Studies in Green Hydrolysis of Waste Wool

By

Parag S.Bhavsar

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Supervisor(s):

Prof. A. Muresan., Supervisor
Prof. S. Maier., Supervisor
Prof. C. Tonin., Supervisor
Prof. G. Rovero., Supervisor
Prof. G. Chen., Supervisor
Prof. G. Jinping., Supervisor

Doctoral Examination Committee:

Prof.PhD.Eng.Antonela Curteza, "Gheorghe Asachi" Technical University of Iasi.	President
Prof.PhD. Eng Augustin Muresan, "Gheorghe Asachi" Technical university of Iasi.	Doctoral Supervisor
Assoc.Prof. PhD. Eng Ada Ferri, Politecnico di Torino, Italy.	Doctoral Supervisor
Prof.PhD. Eng Yan Chen. Soochow University China	Official Reviewer
Prof.PhD. Eng. Ana Galaction, Univ. of medicine and pharmacy Grigore T Popa, Iasi.	Official Reviewer
Research.St.l.Phd.Eng.Marina Zoccola, National Research Counsil, Institute of macromolecular studies Biella, Italy.	Official Reviewer
Assoc.Prof.PhD. Eng. Maier Stelian Sergiu. " Gheorghe Asachi" Technical University of Iasi.	Official Reviewer

Gheorghe Asaci Technical university of Iasi,Romania.
Politecnico di Torino,Italy.
Soochow University,Soochow,China.
2018

Declaration

I hereby declare that, the contents and organization of this dissertation constitute my own original work and does not compromise in any way the rights of third parties, including those relating to the security of personal data.

Parag Bhavsar

I would like to dedicate this thesis to my loving parents

Acknowledgment

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Abstract

A large amount of raw wool, practically unserviceable for textile uses, is generated in Europe from sheep shearing and butchery; this is a byproduct that is either dumped, burned or sent to landfill. Following the European Commission regulations on animal by-product control, unserviceable raw wool is classified as a category 3 special waste materials, and its collection, storage, transport, treatment, use, and disposal is subject to European Union regulations because of a potential risk source to human and animal health. Raw wool has a noticeable chemical potential to conceive and generate a broad category of products, spreading from protein-based scaffold tissues to fertilizers. Considering all these points, raw wool has potential to create a circular economy rather than just wasted as an unserviceable material.

In general, raw wool finds its application in insulation panels, composites, carpets, etc., but needs a complete pre-treatment before use. The problems begin with the use of raw wool is that; it cannot be used as a fertilizer without any previous pretreatment such as washing because of the potential risk of infection and its slow degradation process in the soil environment. For these reasons, fertilization with untreated greasy wool is forbidden by the EU legislation, which strictly provides guidelines for raw wool storage, transportation, and disposal. These costs heavily weigh on the profit of sheep farmers. The primary objective of this study is to develop the cost-effective, sustainable process to use raw wool prior to any pretreatment.

This study aims at

- Converting waste wool into nitrogen fertilizers at a commercial scale for grassland management and cultivation purposes.

- Development of potential novel applications of hydrolyzed wool

In order to achieve the desired aim of fertilizer, the chemical breakdown of wool needs to be done using sustainable way, i.e., chemical-free process.

In general, hydrolysis process is performed using acids, bases, and enzymes. The literature survey on existing hydrolysis processes, their limitations, industrial scale-up viability, sustainability, cost-effectiveness, etc., lead towards the process where chemical transformation is based on a green economically sustainable hydrolysis treatment using only green solvent superheated water. The other advantage of green hydrolysis is that it sterilizes the wool at high temperature, which indirectly overcomes the problem of pretreatment prior to use and infection problem in the application phase.

In order to understand the extent of degradation and industrial viability of the superheated water hydrolysis process with the aim of fertilizer; the development process implies two steps: the first one at laboratory scale (batch process) and the second at semi-industrial scale (continuous process). A set of experiments on batch scale reactors was performed to monitor process parameters and extent degree of hydrolysis on raw wool; to establish the ground for designing and construction of semi-industrial scale reactor. The green hydrolysis process optimization was carried out in batch and semi-industrial scale reactors by varying parameters such as temperature, wool density, material to liquor ratio, time, depending on the extent of degradation of the final hydrolyzed product.

Controlled treatment with superheated water converts wool keratin into simpler compounds. At the end of the process, it is possible to obtain a hydrolyzed product in either solid or liquid phase depending on the extent of hydrolysis parameters implemented. The presence of amino acids, primary nutrients, and micronutrients in wool hydrolyzates, along with a concentration of heavy metals below the standard limit, confirm the possibility of using wool hydrolyzates as nitrogen based ecologically sound fertilizer.

On the way to find the possible application of keratin hydrolyzate other than fertilizer, which overcomes the environmental problem of wool waste and by-products were found to be a foaming agent for dyeing. The foam-forming behavior of the keratin hydrolyzate along with its application in dyeing was studied to develop sustainable and green dyeing process. The surface tension, foam stability, blow ratio, bubble size of the keratin hydrolyzate in aqueous solutions with and without dyeing auxiliaries were determined. The dyeing influential parameter such as wet pickup was studied to identify their effect on dye fixation and color strength. The foam dyeing was compared with conventional cold-pad batch and pad-steam processes for cotton and wool, respectively. The combination of green hydrolysis and the biodegradable keratin hydrolyzate resulted in sustainable green dyeing process.

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P Bhavsar, M Zoccola, A Patrucco, A Montarsolo, G Rovero, C Tonin, "Comparative study on the effects of superheated water and high temperature alkaline hydrolysis on wool keratin" Textile Research Journal, 87 (14),2016, 1696-1705.

Paper II

Parag Bhavsar, Marina Zoccolaa, Alessia Patrucco, Alessio Montarsolo, Raffaella Mossotti, Giorgio Rovero, Mirco Giansetti and Claudio Tonin, "Superheated Water Hydrolysis of Waste Wool in a Semi-Industrial Reactor to Obtain Nitrogen Fertilizers" ACS Sustainable Chemistry & Engineering 4 (12), 2016, 6722-6731.

Paper III

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Paper IV

Parag Bhavsar, Marina Zoccola, Alessia Patrucco, Alessio Montarsolo, Raffaella Mossotti, Mirco Giansetti, Giorgio Rovero, Stelian Sergiu Maier, Augustin Muresan, Claudio Tonin, "Superheated Water Hydrolyzed Keratin: A New Application as a Foaming Agent in Foam Dyeing of Cotton and Wool Fabrics" ACS Sustainable Chemistry & Engineering 5 (10), 2017, 9150-9159.

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Bhavsar P , Zoccola M , Patrucco A, Maier S, Muresan A and Tonin C "Keratin hydrolyzate novel foaming auxiliary for textile dyeing process (wool/cotton)" AUTEX 2017 World Textile Conference, May, 29 – 31,2017 Corfu, Greece.

Chapter 1

Background and objectives

1.1 Background and aim

Since the beginning of humankind wool has been used for various purposes. The wool trade in Europe has taken part in the development of the economy since middle ages where raw material from England was distributed to manufacture in Flanders and middle Italy. Due to the impact of the history and current economic pressure, the sheep distribution in Europe is irregular. Various sheep breeds (around 408) are found in all over the Europe. In general, wool is considered as a by-product from sheep instead of a major source of revenue. The average scouring yield obtained from European wool is less than 50%, which is lower than the world's average. Most of the wool produced in European region has a diameter in the range of 23µm to 40 µm and is contaminated with pigmented fibers and kemp hence finds its application mainly in insulation, carpets, filters, etc.

Over a past decade, the European sheep population is falling continuously. The European Union (EU) area has the second largest world sheep population[1]. In the year 2016 the sheep population in the EU amounted to about 85.7 millions, the majority of them being located in the United Kingdom (27.79 %), Spain (18.62 %), Romania (11.52 %), Greece (10.19 %), Italy (8.50 %), France (8.35 %) and Ireland (4.01 %) are shown in figure 1 [2]. Annual shearing of EU sheep produces 1.5 – 3 kg head of coarse wool considered as a by-product which is mostly disposed off, or illegally thrown over. Indeed, management costs and initial

processing costs (scouring and carbonizing) are economically unsustainable for such poor quality wool.

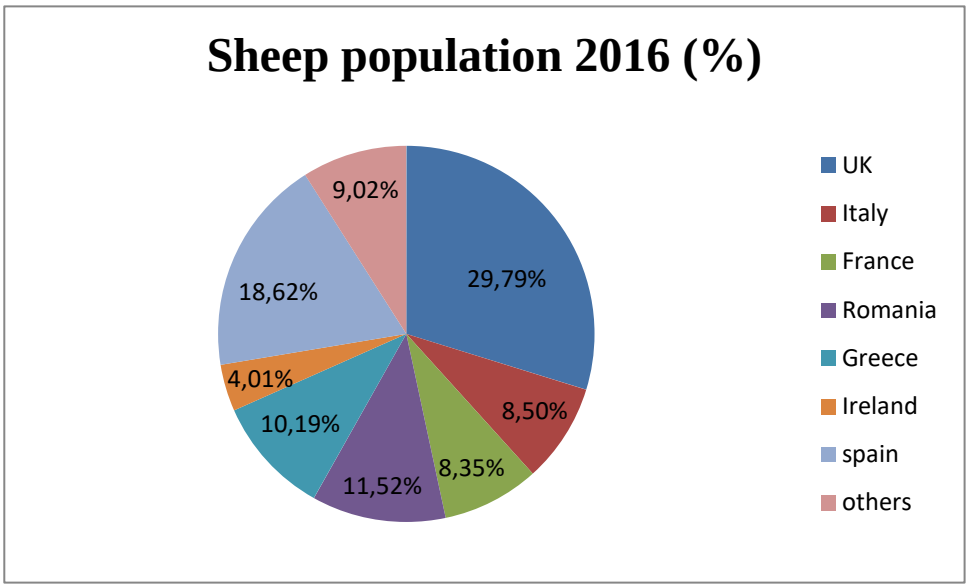

Figure1. Distribution of sheep population in Europe in 2016

The primary purpose of sheep breeding varies according to the geographical location: in northern and temperate EU regions, sheep breeds produce coarse wool and are mainly kept for meat; in southern regions, dairy sheep are breeded[3]. The wool produced within the EU is of low quality if compared to the wool of the major wool producing countries. For many years, sheep farmers are facing the problem of low revenues because of the continual falling of wool prices, due to some extent to an increasing demand for synthetic fibers in the market. Wool economic value is not even sufficient to compensate sheep-shearing cost and wool is often considered as a worthless by-product of sheep farming, with the consequence of leading to massive illegal disposal.

On the other hand, the waste /raw wool consists of peculiar properties such as it's a source of carbon, nitrogen, sulfur and other additional nutrients which play a vital role in plant nutrition. The treatment of raw wool in controlled hydrolysis conditions converts keratins and fats into simple compounds consisting of amino acids, peptides, and hydrolyzed fats. The superheated hydrolysis process allows controlling the speed of release of nutrients to plants and can be used to develop a slow release bio fertilizer, which indirectly helps towards a sustainable bio/organic production of crops.

In order to minimize environmental and health related problems and contemporary device a certain economic benefit, new sustainable technologies must be proven, considering the elemental composition of discarded materials and sustainable transformation processes. Hydrolysis of wool using acid, alkali and enzymes was practiced and studied for long. A current technology of hydrolysis has its own limitations, and advantages and most of them are industrially not applicable. The vast amount of such a valuable waste and lack of industrially viable process is the motivation of this research work to find its solution in the hydrothermal treatment which uses green solvent water. The green hydrolysis of waste wool have been studied using superheated water without the addition of any chemicals.

In this view, energy, and material (quantitative and qualitative) balance drawn from of a combined unit operation scheme provide sound criteria for a novel process assessment. Additionally, it is worth remembering that the success of a process is strictly connected to its structural and operational simplicity. All these issues constituted the guidelines of this study of the hydrolysis of waste wool to find its economic and environmentally friendly applications using sustainable technologies, also considering the logistic distribution of the raw material (chiefly sheared wool) and the ultimate distribution on a territory. Another positive consideration can be addressed to a comprehensive "lean" ecological footprint of waste wool. All these aspects have led to develop a green process characterized by the transformation of non pre-treated greasy raw wool by means of using plain high temperature superheated water.

Objectives of the study

The proposed approach heads towards the establishment of a low cost technology for processing wool wastes from sheep farming and butchery. This byproduct is available in large quantity which is difficult for storage, management, and disposal. Indeed, intermediate short-term objectives were to set up and demonstrate both the technical and the economical viability of a green way of recycling wool wastes into value added organic nitrogen amendment-fertilizers, and novel applications of hydrolyzed product via “green hydrolysis” conversion using superheated water only. These objectives have been pursued through the design and optimization of the process, the construction, and testing of demonstration plants for the hydrolysis of wool wastes, and the chemical, physical and agronomic characterization of the fertilizer (hydrolyzed keratin) and studying possible new applications.

1.2 Structure of thesis

Chapter 1 begins with the background and problem statement of green hydrolysis of waste wool in order to get the general idea and motivation to start research work.

Chapter 2 provides general information about the basic characteristics, bonding, and comprehensive literature on the application of wool in various fields. The use of conventional and green hydrolysis techniques, their wool degradation mechanism was provided along with a summary of all the hydrolysis techniques with treatment parameters.

Chapter 3 consists of all the raw materials used while studying green hydrolysis, detail description of the hydrolysis process, operating condition, optimization parameters related to three different reactors. This chapter also gives detailed information about materials and analysis techniques which were used to analyze wool before and after hydrolysis treatments, analysis of hydrolyzed product for different application purpose, etc.

Onwards chapter 3 each chapter provides the introduction on discussed topics and results related to it.

In chapter 4, the development of a new hydrolysis process begins with understanding the pros and cons of a process and the limitations of reactors. Sequential study of the individual reactor is described.

Depending on the constraints of the reactor, process parameters and characterization of hydrolyzed wool; the design and construction of new reactors were carried out to overcome the previous problems and to achieve the desired primary aim of conversion of waste wool into fertilizer.

In chapter 5, study related to the impact of the superheated water hydrolysis on the photochemical properties of wool and its comparison with a conventional method of alkaline hydrolysis was carried out. This study presents a preliminary understanding of the potential of the new process regarding the extent of hydrolysis of wool with conventional process.

Chapter 6 describes the optimization process of semi-industrial scale reactor on the basis of detail analyses of hydrolyzed product obtained at different parameters. The complete discussion on hydrolyzed keratin regarding its application as a fertilizer and effectiveness of hydrolyzed wool fertilizer in germination study is provided.

After investigating the preliminary aim of waste wool conversion into fertilizer, the other novel application of hydrolyzed keratin (obtained using green hydrolysis) as a foaming agent to develop sustainable and green dyeing process for cotton and wool fabrics using foam dyeing technology was described in chapter 7.

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Chapter 2

Wool properties and hydrolysis techniques

Wool fiber has a complex structure. They mainly consist of a complex mixture of proteins. It is considered as a biological composite made of different chemical and physical components. With the morphological point of view, it is considered as a nano composite with high complexity and clear hierarchy indicating a high degree of self-organization[1]. The details about its morphological structure, chemical composition and different techniques of hydrolysis along with its mechanism are described in the following sections.

2.1 Morphological structure of wool fiber

The sheep wool is a valuable keratinous material which represents a group of fibrous proteins[2]. The keratin is biocompatible, biodegradable and hygroscopic by nature[3-4]. In general wool fibers usually varies in their physical characteristics depending on their fiber length, diameter, and crimps. The chemical composition of wool fibers also varies and consists of 20 amino acids. Wool fibers are also physically heterogeneous.

As shown in the Figure 1 the wool fiber consists of an external cell cuticle (10%), internal cell cortex (86.7%) and cell membrane complex (3.3%). The cuticles are separated from the cortex, and each cortical cell is separated from each other by cell membrane complex. Hence wool fiber is an assembly consisting of cuticles and cortical cells, which are held collectively by a cell membrane complex. In coarse wool fibers, it may consist of another type of cell called as medulla other than present in fine wool. The medulla is the central core of cells having specific

continuous or intermittent arrangements along the fiber axis and function as a maximum thermal insulation[5].

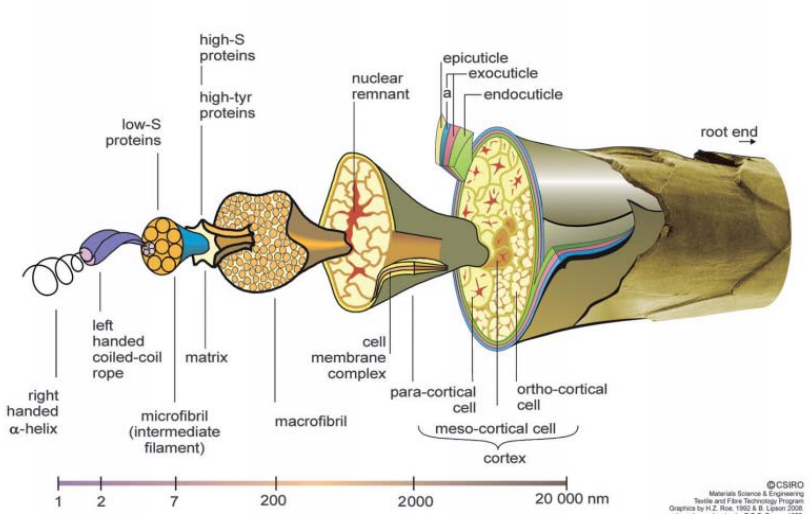


Figure 1. Morphological structure components of wool fiber. (Source CSIRO)

2.1.1 Cuticle

Cuticle cells are mainly divided into two main layers, outer exocuticle (50% ½ cystine) and inner endocuticle (3% ½ cystine) which mainly differ according to the cystine content; it also consists of thin membrane covering the surface of cuticle called as epicuticle (12% ½ cystine). Exocuticle is further divided into two layers such as exocuticle-A (35% ½ cystine) and exocutile-B (15% ½ cystine). Exocuticle is about 0.3 µm thick and represents 60%, endocuticle is around 0.2 µm thick and represents 40%, epicuticle membrane is around 2-7 nm thick and represents 0.1% of total cuticle cells respectively.

2.1.2 Cortex

90% of wool fibers is comprised of the cortex, which is mainly responsible for mechanical properties. The cortical cells are majorly divided into two types, i.e. ortho and para cortical cells. Cortex also consists of the third type of cells which appear in the boundary between ortho and paracortex called as mesocortical cells

which comprise maximum 4% of the fiber. Cortical cells are mostly made of rods like crystalline proteins surrounded by an amorphous matrix called as microfibrils or intermediate filaments having 7 nm diameters and around 1µm lengths. Inside the cortical cells, the intermediate filaments are grouped and form aggregates which are known as microfibrils. Each microfibril consists of a group of 19 intermediate filaments which results in an increase in size and start to occupy the bulk of cortical cells.

2.1.3 The cell membrane complex

In wool fiber, there are intercellular associations present between the main cell types such as within cuticle, cortical, cuticle –cortical and in a case of coarse wool fibers cortical-medulla. These intercellular regions comprise of intercellular material which is known as a cell membrane complex. It constitutes a very small part of the total mass of fiber and it is known to have a large influence on chemical and mechanical properties of wool fibers. It is also considered as a non-keratinous material. Morphological structure components of wool fiber consisting of cuticle, cortical and cell membrane complex are shown in Figure 2. In general, cell membrane complex consists of four main components:

- Modified plasma membranes (b-layers) - they consist of proteinaceous material with low crosslink density
- Intracellular material (d-layer) – it includes mainly lipids
- Inert (resistant membranes) outer boundaries

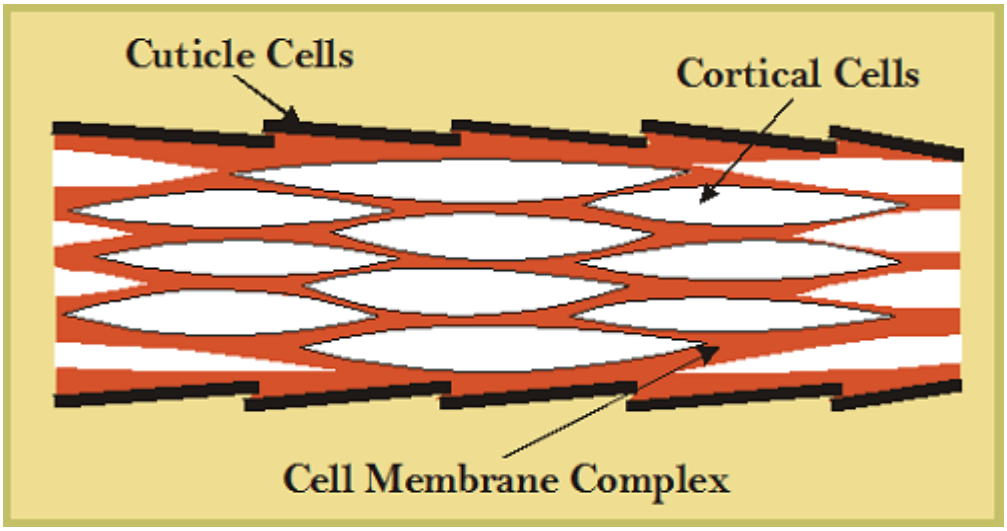


Figure 2. Morphological structure components of wool fiber consisting of cuticle, cortical and cell membrane complex. (Source CSIRO)

2.2 Chemical composition of wool fibers

In general, wool fibers consist of 82% of keratinous protein, which mainly consist of high cystine content. The remaining 17% of wool is composed of proteins know as non-keratinous due to the availability of low cystine content. Wool fibers are also formed of 1% non proteinaceous materials, mainly consisting of waxy lipids and a small quantity of polysaccharides.

Keratins are fibrous proteins which are the main constituent of the wool fibers. Keratins are mainly composed of high sulfur proteins and disulfide bonds, which make them water insoluble and resistant to various chemical agents. The molecular weight of keratin is in the range of 10-60 kDa, obtained after breakage of disulfide bonds with different oxidizing, reducing, enzyme, etc. treatments on wool fibers.

In general, keratins are classified into soft keratins and hard keratins depending on the chemical and physical properties and specifically on the amount of sulfur. The soft keratins have low sulfur content < 3 wt.% and are mainly found in the stratum corneum of the skin, while hard keratins comprise >3 wt.% sulfur and are found in wool, hairs, nails, horns, etc. Keratins are also classified as α-keratin and β-keratin according to x-ray diffraction pattern.

Proteins in wool fiber are composed of 20 different amino acids containing basic amino ($-NH_2$) and acidic carboxyl group (-COOH). The general structure of amino acid is shown in Figure 3. The amino acids mole %, and nature are shown in following Table 1.

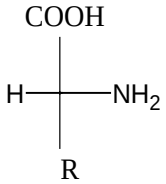


Figure 3. The general structure of amino acids.

Table 1. Amino acid composition of wool fiber (mole %).

Amino acid	Mole %	Nature of side chain
Cysteic Acid	0.20	Sulphur Containing
Aspartic acid	6.70	Acidic
Serine	10.10	Polar
Glutamic acid	13.00	Acidic
Glycine	9.00	Hydrocarbon
Histidine	0.22	Basic
Arginine	7.18	Basic
Threonine	6.23	Polar
Alanine	4.53	Hydrocarbon
Proline	6.60	Heterocyclic
½ Cystine	9.41	Sulphur Containing
Tyrosine	3.57	Polar
Valine	6.27	Hydrocarbon
Methionine	0.46	Sulphur Containing
Lysine	2.80	Basic
Isoleucine	3.33	Hydrocarbon
Leucine	7.31	Hydrocarbon
Phenylalanine	2.70	Hydrocarbon
Tryptophan	0.5	Heterocyclic

2.3 Intermolecular bonds in wool fiber

In wool fiber, individual polypeptides are linked together to shape into other proteins by various types of covalent or noncovalent bonds. Due to the presence of these bonds, wool has its mechanical and morphological properties.

2.3.1 Covalent bonds

Disulphide Bond

The most important bond present in wool is disulfide bond which forms during the growth of fiber by the process known as keratinization. The disulfide bond is a covalent bond and is formed between two particular amino acids cysteine giving rise to a compound called cystine. The presence of disulfide bonds in the wool makes it water insoluble and resistant to chemical and physical damage. These types of bonds can crosslink in same or different interchains and form particular spatial disposition of protein chains such as arcs, rings, and others. Such type of distribution occurs depending on the positioning of the two involved cysteines along the keratin molecule.

Isopeptide bond

Isopeptide bond is another type of covalent crosslink observed in wool fibers, which is formed between amino acids containing acidic or basic groups. These types of bonds are supposed to form a crosslink between polypeptide chains.

2.3.2 Noncovalent bond

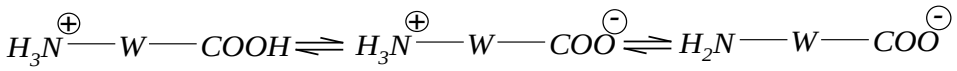
Along with the covalent bonds, noncovalent types of interactions are also observed in the wool fibers. Noncovalent bonds are usually formed between single protein chains or among different chains. These bonds act like crosslink and give a significant contribution towards the fiber properties.

Hydrogen bonds

In wool fiber, hydrogen bonds are formed between the –CO and –NH groups in peptide chains and amino and a carboxyl group in side chains.

Ionic bonds

The basic amino and acidic carboxyl groups present in the wool side chains provide the amphoteric nature to the wool. Due to the presence of these side chains, wool fibers possess a chemical reactivity towards various acids and bases. The strong ionic interactions occurred between ionized amino and carboxyl groups.These ionic interactions are known as ionic bonds or salt linkages, a number of ionic interactions to be formed in fiber is mainly dependent on pH. In fact, the presence of ionic interactions in wool fiber explains the mechanical strength of wool fiber at various pH. Wool chemical structure in acid, neutral and basic media is shown in Figure 4. When the net charge carried by the fiber is zero is called as isoelectric point, above it, carboxyl group forms a cation, and below it, the amino group carries a cationic charge.



For wool

pH <4 *Isoelectric pH 4-5* *pH>5*

Figure 4. Wool chemical structure in acid, neutral and basic media.

2.4 Wool waste disposal problems

According to the Commission Regulation (EU), No 142/2011 unserviceable raw (greasy and unprocessed) wool is subjected to restriction to the collection, storage, transport, treatment, use and disposal, because of a probable source of risks to public and animal health. It is considered as a particular type of waste of class 3 material. EU Control and Implementing Regulation come into force on 4 March 2011 on animal by-product controls, lay many restrictions which are also applicable for unserviceable wool. Inappropriate handling and use of animal by-products have resulted in serious diseases such as foot and mouth disease, classical swine and avian flu, and spread of bovine spongiform encephalopathy. Legislation has been already established and implemented for many years to control these type of risks by setting out the rules for the collection, storage, transport, treatment, use and disposal of animal by-products. Although, instead of these attempts, coarse raw wool from landscaping sheep is mostly disposed in a landfill, or illegally thrown over even having the potential to cause severe ambient threats. Each tonne of raw wool contains around 150 kg of lanolin, 40 kg of suint (soluble contaminants such as potassium salts from perspiration and feces), 150 kg of dirt (soil), 20 kg of vegetable matter, and residues of insecticides, and leaving 640 kg of wool fiber.

Insecticides or insect growth regulators are applied to protect sheep from ectoparasites such as lice, mites, blowfly, tikes, etc. The quantity of pesticide present on wool is varied and depends on the permitted legal usage pattern in each country. As per the new regulations, raw wool has to be washed or treated with a method that ensures that no unacceptable risks to health remain. As per the suggested procedures of world Organisation for Animal Health (OIE) includes: factory or industrial scouring comprising of the immersion of the greasy wool in a continuous baths of warm water (around 50 °C), depilation by method of slaked lime or sodium sulphide; fumigation in formaldehyde in a completely sealed chamber for at least one day; industrial scouring; storage at 18 °C for 4 weeks, or 4 °C for 4 months, or 37 °C for 7-8 day (OIE, World Organisation for Animal Health – (2011), Terrestrial Animal Health Code) (animalhealth.defra.gov.uk).

In general, sheep farmers can carry out these processes by their ownself or delegate to an industrial plant. But scouring of grease wool is expensive (around 1 €/kg) and also it leads to severe environmental problems of safe processing of scouring effluents wastes and resulting sludge. For every kg of clean wool produced by aqueous scouring, some 20 liters of effluent is generated (Eco-Efficient Dry Wool Scouring with total by-products recovery, LIFE+ project, 01-SEP-2012 to 31-AUG-2015) which is remarkably polluting to the environment, consisting of high levels of organic wastes, mineral dirt, and insecticides. The effluent contains a largely concentration of soil particles picked up during grazing; wool grease (lanolin) and sweat (source of potassium); additives of scouring and relevant processes such as detergent residues (anionic or alkylphenolethoxylate non-ionic detergents), pesticide residues. Chemical oxygen demand reaches 100, 000 mg/l, Biological oxygen demand ranges from 20, 000 to 40, 000 mg/l and the disposal of the sludge generated by the effluent treatment is very difficult to manage [6].

Moreover, the Directive 75/442/EEC establishes a waste management hierarchy. The most desirable is waste prevention and minimization of waste generation. This can be supported (in descending order of priority) by reuse of waste, recycling of waste, recovery of waste, use of waste as a source of energy, incineration without energy recovery, landfilling. Also burning wool for fuel is inefficient because it is self-extinguishing (LOI > 21%), and cofiring is polluting due to its high sulphur content (3-4% by weight). Land filling is considered the least desirable waste management option and wool in a landfill not readily degrades.

2.5 Utilization of waste wool

Due to environmental and economic problems related to disposal of waste wool, it is necessary to discover the sustainable utilization of waste wool. The management of unserviceable wool into productive applications with sustainable technologies not only targeting its root to life cycle, but also will improve the economic, environmental and cultural wellbeing of society. The literature suggests that there are various attempts have been carried out in the past to find its productive utilization considering wool is an interesting material and has its unique characteristics such as insulation, proteinaceous material, physiochemical properties, fire resistance, etc.

2.5.1 Insulation panels

Until the mid and late 20th century, the existing insulation materials used in the construction industry do not focus on sheep wool as an insulation material. The conventional insulation materials such as fiberglass, rock wool, etc. have similar thermal properties but different material characteristics which focus on cost, toxicity, water absorption ability and fire protection. Application of sheep's wool in this area is an emerging research topic and finds its place because of a renewable resource, low environmental impact, low cost, hydrophobic-hydrophilic nature, fireproofing, thermal insulation, etc. which makes it an interesting green building material. Symons et al[7]. found that the application of wool shows better thermal insulation and sound absorption properties in comparison with fiberglass and mineral wool respectively. The study also indicates that bulk density of wool fibers is inversely proportional to air flow in pore structures of insulation panels, resulting as an increase in bulk density of wool fibers and leading to greater thermal insulating properties. Zach et al.[8] and Ballagh[9] reported that wool is an excellent acoustic material and it gives maximum sound absorption at 170 mm thickness. Corscadden et al.[10] indicated that application of such waste wool offers a competitive material towards conventional insulation materials and adds benefits to economical and sustainable development in green buildings.

2.5.2 Fiber reinforced composites

Fiber reinforced composites find its application in various fields such as automotive, civil engineering, aerospace, sports equipment industries, etc. In comparison with the metals and unreinforced plastic materials, animal fiber composites offer high strength, flexibility, corrosion protection. Štirmer et al[11]. reported that wool reinforced cement composite mortar provides better workability, small capillary action, better mechanical and compressive strength, thermal conductivity. Use of waste wool in mortar resulted in a decrease in density with increase in fibres volume and makes it better material with less weight, which can be helpful in restoring historical buildings and ceilings. Das et al[12]. developed the waste wool and biochar based hybrid composites which show that use of wool helpful in enhancing the limit oxygen index and some mechanical characteristics such as tensile, flexural strength. Due to charring ability of wool, it

helps in a gradual reduction of heat release and composite shows less smoke release, reduced peak heat release rate in comparison with virgin polypropylene composite. Waste wool can also find its application in making fiber reinforced composite bricks where incorporation of wool resulted in an increase of 37% compression strength. Yükseloğlu and Li et al[13-14] reported that wool waste and wool waste fabric can be useful in making low cost lightweight reinforced composites.

2.5.3 Wool powder films

After alteration in physical properties with some pre-treatment, wool can also be converted in powder form and, due to the presence of high thermal stability, it can be useful in high temperature applications. Depending on the fabrication process and powder properties, the wool powder can be also useful in composite, biomedical and cosmetic fields. The wool powder can also be useful in making biobased films. Wang et al[15]. reported bleaching and colouration of wool powder polypropylene film. He also developed compression molded thermoplastic film by mixing of wool powder with glycerol plasticizer. Film possesses excellent mechanical and tensile strength, and water resistance. This type of bio based film can be useful in food packaging and agricultural applications. Ke[16] and Xu et al.[17] studied the film produced using wool powder and chitosan which possess high affinity towards cationic dyes.

2.5.4 Biomedical applications

Zhang et al.[18] studied that transformation of wool waste into functional protein biomaterial using acid hydrolysis and they used wool as a bio based material in tissue engineering. It can also be combined with synthetic and natural polymers to enhance cell growth. Boulos et al. studied the deconstruction of waste wool to transform it into a bioactive keratin product which can be applied in wound healing and electrospinned for keratin bandages.

2.5.6 Heavy metal and dye absorption

Wastewater generated from most of the industries like textile, paint, metal, electroplating consist of heavy metal particles. These heavy metal particles produces serious health hazard problems if present above the minimum concentration limit. Various processes such as ion exchange, precipitation, etc. employed to remove heavy metal particles have some limitations. Application of biobased materials to remove it opens a new area for research.

Keratin fibers show binding capacity of various types of heavy metals such as (Hg, Pb, Cu, Cr). The adsorption capacity of keratin fibers can be improved with certain chemical treatments which result in more adsorption. Ingrid et al[19]. studied that waste wool can be used a biodegradable, cheap adsorbent for color waste water treatment. With a simple chemical treatment wool can be effective in the removal of acid dyes from wastewater.

2.5.7 Fertilizer

Use of waste by-products as nutrients and amendments for soil for crop production has a long history. Waste sheep wool undergoes landfilling and nutrients present in them are no longer exploited. The best sustainable alternative is to utilize them as a fertilizer. Waste wool consists of a distinct elemental composition of essential plant nutrients such as nitrogen, phosphorus, and potassium. Along with essential primary nutrients, protein hydrolyzate comprising of amino acids which act like a biostimulant. Wool fibers absorb and retain moisture very efficiently; this can be beneficial when applied to soils where it can decrease runoff of contaminants such as pesticides and can aid in water conservation. Previous studies reported that plants utilizes amino acids as a source of nitrogen for growth with a short mineralization process.

In literature, sheep wool has been proposed either as a raw wool or in terms of protein hydrolyzates for fertilizer application. Vončina[20] used sheep wool as a fertilizer without chemical treatment for the asparagus production. Three year of the experimental study shows that, in the second year, use of sheep wool produced the highest content of soil NO_3-N and it resulted in a highest yield of asparagus. In another study, Maria[21] and Ordiales et al[22], used raw wool in form pellets for soil quality improvement and fertilizer respectively for the production of tomato and broccoli. These studies suggest that wool pellets can be an option as an alternative biofertilizer which resulted in a profitable yield of tomato and broccoli in comparison with conventional fertilizers. In a study of Zheljazkov et al[23-24], the addition of unwashed and cut sheep wool in the field as a nutrient source for crops and soil amendments showed positive results with respect to plant growth and productivity of mangold and basil. The plants cultivated with sheep wool for soil amendment yields 40-142% more.

Böhme et al[25]. used a sheep wool pellets containing different amount of starch, casein, and cellulose individually for fertilizer application for vegetables and flowers. In his study, they also reported that wool fertilizer could be used as biofertilizer, but they also suggested that if the use of waste sheep wool as such for fertilizer application is not under the line of EU rules and regulation, then it may be susceptible to human health risk. In his study, MacNeil[26]observed that the application of waste wool carpet as fertilizer for Italian ray grass resulted in an increase in dry matter yield in between 24-82%. The essential elements such as nitrogen, sulphur, and magnesium, etc., were observed to an increased level than control grass. The application of sheep wool for pot cultivation of tomato, sweet pepper, and eggplant was studied by Górecki[27]. In this study, he reported that use of it for pot cultivation resulted in 33% higher yield for tomato and sweet pepper. Application of it resulted in soil amendment and caused changes in nutrients content of substrate and leaves. The application of raw wool in fibrous form has some limitation such as inherent handling problems, lack of ready availability of nutrients, weed problems, and low bulk density[28].

Also, waste sheep wool in hydrolyzate form was used as soil fertilizer and caused an increase in biomass of ryegrass due to increase in the content of K, Mg, and Ca. In this study ryegrass was collected during the first mowing. It also resulted in better seed germination, as well as positive effect on the microbial life of soil [29,30]. Sheep wool hydrolyzate advances growing conditions, by increasing contents of essential elements N, C, and P in the soil[31]. Applied hydrolyzed wool also acts as a chelating agent for microelements available from the soil which are required for plant growth [32]. Overall it is confirmed that waste wool has a potential to be used as a fertilizer, but still, there is need of a sustainable process to overcome all the existing problems and consider its industrial scale application.

2.6 Hydrolysis of wool

Wool is a protein fiber consisting of disulphide bonds, which make wool more stable and chemically resistant. Hence wool have to be processed in harsh conditions in order to break down these bonds and makes it a more process-able material. The hydrolysis of keratin is one of the most promising way to extend the practical application of keratin material because keratin hydrolyzate can be prepared by a different individual or combined hydrolysis treatments. In conventional protein extraction processes such as reduction, oxidation, sulphitolysis etc., molecular weight of proteins remains unchanged, while in the hydrolysis treatment breaking of peptide bonds in wool protein leads to the

formation of low molecular weight peptide, oligopeptide and finally, amino acids. These peptides, oligopeptides and amino acids can be ultimately refined and used as building blocks for various applications. In general hydrolyzed keratin shows better physical and better processing ability than keratin protein. Hydrolysis of wool can be carried out in various processing conditions and using different chemical reagents.

Conventional Hydrolysis

2.6.1 Acid hydrolysis

Acid hydrolysis of wool was studied since the beginning of the 19th century. The first report on acid hydrolysis of wool was by Sakito[33] and deals with the determination of amino acids in a wool protein. Hydrolysis of wool with various mineral acids such as sulphuric acid, hydrochloric acid was studied by many authors. In acid hydrolysis, degradation of wool protein occurs and which depends on various factors such as temperature, pH, time and concentration of acid, etc. The reaction mechanism of acid hydrolysis is shown in Figure 5.

Acid hydrolysis of wool resulted in:

- Free amino acids formation
- The remaining liquor formed in acid hydrolysis consists of ammonium salts, little peptides and dipeptides in greater quantity.
- The bonds between serine and arginine amino acids were observed to be more labile.
- Release of ammonia.
- Weight losses and liberation of end group in wool proteins.

The quantitative determination of amino acids was carried out by strong acids at 6N concentration, 110 °C for 24 h and yields amino acids in a quantitative way where tryptophan is completely destroyed while serine, threonine, and tyrosine are partially damaged respectively. Further quantitative conversion of asparagine to aspartic acid and glutamine to glutamic acid has also occurred. Conventional hydrolysis was performed in mild acidic conditions for a long time 12-48 h using reflux conditions which results in partial hydrolysis of wool with proteins having high molecular weight. Long time of hydrolysis, difficult handling and cost of strong acids make acid hydrolysis an industrially unsuitable process.

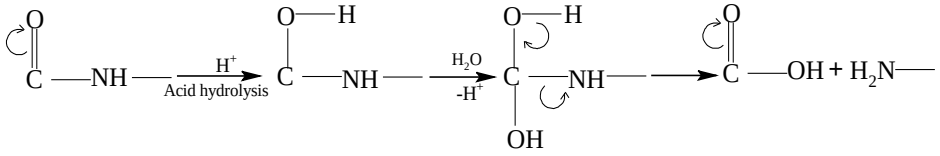


Figure 5. Reaction mechanism of acid hydrolysis[34].

2.6.2 Alkaline hydrolysis

Wool fiber is more sensitive towards alkali. Alkaline hydrolysis of wool was studied by numerous authors using calcium hydroxide, sodium hydroxide, potassium hydroxide, etc. In comparison, use of alkali also leads to degradation of wool fibers, but less specific than acid. In alkaline hydrolysis, alkaline solution binds to the wool resulting in swelling of wool fiber followed by contemporaneous degradation. During alkaline hydrolysis, alkali attached to the wool so firmly that it won't easily washed out using water, and further needs to be removed by neutralizing with acid.

