsorbency properties with remarkable mechanical properties [12,14,203]. Despite the fact the most relevant information available regarding natural-based superabsorbent materials is on polysaccharide materials, table 6.1 summarizes some important works reported to date, where protein-based materials are emphased.

Table 6.1: Relevant works regarding superabsorbent properties of natural materials. A focus is given to protein-based materials. N/R and N/A stands for Not reported and Not Applicable, respectively.

Material	Procedure	Functionalization	Absorption Water , 0.9% NaCl Solution (g/g)	Time for eq. swelling	Reference
Cotton Cellulose	Cellulose dissolved in LiCl/NMP solvent + DMAP and SA	Esterification of the Hydroxyl-cellulose groups	400,100	48 h	[54,196]
CMC/HEC*	Crosslinking of CMC to HEC using DVS	N/A	(pH 7) 110, N/A	24 h	[81,82]
Cellulose/CMC ***	CMC and Cellulose solution crosslinked with Epichlorohydrin	N/A	1000, 200	1 week	[27,194]
Poly(aspartic-ran-succinimide)	Thermal polycondensation reaction of Aspartic Acid to Polysuccinimide and ring opening.	N/A	(pH 8) 269, N/R	6-8 h	[57,190, 196]
Hybrid PVA/Agar/PEG	High energy electron beam (25kGy) crosslinking procedure	N/A	387%***, N/R	24h	[204]
Soy Protein	EDTAD modification and C-L using GA	Acylation	110, 25	25h	[33,205]
Soy Protein/IPN-10%CMC	EDTAD/Soy protein linked with CMC using EGDGE	Acylation and bridging with CMC network	N/R, 12.4****		[206]
Fish Protein	EDTAD modification and C-L using endogenous sulfhydryl groups.	Acylation	526, 12.3	25h	[33,162]
Collagen (gelatin) g-poly(AA-co-NaAA)	AA and NaAA were grafted onto gelatin based protein using APS and MBS as crosslinker	Addition of AA and NaAA onto Gelatin protein	210, 38	90 min	[5,15,90]
Collagen g-poly(AA-co-AAm)	AAm onto collagen using potassium persulfate (KPS) as initiator and methylenebisacrylamide as a crosslinker. Montmorillonite (MMt) introduced as filler	Addition of AA and AAm onto Gelatin protein with Montmorillonite (MMt) 5.6%wt.	952, 70	2 h	[207, 208]
Cottonseed protein	Graft Polymerization of Acrylic Acid and Acrylamide monomers using macroradicals	Carboxylation via graft polymerization	380, 60	24h	[110, 209]
Silk Sericin protein g-(AA-co-AAm)	Graft polymerization of AA and AAm onto silk sericin protein	Carboxylation via graft polymerization	2150, 98	20 min	[192]

**CMC/HEC 3:1 weight ratio as reference.*
***GEL91 (27g CMC + 3g Cellulose and 3mL Epicholorohydrin) as reference*
****Based on gel dry weight et al.*
***** Absorbency under load, 0.3 psi, EDANA ERT 442 – Gravimetric Determination of Absorption under Pressure or Absorbency Under Load [19]*
† Based on 5.6 wt% of nanoclay

6.1. Protein as an emerging SAP candidate: structure/interaction dependent properties

As discussed in the previous section, the knowledge of natural-based superabsorbent materials is strongly biased towards polysaccharides rather than protein-based systems.

Moreover, the studies that discuss protein-related systems have focused on composite systems in which proteins are the minor component present in the material. In addition, several studies/review papers agree that research on fully poly(amino acid) based systems has fluctuated and has not been continuous [5,16,21].

For proteins it is well known that their properties are highly influenced by the amino acid distribution and content, molecular weight, macrostructural stability and the origin of the protein isolate [108,148]. The different amino acids that can make up the primary structure of a protein, together with the very specific interactions that are possible within the protein structure, generate significant heterogeneity in the system[105] (more specific details regarding proteins can be found in Chapter 4). In addition, even using the same type of proteins might result in considerable changes in properties regarding the process history to which the protein was subjected, *e.g.* thermal treatment can lead to denaturation of the protein [210]. Overall, these are possible explanations for why proteins have been less attractive in terms of using them as raw material for superabsorbent polymers.

When it comes to modifying the properties of protein-based systems towards increased water uptake, several different treatments can be applied. Multiple studies have dealt with the functionalization of proteins as a processing route in order to achieve a hydrophilicity that increases the absorbent properties compared to untreated samples [17-19,31,33,161,162,205]. Additionally, hydrogels with high water absorption capacity have been produced either by coupling synthetic PAAc to poly(amino acid) systems, *i.e.* forming semi-IPN networks[206,211], or by grafting/blending polysaccharides onto/with proteins [18,212-214]. Crosslinking type and density[1], and structure of the material have to be taken into consideration when producing natural SAP materials[16,21], the former has been related to the ability to absorb while retaining mechanical stability of the gel and the latter with an increase in the surface area.

