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Hydroformylation assisted by grinded Sardinian wool: a full exploration of a sustainable process.

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Chapter 1

Introduction

1.1 Rules and regulation: the opening to Circular Chemistry

The modern society has a higher awareness about the impact of human activities on the environment, with respect to some years ago. The production of materials, food, fibers, clothes, and the production of chemicals can have dangerous consequences on the environment.

In the mid-20th century, concerns about the environmental impact of chemical and industrial wastes led to specific environmental regulations all over the world. The book Silent Spring, written by Rachel Carson in 1962,[2] report an interesting view of the impact of industrial production both on humans and on the environment. Here she defines the devastation that certain chemicals can cause on the ecosystem. The book has the purpose of being a wake-up call for people and scientists to inspire the modern environmental movement. After it, in 1969, the National Environmental Policy Act (NEPA) recognize the importance of ecological issues. The NEPA goal were the creation and maintenance of the conditions able to let man and nature coexisting in harmony. In 1970, the President Richard Nixon established the U.S. Environmental Protection Agency (EPA). Differently from the NEPA, this is a federal regulatory agency mostly devoted to protecting human health and the environment.[3] The idea of the impact of EPA activities in everyday life is exemplified by the retreatment of DDT from the market together with other common pesticides, used in a large amount in the beginning of '90s and defined toxic after few years. The further decisions have been taken after disasters like the one at Love Canal in Niagara Falls in 1970 and the Seveso disaster in Italy.[4] In the first one, thousands of barrels filled with chemical waste, buried by chemical companies over the previous decades, bring to the leaking toxic chemicals in the soil, contaminating groundwater. After those disasters, EPA and other regulatory bodies, established significant environmental legislation like the "Superfund" act in 1980.[5] More recently, other regulations, have been defined, in Europe and all over the world, such as the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH, regulation in Europe), Globally Harmonized System of Classification and Labelling of Chemicals (GHS, description of the correct labeling of chemical product), Occupational Safety and Health Administration (OSHA, rules about manipulation of chemicals), the Clean Air Act and the Clean Water Act (General US rules about pollution from industries).[6] These guidelines limit and

regulate the release of pollutants and toxic substances into the environment, thus leading to the development of new technologies and approaches to a cleaner chemical manufacturing. Rees, in 2022, says that "Every dream we have about recreating community in the absence of authority will turn out to be a pipe dream in the end ".[7]

Moreover, the final goals of those regulations are:

- **protect human health**: chemicals can have harmful effects on human health, and regulations are put in place to minimize the exposure to those substances.
- **Protect the environment**: reduce the release in nature of toxic substances and pollutants.
- **Ensure product safety**: regulations allows to ensure that chemical products are safe for their use.
- **Promote innovation**: regulations can encourage the development and use of safer chemicals and processes, promoting innovation in the chemical industry.

In the '80s and '90s, new issues related to the development of more sustainable processes start to be considered, moving forward the concept of Green Chemistry This was mainly related to the work of chemists such as Anastas and Warner, who published the 12 principles of Green Chemistry in 1998.[8] These principles provide a framework for designing chemical processes and products that are more sustainable and less harmful for the environment. The 12 principles of Green Chemistry are still related to a classical linear economy model. More recently, the concept of Circular Economy[9], a model of production and consumption which involves sharing, repairing and reusing, refurbishing and recycling existing materials as long as possible, start to be considered as a valuable alternative, looking forward a no waste system.

This new economic model has been recently traduced in the chemical field into the concept of Circular Chemistry.[9]

1.2 An overview on the definitions

The language in the field of sustainability changed pretty fast in the last ten years. This is why it is important to define the following three concepts that are frequently confused:

- **Green Chemistry**: concepts and principles for the protection of people and environment.
- **Sustainability**: concepts for the protection of our planet.
- **Circular chemistry**: concepts and principles to increase the possibility of a sustainable economic growth.

The most important difference can be evaluated between Green Chemistry and Circular Chemistry because both focuses on minimizing the use and generation of hazardous substances and reducing waste also recycling but, Circular Chemistry is based on a circular economic model, respect to Green Chemistry that is based on a linear economic model, and focuses on designing closed-loop systems to minimize waste and reuse subproduct.[10] Sustainability has a definition and principles more superimposed to both the description done above. All these approaches together can help to create a more sustainable and environmentally responsible chemical industry and all of them has a different branch of application that allows the protection of nature and people, in the case of Sustainability and Green Chemistry, but also allow the production of an economical return where the rules of Circular Chemistry are well applied.

1.2.1 Green chemistry

Thanks to growing awareness of environmental sustainability since 1962, Green Chemistry, has become an increasingly important area of research and development, as companies and governments try to reduce the environmental impact of chemical manufacturing and promote sustainable economic growth. Defining this term as reported by Anastas and Warner, Green Chemistry is a set of principles and practices aiming to design chemical products and processes that reduce or eliminate the use

or generation of hazardous substances. Today, those principles, are applied in a wide range of industries.[11] The real application of them,[8] can be traced back to the early 20th century, when chemists first began to recognize the importance of considering the environmental impact of chemical processes. It is a way of thinking of which people have not yet realized the strength and importance but which through proper application can lead to a considerable decrease in environmental impact due to industrial production. The 12 principles of Green Chemistry[12] as defined by EPA are:

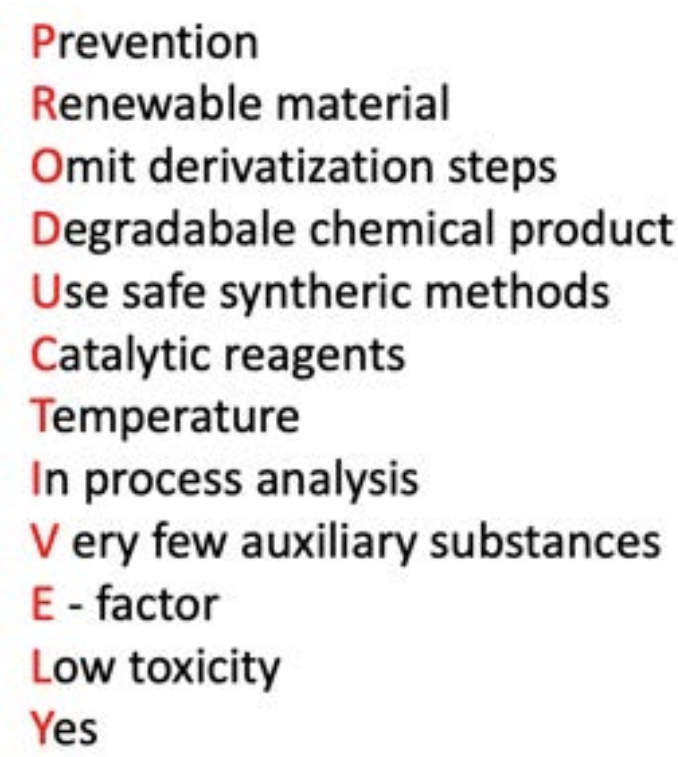
- **Prevention**: it is better to prevent waste in comparison with disposal to landfill highlighting the environmental and economic benefits.
- **Atom economy**: synthetic methods should be designed to maximize the incorporation of all atoms that compose reagents into the final product.
- **Less hazardous chemical syntheses**: wherever possible, synthetic methods should be designed to use and generate fewer or no-toxic compounds for the protection of human health and the environment.
- **Designing safer chemicals**: chemical products should be designed to be fully effective yet have little or no toxicity.
- **Safer solvents and auxiliaries**: the use of auxiliary substances (i. e. solvents, separation agents, gasses, functional derivatives, heating methods etc.) should be made unnecessary wherever possible and innocuous when used.
- **Design for energy efficiency**: energy requirements should be recognized for their environmental and economic impacts and, the energy needed, should be used in the minimal amount.
- **Use of renewable feedstocks**: renewable raw materials and reagents should be used preferentially for the lower impact on the environment.
- **Reduce derivatives**: unnecessary derivatization (use of blocking groups, protection/deprotection, temporary modification of physical/chemical processes, derivatizations) should be minimized or avoided because such steps require additional reagents and can generate waste.
- **Catalysis**: reagents used in a lower quantity respect to reagent used in stochiometric amount, are preferred respect to those used in a stoichiometric quantity, allowing to easily track the others principle.

- **Design for degradation**: chemical products should be designed so that at the end of their function, they biodegraded in an easy and faster manner.
- **Real-time analysis for pollution prevention**: analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances and the final product, allowing to stop the reaction in time, reducing also the energetic lost.
- **Safer chemistry for accident prevention**: substances and the form in witch they are used in a chemical process should be chosen to minimize accidents, including releases, explosions, and fires.

By applying these principles, Green Chemistry can lead to the development of safer and more sustainable chemical products and processes. It has the potential to reduce the environmental impact of the chemical industry.

To facilitate the understanding of all 12 principles, also allowing industries a simpler application in production processes, the term *PRODUCTIVELY* was coined, which includes all the principles just described but with a different language property like described in Figure 1.[13]

Figure 1. The 12 principles of Green Chemistry



The E-factor is a matrix that can be used to integrate the principle of atomic economy. In fact, this factor describes the amount of waste produced compared to the desired product in an industrial process. The value of E. Factor for a production without waste is equal to zero. Although the correct use of the E-factor can give a fair assessment of the environmental impact of the process, today other methods are used favorably and allow a more truthful view for example the Life Cycle Analysis, which will be discussed in Circular Chemistry.

Defining some examples of application of the 12 principles of Green Chemistry, one of the earliest, about catalytic reaction, was pioneered by the chemist Paul Sabatier in 1900, who develop the Hydrogenation reaction using catalyst. Catalyst are important component, generally metals, that are added to the reaction in order to decrease the activation energy and promote the reaction. This allows to have less harsh conditions and that happened in a faster way. This approach was more efficient and produced less waste than previous methods in which stoichiometry is applied, and it is still widely used today in a range of industrial processes and in laboratory.
One another important principle is Atom Economy that mainly is valid for important catalytic processes largely applied in industry such as Hydroamminomethylation[14] or Hydroformylation reactions.[15] Those two processes allow the complete incorporation of all the atoms presents in the starting material into the final product, eliminating atomic wastes. What is important to understand to discriminate between Green Chemistry and Sustainable and Circular Chemistry , is that the linear economy is on the base of this approach. Effectively the use of the 12 principles of Green Chemistry can be done easily and work well only on processes that has the traditional model of production and consumption, where materials are extracted, processed, and manufactured into products that are used and then discarded as waste. In this context the focus is on maximizing economic growth through the production and consumption of goods, without an overview on the reuse and recovery of subproduct or wastes. One of the lack that Green Chemistry has, is the non-consideration of the provenience of goods and starting materials. Effectively the necessary production, the transport, and the cost in terms of pollution is high and important. Because of this a step forward must be done.

1.2.2 Sustainability

In the chemical industry the concept of Sustainability is essential for creating a description of the practice that prevent and safeguard nature, as Green Chemistry do with people. Sustainability have been defined by the Organization for Economic Co-operation and Development (OECD) as "a scientific concept that seeks to improve the efficiency with which natural resources are used to meet human needs for chemical products and services".[16] As Green Chemistry applies the 12 principles to reduce the production and the release of dangerous substances in the environment, Sustainable Chemistry prioritizes production processes that promote increased product value while intersecting the goals of protecting and enhancing human health and the environment.[11]

The goals of this field of chemistry, have been defined by the sustainable Development Goals of the United Nations (Figure 2),[17] that especially in the 12th principle highlights the importance of the resource efficiency for a more sustainable lifestyle also managing the division between economic growth and respect for the environment.[17]

Figure 2. Sustainable Development Goals

Until now, real difference in between Sustainability from Green Chemistry is still not completely clear. Probably, Freeman Dyson was right saying: “further we go into the future, the more differentiation of natural structures we discover, and the more technological diversification we can create” and so now we are not able to differentiate between those two terms, also in the application of the goals in industry. What we can assume is the differentiation done in 1999 by Hutzinger,[18] defining Green Chemistry the “design, manufacture, and use of chemicals and chemical processes that have little or no pollution potential or environmental risk,” while sustainable chemistry reflects the “maintenance and continuation of an ecologically-sound development”. We can also describe the perception that Green Chemistry is a characteristic of the microscopic world, especially in academia, hence with innovative fundamental chemistry, while sustainability is concerned more with the macroscopic domain, more in deep in Industries.

Can we finally introduce now the points that are generally applied for a sustainable process or to switch in this direction?

No, because also in this case we have some trouble in define it in a general manner.

In each field of study are evaluated goals and rules that help in the control of the six environmental indexes of protection that are:

- climate Change;
- environmental corruption;
- innovation toward renewable technologies;
- ethics;
- people.

In Sustainable Chemistry we can evaluate the key principles, almost superimposed with the 12 principles of Green Chemistry, that can be applied:

- waste Prevention;
- use less hazardous chemicals;
- renewable feedstock;
- use safer solvents

- design for efficient degradation;
- injury prevention;
- decrease the use of derivatives;
- improve energy efficiency;

Actually, the overall supply of chemical production suffer of the current war in Ukraine and for the COVID-19 consequences with a highly increasing of prices of important resources such as petrol and gas. In other words, it must be prioritized to identify sustainable building blocks that provide reagents but also substances necessary for industry that can be obtained starting form alternative sources than fossil ones.

1.2.3 Circular Chemistry

The model on which industries based their envelope since 10 years ago was the Linear Economic, where raw materials are collected and transformed into products that consumers use until discharging them. Those are treated like waste with no concern for their ecological footprint and consequences of their presence in the environment. This model is focused on the profit over sustainability. Like easily understandable this causes an important impact also on economy, because no “cash-back” happened so without valorization of the side products and waste in general. To have an economical return, the global development was based on the extraction of finite resources from nature, also with very dangerous and toxic methods for the environment. Over time industries and common people do this in more and more ways to create advanced products also in response to the demographic grown.

Circular Economy can be considered as a new opportunity to build a more sustainable world both socially and economically. Circular Economy is so a model of production which involves sharing, repairing and reusing, refurbishing and recycling existing materials discovering new ways to use it both in chemistry than in another field.

As witnessed by several initiatives at European level and by the role of the European President Ursula von der Leyen, Europe and the European States are moving decisively towards a circular paradigm, that can change radically the view of industries in the design and production of goods, a scenario that requires a solid technical and scientific background, in which chemistry should play an important role. [19]

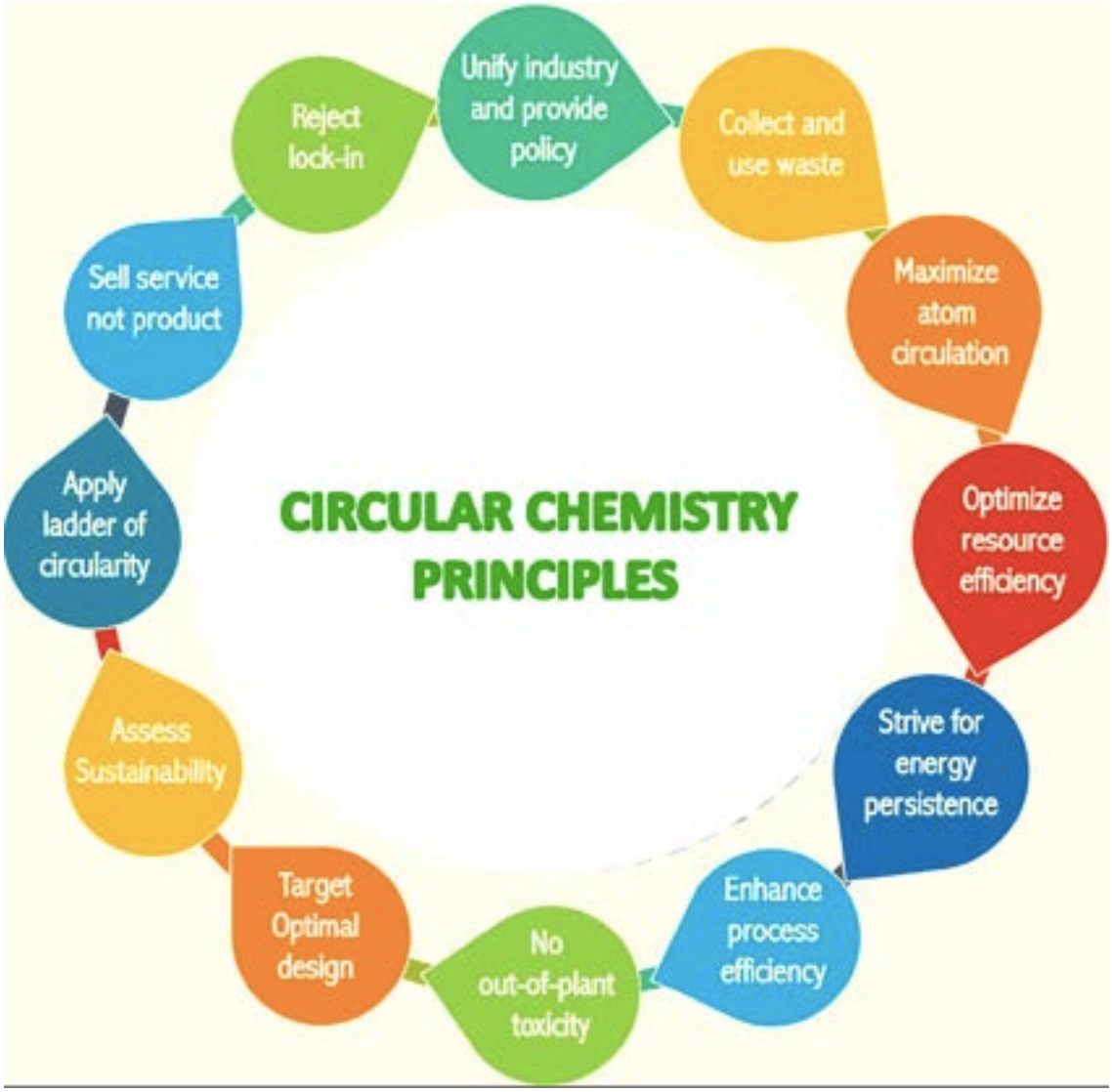
The role of chemistry and chemist is crucial for the development of techniques and synthetic methods that allow the application of principles that can be applied in industry and with the intrinsic admission of them during a process.

Obviously to enter in a Circular Process all the different type of chemist are necessary, from Organic to Inorganic and Physical Chemists because different aspects of the chemical production must be considered.

A further aim of this collaboration is the orientation towards a more sustainable development within a coherent and holistic framework provided by the policies and guidelines of the European Commission, which includes important legislative initiatives.