The wool fiber degradation in alkaline hydrolysis depends on temperature, concentration, etc. Alkali attacks on disulphide bonds, side chain amide bonds, and wool peptide bonds, lead to the destruction of amino acids such as arginine, serine, threonine, cystine, and cysteine.No damage to tryptophan was observed. Alkaline hydrolysis attacks primary on –CONH bond of asparagine and glutamine, and breaks peptide bonds in the main chains leading to racemization of amino acids. In case of mild alkaline hydrolysis, modification of wool keratin through degradation of cystine results in the formation of strong linkages (lanthionine): In addition, wool solubility in alkali decrease as disulphide bonds are replaced with such a strong linkage (lanthionine). As the alkali give rise to new forms of linkages, it can be used for quantitative determination of wool peptides by alkaline hydrolysis[35].

Partial alkaline hydrolysis with controlled hydrolysis conditions for the extraction of particular peptide length appears to be an interesting area of research even though the partial hydrolysis results in ample amount of unhydrolyzed solid residues. Most of the wool proteins get solubilize on the degradation in alkaline hydrolysis, this solubility of wool in standard condition is considered as a measure for chemical changes in wool fiber. The disadvantage of this technique is it needs a higher concentration of alkali and a neutralization step which produce impurities in the form of salts. Due to simplicity and easy availability of reagent this method is still widely used in research.

The following reactions occur in alkaline hydrolysis[35]:

Cysteine -» dehydroalanine + hydrogen sulfide

Cystine -» dehydroalanine + thiocysteine

Dehydroalanine + cysteine -» lanthionine

Dehydroalanine + lysine -» lysinoalanine

Dehydroalanine + ammonia -» aminoalanine

Glutamine -» glutamic acid + ammonia

Asparagine -» aspartic acid + ammonia

Isoleucine -» alloisoleucine.

Green hydrolysis

2.6.3 Enzymatic hydrolysis

Enzymatic hydrolysis is one of the emerging and environmentally friendly processes considering mild processing condition and suitable recovery of hydrolysis proteins which are sensitive to acid and alkaline hydrolysis. The efficiency of enzymatic hydrolysis depends on various parameters such as enzyme loading, substrate concentration, time and surfactant, temperature. These parameters often interact with each other hence their optimization is necessary in order to improve the performance of a process. The group of enzymes that act on keratin is known as keratinases. In enzymatic hydrolysis, enzymes are able to break down keratin in mild conditions such as 25-60 °C and pH of around 8 depending on the type of enzyme used.

Enzymatic hydrolysis of wool using enzyme protease was studied by Yoshimura et al[36]. Protease enzyme is able to hydrolyze the polypeptide of proteins into smaller molecules under mild pH and temperature without using strong chemicals which means that sensitive amino acid cystine in wool remains unaffected. In enzymatic hydrolysis, it is difficult to hydrolyze wool because of its chemical-morphological composition. Therefore this hydrolysis treatment often combines with other pretreatments such as the use of surfactant, ultrasonication, alkali treatments. In a further study, Yoshimura et al.,[37] reported that use of anionic surfactant speed up the hydrolysis process with higher weight loss of wool fibers. The ultrasonication pretreatment prior to enzymatic hydrolysis alters the fiber surface morphology, which allows the protease to penetrate more easily inside the fiber structure. The result is more weight and tensile strength loss of wool fibers. Mokrejs et al,.[38] reported that the use of a two step alkaline-enzyme hydrolysis resulted in a higher hydrolysis in enzyme treatment until 90.8%.

In enzyme hydrolysis, the catalytic activities of enzyme hydrolyze the peptide bonds present in the polypeptide chains. The nucleophilic attack –OH group on enzyme on the carbonyl group of peptide bonds resulted in breakdown of peptide bonds. At the same time, the continuous formation of acyl enzyme intermediate occurred and, hydrolysis of ester linkages bring the second peptide product. The mechanism of enzyme hydrolysis[39] is shown in Figure 6. When protease enzyme hydrolyzes wool fibers, the breakdown of intermolecular hydrogen bonds present

in either amino acid protein side chains or individual polypeptide chains occurred in the aqueous enzyme solution.

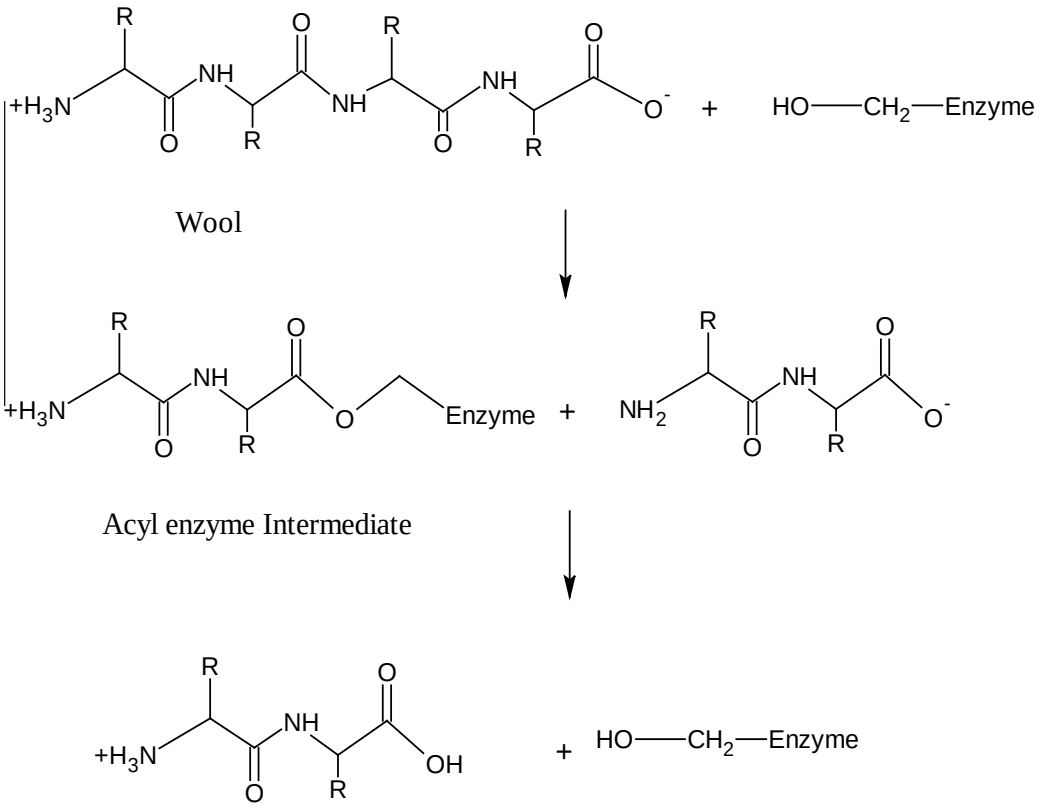


Figure 6. Reaction mechanism of enzyme hydrolysis.

In general the enzyme hydrolysis alone is not effective and it has to be combined with some pre-treatments. It is an ecofriendly process but the cost of enzyme application and the difficulties to reusability restrict its industrial scale application.

2.6.4 Superheated water hydrolysis

Superheated water is defined as the liquid water under pressure in the range of atmospheric boiling point 100 °C and critical temperature 374 °C. The pressure is applied to keep the water in liquid state and it is equivalent to vapour pressure at given temperature or higher temperature. In this temperature range as the temperature increases the density of water molecules decreases. The hydrogen bonds are weakened, and further ionization of water molecules into hydroxonium ions ($H3O^{+}$) and hydroxide ions (OH^{-}) by a combination of oxygen-hydrogen

stretching within a molecule and liberation vibrations between moleculeswas occurred.[40-41]

Properties of superheated water:

- High density
- High solubility
- Polarity
- Diffusivity
- Self ionization of water
- Reduce extraction time and solvent volume
- The extract is more concentrated

Superheated water is a green solvent and replacement of existing organic solvents. The simple word cloud shown in Figure 7 related to it gives clear idea about problems related to use of existing solvent and to overcome it the current solution is superheated water.

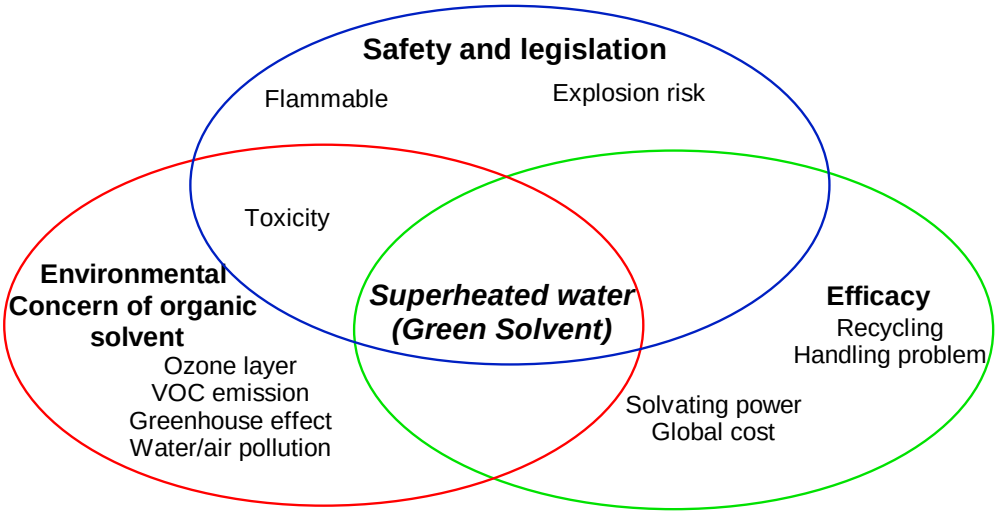


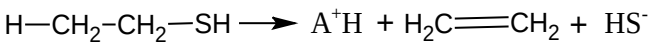
Figure 7. Word cloud of superheated water advantages.

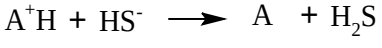
The application of superheated water hydrolysis has been carried out since long in various fields such as food industry for cooking above 100 °C, coffee extraction, paper industry for pulp processing, for waste treatment (called as wet-air oxidation), extraction of flavors and fragrance, extraction of biomass, removing metal and organic compounds from polymers etc.

Superheated water is highly effective in terms of hydrolysis or dissolution of keratin to get oligopeptides. Yin et al.[42] reported that superheated water hydrolysis of a feather at 220 °C for 2 h shows that oligopeptides obtained from hydrolysis have an ability to self-assemble into a crystalline structure, conserve the conformational and crystalline order of native keratins and amino acid sequence of proteins prior to hydrolysis is largely preserved in oligopeptides. In a study of the steam explosion of wool keratin at 220 °C for 10 min Tonin et al[43]. shows that hydrolyzed product obtained exhibits disruption in morphological structure of wool fibers, and loss in molecular weight of proteins. The hydrolyzed product is more water soluble than solid phase and consists of free amino acids, oligopeptides and small proteins with a breakage of disulphide bonds and decomposition of sulphur containing amino acids . Study on steam explosion of wool keratin at different pressures by Xu et al.[44] shows that change in sulphur contains amino acids, destruction in disulphide bonds and damage to wool scales followed by formation of grooves were observed. In high density steam flash explosion of feather keratin, Zhao et al,.[45]. reported disruption of feather keratin in less than 1 s process time and breakage of intermolecular disulphide bonds of protein without causing damage to the protein chain. The hydrolyzed product obtained at the end of the process is highly water and salt solution soluble. Similar studies by Kang and Lamoolphak et al,.[46-47] on the hydrothermal degradation of silk fibroin using superheated water with alkali, acid and without additive show that the hydrolyzed product consists of low molecular weight proteins.

In superheated water hydrolysis, the extent of wool degradation depends on time, temperature, pH of hydrolysis treatment. In the hydrolysis treatment of wool at a temperature about 130-140 °C disappearance of α-keratin XRD pattern with decomposition is observed. During the hydrolysis treatment, the cystine present in wool protein is decomposed and first leads to the liberation of hydrogen sulphide which further attacks on cystine in wool and speed up the decomposition auto-catalytically. The mechanisms of cysteine-cystine self degradation and thiol-disulphide degradation, and self cross linking are shown below[35].

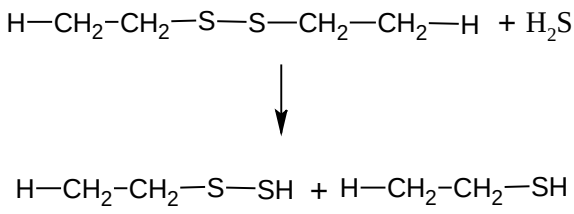
Step 1. Self degradation of cysteine and formation of hydrogen sulfide and hydroalanine residues



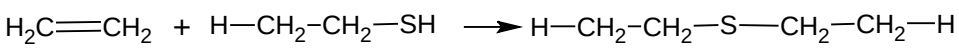


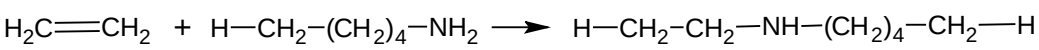
A= Amino groups of histidine or lysine and main chain end groups.

Step 2. Self degradation of cystine due to reduction of disulphide bonds with hydrogen sulphide and followed by formation of cysteine and S-thiocysteine.



Step 3. Self cross linking due to reaction of lysine and cysteine with dehydroalanine and formation of lanthionine and lysinoalanine crosslink.





Superheated water hydrolysis has the following main advantages over other existing processes:

-water as a green solvent instead of organic solvents is used,

-the process is environmentally friendly and economically efficient

- the speed of operation is increased,.
- final purification process such as dialysis is reduced,.
- Purification via crystal formation of proteins allows to further applications, e.g. synthesis of man-made proteins and polypeptides,
- the process is user friendly and no exposure to organic solvent or toxic gasesexists as no chemical are used ,.
- No environmental air/water pollution,
- Non flammable, non explosive process minimizes the potential of accidents.

All these advantages make superheated water hydrolysis of wool an interesting area of investigation in order to develop the process for waste wool minimization into variable applications.

2.6.5 Summary of hydrolysis techniques

Table 2. Summary of hydrolysis techniques

Method	Material	Treatment conditions	Use	Reference
Conventional Process				
Acid	Wool	4 mol/L HCL 95 °C	Wool waste conversion in functional biomaterial	Zhang[48] 2013
Acid	Wool	100 ml/g HCL 40 °C	Amino acid	Blackburn[49] 1953
Acid	Wool	1:1 HCOOH : HCL 40 °C 30-360 min	Amino acid	Menefee[50] 1965
Acid	Wool	5% H_2SO_4	Carbonizing study	Wang[51] 1989
Alkaline	Wool	0.1 N NaOH 24h 60 °C,30 ultrasonication	Wool sponges	Patrucco[52] 2016
Alkaline	Wool	0.1-0.5N NaOH, 80-90 °C 2h	Toxicity analysis	Li[53] 2013
Alkaline-ultrsonication	Wool	4g NaOH in Alcohol/water, 25 °C, 25 min with ultrasonication	Wool powder	Hikima[54] 2008
Alkaline	Keratin and collagen	KOH and NaOH microwave heating 800W	Wool degradation	Gousterova[55] 2005
Alkaline	Wool waste	0.15 m KOH	Biofertilizer	Nustorova[56]

		and 0.05 M NaOH 120 °C 20 min		2006
Alkaline	Wool	0.5 N NaOH, 60-65 °C, 3 h, pH 13.9	Protein sequence study	Cardamone[57] 2010
Green Process				
Enzyme hydrolysis with anionic surfactant	Wool	Protease, 50 °C 3h	Process enhancement	Yoshimura[36] 2012
Enzyme alkaline	Feather	0.2 N NaOH 70-90 °C 30 min, 0.5 g Enzyme 55 °C	Degradation	Dalev[58] 1990
Enzyme hydrolysis with reducing agent	Wool	Trypsin, serine protease 35-40 °C	Digestion of wool	Routh[59] 1938
Enzyme	Wool	0.01N NaHCO3, 2 ml Esperase 8 protease enzyme, 50 °C, 7 days	Chelating agent	Evangelou[60] 2008
Enzyme with alkali	Feathers	0.1-0.3% KOH and 1-5 % proteinase	Feather degradation	Mokrejs[38] 2010
Enzyme-ultrasonic treatment	Wool	Protease, 2 g/l sodium bisulfite, 1 gpl SDS, 55 °C, 96-288 h	Nano particle production	Eslahi[39] 2013
Enzyme-Alkaline	Wool	3-9% $Ca(OH)_2$, 5% Everlase 6.0 T, 6-18 h, 40-60 °C	Wool degradation	Mokrejs[61] 2011
Enzyme	Wool,	1 gpl SDS,	Wool	Eslahi[62] 2013

reducing agent	Feather	2.6% protease savinase, 6.4 gpl sodium hydrogen sulphite. 55 °C	degradation	
Enyme reducing agent	Wool	Pronase and dithioerythritol 39 °C, 72h	Wool degradation	Roper[63] 1984
Alkali-Enzyme		10 g $CaOH_2$, 0.5g proteolytic enzyme Esperase 6.0T 24h, 40-80 °C	Preparation of keratin hydrolyzate	Krejci[64] 2011
Superheated water/steam	Chicken feathers	220 °C 120 min	olegopeptides	Yin[42] 2007
Steam explosion	Wool	220 °C 10 min	Waste minimization by valorisation	Tonin[43] 2006
Steam explosion	Wool	0.2-0.8 mPa	Process optimization wool powder	Xu[44] 2006
Hydrothermal treatment	Wool	100-170 °C	Temperature effect study and proteins residues	Sweetman[65] 1967
Superheated water	Silk fibroin	250 °C, 2-62 min	Amino acids	Kang[46] 2004
High density steam flash explosion	Feather keratin	0.086 sec	Extraction and dissolution of keratin	Zhao[45] 2012
Hydrothermal	Silk waste	160-220 °C, 10-60 min	Proteins and amino acids	Lamoolphak[47] 2008
Superheated water	Hog hair	250 °C, 0-360 min	Amino acids	Esteban[66] 2008
Superheated water	Goose feathers	180-220 °C, 30-120 min	Composite membrane	Liebeck[67] 2017

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Chapter 3

Experimental Section

3.1 Materials

3.1.1 Material for hydrolysis study

Sheep farming in the piedmont region, Italy and Romania is mainly for meat and milk purpose and most of wool is improperly disposed off, land filled or burned because of not suitable for textile grade application. Considering all of these factors the basic raw material waste wool of about 100 kg has been purchased to use in this work. Waste wool obtained is consisting of all the impurities and dirt just after shearing of wool. The same wool was used in all further studies.

Figure 1. Raw grease wool.

3.1.2 Wool hydrolysis preparation for comparative study of superheated water and alkaline hydrolysis

Wool was cleaned with tap water and used for hydrolysis with superheated water, potassium hydroxide (KOH) and Calcium oxide (CaO).

3.2 Equipments

The types of equipment used in this study are represented as equipment used in the development of green hydrolysis technique from small lab scale, final lab scale reactor, semi industrial and industrial scale reactor. The study of the hydrolysis begins with the small lab scale 100 g then 1 kg reactor and it is followed by scale up to semi industrial and industrial scale unit. Further, the equipments were used for the foaming study of hydrolyzed wool product and analytical study. All the hydrolysis reactors used in this study are built by OBEM Spa, Biella Italy.

3.2.1 Small laboratory scale hydrolysis reactor[2]

The small lab scale reactor, as shown in figure 2a and 2b was used to perform the preliminary study of hydrolysis. The reactor 2a is built of stainless steel having 1.39 L volume, minimum capacity of 50 g and maximum of 100 g of wool loading. The design and construction of reactor are allowed to work at a maximum temperature of 165 $^{\circ}$C and 6 bar gauge pressure. Another small scale laboratory reactor as shown in figure 2b having a wool loading capacity of 2-5 g of wool and the maximum operating condition of 185 $^{\circ}$C is used in this study. Both the reactors are not equipped with any external heating system: reactor 2a is used by putting it in glycerin bath with electrical resistance heating and reactor 2b is used by placing inside the laboratory scale oven. The temperature profile of hydrolyzed material inside the reactor is monitored by a temperature controller connected to the reactors.

(a) (b)

Figure 2. Small bench scale reactor (a) 50-100 g Capacity (b) 2-5 g Capacity

A preliminary study of the hydrolysis of greasy wool performed on reactor 2a is carried out at a temperature of 160 $^{\circ}$C for 1 h (total 2h reaction time consisting of 1 h need to reach the desired temperature of hydrolysis and 1 h actual time of hydrolysis at mentioned temperature) in both compressed and uncompressed ways of loading. Another small reactor 2b is used to study for higher temperature hydrolysis of wool at about 170 $^{\circ}$C. The same reactor is used for the preliminary comparative study on the effect of superheated water and alkaline hydrolysis on wool properties.

The preliminary tests performed on greasy wool hydrolysis are shown in table 1.

Table 1. Test performed on greasy wool hydrolysis in a small laboratory scale reactor

Test	Material	Amount (g)	Temperature ($^{\circ}$C)	Material to liquor ratio	Wool loading
1	Greasy wool	25	156	1:3	Uncompressed, incomplete impregnated with water
2	Greasy wool	25	156	1:3	Compressed, well impregnated

3	Greasy wool	25	160	1:3	Uncompressed, well impregnated
4	Greasy wool	25	155	1:6	Uncompressed, well impregnated
5	Greasy wool	25	156	1:3	Compacted in a beaker and well impregnated
6	Greasy wool	25	156	1:2	Compacted in a beaker and well impregnated

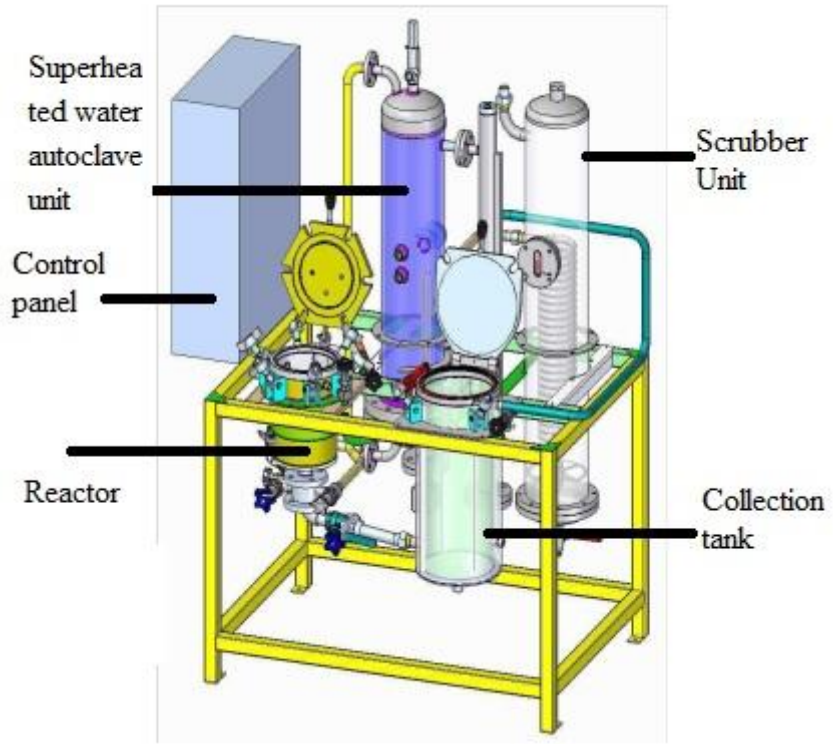
3.2.2 Final laboratory scale hydrolysis reactor

As mentioned above earlier in section 3.2.1 the stepwise progress is necessary in the development of hydrolysis reactor in order to understand its feasibility, process parameters and foreseen properties of the final product. The result obtained from preliminary studies on small laboratory scale reactor 2a is the basis for design and construction of this final laboratory scale reactor as shown in figure 3. The final laboratory scale reactor consists of 5 major parts:

1) Reactor
2) Superheated water provider autoclave unit
3) Hydrogen sulphide scrubber unit
4) Collection tank for hydrolyzed product after hydrolysis
5) Control panel for automatic setting of reactor parameters

The hydrolysis reactor is made of stainless steel having a volume of 10 L and is able to hydrolyze maximum 1 kg of raw wool in a single batch of experiment. The material to liquor ratio set is 1:3 depending on compression of wool on loading, as the wool density varies from 50-400 kg/m^3 depending on its compression. The hydrolysis reactor is designed in a way, having 3 connectors on the top of reactor from which 3 thermocouples can be introduced in 3 different areas: 1st near to the reactor vessel wall, 2nd in bulk of material and 3rd in bulk of the material near to

center of the perforated drum in order to analyze variation in temperature during the progress of hydrolysis reaction. These thermocouples are attached to a data logger to monitor temperature signals. On the basis of preliminary experimental data, 6L is the maximum amount of liquor, which is sufficient to impregnate water in all types of wool densities. The superheated water is prepared prior to beginning of experiments in a 30 L tank and supplied through the superheated water autoclave tank to hydrolysis reactor. The hydrogen sulphide scrubber unit is filled with calcium carbonate in order to provide the chemical desulphurization of the sulphide products. The collection tank in the unit helps to remove and collect the hydrolyzed material at the end of the hydrolysis process by the simple opening of a valve. The control panel helps to ease the setting and monitoring of process parameters during hydrolysis reaction. The temperature and pressure indicators are directly attached to hydrolysis reactor, which is able to provide the temperature inside the rector and inside the superheated water autoclave unit.



(a)

Figure 3. (a) 3D and (b) actual view of final lab scale reactor.

Moreover, some specific features have been evaluated:

1) The relation between physical parameters, reaction yield and product characteristics.
2) The homogeneity of the reaction within the reactor by means of online diagnostics of the process parameters (temperature, pressure).
3) The effectiveness of the desulphurization treatment section.

In all hydrolysis experiments, the same quality of raw greasy wool is used. The wool is loaded in perforated drum and further placed in hydrolysis reactor. The reaction parameters are as mentioned below. At the end of hydrolysis reaction, the reactor is cooled by running room temperature water through the external cooling jacket. The pressure inside the reactor is released at the end of the hydrolysis reaction by manual valve opening which is connected to a hydrogen sulphide scrubber unit where all the gases (H_2S) generated during the hydrolysis treatment

of wool are treated. As soon as the pressure drops inside the reactor the hydrolyzed material is collected in the collection tank. In the study of this type of reactor different parameters are varied and analyzed:

- Material to liquor ratio (1:1.5, 1:2, 1:3, 1:3.5, 1:8)
- Effect of compressed wool loading at different densities 160, 320, 350, 500 kg/m^3.
- Temperatures: 140, 150, 160, 170 $^{\circ}$C
- Time: 30, 60 min

The detailed description of experiments performed on this reactor is shown in table 2.

Table 2. Experimental parameter used during hydrolysis of greasy wool on final laboratory scale hydrolysis reactor

Test	S/L ratio	Density (kg/m^3)	Temperature ($^{\circ}$C)	Time (min)
1	1/8	160	180	60
2	1/3	320	180	60
3	1/2	500	180	60
4	1/2	500	180	60
5	1/1.5	500	180	60
6	1/2	350	160	60
7	1/3	350	160	60
8	1/3	350	170	60
9	1/3	350	170	60
10	1/3	350	170	30
11	1/2.5	350	170	30
12	1/3	350	170	60
13	1/3	350	160	60
14	1/3	350	150	60
15	1/3	350	150	60
16	1/3	350	125	20
17	1/3	350	140	60
18	1/3	350	180	30

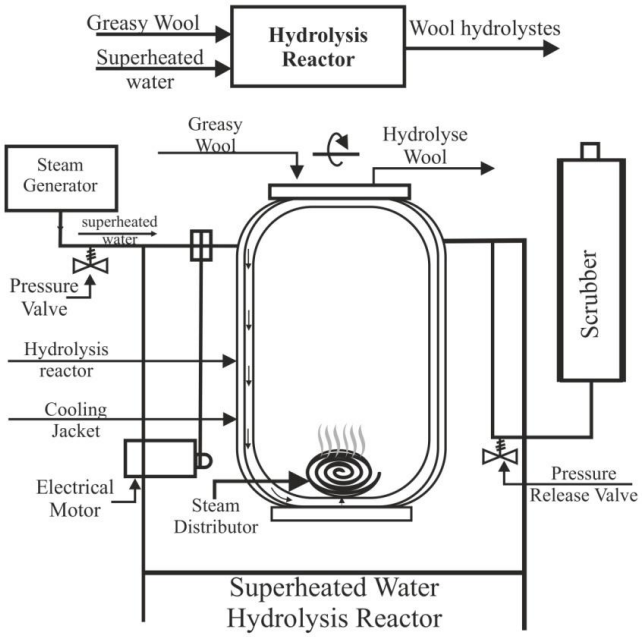
19	1/3.5	350	140	60
20	1/3	350	170	60
21	1/3	350	140	60

3.2.3 Semi-industrial scale rotating reactor

The experimental tests on this reactor were carried out to evaluate the fundamental variables with the following main goals:

1. Optimal reactor rotation speed, able to ensure an adequate liquid-solid mixing;
2. Optimal solid to liquid ratio (S/L) considering the average liquid phase due to steam condensation during the heating and during the treatment;
3. Evaluation of the new heating system carried out by using direct steam, in comparison with the previous electrical resistance based heating.

The hydrolysis of greasy wool was carried out in a semi-industrial scale 100 L reactor specifically designed and built as shown in the figure 4. The reactor is able to hydrolyze from 6 to 20 kg of fibrous material at a maximum temperature of 185 $^{\circ}$C (corresponding to an equilibrium pressure of 10.5 bar); it is equipped with a driving system capable of tumbling the reactor at a variable speed of up to 40 RPM.



(a)

(b)

Figure 4. Schematic (a) and actual representation (b) of semi-industrial scale rotating reactor.

An optimal reactor rotation speed results in a homogeneous impregnation of wool by the hot water. The system is connected to a steam generator, able to supply steam at the maximum pressure of 11 bar, by a feeding supply line through a pressure reducer/regulator for keeping constant the value of the steam pressure regardless of the pressure fluctuations of the boiler. The hydrolysis reactor is equipped with a steam distributor inside to spread the steam over the entire mass. The steam condensates during the heating and hydrolysis reaction provide the sufficient amount of water to complete wool hydrolysis reaction. An outer cooling is provided to lower the temperature of the product before discharge. A drain collection system to collect the hydrolysis products in a proper container, and H_2S scrubbing unit capable of dissolving the discharge steam in an aqueous solution of calcium salts are also provided. A complete control system of this reactor is allowing an automatic and safe working of the reactor.

In all the experiments performed on this reactor, saturated steam was supplied to the hydrolysis reactor at a temperature of 170 $^{\circ}$C corresponding to a pressure of 7.0 bar for different times ranging from 30, 60 and 90 min. Two different amounts of 6.5 and 10 kg of greasy wool were used for the hydrolysis runs. After the hydrolysis treatment, the reactor was cooled down and the wool hydrolyzates were unloaded and characterized.

3.3 Methods

3.3.1 Superheated water and alkaline hydrolysis comparative study

In a comparative study of superheated water and the alkaline wool hydrolysis, the hydrolysis was carried out in a laboratory scale reactor, as shown in figure 2b using superheated water and alkali KOH and CaO respectively. All wool samples were fed to the reactor along with water (fiber to liquid ratio 1:3) and chemicals. The concentrations of KOH and CaO used for alkaline hydrolysis were 5%, 10%, 15% o.w.f. The experiments were carried out at different temperatures: 140 °C, 150 °C, 160 °C and 170 °C for 1 h. Even though hydrolysis was carried out at different temperatures, the main focus was on two extreme temperatures 140 °C and 170 °C. All the analysis was performed on the hydrolyzed product obtained at these two temperatures while a morphological investigation was performed at all the temperatures. Alkali treated samples were dialyzed in a cellulose tube (molecular weight cut off 12.000-14.000) against deionized water for three days at room temperature to remove potassium and calcium salts. The aqueous solutions obtained were filtered (5 μm pore size filters) and then freeze-dried in a PL 3000 (Heto-Holten A/S) freeze-dryer.

Experimental Plan

In all the experiments hydrolysis of wool was carried out for 1h with a material to liquor ratio of 1:3. pH of the alkaline hydrolysis before hydrolysis is in the range of 12-13 and in case of superheated water pH before hydrolysis is in the range of 7-8.The experimental conditions used are shown in the following table 3:

Table 3. Experimental condition of alkaline and super heated water hydrolysis.

No.	Temperature	Chemical	Concentration
1	170 °C	Water	-
2	170 °C	CaO	0.05 g/g
3	170 °C	CaO	0.1 g/g
4	170 °C	CaO	0.15 g/g
5	170 °C	KOH	0.05 g/g
6	170 °C	KOH	0.1 g/g
7	170 °C	KOH	0.15 g/g

8	160 °C	Water	-
9	160 °C	CaO	0.05 g/g
10	160 °C	CaO	0.1 g/g
11	160 °C	CaO	0.15 g/g
12	160 °C	KOH	0.05 g/g
13	160 °C	KOH	0.1 g/g
14	160 °C	KOH	0.15 g/g
15	150 °C	Water	-
16	150 °C	CaO	0.05 g/g
17	150 °C	CaO	0.1 g/g
18	150 °C	CaO	0.15 g/g
19	150 °C	KOH	0.05 g/g
20	150 °C	KOH	0.1 g/g
21	150 °C	KOH	0.15 g/g
22	140 °C	Water	-
23	140 °C	CaO	0.05 g/g
24	140 °C	CaO	0.1 g/g
25	140 °C	CaO	0.15 g/g
26	140 °C	KOH	0.05 g/g
27	140 °C	KOH	0.1 g/g
28	140 °C	KOH	0.15 g/g

3.3.2 Semi industrial scale superheated water hydrolysis of wool

In a study of the superheated water hydrolysis of waste wool in a semi-industrial reactor to obtain nitrogen fertilizers, the hydrolysis of waste wool is carried out as per mentioned in section 3.2.3.

3.4 Characterization

3.4.1 Characterization of raw wool

Moisture Content

The moisture content was determined by drying the greasy wool samples in a ventilated oven at 105 °C up to reaching a constant weight and results were calculated using the formula:

$$\text{Moisture } (\%) = \frac{(P_i - P_f)}{P_i} X\ 100$$

Where:

Pi = Initial weight of the greasy wool sample,

Pf = Final weight of the greasy wool sample.

Washing yield

The washing yield was determined using the laboratory simulating an industrial washing process. The four bath washing process consists of water in the first bath, 1 g/L nonionic surfactant and 5 % Na_2CO_3 in the second bath, 1 g/L non ionic surfactant in the third bath and water in the final bath. All the baths were maintained at 55 °C for 5 min with MLR of 1:50. At the end of washing all the samples were dried at 105 °C for 4 h and washing yield was calculated as:

$$\text{Washing Yield } (\%) = \frac{P_f}{P_i}\ X\ 100$$

Where:

Pi = Initial weight of the dried wool sample,

Pf = Final weight of the dried wool sample.

Ash content

Insert the two crucibles in a furnace and keep at 800 °C for 1 hour, after that remove the crucibles, keep it in desiccators until it cools and then measure the initial weight of the crucibles. Cool down the furnace till 150 °C. 3 g of wool sample is taken for this analysis and keep it in a furnace at 600 °C for 2 hours and measure the weight of the ash. The ash content was calculated by:

$$\text{Ash Content (\%)} = \frac{Pf}{P_i} X\ 100$$

Where:

Pf = Final weight of the ash

Pi = Initial dry weight of the greasy wool sample

Grease and vegetable matter content

The fatty matter content was determined by a soxhlet extraction of the greasy wool with dichloromethane according to the IWTO Standard[4].

The vegetable matter was determined by dissolving the greasy wool sample in a 30 g/l NaOH solution at boiling temperature for 15 min with M.L.R 1:50. The vegetable matter was recovered by filtration, followed by neutralization using 10 gpl acetic acid and dried at 105 °C.

The final results of greasy wool analyses are the mean of four replications.

3.4.2 Characterizations of wool hydrolyzates

Determinations of density, pH and optical microscopy analysis were carried out on the samples after hydrolysis prior to drying, while other determinations were carried out after drying at 55 °C.

Density

The density of the hydrolysis products was determined as weight/volume (g/mL) after the elimination of air bubbles in an ultrasonic bath (Bransonic 1200 E3). Each value reported was determined by the mean of 2 replications.

Acidity (Glass Electrode Method)

The pH value was determined after hydrolysis with a pH meter. (Xs, 300 Euteoh pc Instruments).

Morphological characterization by optical microscopy and scanning electron microscopy (SEM)

The morphological investigation of treated samples before drying and after drying was carried out by an optical microscope (Axioskop, Zeiss) and LEO 135 VP SEM (Leica Electron Optics) respectively. SEM operated with an acceleration voltage of 15 kV, 50 pA of current probe and 30 mm working distance. The samples were mounted on aluminum specimen stubs with double-sided adhesive

tape. Then the samples were sputter coated with a gold layer 20–30 nm thick in rarefied argon, using a sputter coater with a current of 20 mA for 4 min.

Fourier Transform Infra-Red Analysis (FT-IR)

Fourier Transform Infrared spectra were obtained by a Nexus Thermo Nicolet Spectrometer in Attenuated Total Reflectance (ATR) mode with 100 scans, in the range 4000–650 cm^{-1} with a resolution of 4 cm^{-1} and gain of 8.0. The FTIR spectra of dried samples obtained after treatment were compared with the original wool sample. Every spectra was baseline corrected, smoothed with a nine-point Savitsky–Golay function and normalized using amide I as the reference band. Moreover, the amide I band was resolved in Gaussian-shaped bands corresponding to different secondary structures of the proteins and oligopeptides using the Marquardt method[5]. The fitting procedure was done between 1600 and 1750 cm^{-1}, using the fitting module of the ORIGIN Pro 2015 software (OriginLab Corporation). The numbers of the fitting peaks were defined by second derivative analysis. The area of each fit peak was integrated and normalized over the total area of the amide I band in order to evaluate the contribution of each peak to the amide I band.

Amino acid Analysis

Original wool and wool samples obtained after treatment (40 mg) were hydrolyzed with HCl (6N) at 110 °C for 24 h under nitrogen atmosphere. Free amino acid residues were derivatized with 6-aminoquinolyl-N-hydroxysuccimildyl carbamate (AQC by Waters) and eluted through a reversed-phase column (Waters) using a ternary gradient of water, acetonitrile and acetate buffer. An Alliance (Waters) high-performance liquid chromatography (HPLC) was used and the eluate was detected at 254 nm. The quantitative amino acid composition was determined by calibration with the Amino Acid Standard H (Pierce), cysteic acid and lanthionine (TCI Europe) as external standards, and α-aminobutyric acid as the internal standard.

Molecular weight distribution

The molecular weight distribution of the proteins (freeze-dried powder) was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), according to Laemmli's method[6]. The original wool and the freeze-dried powders were dissolved in Tris/HCl (550 mM, pH 8.6), dithiothreitol (DTT, 140 mM), ethylenediaminetetraacetic acid (EDTA, 5 mM) and urea (8 M) overnight under nitrogen atmosphere[7]. Samples were dissolved in a sample buffer

containing NuPAGE LDS Sample Buffer and NuPAGE Sample Reducing Agent, as recommended by Invitrogen protocol. The SDS-PAGE was performed using a Xcell SureLock Mini-Cell (Invitrogen), on 4–12% polyacrylamide pre-cast gel (Invitrogen) using NuPAGE MES SDS Running Buffer, suitable for proteins with molecular weights from 188 to 3 KDa, referring to myosin, bovine serum albumin, glutamic dehydrogenase, alcohol dehydrogenase, carbonic anhydrase, myoglobin, lysozyme, aprotinin and insulin B chain as the molecular weight markers.

Chemical analysis of wool hydrolyzates nutrients

Organic carbon (C), total nitrogen (N), ammonium nitrogen ($NH4^+$), total phosphorus (P) and total potassium (K) were determined according to UNI EN 13137:2002[8], UNI 10780:1998[9], CNR IRSA 7 Q 64 Vol 2198, CNR IRSA 9 Q 64 Vol 3 19, standards respectively. Microelements (Br, Co, Cr, Mn, Hg, Ni, Pb, Cu and Zn) were determined according to the United States environmental protection analysis methods (EPA 3050, 3052, 3051 B 1996 and EPA 6010C 2007, EPA 7470A 1994).

Germination Rate

The test was performed by evaluating the germination of a plant Lepidium Sativum. The preparation involves the laying of Lepidium Sativum seeds, previously left to swell in the water on an absorbent paper sheet. The paper disks were placed in petri dishes and soaked with 1 ml of distilled water (for control) and with 1 ml of the diluted hydrolyzed wool sample at various concentrations. The concentrations used for the examination were: 1 g/L and 10 g/L; in particular the concentration of 1 g/L was assumed taking into account the use instructions of commercial products with similar characteristics used for foliar applications (N90 ILSAMIN of ILSA Spa). The sample used in the tests was obtained from the hydrolysis of 6.5 kg of greasy wool for 60 min due to short process time, limited process energy duty and production cost of any future industrial development. The germination investigation was stopped after 36 h, by counting the number of germinated seeds and root length. These values are used to determine the Germination Index (GI %) in relation to the control. The Germination Index was calculated through the formula

$$\text{Germination Index (GI) (\%)} = \frac{(G_s \text{ X } L_s)}{(G_c \text{ X } L_c)} \text{X } 100$$

Where:

GI= Germination Index,

Gs = Average number of sprouted seeds -hydrolyzed wool sample,

Gc = Average number of sprouted seeds -control sample,

Ls = Average root length -hydrolyzed wool sample,

Lc = Length radical media- control sample.