6.1.1 Functionalization and modification of proteins

One of the most common and successful methods for tailoring the properties of natural materials towards gaining superabsorbent-like behaviour (*i.e.* high absorption within short periods of time, gel strength, and good spreading properties) is chemical functionalization. Covalent attachment of functional groups onto cellulose structures has been reported by many researchers (Table 6.1). For the natural materials that have been used as raw materials for SAP applications, the absorption mechanism for polysaccharides such as cellulose, regardless of

whether it refers to polyelectrolyte absorption onto substrates, charged networks (Layer-by-Layer deposition) or water uptake under different conditions, is well understood [96,215].

To some extent, acylation is a preferred mechanism for functionalizing surfaces by using available hydroxyl groups. Acylation is a covalent inclusion of an acyl group (-COR) that forms ester links between the acyl reagent and the hydroxyl moiety of the material [216]. The general mechanism that drives the reaction is illustrated in figure 6.2. The chemical modification by the use of acylation is widely implemented when the –R element of the acyl consists in a hydroxyl group, which leads to the formation of carboxylic-like pendant groups.

Several reactants can be used to produce chemical reactions that give rise to the formation and inclusion of acyl groups. Yu *et al*. and Hokkanen *et al.* respectively analysed the use of Succinic Anhydride (SA) as acylation agent for cellulose nanocrystals (CNC) and mercerized nanocellulose (MNC) [217,218]. The absorption capacity of the acetylated CNC was stretched by using solutions containing heavy metal-divalent ions (*e.g.* Pb^{+2} and Cd^{+2}), resulting in 367.6 g/g of free absorption [217]. Furthermore, metal-ion retention of MNC structure was evaluated by centrifugation of the samples to pull the water out. The depletion in the metal concentration after the centrifugation process showed that it is possible to clean/remove the water from metal ions [218]. Corroboration of the binding of the acyl-donating group on the material structure can be achieved by using Fourier transformed-Infrared Spectroscopy (FT-IR) and Nuclear Magnetic Resonance (NMR), whereas the extent of carboxylation is calculated using a titration method with phenolphthalein by means of Equation 7 [217]. Furthermore, for the evaluation of the general properties of the functionalized material, Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) have been used as well [183,219]

$$[COOH] = {(V_{Base} * [Base] - V_{acid} * [Acid])}/{m} \quad \textbf{(Equation 7)}$$

Where V is volume, [ ] molar concentration and m denotes mass of the sample

Although Succinic Acid serves as an easy and successful technique for acylation of natural fibres, to our knowledge it has not been tested on protein materials for addressing super absorbent properties [303]. Experiments have been carried out by Bräuer *et al.*, using gluten, soy, and zein protein as raw materials and functionalizing these materials by the use of palmitic acid chloride and succinic anhydride. The material was evaluated from the mechanical properties perspective only, obtaining biobased plastics that were possible to extrude with the addition of 10% of glycerol [220]. However, the most successful functionalization mechanism *via* acylation established so far for addressing superabsorbent properties in protein-based materials has been

reported by Damodaran *et al*. For the acylation process, Soy Protein and Fish Protein from food waste have been used as raw materials. The principle consists of the chemical reaction of lysyl-like residues (-NH_2) with Ethylenediaminetetraacetic Dianhydride (EDTAD), forming stable amide bonds, of the same nature to those obtained in polyamides (nylons) [221]. The opening of one of the highly reactive furodianone group present in the EDTAD as a result of the reaction with the lysyl moiety, together with the interaction with the alkali media (excess of OH ions), give rise to the inclusion of three carboxyl groups per mol of group reacted [31,205,206]. The reaction is illustrated in figure 6.3.

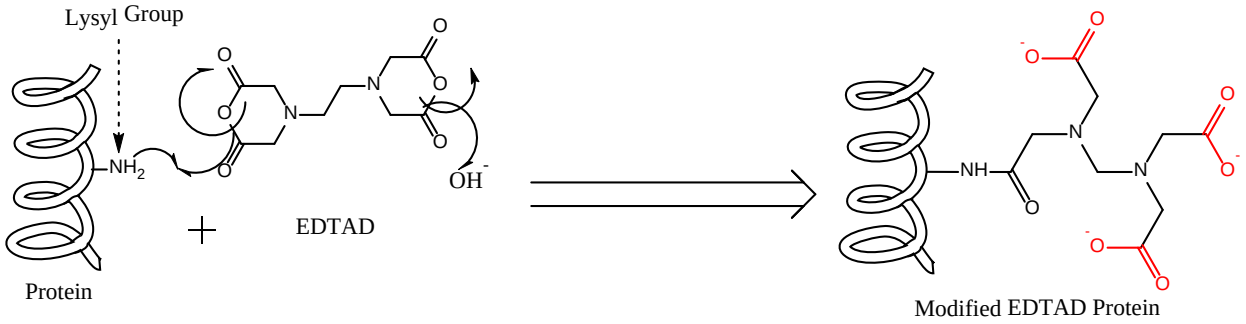

Figure 6.3: Reaction mechanism of functionalization of lysyl groups by using EDTAD. Adapted from Damodaran *et al.* [31].