While principles of Green Chemistry focused exclusively on the sustainability assessment of chemical reactions, Circular Chemistry introduces the consideration of the entire lifecycle of the whole "value chain".[20] In their Nature paper, Slootweg et. all. define the 12 principles of Circular Chemistry (Figure 3).[21]

Figure 3. Circular Chemistry Principles

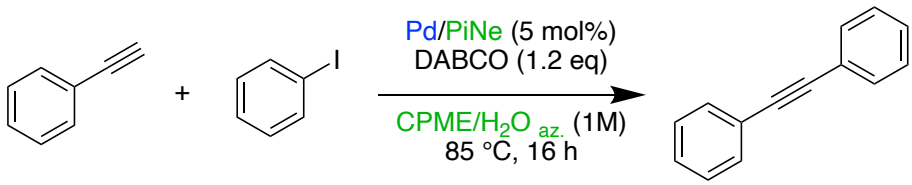


Interestingly, these twelve principles of Circular Chemistry are covering aspects of chemistry, but also aspects of the economy, policy, and environmental science highlighting the importance of the interconnectivity between these areas and the need for trans- and multidisciplinary approaches, research, and practice.[10]

Evaluating the impact of this knowledge on the economy and therefore Circular Chemistry we can observe that the number of publications that carry this "key-word" increased from 900 to 2500 in around 10 years (period 2010-2019),[22]. Some of these publications have contributed to the evolution and dissemination of Circular Chemistry concepts, guiding scientists, policymakers, and industry leaders toward the development of sustainable chemical practices. Looking at examples that can explain the possible use of wastes in the organic synthesis we can focus our attention on different aspects. Waste materials can come from industrial processes or from a completely natural origin. Based on their properties they can be used as solvents (i.e., valerolactone) or as adjuvants or supports for catalysts. In this last field, depending on the properties and origin of the waste used, these substances can be used directly as catalysts and as pre-catalysts, or can be modified and used as supports. Just thinking about one of the most common waste from the steel industry, the red mud, is the result of the digestion in caustic soda of bauxite for the production of aluminum with the so-called process Bayer.[23] It is composed of a varied mixture of metal oxides, such as Fe_2O_3, Al_2O_3 and small amounts of phosphorus.[24] In hydrogenation reactions of variously substituted olefins, this material is used as a catalyst capable of activating H_2.

The application of the principles of Circular Chemistry a key role is played by waste materials that can be considered as a valuable additive and starting materials for chemical transformations. Many efforts have been made by different groups on this field, including the use of Pine needles to support Pd for a Sonogashira cross coupling (Scheme 1).[25]

Scheme 1. Sonogashira reaction



The use of this Pd supported on biochar in Sonogashira's reaction has made it possible to make a reaction already very efficient even much more sustainable and economic. In fact, this approach allows the minimization of waste, is somehow atomic economical and use a supported catalyst that is recovered and regenerated at the end of the reaction and can be reused (**Figure 4**).

1.3 Sardinian Wool

Among the various waste materials identifiable in the category of natural origin, wool and product from the textile industry are very important as Italy is a leader in the production of yarns.

In this world, the wool that can finds application can be of a suitable quality and is usually produced by selected sheep of a stablished races.[26] The fibers that are used outside textile industry mentioned above are called "High Performance Fibers".[27] The differentiation between sheep reared to produce yarns and those used for the manufacture of dairy products is fundamental in understanding the origin of waste. Indeed, for the welfare of the animal shearing is important in both cases but, the strict rules defined previously to ensure the goal of the textile industry, leads to a large amount of wool that would simply be discarded.

This is not the only evaluable problem of the wool waste generation. Between the 17th and the first half of the 20th centuries, the wool textile sector, has a rinsing demand in the global industry, but competition from synthetic fibers, combined with consumer requirement considering the high cost of wool yarns product, shift in favor of lighter weight and casual products the popular demand, negatively affecting the waste production.

Just to have some ides, the wool used in industry pass from 9.9 % in 1960 to 3.2 % in 2000. In the 2011/2012 season, when wool production fell again to reach the lowest value in modern times, the global market was 1.3%, while the synthetics share was 61.4%. In 2013, world wool production lifted by 3% and has kept this timid value.[28] We can definitively say that wool is currently perceived as a marginal fiber, reserved mainly for luxury products.

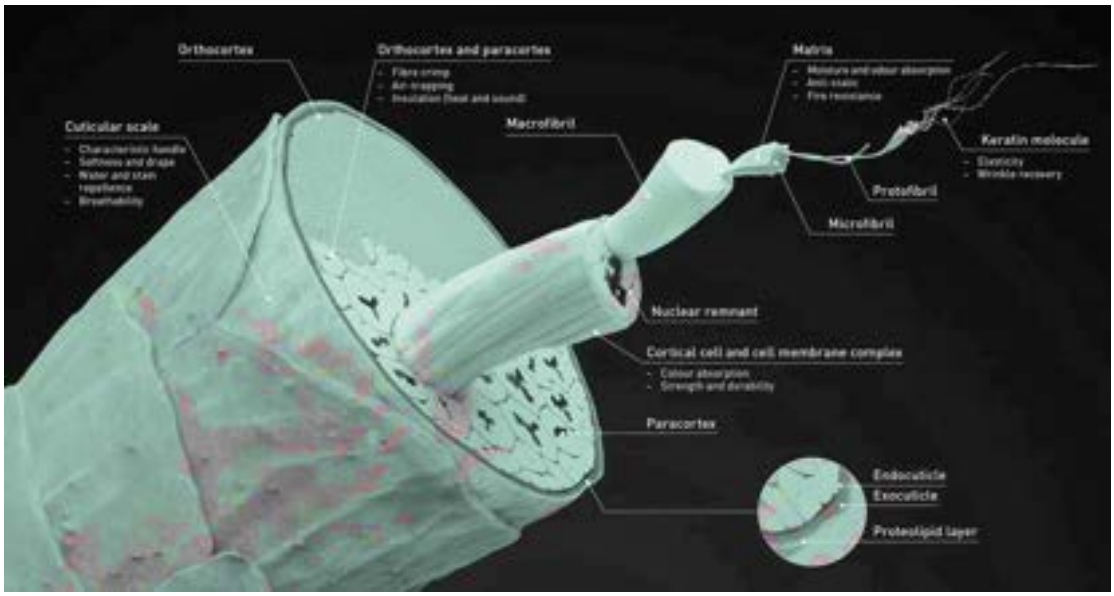
Despite the above, it is important to stress that all sheep used to produce meat, textile fibers or dairy products must be sheared. That is why the amount of waste is important like find possible use of it for further productions and uses.

Wool fibers are classified on the base of their chemical and structural properties.

The histological structure of wool fiber comprises: the cuticle (external region) the Cortex (internal part), enriched by the cell membrane complex. Further inside, the inner channel named Medula exists only in coarse and medium grade fibers where

are located macrofibrils and an intermediate filament (microfibrille and proteins) consisting of a matrix, a twisted molecular chain and a helical spiral (Figure 4).[28-31]

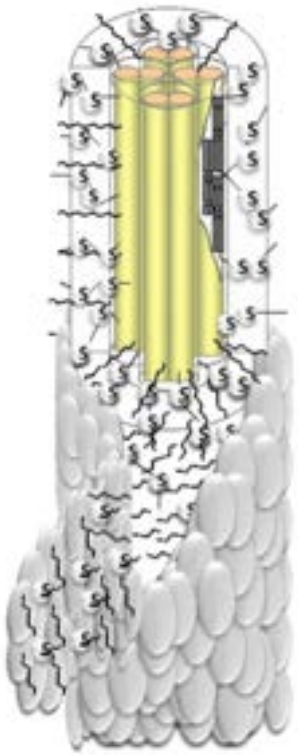
Figure 4. Schematics of wool fiber morphology and hierarchical organization of keratin macromolecule.[32]



The cortex is a major part of this fiber, concerning differences between races of sheep, and is composed of many elongated helical-shaped cells, that contain keratin in up to 93 % by weight.[33] Transmission electron microscopy (TEM), a form of microscopy in which a beam of electrons transmits through a thin layer of a material and interacts with the specimen when passing through it is generally used for the characterization of the cross-section of cortical cells. This allows to have a clear image of wool fiber morphology and hierarchical organization of keratin, the presence of macrofibrils and microfibrils embedded in the matrix. Wool proteins are all α-keratins, fibrous proteins insoluble in H_2O as well as collagen and elastin, and are classified according to their aminoacidic composition. The most widely accepted structural model is that proposed by Astbury and Bell (1941), which describes a generally helical conformation of keratin.[29] The resulting structure generates very large molecules with a molecular weight of 60000 Atomic Mass Unit (AMU). More in detail, the primary structure of keratin comprises 18 α-aminoacids, whose relative

ratios, sequencing and side chains reactivity dictate the chain conformation and length. The particular amino acid in keratin is cysteine, which has disulfide bridges that link adjacent polypeptide chains generating highly-ordered supramolecular aggregates. Those bonds give the high structure stability and the chemical and mechanical resistance of wool (Figure 5). Several treatments are possible, both chemical (i.e. alkaline, reducing or oxidizing agents, keratinolytic enzymes) and mechanicals (Mechanochemistry)[34] for the destruction of the disulfide bridge.

Figure 5. 3-D structure of hard-alpha keratin intermediate filament embedded in the matrix highlighting cysteines and disulphide bridges.



Other aminoamides are present in the wool and so keratin structure: glutamic acid, serine, leucine, glycine and proline are the major ones. Four main classes of proteins are found in wool fibers, depending on the aminoacidic composition:

- low sulfur proteins, “alpha-keratins” (LSP);
- high sulfur proteins (HSP);
- ultra-high sulfur proteins (UHSP);
- high Tyrosine-Glycine Proteins (HYGP).

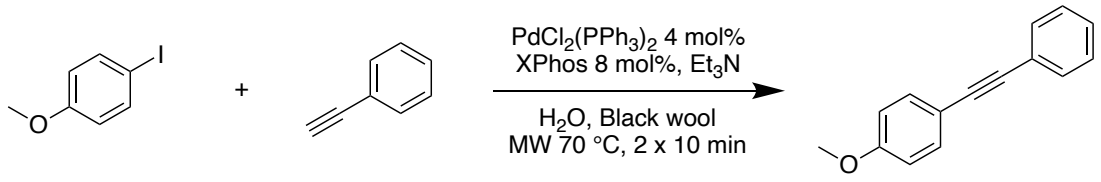
A further classification can be done regarding the molecular weight of those proteins:

- type II keratins (≈52 kDa);
- type I keratins (≈47 kDa);
- keratin-associated proteins (Low molecular weight).

One of the characteristics of wool, that give also the high applicability of this in yarns is that the external structure gives the repellent property to dirty and water but, more important for the chemical applicability, it is the ability to absorb water vapors.[35] This is obviously related to the proteomic profile of the fiber.Another point that must be considered for the application of wool in chemistry and more in detail in catalysis, it is represented by the possible interactions. The interaction of the catalyst with the lateral chains of aminoacids with lateral groups (amines of lysins, thiol of cysteines) or week interactions have to be considered. Keratin macromolecule acts as an amphoteric compound whose overall charge depends on the pH of the surrounding aqueous media. When the molecule has a neutral charge at a certain pH, this value is called the isoelectric point (pI). Generally, this is a range, whose value for wool proteins is 4.7–6.1.[36] When the pH is higher than the pI, the wool fiber surface carries negative charges with affinity for positively charged ions in solution. When the pH is lower than the pI, the wool surface carries positive charges, which enhance the affinity for negatively charged ions in solution.[28] This property is essential for the behavior of wool and keratin-based protein forms in solution, and interaction with other charged species like metals and complex used in catalyzed reactions.

In recent years, Petricci's lab exploit different waste materials, such as micronized white and black Sardinian wool sheep, chicken feathers and waste from the tanning industry, to optimize catalytic transformations. Those projects are in collaboration with Prof. Porcheddu's group at the University of Cagliari. The micronized black Sardinian wool sheep was used in Sonogashira coupling,[36] a reaction catalyzed by Palladium leading to the formation of a covalent bond between a terminal Alkene and an Aryl or Alkenyl halide. Through the identification of optimal reaction conditions, it was possible to obtain a conversion of the 95%, after only twenty minutes of irradiation with microwave, using water as the solvent and in the absence of copper (Scheme 2)

Scheme 2. Sonogashira reaction catalyzed by Pd with Black Wool in the reaction environment.



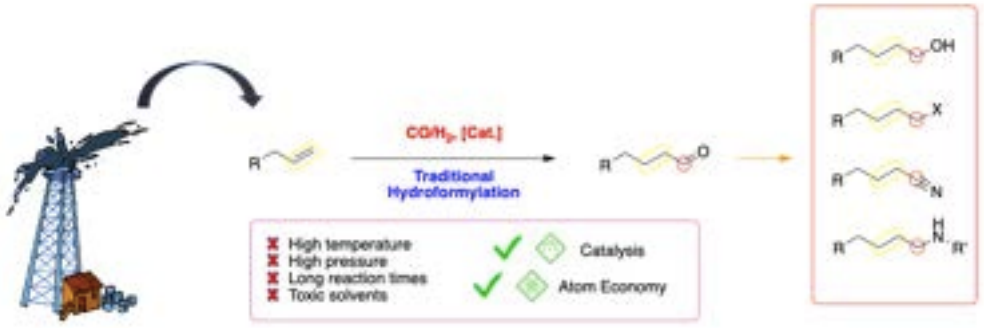
In this example, wool participation suggests an interaction between the aminoacids on the surface of the wool and Pd either through weak interactions, such as hydrogen bonds or Van Der Waals interactions, or stronger, such as ionic bonds. Studies on the interactions between Pd and wool in a similar reaction, showed that coordination or ionic bonds are formed by the connection of N atoms (in -NH_2) and S atoms (in -SH and -S-S-) with Pd atoms.[37]

1.4 Hydroformylation

Remaining within the framework of catalysis and considering the most industrially applied reactions in the world, hydroformylation (HF) is suitable transformation for the use of solid waste in catalysis. HF allows to synthesize aldehydes from olefins and mixtures of CO and H_2 known as syngas. This transformation sins to be suited for our purpose. Those products are very versatile and reactive functional groups usually used as intermediates for further transformations into alcohols, amines or condensation products, by also using domino and tandem protocols.[16] Classical hydroformylations require high pressures (10–100 bar) of H_2 and CO mixtures (syngas) in different ratios (i. e. 1 : 1, 2 : 1, 4 : 1) in stainless steel autoclaves, for long reaction times (1–4 days), at high temperatures (80–200 °C), in not properly eco-friendly media such as toluene or THF some Green Chemistry principle are not respected .

Otto Roelen was the first that define this reaction as Oxo Process.[38] The importance of it in Chemical industries can be defined both for bulk and fine chemical industries (Figure 6). HF is in fact pivotal for the synthesis of Active Pharmaceutical Ingredients [39-40] (*i. e*. Naproxen,[41] Omapatrilat [42]), fragrances (*i. e*. linalool, β-citronellene), detergents and natural products. [43]

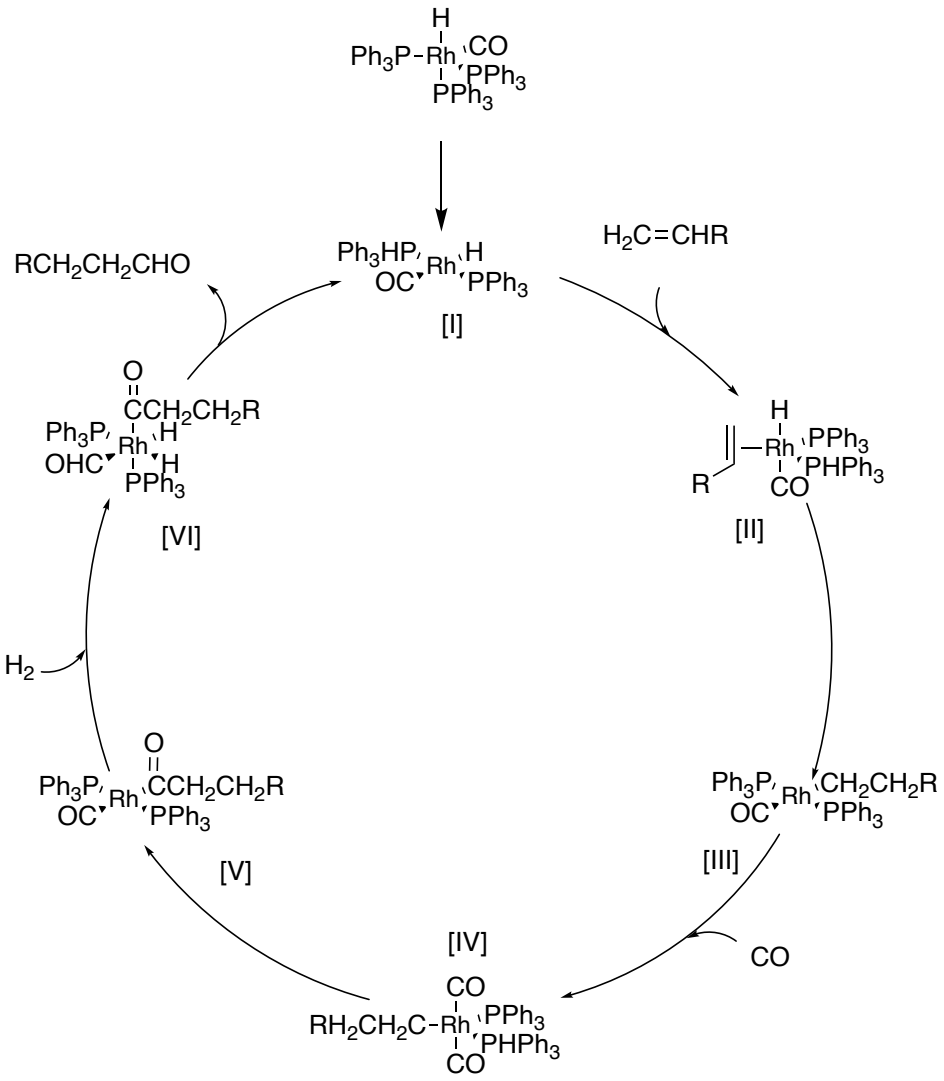
Figure 6. General provenience and transformation in hydroformylation with an overview on the principles of Green Chemistry followed.



HF is an atom economic process catalyzed by different transition metals such as Co,[44] Ru,[45] Pt,[46] Fe[47] and Rh.[48] The presence of Ligands may impact both on regio- and

chemoselectivities.[49] The proposed HF mechanism (Figure 7) initially provides for the generation of the active form of the catalyst-ligand complex, thanks to the transition from a trigonal bipyramid geometry to the square planar one (**I**) due to the loss of a phosphine ligand. At this point, the transition binding of olefins with the active organometallic compound formed in situ happened, through oxidative addition. Olefin initially coordinates the metal in η^2 binding mode, an intermediate binding of double bonds, leading to the formation of a new trigonal bipyramid (**II**) intermediate. There, the formed complex, have a switch to a η^1 binding mode through a migratory insertion 1,2 (**III**).

Figure 7. Catalytic cycle of hydroformylation with general catalyst and ligand



The addition of CO to the activated complex (**IV**), followed by migratory insertion 1,1 leads to the formation of acyl carbon (**V**) the one present in the product. With the oxidative addition of H_2, one of the two hydrides is transferred from the metal to the acyl carbon (**VI**) and the aldehyde is finally obtained by reductive elimination. Like all catalyst the process led to the precursor (**I**) and the release of the final product, allowing the next catalytic cycle.