Surface tension

Different concentrations of keratin hydrolyzate solutions were prepared ranging from 0.00001 to 50% w/v, and the surface tension was measured by using a Sigma 700 force tensiometer (KSV Instruments).

3.5 Foam dyeing application of hydrolyzed keratin

3.5.1 Materials

Wool was obtained from a local supplier in Romania. Levafix Navy CA (Hetero-bifunctional reactive dye[1], DyStar Germany), Nylosan Navy S-3G SGR (Acid dye, Clariant) were used. A bleached and mercerized 100 % cotton fabric having 110 GSM, and wool fabric 150 GSM were obtained from Ausiliari Tessili Srl.

3.5.2 Equipments

3.5.2.1 Foaming hand mixer

In foaming study of keratin hydrolyzate and its application for foam dyeing, the foam is generated by using high-speed Philips HR1459 hand mixer as shown in figure 5. The whipping type of blades is used with a maximum speed of the equipment.

Figure 5. Foam producing hand mixer Philips HR1459.

3.5.2.2 Padding mangle

In foam dyeing study the dyeing of cotton and wool fabric was carried out using padding techniques. The padding mangle build by Matis (Switzerland) was used for padding application as shown in Figure 6. The padding mangle is equipped with the pressure control system to control the pressure between two squeezing rollers, which will be beneficial to adjust the desired wet pick up on wool and cotton fabric. The padding mangle has the facility to adjust the speed of the roller. The padding mangle consists of two rollers, one of them is built of heavy metal, steel and another is made of rubber.

Figure 6. Universal padding mangle Matis (Switzerland).

3.5.3 Methods

3.5.3.1 Foam Dyeing of Cotton and Wool Fabrics

In a study of the application of superheated water hydrolyzed keratin as a foaming agent and its use in foam dyeing of wool and cotton; the hydrolyzed keratin was produced by the same method as mentioned in section 3.2.3.

Dyeing

The foam was generated with mechanical agitation using high-speed Philips HR1459 hand mixer, for 3 min. All foam dyeing was performed on laboratory scale horizontal padding mangle (Matis, Switzerland). The wet pickup was determined for cotton and wool fabrics as the amount of water absorbed by the fabrics after padding at different pressure values. All foam dyeing tests were performed at a wet pickup of 40%. In the case of conventional dyeing which is used as a reference, dyeing was performed at a wet pickup of 70%. Dyeing liquor for conventional dyeing contains similar auxiliaries and dyes as mentioned below for cold pad batch reactive dyeing of cotton and for pad-steam acid dyeing of wool respectively, excepting the keratin hydrolyzate. The percentage dye fixation for both the foam and conventional dyed fabric samples was determined using the following equation.

$$\text{Dye fixation (\%)} = \frac{\left(\frac{K}{S}\right) \text{Value after soaping}}{\left(\frac{K}{S}\right) \text{Value before soaping}} \text{X } 100$$

Cold-pad batch reactive dyeing of cotton

Foam dyeing of cotton samples was carried out using reactive Levafix Navy CA. The dyeing liquor composition includes: 20 g/L keratin hydrolyzate as a foaming agent, 1-5% dye, 100 g/L urea, 65 g/L sodium silicate, 50% mL/L sodium hydroxide, depending on the amount of dye present in the dye solution. The recipe was obtained from the standard cold-pad-batch (sodium silicate) process of DyStar. The dye fixation was carried at room temperature, for 6 h batching on a slowly rotating beam. After dyeing, fabric samples were washed with cold water and then with hot water, followed by soaping treatment using 2g/L non-ionic soap

at boiling. Ten different samples were obtained at 1, 2, 3, 4, and 5 % dye concentration, both for foam and conventional dyeing.

Pad-steam, acid dyeing of wool

Prior to wool dyeing, the wool fabric was processed with Grinp P20 dielectric barrier discharge plasma (Grinp Srl, Italy), using helium (He) gas at a flow rate of 9 mL/min. The plasma treatment was carried out at a discharge power of 1000 W, and the fabric was passed under the plasma with a speed of 1 m/min[3]. Foam dyeing of wool samples was carried out using acid dye Nylosan Navy S-3G SGR. Dyeing liquor consists of 20 g/L keratin hydrolyzate as a foaming agent and 1-5% dye. The pH of the dyeing solution was adjusted to 4.5 using acetic acid. The dye fixation was carried out by steaming of the dyed samples, for 30 min, at 100-105 °C. After steaming, all fabrics were washed with cold water, followed by soaping treatment using 2 g/L non-ionic soap at 40 °C and rinsing with cold water. 10 different samples were obtained at 1, 2, 3, 4, and 5 % dye concentration, both for foam and conventional dyeing.

3.5.4 Characterization of keratin hydrolyzate foam

Blow ratio and foam stability of keratin hydrolyzate and dyeing solution

Volumes of 50 mL solution of both hydrolyzed keratin at different concentrations and hydrolyzed keratin along with dye and auxiliaries were stirred for 3 min. As soon as the stirring stops the half-life ($t_{1/2}$) and blow ratio were determined. The stability of foam was expressed in terms of its half-life, the time required to half its initial volume. Blow ratio was determined by the weight ratio of 50 mL volume of foaming solution to its equivalent volume of foam generated as mentioned in following equation[10].

$$\text{Blow ratio} = \frac{\text{Mass of 50 ml unfoamed solution}}{\text{Mass of 50 ml volume of foam}}$$

Bubble size

All the microscopic images were processed using the ImageJ v1. 51k software application. The image analysis was performed on all microscopic images; the images were calibrated using standard microscopic 1 mm length slide at the same magnification. In order to measure the bubble size accurately, the global scaling

factor was set as per standard 1 mm length image at 25X magnification. Image processing steps were the following: image conversion into gray, enhancement of contrast and edges, image conversion to binary by thresholding, circles fitting into the segments and overlapping of circles. Larger circles were kept and missing bubbles were selected manually in the image and bubble size was measured[11-12]. The processing steps of bubble micropgrah are shown in figure 7 and figure 8.

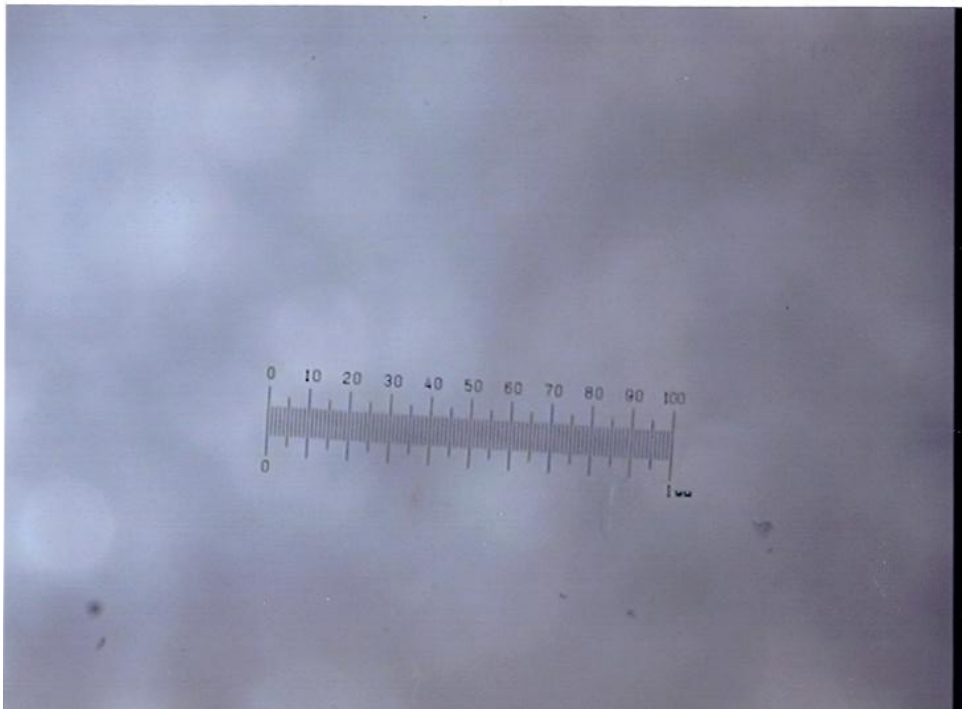


Figure 7. Image processing of bubble micrographs,1mm,25X standard length

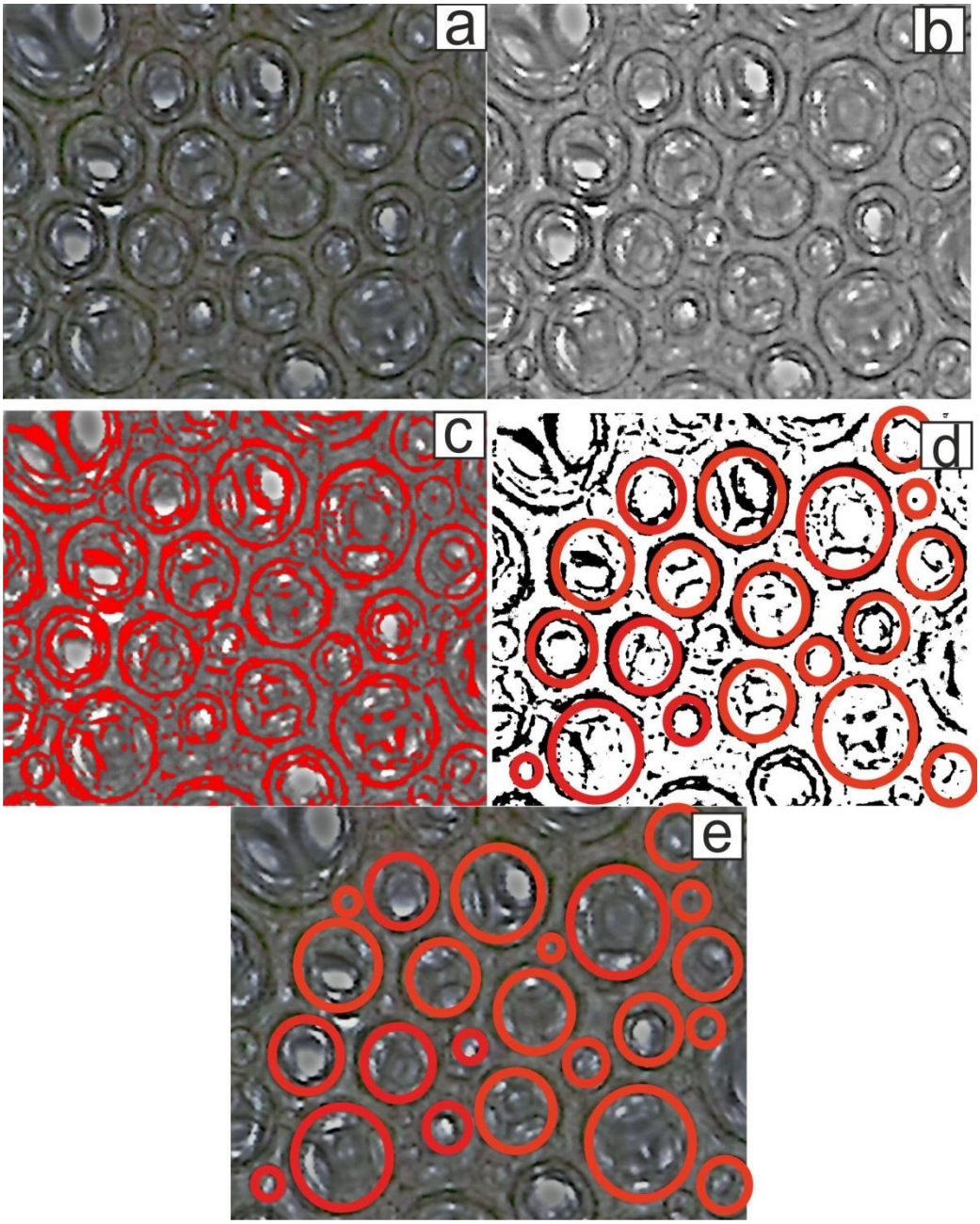


Figure 8. Image processing of bubble micrographs a) Image of bubbles (25x) b) image conversion into gray and the enhancement of contrast and edges c) Image conversion to binary by thresholding d) circles fitting into the segments and e) overlapping of circles

3.5.5 Characterization of Cotton and wool dyed fabrics

Cross section of dyed wool fibers

Yarns removed from wool fabrics were embedded in a resin (celloidin), further cut into slices about 10-20 µm thick cross sections using a microtome (2030 Mot Biocut, Reichert Jung) as shown in Figure 9 and mounted on microscopic slides for optical microscopy observation[13].

Figure 9. Microtome.

Color strength evaluation

The color strength (K/S) of foam and conventional dyed samples was measured using a Data Color Spectroflash SF 600X. The K/S values are based on the Kubelka–Munk theory, where equation mentioned below defines the relationship between the absorption coefficient, K, the scattering coefficient, S, and the reflectance of fabric at maximum absorption, R.

$$\frac{K}{S} = \frac{(1-R)^2}{2R}$$

Fastness Testing

The color fastness to washing of the dyed samples was tested according to UNI EN ISO 105 C06 standard[14]. Dry and wet rubbing were checked by UNI EN ISO 105 X 12 standards[15].

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Chapter 4

Green hydrolysis process and design optimization

4.1 Introduction

The green hydrolysis of greasy waste wool is a novel process, especially for treating the raw greasy waste wool, due to its structure and the impurities and contaminants present in raw greasy wool fibers. This hydrolysis process progresses from hydrolysis in small batch scale process to semi industrial scale rotating reactor, as process parameters and reactors details described in chapter 3. The process optimization and output of the hydrolysis product from every reactor is the basis for designing and construction of each type of reactor. These results are described in this chapter in order to understand the green hydrolysis process and design optimization. The first step of the process is the collection and analysis of raw greasy wool.

4.2 Results and discussion

4.2.1 Characterization of raw greasy wool

The 100 kg of raw greasy wool was stored in 3 different bags; prior to perform this analysis on raw greasy wool, the wool was picked from a small quantity of three different bags and made homogenous manually.

Table 1, 2, 3, 4 and 5 shows the percentages of moisture content, washing yield, vegetable matter, fatty matter and ash content present in greasy wool, prior to hydrolysis. For each testing 4 replications are carried out in order to get exact values. The values are refer to the dried material. The moisture content of wool was 12-13% w/w. In general, the moisture content of wool can vary considerably depending on the environmental conditions of storage.

The washing yield, indicating the percentage of clean, dry wool, was higher than 60 %, while the ash content of the wool was about 14 %, mainly due to the presence of inorganic (soil) and extraneous material, collected by each animal during grazing or in the farm, as well as dirt (solidified faeces and urine). Fatty substances (obtained from dichloromethane, DCM extraction) and vegetable matter were present in small quantity (about 5%). The results obtained are in agreement with the results obtained by other authors and referred to coarse wool, which have in general less contaminants compared to fine wool[10]. This characterization of the greasy wool is helpful as a reference value to better interpret the results from hydrolysis.

Table 1. Moisture content of greasy wool

Sample No	Initial weight (g)	Dry weight (g)	Humidity Content %
1	20.10	17.32	13.83
2	20.02	17.33	13.43
3	20.06	17.33	13.60
4	20.02	17.53	12.43

Table 2. Washing yield of greasy wool

Sample No	Initial weight (g)	Dry weight (g)	Washing Yield %
1	20.12	12.57	62.47
2	20.02	13.22	66.03
3	20.02	13.25	66.18
4	20.01	13.31	66.51

Table 3. Vegetable matter content of greasy wool

Sample No	Initial weight (g)	Dry weight (g)	Vegetable matter (g)	Vegetable matter %
1	20.02	17.42	0.2309	1.32
2	20.03	17.30	0.3157	1.83
3	20.02	17.45	0.3385	1.93
4	20.06	17.40	0.7124	4.09

Table 4. Grease content of greasy wool

Sample	Initial Weight of flask (g)	Final Weight of Flask (g)	Initial Wool weight (g)	Final wool weight (g)	Grease content.
1	108.70	109.40	20.06	16.60	5.60%
2	107.72	108.66	20.09	16.64	4.23%
3	107.60	108.34	20.04	16.34	4.56%
4	110.85	111.75	20.06	16.33	5.53%

Table 5. Ash content of greasy wool.

Sample	Initial Weight (g)	Wool (g)	After weight (g)	Ash Content
1	10.1487	3.059	10.5783	14.08%
2	10.2241	3.059	10.5908	12.02%
3	102228	3.029	10.5940	12.29%
4	10.1466	3.050	10.4554	10.12%

4.3 Small laboratory scale hydrolysis reactor

The preliminary hydrolysis of waste wool was performed on a small laboratory scale reactor in order to understand hydrolysis parameters (time, temperature, etc.) and the behavior of wool under hydrolysis treatment such as viscosity, moisture content of hydrolysis product, morphological properties etc. prior to develop the final laboratory scale hydrolysis reactor.

The objective of the hydrolysis of wool on small laboratory scale reactor is to determine the basic parameters for theoretical designing of the lab scale reactor:

- Material to liquid ratio (wool to water).
- Wool loading techniques such compressed or uncompressed loading.
- Physical properties of the final hydrolyzed product and effect of loading and handling parameters on it.

The several hydrolysis tests performed in this reactor are mentioned in chapter 3. The wool loading in a different way is clearly shown in following figure 1. The wool was fed into the reactor in two different ways where uncompressed wool has a bulk density of 70 kg/m^3, and with compression of wool it was possible to obtain wool density of 780 kg/m^3.

(a) (b)

Figure 1. Wool loading a) uncompressed wool b) compressed wool.

This type of reactor hasn't the possibility to obtain desired 180 °C temperature, hence the hydrolysis reaction are limited to maximum attainable temperature of 160 °C. As mentioned in chapter 3.2.1 the hydrolysis reaction was performed for total 2 h, where the time required to heat the reactor to reach desired temperature is included. In test 1 the wool loaded inside the reactor is uncompressed and the water is simply poured onto the wool fibers without proper impregnation. The results obtained from the hydrolysis shows that at a temperature of 156 °C with mentioned type of wool loading the incomplete hydrolysis of wool, and change in color to brownish occurred, where some of the fibers show still its fibrous structure. The hydrolysis product obtained is consisting of excess water at the end of reaction due improper wetting of wool inside the reactor due to the hydrophobic nature of greasy wool. The wool hydrolysis product at end of test is shown in figure 2. In figure 3 no effect of hydrolysis on wool structure is clearly visible.

Figure 2. Wool hydrolyzate obtained from test1.

On the contrary to the results obtained from test 1, the results obtained from test 2 are more promising in terms of degree of hydrolysis. In test 2 effect of wool loading on end hydrolyzed product is clearly visible in figure 4. The wool prior to hydrolysis is compressed and added water is well impregnated inside the greasy wool fiber to improve its contact with water during hydrolysis reaction. The hydrolyzed product from test 2 shows a higher degree of hydrolysis than test 1 and is appearing in black/dark color, no fibrous appearance of wool and

hydrolyzed wool consisting of water and appeared to swollen in the form of dough. The 10.5 g of hydrolyzed product is further dried in oven at 105 $^{\circ}$C in order to estimate its water content.

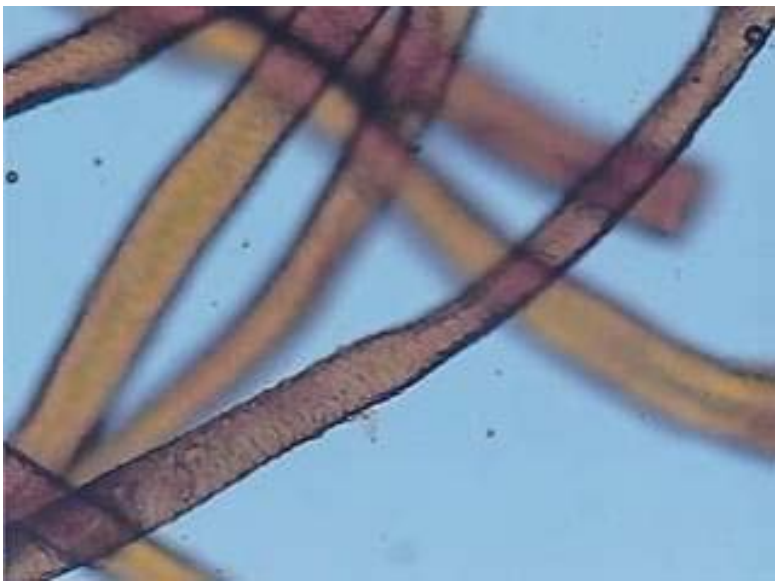


Figure 3. Optical micrograph (200X) of hydrolyzed wool from test 1.

The results obtained from drying show that it is easy to crush and make powdered shown in figure 5 and has dry mass of 3.6 g which means residual water is 66 % by weight of hydrolyzed product. The effect of hydrolysis on wool morphology is shown in figure 6 where wool fibers appeared to be swollen and change in its morphology

(a) (b)

Figure 4. a) Greasy wool compressed and well impregnated with water prior to hydrolysis and b) hydrolyzed wool after hydrolysis in test 2.

Figure 5. Dry hydrolyzed wool product from test 2.

Figure 6. Optical micrograph (200X) of hydrolyzed wool from test 2.

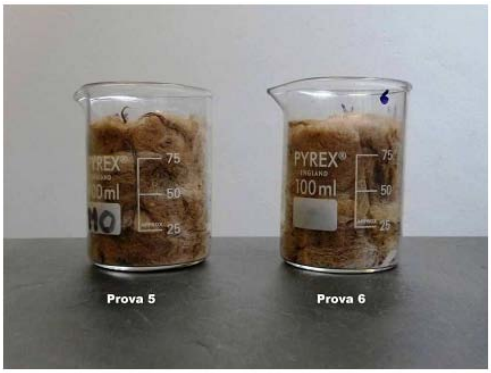
The same material to liquor ratio as mentioned in test 1 and test 2 is used in test 3, 1:3 (MLR) but the wool loading is varied. In test 3 wool is completely wet with water prior to hydrolysis but kept remain uncompressed, the result of hydrolysis shows that its unable to obtain a sufficient degree of hydrolysis even though wool is wet and liquor ratio is maintained same. The hydrolyzed product obtained at the end of the reaction has the same behavior as shown in test 1 which is brown in color and remained in its fibrous nature. The effect of hydrolysis on wool morphology results in no damage to wool morphology is shown in figure 8.

(a) **(b)**

Figure 7. a) Greasy wool uncompressed and well impregnated with water prior to hydrolysis and b) hydrolyzed wool after hydrolysis in test 3

Figure 8. Optical micrograph (200X) of hydrolyzed wool from test 3.

In test 4 prior to hydrolysis the wool is loaded uncompressed and the material to liquor ratio change to 1:6. The hydrolyzed product obtained from hydrolysis looks similar to results obtained from test 2 and consists of excess amount of water. These results suggest that a higher amount of water is required to the hydrlozed uncompressed state of wool to obtain a sufficient degree of hydrolysis. In test 5 and 6 the wool loading is performed in a beaker. The wool is wet and compacted in a beaker and then these beakers are introduced in hydrolysis reactor. The material to liquor ratio is varied such as 1:3 and 1:2 for test 5 and 6 respectively. The wool loading in a beaker, placement in a reactor and hydrolyzed product are shown in figure 9.



(a)

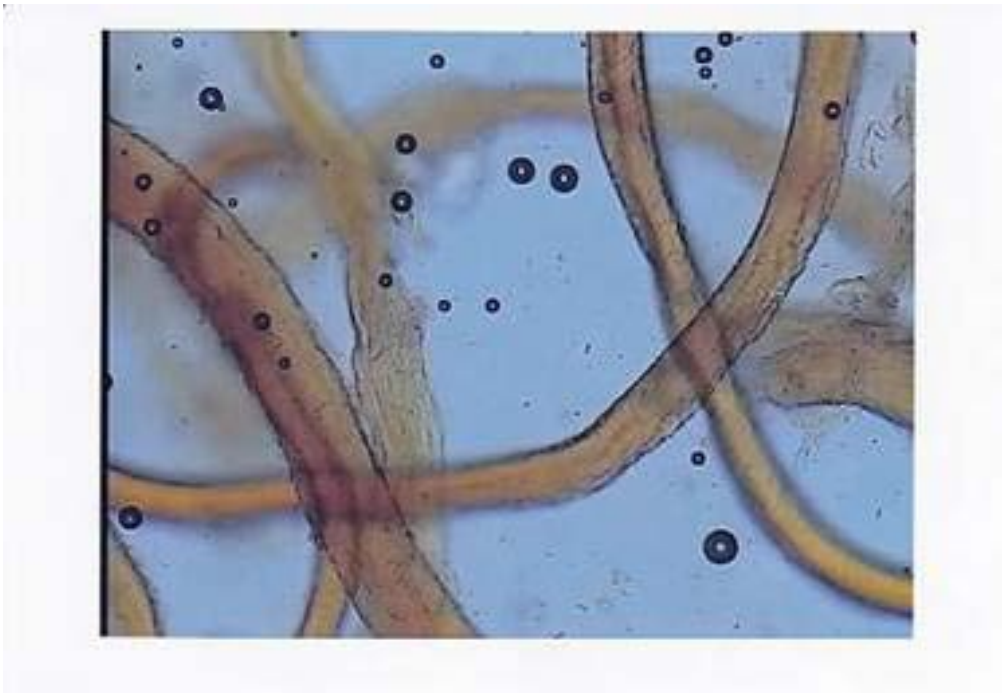
(b)

(c)

Figure 9. a) Greasy wool compressed and well impregnated with water prior to hydrolysis and b) place of beakers inside the reactor and c) hydrolyzed wool from test 5 and test 6.

The hydrolysis results from test 5 and 6 show that hydrolyzed wool have same material consistency. The hydrolyzed product appeared to be in viscous/paste form and disappearance of fibrous structure was found. Before removing the samples from the reactor at the end of hydrolysis, it was observed that the effect of hydrolysis was more at the bottom part where wool is in more contact with water. The material to liquor ratio of 1:3 is sufficient to obtained paste form hydrolyzed product in comparison with material to liquor ratio of 1:2. The optical micrograph results of test 5 and test 6 are shown in figure 10 where wool fibers obtained from both hydrolyzed product from test 5 and 6 appeared to be damaged.

(a)



(b)

Figure 10. Optical micrograph (200X) of hydrolyzed wool from a) test 5 b) test 6.

In general, it was observed from the results that presence of steam is not enough to ensure a consistent degree of hydrolysis. Compressed wool samples can be completely wet and impregnated with lower amounts of water, with respect to the non-compressed samples, showing the best results in terms of hydrolysis. The optical microscope analysis demonstrated that original wool fibres are fragmented and totally swollen, giving origin to a slurry-type product, very dense and viscous. The solid to liquid ratio has been evaluated as well. The optimal conditions, which minimize the quantity of water needed, but allow to reach a consistent hydrolysis degree, have been established considering a ratio “1:2” and “1:3”. These results have been extremely useful for the designing and testing of the laboratory-scale unit.

4.4 Final laboratory scale hydrolysis reactor

On the basis of results obtained from small laboratory scale reactor the designing and construction of the final laboratory scale reactor was carried out. The points taken into consideration on the basis of previous reactor hydrolysis are:

- Pressing or compactness of wool is a preliminary stage prior to start hydrolysis.
- Compactness of wool with low material to liquor ratio performed better in hydrolysis process, as pressing of wool results in homogenous wetting of wool in comparison with uncompress or loose wool.
- Application of compactness allows us to process large amount of wool in comparison with uncompressed wool, while use of low liquor will be beneficial in terms of energy where low energy is required to heat less amount of water.
- The hydrolyzed product with low liquor ratio results in paste or viscous means more concentrated product which will be helpful not only in the transportation of hydrolyzed material, but also in case of drying of product where less energy and time are consumed.
- It was confirmed that only superheated water is able to hydrolyzed wool, which is beneficial in terms of cost saving in case of use of additional reagents (such as acid, base, enzyme etc).

Among all the experiments performed on this reactor, results obtained from key experiments are reported in this section which helps to understand and identify better hydrolysis process conditions. It was observed from the results that the reaction temperature plays an important role in hydrolysis process. The hydrolyzed product obtained at the end of the test carried out at 180 $^{\circ}$C for 1 h with material to liquor ratio of 1:8 and wool density of 160 kg/m^3 is completely in liquid phase, which is collected in a collection tank; the material remaining in perforated hydrolysis vessel consists mostly of vegetable matter present in raw wool and some partially hydrolyzed wool as shown in figure 11.

(a)

(b)

Figure 11. Hydrolyzed product at 180 °C, 1:8, 60 min a) liquid phase b) solid residues mostly vegetable matter.

In following hydrolysis tests the temperature of hydrolysis is kept constant at 180 °C but the change in hydrolysis time (30, 60 min), liquor ratio from 1:8 to 1:3 and wool density from 160 to 350 kg/m^3 is implemented. The results obtained at the end of 30 min and 60 min of hydrolysis reaction are similar as shown in figure 11. The hydrolyzed product is in liquid phase while some solid residues from vegetable matter and partially hydrolyzed fibers are observed, while lowering the hydrolysis time yields in slightly more solid phase residues in comparison with 60 min of hydrolysis time.

In following test wool density increase from 350 to 500 kg/m^3 and liquor ratio reduce to 1:2; 1:1.5 is implemented keeping the same temperature 180 °C and time 60 min. The results show that even lowering the liquor ratio the hydrolyzed product recovered in liquid phase is similar to shown in figure 11 and solid phase where it shows higher extent of hydrolysis and appeared to be in paste /viscous form as shown in figure 12. The yield obtained from this test is shown in table 6.

(a)

(b)

Figure 12. Hydrolyzed product at 180 °C, 60 min a) Solid phase obtained using liquor ratio 1:2 b) Solid phase obtained using liquor ratio 1:1.5.

Table 6. Effect of hydrolysis parameters on yield.

Material to liquor ratio	Wool density (kg/m^3)	Temperature (^0C)	Yield
1:2	500	180	846 g solid, 2.9 L liquid
1:1.5	500	180	940 g solid, 1.6L liquid

The next set of hydrolysis tests are performed maintaining constant temperature at 170 $^{\circ}C$ and wool density of 350 kg/m^3. The change in hydrolysis time (30, 60 min) and liquor ratio 1:3, 1:2.5 is implemented. The hydrolysis performed maintaining the parameters as mentioned above and varying liquor ratio 1:2.5, 1:3 for 30 min, 60 min resulted in liquid phase and solid phase while some solid residues from vegetable matter are also observed. The decrease in the hydrolysis time yields in slightly more solid phase residues in comparison with 60 min of hydrolysis time, but the extent of hydrolysis of the end product is observed to be consistent. One test was performed using parameters, temperature 170 $^{\circ}C$, density 350 kg/m^3, liquor ratio 1:3 and time 60 min. It was observed at the end of the hydrolysis process that the higher extent of hydrolysis occurred at the wall of reaction vessel in comparison to the central part of a vessel as shown in figure 13. This phenomenon is explained in section 4.3.2. The effect of wool hydrolysis time on wool morphology is shown in figure 14 and 15 and wool appeared swollen and damaged at the end of 30 and 60 min of hydrolysis.

Figure 13. Solid residues at the end of hydrolysis, vary from wall of reactor vessel to the center part.

Figure 14. Optical micrograph (100X) of hydrolyzed wool from test 170 °C, density 350 kg/m^3, liquor ratio 1:3 and time 60 min.

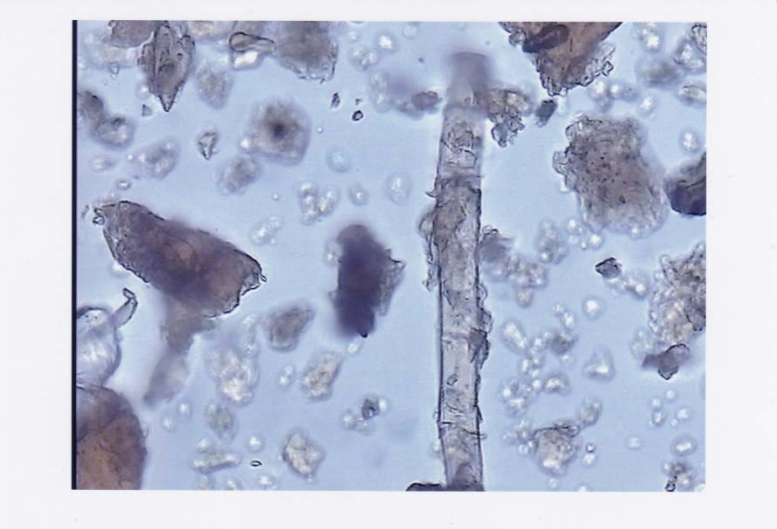


Figure 15. Optical micrograph (100X) of hydrolyzed wool from test 170 °C, density 350 kg/m^3, liquor ratio 1:3 and time 30 min.

Later on tests was performed with decrease in hydrolysis temperature from 160 °C, 150 °C to 140 °C. It was observed that as the temperature of hydrolysis decreases, there is an increase in the solid fraction in hydrolyzed product. The variation in temperature is able to obtain more solid or liquid part in the end

hydrolyzed product. The material to liquor ratio also plays an important role in order to obtain the homogenous hydrolyzed product.

4.4.1 Effect of material to liquor ratio

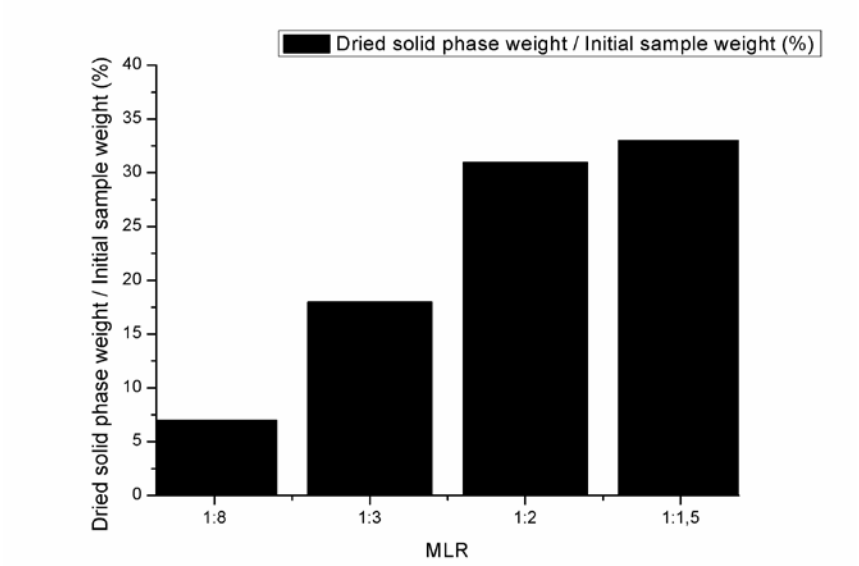


Figure 16. Solid phase dried weight compared to the weight of the initial sample.

The different test performed at temperature of 180 $^{\circ}$C by varying the material to liquor ratio was shown in figure 16. The results show that at higher liquor ratio 1:8 the hydrolyzed product contains only 5% solid part while rest is obtained in liquid phase. As the liquor ratio decreases from 1:8 to 1:1.5 the percentage of solid phase starts to increase. Even at high temperature of hydrolysis, liquor ratio plays a significant role to obtain a different amount of solid or liquid phase. The liquor ratio of 1:3 seems to be an optimal liquor ratio in order to obtain certain amount liquid parts, use of low liquor ratio will be helpful in a way that less amount of energy will be required to heat less amount of water.

4.4.2 Effect of wool density on heat transfer during hydrolysis

The complete study of wool bulk density and heat transfer modeling of hydrolysis reactor was carried out by Giansetti et al. The authors observed that wool bulk density is important in terms of energy saving where the less amount taken by the wool and to have an effective impregnation, it needs to be optimized because high

bulk density resulted in heat transfer problems from outside to inside, near to center of reaction vessel resulting in the non homogenous hydrolysis of wool fibers. Wool is a natural insulating material as the bulk density increases it results in strong insulation and affects the heat transfer phenomenon inside the reactor. The wool hydrolysis is an endothermic reaction. In this reactor the heat transfer is one directional, heat enters from the wall of the reactor (where heating element is arranged) towards the center of spindle. The static system of reactor does not contribute in heat transfer phenomenon inside the reactor as it does not equipped with any stirring or mixing accessory in it.

Hence, in order to overcome this problem the modification of perforated reaction vessels and the introduction of new perforated spindle to it was carried out. In case of reaction vessel modification the diameter of initial vessel is increased and the distance from the bottom was minimized in order to decrease the "dead" areas in the reactor, i.e. those occupied only by the water. The introduction of central perforated spindle shows the remarkable behavior of liquid circulation through the central part of reaction vessel results in increase in heat transfer phenomenon. The study was carried out by Giansetti et al. on two different densities 350 kg/m^3 and 500 kg/m^3. With the modification as mentioned above it is possible to process 350 kg/m^3 wool during hydrolysis with homogenous results.

This study of heat propagation with and without perforated spindle was carried out using thermocouples. To understand the heat transfer inside the hydrolysis reactor during hydrolysis, thermocouples are introduced to monitor the temperature of the water within compact wool. In initial tests, the two thermocouples were placed respectively in the area of liquid between the reactor vessel and the reactor wall and inside (in the center of) compact wool. These two thermocouples PT-100 type are placed on the wall of the reactor, near the bottom. In this test, hydrolysis reaction was performed using parameters such as, temperature 160 $^{\circ}$C, liquor ratio 1:3, time 60 min and with wool density 350 kg/m^3. However, in this case, in order to improve homogenization the initial impregnation of materials took place in the reaction vessel using 2 liters of water for 1 kg of wool, and then proceed with pressing and gradual inclusion of 1 liter of remaining water. The placement of thermocouple and compression of wool is shown in figure 17, 18.

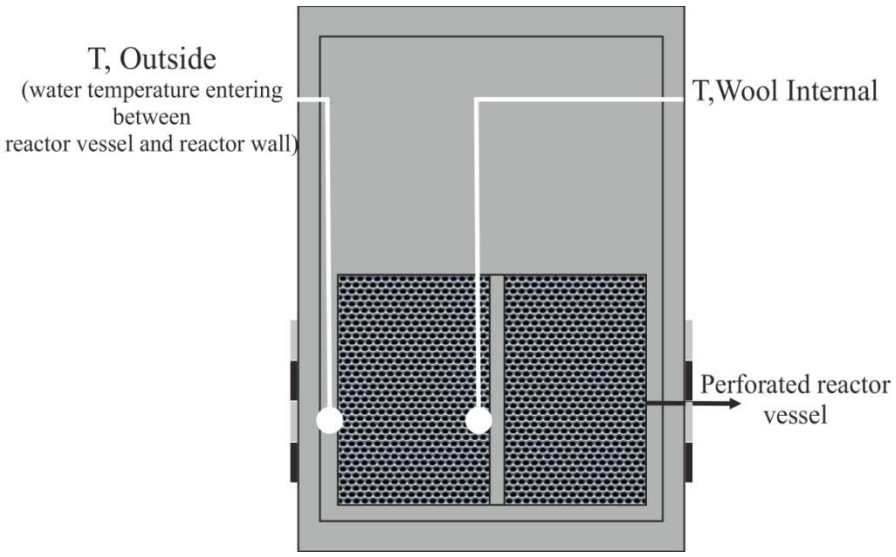


Figure 17. Schematic diagram of placement of thermocouples inside reactor without spindle.