Using this functionalization method, the total net charge induced on the protein backbone upon reaction with the EDTAD was enough to cause an important protein unfolding due to a high electrostatic repulsion between the free carboxyl groups. Furthermore, the treated Soy Protein system was able to retain *ca.* 300 g/g of pure water even after centrifugation of the swollen gel at 214 RCF [33,161]. The polyanionic feature given to the protein upon functionalization (polyelectrolyte-like behavior) increases the binding bridges with water, giving rise to the highly absorbent character observed through the method previously described [222]. Hwang *et al.* also reported a similar successful functionalization procedure by using fish protein food waste. The results showed the possibility of obtaining a water retention of *ca.* 540 g/g of water, even after centrifugation at 214 RCF [162]. Using the Trinitrobenzenesulfonic Acid (TNBS) method for calculating the relative amount of carboxyl groups formed and lysyl residues available upon the functionalization reaction, the results showed an increase from 149 to 295 mole of COO-/10^5 mole of protein and a depletion of lysyl moieties from 41 to 4 mole/10^5 mole of protein after chemical treatment [161]. This outstanding functionalization route was enough to encourage a number of patents where the successful modification of fish and soy protein by acylation through EDTAD has been reported [31,47,57,205,206].

The advantages of using EDTAD as a functionalization agent can be listed in terms of the low toxicity of the salts produced after the acylation reaction and the relatively fast reaction given by this method [21]. However, parameters such as protein concentration and amount of EDTAD added should be taken into account due to the possible impairing of the high water absorbency due to the excessive formation of crosslinks between the carboxyl groups formed and unreacted lysyl moieties in the protein [33,162,161]. Optimal conditions for such a procedure have been found to be located at *ca.* 1% w/v protein concentration in solution, 50% m/m of EDTAD/Protein and 3 h reaction [31,206].

However, the post-functionalization concentration and drying processes should also be taken into consideration as an important industrial factor in the production of bio-based SAPs. Early results obtained for EDTAD-protein modification were based on acid precipitation at pH *ca.* 4.5; lyophilisation and dialysis steps in order to concentrate, and remove the water and the salt from the systems, respectively [33,161,162]. As mentioned in Section 2, presence of counter-ions (*i.e.* salts) is a critical factor influencing the absorbency characteristics of SAP. Nonetheless, both processes are not attractive in a scale-up, from lab to industry.

Functionalized proteins have been post-treated with ethanol in an attempt to speed up and simplify drying and cleaning process, without affecting the absorbency properties. Rathna *et al.* have precipitated functionalized soy protein, reducing the pH of the reaction suspension (pH 12) to pH 4.5, and washed the pellet vigorously with ethanol. After drying of the ethanol-washed particles, the absorbency in MilliQ® water was *ca.* 425 g/g, whereas for non-ethanol systems the absorbency resulted in 200 g/g. The increase in absorbency relied on an increase in the denaturation of the soy protein after the ethanol treatment. The results were confirmed by the increase in the concentration of α-helix structure in the protein compared with control samples [17].

Egg white albumins have also been functionalized by means of alkali EDTAD acylation of lysyl residues following a similar experimental setup as Rathna *et al*. The experimental results showed that the addition of acetone as a post-treatment of the modified-albumin egg white, increases the absorbency of pure water from 12 to 16 g/g [223]. The alkali acylation using EDTAD has also been compared with denaturation of the protein in acid media. Guimaraes *et al.* proved that by solubilizing fish protein in acid media the water uptake increased to 103 g/g from 79.42 g/g in the case of alkali solubilisation. The cause of the increase in water uptake was attributed to a higher modification of lysyl residues with the EDTAD [224].

The feaures that these EDTAD-functionalized proteins display have been evaluated from many different perspectives. For instance, the absoption and extraction of heavy metals from contaminated water, *e.g.* zinc, mercury and lead, have been studied. In that sense, EDTAD-modified soy protein has shown a retention of 0.65; 0.95; and 0.70 mmol of Zn, Hg and Pb/g of dry gel, respectively [18,225]. The pH influence on the absorption was also tested by Hwang *et al.*, showing that the EDTAD-modified protein systems increase the water absorption even at pH 10, with a liquid uptake of 140 g/g [33,161].