The synthesis of aldehydes generally requires the use of oxidizing and reducing agents, strong acids and bases, often responsible for the formation of many by-products and dangerous because toxic and highly reactive. However, the main limitation of the developed protocols is still represented by the use of toluene as solvent, in diluted conditions (i. e. 0.1 M), unsuitable for industrial applications.[50]

HF developed by Otto Roelen and Heck, allows a comprehensive study of the process using an innovative catalyst ($Co(CO)_4H$) that allow excellent yield but with drastic conditions, poor control on the selectivity and really expensive.[51] To overcome the problem of cost and low regioselectivity in 1960 Wilkinson developed the first hydroforming processes catalyzed by Rh-based complexes containing ligands. Those Organophosphons can produce the reaction in milder conditions with a good regioselectivity. The structure of the catalyst, $Rh(CO)H(PPh_3)_2$, in the presence of a phosphine ligand, generates a stable intermediate that allow to improve the reaction rate.[52] The pentacoordinate intermediate structure of the catalyst $HRh(P\text{-}P)(CO)_2$ (P-P = phosphine, R = olefines) play a key role in the reaction control.

The most common ligands used in [Rh] catalyzed reactions are derived form Xanthene, whose structure and substituent generate an environment, in terms of electronic interaction and steric hindrance, that allow the control of the stereo-chemoselectivity. Most of those reagents has a really high cost and are difficult to be produced. Taking in example 6-DPPON and SulfoXantphos has a cost around 200 €/g.[53-54]. In some cases, like described before, the process is just apparently green because solvents like Et_2O and CH_2Cl_2 have to be used for purifications and extractions procedure, no catalyst can be recover and recycle is investigated, thus negatively impacting in the *E-Factor*, generally used for those reactions sustainability evaluation.

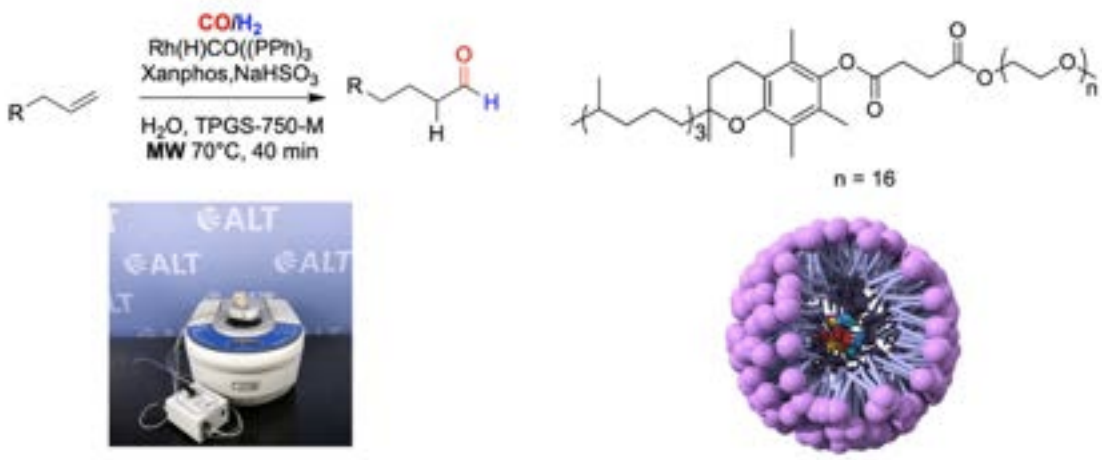
Several steps toward a more sustainable process have been done in the research group in which I have worked for this thesis. The olefin hydroformylation in mild and more sustainable conditions, at low pressure of syngas, including taking advantages of microwave (MW) irradiation is the milestone in this field. These transformations have been successfully extended to heterogeneous catalytic systems as well as to tandem and domino processes.[55-56] Water is a safe and non-toxic solvent used in a few transformations because of the low solubility of most organic compounds in it because of this micellar catalysis was developed and used for HF in the research lab in which this thesis work was done.[16] This is a surfactant generating supramolecular aggregates, such as micelles, able to solubilize organic lipophilic molecules in water, working like a nanoreactor in the reaction media.[57] Despite the excellent results obtained with the micellar catalysis, both in terms of yield, regioselectivity and the ability of recovery Catalyst, Ligand, Solvent and obtain the pure product, the surfactant TPGS-750 M required, is expensive and can present some problems of high degradability.[58]

1.5 Aim of the thesis

The purpose of this thesis is to define the use of Sardinian sheep's wool in the HF reaction catalyzed by Rh, using dielectric heating thanks to the use of MW.

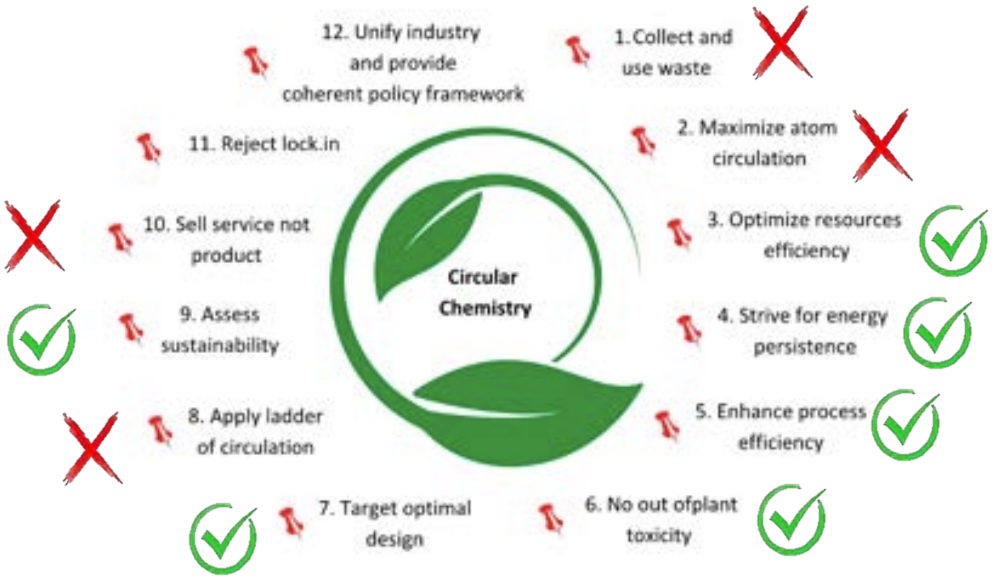
The previously developed HF processes,[16,55] present a series of solutions to problems related to the operating conditions of the process (i.e. high temperature, high pressure long reaction time). MW irradiation allow to archive the reaction providing excellent results in terms of conversion and regioselectivity in very short reaction times (4 min) and at very low pressures (2.8 bar) compared to conventional autoclave hydroformylation reactions, which generally require more drastic conditions.[55] Although, the use toluene like solvent, ionic liquid [bmim][BF_4] to reach the optimum required temperature of 110 °C are required and remains unsolved problems. Because of this, micellar catalysis was used and this rise the sustainability of this reaction. This protocol allows to obtain the desired product without any purification thanks to the use of sodium bisulphite, performing the reaction with 9 bar of Syngas, obtaining the linear aldehyde as the main regioisomer in 40-60 minutes at 60 °C and using [$Rh(CO)H(PPh_3)_3$]/Xantphos like the perfect Catalyst-Ligand couple also from the economical point of view (Figure 8).

Figure 8. Microwave assisted micellar hydroformylation process.



Analyzing the HF process on the base of the transformation demonstrated to be green. However, from a Circular Chemistry point of view most of the principles are not considered at all. (Figure 9).

Figure 9. Circular Chemistry principles followed by reported HF processes.



Here is reported the last efforts toward more sustainability HF protocols involving the use of mechanically micronized Sardinian sheep wool produced in the Prof. Porcheddu's group in an aqueous environment, without the use of surfactants or ionic liquids. In addition, to contributing to the reduction of costs and energy expenditure necessary for the production and purchase of surfactant, this process allow to define a HF reaction closer to the 12 principles of Circular Chemistry under mild conditions of syngas pressure and temperature and using a waste material in the catalytic system. Defining the effective method for the synthesis of aldehydes, the reaction mechanism will be also investigated as recent studies on the micellar and MW catalyzed HAM demonstrated a switching in the reaction pathway.[15] Instead of having a classical HF with addition of CO/H_2 to the double bond, there is first a carbonylation of the double bond with the formation of the corresponding intermediate which then undergoes a hydrogenation of the double bond obtaining the final ammine with regioselectivity in favor of the linear product. In this work is reported the full analysis of the process, passing from the optimization of the reaction trough the substrate scope and the characterization of the biomass, using analytical and biological methods.

Chapter 2

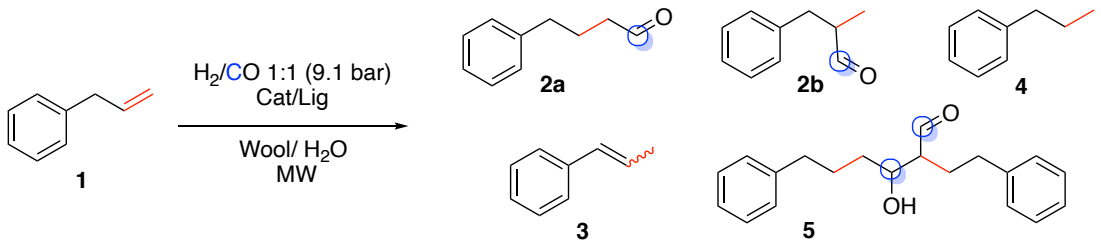
Result and discussion

2.1 RESULTS AND DISCUSSION

2.1.1 Optimization of the reaction conditions

This study starts from a previous work, HF under micellar catalysis conditions,[14] indicated that the best conditions for an effective conversion require the addition of $NaHSO_3$ to the reaction thus producing the Bertagnini's salt. This plays a key role for obtaining linear aldehydes in excellent yields, preventing the typical aldol condensation occurring in HFs in aqueous media.[3] With these data in hand, it was natural to start this project using Allylbenzene **1** as the model substrate for optimizing the HF process in water in the presence of micronized wool: a suspension of **1** in H_2O is irradiated with MW in the presence of Wool, $NaHSO_3$, a 1:1 CO/H_2 mixture, $Rh(CO)H(PPh_3)_3$, Xantphos at 70 °C for 40 min obtaining a non-conversion into the corresponding aldehyde (Table 1, entry 1). The addiction of wool in the reaction mixture generates a suspension in H_2O given also by the resulting $NaHSO_3$ not fully solubilized.

Table 1. Optimization of the reaction conditions

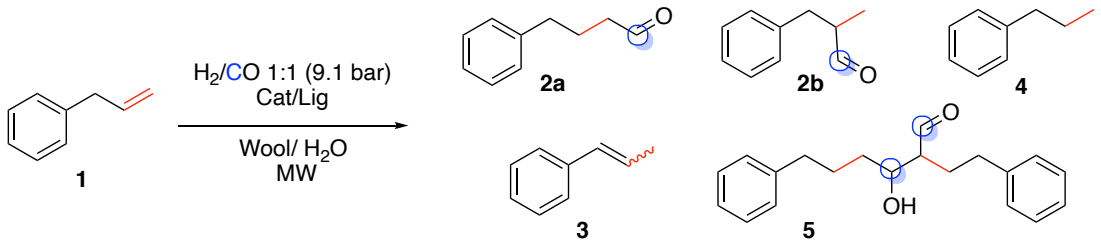


Entry	Cat., Ligand., additive, syngas (P)	T, time	Conv. [%][a]	**2** [%][a] (**2a/2b**)	**3** [%][a]	**4** [%][a]	**5** [%][a]
1[b]	[$Rh(CO)H(PPh_3)_3$] 1 mol%, Xantphos (4 mol%), $NaHSO_3$, Wool (cat/wool 2:1), CO/H_2 (9.1 bar)	MW, 70 °C, 40 min	-	-	-	-	-
2[b]	[$Rh(CO)H(PPh_3)_3$] 1 mol%, Xantphos (4 mol%), $NaHSO_3$, Wool (cat/wool 4:1), CO/H_2 (9.1 bar)	MW, 70 °C, 40 min	58	35 (14:1)	19	1	3
3[c]	[$Rh(CO)H(PPh_3)_3$] 1 mol%, Xantphos (4 mol%), Wool, $NaHSO_3$, (cat/wool 8:1), CO/H_2 (9.1 bar)	MW, 70 °C, 40 min	26	8 (30:1)	15	-	3
4[c]	[$Rh(CO)H(PPh_3)_3$] 1 mol%, Xantphos (4 mol%), Wool (cat/wool 4:1), CO/H_2 (9.1 bar)	MW, 70 °C, 40 min	96	64 (16:1)	27	-	5
5[c]	[$Rh(CO)H(PPh_3)_3$] 1 mol%, Xantphos (4 mol%), Wool (cat/wool 8:1), CO/H_2 (9.1 bar)	MW, 70 °C, 40 min	39	-	35	2	2

[a] Conversion of **1** into **2, 3, 4** and **5** determined by GC/MS as reported in SI. [b] **1** (0.75 mmol), [$Rh(CO)H(PPh_3)_3$] (0.01 mmol), xantphos (0.04 mmol), $NaHSO_3$ (1.12 mmol), CO/H_2 (9.1 bar), Wool, H_2O (3 mL), MW. [c] **1** (0.75 mmol), [$Rh(CO)H(PPh_3)_3$] (0.01 mmol), xantphos (0.04 mmol), CO/H_2 (9.1 bar), Wool, H_2O (3 mL), MW.

Different ratios between Wool/Catalyst have been screened (Table 1, entry 1-3). These results being obtained with a 2:1 ratio of Wool/Catalyst in H_2O at 70 °C (Table 1, entry 3). A 26% conversion with respect to **1** was observed with an acceptable regioselectivity toward the linear amine (**2a/2b**: 16/1). The low conversion can be probably related to the use of $NaHSO_3$ that should bring to Bertagnini's salt formation interfering with the wool mediated catalytic process. In the absence of $NaHSO_3$, the best conversion in to **2** with a 1:4 ratio of Cat/wool was indeed observed (Table 1, entry 4) Using a higher quantity of wool (Cat/Wool 8:1) a lower conversion with a higher regioselectivity was detected (**2a/2b** 30:1 (Table 1, entry 5). This data suggests that wool plays effectively a role in the regioselectivity of the final product, favoring the linear aldehyde. Low temperature brings to the formation of an higher amount of the product **3,** generating in the and a lower conversion in to the desired product (Table 2, entry 1). Higher temperature (80 °C) produces 86% conversion and the formation of aldehyde **2** in 73% of yield, but with a lower regioselectivity (Table 2, entry 2), instead of it 90 °C cause the formation of the subproduct **5** in 14 %. (Table 2, entry 3).

Table 2. Optimization of the reaction time and temperature



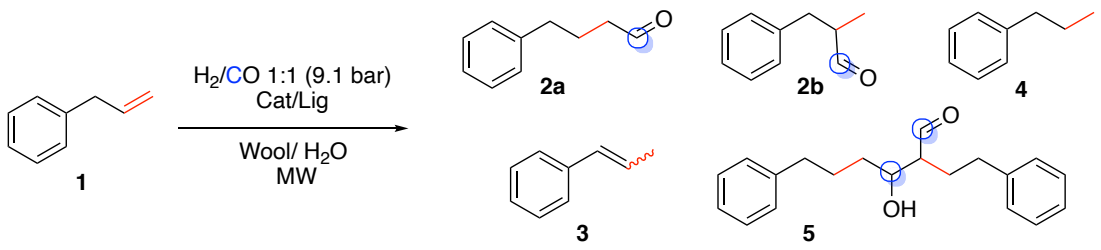
Entry	Cat., Ligand., additive, syngas (P)	T, time	Conv. [%][a]	**2** [%][a] (**2a/2b**)	**3** [%][a]	**4** [%][a]	**5** [%][a]
1[b]	[Rh(CO)H(PPh$_3$)$_3$] 1 mol%, Xantphos (4 mol%), Wool (Cat/Wool 1:4), CO/H$_2$ (9.1 bar)	MW, 60 °C, 40 min	77	41 (18:1)	25	3	8
2[b]	[Rh(CO)H(PPh$_3$)$_3$] 1 mol%, Xantphos (4 mol%), Wool (Cat/Wool 1:4), CO/H$_2$ (9.1 bar)	MW, 80 °C, 40 min	86	73 (16:1)	11	-	2
3[b]	[Rh(CO)H(PPh$_3$)$_3$] 1 mol%, Xantphos (4 mol%), Wool (Cat/Wool 1:4), CO/H$_2$ (9.1 bar)	MW, 90 °C, 40 min	88	28 (18:1)	40	6	14
4[b]	[Rh(CO)H(PPh$_3$)$_3$] 1 mol%, Xantphos (4 mol%), Wool (Cat/Wool 1:4), CO/H$_2$ (9.1 bar)	MW, 80 °C, 30 min	79	51 (13:1)	19	-	9
5[b]	[Rh(CO)H(PPh$_3$)$_3$] 1 mol%, Xantphos (4 mol%), Wool (Cat/Wool 1:4), CO/H$_2$ (9.1 bar)	MW, 80 °C, 50 min	93	47 (29:1)	35	7	4

[a] Conversion of **1** into **2, 3, 4** and **5** determined by GC/MS as reported in SI. [b] **1** (0.75 mmol), [Rh(CO)H(PPh$_3$)$_3$] (0.01 mmol), ligand (0.04 mmol), CO/H$_2$ (9.1 bar), Wool (Cat/Wool 4:1), H$_2$O (3 mL), MW.

Irradiating for shorter reaction time (30 minutes) 79% conversion was observed with just a 51% yield of **2** in reduced regioselectivity (Table 2, entry 4). On the contrary irradiating for 50 min higher conversion (93%) was observed moreover with the formation of **3** the product of isomerization of the double bond in large quantity (Table 2, entry 5).

Using a higher quantity of the catalyst (2 mol%) **3** was obtained as the major reaction products (Table 3, entry 1). Doubling both the quantity of $[Rh(CO)H(PPh_3)_3]$ and Xantphos, the product **3** is not detected but, the starting material remain in high concentration in the reaction environment (Table 3, entry 2).

Table 3. Optimization of reaction conditions for the reported HF protocol.