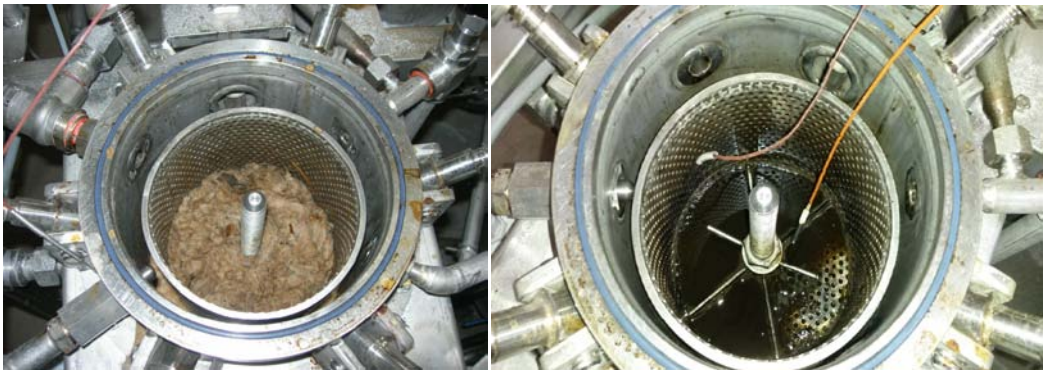
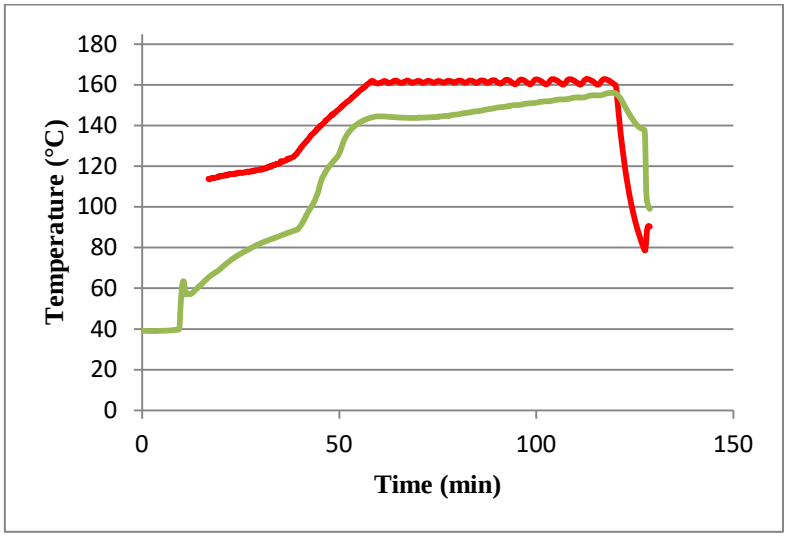
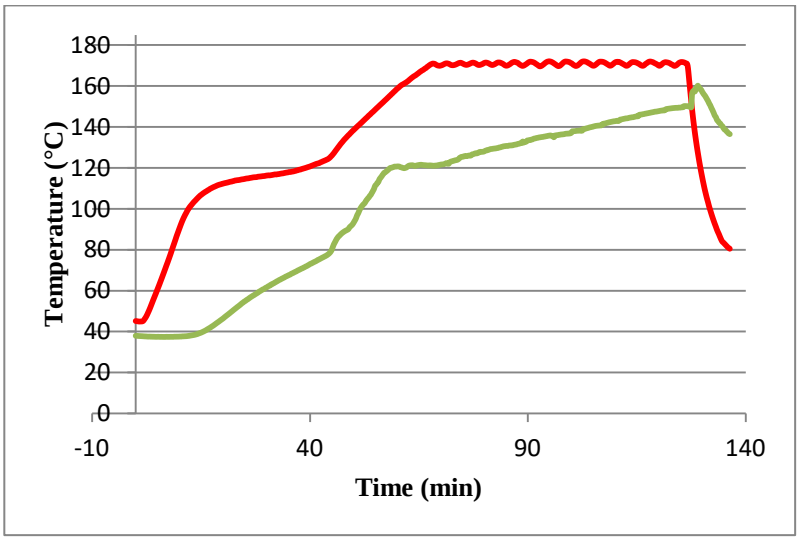


Figure 18. Compression and wetting of wool and placement of thermocouples inside reactor vessel.

The temperature profile of hydrolysis shown in figure 19, the attainment of the set temperature (160 °C) into the liquid came in about 60 min; however, there is a significant difference between water temperatures coming inside the reactor and those of the interior. This is indicative of the fact that the temperature front has difficulty moving from outside to inside, most likely due to the substantial degree of compaction (wool is an insulator) and the endothermic reaction. In particular, it was noticed that the temperature inside the wool never reaches the reaction temperature. The similar results are obtained even using the same parameters and increasing the hydrolysis temperature to 170 °C.



(a)



(b)

Figure 19 -Temperature profiles within the hydrolysis reactor (without spindle). Red line shows outside temperature (water temperature entering between the reactor vessel and reactor wall) and green line showing internal wool temperature. a) 160 °C b) 170 °C.

The previous test highlighted the problem of temperature difference from the outside to the inside of the material. The problem was overcome in this test with

the use of perforated spindle and modified reaction vessel. In this test, two thermocouples were used, inserted in the middle and central (near the rod) part of compact wool shown in figure 20.

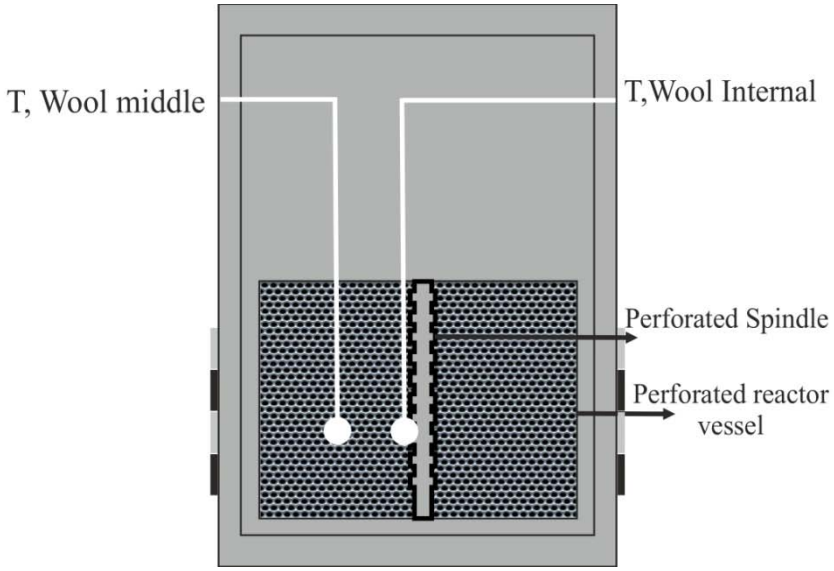


Figure 20. Schematic diagram of placement of thermocouples inside reactor with spindle.

The compression of wool was carried out as mentioned in the previous test and process parameter set to 170 $^{\circ}$C, 1:3, 350 kg/m^3 wool density and time 60 min. The temperature profile shown in Figure 21, in this case, the uniformity of treatment proves to be very good. As demonstrated by the behavior of the monitored temperatures, the preset temperature (170 $^{\circ}$C) is reaching into the liquid was approximately in 60 min. In particular, it was noticed that the temperature inside of the wool reaches to desired set temperature, which results in proper functioning of the reactor.

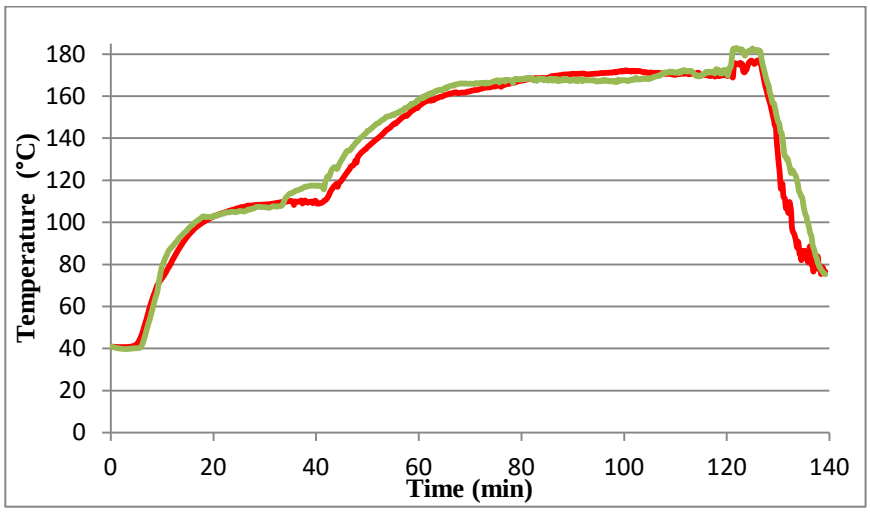


Figure 21 -Temperature profiles within the hydrolysis reactor. Red line shows the temperature of the middle part of wool and green line showing internal wool temperature. a) 160 °C b) 170 °C.

Further trial was performed using additional thermocouple which was inserted in a corner of reaction vessel as shown figure 22; the temperature profiles obtain showing similar results as shown in figure 21. The modification of reaction vessel with central perforated rod resulted in proper heat transfer from outside to inside the compressed wool results in homogeneous hydrolysis.

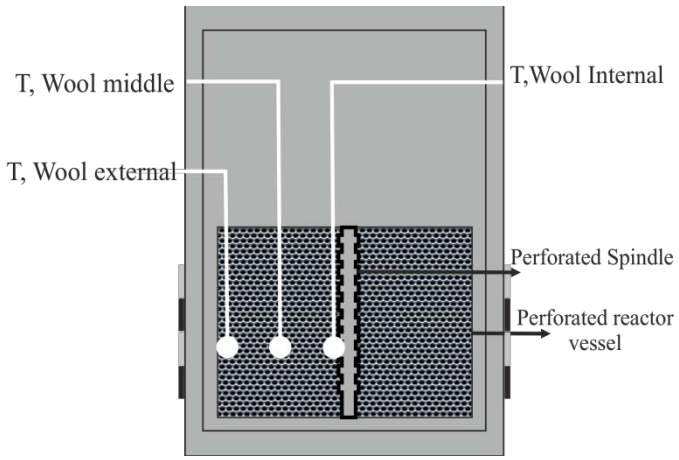
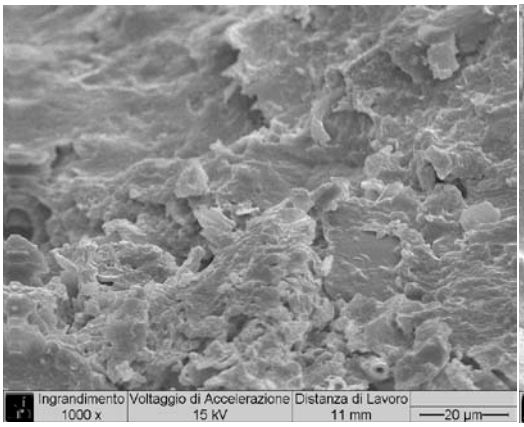
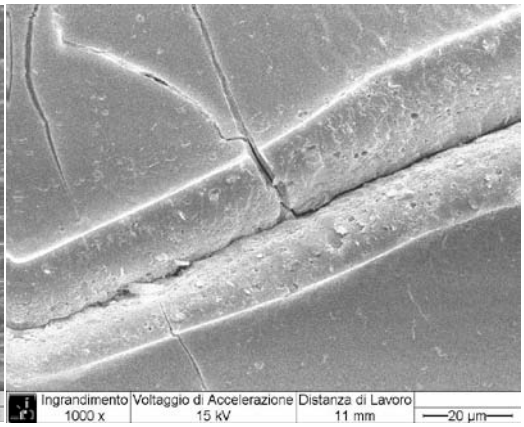


Figure 22. Schematic diagram of placement of thermocouples inside reactor with spindle.

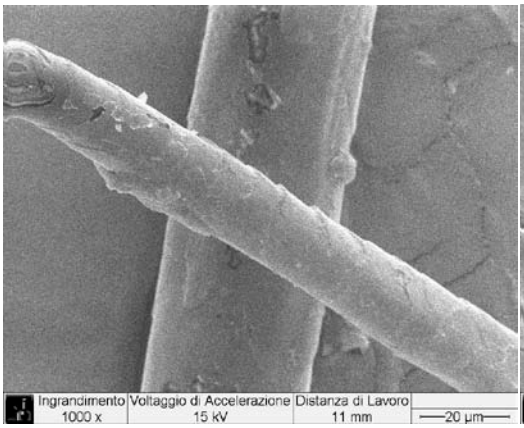
4.4.3 SEM analysis



(a)



(b)



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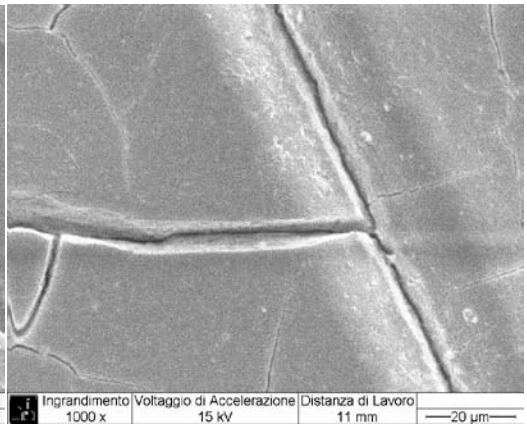

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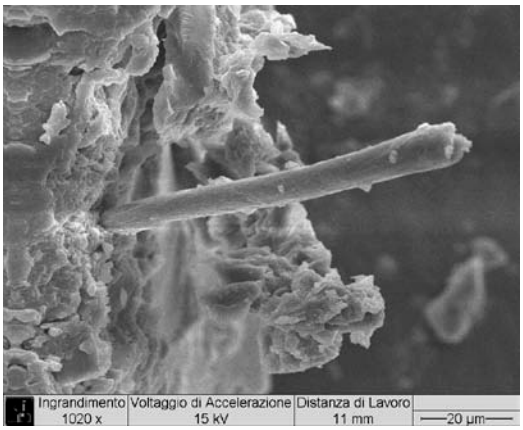

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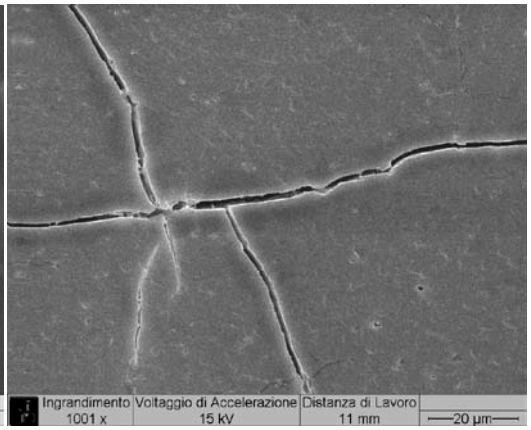
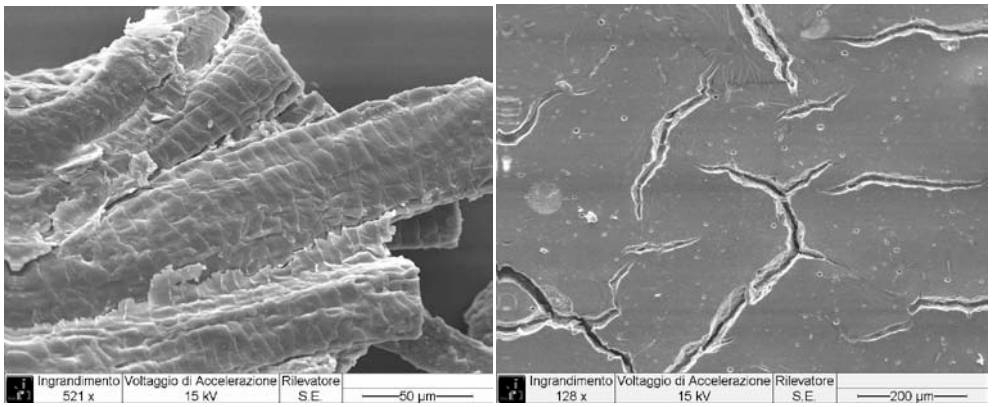
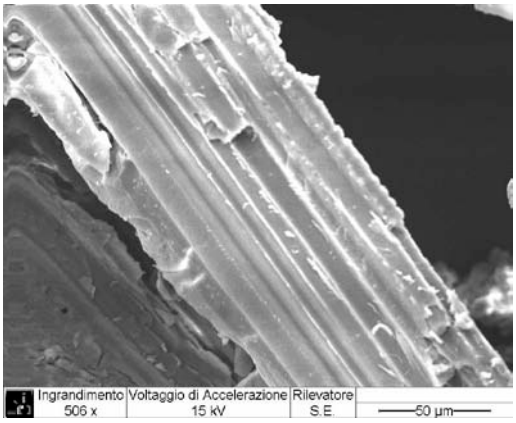


(f)



(g) (h)



(i)

Figure 23. SEM analysis of the hydrolyzed product obtained from different test having same liquor ratio 1:3, hydrolysis time 60 min and wool density 350 kg/$m^{3.}$ On the left solid phase and on the right is liquid phase a) 170 $^{\circ}$C solid phase b) 170 $^{\circ}$C liquid phase c) 160 $^{\circ}$C solid phase d) 160 $^{\circ}$C liquid phase e) 150 $^{\circ}$C solid phase f) 150 $^{\circ}$C liquid phase g) 140 $^{\circ}$C solid phase h) 140 $^{\circ}$C liquid phase i) vegetable matter

All the hydrolyzed products obtained from different tests in liquid phase were dried in an oven at 50 $^{\circ}$C and are in the form of films. Microscopic investigations by SEM shows undifferentiated structures not attributable to particular materials. All dried liquid phases show the same characteristics at the different temperatures of hydrolysis. In SEM image 23a from solid phase shows only shapeless aggregates produced at 170 $^{\circ}$C in the process of hydrolysis. Some fibers show no cuticular layer and are morphologically more damaged. More dissolution of wool fiber at this temperature indicates abundance of vegetable matter because the

dissolution temperature of vegetable matter is more in comparison with the wool. In the sample treated at 160 °C, wool fibers still retain their elongated shape morphology but cuticles are completely removed. Furthermore, the fibers generally have a bent, twisted morphology with fragile breaks. The solid phase of the sample treated at 150 °C shows less damaged fibers. Samples treated at 140 °C, in solid phase the appearance of wool fiber shows morphological characteristics of wool with cuticular scales. Fiber appearance is sticky in nature with each other because of foreign materials or proteins. The fibres shown in SEM image 23g are more bent in comparison with the normal wool fiber. The fracture of wool fiber is smooth; it indicates brittleness of treated fibres. It is observed a certain amount of vegetable substances in a hydrolyzed product shown in image 16h, due to their concentration in the solid phase by dissolution of the wool at these temperatures. As regards the solid phases, their morphology depends on the temperature of hydrolysis.

4.4.4 Molecular weight analysis

The gel electrophoresis patterns of hydrolyzed product obtained in the form of solid phase and liquid phase at different temperatures are shown in figure 24. The electrophoresis patterns are compared with molecular weight patterns of original wool. The electrophoresis patterns of original wool keratin fractions (lane 2, 3) indicate mainly low sulphur (LS) proteins of the intermediate filaments having a molecular weight in the range of 67–43 kDa and the high sulphur (HS) proteins, whose molecular weight falls in the range of 28–11 kDa. The electrophoresis patterns of liquid phase hydrolyzed product show strong bands of low sulphur proteins are disappeared. Hence, the protein having a molecular weight in the range of 8-3 kDa appeared with strong signals. The product obtains in the form of solid phase at low temperature hydrolysis treatment also shows a reduction in molecular weight of proteins. The effect of hydrolysis treatment is clearly visible in electrophoresis patterns.

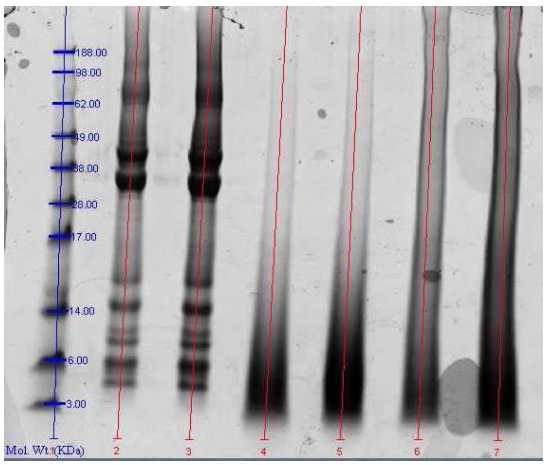


Figure 24. Lane 1 standard molecular weight of proteins; lanes 2 and 3 original wool, lanes 4 (170 °C) and 5 (160°C) liquid phase; lanes 6(170 °C) and 7(160°C) solid phase liquor ratio 1:3.

4.4.5 Amino acid analysis

Amino acid analysis of hydrolyzed wool obtained in solid and liquid phase at 170 °C for 1 h liquor ratio 1:3 are shown in table 7. In the solid and liquid phase hydrolyzed product, prominent changes were occurred in comparison with the original wool are the loss of ½ cystine residues and formation of lanthionine residues. The loss occurred in ½ cystine residues from 8.66 mole% to 0.38 and 0.25 mole % for liquid and solid phase respectively.

Table 7. Amino acid analysis of hydrolyzed wool liquid and solid phase

Amino Acid	Wool (Mole %)	Liquid Phase	Solid Phase
CYA	0.20	0.19	0.28
ASP	9.05	9.11	9.66
SER	10.95	7.18	8.59
GLU	15.54	19.09	17.51
GLY	6.53	11.91	11.50
HIS	0.61	0.80	0.90
ARG	6.25	5.85	5.88
THR	6.04	3.14	3.78
ALA	5.84	9.09	8.44
PRO	6.61	7.04	7.08
LANT	0.62	0.77	1.47
1/2CYS	8.66	0.38	0.25
TYR	2.46	2.50	2.34
VAL	5.91	6.74	6.38
MET	0.43	0.52	0.40
LYS	3.68	3.18	3.57

ILE	3.15	3.57	3.32
LEU	7.23	8.28	7.66
PHE	1.97	2.19	2.19

The amount of lanthionine residues increased from 0.62 mole % to 0.77 and 1.47 mole % in liquid and solid phase respectively. It is clear that the effect of high temperature hydrolysis in case of solid and liquid phase results in high degradation of cystine and breakage of disulphide bonds, which makes wool keratin particularly stable and difficult to degrade in soil. The hydrolysis treatment allows faster degradation compared to untreated wool, and such degradation can be modulated according to the extent of hydrolysis (degree of degradation) of the protein.

4.4.6 Agronomical properties of hydrolyzed product

The chemical and agronomical test was performed by UPTOFARM Srl. in order to understand the fertilizer properties of hydrolyzed product. In their preliminary analysis they found that hydrolyzed product obtained using different hydrolysis treatment and final laboratory scale reactor consists of primary and non nutrients such as carbon, nitrogen, potassium etc as shown in table 8. The availability of these nutrients in a hydrolyzed product is a positive sign to find its application as an agricultural bio/organic fertilizer. Use of the hydrolyzed product having nitrogen and other nutrients will be beneficial of plant growth and nourishment.

Table 8. Composition of nutrient elements presents in the liquid phase of different temperature hydrolyzed samples.

Sample Temperature °C, MLR 1:3, 60 min	C (%)	N (%)	P (%)	K (%)	Ammonium nitrogen (%)	C/N Ratio
Greasy wool	32.52	7.62	0.05	2.33	1.22	4.26
140	12.26	3.20	0.005	0.89	0.20	3.83
150	8.06	2.08	0.017	0.49	0.26	3.87
160	9.09	2.28	0.018	0.53	0.34	3.98
170	15.21	3.01	0.027	0.96	0.18	5.05

The liquid phase obtained from the hydrolysis process appears to be turbid color suspension from light brown to very dark, with odor varies depending on process conditions. Suspended solids increase with the degree of hydrolysis, as well as the nitrogen and carbon content. The pH of the suspension is always alkaline, predisposing factor for the loss volatilization of ammonium nitrogen. In general, the liquid phase resulting from the hydrolysis process is homogenous and stable over a time; it seems to be a promising start to investigate in depth on his abilities to support the plant growth.

The analysis of the solid phases derived from the various processes shows first of all a considerable non-homogeneity of the products, obtained according to the process conditions, especially the degree of hydrolysis related to the temperature applied. One option may be a further drying step which would yield a product with a high content of nutrients, stable over time and easily transportable. The amount of total nitrogen varies about 3-4%. A commercial fertilizer comparable with the product obtained by this process, it could be the dried manure, which generally presents carbon values around 20-25% and of about 3% nitrogen.

4.5 Semi industrial scale hydrolysis reactor

On the basis of experimental parameters and physiochemical characterization of hydrolyzed product performance using final laboratory scale reactor the design and construction of semi-industrial scale rotating reactor has been carried out. The final laboratory scale reactor needs some minor changes in order to improve the evenness in heat propagation inside the reactor, product homogenization, homogenous wetting of wool inside the reactor. The determination of optimal hydrolysis time of 60 min and liquor ratio (1:3) allow the industrialization of the process, by varying solid and liquid phase within a fairly broad range of temperature variation (140 $^{\circ}$C to 170 $^{\circ}$C).

To overcome all these limitations and considering optimal parameters of previously build reactor is the basis for designing and construction of semi industrial scale rotating reactor to hydrolyze large volume of raw wool.

4.5.1 Wool wetting

The tuning of the reactor begins with the basic problem of homogenous wetting of wool fibers inside the reactor. The preliminary test was performed using 6 kg raw

wool, liquor ratio 1:3 and a rotation speed of reactor maintained at 14 rpm. After loading of wool inside the reactor, it was noticed that the volume of the reactor is completely taken by wool. Then water is introduced in the reactor, which fill-in empty areas within the wool fibers. The wool wetting test was performed for 20 min and within the interval of 5 min of rotation, a volume taken by wool fibers was measured by removing the water from the valve placed at the bottom of the reactor. After each measurement the water was again introduced in the reactor and the cycle continues until 20 min. The results obtained at the end of each rotation cycle is shown in table 9.

Table 9. Water volume recovery at different time interval of rotation in semi industrial scale reactor.

Total rotation time (actual) (min)	Rotation time (single cycle) (min)	Recovered water volume (L)
5	5	9
10	5	7
15	5	5,4
20	5	6,2

Visual examination of wool samples inside the reactor was done at the end of each cycle in order to observe the wetting, impregnation, compaction and mixing of material with water simply by opening the flange at the top of the reactor. It was observed from the visual examination that at the end of first two rotation cycles corresponding to time of 10 min, wool fibers appeared to significantly wet and compact. The results obtained at the end of fourth cycle suggest that at the end of 20 min wool fibers are unable to absorb more water anymore. The amount of water which remains inside the reactor and impregnating the wool fibers is 12 L.

At the end of the wool wetting test the wool samples were collected from different area of reactor such as top and middle of the reactor in order to find out the moisture regain due to the wetting and impregnation of wool fibers during wetting. The wool sample was weighed after removal from the reactor and the totally dried in the oven and further dry weight measurement was takes place. The result of moisture regain by wool fibers is shown in table 10. It can be seen from the table that the top of the reactor, which generally consist of external areas of

the wool fibres agglomerates has higher moisture regain with respect to the middle of the reactor. Despite that, the total amount of water which can be trapped among fibres agglomerates or absorbed into the fibres is relatively high, demonstrating the good impregnation obtained with the rotating mechanism.

Table 10. Moisture regain of wool fibers in different areas of reactor due to rotational mixing in the semi industrial scale reactor

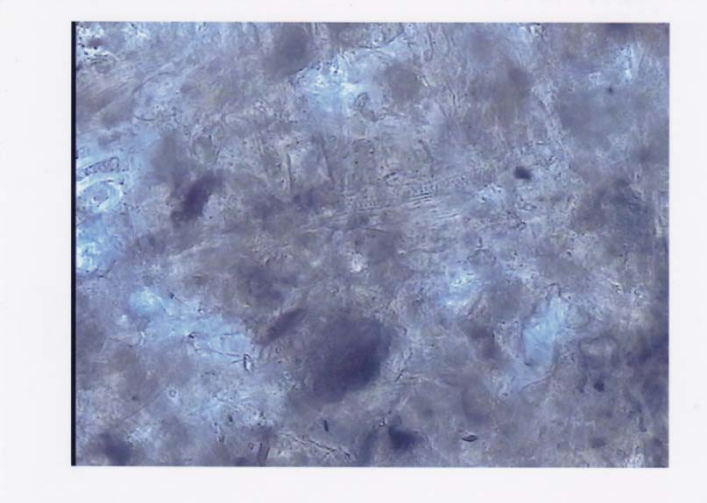
Sample No	Area of reactor	Wet weight (g)	Dry Weight (g)	Moisture regain (%)
1	Top of reactor	181.65	52.77	244
2	Top of reactor	154.87	46.67	231
3	Middle of reactor	88.89	33.62	164
4	Middle of reactor	41.29	16.28	153

4.5.2 Wool density

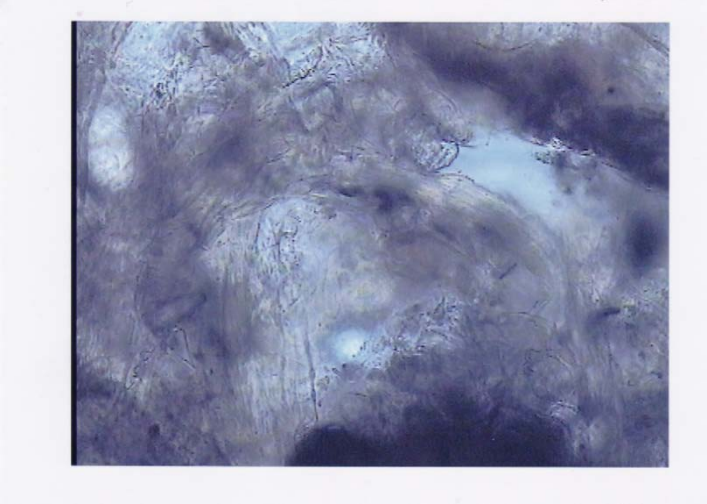
As we know from the previous rector's study, wool density also plays important role in resulting homogenous hydrolysis. In order to understand its influence on this type of reactor, the hydrolysis of raw wool was performed on this reactor by loading two different of wool inside the reactor. The process parameters used during the test are raw wool 6.5 kg (bulk density 64 kg/m$^{3)}$ and 10 kg (100 kg/m^3), liquor ratio 1:1, speed 8 rpm, temperature 160 $^{\circ}$C and time 85 min.

The test performed using manually pressing of wool inside the reactor, resulting in mentioned wool bulk density. Raw wool and water are allowed to mix inside reactor need 5 min of rotation in order to get homogenous impregnation. Successively, steam (coming from a steam generator) was supplied at 8.5 bar gauge in the reactor until the end of the test, while the rotation speed was maintained at 8 rpm. A temperature of 130 $^{\circ}$C was reached after 4 min; while after 2 more minutes the desired reaction temperature of 160 $^{\circ}$C (corresponding to a pressure of about 7 bar gauge) was reached. At the end of the reaction time of 85 min the solid and liquid phase was collected from the reactor. Wool appeared to

be well hydrolyzed, indeed in the solid phase, there is no appearance of fibres, but it shows the morphology of shapeless aggregates, as can be seen from the microscopic analysis reported in the following figure 25a. During the test performed with 10 kg of wool it was observed that the maximum loading limit of wool with manually pressing inside the reactor is 10 kg. The similar results are obtained from hydrolysis as mentioned above, wool fibers after hydrolysis appeared to be in shapeless aggregated in solid phase are shown in figure 25b.



(a)



(b)

Figure 25. Optical micrograph (100X) of hydrolyzed wool solid phase a) 6.5 kg flash cooling operation, b) 10kg external cooling.

Along with the study of wool bulk density, in two tests the different way of cooling at the end of hydrolysis was implemented. The test performed with 6.5 kg wool, the reactor cool down at the end of hydrolysis with flash operation where the temperature of the reactor was cool down to 100 $^{\circ}$C by releasing pressure from the pressure valve manually. In another test with 10 kg of wool cooling take place by progressive cooling using fresh water as a cooling medium. The results obtained show that with flash operation, cooling took place fast, but the material recovered is in concentrate form and 25% of total volume of the reactor with a decrease of 75% of the initial volume. In progressive cooling using fresh water the hydrolyzed product obtained was the total volume of the final product was half of the entire reactor capacity, with a decrease of the 50% of the initial volume. The progressive cooling was resulted in condensation of existing steam inside reactor results in higher recovery of material. The wool density of 64 kg/m^{3}and 100 kg/m^3 does influence the hydrolysis process and resulted in homogenous hydrolysis treatment. Also a material to liquor of 1:1 resulted in complete hydrolysis of wool in this type reactor. The process temperature, time optimization and agronomical analysis of hydrolysis is explained in detail in chapter 6.

4.6 Conclusions

The optimization phase carried out on different types of reactors allowed to demonstrate the flexibility of the process and the equipment, working in a wide range of conditions and obtaining different products. Moreover, the experimental trials highlighted also the critical issues of the process, particularly concerning the difficulties of having a homogeneous treatment without an effective mixing of the reagents within the reactor. The results and considerations obtained in different phases have been of fundamental importance for the design and construction of an intermediate-scale unit and for the demonstration unit.

The characterization and tuning of wool hydrolysis by lab-scale plant highlighted problems of wetting, homogeneity of treatment and temperature evenness during processing. To overcame these problems, a rotating intermediate reactor has been built. To reduce the time to fine tuning and have a perfectly working demonstration reactor since its initial operation was decided to build a reactor in intermediate scale, having a capacity of about 5-8 kg, working with the principle described earlier, with which to perform all tests necessary to test the "dynamic" process already during the functional design and construction of the demonstrative prototype.

Moreover, the new heating system, with steam supplied directly in the reactor chamber, allows to reduce the time needed to reach the hydrolysis temperature (heating time) and contributes to supply a considerable amount of water (thanks to its condensation) necessary to reach the desired solid to liquid ratio.The characterization of hydrolyzate resulted towards our aim to be applicable as a biofertilizer.

References

1. Giansetti M., Pezzin A., Sicardi S., Rovero G. Modeling of a Wool Hydrolysis Reactor. COMSOL Conference 2014, Cambridge (UK), 17 – 19 September 2014

Chapter 5

Comparative Study on the Effects of Superheated Water and Alkaline Hydrolysis on Wool Keratin

5.1 Introduction

The wool hydrolysis process by water, acids, bases and enzymes involves breakage of peptide bonds and disulfide bonds of proteins, which results in the formation of oligopeptides and finally amino acids (dissolution of keratins). These proteins and amino acids can eventually be refined and used as building blocks for various applications.[1] The alkaline hydrolysis of wool with strong hydroxides such as sodium hydroxide, potassium hydroxide etc. resulted in breakage of disulfide, peptide and side-chain amide bonds while the degradation of cystine, leading to the formation of lanthionine, which decreases the solubility of wool in alkali, it was observed in mild alkaline treatment.

In a previous study, poultry feathers and cow hairs were transformed into keratin hydrolyzates in an autoclave reactor using CaO at 150 $^{\circ}$C for 3 h and resulted in 90% of keratin that was hydrolyzed. Keratin hydrolyzate obtained by this process can be used as supplements for ruminants.[2] In a different study, the alkaline hydrolysis of wool waste using KOH and NaOH were carried out at 120 $^{\circ}$C for 20

minutes. The results showed that the wool hydrolyzates improved the soil characteristics and could be used as an alternative bio-fertilizer.[3] Polypeptides extracted from wool treated with NaOH 0.5 N at 95 °C for 2 h were used as reinforcement for polymer composite fibres.[4] The superheated water has a strong potential towards the dissolution of feathers at 220 °C for 120 min resulting in the destruction of cystine and cystine. High-density steam flash-explosion of the feather and wool at 220 °C for 10 minutes resulted in the breakage of disulfide bonds and dissolution of keratin in water. [5-6]

At room temperature and atmospheric pressure, water is a strong polar solvent due to the presence of hydrogen bonds in its structure, while at higher temperature and pressure it behaves like an organic solvent which dissolves leading to degradation of wool. During the wool hydrolysis, the hydrogen bond presented in the water may replace the amide groups of wool as the hydrogen bond donors and the oxygen atom as the carbonyl oxygen acceptors.[7] The superheated water hydrolysis sterilizes the wool and makes it more biodegradable, which advances its mineralization process in the soil.

The acid and alkaline hydrolysis have some limitations such as high cost of acid and alkali, the need for recovery, difficulty to handle, excessive damage to keratin structure, etc. The enzyme hydrolysis is an eco-friendly process, but it also has limitations such as the cost of enzyme and the time of hydrolysis. In comparison with all the processes, superheated water is a cost effective and green process, which overcomes the limitations of other hydrolysis process. Therefore, the primary purpose of this study is to investigate the influence of the superheated water hydrolysis on the chemical properties of wool and to compare it with the conventional method of alkaline hydrolysis.

5.2 Results and Discussion

5.2.1 pH

The pH of the wool hydrolysates obtained by superheated water hydrolysis at 140 °C and 170 °C was 7.90 and 5.16 respectively. These lower pH values were compensated with the wool pH in water, which is slightly alkaline (pH 8.16). The pH of the alkaline hydrolyzed samples was in the basic range (pH 8-10) in comparison with the superheated water hydrolyzed samples; (Table 1). The higher pH values of alkaline hydrolysis lead to swelling of wool fibres due to deprotonation of the carboxylic group and the amino acids produce net negative

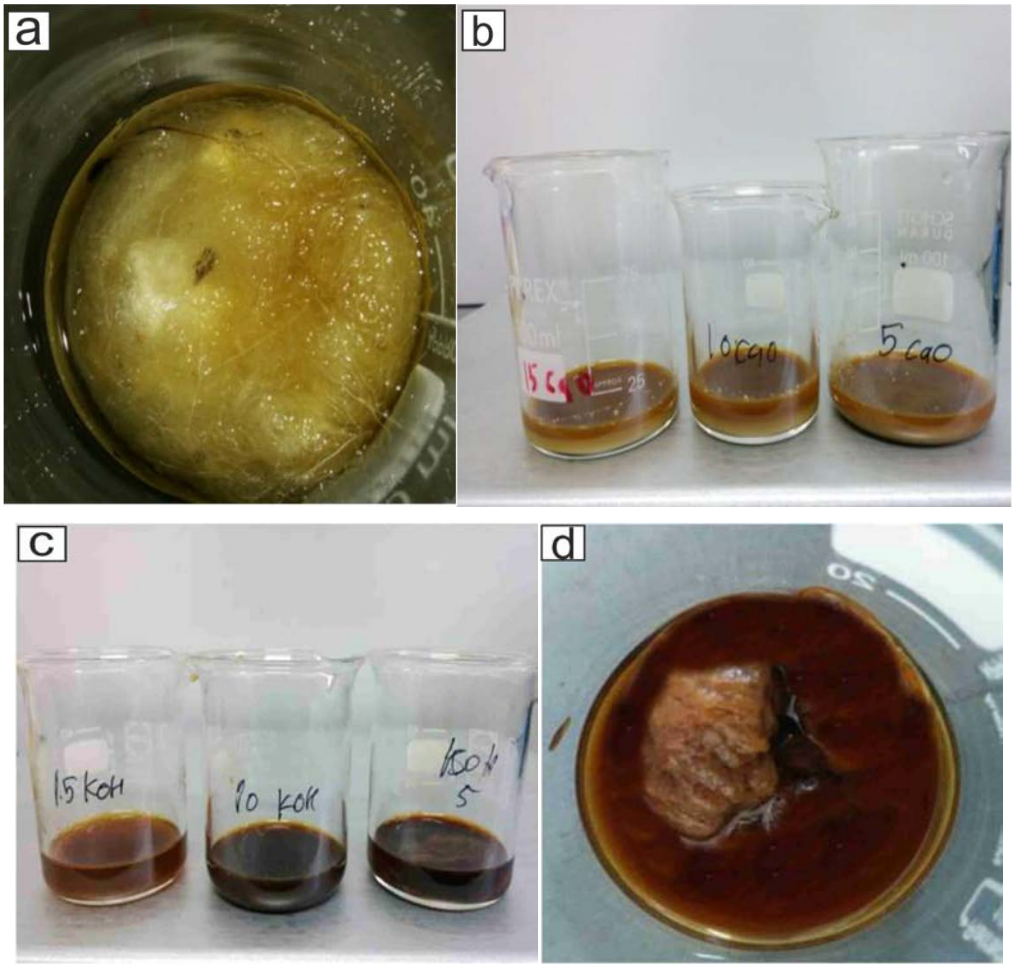
charges on proteins.[8] The swelling effect is prominently evidenced in optical microscopy. The shifting of pH towards the neutral range in the superheated water hydrolysis avoids the neutralization process which was essential in case of alkaline hydrolysis due to the use of potassium hydroxide and calcium oxide, for further use of keratin hydrolysates.

Table 1. pH of the superheated water and alkaline hydrolyzed wool samples.