Another mechanism widely used for giving superabsorbent-like characteristics to protein-based materials is the conjugation/grafting of secondary agents onto the protein backbone structure. Paujarvadini *et al.* have worked with gelatin and radical polymerization-grafting of hydrophilic components which increases the water uptake of the system. Acrylic acid (AA) monomer units have been widely used as a grafting agent due to the contribution of carboxylic groups to the system. The radical polymerization of 70% AA onto the gelatin backbone, combined with crosslinking of the gel with N,N'-methylene bisacrylamide (MBA) has led to a water uptake of 360 g/g and 80 g/g in pure water and 0.9% saline solution, respectively (Pourjavadi *et al.*, 2007b; Pourjavadi *et al.*, 2007c). In addition, different types of hydrophilic monomers have been grafted onto the gelatin backbone, such as Acrylamide (AAm) or mixtures of AAm and AA. For instance, a grafting reaction of 4:1 AA/AAm (molar ratio) onto gelatin using MBA as crosslinking agent obtained 210 g/g of water take [5,15]. The grafting reaction was also tried with cottonseed protein isolate by Zhang *et al*. On these studies, 1:10 (mass ratio) of cottonseed to Acrylic Acid monomer and MBA showed an absorbency of 900 g/g. In addition, the water retention was *ca.* 60% after 10 hours treatment of the swollen gel at 80 ºC [110,209,226]. A general scheme of the grafting of AA monomer onto gelatin is exposed in figure 6.4 (Note that addition of AAm or AA/AAm follows the same mechanism).

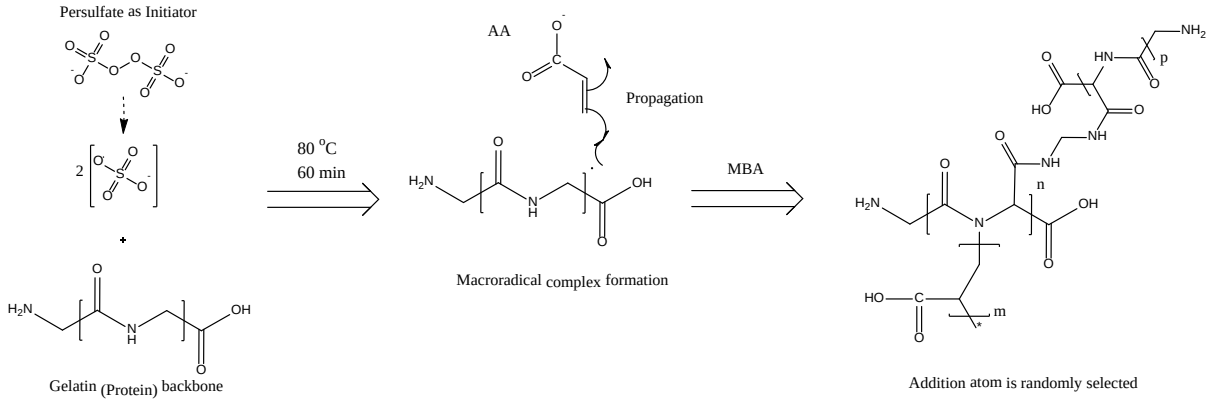

Figure 6.4: Scheme showing grafting polymerization of a protein backbone (*e.g.* gelatin). Inspired by Pourjavadi *et al.* and Zhang *et al.* [5,110]

Hu *et al*. have achieved a water absorption of 2150 g/g after grafting AA and AAm onto silk sericin protein (mass ratio of 6:4:1 related to protein content). In his work, 8 mmol/L of Potassium Persulfate (KPS) as initiator and 2.5 mmol/L of MBA as crosslink agent, 6h at *ca.* 60 ºC were implemented [192]. However, new trends are including the use of nanomaterial in the reaction recipe, towards the improvement of the already existing reaction mechanism. Reactions using grafting of monomer AAm and AA onto collagen (KPS and MBA were used as well) were performed by Marandi *et al.* In this study, 5.6% wt. of montmorillonite was selected as additive. The results showed a high water retention (*ca.* 950 g/g) with important enhancement in mechanical properties of the gel [207]. The addition of montmorillonite provides ionization to the system, increasing the internal osmotic pressure, hence contributing to the water absorbency of the nanocomposite material.

Finally, use of polyethylene glycol (PEG) and related compounds under the method called "PEGylation" have been used in medical applications for adding targeting properties onto polypeptide-like structures [227]. Although adding carboxylic groups within the chain throughout PEGylation is possible, water absorbency was not studied in their literature review.

6.1.2 Crosslinking of Proteins

One critical factor to consider in order to produce superabsorbent networks are crosslinks. The concept of forming a 3D network out of free-polymer chains involves the formation of crosslinks, no matter whether referring to thermosets, hydrogels, elastomers, etc[56]. The addition of covalent bridges between hydrophilic polymer chains reduces the maximum water absorbency of the material, due to restricting the chains from expanding and interacting with more water molecules [195]. Although it reduces swelling and water absorption, crosslinking is needed for avoiding dissolution of the polymer [228]. Furthermore, crosslinks are of extreme importance when mechanical strength or geometry stability of the gel once in swollen phase is needed [62,184]. Figure 6.5 illustrates the network expansion of a hydrophilic crosslinked polymer upon interaction with water.