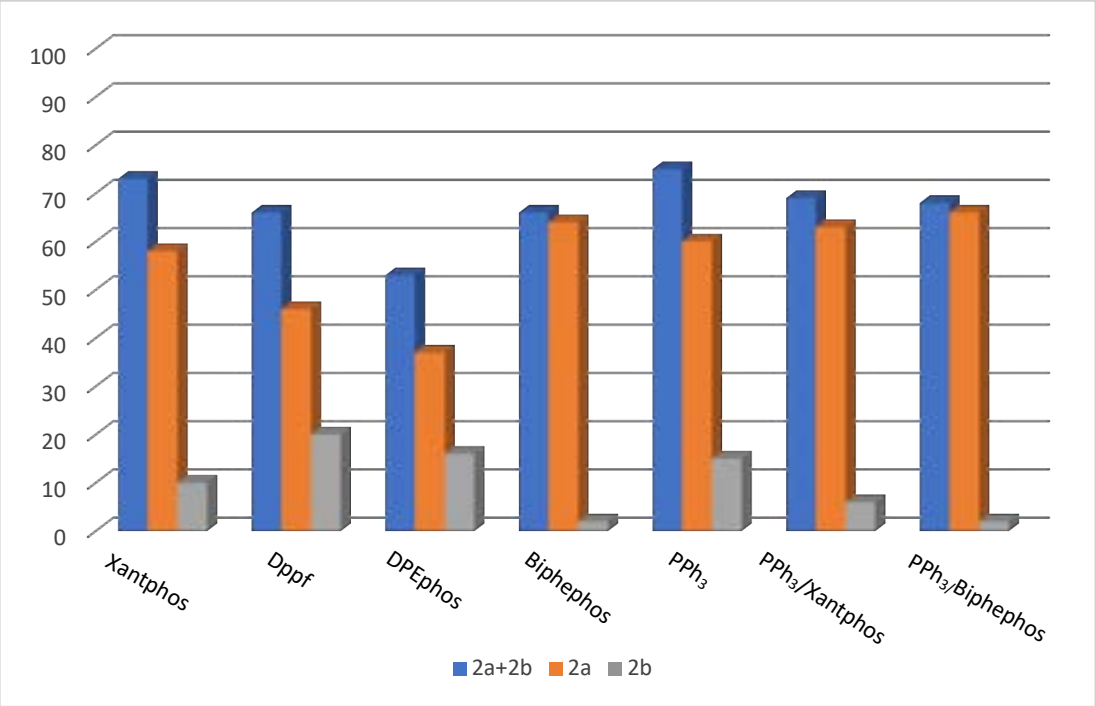
Entry	Cat., Ligand., additive, syngas (P)	T, time	Conv. [%][a]	**2** [%][a] (**2a/2b**)	**3** [%][a]	**4** [%][a]	**5** [%][a]
1[b]	$[Rh(CO)H(PPh_3)_3]$ 2 mol%, Xantphos, Wool (Cat/Wool 1:4), CO/H_2 (9.1 bar)	MW, 80 °C, 40 min	90	63 (9:1)	25	-	2
2[c]	$[Rh(CO)H(PPh_3)_3]$ 2 mol%, Xantphos, Wool (Cat/Wool 1:4), CO/H_2 (9.1 bar)	MW, 80 °C, 40 min	50	43 (12:1)	-	3	4
3[d]	Wool (Cat/Wool 1:4), CO/H_2 (9.1 bar)	MW, 80 °C, 40 min	3	-	2	1	-
4[e]	$[Rh(CO)H(PPh_3)_3]$ 2 mol%, Xantphos, Wool (Cat/Wool 1:4)	MW, 80 °C, 40 min	3	-	-	3	-
5[f]	$[Rh(CO)H(PPh_3)_3]$ 2 mol%, Xantphos, CO/H_2 (9.1 bar)	MW, 80 °C, 40 min	10	5	2	3	-

[a] Conversion of **1** into **2, 3, 4** and **5** determined by GC/MS as reported in SI. [b] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.02 mmol), Xantphos (0.04 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), H_2O (3 mL), MW. [c] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.02 mmol), Xantphos (0.08 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), H_2O (3 mL), MW. [d] **1** (0.75 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), H_2O (3 mL), MW. [e] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.02 mmol), Xantphos (0.08 mmol), Wool (Cat/Wool 4:1), H_2O (3 mL), MW. [f] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.02 mmol), Xantphos (0.08 mmol), CO/H_2 (9.1 bar), H_2O (3 mL), MW.

The best reaction conditions observed right now were obtained using $Rh(CO)H(PPh_3)_3$ and Xantphos (1 mol% and 4 mol% respectively) H_2O in presence of wool as an additive irradiating for 40 minutes at 80: conversion 73% with a 16:1 ratio of **2a/2b**. As expected, in the absence of Syngas, Rh catalyst or wool, only the starting material and some traces of its isomer **3** and propilbenzene **4** were recovered.

The impact of different ligands in conversions and regioselectivities has been also evaluated (Graphic 1**)**.

Graphic 1. Use of different ligands in HF reaction



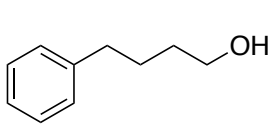
Exploration of different gasses parameters: [a] Conversion determined by GC/MS as reported in SI. [b] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.01 mmol), Xantphos (0.04 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), H_2O (3 mL), MW, 80 °C, 40 min. [c] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.01 mmol), ligand (0.04 mmol), CO/H_2 (9.1 bar), CO_2, Wool (Cat/Wool 4:1), H_2O (3 mL), MW, 80 °C, 40 min.

Using Dppf poor 3:1 regioselectivity is observed. The Ferrocene like ligand (Dppf), show the higher conversion, probably because the Bidentate coordination of Rh, increase the reactivity of active catalytic species blocking the formation of an isomer during the migration-insertion process (Figure 8), impacting in the final regioselectivity. Dppf is usually reported to obtain high regioselectivity together with low conversions.[59]

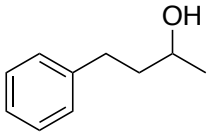
Using DPEphos bad results are obtained, not comparable with other ligands (**2** 53%, **2a/2b** 3:1). Biphephos showed the best selectivity towards aldehyde **2a** (**2a/2b** 32:1), unfortunately with the worse conversion observed so far. This result, consistent with what already observed in micellar catalyzed hydroformylation,[60] is showing that good regioselectivity are mainly influenced by steric hindrance of the ligand used, but also by the presence of different functional groups that can change the electronic properties of the active catalytic species formed in situ. π -staking interactions can be effective in those reactions, because of his role in stabilizing catalytic intermediates. In the end, the best conversion was finally observed with PPh_3, with not very good regioselectivity. Use a mixture of two different ligands such as PPh_3/Xantphos a conversion of 69% in to **2** (**2a/2b** 12:1), was observed, while with PPh_3/Biphephos a good conversion (68%) with very high regioselectivity (**2a/2b** 32:1) was obtained.

The influence of syngas composition was also evaluated together with the possible effect of CO_2 traces present in H_2O. Firstly, a 4:1 ratio of CO/H_2 was used lowering down the formation of side product 4 but also observing a low conversion into the aldehyde. This is a general trend observed with an higher quantity of CO with respect to H_2 where the influence of CO/H_2 ratio from 3:1 to 1:1 is minimal both on the conversion of **1** to the homologous aldehyde superior **2**, and on regioselectivity. The best result is the one obtained with CO/H_2 ratio of 1:1. By using higher quantities of H_2, the formation of alcohol is observed (Scheme 3). With a CO/H_2 ratio of 1:2, 1:3 and 1:4 we obtain respectively 6%, 9%, 14% of **6**.

Scheme 3. Representation of alcohol subproduct



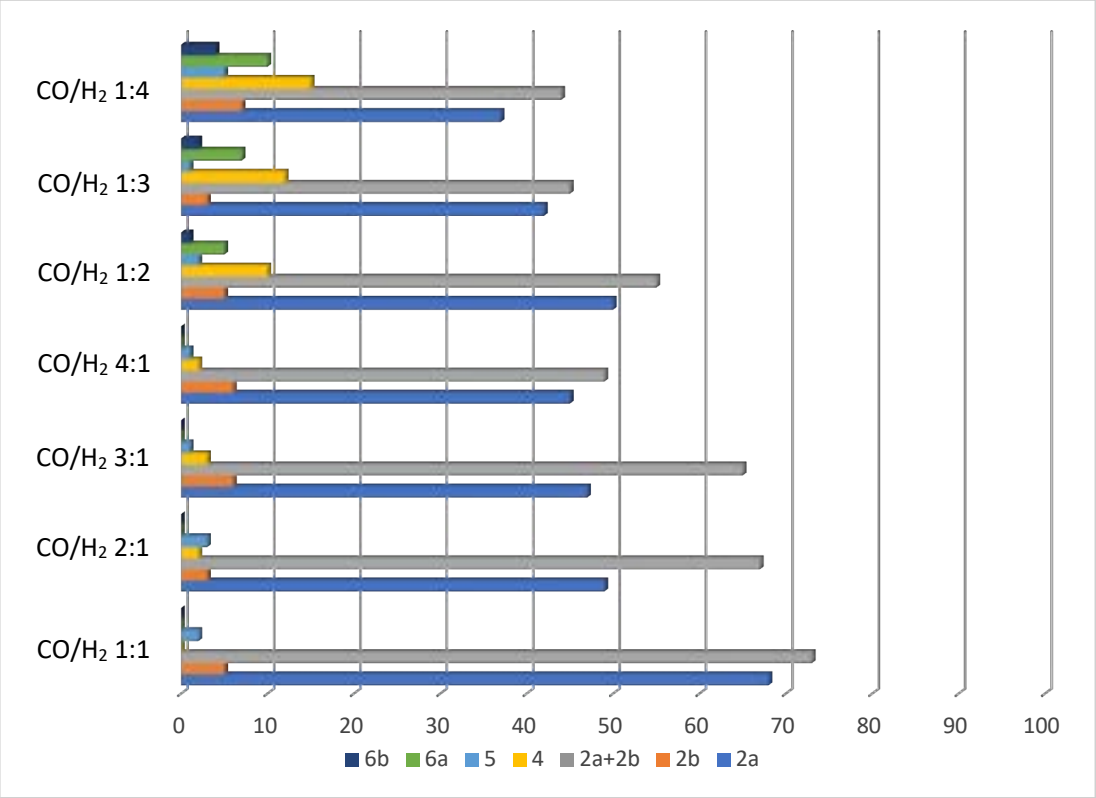
6a



6b

The data are really important and can open this research toward the preparation of these alcohols in a single step from olefines. It is interesting to note that the branched alcohol was obtained only in traces by ^{1}H-NMR analysis. In these conditions, CO/H_2 ratio of 4:1 (Graphic 2), a higher quantity of **4** was also observed.

Graphic 2. Use of different gasses in HF reaction



Exploration of different ligand: [a] Conversion determined by GC/MS as reported in SI. [b] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.01 mmol), ligand (0.04 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), H_2O (3 mL), MW, 80 °C, 40 min. [c] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.01 mmol), ligand A (0.02 mmol), ligand B (0.02 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), H_2O (3 mL), MW, 80 °C, 40 min.

By degassing the reaction mixture before the irradiation (Table 4), no changes in both conversion and regioselectivity were noted. By adding CO_2 **4** was obtained in higher quantities together with the product of isomerization.

Table 4. Gas Variation in the reaction mixture

Entry[a]	Conditions	Conversion [b]	2a/2b	Side-product		
				3	4	5
1	CO/H_2 1:1	73 %	19:1	13%	-	3%
2	Degassed Solution CO/H_2 1:1	67 %	19:1	21%	2%	-
3	CO_2 CO/H_2 1:1	49%	12:1	32%	5%	2%
4	CO/H_2 1:1 Tap Water	70 %	19:1	14%	-	4%

[a] $Rh(CO)H(PPh_3)_3$ 1 mol%; Lig/Cat 4:1; Lan/Cat 4:1; 40 min, 80 °C; [b] Calculated by GC-MS.

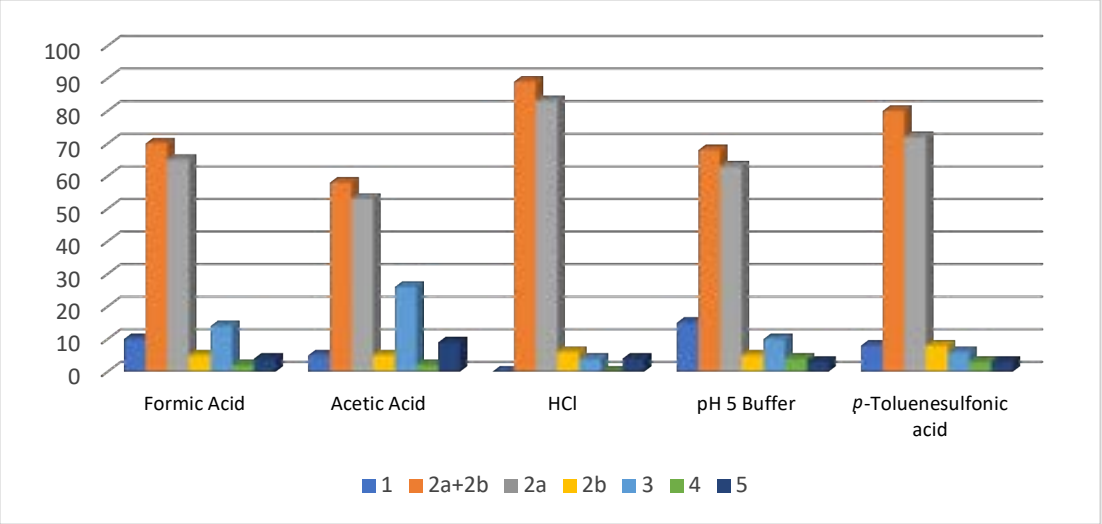
Those data allow to see that degas the solution or treat it with CO_2 bring to obtain a worst conversion respect to the best conditions obtained with only Syngas. Another important observation was done using water from the sink. In this case the conversion is equal to the one obtained using water for reaction sold by chemical suppliers, allowing to hypothesize the use of wastewater for HF reaction. This will increase the sustainability of this process.

With these data in hand and based on our previous experience finding on HF and HAM under micellar catalysis conditions, the possible impact of an acidic environment in HF was also investigated. The acidic medium should be responsible for both activation of the wool by protonating aminoacidic residues and/or from different active catalytic species. In fact, wool is composed by proteins that at different pH, can be positively or negatively charged on the base on the isoelectric point. This can obviously affect the behavior of this fiber in the reaction thus impacting the coordination of the metal in catalyst formation and reactivity.

From a catalyst point of view the presence of ions in the reaction media can affect the catalytic cycle because of the exchange with ligands, over the catalyst thus changing the reaction outcome as observed in HAM reaction.[14]

The results obtained by adding acids in the reaction environment, are summarized in **Graphic 3**.

Graphic 3. Use of different acids in HF reaction



Exploration of acids in the reaction: [a] Conversion determined by GC/MS as reported in SI. [b] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.01 mmol), Xantphos (0.04 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), Acid (0.1 mmol) H_2O (3 mL), MW, 80 °C, 40 min. [c] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.01 mmol), Xantphos (0.04 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), AcOH (0.05 mmol), AcONa (0.05 mmol), H_2O (3 mL), MW, 80 °C, 40 min.

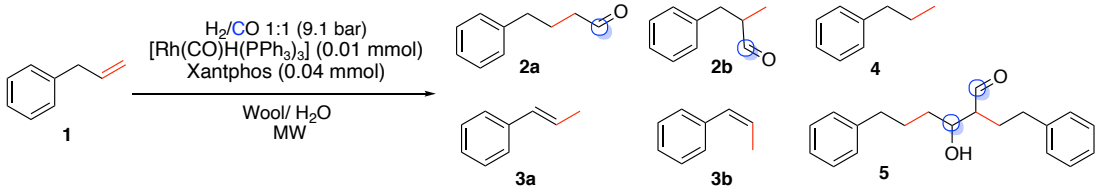
The use of a weak acid, such as formic acid, leads to a decrease in conversion to the aldehyde with an increase in by-products. As strong acid, HCl (0.1 mmol), we note a strong increase in conversion obtaining **2** in 89% yield with excellent regioselectivity (Graphic 3). In the presence of a catalytic amount of HCl the formation of an emulsion accurses. In both cases, the degradation of wool was observed as indicated by SEM analysis in **Paragraph 2.5.2**. A pH 5 AcOH/AcONa buffer and *p*-toluensulfonic, allows to observe that strong acids are required for a good conversion. In the case of AcOH/AcONa buffer a conversion of 68% is obtained while with *p*-TSA the conversion rises to 80%, but with worse regioselectivity.

2.2 Reaction in neat conditions: a further optimization

Looking at the green and circular chemistry principles, the attention was focussed on minimizing wastes production and release. For this aim, the use of lower amount of water as a solvent have been investigated. H_2O is not a critical part of the process, although it may partially compromise a possible recovery and reuse of the catalyst and wool.

The main issue should be represented by the solubility of syngas in the reaction mixture that in highly concentrated an heat conditions should hamper the reaction.

Table 5. Optimization of reaction conditions without solvent for the reported HF protocol.



Entry	Cycle 1	Cycle 2	Cycle 3	Conv. [%][a]	**2** [%][a] (**2a/2b**)	**3** [%][a]	**4** [%][a]	**5** [%][a]
1[b]	HCl (0.1 mmol), MW 80 °C, 40 min	-	-	32	28 (16:1)	-	-	4
2[c]	HCl (0.1 mmol), H_2O (100 µL), MW 80 °C, 40 min	CO/H_2 (9.1 bar), MW 80 °C, 40 min	-	62	58 (19:1)	1	-	3
3[c]	HCl (0.1 mmol), H_2O (100 µL), MW 80 °C, 40 min	CO/H_2 (9.1 bar), MW 80 °C, 40 min	CO/H_2 (9.1 bar), MW 80 °C, 40 min	100	-	2	-	98
4[b]	HCl (0.1 mmol), MW 80 °C, 90 min	-	-	85	84 (19:1)	-	-	1
5[b]	HCl (0.1 mmol), MW 80 °C, 115 min	-	-	93	78 (19:1)	-	-	15
6[b]	HCl (0.1 mmol), H_2O, MW 80 °C, 90 min	-	-	60	-55 (10:1)	2	3	-
7[b]	HCl (0.1 mmol), H_2O, MW 80 °C, 115 min	-	-	5	-	2	3	-

[a] Conversion of **1** into **2, 3, 4** and **5** determined by GC/MS as reported in SI. [b] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.01 mmol), xantphos (0.04 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), HCl (0.1 mmol), MW. [c] **1** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.01 mmol), xantphos (0.04 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), HCl (0.1 mmol), H_2O (100 µL), MW.

Performing the reaction in the same conditions obtained until now, irradiation for 40 minutes at 80 °C in solvent free conditions with catalytic amount of HCl in the reaction mixture very bad results have been observed (Table 5, entry 1). This can be related to the low homogeneity obtained. Better results have been obtained applying 2 cycles of irradiation of 40 minutes each in the presence of 100 μL of H_2O (Table 5, Entry 2). Again, the conversion in **2** is still too low. In these conditions, the starting material **1** didn't react. Thinking about the reactivity of the reagent in absence of the solvent, we decide again to increase the number of cycles. In this case only the side product derived from the aldolic condensation has been obtained (Table 5, Entry 3). Performing the reaction at 80 °C for 90 minutes in absence of water with 0.1 mmol of HCl, a good conversion of 84% into the corresponding aldehyde with no byproduct occurred (Table 3, entry 4). By increasing the reaction time, the formation of the aldehyde as **5** is the main reaction product (Table 2, entry 5). It's interesting to note that performing multiple cycle or a single longer irradiation do not impact in the reaction outcome. The addition of water in catalytic quantity consent to recover a higher amount of starting material in the reaction mixture. Concluding that one cycles of 90 min of irradiation at 80 °C in the presence of wool are beneficial for our purposes and that good results can be obtained by using a catalytic amount of HCl (0.1 eq) with respect to allylbenzene **1** (0.75 mmol). The work-up of this transformation hasn't been yet optimized but a simple extraction with AcOEt,[61] allowed to obtain the pure final product without further purifications needed.