No.	Temperature (°C)	Chemical	Concentration (%) o.w.f	pH after hydrolysis	State after hydrolysis
1	170 °C	Water	-	5.16	Mostly liquid very less solid
2	170 °C	CaO	0.05	8.61	Liquid
3	170 °C	CaO	0.1	9.12	Liquid
4	170 °C	CaO	0.15	9.56	Liquid
5	170 °C	KOH	0.05	7.82	Liquid
6	170 °C	KOH	0.1	9.01	Liquid
7	170 °C	KOH	0.15	9.55	Liquid
8	160 °C	Water	-	5.01	Solid
9	160 °C	CaO	0.05	7.90	Liquid
10	160 °C	CaO	0.1	9.39	Liquid
11	160 °C	CaO	0.15	10.13	Liquid
12	160 °C	KOH	0.05	6.75	Liquid
13	160 °C	KOH	0.1	8.92	Liquid
14	160 °C	KOH	0.15	9.14	Liquid
15	150 °C	Water	-	7.13	Solid
16	150 °C	CaO	0.05	8.52	Liquid
17	150 °C	CaO	0.1	9.33	Liquid
18	150 °C	CaO	0.15	10.13	Liquid
19	150 °C	KOH	0.05	6.87	Liquid
20	150 °C	KOH	0.1	9.17	Liquid
21	150 °C	KOH	0.15	9.85	Liquid
22	140 °C	Water	-	7.90	Solid
23	140 °C	CaO	0.05	9.04	Mostly liquid very less

					solid
24	140 °C	CaO	0.1	9.80	Liquid
25	140 °C	CaO	0.15	10.22	Liquid
26	140 °C	KOH	0.05	8.07	Mostly liquid very less solid
27	140 °C	KOH	0.1	9.29	Liquid
28	140 °C	KOH	0.15	9.75	Liquid

The physical appearance of the hydrolyzed product obtained after the hydrolysis process is shown in figure 1.



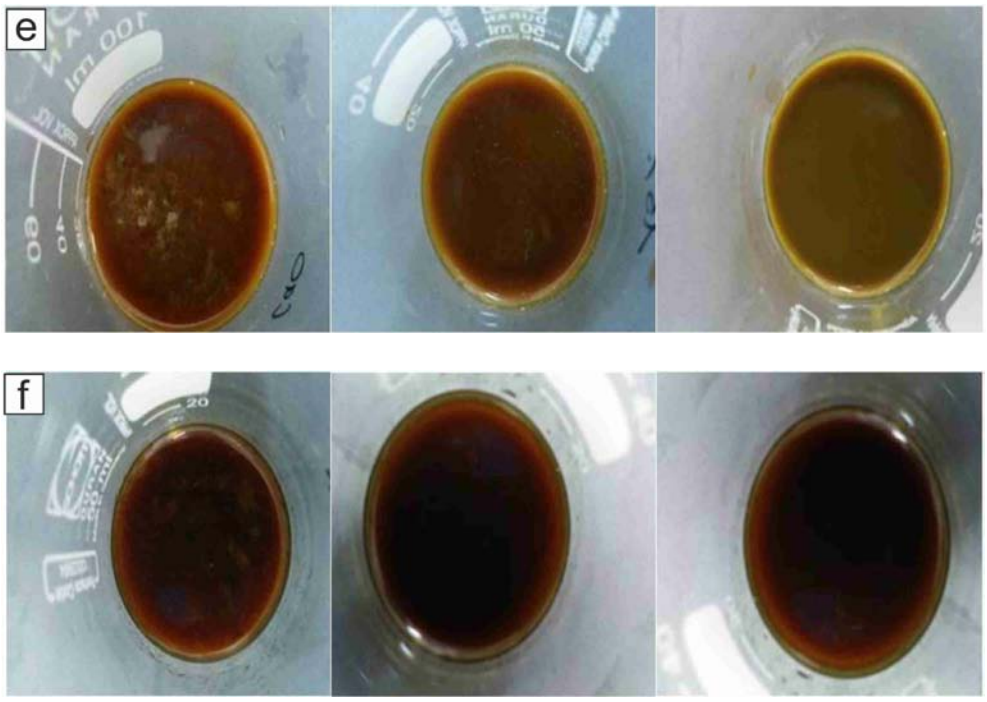
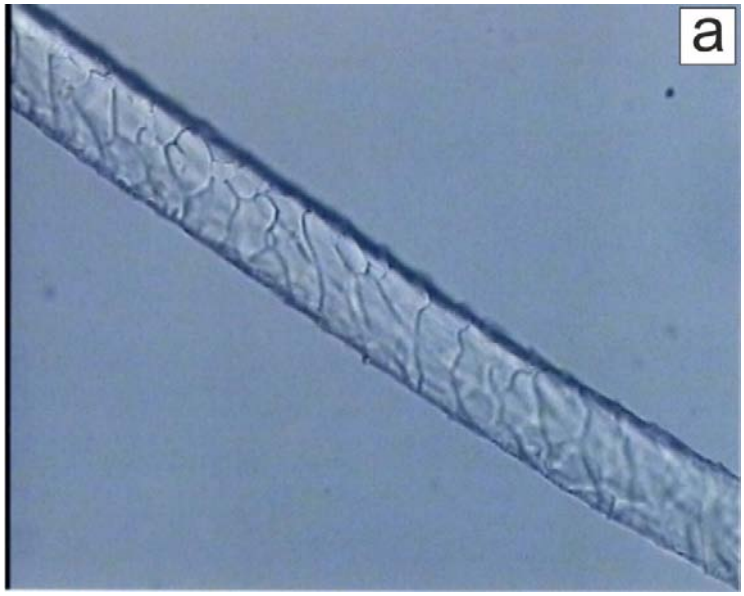


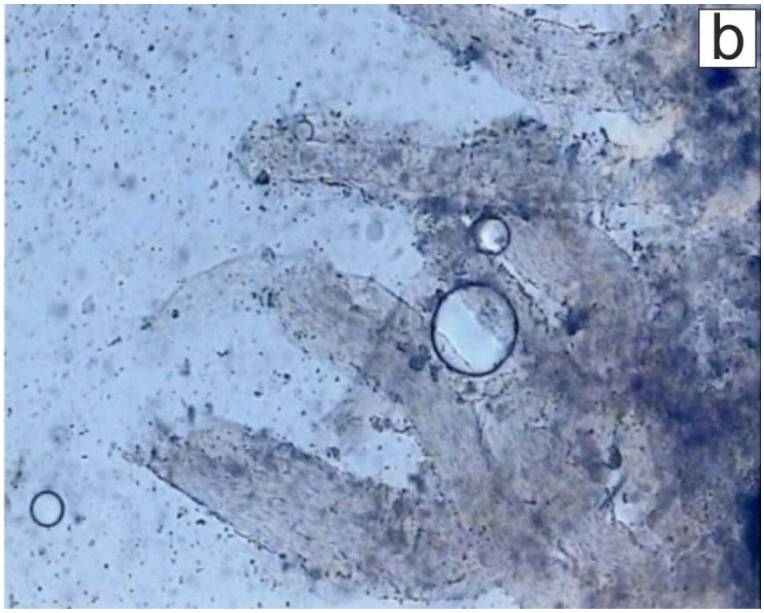
Figure 1. Hydrolyzed wool obtained at two different temperatures a) 140 °C superheated water b) 15%, 10%, 5% 140 °C CaO (from left to right) c) 15%, 10%, 5% 140 °C KOH (from left to right) d) 170 °C superheated water e) 5%, 10%, 15% 140 °C CaO (from left to right) f) 5%, 10%, 15% 170 °C KOH (from left to right)

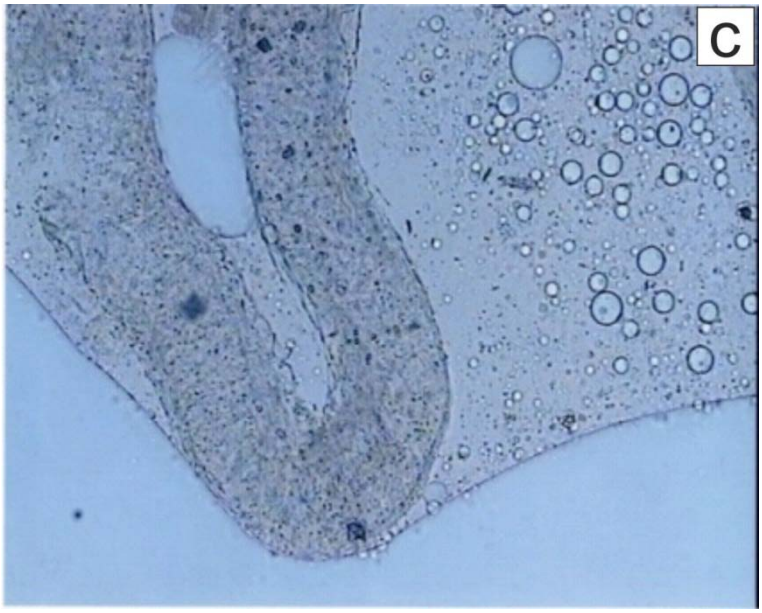
5.2.2 Morphological characterization by optical microscopy and scanning electron microscopy (SEM)

After the hydrolysis, most of the samples are in a liquid phase except for samples treated with the superheated water, 5% o.w.f CaO and KOH at a lower temperature (140 °C). The optical micrographs of hydrolyzed samples are shown in Figure 2. The sample hydrolyzed at 140 °C and 150 °C with superheated water (Figure 2a, 2h) shows that wool fibres are mostly in the solid phase and less-damaged with a normal cuticular structure on the fibre surface. The samples hydrolyzed with both alkali at 140 °C appeared in a viscous phase, very swollen, degraded and no appearance of cuticle cells on the fibre surface. The wool fibres

hydrolyzed with the superheated water at 160 °C and 170 °C were observed in shapeless protein aggregates. (Figure 2f, 2g). The optical microscopy results suggest that the superheated water hydrolysis has a promising potential towards degradation of wool fibres in comparison with the alkaline hydrolysis.



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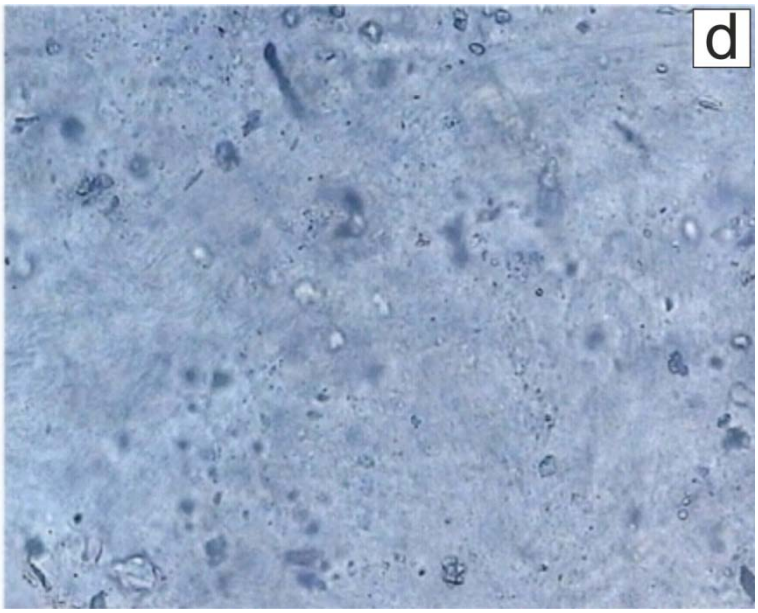
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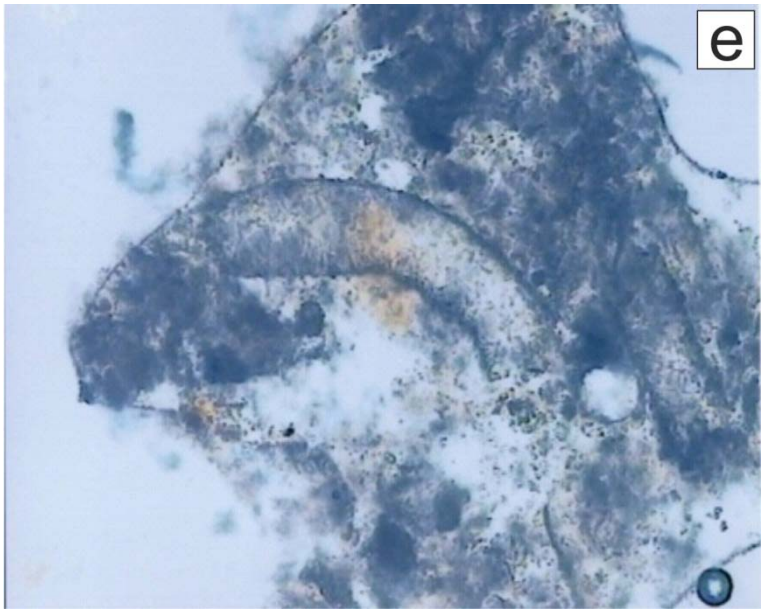
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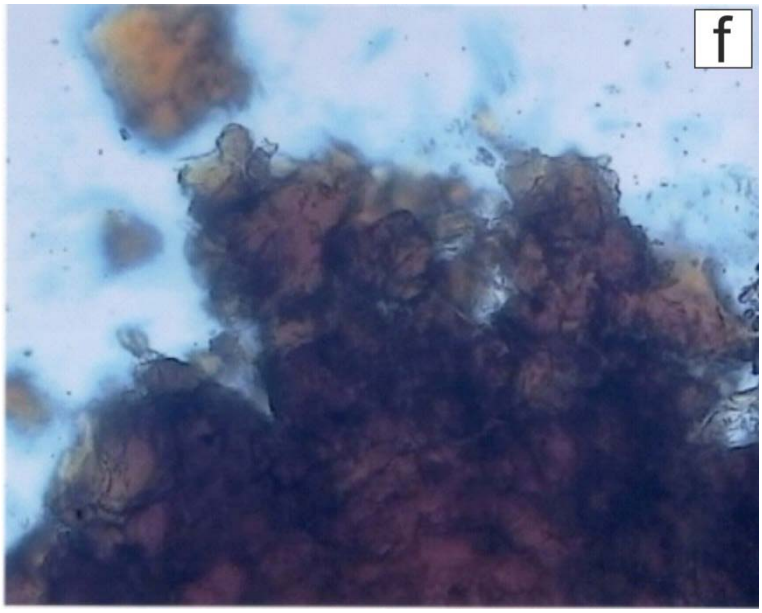
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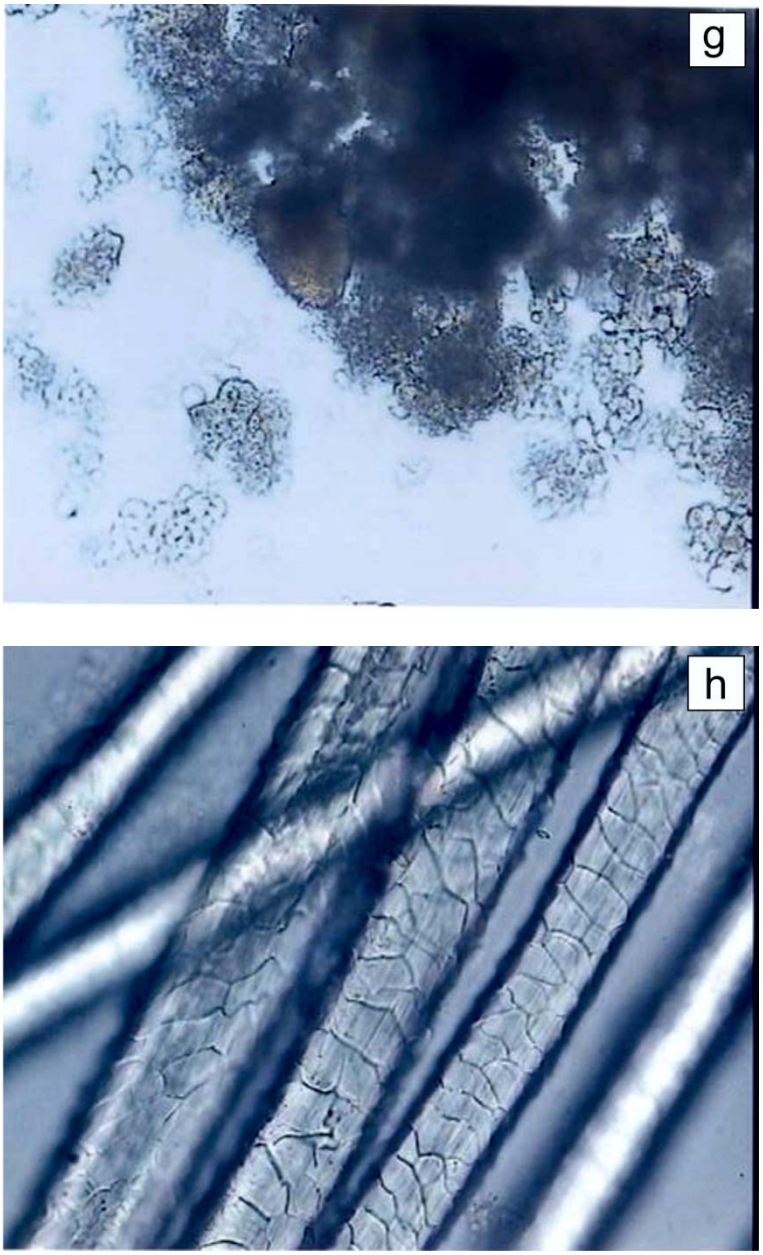


Figure 2. Optical microscopy pictures of hydrolyzed samples a) 140 °C superheated water b) 5 % o.w.f CaO 140 °C c) 5 % o.w.f KOH 140 °C d) 10 % o.w.f CaO 140 °C e) 10 % o.w.f KOH 140 °C f) 170 °C superheated water g) 160 °C superheated water h) 150 °C superheated water.

The SEM micrograph of wool fibres obtained from the superheated water and the alkaline hydrolysis revealed the morphological changes in wool fibres. The effect of temperature and alkali concentrations (5-15% o.w.f.) on wool fibres are also

visible in Figure 3. In the superheated water hydrolysis treatment at 140 °C, the appearance of wool fibres shows morphological characteristics of wool with less or no cuticular cells. After hydrolysis, fibres appeared sticky in nature. The fracture of wool fibres is smooth indicating the brittleness of treated fibres. In the case of superheated water hydrolysis treatment at 170 °C, SEM images reveal only shapeless aggregates produced during the process. Some fibres appeared morphologically more damaged with no cuticular layer. The treatment of wool fibres in superheated water resulted in the hydrolytic damage to the cuticle and cortical cell along with the loss of surface integrity.[9] SEM micrograph of all the keratin hydrolysates obtained from alkaline hydrolysis (5-15% o.w.f, KOH and CaO) at both temperatures reveal that all keratin hydrolysates were observed in shapeless aggregates without any significant differences because of complete degradation and dissolution of wool in the presence of alkali and temperature. The SEM micrographs clearly signify that the superheated water at 170 °C and the alkaline hydrolysis at 140 °C show the degradation of wool fibres.

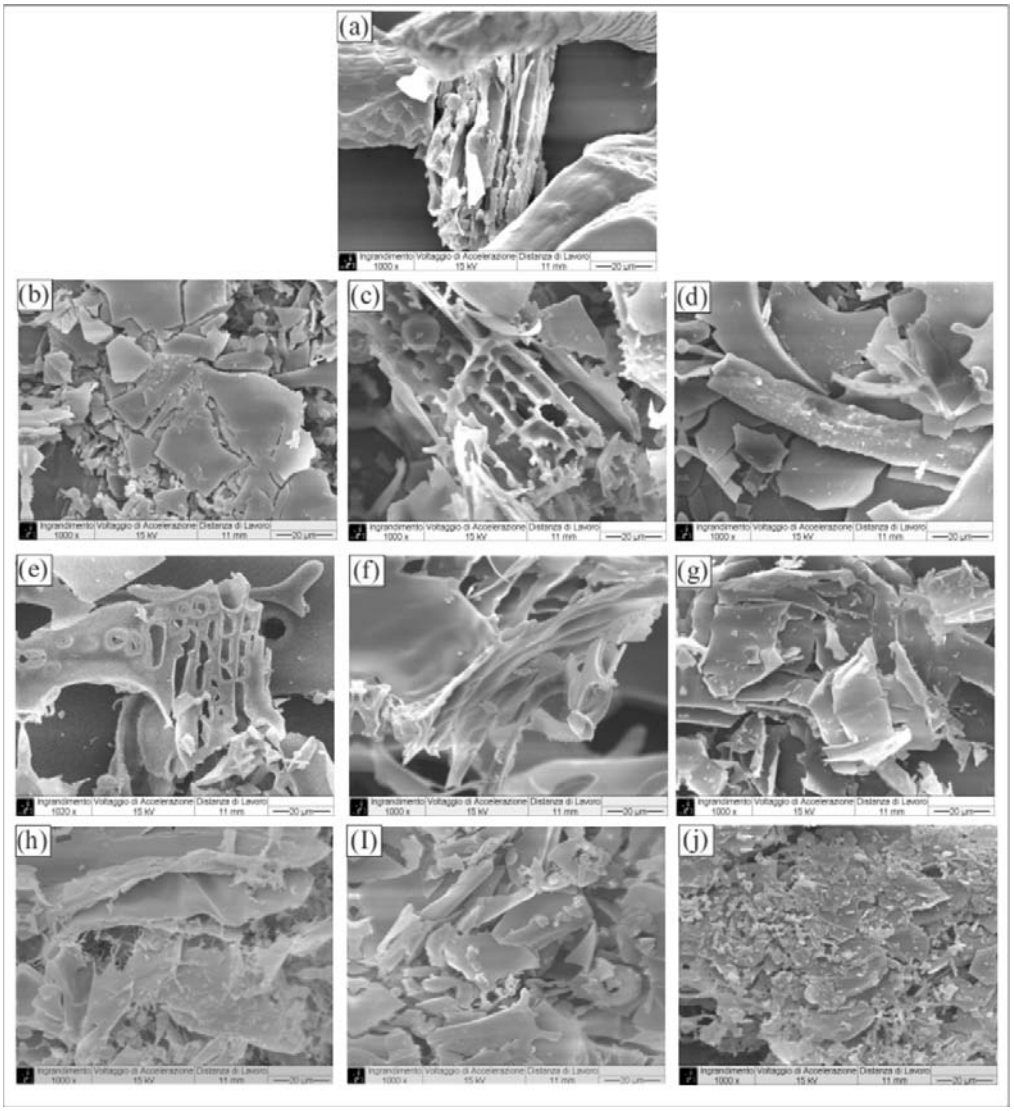


Figure 3. Scanning electron micrographs of hydrolyzed samples a) 140 °C superheated water b) 140 °C 5 % KOH c) 140 °C 10 % KOH d) 140 °C 15 % KOH e) 140 °C 5 % CaO f) 140 °C 10 % CaO g) 140 °C 15 % CaO h) 170 °C water i) 170 °C 15 % KOH j) 170 °C 15 % CaO .

5.2.3 Amino acid analysis

The amino acid contents of wool keratin hydrolysates obtained after the superheated water and the alkaline hydrolysis treatment at different temperatures for 1 h are shown in Table 2 and 3 respectively. In the superheated water and alkaline hydrolysis, prominent changes occurred in comparison with the original

wool are the loss of ½ cystine residues and formation of lanthionine residues. In the superheated water hydrolysis, the loss occurred in the case of ½ cystine residues from 10.97 mole % of original wool to 8.22 mole % and 0.62 mole % at 140 °C and 170 °C respectively. In alkaline hydrolysis as the temperature of hydrolysis and concentration of CaO and KOH increases, the loss occurred in case of ½ cystine residues from 10.97 mole % of original wool to 0.255 mole % (15 % CaO), 0.335 mole % (15 % KOH) at 140 °C and 0.105 mole % (15 % CaO), 0.105 mole % (15 % KOH) at 170 °C. The amount of lanthionine residues increased from 0.12 mole % of original wool to 0.82 and 1.625 mole % at 140 °C and 170 °C respectively in superheated water hydrolysis. The samples hydrolyzed at 140 °C with 5 % KOH and CaO resulted in the increase of lanthionine residues from 0.12 mole % in original wool to 2.015 mole % and 2.33 mole % respectively.

The optical microscopy examination of the same samples evidenced that fibres were swollen, sticky in nature but not completely degraded. In the case of alkaline hydrolysis as the concentration of alkali increases, there is a decrease of lanthionine residues. It confirms that the effect of higher temperatures results in the disruption of S-S bonds and degradation of cystine residues and also leads to the formation lanthionine through the creation of dehydroalanine and reactive sulfur-containing residues.[10] Carr[11] found that alkali hydrolyzes the peptide bond and leads to the gradual dissolution of the fibre as well as the elimination of sulphur as sodium sulphide. The degradation effect of alkali on wool sulphur bonds increases by rising both temperature and alkali concentration.[12-13]

In the superheated water hydrolysis, loss of aspartic acid and serine residues were observed from 7.46 mole % aspartic acid in original wool to 5.56 mole % and from 8.99 mole % of serine in original wool to 6.83 mole % at 170 °C. The amount of glycine residue increased from 7.27 mole % to 8.975 mole % at 170 °C. The amount of aspartic and glutamic acid residues increased in alkaline hydrolysis, due to the increase of hydrolysis temperature and alkali concentration. Small changes observed in the concentration of other amino acids can be related to their dissolution in water and thermal stability. The results obtained are in agreement with Horio et al[14] who observed the loss of aspartic acid and serine amino acids in hydrolysis performed at high temperature. In alkaline hydrolysis asparagine and glutamine residues hydrolyzes to aspartic and glutamic acid residues.[15] Norton and Nicholls [16] reported that when the wool is exposed to the alkali; amino acids cystine, tryptophan, threonine, arginine and lysine are modified and the breakdown of some main chains also occurred. A large amount of lanthionine is formed from the cystine residues when the wool is heated in

boiling water for a long period of time; also during the alkaline hydrolysis cystine residues in wool are attacked by alkali and two new crosslinks lanthionine and lysinoalanine are formed.[28]

Table 2. Amino acid analysis of freeze dried wool hydrolysates obtained from superheated water hydrolysis at 140 °C and 170 °C compared with the amino acid composition of original wool (mole%).

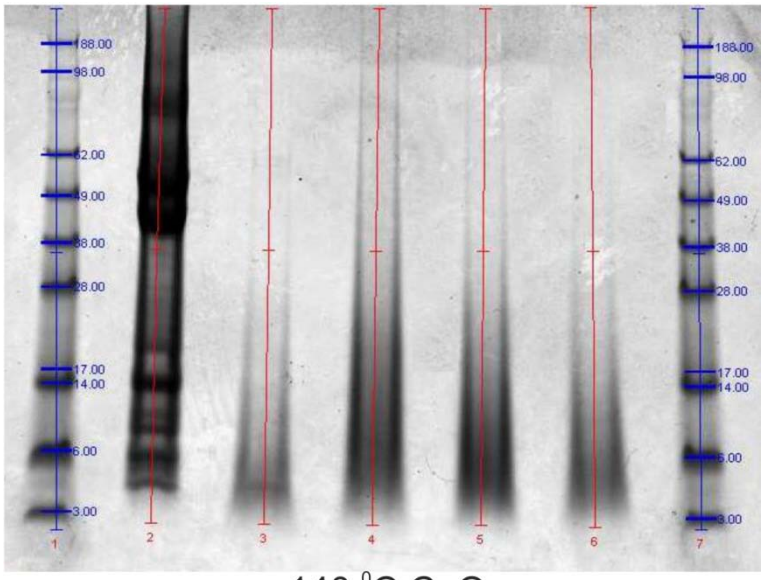
Name	Wool Standard	Wool superheated water 140 °C	Wool superheated water 170 °C
CYA	0.08	0.24	0.265
ASP	7.46	7.48	5.26
SER	8.99	9.1	6.83
GLU	13.46	13.94	20.835
GLY	7.27	7.79	8.975
HIS	0.84	0.98	0.705
ARG	6.9	7.25	5.595
THR	5.28	5.29	4.475
ALA	5.47	5.78	7.995
PRO	6.73	6.69	6.58
LANT	0.12	0.87	1.625
1/2CYS	10.97	8.22	0.62
TYR	2.87	1.86	2.175
VAL	6.78	6.89	7.965
MET	0.27	0.4	0.31
LYS	3.42	3.2	6.305
ILE	3.47	3.58	3.895
LEU	7.4	7.77	8.075
PHE	2.22	2.65	1.5

Table 3. Amino acid analysis of freeze dried wool hydrolysates obtained from alkaline hydrolysis using (5-15% o.w.f) KOH and CaO, at 140 °C and 170 °C compared with the amino acid composition of original wool (mole%).

Name	Original Wool	5% CaO 140 °C	10% CaO 140 °C	15% CAO 140 °C	5% KOH 140 °C	10% KOH 140 °C	15% KOH 140 °C	5% CaO 170 °C	10% CaO 170 °C	15% CaO 170 °C	5% KOH 170 °C	10% KOH 170 °C	15% KOH 170 °C
CYA	0.08	0.045	0.88	0.63	0.425	0.255	0.63	0.365	0.69	0.44	0.355	0.375	0.475
ASP	7.46	6.03	10.685	11.845	9.385	10.66	11.06	9.665	9.515	9.57	5.315	5.435	9.01
SER	8.99	10.17	1	0.395	7.655	4.43	1.3	1.22	3.205	0.68	4.48	2.025	0.575
GLU	13.46	8.37	21.575	25.185	18.305	19.875	22.195	22.53	12.345	24.42	18.095	18.645	23.55
GLY	7.27	10.2	8.005	6.71	8.25	9.215	8.945	9.62	10.6	7.265	12.36	12.825	9.515
HIS	0.84	1.245	1.315	1.015	0.47	0.17	0.53	1	1.29	0.795	0.985	0.96	0.97
ARG	6.9	7.97	5.425	3.99	6.63	5.575	5.115	3.585	6.27	2.875	6.57	5.39	3.73
THR	5.28	6.555	0.935	0.195	4.275	1.92	0.765	0.845	1.45	0.725	2.99	1.35	0.315
ALA	5.47	6.77	8.96	9.3	7.025	8.295	8.62	8.915	8.25	9.07	7.875	8.565	8.6
PRO	6.73	8.39	4.515	3.865	6.07	5.54	4.375	4.34	6.07	4.195	5.895	5.655	4.47
LANT	0.12	2.33	0.85	0.425	2.015	1.775	0.755	0.285	1.445	0.18	0.335	0.17	0.145
1/2CYS	10.97	0.01	0.32	0.255	0.865	0.585	0.335	0.155	0.01	0.105	0.15	0.04	0.105
TYR	2.87	3.485	3.025	2.48	1.725	2.61	3.045	2.76	0.605	2.965	3.245	3.34	2.955
VAL	6.78	1.595	8.22	8.33	7.305	7.625	8.19	8.36	2.49	9.33	8.04	8.725	8.855
MET	0.27	7.32	0.505	0.675	0.435	0.515	0.54	0.825	7.155	0.935	0.555	0.75	0.77
LYS	3.42	0.39	4.79	5.715	4.44	4.655	4.755	5.805	0.47	5.415	5.805	6.43	6.175
ILE	3.47	3.425	4.875	5.015	3.96	4.25	4.81	4.96	2.61	5.62	4.4	5	5.215
LEU	7.4	4.41	11.595	11.815	8.71	9.895	11.5	12.105	14.5	12.935	10.095	11.44	12.255
PHE	2.22	8.09	2.505	2.145	2.045	2.155	2.52	2.64	8.415	2.465	2.44	2.87	2.3

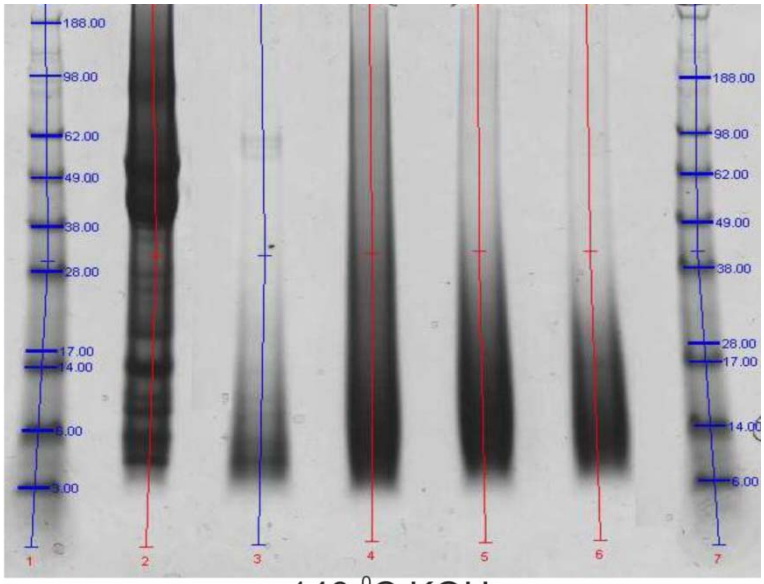
5.2.4. Molecular weight distribution

The gel electrophoresis patterns of freeze dried keratin hydrolysates obtained from the superheated water and the alkaline hydrolysis at 140 °C and 170 °C are shown in Figure 4.



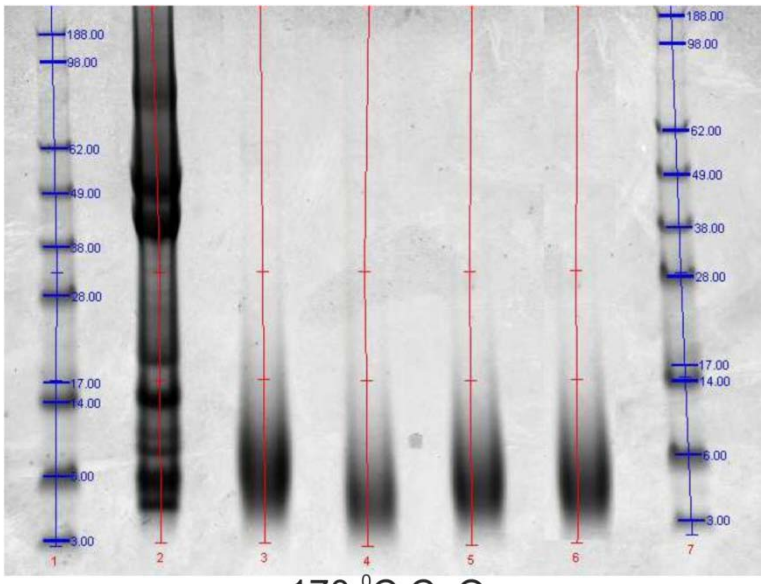
140 ^{0}C CaO

(a)

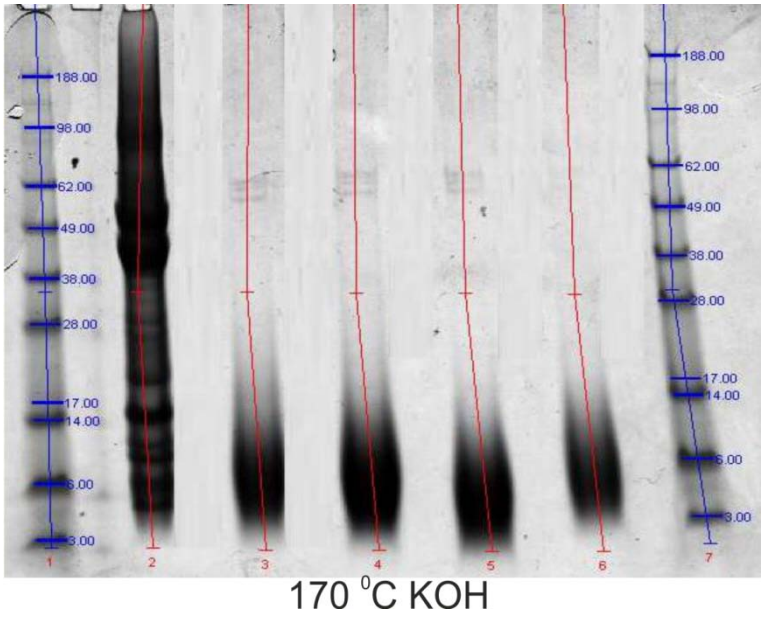


140 ^{0}C KOH

(b)



(c)



(d)

Figure 4. Gel-electrophoresis patterns of superheated water and alkaline hydrolyzed freeze dried wool keratin powder at140°C (a) and (b) 170°C (c) and (d). Lane (1) Molecular weight standard (2) Original wool (3) superheated water hydrolyzed wool keratin(4) 5 % (5) 10 % (6) 15 % (o.w.f) Alkaline hydrolyzed wool keratin (7) Molecular weight standard.

The electrophoresis patterns are compared with the molecular weight pattern of the original wool. The electrophoresis patterns of original wool keratin fractions (lane 2) indicate mainly low sulphur (LS) proteins of the intermediate filaments having a molecular weight in the range of 67–43 kDa and the high sulphur (HS) proteins, whose molecular weight falls in the range of 28–11 kDa. The electrophoresis patterns of wool hydrolyzed with superheated water at 140 °C and 170 °C (lane 3) shows that the strong bands of LS proteins disappeared. Hence, the protein having a molecular weight in the range of 8-3 kDa appeared with strong signals. The effect of temperature and concentration of alkali on molecular weight distribution is clearly visible in electrophoresis patterns. As the concentration of alkali and temperature of hydrolysis increase, there is an increase of low molecular weight proteins around 8-3 kDa. The electrophoresis patterns of wool hydrolysates treated with alkali at 140 °C resulted in a gradual decrease of the molecular weight of proteins with the increase in alkali concentration indicating the effect of alkali concentration on wool degradation; this effect wasn't observed at higher temperatures. In comparison with the electrophoresis data of alkaline hydrolysis, data obtained from the superheated water hydrolysis (lane 3) shows equivalent potential towards loss of high molecular weight proteins indicating that the high temperature of superheated water hydrolysis affects the chemical structure of keratin.

5.2.5Fourier Transform Infra-red Analysis (FT-IR)

The absorption bands in the spectra are mainly assigned to peptide bonds present in the wool.

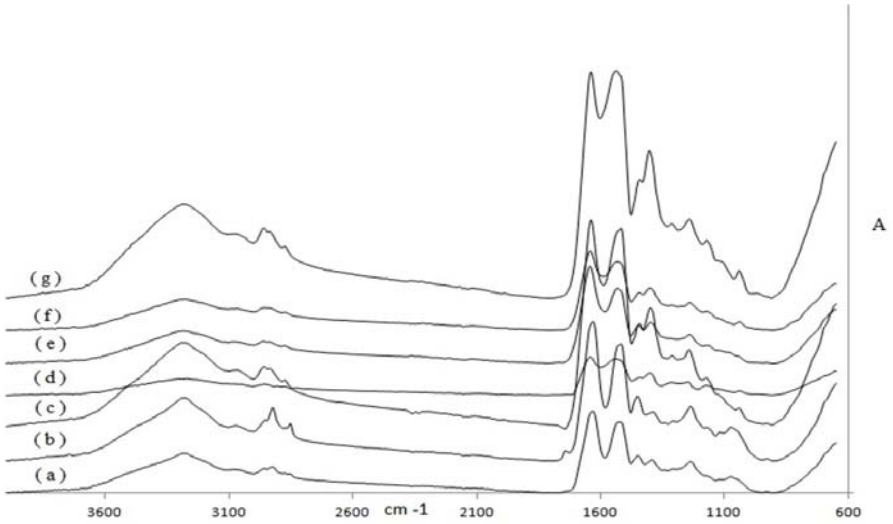


Figure 5. FTIR spectra of original wool, superheated water and alkaline hydrolyzed wool and freeze dried keratin powders.(a) Wool (b) 140 °C superheated water (c) 15 % KOH 140 °C (d) 15 % CaO 140 °C (e) superheated water (f) 15 % KOH 170 °C (g) 15 % CaO 170 °C.

The broad absorption band in the region 3500 to 3200 cm^{-1} can be assigned to the stretching vibration of O-H and N-H bonds. The vibrations in the peptide bonds are mainly Amide A, I, II. The Amide A band at 3282 cm^{-1} is attributed to the stretching vibration of N-H bonds. The absorption bands in the range of 3000–2800 cm^{-1} are related to C-H stretching. The Amide I band that occurs in the range of 1700-1600 cm^{-1} is due to the C-O stretching vibration while the Amide II falls in the range of 1580–1480 cm^{-1} and it is attributed to N-H bending and C-N stretching vibration. Some changes in the absorption bands intensity mainly for the hydrolyzed fibre were observed in the range of 1700-1100 cm^{-1} in comparison with untreated fibres. Variations in peak intensity and position of amide groups correspond to the changes in the keratin structure. The changes observed in the range of 1300-1000 cm^{-1} which includes both the disulfide oxidative intermediates and Amide III band are in accordance with the modification of disulfide bonds as seen in amino acid analysis. The Amide I absorption, in the1700–1600 cm^{-1} range, are known to be sensitive to the secondary structure of proteins. A Gauss-shaped band fitting was done in the range of 1750-1600 cm^{-1}. As reported in the literature, α-helix falls in the region of 1657–1651 cm^{-1}, β-sheet 1631–1621 cm^{-1} and 1680–1694 cm^{-1}, and disordered regions 1697–1670 cm^{-1}. [15,17-19]

The secondary structure assignment and its percentage are shown in Table 4. The polypeptides extracted with the superheated water hydrolysis treatments at a temperature of 140 °C and 170 °C resulted in more β sheets and random coil structure. In the case of alkaline hydrolysis at 140 °C and 170 °C, the presence of more random coil structures along with a marginal amount of β sheets and absence of α helix was observed. These results are consistent with HPLC and SDS-PAGE electrophoresis analysis carried out on the same samples. Probably the higher content of polypeptides of molecular weights around 3 kDa hinders the formation of organized secondary structures as α-helices or β-sheets. The molecular weights of the protein fraction are probably not high enough to allow the formation of crystalline structures.