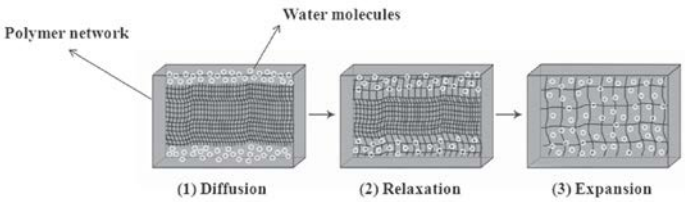

Figure 6.5: Illustration of diffusion of water molecules into a hydrophilic crosslinked polymer. Reproduced under permission from Entezan *et al.* [204].

One of the most common protein crosslinking agents found in the literature are aldehyde compounds. For instance, the simplest aldehyde form (*i.e.* formaldehyde) can be used in

proteins for crosslinking two chains through the condensation of the compound with any available hydrogen in the protein and a further release of water molecules [229,230]. Glutaraldehyde (GA) has been widely used as a protein-based material cross-linker due to the fast crosslinking reactions that it conveys, regardless of whether the material is in solution or solid state [36,176,231-233].

Superabsorbent materials obtained from fish protein waste and soy protein have been crosslinked with GA [31,33,161,205,206]. As shown in figure 6.6, glutaraldehyde possesses double functionality which increases its reactivity compared to formaldehyde. Unfortunately, GAs high reactivity also attributes to its main downsides: first of all, it is not specific to one single protein residue (*e.g.* it also reacts with –OH) nor other elements present in the system creating non-homogeneous crosslinks [234], and secondly it can self-polymerize giving rise to a chain size distribution and thus differing distances among the joints formed [235-239]. Other bifunctional compounds such as diamines have been used for crosslinking wheat gluten hot-pressed composites. Wretfors *et al.* have crosslinked wheat gluten protein with Jeffamine® (a diamine), showing that an increase in the network polymerization is possible without compromising the mechanical properties of the material [240].

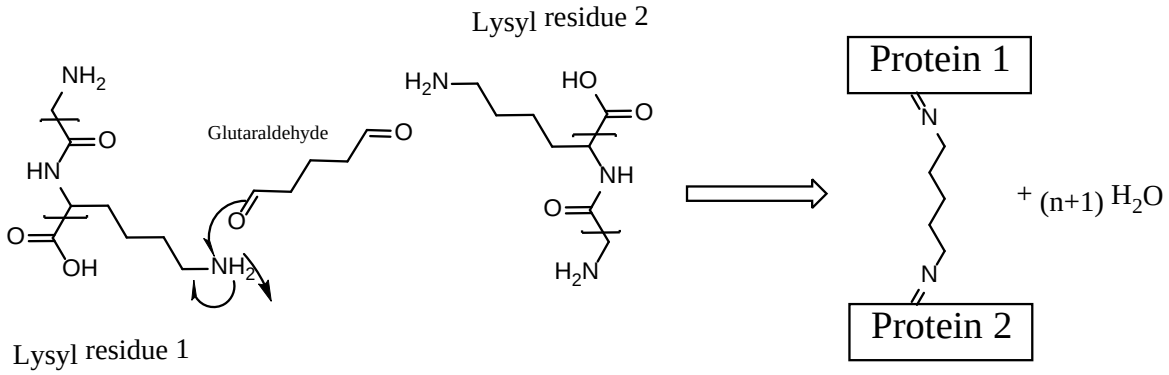

Figure 6.5: Crosslinking of protein throughout Lysyl group using GA. Inspired by Asma *et al.* [234]

Therefore, some authors have studied other alternatives for enhancing the crosslinking properties of the gel network while preserving superabsorbent properties. Zhang *et al.* used N,N-methylene bisacrylamide (MBA) as a crosslinking agent. The authors have shown that the molar amount of C-L agent is critical and very sensitive for the water uptake, obtaining a reduction of 30% in uptake when increasing MBA to AA monomer molar ratio from 0.0625 to 0.075 [110,209,226]. Similarly, superabsorbents obtained from grafting AA onto gelatine have been crosslinked with MBA, showing a maximum swelling of 350 g/g when 0.01 M of the C-L agent was used, whilst the swelling decreases exponentially with increasing MBA content [5,15,89,90,193]. Other divinyl-like species have been used to crosslink hydrogels, *e.g.* ethylene

glycol dimethacrylate (EDGMA), producing similar results as those obtained from MBA recipes [4,241]. Interestingly, Hua *et al.* showed that an excess of AA monomer in the production of synthetic SAP can produce self-crosslinks, bringing about the possibility of avoiding extra components for obtaining C-Ls [11,310]. Self-polymerization behaviour has also been observed in naturally-based proteins such as milk (*i.e.* casein). Schorsch *et al*. have presented theories in which a combination of hydrophobic forces, disulphide bonds and hydrogen bonds could be an important driving factor for gelatination of casein, when changes in temperature are introduced. This allowed the "selection" of the type of intermolecular force acting by modifying the temperature, giving rise to different behaviour at a gel phase [198].