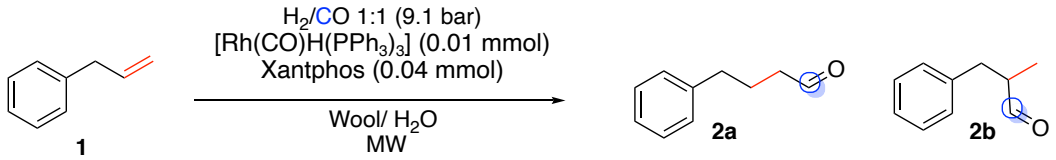
2.3 Reaction with other biomasses

Other micronized solid wastes have been also evaluated as additives for this HF process: micronized chicken feathers, black wool of Sardinian and Australian sheep, white wool from Lombard sheep and vegetable tanned leather scarfs. In the latter case, the leather has been previously subjected to different extraction cycles using EtOH, acetone and petroleum ether to remove tannins residues.

White wool from Sardinians sheep demonstrated to be the most effective additive for this transformation (Table 6). Using all the other matrices, worst results have been obtained in the same conditions, probably because of the different interactions between catalyst and the amino acid residues/functional groups present on the biomass.

Further characterization of protein composition of these biomasses confirms these hypothesis.

Table 6. Reaction with different wastes.



Entry [a]	Conditions	2 [%][b]	(2a/2b)
1	Black wool	48	8:1
2	Black Australian Wool	40	8:1
3	White wool "Lombarda"	65	20:1
4	Chicken feathers	69	19:1
6	White Wool	73	16:1

[a] $Rh(CO)H(PPh_3)_3$ 1 mol%, Rapporto Lig/Cat 4:1, Rapporto Additivo/Cat 4:1; [b] Calcolati attraverso GC-MS.

Australian and Sardinian black wool showed similar results in terms of conversion and regioselectivity (Table 6, entry 1-2). With the Sardinian one we observe a conversion into the corresponding aldehyde of 48% and the regioselectivity of 8:1 (Table 6, entry 1), while with the Sardinian black sheep wool we had obtained, in the same conditions, 40% conversion with a **2a/2b** ratio of 8:1 (Table 6, entry 2). The origin and the lifestyle of the sheep are pivotal for the differentiation of races but, from a chemical point of view are very similar.

Observing the result obtained by using the "Lombard" sheep's wool we can see a great similarity with the white wool and above all an excellent regioselectivity (Table 6, entry 3). This is best achieved by using additives other than the wool used in the optimization. The conversion into the corresponding aldehyde is of 65% and a regioselectivity of 20:1 in favor of the linear aldehyde. A similar result is obtained using Chicken feathers (Table 6, entry 4).

2.4 Substrate scope

The optimized reaction in H_2O using wool in the reaction mixture and MW dielectric heating allow a conversion of 73% into the desired aldehyde with an irradiation at 80 °C for 40 minutes and 89% using a catalytic amount of HCl, in both cases with an optimal regioselectivity. The reaction without HCl can be successfully applied to different olefins demonstrating a high versatility and group tolerance (Table 7). The use of different alkenes is demonstrating a high regioselectivity starting from long chain olefins or decorated alkenes. Only terminal alkenes have been tested because of the impossible use of higher pression of Syngas.

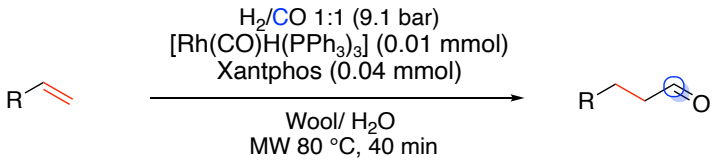
It is worth noting that the HF of industrially valuable long chain olefins, such as 1-octene (**7**), occurs very efficiently producing linear nonanal in 70% isolated yields with a high regioselectivity for the product **15** (Table 7 entry 1).

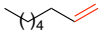
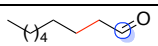
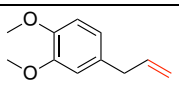
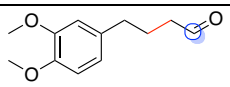
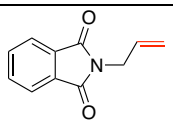
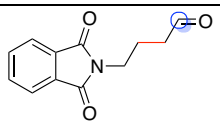
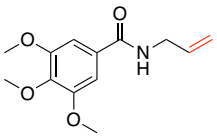
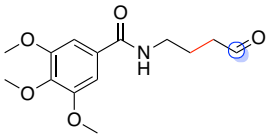
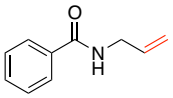
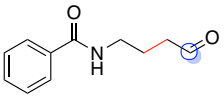
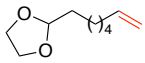
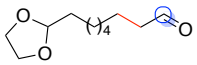
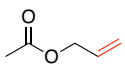
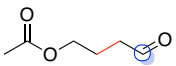
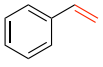
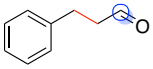
Good results were obtained for the synthesis of 4-(3,4-dimethoxyphenyl)butanal (**16**) and the product **17** with a conversion of 58% and 65 % respectively. The regioselectivity also in this case is in favor of the linear aldehyde (Table 7, entry 2-3).

The substituents seem to have a rather small impact on the isolated yields, however, in terms of solubility we can have some problems. Using H_2O in the absence of HCl, is not very efficient when solid water insoluble olefines, such as **10** and **11**, are used (Table 7, entry 4-5). Those HF reaction allow to obtain a conversion into the corresponding aldehyde of **10** and **11** of only 20% and 18% with a regioselectivity **18a/18b** of 2:1 and **19a/19b** of 5:1.

The Ketal **12** and the ester **13** (Entry 6-7) react with total conversion into the corresponding product (98 % yield) and about the regioselectivity, in the first case, were acceptable, instead, for the ester, only the linear product was obtained (**20a/20b** 2:1 and **21a/21b** 5:1). [62-64]

Table 7. Substrate Scope



Entry[a]	Alkene	Product	Yield [%] (l:b)
1	 **7**	 **15**	70 (40:1)
2	 **8**	 **16**	58 (40:1)
3	 **9**	 **17**	65 (60:1)
4	 **10**	 **18**	20 (2:1)
5	 **11**	 **19**	18 (5:1)
6	 **12**	 **20**	98 (19:1)
7	 **13**	 **21**	98 (99:1)
8	 **14**	 **22**	70 (2:1)

[a] **Alkene** (0.75 mmol), $[Rh(CO)H(PPh_3)_3]$ (0.01 mmol), Xantphos (0.04 mmol), CO/H_2 (9.1 bar), Wool (Cat/Wool 4:1), H_2O (3 mL), MW, 80 °C, 40 min.

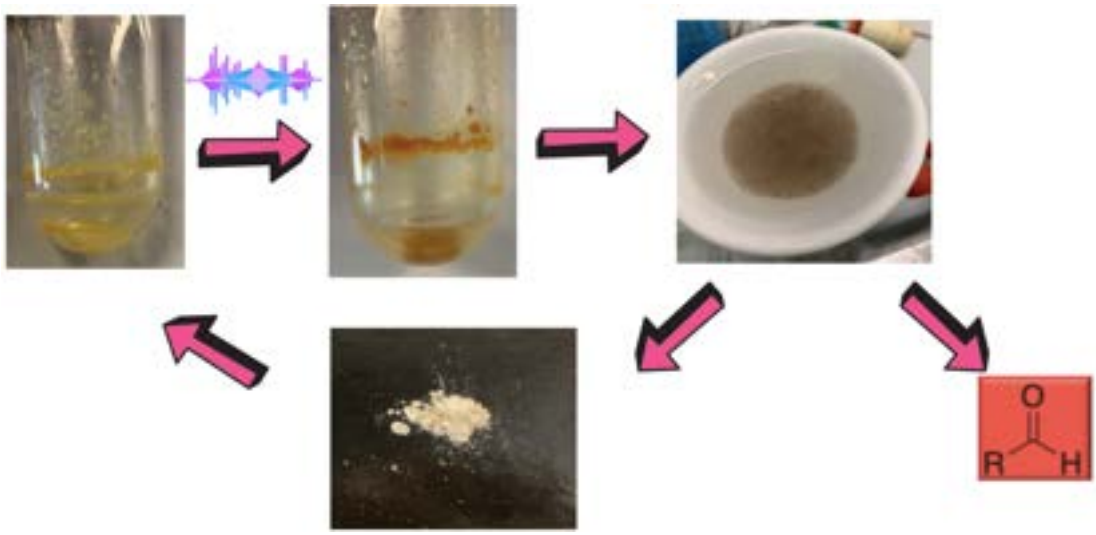
A good surprise was the hydroformylation of styrene **8** that furnish the linear aldehyde as the main reaction product in good yields (**Entry 8**). This is a surprising result because in classical HF processes of styrene derivatives, the branched aldehyde is the main product. The evaluation of this new result, comparable to the one obtained in another process, such as micellar catalyzed HAM,[14] suggest a possible reaction mechanism different than the one of classical HF reaction. Some studies are in progress for the evaluation of the presence of reaction intermediates and Rhodium complexes that can lead to the affirmation of the effectiveness of this hypothesis that would allow to open a new chapter in the history of HF.

2.5 Workup optimization

To obtain a sustainable process, once developed the best reaction conditions, a careful set-up of work-up procedures was needed with a special focus on catalyst recovery and on minimizing the use of toxic organic solvents. In this protocol the work-up is represented by filtration, recovering the wool, an extraction of the water with a sustainable organic solvent as AcOEt.[3] The content in metal of all the phases, as well as the characterization of the wool fiber recovered has been investigated. In all the protocols an important blanching of the wool was observed with a. yellow/orange color of the matrix observed before the irradiation, with the consequent addiction of the catalyst. After irradiation and filtration, the color of wool reverts to pale white/yellow color.

(**Figure 10**).

Figure 10. Workup performing Filtration and extraction.

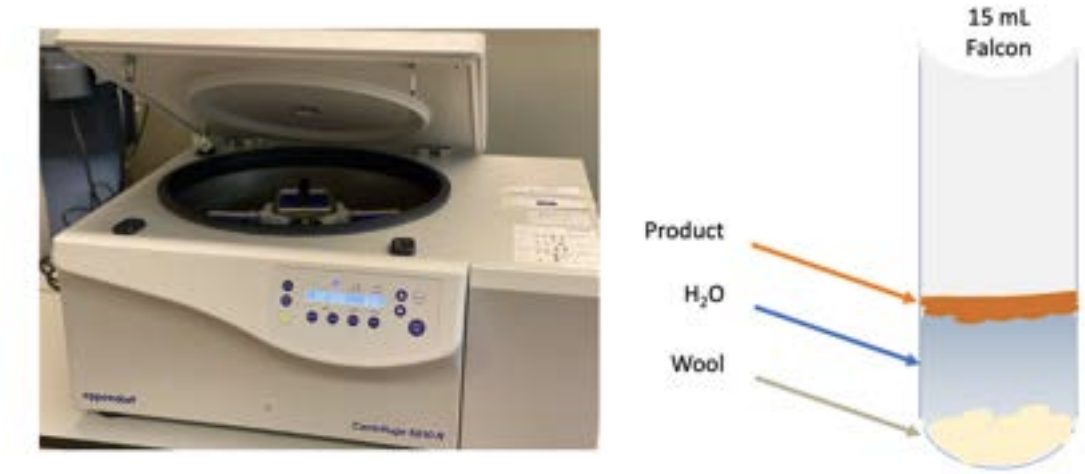


The colour changes observed on wool suggested that the catalyst should be retained in small quantity by the matrix. A dark colour of the wool after the irradiation has usually been observed in conditions producing HF by-products.

With the aim to minimize the use of organic solvents and to facilitate the separation of the solid matrix from water, aldehyde liquid phase and to understand where

Catalyst and Ligand remain after the reaction, after the irradiation the tube was subjected to centrifugation at 3500 rpm for 30 minutes (**Figure 11**).

Figure 11. Workup performed with centrifuge



.

After it, all the phases are separated and, using a pipette, the organic phase composed by the pure aldehyde has been recovered, the H_2O and wool were directly reused by adding allylbenzene for further HF cycles.

To have an opinion about the sustainability of this method of workup respect to the classical one performed with the solvent, an LCA analysis can be performed.

2.6 Analysis and Composition of wool

Sheep's wool has many different properties directly related to the origin of the animal and the consequent composition of the wool produced. To fully understand the behavior of this fiber within the reaction environment and how it participates in the HF of terminal alkenes, analysis of proteins and wool structure have been performed.
In Prof. Porcheddu's laboratory of the wool is washed with H_2O and soap to remove the coarser impurities derived from the deposit of the wool following the shearing of the animal. Then a washing with acetone and hexane (50 ml/10 g of wool) takes place to eliminate the polar and apolar components.
After drying under an IR lamp, the wool is chopped manually to eliminate the possible formation of aggregates in the next step.
The micronization takes place in a zirconia jar mill [65] whose operational specifications are:

- 30 Hz for 20 minutes grinding
- 2 zirconia sphere 12 mm
- 2 zirconia jars diameter 50 mL
- An eventual second step of grinding of 5 minutes

The wool is than used directly in the reaction. Sodium Dodecyl sulfate (SDS-page) analysis[66] Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM-EDS),[67] Scanning Transmission Electron Microscopy (STEM),[68] Inductive coupled Plasma Spectrometry (ICP)[68] have been performed on the fiber.

2.6.1 Analysis of wool proteins

To evaluate the composition of the different types of wool available to the laboratory where this thesis work was conducted, we decided to carry out biological tests to define the content and topology of proteins that make up the fibers. Like previously described we can differentiate between:

i. Low sulfur proteins, “alpha keratins” (LSP)
ii. High sulfur proteins (HSP)
iii. Ultra-high sulfur proteins (UHSP)
iv. High Tyrosine-Glycine Proteins (HYGP)

And based on the molecular weight of those proteins:

i. type II keratins (≈52 kDa)
ii. type I keratins (≈47 kDa)
iii. keratin-associated proteins (Low molecular weight)

In the laboratory we have wool coming from Sardinia, native breed, whose difference lies in the color, white wool and black wool. The second one, has a different origin from the white one: it is in fact a wool derived from African sheep imported in the region of interest many years ago, in a time not well defined. Another interesting comparison concerns the proteins concentration of micronized chicken feathers compared to the fibers described above. This waste has been used in the hydroforming reaction leading to results almost comparable with white wool.

After the protein extraction, 1.5 ml aliquots from each sample were centrifuged at 14000 rpm for 15 mins at room temperature and aliquots of the supernatants were concentrated and submitted to Bradford’s protein assay and SDS-PAGE.[14]

We can see that black wool has a concentration of proteins lower respect white wool with a ratio of around 1:4 **(Table 8).** Even if all the wool samples were submitted to the same protein extraction protocol, results of the Bradford's assays showed clear differences in the obtained protein yields. In particular, the black wool sample had a protein concentration four times lower than the white wool sample. This may suggest a worse extraction potentially due to the necessary optimization of the method.

Table 8. Bradford's protein assay results

Entry	Name	Absorbance 595 nm	Protein Concentration (μg/μL)
1	White Sardinian wool	0.66217	6.1559
2	Black Sardinian wool	0.39751	1.4957
3	Chicken feathers	0.34464	0.9642
4	HF reaction	0.88967	4.0135
5	HF + Reused wool	0.49769	2.5026
6	HF reaction +HCl	0.66261	4.1603
7	Vehicle	0.0031047	0.031207

The results show the significant difference between the amount of proteins present in white wool and black wool. A further data concerns the similar content between the black wool and the chicken feathers making presuming a possible concomitance in the use in chemical reactions. The evaluation of the protein content following the hydroformylation reaction, Table, allows to see a comparable result after the reaction. Only in the case of the reaction of hydroformylation with reused wool, because used already once in the reaction itself, the result is lower than the initial data. This may be related to partial fiber degradation.
Analyzing fibers obtained from sheep of different races and from a different country the result can change but with slight differences (**Table 9**).

Table 9. Bradford's protein assay results for other wools.

Entry	Name	Protein Concentration (μg/μL)
1	White Sardinian wool	8.3259
2	White wool "Lombarda"	10.3557
3	Black Sardinian wool	3.0730
4	Black Australian wool	9.7430

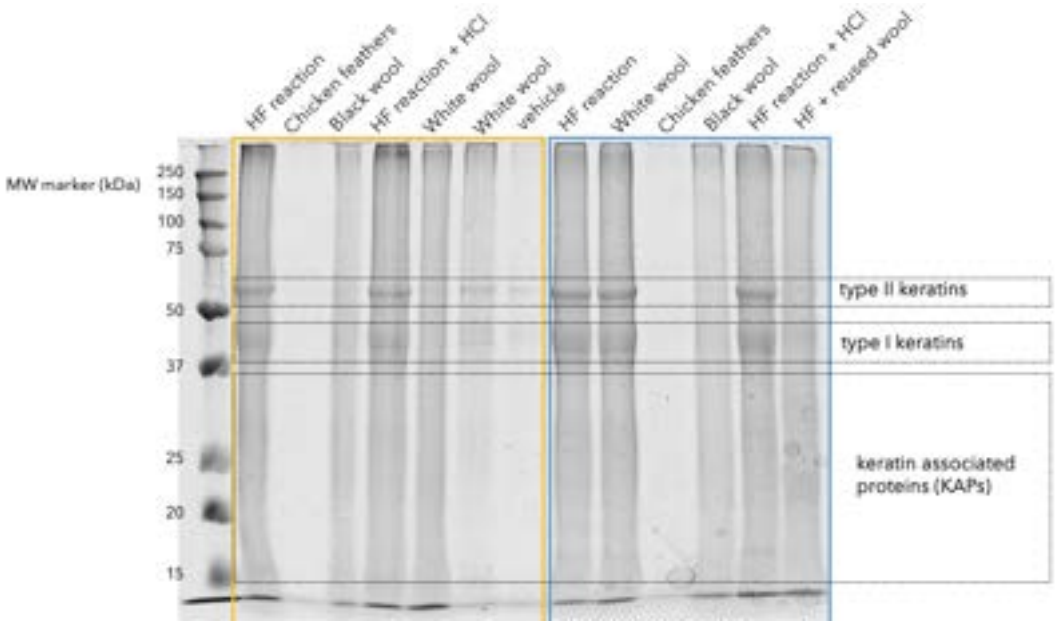
There we can also see a little difference between white wool in this round of analysis respect to the previous one, this is correlated with the ability of performing the extraction of proteins. The results are otherwise comparable.

Then, SDS-PAGE was carried out to analyze and compare the protein profiles of the different samples. In a first attempt, the SDS-PAGE gel was stained with Coomassie blue **(Figure 13).** A preliminary protein identification was carried out by comparison

(gel-matching) with previously published reports[70] and by confirming the protein molecular weight with what indicated in the protein database UniProt KB (https://www.uniprot.org/).