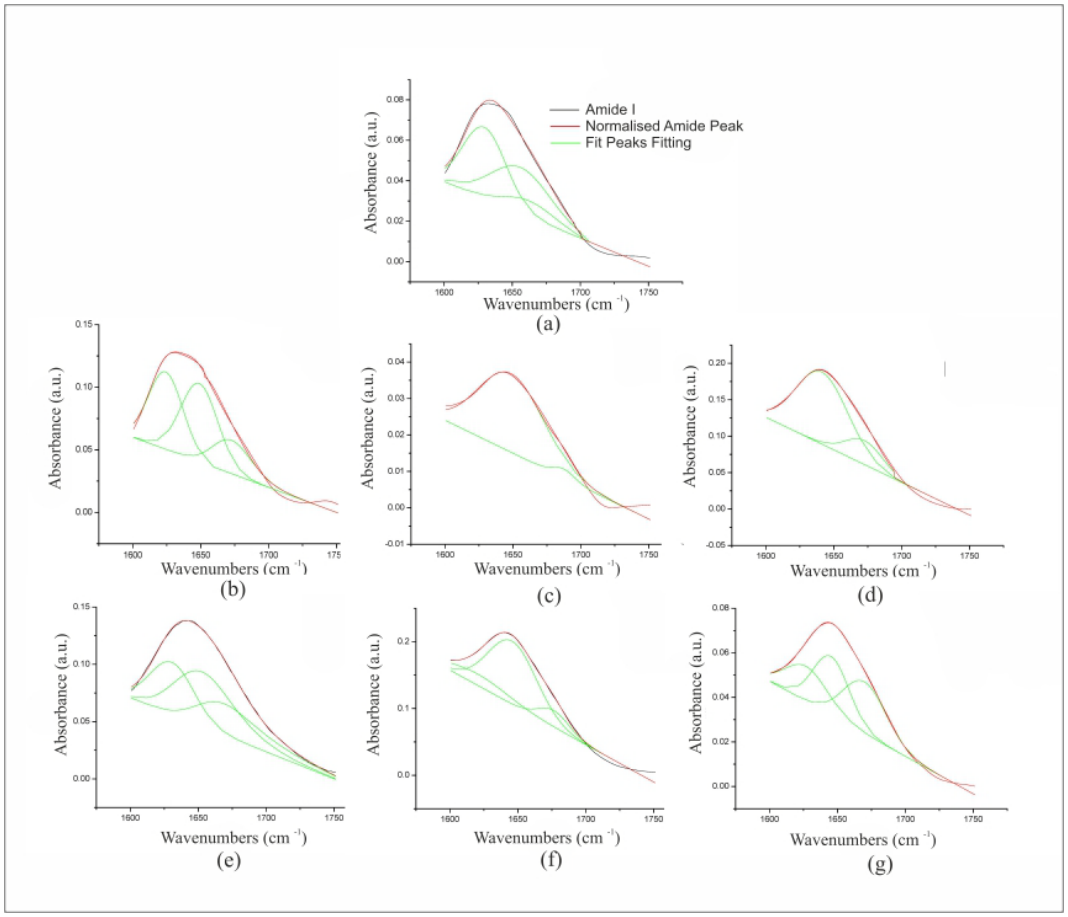


Figure 6. Amide I curve fitting of wool keratin after superheated water and alkaline hydrolysis (a) Wool (b) 140 ˚C superheated water (c) 15 % CaO 140 ˚C (d) 15 % KOH 140 ˚C (e) 170 ˚C superheated water (f) 15 % CaO 170 ˚C(g) 15 % KOH 170 ˚C.

Table 4. Characteristics of the amide I absorption bands

Sample	Peaks (cm^{-1})	Assignment	%	R^2
Original Wool	1629	β sheet	46.40	0.9920
	1655	α-helix	41.18	
	1661	β sheet/ Random coil	12.40	
140 °C water	1623	β sheet	38.80	0.9909
	1648	Random coil	42.08	
	1666	Random coil	19.10	
170 °C water	1630	β sheet	29.14	0.9989
	1640	Random coil	42.63	
	1653	α-helix	28.21	
140 °C 15% KOH	1643	Random coil	96.31	0.9949
	1683	β sheet	3.68	
140 °C 15% CaO	1645	Random coil	96.34	0.9727
	1687	β sheet	3.65	
170 °C 15%KOH	1621	β sheet	25.67	0.9955
	1644	Random coil	36.17	
	1666	Random coil	38.15	
170 °C 15% CaO	1620	β sheet	15.94	0.9875
	1645.	Random coil	72.08	
	1676	Random coil	11.96	

5.3 Conclusions

In this work, the superheated water and the alkaline hydrolysis of wool with different concentrations (5%, 10%, 15% o.w.f.) of KOH and CaO at different temperatures (140 °C and 170 °C) were performed. Keratin obtained from both hydrolysis treatments is composed of low molecular weight polypeptides and proteins with a low amount of cystine and a mainly disordered secondary structure. The optical and SEM micrographs indicate that the superheated water hydrolysis at higher temperature is as effective as the alkaline hydrolysis towards degradation of wool. The superheated water hydrolysis is a more eco-friendly process compared to alkaline hydrolysis. The superheated water hydrolysis at 170 °C is a very effective method in terms of keratin dissolution also at low L.R. (1:3) and short time (1 h). The low liquor ratio ensures the uniform impregnation of the wool and a complete hydrolysis reaction and the temperature variation allows obtaining more liquid or solid products. The superheated water hydrolysis treatment has the potential for low-cost commercial application. The protein hydrolysates obtained from the superheated water hydrolysis could find applications such as food supplements, bio-stimulants and fertilizers production.

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Chapter 6

Superheated Water Hydrolysis of Waste Wool in a Semi-Industrial Reactor to Obtain Nitrogen Fertilizers

6.1 Introduction

This process is an example of a well sustainable circular economy, which starts from grazing and returns the fertilizer back to the lands which produced sustenance to sheep. The product devised is expected to perform a tunable nitrogen release. Depending on the hydrolysis degree, the fertilizer obtained from wool can be positioned between fast dissolution (typical of synthetic mineral fertilizers) and extremely low releasing nitrogen-based substances. The aim of this study is to use greasy wool without prior washing or any cleaning step for the hydrolysis treatment to obtain a nitrogen fertilizer. Hydrolysis treatment was performed in the presence of superheated water in a semi-industrial reactor to get a complete dissolution of greasy wool and a complete characterization of wool hydrolyzates was carried out.

6.2 Results and discussion

6.2.1 Density

The hydrolysis of wool was carried out using lots of 6.5 kg and 10.0 kg of greasy wool for different treatment time intervals (30, 60 and 90 min) at 170°C : different viscosities of the produced material were obtained. When the hydrolysis was carried out for 30 min the protein hydrolyzates appeared as semisolid gelly phase, while as the hydrolysis time was increased from 30 min to 90 min, the hydrolyzates appeared to be more liquid. The different consistency depends on the amount of superheated water brought into contact with the fibrous matter: higher was the hydrolysis time, larger amounts of steam were fed to the reactor and transformed into superheated water, and also on the initial amount of greasy wool, more is the amount of greasy wool more is material obtained in semi-solid phase. The different phases obtained from hydrolysis treatment are shown in figure 1.

Figure 1. a) Greasy wool b) hydrolyzed solid product c) hydrolyzed liquid product d) 6.5 kg 170 °C 30 min e) 6.5 kg 170 °C 60 min.

The hydrolyzed product obtained at the end of hydrolysis treatment was observed to be in dark brown color with the presence of vegetable matter. The smell of the hydrolysis product is pungent, similar to volatile compounds such as ammonia and hydrogen sulfide. As shown in figure 1d the sample hydrolyzed for 30 min consists of paste like viscous material similar to wet animal dung (cow dung) with high moisture content. The sample hydrolyzed for 60 min is in the liquid phase as shown in figure 1e and having similar dark brown to black color and pungent smell. The density of hydrolyzates varies from 1.00 to 1.15 g/mL (Table 1). It should be noted that the hydrolysis treatment transforms the greasy wool from a fibrous solid into a semisolid or high viscosity liquid phase. The apparent density of greasy wool, which is characterized by a high void degree, is significantly increased from an initial value of 0.07-0.08 g/mL. to 1.00-1.14 g/mL (Table 1). The product can be stored in a much smaller volume, is easily mixed with soil without originating segregation, typical phenomenon originated when a bulky materials (wool) is combined to a dense phase (earth).

Table 1. Consistency, density and pH of greasy wool after hydrolysis with superheated water.

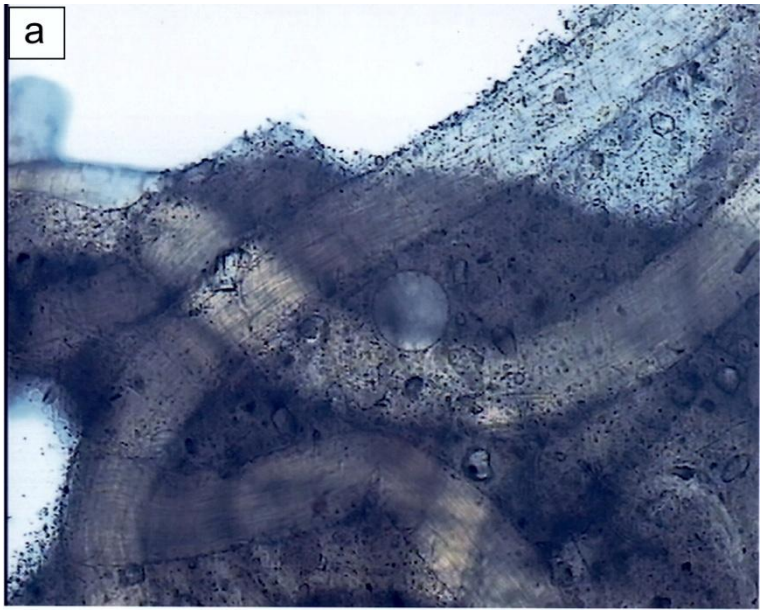
Sample Batch mass (kg)-Process time (min)	Consistency aspect	Density g/mL	pH
Greasy wool	fibrous	0.07-0.08	9.5-10
6.5 - 30	semisolid	1.07	8.3
6.5 - 60	liquid	1.13	7.78
6.5 - 90	liquid	1.07	7.83
10.0 - 30	semisolid	1.02	7.7
10.0 - 60	semisolid	1.00	7.5

10.0 - 90	liquid	1.14	7.25

6.2.2 Acidity

The pH of the hydrolyzed samples resulted almost constant, ranging around neutrality between 7.2 to 8.3. The acidity of greasy wool is in the basic pH range between 9.5 to 10.(Table 1) A neutral pH confers solubility of all nutrients easily available for plants; hence, the fertilizer having a neutral pH can be used for a wide variety of crops

6.2.3 Morphological characterization by Optical Microscopy and Scanning Electron Microscopy



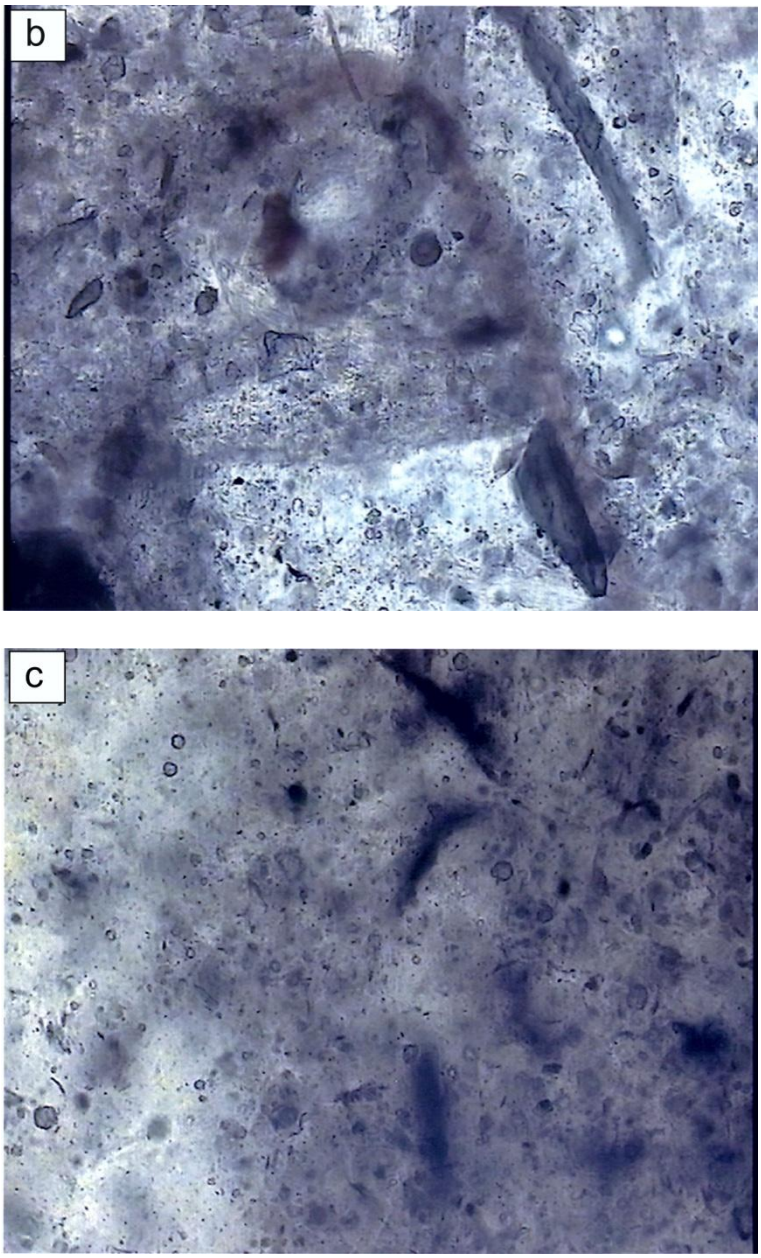
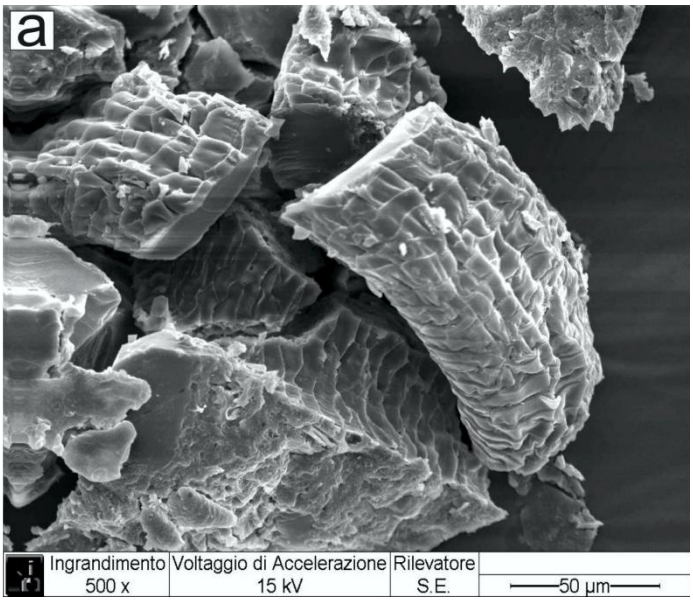


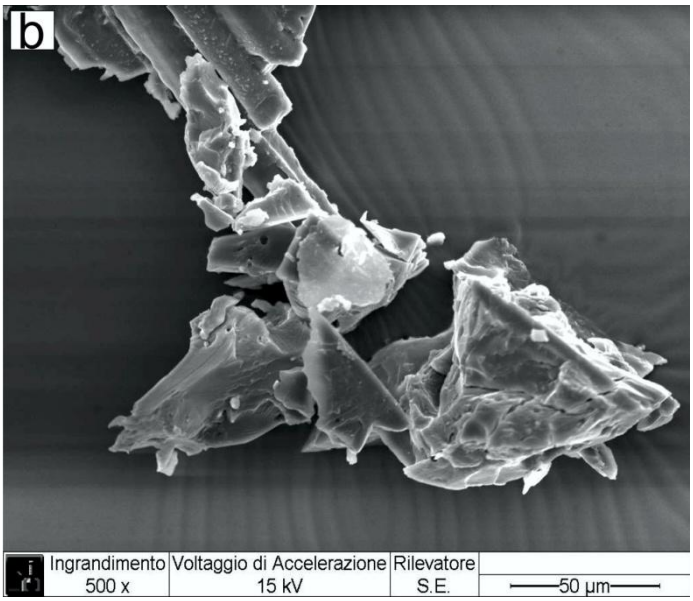
Figure 2. Optical Micrograph (100X) of greasy wool hydrolyzed at 170 °C for a) 30 min b) 60 min c) 90 min.

Maintaining a constant temperature of 170 °C, the hydrolysis time was the factor which mostly affected the morphology of product whereby the amount of steam

used. The residual fibres existing in the viscous product were very swollen and degraded. The fibre surface appeared quite rough, uneven and without the cuticle cells at 30 min treatment (Figure 2 a). When the hydrolysis was carried out for 60 min and 90 min fibres appeared as shapeless protein aggregates which resulted from the complete dissolution of wool fibres, as shown in the images of Figures 2 b and 2 c, respectively.

Scanning Electron Microscopy of hydrolyzed greasy wool at 170 ^{o}C for different time intervals are shown in Figure 3. The wool fibres were extensively degraded by the superheated water and appear as shapeless aggregates.




b
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500 x
Voltaggio di Accelerazione
15 kV
Rilevatore
S.E.
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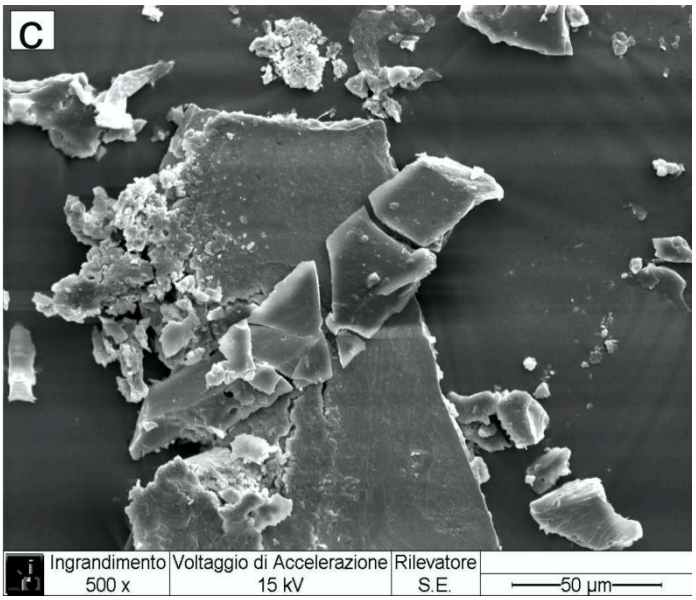
c
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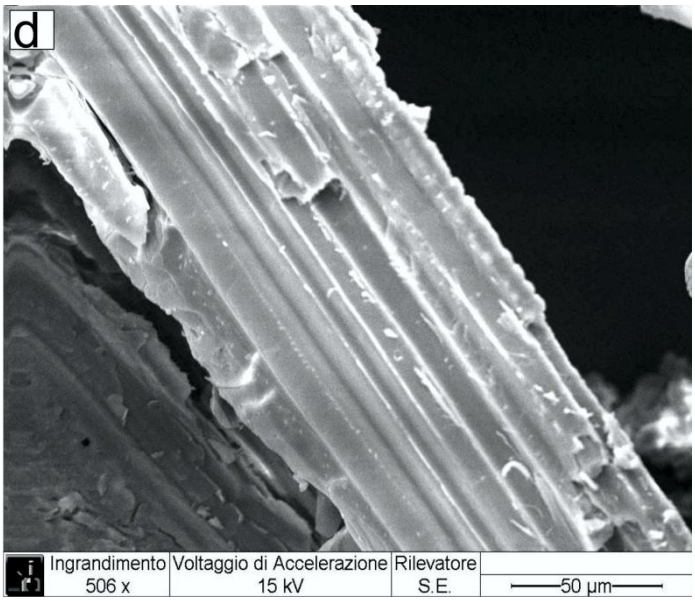


Figure 3. Scanning electron microscopy image (500X) of grease wool hydrolyzed at 170 °C for a) 30 min b) 60 min c) 90 min d) Vegetable matter.

The greasy wool hydrolyzed for 30 min shows fused cuticular layer and fibres were morphologically severely damaged (Figure 3 a). The duration of the hydrolysis treatment had a major impact on fibre morphology; fibres were completely solubilized in shapeless aggregates without any residual morphological similarity to wool after 60 min and 90 min of hydrolysis treatment (Figure 3b and 3c). All samples appeared as shapeless aggregates of proteins, with some exception of the short time sample, whereby a smaller amount of superheated water came in contact with greasy wool. The residues of extraneous matter (vegetable or inorganic) remained undissolved at the end of hydrolysis (Figure 3d). This analysis shows that a 60 min hydrolysis is sufficient to obtain a complete morphological breakdown of greasy wool into shapeless protein aggregates.

6.2.4 Fourier Transform Infrared Spectroscopy

FTIR spectra of greasy and hydrolyzed wool treated for different time are shown in Figure 4. There are no new chemical groups or free residues formed from the hydrolysis treatment. The sharp absorption peaks at 2850–2965 cm^{-1} are seen in the hydrolyzed wool samples in comparison with untreated wool. Bands that fall

in the 2800-3000 cm^{-1} range are related to C-H stretching modes. The absorbance peak at 1630–1650 cm^{-1} is associated with Amide I which is attributed to stretching vibrations of the C=O bond, the peak at 1530–1550 cm^{-1} belongs to Amide II bending vibrations of N–H bonds and the peak around 1233 cm^{-1} is attributed to Amide III. Amide A (3282 cm^{-1}) and Amide B (3065 cm^{-1}) bands correspond to the frequency of N-H stretching vibrations. C=O and N-H bonds take part in hydrogen bonding.[26-27]Type and a number of hydrogen bonds in the chemical structure are responsible for the peak shapes. The wedge shape peaks of Amide I and II were observed in the hydrolyzed samples. The transformation of smooth amide peaks of greasy wool into wedge shape peaks may be attributed to the bonds breakage due to the hydrolysis treatment.[28]

Disulfide bonds in wool fibre greatly contribute to its physical and chemical properties. In the range from 1300 cm^{-1} to 1000 cm^{-1}, the spectra are characterized by the presence of medium-to-high intensity bands attributed to different sulphur containing chemical groups of wool.[29] A closer examination of the spectra in this range revealed some changes between different samples: these changes around 1250 cm^{-1} are shown in Figure 4. The absorbency of treated samples increased in comparison to untreated wool, mainly due to transformation or loss of some sulphur containing groups during the treatment.

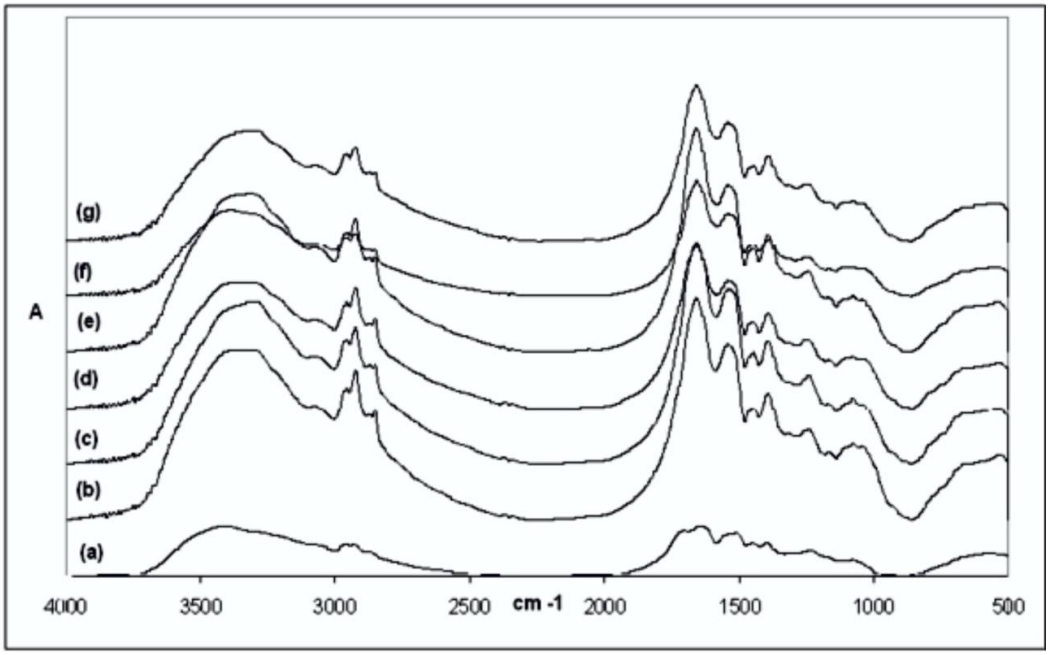


Figure 4. FTIR spectra of greasy and hydrolyzed wool samples. (a) Greasy Wool (b) 6.5 kg- 30 min (c) 6.5 kg - 60 min (d) 6.5 kg - 90 min (f)10 kg - 30 min (g) 10 kg - 60 min (h) 10 kg - 90 min.

In hydrolysis process at high temperature (170 ^{o}C) and elevated steam pressure (7 bar), most of the disulphide bonds in the fibre were broken. This destruction was confirmed by changes in the molecular weight distribution and amino acid composition such as the decrease of the cystine amount that witnessed changes in the protein chain rearrangements.

6.2.5 Amino acid analysis

Table 2 shows the amino acid composition of various hydrolyzed samples compared to the greasy wool. It is evident that degradation of cysteine residue was found in all the samples due to the hydrolysis treatment. About 1 mole % cysteine were present in the samples treated for 30 min and 0.5 mole % in the samples treated for 60 and 90 min in comparison with 9.41 mole % found in original wool. A low amount of lanthionine formation was observed after hydrolysis;

Table 2. Amino acid distribution (mole %) in hydrolyzed samples compared to amino acids of wool.

Amino Acid	Wool	6.5-30	6.5-60	6.5-90	10.0-30	10.0-60	10.0-90
		Batch mass (kg) – Process time (min)					
CYA	0.20	0.16	0.15	0.18	0.13	0.20	0.17
ASP	6.70	8.48	7.58	8.34	7.53	7.64	8.10
SER	10.10	9.93	9.25	9.29	10.01	9.24	9.26
GLU	13.00	15.90	15.26	15.70	14.86	15.18	15.72
GLY	9.00	10.74	12.65	12.27	10.75	12.60	11.76
HIS	0.22	0.33	0.645	0.58	0.66	0.79	0.84
ARG	7.18	6.60	7.40	6.875	7.40	7.20	7.23
THR	6.23	5.53	4.69	4.77	5.58	4.70	4.83
ALA	4.53	5.74	6.03	6.13	5.69	5.86	6.35
PRO	6.60	7.09	7.19	7.18	7.10	7.47	7.22
LANT	0.34	2.53	1.33	1.43	2.39	1.10	1.11

1/2CYS	9.41	1.07	0.55	0.57	1.06	0.50	0.53
TYR	3.57	2.64	3.17	2.76	3.04	3.18	3.15
VAL	6.27	6.59	6.81	6.80	6.70	7.01	6.77
MET	0.46	0.39	0.48	0.41	0.46	0.47	0.46
LYS	2.80	3.03	2.68	2.78	2.87	2.62	2.66
ILE	3.33	3.46	3.62	3.58	3.53	3.63	3.51
LEU	7.31	7.60	7.86	7.85	7.67	7.92	7.81
PHE	2.70	2.10	2.59	2.47	2.50	2.65	2.47

this substance increased its concentration from 0.345 mole % in untreated wool to 2.5 mole % in the samples originated by for the shortest hydrolysis time. The amount of tyrosine decreased in hydrolyzed samples in comparison with the untreated wool, while minor or no change at all was observed for phenylalanine. The loss of aspartic acid and an amount increase of glycine residues were observed. The concentration changes of the other amino acids can be attributed to their solubility and thermal stability.

The ratio of temperature stable to temperature sensitive increased to a maximum value of 2.22, as the hydrolysis time was prolonged from 30 min to 90 min in comparison with a reference value of 1.64 found in wool. (Table 3) The disulphide bonds which make wool keratin particularly stable and difficult for soil degradation are broken apart during the hydrolysis process, which leads to a faster degradation of hydrolyzates in the soil compared to untreated wool. The main concentration changes in cysteine, lanthionine and other amino composition was found by extending the process from 30 min to 60 min.

Table 3. Ratio of temperature-stable and temperature-sensitive amino acids in greasy wool and hydrolyzed samples at different time.

Sample	Wool	30 min*	60 min*	90 min*
T- stable (Glu, Gly, Pro, Ala, Val)	39.42	45.59	48.04	47.96
T- sensitive (Arg, Met, Thr, Ser)	23.99	22.96	21.73	21.57
T-stable / T-sensitive ratio	1.64	1.99	2.21	2.22

It is interesting noting that the amount of amino acids in untreated wool and hydrolyzed products varied greatly: this is a promising qualitative indication since the amino acids can act as bio-stimulants for plants. The categories of biostimulants include protein hydrolyzate, which consists of a mixture of peptides and amino acids of animal origin. The main amino acids (including alanine, arginine, glycine, proline, glutamine, valine and leucine) stimulate the growth of the plants and appear to be distinguished in terms of nutritional effect by higher amounts of nitrogen.[30-31]

6.2.6 Molecular weight distribution

The molecular weights of proteins extracted from the hydrolyzed samples were determined and compared to molecular weights of greasy wool. The electrophoresis pattern of wool consists of major protein fractions visible in the lane 2 of Figure 5. In these patterns, molecular weights around 43 to 67 kDa correspond to the low sulphur protein content of intermediate filaments of cortical cells, while molecular weights in the range of 11 to 28 kDa belong to the high sulphur content proteins.[17] The effect of hydrolysis treatment is clearly visible in Figure 5 all samples, even those treated for 30 min, shows the absence of low sulphur proteins. As the hydrolysis time was increased from 30 to 90 min, the molecular weight distribution remained unchanged. The products obtained had molecular weights varying approximately from 3 to 10 kDa. The 3 kDa is the lower limit of detection of the analytical technique adopted. The effect of hydrolysis on the degradation of protein molecular weight was unaffected by the initial amount of material used for the treatment, 6.5 kg or 10 kg. The loss of high molecular weight proteins is attributed to peptide bond hydrolysis because of water temperature.

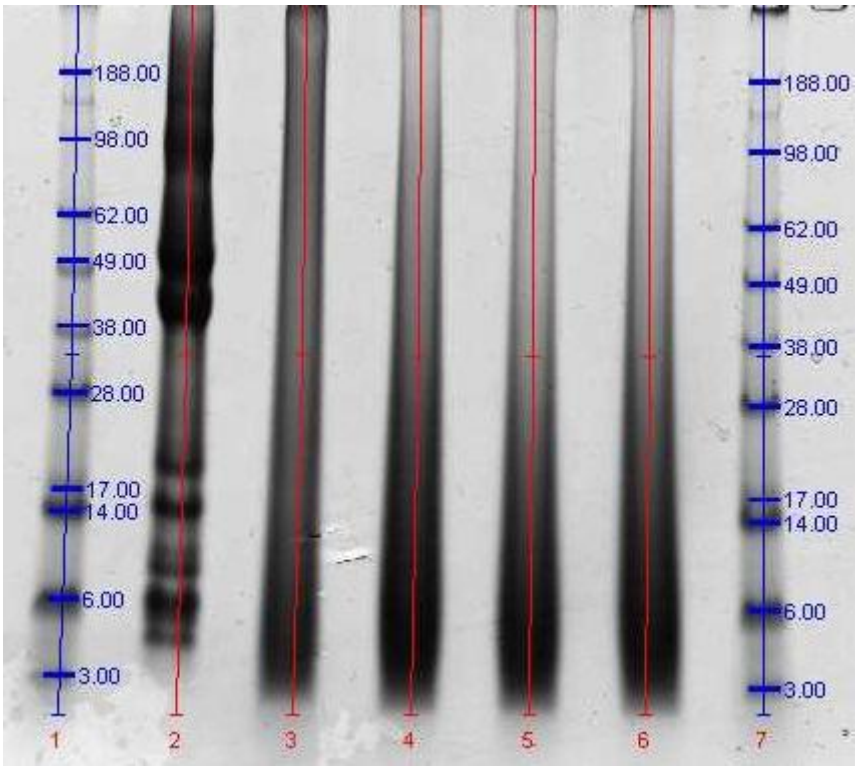


Figure 5. Electrophoresis pattern of proteins from hydrolysis 1) and 7) Standard reference proteins of different molecular weight 2) Greasy Wool 3) 6.5 kg - 30 min 4) 6.5 kg - 90 min 5) 6.5 kg - 60 min 6) 10 kg - 60 min.

6.2.7 Elemental analysis of protein hydrolyzates

The essential primary and non-mineral nutrients: nitrogen, phosphorus, potassium, carbon and ammonia are present in the final hydrolyzed products. The presence of nutrients are in favour of proposing a positive application of hydrolyzates in agriculture as a fertilizer. Nitrogen is the main important component of proteins, necessary for plant growth and their development. The amount of nitrogen found in protein hydrolyzates was a function of both the amount of wool loaded initially into the reactor (6.5 or 10.0 kg) and time (30, 60 or 90 min). It was found to be in a range between 3.9 to 5.09 % in the hydrolyzed samples.(Table 4)

Table 4. The composition of nutrient elements presents in different hydrolyzed samples.

	Nutrient elements					
Sample batch mass (kg) – process time (min)	C (%)	N (%)	P (%)	K (%)	Ammonium nitrogen (%)	C/N Ratio
Greasy wool	32.52	7.62	0.05	2.33	1.22	4.26
6.5-30	14.19	5.09	0.03	0.55	0.17	2.78
6.5-60	8.52	3.9	0.02	0.58	0.20	2.18
6.5-90	8.95	3.9	0.02	0.56	0.22	2.29
10.0-30	15.08	4.21	0.03	1.25	0.25	3.58
10.0-60	16.47	4.88	0.02	1.58	0.34	3.375
10.0-90	15.00	4.22	0.02	1.36	0.27	3.55

The ammonium nitrogen values were found in a range between 0.17 to 0.34 % w/w, with very uniform values around 0.20 % for the semiliquid products. Ammonium nitrogen is a source of nourishment promptly available for crops, while the nitrogen bound to organic molecules must undergo a process of mineralization by soil microorganisms. The amount of phosphorus found varied in the range between 0.015 to 0.025 %. The amount of potassium total detected varied from 0.55 to 1.58 %. The threshold limits for standard organic fertilizer set by the European Consortium of the Organic based Fertilizer Industry (ECOFI) are C_{org}= 15 % for solid and 5 % for liquid organic fertilizer, ≥ 2,5% N_{tot} and/or ≥ 2% $P_2O_{5\ tot}$ and/or ≥ 2 % K_2O water soluble for solid type of fertilizer and ≥ 2% N_{tot} and/or ≥ 1 % $P_2O_{5\ tot}$ and/or ≥ 2 % K_2O water-soluble for liquid fertilizer.[32] The amount of nitrogen present in protein hydrolyzates exceeded the boundary limit so the hydrolyzed product can be included into the organic fertilizer category.

Table 5. Metallic amount of different hydrolyzed samples.

Metal	Technical regulation, DCI 27/07/'84 (MSWC)	Law on fertilizers (L 748/'84 and 03/'98) BWC/GC/SSC	6.5 kg-30 min (mg/kg d.b.*)	6.5 kg-60 min (mg/kg d.b.*)	6.5 kg-90 min (mg/kg d.b.*)
B	-	-	< 0.5	< 0.5	0.8
Cd	10	1.5	0.174	0.169	0.17
Co	-	-	1.1	0.8	< 0.5
Cr	500	0.5	< 0.2	< 0.2	< 0.2
Mn	-	-	84.5	74.5	72.1
Hg	10	1.5	< 0.05	< 0.05	< 0.05
Ni	200	50	4	4	4
Pb	500	140	< 1	< 1	< 1
Cu	600	150	8	8	7
Zn	2500	500	44	44	44

* d.b. =dry basis

The C/N ratio is an important parameter which gives information regarding decomposition of organic matter in the soil because a microorganism that decomposes the organic matter uses carbon as a source of energy and nitrogen for building a cell structure. Materials with a high C/N ratio (> 10) facilitate the processes of humification, which enriches the soil of re-synthesized organic molecules of high molecular weight, representing an important factor for increasing fertility and structural quality of soil.[33] On the contrary, low C/N ratios (< 10) strongly stimulate the process of mineralization of organic matter, providing large amounts of available nitrogen; this modifies the overall process more toward a prompt mineralization, accentuating the function of fertilizer and productive with respect to improving the soil quality. The analyzed samples displayed a very low C/N ratio, ranging between 2.18 to 3.5. These values are in agreement with the data obtained from a previous study regarding fertilizers of similar origin.[34]

Micronutrients (B, Mn, Ni, Cu, Zn) in general are available in very small quantities in most soils and plants. These micronutrients are as essential as the primary or secondary nutrients and its deficiency leads to a severe limitations in plant growth, crop yield and quality.[35] In our study the elemental analysis of 6.5 kg wool samples hydrolyzed for 30, 60 and 90 min was carried out.

According to the European compost standards[36], heavy metals such as Cd, Cr, Cu, Hg, Ni, Pb, Zn are considered as potential toxic elements (PTEs). These PTEs are the most important factors of concern because a high concentration of PTEs can penetrate the soil-plant system and affects the natural root-microorganism mechanisms which regulate the transport and accumulation from soil ending up in the edible part of vegetables. Through the food chain, PTEs may accumulate inside human or animal organs leading to poisoning effects, cancer, etc. Hence, PTEs indirectly pose a potential risk for human health.[37-38] The concentrations of heavy metals present in all the fertilizers produced during this study were far below than limits mentioned in Italian technical regulation standards, DCI 27/07/'84 (MSWC) and law on fertilizers (L 748/'84; amd: 03/'98) BWC/GC/SSC.(Table 5) The low concentration of micronutrients and heavy metals in the hydrolyzed products guarantee the potential application of these hydrolyzates as a fertilizer.

6.2.8 Nutrient release dynamics

The parameters that affect the dynamics of mineralization are related to both the nature of the soil (pH, texture, nature of the microbiome present) and to the added organic fertilizer (nature of the molecules present, contributions of microorganisms and other substances, i.e., enzymes). All experiments were carried out in an incubation growing chamber. Hydrolyzed wools refer to the following samples: 5R liquid (6 kg, 90 min); 9R solid (10 kg, 60 min); 12R liquid (6.5 kg, 90 min); 13 R liquid (6.5 kg, 60 min). Samples were compared with the organic fertilizer of common use (digested manure and slurry from a pig) with a mineralization rate comparable to the wool hydrolyzate (C/N 4.66), commonly used nitrogen fertilizer (urea), and reference sample not fertilized. Soil used was collected from an aeolian hill near Turin. The experiment was set up in mesocosms equipped with porous cups for the sampling and analysis of the circulating solution and stoppers fitted with devices for the sampling of the gaseous emissions. The pots were then placed in a cell at 20 $^{\circ}$C, and the fertilizer was added to ensure a level of fertilization of 200 kg N/ha. Soil solution was analyzed for ammonium, nitrates, chlorides, sulphates, phosphates and the amount of gas emission (CO_2, N_2O).

Nitrates and sulphates showed the most interesting and important results. The presence of nitrates in the circulating solution is related to the dynamics of the degradation of the organic substance and the consequent action of nitrifying bacteria that progressively oxidize the ammonium ion to nitrate ion. Comparison with the not fertilized reference enhanced the good potential fertilizer of hydrolyzed wool. The presence of nitrates is important for the availability of readily assimilable nitrogen by crops, but nitrates can percolate in groundwater, or be dispersed in the atmosphere in the form of nitrous oxide.