As an alternative to MBA as a cross linker, Esposito and Sanino *et al.* used Divinylsulphone (DVS) in superabsorbent cellulose systems [81,82,242]. Once more, the results showed that the increase in C-L density conveys a decrease in maximum water uptake due to an increase in the elastic force which counteracts the expansion of the network (Fig. 6.5). Nonetheless, the most remarkable conclusion regarding C-L density was drawn by Sanino *et al.;* when a molecular spacer was added at both ends of the DVS molecule (polyethyleneglycol (PEG)), the water sorption capacity increased for similar C-L densities as to reference samples [16,81,82]. Unfortunately, DVS traces both in the SAP and in side stream water are regarded as an environmental and health hazard due to the toxicity of DVS. However, Marci *et al.* have been able to stablish a method where water was irradiated with polycrystalline TiO_2 resulting in a cleaning of all the unreacted DVS [243].

Recently, another candidate Epichlorohydrin (ECH), has been described as suitable for crosslinking natural-based hydrogels. Chang *et al*. reached a free water swelling of 100 g/g based on CMC/Cellulose composites by using ECH as binding agent [27,181,194,195]. The results showed that the system might be a positive alternative to MBA and DVS thanks to an improvement in cellulose hydrogel solubility in aqueous and organic solutions [194].

The absorption under load (AUL) for modified fish and soy-based proteins is still too low compared to synthetic SAP. This property is tightly related with the surface crosslink of the material [3,39]. Hence, bifunctional agents have been studied by Damodaran *et al.*, aiming for the addition of less toxic C-L species as well as the increase in the surface crosslinking of their modified fish and soy proteins. Ethylene glycol diglycidyl ether (EGDGE) was used at 1% concentration in a soy protein/CMC hybrid hydrogel modified with EDTAD [206]. This allowed the authors to increase the AUL from 6 to 9.8 g/g in 0.9 wt% NaCl solution. However, Hwang *et al.* showed that EDTAD-modified hydrogels have the ability to form intramolecular

crosslinks by native disulphide bonds and endogenous sulfhydryl groups giving rise to insoluble and less toxic hydrogel networks [162].

This literature review brought the opportunity to explore an old and health-friendly mechanism for crosslinking proteins, such as enzymatic catalysis in food grade applications [244]. One of the main protagonists has been Transglutaminase, a divalent Ca^{+2} dependent enzyme. By using Transglutaminase it is possible to catalyze intramolecular C-L through the formation of covalent bonds known as γ-glutamyl-ε-lysine [199]. Despite the mentioned possibility of some protein systems (*e.g.* casein) to self-crosslink by means of weak-interaction complexes forming at elevated temperature. The induction of C-L with transglutaminase gives rise to the formation of covalent junctions that are stronger than previously reported works [198]. Other catalytic reactions used might include lysyl oxide, *i.e.* the family of amine oxidases, giving rise to lysyl-aldehyde derivates in gelatine and collagen protein, which act as a crosslinker precursor [244,245-248].

Recent studies have revealed the applicability of using nanofillers in highly absorbent hydrogels, *e.g.* graphene oxide (GO)[203] and clay nanosheets [14]. According to this research track, next to inducing a tremendous mechanical strength to the gel, the nanofiller might also function as a crosslink bridge, having a high efficiency due to the great surface area that these materials potentially provide.

6.1.3. Porous and non-porous systems

Another important parameter to consider when talking about SAP materials is the morphology. Although, it seems the least processing parameter to take into account, the swelling properties, swelling rate and diffusion efficiency can be fine-tuned when the morphological features of the material are altered, even if they have the same matrix [241]. Usually, absorption involves the understanding of diffusion/transport of a liquid phase within a hydrophilic matrix (not discriminant characteristic). However, the presence of a porous media facilitates the diffusion of the liquid molecules in the matrix and, therefore, has the ability to modify the overall absorbent properties [7]. These morphological factors include everything from the study of porous and non-porous matrices to the final mesh size of the samples (*e.g.* particle size of the SAP product) [90,193]. In the following subchapters, a short literature description will address the morphological characterization of the SAP matrix, regarding its relationship with the absorbency properties and new trends on this topic.