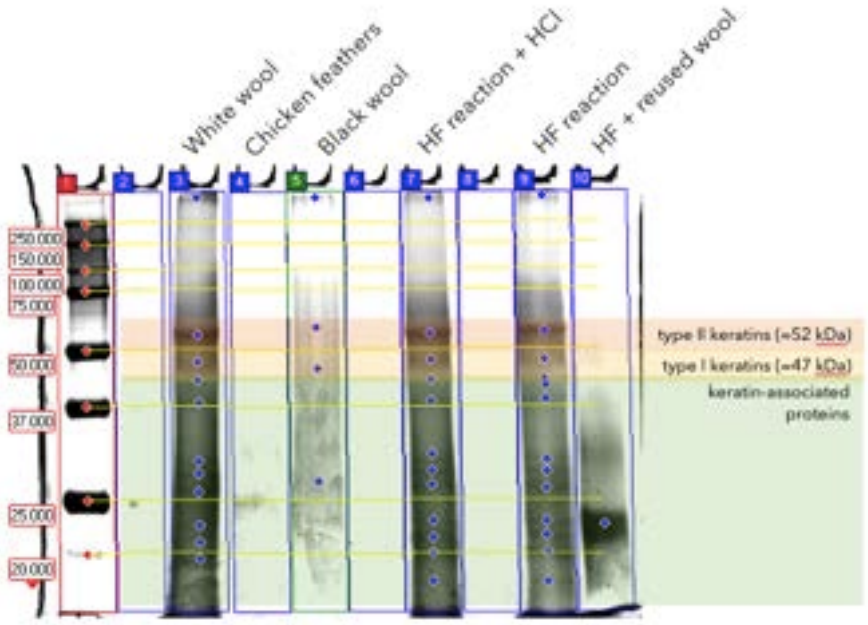
Thanks to analysis performed on similar samples, the SDS page was constructed the best way from the first attempt **Figure 12**).

Figure 12. 12% SDS-PAGE gel stained with Coomassie Blue.



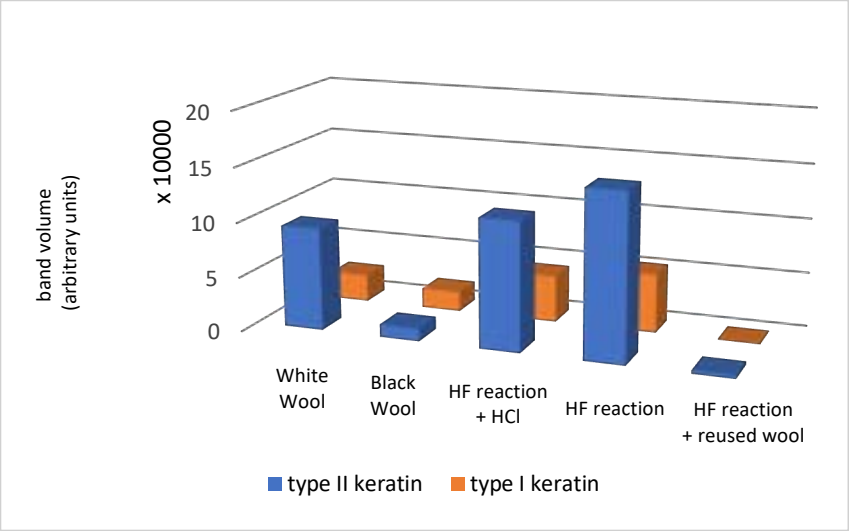
In this first analysis, we could confirm the presence of type I and type II keratins, whose bands were clearly visible in the gel. On the contrary, due to its lower sensitivity, the Coomassie staining could not reveal any band in the keratin associated proteins (KAPs) region. Also, high glycine-tyrosine protein bands (MW≤10kDa) were not properly resolved. Therefore, a novel 12% acrylamide SDS-PAGE experiment was run, and the gel submitted to the more sensitive silver staining (**Figure 13**). This allowed a proper visualization of KAPs along with type I and II keratins in the samples. However, for a better resolution of high glycine-tyrosine proteins, an additional 18% acrylamide SDS-PAGE gel was run and submitted to silver staining as well (**Figure 14**).

Figure 13. 12% SDS-PAGE gel stained with Silver.



The main finding stemming from Figure 17 and Figure 18 and the semi-quantitative analysis of the protein bands is that similar profiles were obtained for the Sardinian white wool before and after the reaction ("white wool" in comparison with "HF reaction"), suggesting no significant protein changes and, possibly, maintenance of the fiber composition. To fully characterize the protein profiles of all the analyzed fibers, and to highlight differences before and after the reaction but also after double use, the semiquantitative analysis of the protein profiles found in **Figure 13** is presented in **Figure 14**.

Figure 14. Abundance of type I and type II keratin in the protein samples resolved in Figure 18. The volume of each resolved band (arbitrary units) was obtained by performing an image analysis with the software ImageQuant as described in Materials and Methods.



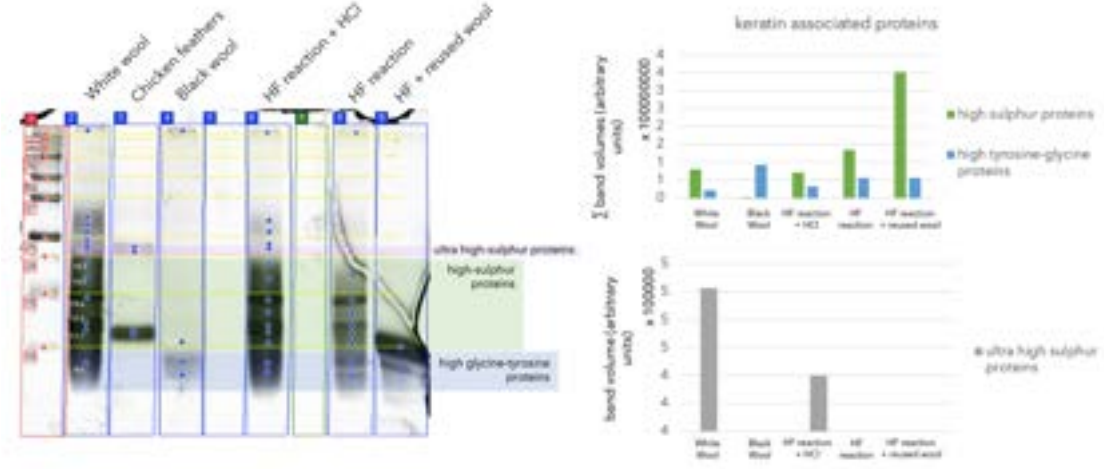
We can easily notice the presence of higher quantities of type II keratin compared to type I in white wool, while similar amounts were found in the black wool sample (**Figure 14**). Unfortunately, probably due to problems correlated with the protein extraction process for the low quantity of this sample, no type I and II keratins were found in the sample of wool used two times ("reused wool"), for which further analysis was necessary.

Thanks to the classic online portals for the evaluation of proteins like UniProt KB,[71] the classification of proteins based on the molecular weight also allow the evaluation of the aminoacidic composition. In fact, we can see those proteins with a high content of Cysteines and so sulfur atoms are in the upper region of SDS-page respect to proteins with wide content of glycine and tyrosine, presents in the lower region of the separating gel. Based on the sulfur content we can have a further differentiation: ultra-high sulfur proteins and high sulfur proteins.[14]

Figure 15 shows that white wool has a high concentration of proteins rich in cysteines, while black wool has a higher tyrosine-glycine protein content. The difference between the fiber before and after the reaction is identified. Looking at

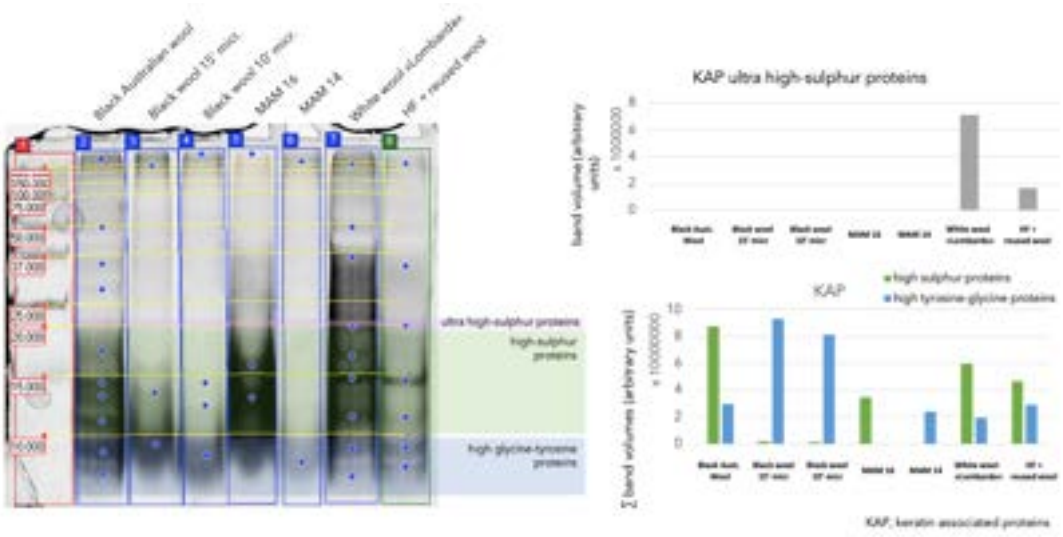
the content of sulfur atoms, in both the samples are in high concentration but, proteins are different. Probably a reduction of S-S bridge can bring to a different classification for the proteins and this is also visible in the separation bands in which dimers are visible in both cases, correlated to the possible presence of isoforms (**Figure 15**).

Figure 15. Classification of the separated proteins based on the aminoacidic composition.



The same analysis was carried out with the other wool available to the laboratory, also making a comparison between before and after the reaction using the black wool of Sardinian sheep used in the reaction of Sonogashira (These results will not be discussed here) (**Figure 16**).

Figure 16. Classification of new wool and fibers based on the separated proteins based on the aminoacidic composition.



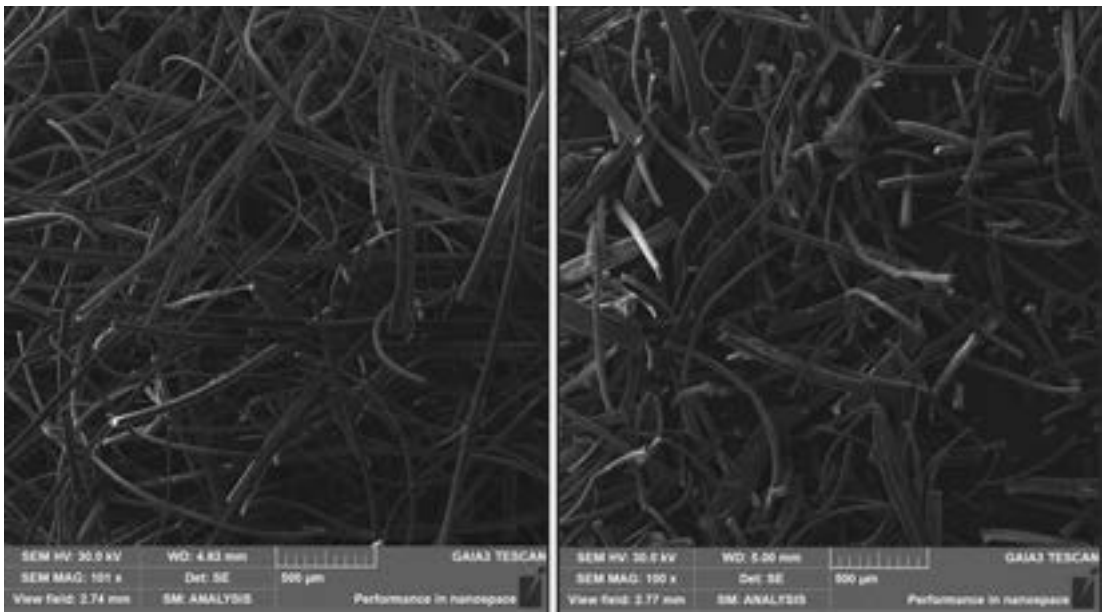
Unlike wool of Sardinian origin, Australian black wool has a protein composition more similar to the white one. This has allowed to hypothesize its possible use in the reaction of hydroformylation. This result can be extended to Lombard wool. The results of the reactions have been previously reported in the chapter.

2.6.2 Analytical analysis of wool

Based on the observations made so far on wool samples and starting from the difference in protein composition observed, a further characterization of the fibers was performed. Previously we noticed the similarity of white wool before and after the reaction. Some interesting investigations on further differences in the structure of the matrixes have performed by ICP, SEM-EDS and STEM analysis in collaboration with Dr. Massimo Calamante of the CNR in Florence. Those have been than compared to the results obtained from the biologicals data in order to have a full characterization of the wastes.

In **Figure 17** white wool and black wools are compared showing that the first one, has larger fibers section and size than black wool which is more needle-shaped.

Figure 17. Black wool (left) and white wool (right) in comparison.

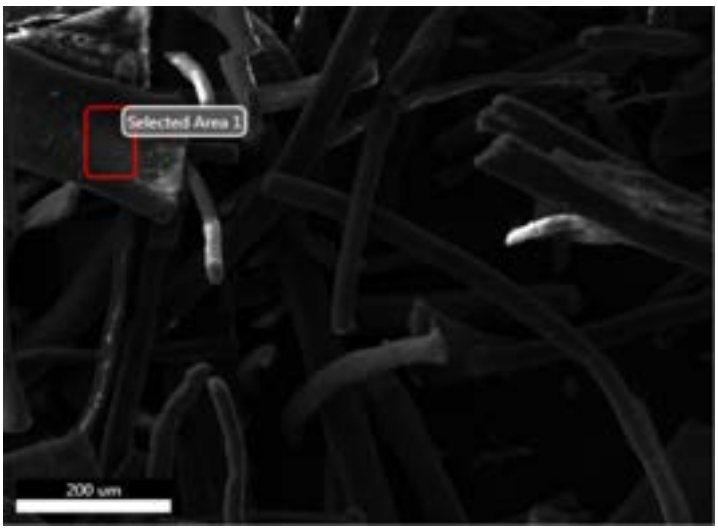


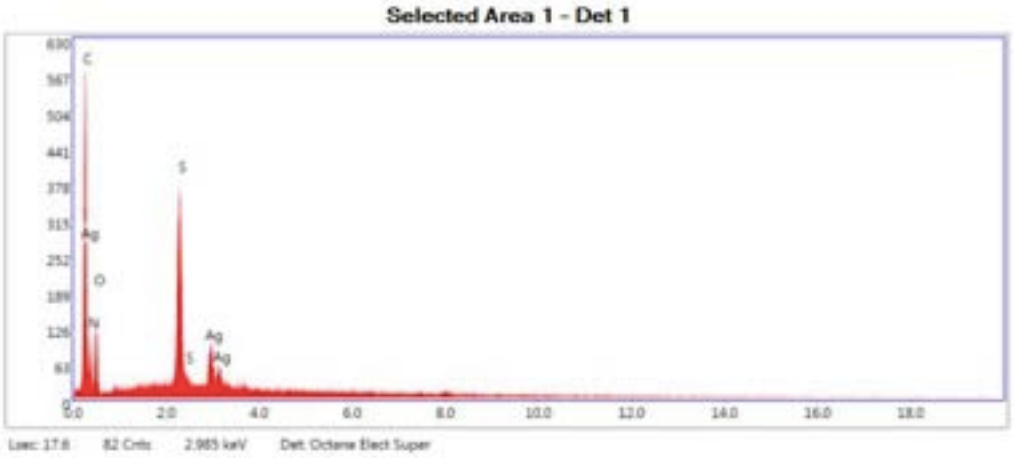
Making a speculation on the data in our hand, we can hypothesize that the white fiber has higher content in Sulfur Proteins than the black one bring responsible for the formation of thicker and roundish filaments.

A similar result that reinforces our hypothesis comes from the elemental analysis of the two samples. Looking at the two graphs in the figure, we can see the selected are

for the analysis and in the table the results for the atomic composition for both white and black wool (**Figure 18 and 19**)

Figure 18. Elementary analysis of white wool

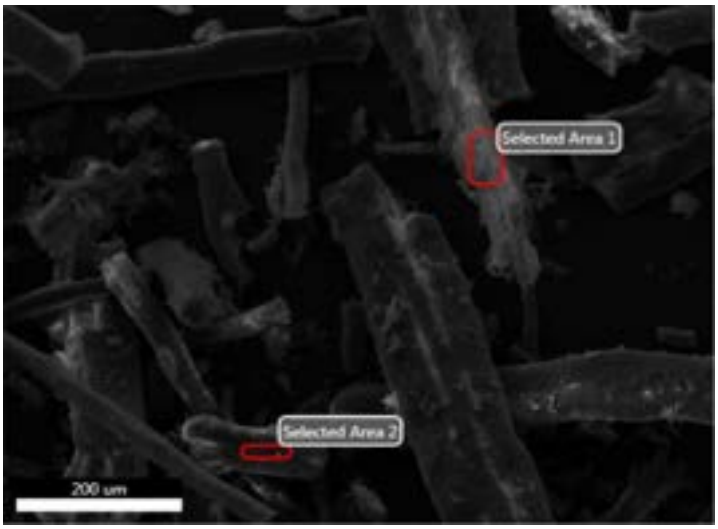


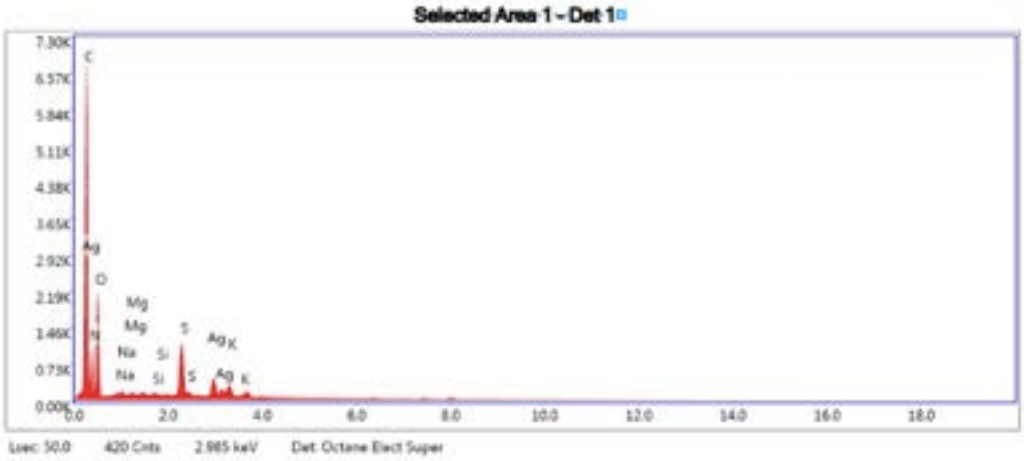


eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	54.26	62.25	389.26	8.21	0.2342	1.0223	0.4224	1.0000
N K	20.53	20.19	50.07	20.48	0.0177	1.0022	0.0859	1.0000
O K	17.55	15.12	79.43	16.61	0.0151	0.9846	0.0873	1.0000
S K	4.84	2.08	413.15	3.90	0.0414	0.8907	0.9502	1.0118
AgL	2.82	0.36	110.51	18.53	0.0263	0.7058	1.3199	0.9998