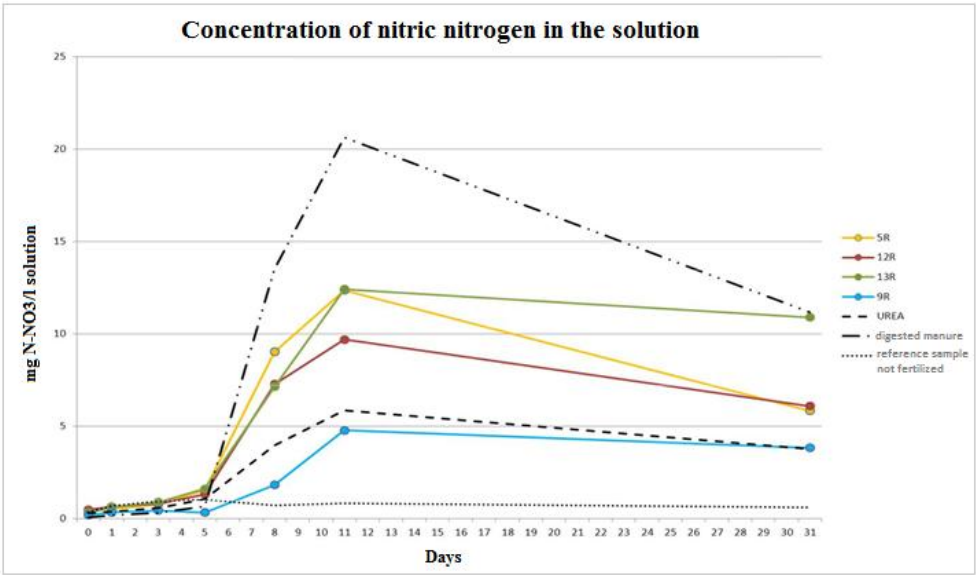


Figure 6. The release of nitric nitogen concentration in fertilizer solution over time.

As shown in figure 6, detection of nitrates in groundwater started about 5 days after application of the fertilizers to the ground, because the production of nitrates is delayed by the presence of ammonium precursor ions, which are released gradually by the samples. In general, these samples show the release behaviour and the typical dynamics of the organic substance used as a fertilizer or soil improver, presenting a gradual release of nutrients over time. The similar dynamics of mineralization is observed for the digested manure and urea. Mineralization dynamics of sulphur compounds are fully consistent as shown in figure 7: samples that have been treated with higher hydrolysis time (90 min) shows the higher release speed of sulphate. Below sample treated with a shorter

hydrolysis process (60 min) also shows a high alteration of the organic molecules from the matrix.

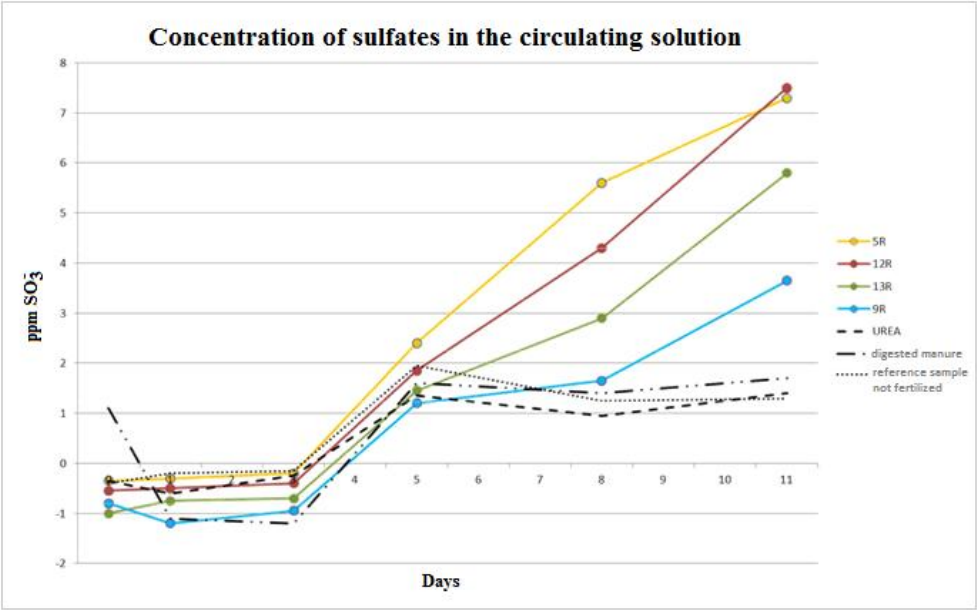


Figure 7. The release of sulphate concentration in fertilizer solution over a time.

CO_2 emission

CO_2 emission from agricultural soils are the expression of the soil microbiome towards organic matter. Since CO_2 is the final product of the mineralization dynamics, CO_2 emissions are indicative of both the quantity and quality of organic matter in soil, the quality of micro-organisms, and the speed of availability of nutrients from organic matter to the crops. Figure 8 shows that the addition of fresh organic substance increases the production of CO_2 especially in the first 15 days after fertilization, then the production of CO_2 returns to the level of sample not fertilized. Sample 5R (liquid) showed greater susceptibility to mineralization of organic substance and could support active microbial processes for a longer time.

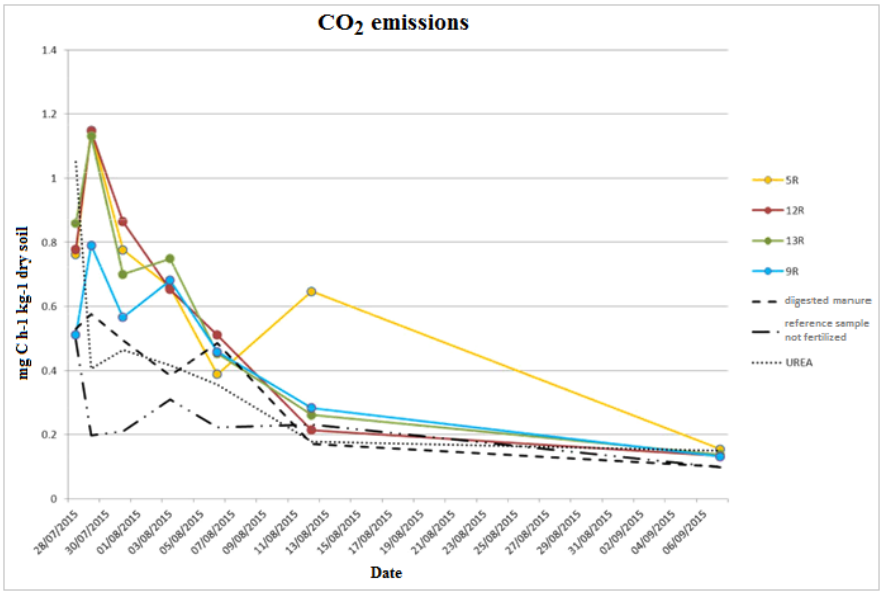


Figure 8. The CO_2 emission from soil due to addition of different fertilizer over an interval of time.

N_2O emission

Nitrous oxide (N_2O) is a product of nitrification and denitrification microbial processes.

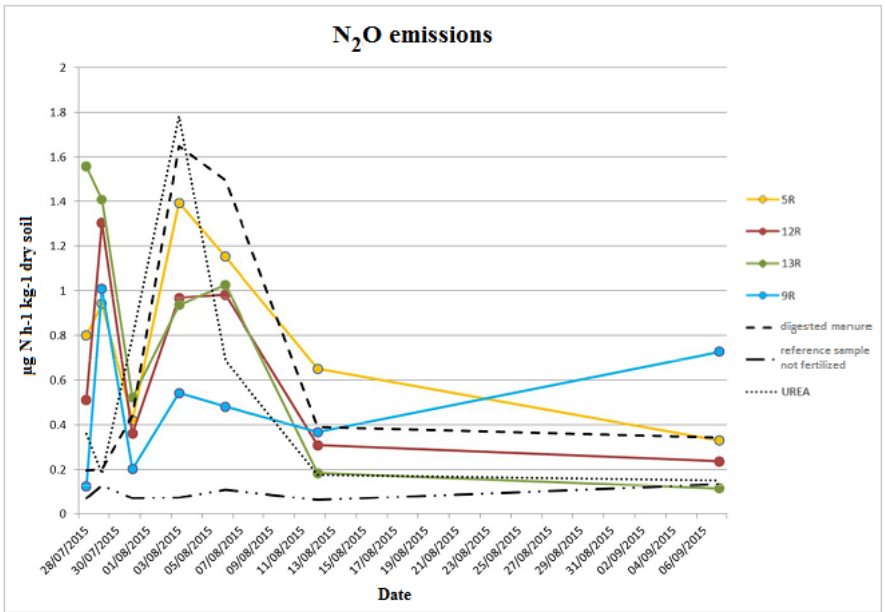


Figure 9. The N_2O emission from the soil due to the addition of different fertilizer over an interval of time.

Figure 9 shows that the emission of N_2O is related first to the ammoniacal nitrogen already available in the product itself. Then it shows a general decrease with the depletion of this initial amount of ammoniacal nitrogen, and finally, it increases because of the availability of new ammonia nitrogen released by the progressive mineralization of organic matter. The solid sample 9R showed a weaker dynamic of emission in the first period; however, it had a much higher rate of emission in the following period, due to a lower hydrolysis of the original protein.

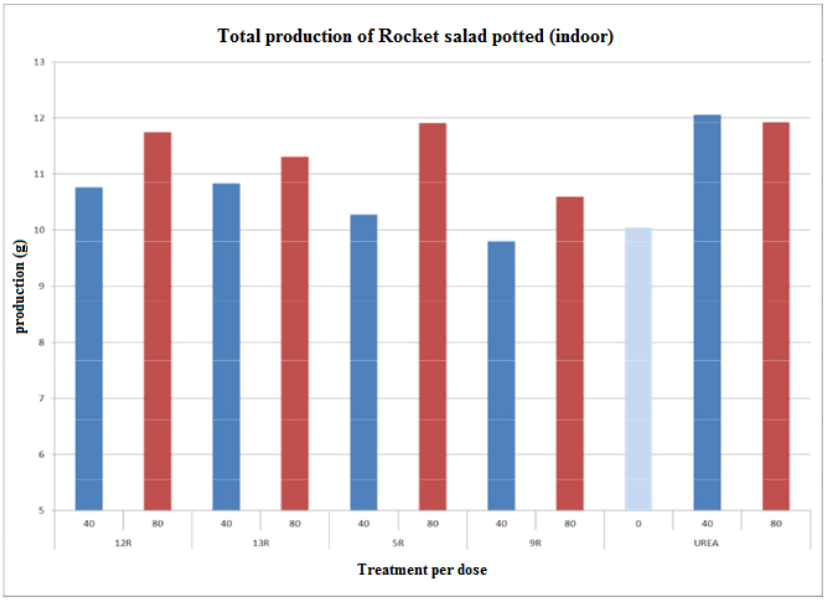
6.2.9 Interaction between fertilizer, soil and plants: pot trials with hydrolyzed wool

The cultivation pots test has been carried out to determine the production levels of plants fertilized with different organic fertilizer compared with hydrolyze wool. Wool hydrolyzates used were the same that have been subjected to previous mineralization tests. Two plants were used for the tests:

- Rocket salad (Eruca sativa L.) cultivated indoor in a thermostated greenhouse.
- Italian Ryegrass (Lolium multiflorum L.) cultivated outdoor.

The amount of fertilizer used was calculated in order to add the soil with a suitable nitrogen amount (40 and 80 kg/ha for rocket salad and 100 and 200 kg/ha for Italian ryegrass). Production level was evaluated on the dry ground biomass collected at the end of the tests.

(a)



(b)

Figure 10. (a) Pot preparation for cultivation using wool hydrolyzate (b)Results from pot trials of racket salad production.

Rocket salad indoor

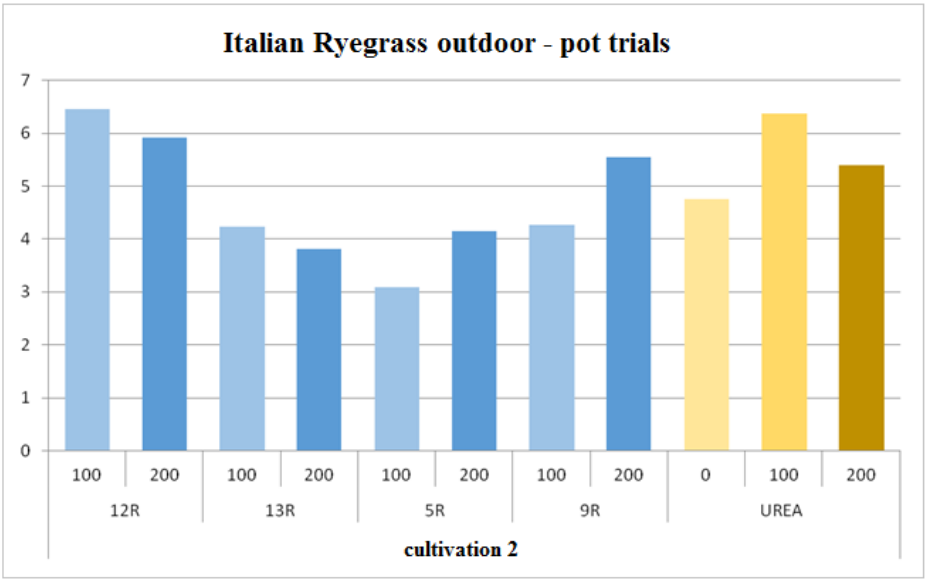
As it can be seen in figure 10, all plants were responded well to fertilization, differentiating two production levels depending on the fertilizer amount. The production level of urea was slightly high than the production level of wool hydrolyzate. In case of wool hydrolyzate fertilizer the best performance was given by the sample 5R, due to the high capacity of mineralization, which allowed constant intake of nourishment to plants and also comparable to reference fertilizer urea. The solid sample 9R for its characteristics of slow degradability seemed not to be able to support the nourishment of crop growth in full potential. The fertilizers 12 and 13R were placed in an intermediate production level

Italian Ryegrass outdoor

The analysis of the total biomass shows that, even if a part of the experimental samples showed productive results below the unfertilized control, the 12R sample (the higher hydrolysis degree) gave production levels equal or superior to the mineral fertilizer (urea).

Data obtained from pot trials of rocket salad and Italian ryegrass show complex results in terms of response from crops of different types of fertilizer. The results show the possibility to optimize different nutrient solutions starting from the hydrolysis treatment for the application on different types of crops.

(a)



(b)

Figure. 11 (a) Pot trials for Italian raygrass and rocket salad (b) Results from pot trials of Italian ryegrass production

6.2.10 Germination test

A germination test is used for evaluating the phytotoxicity degree of a product. In this study, the percentage of germination was around 100 % indicating that the wool hydrolyzate did not display any phytotoxic effect. Moreover, the product examined and diluted with water at a concentration of 1 g/L showed a higher seed germination rate in comparison with the control specimen and with samples treated with a concentration of 10 g/L. The possible explanation is ascribable to a bio-stimulating effect resulting from molecules containing promptly available nitrogen (such as proteins, amino acids) and also to the presence of microelements in the fertilizer. This effect appeared to be depressed at the higher concentration, because of problems most likely related to the excessive osmotic salt concentration around a seed. The percent germination rate (GI %) reached the values of 177 % and 90 % at 1g/L and 10 g/L, respectively.

Figure 12. Germination test of Lepididum sativum.

Table 6. Number of sprouted seeds and average root length of control samples and samples treated with 1g/L and 10g/L of wool hydrolysate

Number of seeds	Control samples (root length, mm)					Average C	Hydrolyzed wool sample 1 g/L (root length, mm)					Average H1	Hydrolyzed wool sample 10 g/L (root length, mm)					Average H10
	1	2	3	4	5		H 1_1	H 1_2	H 1_3	H 1_4	H 1_5		H 10_1	H 10_2	H 10_3	H 10_4	H 10_5	
1					0		2	7	12	12	2		4	5	8	8	0	
2				0			2	9	9	10	3		5	6	0	1	0	
3							10	8	9	6	9		6	5	2	0	11	
4							4	6	3	0	9		8	4	0	7	7	
5	0				2		8	8	6	8	9		7	0	7	1	7	
6					1		11	6	10	10	13		7	10	8	0	1	
7							12	1	0	9	13		1	6	6	5	2	
8							12	13	10	1	10		8	1	6	6	0	
9							0	5	8	10	8		8	5	4	2	0	
10	0						4	8	9	6	7		8	5	5	1	0	
Number of sprouted seeds						**8.4**	9	10	9	9	10	**9.4**	10	9	8	9	5	**8.2**
Average root length	.2	.7	.1	.5	.7	**4.64**	6.5	7.1	7.6	7.2	8 .3	**7.34**	6 .2	4.7	4.6	3.1	2.8	**4.28**

6.3 Conclusions

The technology presented in this study, which suggests wool hydrolysis by superheated water, demonstrated the feasibility of a well sustainable circular economy. The process can be defined entirely "green," as no chemicals at all contribute to the reaction. The tests presented here are illustrated regarding physical, chemical and morphological properties of the fertilizer produced. The elemental analysis was addressed to measure both the concentration of nutrients and heavy metals. Altogether, this evaluation has demonstrated that the hydrolyzate fulfills the standards according to the Italian law on organic fertilizers.

The optimal process parameters of the hydrolysis process range around 170 ^{o}C and 60 min with a greasy wool to water mass ratio close to unity. In this view, a major reaction requirement is represented thorough an impregnation of the solid phase, as demonstrated by preliminary proof-of-principle tests. A second hydraulic condition is given by an effective solid to liquid mass transfer obtained by the tumbling motion of the hydrolysis reactor. The optical and the scanning electron microscopy revealed the effectiveness of hydrolysis treatment in wool degradation. The wool hydrolyzates obtained consisted of low molecular weight proteins and peptides along with a low amount of cysteine residues. The content of nitrogen, amino acids, micronutrients and a neutral pH suggest a direct application of the hydrolyzate as a bio-stimulant of soil microbial activity through fertirrigation and as a stimulant for plant growth. A series of germination tests showed that a very high germination index can be obtained by applying a low concentrated solution which clearly indicates that the product has no phytotoxic effect.

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Chapter 7

Superheated Water Hydrolyzed Keratin: a New Application as a Foaming Agent in Foam Dyeing of Cotton and Wool Fabrics

7.1 Introduction

Conventional textile wet processing generates large volumes of wastewater and is highly energy consuming in processing, leading to negative environmental impact. To minimize this impact, different textile dyeing processes are developed such as low liquor dyeing, waterless supercritical dyeing, solvent dyeing etc- . The dyeing with low liquor needs highly soluble and stable dyestuffs, while solvent dyeing and supercritical dyeing technologies are applicable to a limited range of dyes and involves high capital investment as well as an efficient solvent recovery system.

Foam technology is an effective, low add-on technology, due to expanded volume and large internal surface area of the foam bubbles. In this technology, the foam is used as a driving tool to apply chemicals and dyes on the textile substrate where water is replaced with air. Low wet pickup (in the range of 20-40%) in foam dyeing rather than conventional pad dyeing (60-100%) results in economic advantages such as low drying time, less of water and energy utilization, improvement of the productivity and lower load on effluents. Due to its exceptional performance, foam technology finds its application in different textile

wet processing such as sizing, dyeing, mercerization, finishing, and printing[5-10]. Foam dyeing of cotton fabric using reactive dyes was studied by Yu et al., and the resulting foam dyed fabric has shown that excellent fastness, moreover better build up properties of dyestuff can be achieved through this procedure.

In general, a wide range of synthetic surfactants were used as a foaming agent such as sodiumdodecyl benzene sulphonate, dodecylamine hydrochloride, poly(ethylene oxides), dodecyl [11-12] belaine etc - . Proteins are similar to the amphiphilic synthetic surfactants because they contain hydrophobic and hydrophilic, anionic and cationic amino acids, which afford them a certain[13] degree of surface activity. Renewed interest in protein-based surfactants has occurred not only as products based on renewable raw materials, but also as a solution for the waste disposal of animal and vegetable protein by-products.

Keratins are fibrous proteins which are the main constituent of wool fibers. Keratin is abundantly available in nature and also available as a by-product from wool industries, slaughterhouse, poultry farms and tanneries[14].In previous studies, proteins such as hydrolyzed collagen and keratin were used as a modifier of cotton fabric and leather substrates. Dyeing of leather in the presence of collagen hydrolyzate as an additive resulted in better dyeing properties. Also, keratin hydrolyzate has been used as an exhausting agent in the reactive dyeing of cotton fabric[15-16]. Hydrolyzed keratin was used as a foaming agent in an application such as fire fighting foam[17]. In this study, for the first time, keratin hydrolyzate was used as a foaming auxiliary in the textile dyeing process. Application of keratin as a foaming agent will be beneficial over synthetic surfactants not only in terms of higher biodegradability and less toxicity but also in wastewater processing of dyeing effluents[18].

Extraction of keratin from wool was carried out by various processes such as hydrolysis (with acids, bases, and enzymes), steam explosion, acoustic cavitation, sulfitolysis, enzyme degradation etc. In our paper, keratin hydrolyzate was produced by using superheated water hydrolysis which is an eco-friendly and economical process, as it is carried out using water as a solvent. The superheated water hydrolysis treatment sterilizes the wool and makes it more biodegradable, hence suitable for different applications. Studies revealed that keratin hydrolyzate obtained at the end of such a treatment consisting of low molecular weight oligopeptides and amino acids, and resulted in the dissolution of wool fibres[19-24].

In this chapter, the study has been carried out in order to use hydrolyzed keratin as a foaming agent in the reactive dyeing of cotton and acid dyeing of wool fabric.

Foamability and foam stability were used to analyze foaming properties of hydrolyzed keratin. Foam dyeing properties such as color strength, fastness etc., were compared with the conventional dyeing processes.

7.2 Results and discussion

Keratin hydrolyzate

7.2.1 Surface tension

The surface tension decrease with the increase in keratin concentration is observed in Figure 1. Below a concentration of 0.005% w/v, a slight reduction in the surface tension is observed. As the concentration of keratin hydrolyzates increases from 0.005% to 1% w/v a progressive nonlinear reduction is observed. Further increase in concentration beyond 2% of keratin hydrolyzates results in a relatively small reduction in surface tension. This fact is attributed to the saturation of the adsorbed keratin hydrolyzates at interfaces, because of anomalous behavior of protein-based surfactant which gradually reaches an equilibrium value of surface tension. This feature of protein surfactants is exhibited in the very slow setting of the equilibrium value of surface tension. The slow formation of the equilibrium adsorption layer was attributed to diffusion of globular molecules to interface and formation of peptide chain on the surface[33-34].

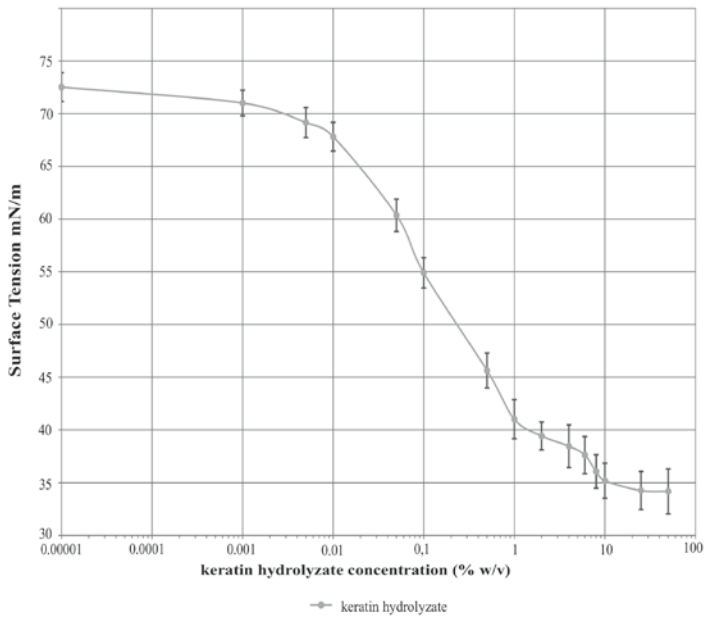


Figure 1. Surface tension of keratin hydrolyzates at various concentrations.

7.2.2 Molecular weight distribution

The molecular weight distribution of keratin hydrolyzate obtained from superheated water hydrolysis treatment was compared with wool samples. The keratin hydrolyzates obtained from hydrolysis consist of a mixture of various protein fractions having near values of molecular weight. As seen in the lane 2 of Figure 2, unhydrolyzed wool is composed of various major fractions of proteins while the effect of hydrolysis is clearly seen in lane 3. The absence of low sulfur proteins is clearly visible in keratin hydrolyzate. The keratin hydrolyzate used in this study consists of proteins with a molecular weight in the range of 3 to 14 kDa, where 3 kDa is the lowest limit of detection of molecular weight for the technique we have used.

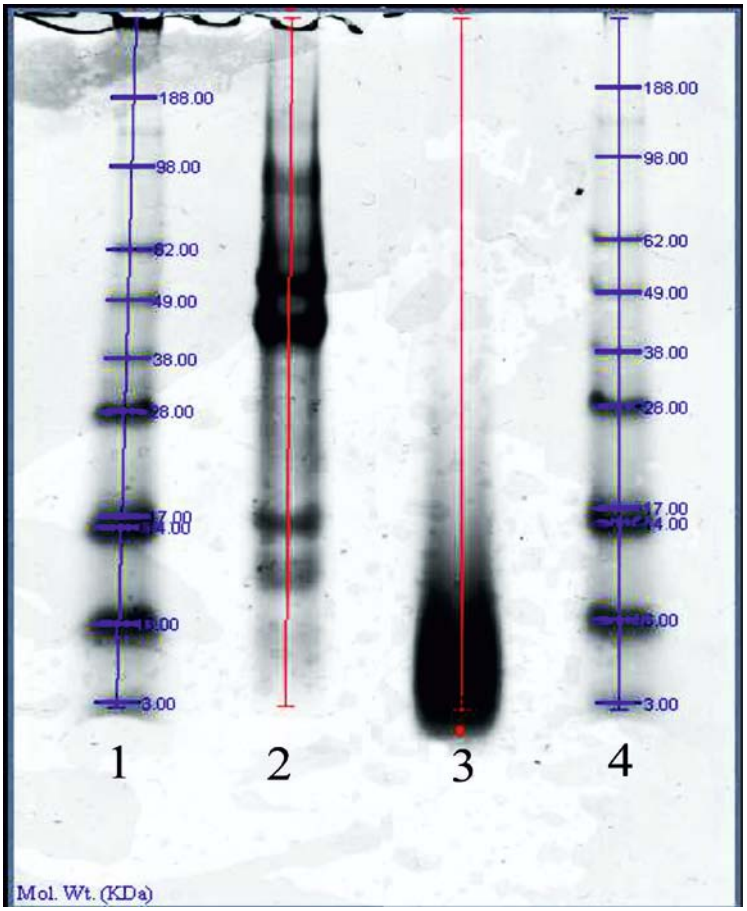


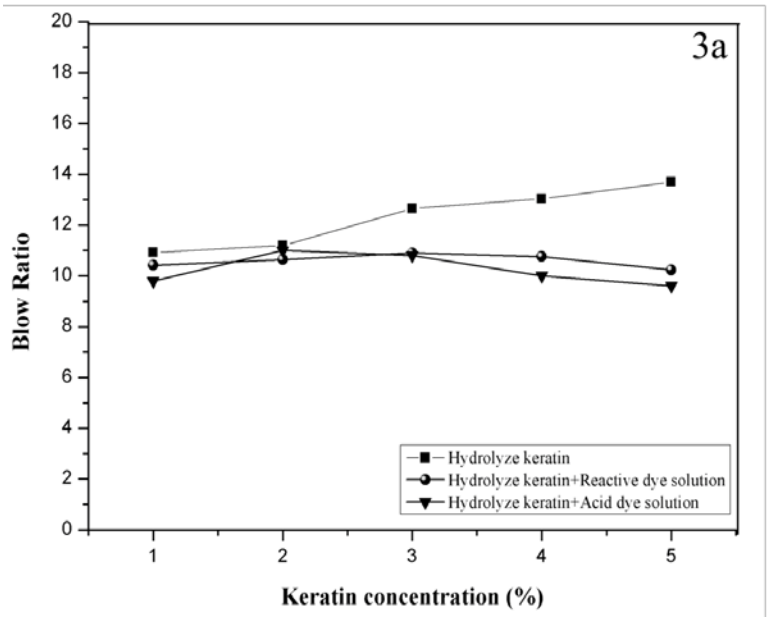
Figure 2. Electrophoresis pattern of keratin hydrolyzate obtained from hydrolysis lane 1) and 4) standard reference proteins of different molecular weights, lane 2) wool, lane 3) keratin hydrolyzate.

The molecular weight of keratin hydrolyzate is an important parameter in case of dyeing, especially for wool fibers, due to the fact that the adsorption of proteins into fibers depends on its molecular weight. Low molecular weight keratin hydrolyzate is adsorbed more, in comparison with high molecular weight[38]

Foam

7.2.3 Blow ratio and foam stability of keratin hydrolyzate and dyeing solution

As shown in Figure 3 a, b, foamability of keratin hydrolyzate was measured in terms of its blow ratio. Five different concentrations were used to determine foaming ability and foam stability of keratin hydrolyzate solutions at neutral pH (7.16), keratin hydrolyzate liquors with reactive dye, acid dye and respective auxiliaries required for dyeing solution. In the case of keratin hydrolyzate only, the prepared foams show that, as the concentration of keratin hydrolyzate increases, there is an increase in blow ratio and foam stability.



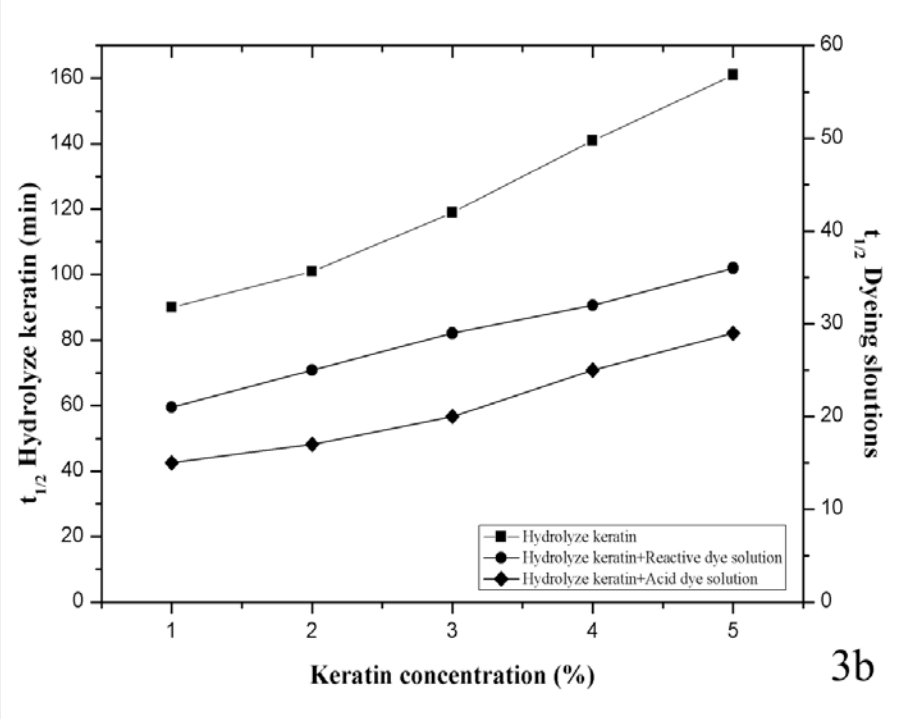


Figure 3. (a) Blow ratio (b) foam stability of keratin hydrolyzates at neutral pH and acid and reactive dye solution at 1-5 % of keratin hydrolyzate concentration.

The blow ratio of keratin hydrolyzate containing reactive dye and acid dye solution increases initially with the increase in concentration up to 2% w/v. Beyond this value, a further increase in concentration resulted in a gradual decrease in blow ratio. The decrease in the blow ratio in the case of dyeing solution is attributed to the presence of dyeing auxiliaries and dyes. The foam stability of dyeing solution increases with the increase in keratin hydrolyzate concentration. The blow ratio of 2 % w/v keratin hydrolyzate including reactive and acid dye solution is observed to be 10:1 and 11:1 respectively in agreement with the blow ratio required for foam dyeing (6:1-12:1) for woven fabric[36]. The compatibility of dyeing auxiliaries with a foaming agent is necessary for foam technology. The dyes and dyeing auxiliaries selected for dyeing recipe show a good compatibility with keratin hydrolyzate in terms of foamability and foam stability.

7.2.4 Morphological characterization of foam by optical microscopy and bubble size

The bubble size is an important characteristic in foam behavior. In general, foams with small bubble sizes are more stable. The micrographs of the foam obtained using keratin hydrolyzate, reactive and acid dyeing solutions for cotton and wool fabric dyeing are shown in Figure 5 a, b and c, respectively. The microscopic analysis reveals the bubble shape and size produced from the three different solutions. From the micrographs, it was observed that all the foams produced using the three different solutions have similar morphology.

As shown in the histograms in Figure 5, the bubble size for keratin hydrolyzate is in the range of 0.05-0.1 mm diameter, and for reactive dye and acid dye solution the maximum numbers of bubbles are falling in the region of 0.025-0.1 mm diameter. The bubble size with a diameter in the range of 0.05 mm to 0.1 mm is essential to perform textile wet processing[11]. The use of keratin hydrolyzate as a foaming agent is able to produce a bubble diameter with above-mentioned sizes, which can be applicable in textile wet processing.

Figure 4. Representation of foam produced from keratin hydrolyzate along with dye auxiliaries.

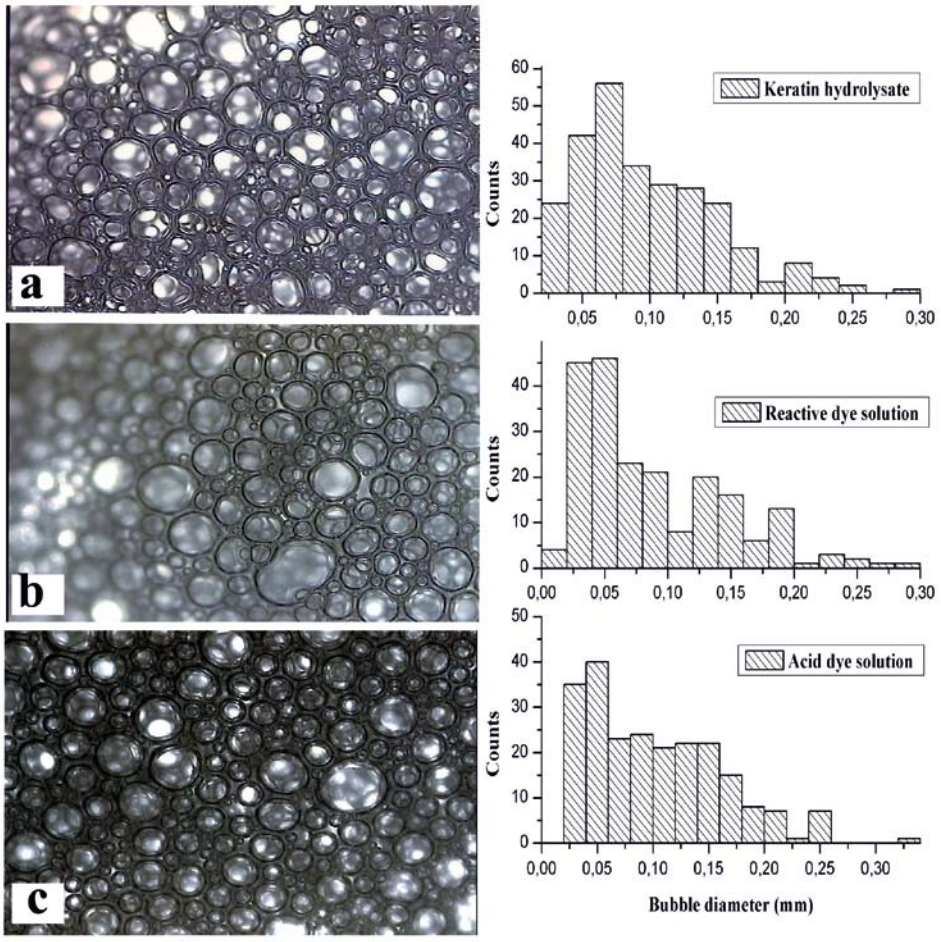
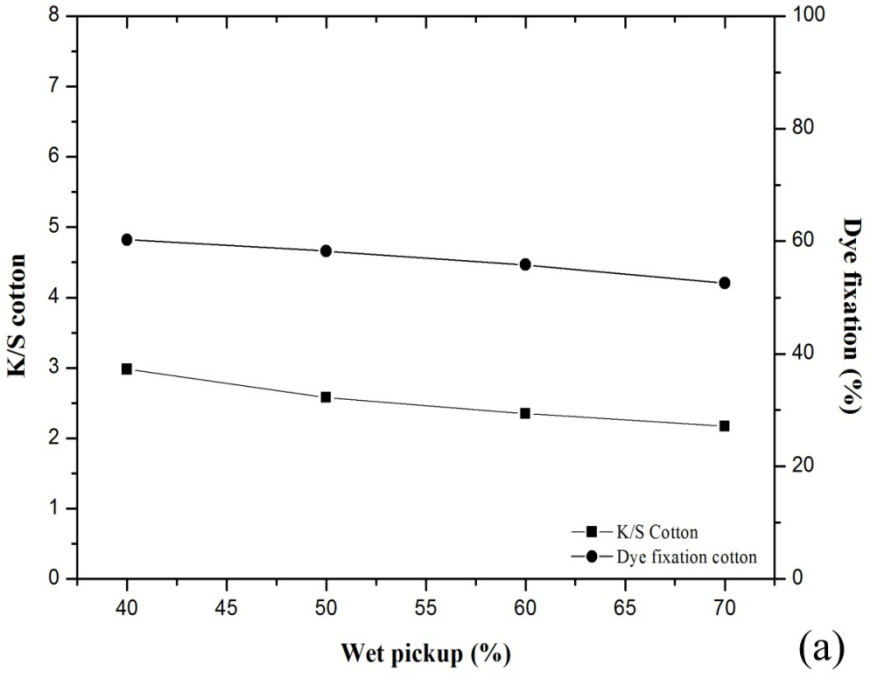


Figure 5. Microscopic images 25X and bubble size of foam produced from (a) keratin hydrolyzate (b) reactive dye solution (c) acid dye solution.

7.2.5 Wet pickup

The wet pickup is an important parameter affecting coloring properties of the dyed fabric. To study the effect of different wet pickups on dyeing properties is essential in order to deliver the desired quantity of dye onto the fabric. As shown in Figure 6 (a) and (b), wet pickup higher than 40% resulted in a gradual decrease in K/S, with insignificant changes in dye fixation rate of both reactive and acid dyeing of cotton and wool fabrics respectively. As the increase in the wet pickup of reactive and acid dyeing from 40 to 70%, the maximum value of K/S was

observed at 40%, which is adequate for the reactive and acid dyes to penetrate and diffuse consistently in cotton and wool fabrics. The color strength of cotton and wool fabrics at 40% wet pickup is larger in comparison to the wet pickup at 70%, while dye fixation rate is slightly smaller in comparison to the wet pickup of 40%. This is due to the availability of free water at a higher wet pickup, which resulted in the saturation of dye molecules inside the cores of fibers leading to decrease in K/S and dye fixation further.



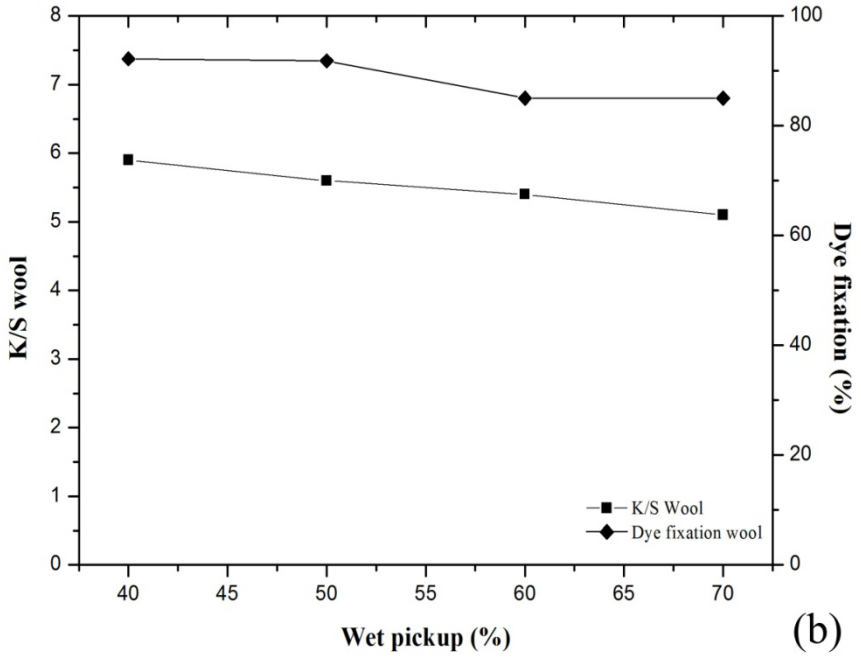


Figure 6. Effect of wet pickup on K/S values and dye fixation of (a) reactive dyeing of cotton and (b) acid dyeing of wool.