6.1.3.1 Foam structures

Foam structures are another route for absorbent materials, they are generally attractive due to the easy and mild processing, important Laplace Capillary forces involved and a broad material selection available [108]. Furthermore, the water uptake of foams as a consequence of either hygroscopic properties of some systems and hydrophilicity (if any), can be maximized by the high surface area provided by the high porosity [249]. Foams are one of the oldest absorbent structures studied, especially since they have many potential uses in food and daily-life applications. Proteins are attractive as a foaming raw material due to its stability when foamed, a consequence of its viscoelasticity[250], amphiphilic nature, and biocompatibility [108,251,252]. In addition, the molecular flexibility of protein chains stabilizes the foaming of the matrix, although cohesiveness and reasonable intermolecular interaction properties must be taken into account when dealing with polypeptide-based materials and/or other biopolymers for producing bio-based foams [253-258].

Aulin *et al.* worked with the selective absorbance feature of nanocellulose aerogels, treated with vapor deposition of silanes onto the porous structure [48,201,202]. This allowed the authors to tune the affinity of the cellulose porous structure towards specific substances. As an example, perfluorodecyltrichlorosilane (PFOTS) was used as a coating agent of the cellulose, resulting in hydrophobic behaviour for a selective and efficient oil absorption from water suspensions [202]. Furthermore, stable protein-based foams from wheat gluten (WG) coated with tetraethylorthosilane (TEOS) have been achieved by Wu *et al.* through a freeze-drying process of WG suspensions [35,36,231]. The generally hydrophobic WG, showed a fast water absorption when the foam was placed in pure water [36]. Obviously, the high loss of water upon mechanical compression of the foams described previously, makes the material an unsuitable option for superabsorbent applications without applying a further modification [255-258].

6.1.3.2 Hydrogels

The first important differentiation concerning absorbent materials is the setting of the borders between a hydrogel and a SAP. Even though both might have a water absorbency of several hundred g/g, the high mechanical stability and relatively low absorbency rate of the hydrogels is what determines the difference compared to SAP [7]. Hydrogels can be defined as hydrophilic polymeric chains which have been joined by a number of crosslinks, resulting in the formation of a stable 3D network. In that sense, the crosslink density is what determines the behaviour of the material. Evidently, if mechanical stability is key for the final application of such materials,

more than the minimum crosslinks necessary for avoidance of polymer dissolving must be assessed [7,10], the latter being a fundamental parameter for SAP materials [3].

In the literature, an important focus has been put on hydrogels based on natural resources. Collagen has been especially selected as the raw material in many papers. Pourjavadi *et al.* worked with hydrolyzed collagen protein as a backbone material, proceeding with grafting of AA and 2-acrylamido-2-methylpropanesulfonic (AMPS) monomers [5,58,90,193]. Compared to similar work, their results showed a relatively fast absorption rate for this hydrogel systems and a maximum swelling in pure water of 352 g/g [21,90,184]. However, by the use of silk-sericin instead of collagen as a protein backbone and following a similar grafting polymerization recipe, a superabsorbent hydrogel can be obtained with water absorbency of 2150 g/g [192]. Yet, the crosslink agent concentration (MBA) used by Hu was at least 1 order of magnitude lower than Pourjavadi *et al*., considerably too low for hydrogels.

Another often used natural material for producing biocompatible hydrogels is cellulose, with carboxymethylcellulose (CMC) as a favorite candidate. By using DVS as a crosslinking agent, a mechanically stable hydrogel can be produced with a water uptake of *ca.* 110 g/g in neutral water. This allowed the authors to produce systems suitable for biomedical applications such as water elimination and tissue cell growth [81,82.259]. CMC fibers have also proven to be a suitable material for specific polyelectrolyte absorption, giving rise to an attractive naturally-sourced material for layer-by-layer deposition systems [294]. The approach pursued very specific surface behavior having the ability to tune the same base material for many different purposes [96,215]. Hydrogel composites of carboxymethyl-chitosan and gelatin have appeared in the literature as well [295,302]. Chen *et al.* showed a system with 60 g/g of absorption with a particular ampholytic character (*i.e.* polymer containing both positive and negative charged groups [260]), making the biocompatible material a suitable candidate for selective drug delivery systems [203,234,296-298].

The morphological structure of the hydrogel is also considered and studied intensively in several papers. Although it seems less important compared to, for instance, the chemical nature of the material, many different properties can be adapted by modifying porosity of the hydrogel, type of porosity and particle size of the dry gel [261-264]. Large open-cell pores of 100 μm and 80% porosity can be introduced into the hydrogels to obtain what is considered in the literature as super porous hydrogel structures (SPH) [263,265]. Such super porous structures have been created through physical techniques such as lyophilization [265,266], emulsion polymerization[267] and chemical dissolution of sodium bicarbonate in acidic media[80,261,263,268,269], with the latter

as the most preferable technique. Additionally, the combination of an important capillary effect brought about by the high pore size and open-cell structure, together with the hydrophilic nature of the matrix, give rise the possibility to tune a material which can absorb faster than a typical SAP (reported swelling time of *ca.* 2-8 min [261]), but with a low water retention [241,263,264].