Figure 19. Elementary analysis of Black wool





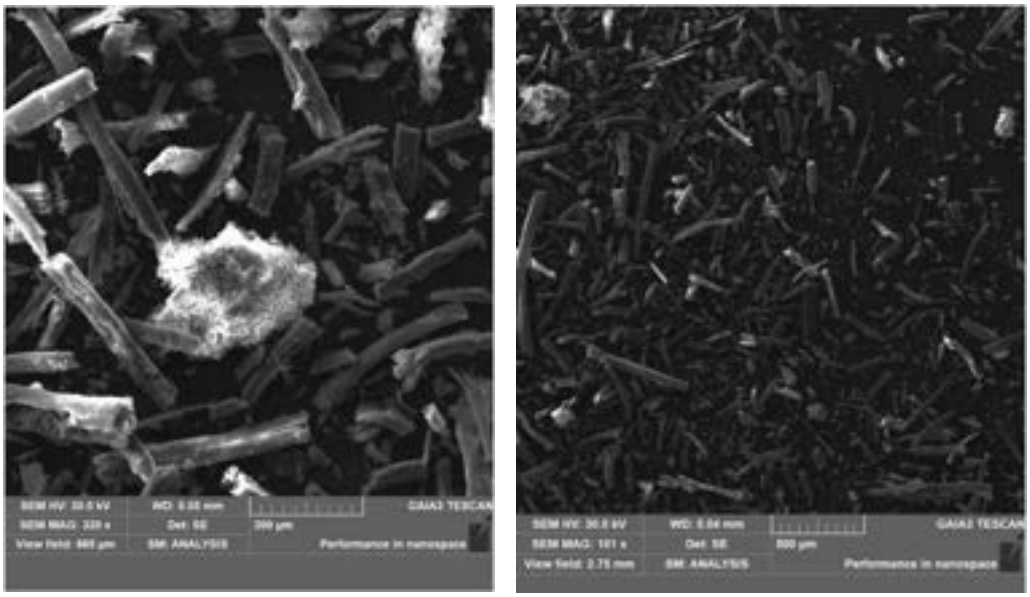
eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	49.35	56.23	1500.82	5.91	0.2641	1.0198	0.5250	1.0000
N K	19.66	19.21	183.71	13.65	0.0190	0.9995	0.0965	1.0000
O K	26.87	22.99	464.72	11.09	0.0259	0.9818	0.0981	1.0000
NaK	0.92	0.55	54.09	12.62	0.0020	0.8979	0.2404	1.0013
MgK	0.16	0.09	19.97	20.78	0.0006	0.9149	0.3775	1.0023
SiK	0.05	0.03	13.67	33.57	0.0003	0.9033	0.7064	1.0069
S K	1.49	0.63	419.92	2.75	0.0124	0.8873	0.9319	1.0129
AgL	1.14	0.15	155.89	8.98	0.0109	0.7030	1.3596	1.0003
K K	0.36	0.13	93.13	6.09	0.0033	0.8434	1.0362	1.0224

There are important differences in the amount of Sulphur present in each sample. Black wool has a lower Sulphur (S) content (**Figure 19**) than white wool (**Figure 18**). Based on the point at which it is chosen to conduct the analysis, some variation in the data is found but without degrees differences.

Wanting to investigate in detail the role of wool in the hydroforming reaction and the eventual possibilities of recovering the fiber for further reactions, these analyses were conducted on the fiber before and after it. Those have been done both on the classical Hydroformylation performed in water and in the case of the addiction of HCl. In the two images below obtained at 500 and 200 micrometers (**Figure 20**), we can see a significant fragmentation of around the 40 % of the wool following the reaction of hydroformylation in H_2O. Those data are important to explain the variation in the aldehyde conversion of compound **1**, when wool is recycled. Fortunately, the variation in product formation is low. The ICP analyses showed that the atomic composition is almost equal but, a small percentage of Rh used in the reaction remain on wool. The other amount of the catalyst is almost equally distributed between water and the organic phase but, eliminating the use of solvent during the workup, the situation in better and a possible recovery of the Catalyst and Ligand should be possible.

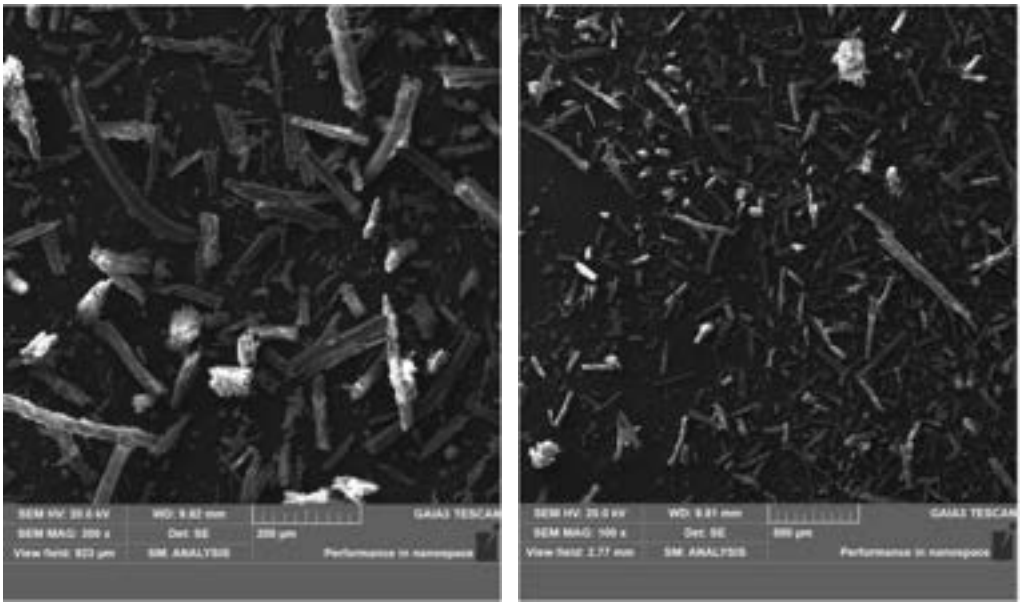
Figure 20. White wool before the hydroformylation reaction



A similar comparison was made between the micronized wool and it after the hydroformylation reaction with HCl within the mixture. The most important problems observed even with eyes is that after an emulsion is formed that does not allow the correct recovery of the fiber. In fact, observing the figure below (**Figure 21**) we can

see the almost total decomposition and rupture of the fibers, which then leads to their failure of recovery. Despite this, however, the reaction leads to the best result obtained so far, somehow activating the protein residues and thus activating the catalytic cycle of HF.

Figure 21. Wool after hydroformylation with catalytic HCl in the reaction mixture



Unfortunately, we have not until now been able to analyze the difference between the wool of Sardinian sheep and those of different origin whose analysis SDS-page had led to important explanations. In the future possible results can be obtained using those techniques.

Chapter 3

Conclusions

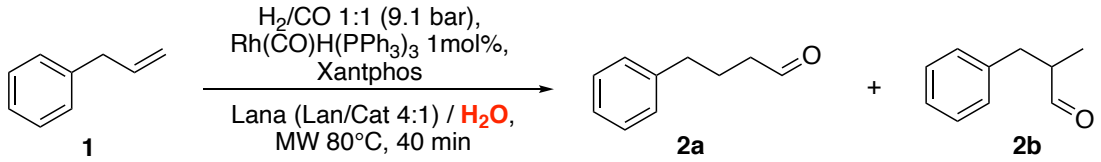
3 Conclusions

In this thesis a protocol for the synthesis of aldehydes from terminal alkenes with the use of micronized sheep wool have been investigated. The results of this study furnish the conditions for a sustainable HF process respecting several principles of Circular Chemistry, thus using non-toxic or dangerous natural waste materials. The work open a new perspective on the most industrially applied reaction in the world (Figure 22).

Figure 22. Circular chemistry principles for HF with wool

The synthetic process developed is divided into three different conditions: the first with the use of H_2O as a solvent, the second without any solvent and the third using HCl in the reaction environment to have an increase of the conversion to the desired product **2**. Evaluate the best possible conditions, we obtained a maximum conversion of 73% to aldehydes **2** with a linear/branched aldehyde ratio **2a/2b** of 16:1, defining the effective applicability and regioselectivity of synthesis (Scheme 4).

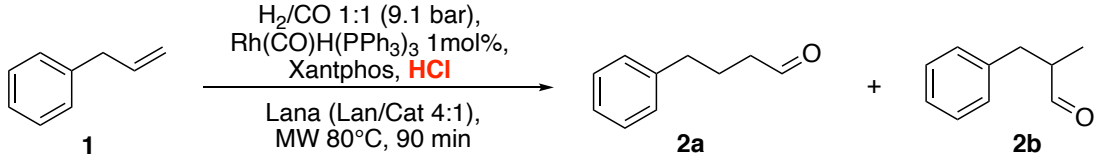
Scheme 4. Optimized Hydroformylation reaction in H_2O



1 2a 2b

For the solvent-free reaction we obtained a conversion of 84% to aldehydes with a **2a/2b** ratio of 19:1 (Scheme 5). The possibility of conducting the reaction in these conditions is advantageous given the absence of solvents.

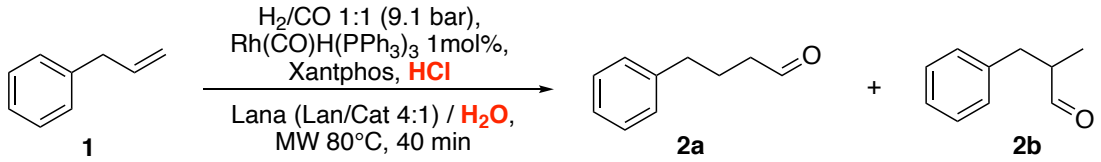
Scheme 5. Optimized Hydroformylation reaction without solvents



1 2a 2b

With the addiction of HCl (0.01 mmol) in the same conditions of the reported HF in H_2O, we obtained a best conversion of 89% to aldehydes **2** with a linear/branched aldehyde ratio **2a/2b** of 14:1 (Scheme 6).

Scheme 6. Optimized Hydroformylation reaction with HCl in the reaction environment



1 2a 2b

HCl in the reaction environment leads to a kind of wool activation, such as to allow a better conversion into the desired product selectively. The result was attributed to the protonation of the amino acid residues of the wool leading to an improvement in synthesis. Despite this it has been seen that following the reaction the wool is "destroyed" preventing its recovery and reuse.

Thanks to the optimization of the workup, which takes place through a separation by means of centrifugation, the use of organic solvent is completely eliminated, bearing greater environmental sustainability and a significantly lower cost of reaction.

These deductions and the most important differences regarding the different types of wool and waste are supported by SEM-EDS, ICP and STEM analysis, as well as protein composition analysis through SDS-page. White wool has proved far more effective than all other waste. Following elemental analysis, it was found that the supporting capacity in the MW-assisted HF reaction could be related to the amounts of Sulphur present in wool samples, and then ultimately to the abundance of the amino acid cysteine or in general the perfect protein composition of the fiber for this application.

It has been seen that the reaction leads to fair yields regarding most of the alkenes used but, in some cases, solubility leads to worse yields, such as in the case of aldehydes **17** and **18**. For this reason several conditions such as increased H_2O quantity, increased stirring time before irradiation and different conditions will be explored.

It has been shown that Sardinian wool, which is currently not used economically and must be disposed of in waste-to-energy plants, has potential in the field of chemical synthesis, and the method proposed by us could be able to reuse the same material for many production cycles, greatly increasing the life of its life.

Chapter 4

Materials and methods

4 Material and methods

4.1 Equipment used.

NMR spectrometer

^{1}H NMR and ^{13}C NMR spectra were recorded on 400 MHz 101 MHz and 600 MHz Bruker Advance NMR spectrometers. Deuterated chloroform and methanol were used as the solvents and chemical shift values (δ) are reported in parts per million (ppm) referring to the signals of TMS (tetramethylsilane) and the deuterated solvent (δ 7.26 for 1H and δ 77.6 for ^{13}C in $CDCl_3$, δ 3.34 for ^{1}H and δ 49.00 for ^{13}C in CD_3OD). Data are represented as follows: chemical shift, multiplicity (s= singlet, d= doublet, dd= doublet of doublets, d t= doublet of triplets, t= triplet, q= quartet, m= multiplet or multiple resonances, bs= broad singlet), coupling constant (J) in Hertz and the integration in ppm.

Gas Chromatography/Mass spectrometry

GC/MS analyses were conducted with a Varian-GC gas chromatograph equipped with a CP 8944 column containing the VF-5ms [30 m x 0.250 mm x 0.39 µm] as a stationary phase [L(m) x ID (mm) x OD (mm)]. Method A: 40 °C, 3 min, 0 °C/min - 200 °C, 1 min, 10 °C/min - 240 °C, 3 min, 20 °C/min. The above method starts the acquisition after 2 minutes of stabilization and from 3 minutes run.

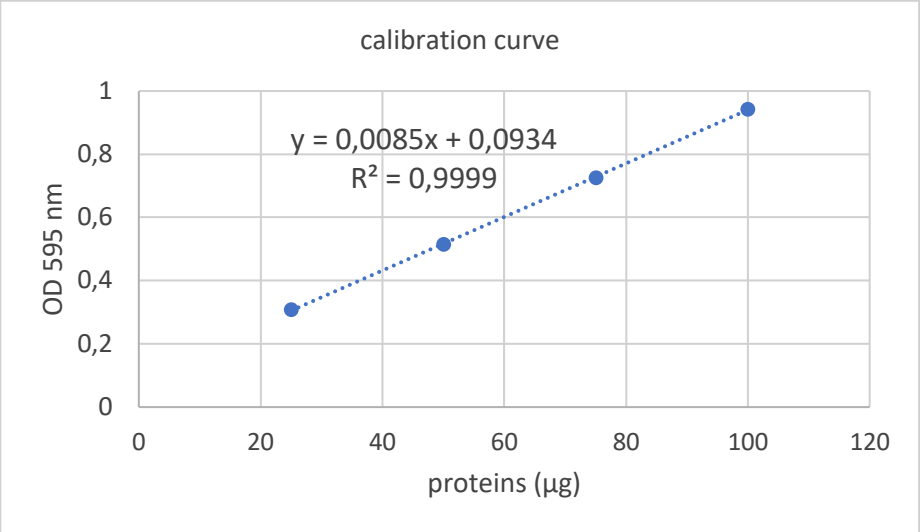
SDS-page and Bradford ‘s protein assay

Protein content in the extracted protein samples was assessed according to Bradford (1976).[72]

The protein extraction is performed using a mixture of chloroform/methanol solution (5 ml) to wash the fiber and a mixture containing 25mM of Tris–HCl at pH 8, 2.6 M thiourea, 5 M urea and 5% 2-mercaptoethanol (2-ME), 1.5 ml aliquots from each sample were centrifuged at 14000 rpm for 15 mins at room temperature. 0.5 mL aliquots of the supernatants were concentrated and submitted to Bradford’s protein assay and SDS-PAGE.[14]

Here is reported the calibration curve for the Bradford’s protein assay (**Figure 26**), hose results are reported in the upper chapter.

Table 10. Calibration curve and results for Bradford's assay



Entry	Name	Absorbance 595 nm	µg (10µL sample + 25µg BSA)	µg (10µL sample	Protein Concentration (µg/µL)
1	White Sardinian wool	0.66217	66.55900	41.55900	6.1559
2	Black Sardinian wool	0.39751	39.95700	14.95700	1.4957
3	Chicken feathers	0.34464	34.64200	9.64200	0.9642
4	HF reaction	0.88967	85.135000	60.13500	4.0135
5	HF + Reused wool	0.49769	50.02600	25.02600	2.5026
6	HF reaction +HCl	0.66261	66.60300	41.60300	4.1603
7	Vehicle	0.0031047	0.31207	0.31207	0.031207

Sodium-Dodecyl-Sulphate Gel Electrophoresis (SDS-PAGE)

Protein samples prepared as described in last chapter, were submitted to SDS-PAGE in 12% home-made acrylamide gels. Bis-acrylamide was chosen as the crosslinker for Coomassie stained gels, but it was replaced with piperazine di-acrylamide (PDA) for silver-stained gels.

20 µg of proteins of each sample were separated by using a two-step protocol for electrical conditions: 50V for 15 min, then 150V until migration front reached the lower portion of the separating gel (total time: about 90 minutes). At this point, gels were gently removed and submitted to one of the following staining procedures.

Coomassie Blue staining

Gels are soaked in a solution containing Coomassie blue R 250 dye [0.1% (w/w) Coomassie blue R-250 in 40% (V/V) methanol, 10% (V/V) acetic acid] for 45 minutes, and then the background staining is removed by incubation in a de-staining solution [40% (V/V) methanol, 10% (V/V) acetic acid].

Silver staining

The gel is washed for 5' and then fixed for 60' with a solution of:

Ethanol	40%
Acetic Acid	10%
Water	50%

Than overnight:

Ethanol	5%
Acetic Acid	5%
Water	90%

The sample is than treated:

10 'in water at 4˚C, 30' in Glutaraldehyde 2% + Sodium Acetate 0.5 M at 4 ˚C, 3 washes for 10' with H_2O at 4˚C, 2 washes of 30' each with 2-7 NAPHTHALENESULFONIC acid 0.05% at 4°C, 4 washes of 15' each with WATER at 4°C.

Prepare the following solution: 6 g of nitrate silver in 30 mL of water; in a 1000 mL cylinder place 160 mL of water, 1.5 mL of NaOH 10 N and 10 mL of concentrated ammonia, slowly add the silver nitrate solution and make up to 750 mL with water.

Soak the gel for 20 minutes in the aforementioned solution, 3 washes of 5' each with Water.

Soak the gel in the following solution:

H_2O	2000 mL
Citric Acid	0.2 g
Formaldehyde 37%	2 mL

Check the development of staining. Once stained, vacuum the solution. Time needed for development about 12'. The development reaction is stopped by immersing the gel for 15' in a solution of ACETIC ACID 5% in water.

Digitization and image analysis

Commassie- and silver- stained SDS-PAGE gels were scanned with the Image Scanner III using the LabScan software, using a 300dpi resolution and a suitable scanning filter. Gel images were saved as ".tif" files and then analyzed by ImageQuant TLincluding the following steps:

- manual detection of the lanes
- background subtraction with a rolling ball method
- automatic detection of bands and manual correction, if necessary, of "minimum slope" and "noise reduction"
- automatic calculation of the molecular weight by calibrating with a suitable standard (Precision Plus Protein Dual Color Standards Bio-Rad).

Numerical datasets were then exported as ".xls" files for comparative semiquantitative analyses, while images were exported as ".bmp" files.

Analytical methods

ICP instrumentation for elementary analysis was performed using an MP-AES 4210 Agilent Technologies instrument. The preparation of the sample is divided based on the state of it.