The results obtained in this study are in agreement with the wet pickup study in foam dyeing. In comparison with the different wet pickup values, working at 40% resulted in good dyeing properties, hence the same wet pickup was implemented in further study of reactive and acid dyeing of cotton and wool fabrics respectively.

Cotton and wool dyed fabrics characterization

7.2.6 Fourier transform infrared spectroscopy

FT-IR spectra of keratin hydrolyzate, control cotton fabric, foam dyed cotton fabric before and after washing are shown in Figure 7. The absorption spectra of keratin hydrolyzates show the classic three different regions named amide I, amide II and amide III, among which amide I is the most intense absorption band of proteins. The absorption frequency of amide I (1630-1650 cm^{-1}) is associated with stretching vibrations of the C = O bond. Amide II is found in the region of 1530-1550 cm^{-1} and belongs to bending vibrations of N-H bonds. The absorption frequency in the region of 1220-1240 cm^{-1} is attributed to the amide III. The

absorption frequency of amide A and amide B at 3282 and 3065 cm^{-1} respectively corresponds to N-H stretching vibrations.

In the case of cotton fabric, the absorption frequencies in the region 3100-3600 cm^{-1} and 2800-3100 cm^{-1} correspond to -OH and -CH stretching, respectively. The absorption frequency in the region 1633-1650 cm^{-1} corresponds to H-O-H bending due to absorption of water molecules. In the analysis of FT-IR peaks, the presence of a small peak at 1535 cm^{-1} in the unwashed dyed fabric is observed, in comparison with the dyed fabric after washing. This peak is due to the presence of small amount of keratin hydrolyzates which disappears after washing of dyed fabric and shows that keratin hydrolyzate used as foaming agent only act as dye carrier.

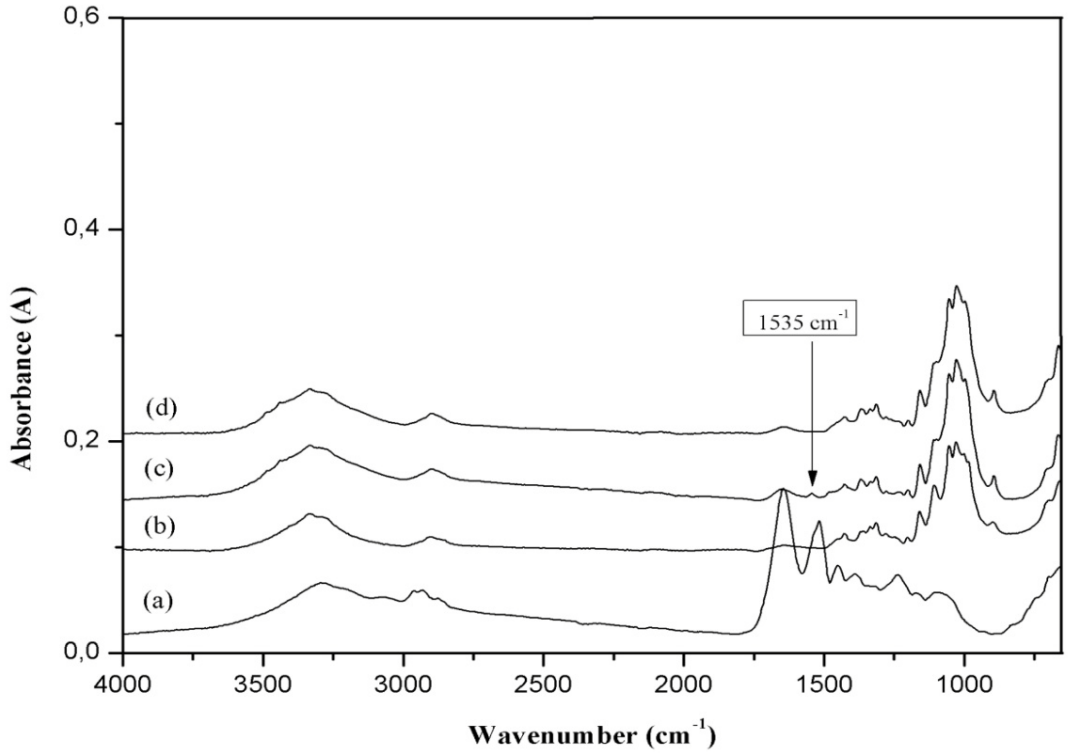
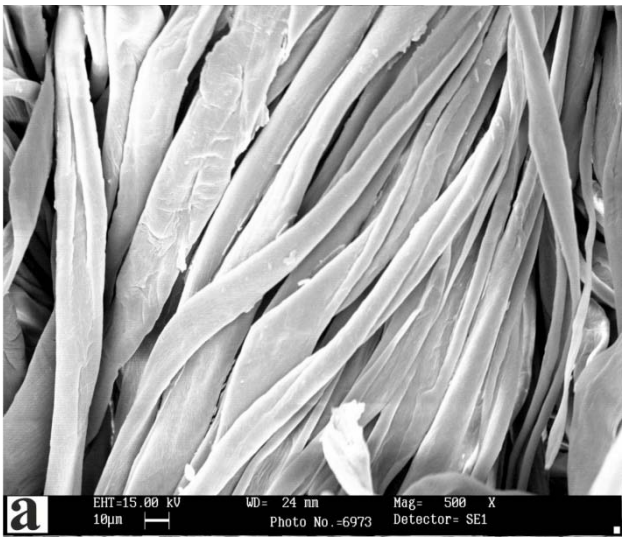
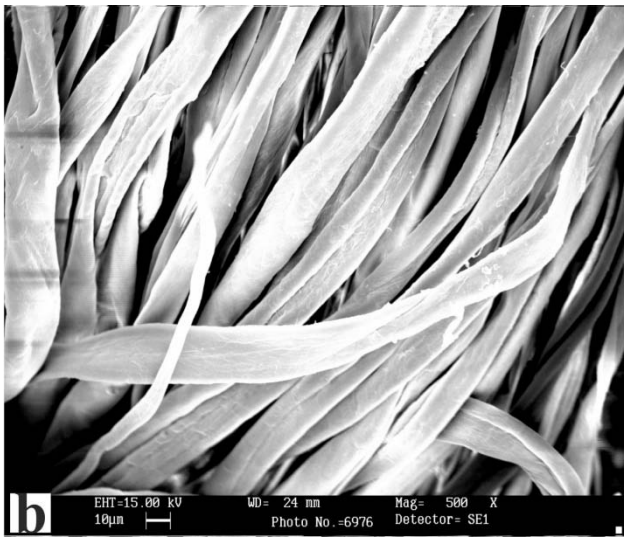


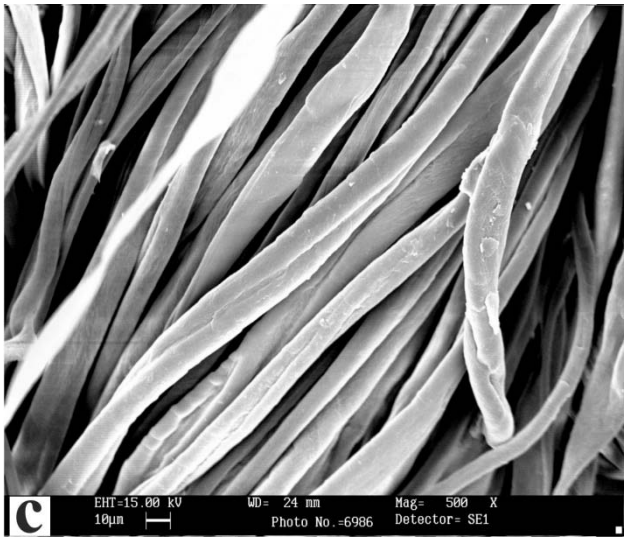
Figure 7. FT-IR of (a) keratin hydrolyzate (b) control cotton fabric (c) foam dyed cotton fabric before washing (d) foam dyed cotton fabric after washing.

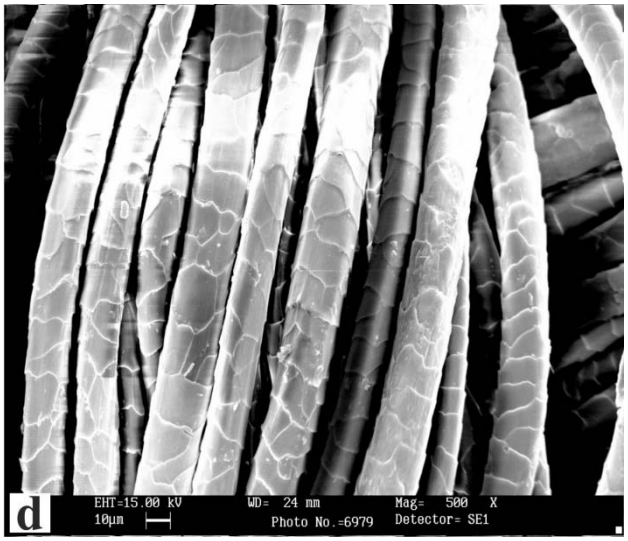
7.2.7 Morphological characterization of dyed samples by SEM analysis

In Figure 8, SEM pictures of foam and conventional dyed cotton and wool samples along with plasma treated wool sample are shown, in order to observe the presence of the keratin hydrolyzate after foam dyeing and the effect of plasma treatment on wool fibers.






c
EHT=15.00 kV
WD= 24 mm
Mag= 500 X
10µm
Photo No.=6986
Detector= SE1


d
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WD= 24 mm
Mag= 500 X
10µm
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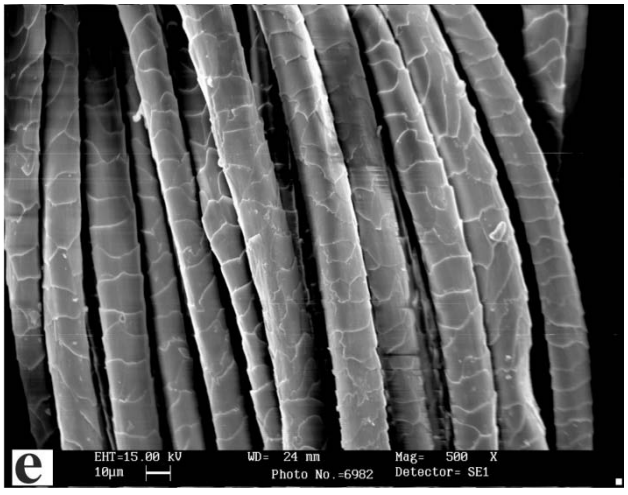
Detector= SE1


e
EHT=15.00 kV
WD= 24 mm
Mag= 500 X
10µm
Photo No.=6982
Detector= SE1

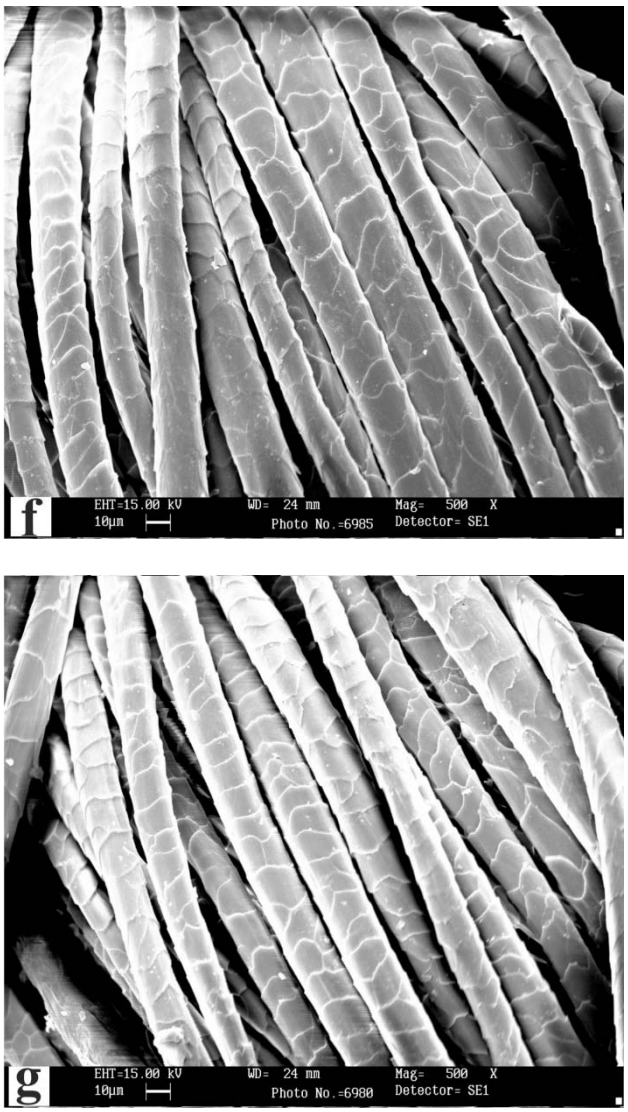


Figure 8. SEM images (500X) of a) reference cotton b) conventional dyed cotton c) foam dyed cotton d) reference wool e) conventional dyed wool f) foam dyed wool g) plasma treated wool.

It is clear from images 8.c (foam dyed cotton) and 8.f (foam dyed wool) that there is no presence of keratin hydrolyzate on cotton and wool fabrics after foam dyeing. Hence the absence of bonding of the keratin hydrolyzate with cotton and wool, already observed by FT-IR, is confirmed. The results show that SEM images of foam dyed samples are similar to conventional dyed samples, no morphological differences are observed due to the use of keratin hydrolyzate. Figures 8.d and 8.g shows the effect of helium plasma on untreated and treated

wool fabric. In comparison with the untreated wool, the plasma treated wool sample appears to have similar morphology. The short contact time in the plasma region (6 seconds, considering the fabric speed of 1 m/min) has not resulted in damage to the wool fibers. The morphology of plasma untreated and treated wool fibers shows insignificant changes which might be due to uniform and thin etching of the surface of wool fibers resulting in an increase of wettability of wool fibers.

7.2.8 Cross section of dyed wool fibers

Microscopic images of cross section of foam and conventional dyed wool fibers are shown in Figure 9 a, b. The plasma treated wool fibers resulted in good dye penetration due to the removal of lipid layer from the outer surface of the wool fiber, giving access to the inner hydrophilic protein layer and also because of oxidation of cystine disulfide bonds to cysteic acid, resulted in increasing hydrophilicity of wool fibers[43]. As shown in Figure 9.a and 9.b, the diffusion of dye molecules occurred not only on the outer ring of the fiber but also inside the core of the fiber. This demonstrates that foam dyeing with keratin hydrolyzate resulted in a comparable dye diffusion respect to conventional dyeing, i.e. dye penetrates uniformly from the outer ring to the center of the fiber. Keratin hydrolyzate foam dyeing technique resulted in similar dye penetration in comparison with the conventional dyeing process.

Figure 9. Optical micrograph (100X) of fiber cross sections of wool a) conventional dyed and b) foam dyed.

Keratin hydrolyzate foam dyeing technique resulted in similar dye penetration in comparison with the conventional dyeing process.

7.2.9 Color strength of foam and conventional dyeing

As shown in Figure 10, a comparative study of color strength was carried out between fabrics dyed with conventional and foam dyeing. The role of keratin hydrolyzate as a foaming agent in foam dyeing process was also investigated. The isoelectric point of keratin hydrolyzate is in the range of 4.7-5.4[44], while change in its ionic charge depends on the pH: below the isoelectric point it is cationic and above it is anionic. In reactive dyeing of cotton, at alkaline pH, the keratin hydrolyzate has an anionic charge and it acts as a carrier for dye molecules onto the fabric, without any interaction with the fabric. Figure 10.a shows the K/S values of the cotton fabric dyed with foam dyeing compared with conventional pad batch reactive dyeing. It is obvious that conventional dyed samples show slightly higher K/S values for 1%, 4%, and 5% dye concentrations and for 2% and 3% dye concentrations the foam dyeing with keratin hydrolyzate shows higher color strength values. As compared to conventional dyeing where dyeing was carried out at 70% wet pickup, the foam dyeing of cotton with reactive dye shows comparable results. This is due to the fact that, in alkaline pH, keratin hydrolyzate

and dye molecules have anionic charges. In this case, the hydrolyzed keratin can be physically adsorbed and dye is able to be delivered into the cotton fabrics[16].

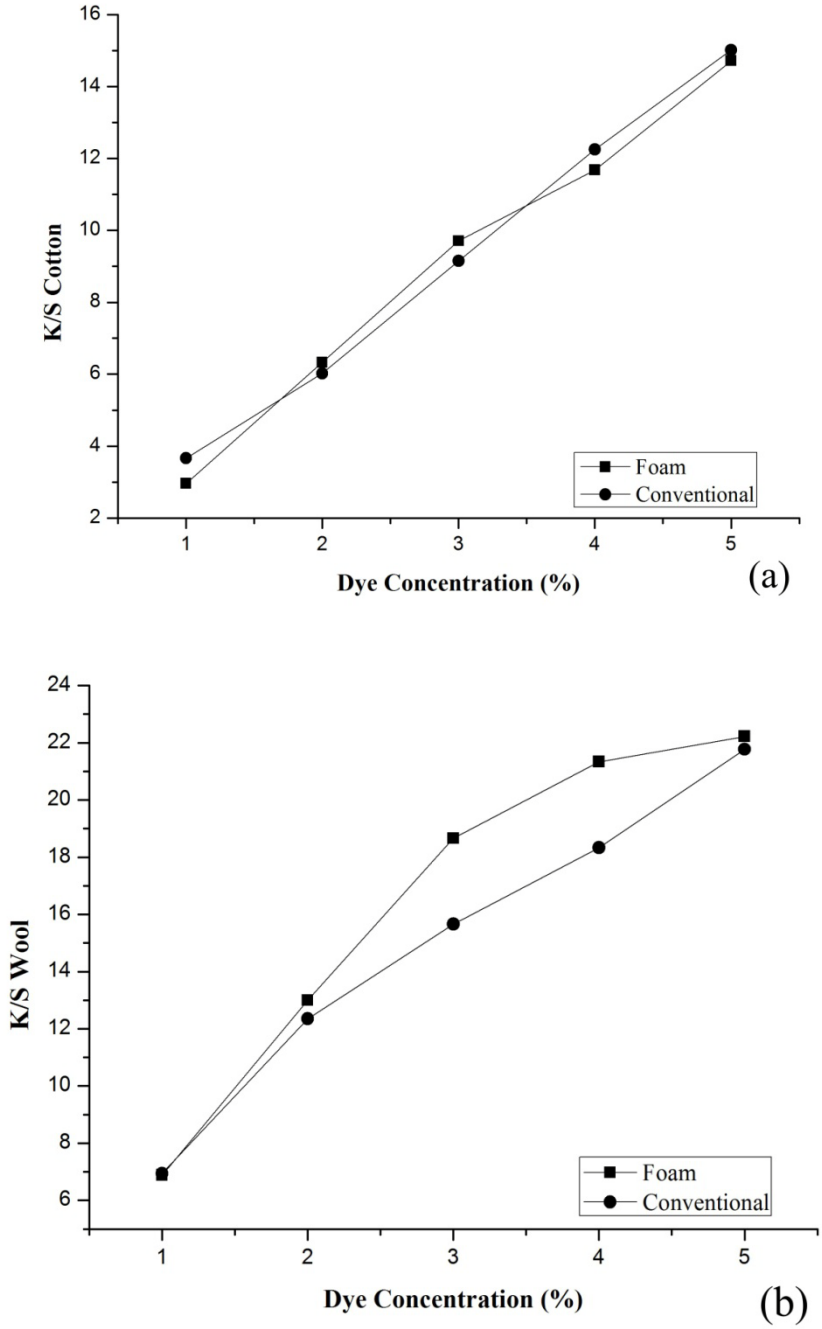


Figure 10. K/S values of foam and conventional dyeing of (a) reactive dyeing of cotton and (b) acid dyeing of wool fabric.

The dyeing of wool fabric with acid dye was performed at pH 4.5. Below to its isoelectric point of 4.8, the wool fabric has cationic charge. The keratin hydrolyzate used as a foaming agent also has cationic charge at pH 4.5, hence we proposed that it will act as a dye carrier to the fabric and does not interact with the fabric. As both the wool and keratin hydrolyzate have cationic charges, it leads to repulsion, even though there is a possibility that some of the hydrolyzed keratin gets adsorbed or physically deposited on the surface of wool fibers. The dyeing mechanism is confirmed by the SEM analysis as in the case of foam dyeing no deposit of keratin hydrolyzate was observed onto the wool fibers. Instead of repulsion between wool fibers and keratin hydrolyzate, the dye anions are equally attracted to wool and hydrolyzed keratin resulting in minimizing the repulsion and increasing dye uptake of fabric. As shown in Figure 10.b, the K/S values of wool fabric dyed with foam dyeing resulted in higher values in comparison with the conventional dyeing. The synergy between keratin hydrolyzate and wool resulted in an increase in color strength and levelness of wool dyeing. The hypothetical presentation of foam dyeing of cotton and wool fabric using keratin hydrolyzate was shown in figure 11. The foam dyed fabric samples compared with conventional dyeing shown in figure 12.

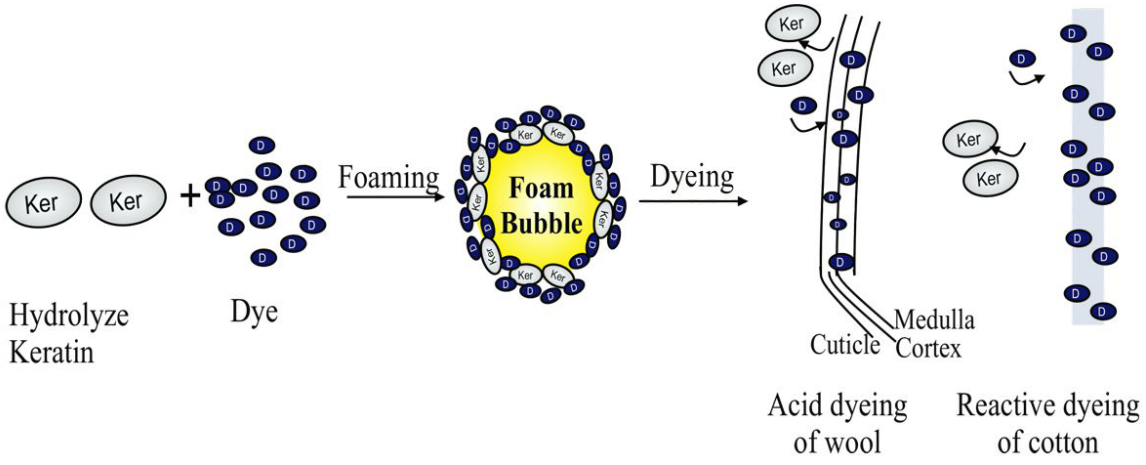
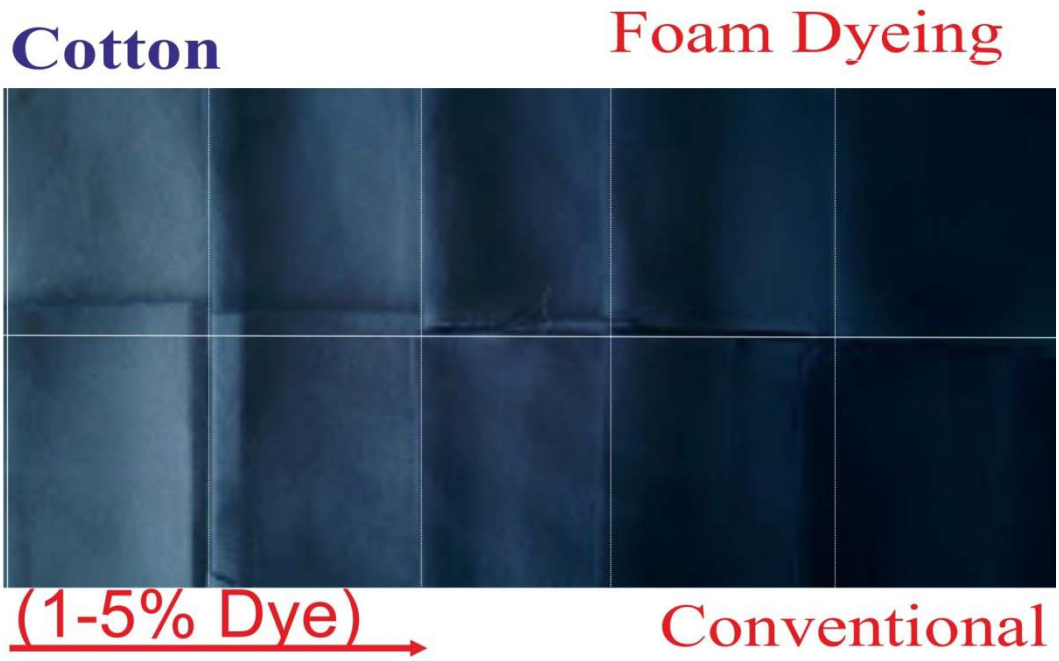
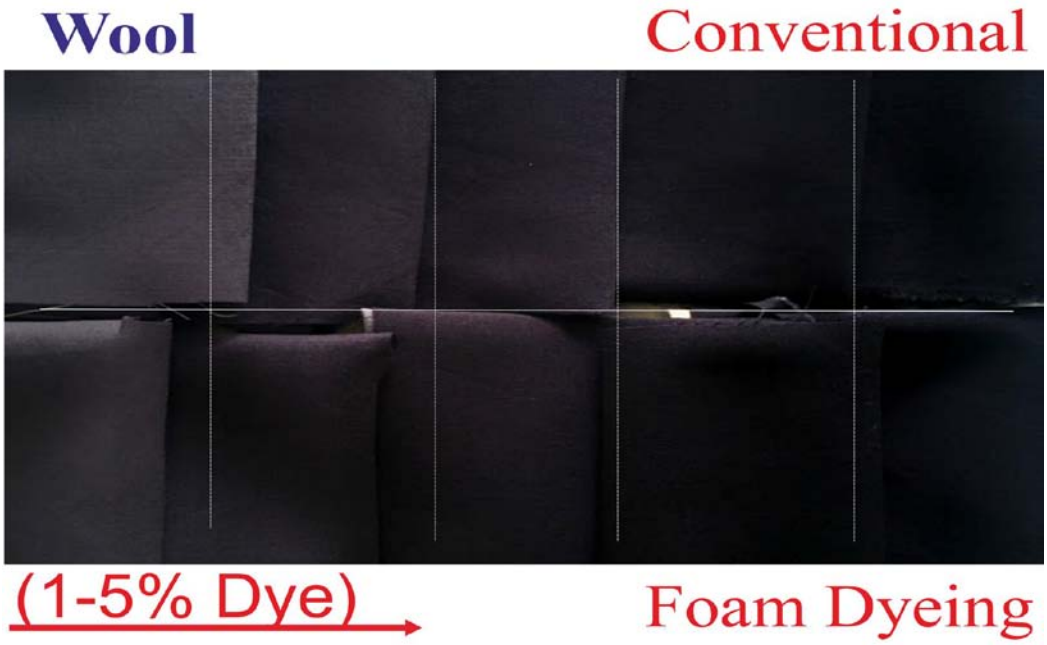


Figure 11. The hypothetical presentation of foam dyeing of cotton and wool fabric using keratin hydrolyzate.



(a)



(b)

Figure 12. Foam dyed fabric samples of cotton (a) and wool (b) compared with conventional dyeing process.

7.2.10 Fastness Testing

Rubbing and washing fastness

Tables 1 and 2 show the wet and dry rubbing fastness and Tables 3 and 4 present the washing fastness of cotton and wool fabrics dyed using foam and conventional dyeing processes. As shown in Tables 1 and 2, foam dyed cotton and wool fabrics have relatively similar rubbing fastness properties as compared with conventional dyeing process. As observed in Tables 3 and 4, all samples of cotton and wool fabrics dyed with foam resulted in good washing fastness, equivalent to convention dyeing processes. In general, all foam dyed samples show good fastness properties in washing and rubbing. It is concluded that the use of the keratin hydrolyzate as a foaming agent can be successfully implemented in the dyeing of cotton and wool fabrics.

Table 1. Dry and wet rubbing fastness of conventional and foam dyed cotton samples.

Dye Concentration (%)	Conventional		Foam dyeing	
Dry	Dry	Wet	Dry	Wet
1	5	5	4/5	5
2	5	5	5	5
3	5	4/5	5	5
4	4/5	4/5	4/5	4/5
5	4/5	4/5	4/5	4/5

Table 2. Dry and wet rubbing fastness of conventional and foam dyed wool samples.

Dye Concentration (%)	Conventional		Foam dyeing	
	Dry	Wet	Dry	Wet
1	4/5	4/5	4/5	4/5
2	4/5	4/5	4/5	4/5
3	4/5	4	4/5	4
4	4/5	4	4/5	4/5
5	4/5	4	4/5	4

Table 3. Washing fastness of conventional and foam dyed cotton samples.

Dye Concentration (%)	Conventional			Foam dyeing		
%	Change in color	Cotton	Wool	Change in color	Cotton	Wool
1	4/5	5	5	4/5	5	5
2	4/5	5	5	4/5	4/5	5
3	4/5	4/5	5	4/5	4/5	5
4	4/5	4	5	4/5	4	5
5	4/5	4/5	5	4/5	4	5

Table 4. Washing fastness of conventional and foam dyed wool samples.

Dye Concentration (%)	Conventional			Foam dyeing		
	Change in color	Cotton	Wool	Change in color	Cotton	Wool
1	5	5	5	5	5	5
2	5	5	5	5	5	5
3	5	5	5	4/5	5	5
4	5	5	5	4/5	5	5
5	5	5	5	5	5	5

7.3 Conclusions

The use of hydrolyzed keratin in foam dyeing is a novel concept. It was observed from the study that keratin hydrolyzate has an excellent surface activity. Keratin hydrolyzate used as foaming agent shows good foaming ability and foam stability for reactive and acid dye solutions. The blow ratio of 10:1 and 11:1 is suitable for even reactive dyeing of cotton and acid dyeing of wool fabric. The bubble diameters for both reactive and acid dyeing were found in the range of 0.02¬0.1 mm, which leads to the promising application of the keratin hydrolyzate as a foaming agent.

The low wet pickup of 40% was found to be sufficient for reactive and acid dyeing. It was observed that both reactive and acid dyeing performs differently in hydrolyzed keratin foam dyeing depending on its pH, which increases or reduces the dye affinity onto the fibers. The results of foam dyeing of cotton fabric show promising results in comparison with the conventional pad-batch process. The K/S value of the foam dyed cotton fabric was found close to conventional padding samples. In the case of wool dyeing, foam dyed wool samples show the best results in comparison with the conventional pad-steam process, with higher color strength values. In comparison with conventional padding processes, foam dyeing of cotton and wool samples resulted in equivalent rubbing and washing fastness properties, which clearly indicate that the use of the keratin hydrolyzate as a foaming agent has no impact on fastness properties.

Foam dyeing is an energy and water efficient technology. The use of keratin hydrolyzate, which is a biodegradable and eco-friendly by-product in nature, not only resulted in an increase in dye uptake of wool fibers but also will be helpful in the reduction of load on effluent. The use of the keratin hydrolyzate in cotton and wool, foam dyeing made it completely sustainable and green dyeing process.

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Chapter 8

Conclusion and future work

8.1 Conclusions

The problems related to the waste minimization and productive utilization of waste wool in order to create a sustainable circular economy is the motivation of this study. The problem statement allows us to understand the difficulties in the utilization of grease wool such as slow degradation and potential cause to occur infection during storage and disposal on the basis European rules and regulation related to the handling of raw wool. The quantification of availability of raw wool in the European region and chemical potential of it on the basis of literature provides a clear idea that this material is not just a waste by product but having the potential to establish a circular economy by converting it into organic fertilizer. The first part of study allows us to understand that wool is a complex, layered structure which is considered as a composite made of cuticles, cortex, medulla etc., and chemical bonding present in wool fibers specifically cystine disulfide bonds which give them mechanical strength are difficult to break down. In order to achieve the desired aim of the transformation of raw wool into organic fertilizer in a sustainable way, there is need of clean and green process. The chemical breakdown of wool with the help of acids, bases and enzymes has been studied by so many authors, but the existing processes have some limitations such as the cost of material, process handling, a time span of reaction etc. The choice from the various processes available is made according to effectiveness, profitability and the level of risk of environmental pollution produced, leads us to

implement the superheated water green hydrolysis process where superheated water is the only green solvent which hydrolyzed the raw wool.

Indeed, in order to establish the green hydrolysis process, the process begins with the preliminary examination of raw wool which provides the information related to moisture content (12-13%), washing yield (62-64%), grease content (4-5%), vegetable matter (1-4%) and ash content (10-14%) which are helpful in understanding of interpretation of hydrolysis results. The step by step approach was carried out from batch scale reactor (batch process) to semi industrial scale reactor (continuous process) to optimize varying parameters which play important roles in the hydrolysis process such time, temperature, wool density, material to liquor ratio.

The design and construction of the small batch scale reactor allow to hydrolyzed 2-50 g of material at a maximum temperature of 160 $^{\circ}$C. This reactor provides the preliminary understanding of hydrolysis parameters and points out that wool density also plays an essential role in the extent of hydrolysis where compressed wool shows better wetting of wool than uncompressed wool. The material to liquor ratio needs to be exact to obtain uniform hydrolysis treatment and impregnation of wool was found in the range of 1:1.5 to 1:3. The effectiveness of green hydrolysis treatment was compared with alkaline hydrolysis using calcium oxide and potassium hydroxide. This study revealed that green hydrolysis has a similar potential as compared to alkaline hydrolysis to obtain low molecular weight proteins, destruction of disulfide bonds and effective degradation of wool fiber at a higher temperature of 170 $^{\circ}$C with material to liquor ratio of 1:3.

To overcome the limitations of the small batch scale reactor, process large amount of material, understand the relation between processing parameters and the homogeneity of hydrolysis process; design and construction of the final batch scale reactor were carried out. The application of thermocouples in order to understand the homogeneity of the hydrolysis process inside the reactor shows that wool density affects the heat transfer phenomenon from outer to inner region towards the central shaft of the reaction vessel. To overcome the problem further modification in the design of reaction vessel implemented such as an increase in diameter of a reaction vessel and insertion of a central perforated rod, which allows even heat distribution from outer to the inner region of a reaction vessel and uniform hydrolysis effect at a wool density of 350 kg/m^3. As the increased material to liquor ratio in a range of 1:3 to 1:8, temperature from (140 $^{\circ}$C- 180 $^{\circ}$C) and time (30-60 min) there is an increase in the liquid phase and less solid phase

mostly consist of unhydrolyzed vegetable matter. The liquor ratio of 1:3, temperature 170 °C and time 60 min seems an optimal parameter to obtain certain amount liquid parts, use of low liquor ratio will be helpful in terms of less energy consumption will take place for water heating. The physiochemical analysis shows that high temperature hydrolyzed wool consists of low molecular weight proteins with a significant reduction in ½ cystine amino acid and morphology of shapeless protein aggregates. The preliminary agronomical analysis resulted in the presence of 3-4% nitrogen in hydrolyzed wool, which indicates that the hydrolyzed product having nitrogen and other nutrients will be beneficial for plant growth and nourishment.

The characterization and tuning of wool hydrolysis by lab-scale reactor highlighted problems of wetting, homogeneity of treatment and temperature evenness during processing. To overcome these problems, a rotating intermediate semi industrial scale reactor has been built, having a capacity of about 5-8 kg. The first experimental trials, realized with the intermediate-scale unit, have already brought to very good results in terms of final product homogeneity. Moreover, the new heating system, with steam supplied directly into the reactor chamber, allows to reduce the time needed to reach the hydrolysis temperature (heating time) and contributes to supply a considerable amount of water (thanks to its condensation) necessary to reach the desired solid to liquid ratio. The optimal process parameters for this reactor are temperature 170 °C and time 60 min with the material to liquor ratio of about 1:1. An effective to solid to liquid mass transfer obtained due to tumbling motion of hydrolysis reactor. The physiochemical study suggests that the hydrolyzed wool obtained at the end of 30, 60 and 90 min of the hydrolysis process consist of low molecular weight proteins and morphology with shapeless protein aggregates. The agronomical study shows the at low concentration application (1g/l) of hydrolyzed wool resulted in higher germination rate without any phytotoxic effect. The hydrolyzed product obtained in solid phase behaves like a slow release fertilizer where low concentration liquid phase with the appropriate amount of nutrients are readily available for crops.

The nitrogen amount is comparable with similar products (protein hydrolysates from the waste of slaughter and tanning process) and the presence of nutrition and trace elements (potassium, sulphur, iron) is a valuable aspect. The pots and on field tests have shown a good performance of the fertilizer tested, in some cases comparable with mineral fertilizers. However, it seems necessary to consider the peculiarities of the various products obtainable in order to take advantage of the

nutrient release properties considering the type of crops, the doses and methods of use.

Similar hydrolyzed wool, which consists of low molecular weight proteins and a hydrophobic hydrophilic mixture of amino acids shows a reduction in surface tension which confirms its behavior like a surfactant. The foaming ability and foam stability of hydrolyzed keratin are an effective, even in the presence of dyeing auxiliaries. The effective bubble size of foam allows uniform dyeing of wool and cotton fabrics. The hydrolyzed keratin plays a role of dye carrier in foam dyeing and resulted in better dyeing properties of wool fabric in comparison with the conventional process. The dyeing performed at low wet pick using hydrolyzed keratin, not only resulted in saving of water, but also effective in terms of energy and chemicals saving. Thanks to its biodegradability and dye/metal chelating property make it beneficial in water treatment over synthetic surfactants.

Summary of conclusion:

- Green hydrolysis process at high temperatures and pressures is a simple, fast and relatively inexpensive process to obtain organic fertilizer and further potential application such as a foaming agent.
- Standardization of optimal condition allows obtaining homogeneous hydrolysis. It is mainly dependent on the amount of wool loaded in the final reactor and material to liquor ratio, which allows you to obtain solid and liquid phase material.
- The consistency of the hydrolyzed wool is an important factor to be considered as varying process condition resulted in solid and liquid phase product. The liquid phase product seems to be promising to be considered for production of the final fertilizer product. The liquid phase product is more homogenous in nature, easily diluted, transported and distributed for fertilizer application. The shelf life of liquid phase observed to be more longer in comparison with a solid product which is difficult to handle due to its sticky paste form nature.
- The C / N ratio, which stimulates the process of mineralization of organic matter and providing a large amount of available nitrogen is found in an appropriate range in this hydrolyze wool organic fertilizer.
- The annex of legislative decree 75/2010 confirms the application of hydrolyzed wool as an organic fertilizer in the category of nitrogen fertilizers allowed in organic farming.

- Foaming behavior and its application in wool dyeing where foam dyeing resulted in good dyeing properties in comparison with conventional process confirm its contribution to the development of sustainable green dyeing.

8.2 Future work

In recent years, there have been several reports on the use of keratins extracted from wool to produce films, sponges, and hydrogels for potential applications in tissue engineering or medicine. Keratin proteins are known to have cell adhesion motifs such as Arg-Gly-Asp (RGD) and Leu-Asp-Val (LDV) which mainly induce cell-matrix interaction. Their environmental stability, biocompatibility, and unique properties provide an important basis for using this natural protein in biomedical applications. The major drawback of keratin film and scaffolds is their fragile nature. So to overcome this drawback and to use keratin successfully in various biomedical applications, it is blended with natural as well as synthetic polymers. Based on the literature review there will be the possibility of future work mentioned below which has not yet be reported can be carried out.

- Preparation and characterization of a seaweed polysaccharide (K-carrageenan) cross linked superheated water degraded keratin/keratin cap inorganic nano particle using glycerol bio composite films for packaging application
- In another work, I would like to propose, In situ synthesis of hydrogel scaffolds using Tamarind Seed Xyloglucan and keratin. Wound healing drugs were also incorporated in the natural polymeric scaffold. Selection of drug on the basis, having its inherent antibacterial property which will be an additional benefit. So our present study reveals a novel combination of two natural polymers enriched with wound healing drug to be used as in situ hydrogels to obtain enhanced wound healing properties.
- Layer by Layer finishing of keratin on Polyester fabric.
  Till now the literature on keratin finishing of polyester (after surface modification of polyester) using a coating or conventional pad dry cure process is available, but designing a new protocol for layer by layer is a challenging and a cost effective process will open a door for various applications. Based on the literature keratin can act as polyanion or polycation depending on pH (> or < pH 3.8). Selecting the corresponding

polyelectrolytes for this protocol will help to apply keratin on polyester without prior surface modification.