The possibility of introducing different pore sizes within the polymer network has allowed the development of applications in the biomedical field which are unusual for bio-based superabsorbent foams [266], such as blood and plasma retention [270], pH sensitive controlled drug release[5,15,212,228,269] and tissue engineering [271,272]. Nevertheless, there are different processing routes for obtaining networks that can function as superabsorbent hydrogels [304-306]. In that sense, IPNs have been shown to be able to form stable networks with similar water uptake as crosslinked hydrophilic materials. IPNs permit the formation of: i) physical entanglements as in rubber materials, ii) crystals as in semi-crystalline plastic matrixes, iii) ionic bonds and/or iv) intermolecular interaction through weak forces [62,228]. Some authors have prepared hydrogels having an IPN structure in order to study the influence of this type of 3D structure in the water absorbing capacity. Chatterji *et al.* used gelatine and CMC composites obtaining a free swelling of 11.5 g/g in 0.9% saline solution with an elongation at break of *ca.* 100% [273,274]. One of the main focuses when studying hydrogels is to mimic important tissue mechanical properties, such as for cartilage and joints [62,110,184,209,273].

The focus on mechanical stability of the gels in the equilibrium swelling state has led researchers to develop interesting results regarding IPN networks [275,276]. The main down-side of using interpenetrating networks is that each individual polymer used, preserves their own properties when mixed and crosslinked simultaneously [62,189,277,278]. In order to promote a synergic effect in the mixing of 2 different macromolecules, semi-interpenetrating networks (Semi-IPNs) are used. For instance, one can promote the polymerization of acrylic acid (AA) monomers into a polyaspartic acid (PAsp) matrix forming a semi-IPN, which gives rise to an increase in the entanglement density and therefore improvements of mechanical responses at the macroscopic level while retaining high water uptake [62,189,279,280].

Recent work has explored the utilization of nano-clay, aiming for a combination between high mechanical properties together with super swelling properties [262,281-286]. Bagheri *et al.* enhanced the water uptake of collagen-graft-AA hydrogels by introducing 0.3%wt. of Sodium-Montmorillonite from 450 g/g to 950 g/g [207]. Increasing the clay content beyond the percolation threshold gives rise to a decrease in water swelling due to a possible increase in the crosslink density from the particle-particle interactions imposed by the clay [207,278,283]. The use of nano-fillers in

hydrogel systems has resulted in mechanical strenght of *ca.* 4 MPa[14] as well as self-healing materials [203,287,288].

7. Challenges, perspectives and approaches

As described in previous chapters, protein-based absorbent materials have been created either by grafting synthetic hydrophilic monomers or by chemical treatment of the polypeptide. Still, the bio-based materials reviewed in this paper do not fulfill all the requirements needed for competing with petroleum-based SAP. Additionally, the use of petroleum-based monomers in the recipe for treating biopolymers is not a sustainable solution for the production of a "green" SAP. The main challenge is to equate the swelling properties of protein-based absorbent materials to those of petroleum-SAP and to establish a protein matrix candidate without the addition of petroleum-based material such as AA.

A concern is brought about when agricultural resources are used as feedstocks for the production of bio-based materials, *e.g.* wheat, oat, soy bean, etc. These raw materials are mostly used as human and animal food. Therefore, the use of these as protein feedstock for fabricating bio-based SAP have to compete with the food industry [270,289-292]. As a conclusion, it could be thought that proteins are not suitable as raw material feedstocks for the industry. Nonetheless, the protein concentrates that have been suggested in this review paper as candidates for the production of protein-based SAP are sidestreams from different industrial processes. Consequently, there is an enormous potential for using these protein concentrates since they can provide a solution for the non-sustainable synthetic SAP and contribute towards a higher monetary value to industrial sidestreams [293].

In terms of manufacturing processes, two main considerations have to be taken into account when natural based materials are considered as a replacement for synthetics: the effective adaptation of the already acquired equipment and technology for the development of a novel industry, and the storage of a product that contains proteins [26]. Regarding the first issue, protein-based materials have been efficiently produced by common polymer processing techniques such as i) extrusion [152], ii) injection moulding [299], iii) compression moulding[40,147], iii) solution casting [212,300,301], and iv) grinding from big batches (similar to petroleum-based SAP technologies) [31,205,206,308]. Thus, it is possible to think of feasible processing routes for protein-based SAP. Lastly, the long-term storage of these protein-based materials could be considered problematic. For instance, microbial growth could be a threat for these materials in high humidity environments. However, Wu *et al.* and Ture *et al.* have shown that wheat gluten

biomaterials, containing non-toxic antimicrobial agents (*e.g.* Lanasol), can address this problem efficiently [36].

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