Solutions: The solutions were concentrated at small volume and then treated with aqua regia reaching a volume of 10mL. Specifically, once the solution is dry, 3 ml of concentrated HNO_3 and HCl (6ml) are added. The solution is left to boil for 1 hour and then make up to volume with concentrated hydrochloric acid (the whole process is done in a flask). The resulting sample is filtered on paper to remove any precipitate and then analyzed by infusion into the ICP.

Solid: Wool samples are treated in the same way. Obviously, the wool samples already dried do not need to be dried and are directly attached with aqua regia with the same procedure mentioned above.

SEM and STEM analysis (for nanoparticles) have been performed with Gaia 3Tescan focused ion column complete field emission electron microscope with Edax

microanalysis (**Figure 23**). Samples in this case are deposited on the sample holder and air dried (in the case of solutions) and analyzed directly.

Figure 23. Gaia 3Tescan focused ion column complete field emission electron microscope with Edax microanalysis

Microwave

An CEM Discover microwave is used with an 80 ml tube for reactions under pressure. This glass tube, resistant up to 250 psi (17 bar), is equipped with a connection, through a screw cap fitted with a tube, to an external pressure control equipped with a valve and an output tube for venting at the end of the reaction. When the valve of the pressure control system is closed, the reactor vial is under pressure that can be controlled using the valve in the upper region of the system (**Figure 24**). When the valve is open the vial is connected to the output tube to vacuum or to the gas mixture. We connected this outlet tube to a cylinder containing the CO/H_2 1:1 mixture (syngas) via a three-way tap equipped with two taps. [24,25] Once the syngas has filled the 80 ml tube to the desired pressure, the tap connected to the cylinder and the tap of the external pressure control system (located on Pressurized) are closed. At this point,

microwave radiation is activated until the desired temperature is reached. After the reaction, the system is cooled in nitrogen flow and the syngas is removed. We perform 3 steps of vacuum/syngas in the beginning of the reaction to eliminate the eventual air present in the tube.

Figure 24. CEM modified microwave with external pressure control system.

Purification methods

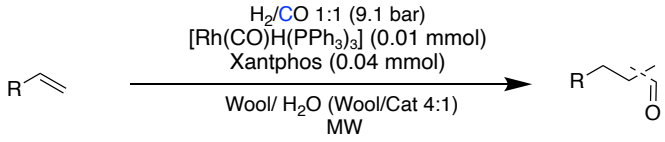
The purification was carried out through a normal phase Flash chromatography with instrumentation and procedure described by W.C. Still (st al.). 36 It is used as a stationary phase silica gel 60 with particle size 230-400 mesh, purchased from Sigma-Aldrich.Thin-pile chromatography (TLC) was carried out using aluminum plates coated with silica gel 60 F254 to characterize the substances using the UV lamp at 254 nm. Chemical detectors used solutions of $KMnO_4$ (yellow spots on purple background), p-anisaldehyde (yellow/red spots, blue on pink background)

4.2 Procedure and characterizations

The wool used is of Sardinian origin and was supplied by Professor Andrea Porcheddu of the University of Cagliari. The material has undergone a process of mechanical micronization via mechanochemical.30 The processing has led to the obtaining of a homogeneous powder and structurally different from the starting fiber.

Reagents and laboratory equipment were purchased from common chemical supply sites such as TCI, Sigma Aldrich, Carlo Erba.

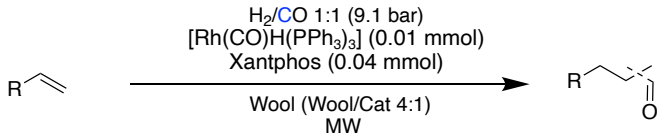
MW-assisted HF procedure in H_2O



3 ml H_2O for HPLC is added to an 80 ml MW tube, equipped with a magnetic hook, which is subjected to 3 vacuum cycles/N_2. Allylbenzene (0.132 ml, 1 mmol), wool (40 mg,) and HCl at 37% w/w (0.003 mL, 0.1 mmol) are added and the solution is stirred for 2 minutes, 3 more vacuum/N_2 cycles are performed and Xantphos (22 mg, 0.04 mmol) and $Rh(CO)H(PPh_3)$ are added (10 mg, 0.01 mmol). The reaction mixture is stirred for additional 2 minutes. The tube is placed in the appropriate chamber in the MW and subjected to three vacuum cycles/ Syngas. When the syngas pressure of 9.1 bar is reached, the pressure control is switched to pressurized and the reaction mixture is irradiated with microwaves at 80 ºC for 40 minutes, after which it is cooled with N_2 to 30 ºC. After cooling, the syngas is removed from the mixture by connecting the chamber to the vacuum and the tube is removed from the MW.

The workup is performed trough centrifugation at 3500 rpm for 30 minutes. This allows the separation of the organic phase, H_2O and wool. This allows to obtain the desired product through a simple withdrawal. H_2O and wool can be than transferred to perform another reaction.

Microwave-assisted hydroformylation procedure without solvent



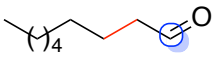
Wool (40 mg) is placed in an 80 ml microwave tube, equipped with a magnetic hook, and kept for 16 h in an atmosphere saturated with water vapor, so that the fiber absorbs the minimum amount of H_2O.

After 3 vacuum cycles/N_2, allylbenzene (0.132 ml, 1mmol) and HCl at 37% w/w (0.003 ml, 0.1 mmol) are added and maintained under stirring for 2 minutes, after which vacuum/N_2 cycles are repeated.

Ligand (22 mg, 0.04 eq) and $Rh(CO)H(PPh_3)$ are added successively (10 mg, 0.01 eq) and the system is kept under stirring for additional 2 minutes, after which the tube is placed in the appropriate chamber in the microwave. Three vacuum/Syngas cycles are carried out and the desired pressure is reached. After the switch of the pressure control on pressurized, the reaction mixture is irradiated at 80 °C for 90 minutes, after which it is cooled with N_2 to 30 ºC. After cooling, the syngas is removed from the mixture by connecting the chamber to the vacuum and the tube is removed from the microwave. AcOEt (5ml) is added to the mixture and the solution is kept under magnetic stirring for 2 minutes. The solution is then filtered onto büchner to recover the fiber. The solvent is then evaporated under vacuum.

Characterizations

Nonanal (14 a).

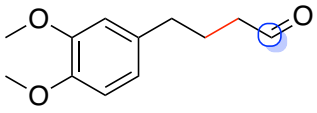


14

Yield: 68% **GC/MS** (m/z): 142; Rt**=** 10.818 min (Method A). **^{1}H NMR** (400 MHz, CDCl3) δ 9.73 (s, 1H), 2.38 (td, *J*= 7.3, 1.7 *Hz*, 2H), 1.61–1.57 (m, 2H), 1.26–1.23 (m, 10H), 0.84 (t, *J*= 6.3 *Hz*, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 203.1, 44.0, 31.9, 29.4, 29.3, 29.2, 22.8, 22.2, 14.2.

2-methyloctanal (14 b). Yield: 2% **GC/MS** (m/z): 142; Rt**=**9.518 min (Method A). **^{1}H NMR** (400 MHz, CDCl3) δ 9.82 (d, J=6,2 Hz, 1H), 2.37 (m, 1H), 1.5-1.46 (m, 2H), 1.25–1.22 (m, 8H), 1,12 (d, J=6,8 Hz, 3H) 0.86 (t, J=8.2 Hz, 3H). **^{13}C NMR** (101 MHz, CDCl3) δ 204.3, 45.0, 31.9, 29.4, 29.2, 26.2, 22.8, 15.2, 14.2.

4-(3,4-Dimethoxyphenyl)butanal (15 a).

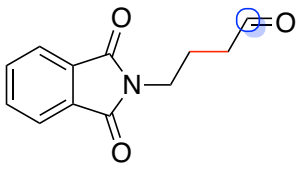


15a

Purification: 10% EtAOc in petroleum ether. **Yield:** 56%. **GC/MS** (m/z): 208; Rt= 18.345 min (Method A). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.76 (s, 1H), 6.80 (d, *J*= 7.9 *Hz*, 1H), 6.71 (d, *J*= 9.1 *Hz*, 1H), 6.70 (s, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 2.61 (t, *J*= 7.5 *Hz*, 2H), 2.61 (t, *J*= 7.5 *Hz*, 2H), 1.95 (p, *J*= 7.3 *Hz*, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 202.4, 148.9, 147.4, 133.9, 120.3, 111.7, 111.3, 56.0, 43.1, 34.6, 23.8.

3-(3,4 Dimethoxyphenyl)-2-methylpropanal (15 b). Purification: 10% EtAOc in petroleum ether. **Yield:** 2%. **GC/MS** (m/z): 208; Rt= 17.359 min (Method A). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.64 (s, 1H), 6.87 (d, *J*= 6.5 *Hz*, 1H), 6.75 (d, *J*= 9.9 *Hz*, 1H), 6.66 (s, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 2.13–2.04 (m, 1H), 1.77–1.70 (m, 1H), 1.67 (q, *J*= 8 *Hz*, 1 H), 0.91 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 201.0 121.1, 119.7, 111.6, 111.1, 109.7, 60.4, 55.9, 22.8, 11.7, 10.3.

4-(1,3-Dioxoisoindolin-2-yl)butanal (16 a).

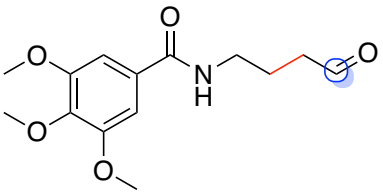


16

Yield: 62%. **GC/MS** (m/z): 217; Rt**=** 21.046 min (Method A). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.77 (s, 1H), 7.87–7.83 (m, 2H), 7.74–7.71 (m, 2H), 3.74 (t, *J*= 6.6 *Hz*, 2H), 2.54 (t, *J*= 6.8 *Hz*, 2H), 2.05–1.98 (m, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 201.2, 168.7, 134.4, 132.3, 123.6, 41.4, 37.4, 21.5.

3-(1,3-dioxoisoindolin-2-yl)-2-methylpropanal (16 b). Yield: 3%. **GC/MS** (m/z): 217; Rt= 21.046 min (Method A). **^{1}H NMR** (400 MHz, CDCl3) δ 9.72 (d, J=6.23 Hz, 1H), 7.85–7.83 (m, 4H), 4.03–3.77 (m, 2H), 2.92 (dt, J=7.2 Hz, 6.8 Hz), 1.28 (d, J=7.2 Hz, 2H). **^{13}C NMR** (101 MHz, CDCl3) δ 203.2, 164.7, 136.4, 123.3, 102.6, 43.4, 40.4, 20.5.

3,4,5-Trimethoxy-N-(4-oxobutyl)benzamide (17 a).

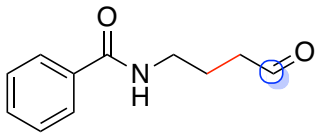


17

Purification: 14% MeOH in CH_2Cl_2. **Yield:** 60%. **^{1}H NMR** (400 MHz, CDCl3): δ 9.79 (s, 1H), 6.98 (s, 2H), 3.87 (s, 3H), 3.83 (s, 6H), 3.44 (t, *J*= 6.3 *Hz*, 2H), 2.61 (t, *J*= 6.6 *Hz*, 2H), 1.94 (q, *J*= 6.7 *Hz*, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 200.0, 167.1, 153.3, 141.4, 129.1, 104.6, 60.9, 60.3, 56.3, 39.9, 23.2, 22.2.

3,4,5-Trimethoxy-N-(2-methyl-3-oxopropyl)benzamide (17 b). Purification: 2% MeOH in CH_2Cl_2. Yield: 6%. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.68 (s, 1H), 7.05 (s, 2H), 3.91 (s, 6H), 3.88 (s, 3H), 1.99 (ddt, J= 169.1, 14.3, 7.6 Hz, 2H), 2.05–1.91 (m, 1H), 1.02 (d, *J*= 7.4 *Hz*, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 199.3, 167.1, 153.3, 141.3 129.2, 104.6, 60.9, 60.3, 56.4, 29.7, 14.1, 9.5.

N-(4-Oxobutyl)benzamide (18 a).

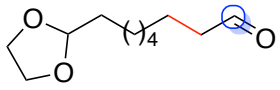


18

Purification: 10% EtAOc in petroleum ether. **Yield:** 15%. **GC-MS** (m/z): 191.2, Rt=15.050 min (Method A). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.42 (s, 1H), 7.81 (d, *J*= 8.3 *Hz*, 2H), 7.72 (d, *J*= 8.4 *Hz*, 2H), 7.47 (dd, *J*= 7.4, 7.4 *Hz*, 1H), 7.44 (dd, *J*= 7.4, 7.4 *Hz*, 1H), 7.42 (dd, *J*= 7.5, 7.4 *Hz*, 2H), 7.34 (dd, *J*= 7.8, 7.7 *Hz*, 2H), 6.70 (bs, 1H), 6.64 (dd, *J*= 7.5, 7.4 *Hz*, 1H), 6.44 (bs, 1H), 3.54 (dd, *J*= 6.5, 6.5 *Hz*, 2H), 3.53 (dd, *J*= 6.4, 6.4 *Hz*, 2H), 2.65 (dd, *J*= 6.0, 6.0 *Hz*, 2H), 2.59 (dd, *J*= 7.5, 7.5 *Hz*, 1H), 2.58 (dd, *J*= 7.0, 7.0 *Hz*, 1H), 1.92 (ddd, *J*= 7.0, 7.0, 6.9 *Hz*, 2H), 1.44 (bs, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 202.1, 170.9, 167.6, 135.7, 134.3, 131.2, 130.3, 128.4, 128.3, 127.1, 126.7, 82.3, 49.1, 41.4, 39.3, 32.1, 23.6, 21.7.

N-(2-Methyl-3-oxopropyl)benzamide (18 b). Purification: 10% EtAOc in petroleum ether. **Yield:** 3%. **GC-MS** (m/z): 191.3, Rt= 16.716 min (Method A). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.69 (s, 1H), 7.70 (d, *J*= 7.0 *Hz*, 2H), 7.45 (d, *J*= 7.3 *Hz*, 1H), 7.41–7.36 (m, 2H), 3.78–3.43 (m, 2H), 2.77 (td, *J*= 7.6, 4.3 *Hz*, 1H), 1.19 (d, *J*= 7.5 *Hz*, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 204.4, 167.6, 134.2, 131.6, 128.6, 126.9, 46.8, 39.8, 11.5.

11-(1,3-Dioxolan-2-yl)undecanal (19 a).

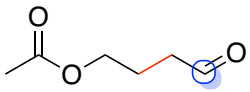


19

Purification: 96% EtAOc in petroleum ether. **Yield:** 90%. **GC/MS** (m/z): 242; Rt=20.425 min (Method A). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.76 (s, 1H), 4.84 (t, *J*= 4.8 *Hz*, 1H), 3.96 (t, *J*= 6.8 *Hz*, 2H), 3.84 (t, *J*= 6.8 *Hz*, 2H), 2.41 (t, *J*= 7.2 *Hz*, 2H), 1.63 (dp, *J*= 17.5, 7.0, 6.3 *Hz*, 2H), 1.41 (p, *J*=7.1 *Hz*, 2H), 1.35–1.23 (m, 14H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 203.0, 104.7, 64.8, 43.9, 33.9, 29.5, 29.4 (2 C), 29.3 (2 C), 29.12, 24.1, 22.1.

7-(1,3-dioxolan-2-yl)-2-methylheptanal (19 b). Not Isolated

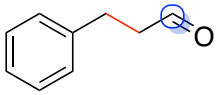
4-Oxobutyl acetate (20).



20

Yield: 98%. **GC/MS** (m/z): 130; Rt= 8.655 min (Method A). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.80 (s, 1H), 4.11 (t, *J*=6.2 *Hz*, 2H), 2.55 (t, *J*= 6.9 *Hz*, 2H), 2.05 (s, 3H), 1.98 (q, *J*= 6.4 *Hz*, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 201.2, 171.0, 63.4, 40.5, 21.3, 20.9.

3-Phenylpropanal (21 a).



21

Purification: 10% EtAOc in petroleum ether. **Yield:** 46%. GC-MS (m/z): 134.4, Rt= 11.527 min (Method A). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 7.28 7.23 (m, 2H), 7.21–7.17 (t, *J*= 8.1 *Hz*, 3H), 2.88 (t, *J*= 7.54 *Hz*, 2H), 2.75 (t, *J*= 7.3 *Hz*, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 201.6, 140.6, 128.7, 128.5, 126.3, 45.7, 28.2.

2-Phenylpropanal (21 b). Purification: 10% EtAOc in petroleum ether. **Yield:** 23%. **GC-MS** (m/z): 134.5, Rt= 10.405 min (Method A). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.72 (d, *J*= 1.4 *Hz*, 1H), 7.44–7.37 (m, 2H), 7.30 (tt, *J*= 7.4, 2.1 *Hz*, 1H), 7.24–7.18 (m, 2H), 3.65 (qd, *J*= 7.2, 0.8 *Hz*, 1H), 1.41 (d, *J*=7.1 *Hz*, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 201.2, 137.9, 129.2, 128.4, 127.7, 53.1, 14.7.

Chapter 5

Abbreviations

5 Abbreviations

^{1}H-NMR	Nuclear magnetic resonance of Proton atoms
^{13}C-NMR	Nuclear magnetic resonance of Carbon atoms
AcOEt	Ethyl acetate
AcOH	Acetic acid
AcONa	Acetato di sodio
AMU	Atomic Mass Unit
Biphephos	6,6'-[(3,3'-Di-tert-butyl-5,5'-dimethoxy-[1,1'-biphenyl]-2,2' diyl)bis(oxy)]bis(6H-dibenzo[d,f][1,3,2]dioxaphosphepine)
bs	broad singlet
cat	Catalyst
d	Doublet
DCM	Dichloromethane
DPEPhos	Bis[(2-diphenylphosphino)phenyl] ether
Dppf	**(**Ferrocene-1,1'-diyl)bis(diphenylphosphane)
E.F.	E-factor
Et_2O	Diethyl ether
EtOH	Ethanol
GC-MS	Gas chromatography
H_2O	Water
HCl	Hydrochloric acid
HCO_2H	Formic acid
HF	Hydroformylation

HAM	Hydroamminomethylation
Hz	Hertz
J	Coupling constant (Hz)
LCA	Life Cycle Assessment
lig	Ligand
m	Multiplet
MeOH	Methanol
min	Minutes
MW	Microwave
$NaHSO_3$	Sodium Bisulphite
NEDA	National Environmental Policy Act
PE	Petroleum Ether
***p*-TSA**	*p*-toluenesulphonic
Rt	Retention time
s	Singlet
t	Triplet
THF	Tetrahydrofuran
TLC	Thin-Layer Chromatography
TPGS-750-M	DL-alpha-Tocopherol methoxypolyethylene glycol succinate
Xantphos	(9,9-Dimethyl-9H-xanthene-4,5-diyl)bis(diphenylphosphane)
δ	Chemical shift

Chapter 6

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6 Bibliography

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