EnXylaScope

Unleashing Xylan's Potential with Enzymes for a Scope of Consumer Products

D8.9

Policy and regulatory framework report

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1 Executive Summary

EnXylaScope will discover novel enzymes for debranching xylan, a highly abundant polymer in plants. Productions systems for these enzymes will be optimised and the enzymes will be applied to produce a debranched (water-insoluble) form of xylan that has properties which make it suitable as ingredients in a scope of consumer products. The debranched xylan will be further modified, using available enzymes, to confer functionalities that expand the range of potential consumer products. In total 3 types of enzymatically modified xylan will be made and will be application tested for 6 consumer products. These product span 3 sectors (cosmetics, personal care, nutraceuticals). Advanced techniques will be used for the discovery, production, and formulation of these enzymes and the project is designed so that maximal research outputs are achieved in the period and that the post-project timeframe for launching these products on the market is significantly reduced.

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2 Objective of the deliverable

The main objective of the task (T8.5) performed is to facilitate the acceptance and utilization by the market of the developed products and processes.

Other objectives are to provide starting information for other WPs, ensure compatibility and interoperability with what already exists in the market through standards, as well as to use the standardisation system as a tool for dissemination of the project results and interaction with the market stakeholders.

This deliverable includes a summary of the health and environmental impacts of cosmetic and personal care products which set the base line for the project. Alternatives are being developed within the project (xylans) and the regulatory framework for these products as well as the EU Biodiversity policy are explained and linked to the project.

An update of this deliverable will be submitted in M48 (D8.10).

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3 Introduction

3.1 Current situation

The Bioeconomy – encompassing the sustainable production of renewable resources from land, fisheries and aquaculture environments and their conversion into food, feed, fibre, bio-based products, and bioenergy as well as the related public goods[1] – is an important element of Europe's reply to the challenges ahead.

Many countries have a tradition of biotechnology and biofuel policies, and bioeconomy policy in many of these countries is restricted to strategies for these[2]. The number of countries with an integrated bioeconomy strategy including all facets of the bioeconomy is restricted. In the EU, the number of countries with bioeconomy integrated strategies are increasing in the last years, for example Finland, Flanders, Germany, and Sweden[3], and more recently also France and Spain.

With the development of a bioeconomy strategy in 2012, a distinct policy field has been established. In this strategy, the EU-Commission defined guiding principles for the bioeconomy in Europe, with major emphasis on research and innovation. In the course of 2017 and 2018, the strategy was reviewed leading to the publication of an updated document.

The year 2015 was a turning point: the 2030 Agenda for Sustainable Development and its Sustainable development Goals (SDGs) were adopted, and the Paris Agreement on climate change came into effect. These sent out a global political message on the way forward to transform our economic system to end poverty, protect the planet, and ensure prosperity for all.

[1] Bioeconomy | Horizon 2020 (europa.eu)
[2] German Bioeconomy Council. Bioeconomy Policies around the World. Available online: http://www.biooekonomierat.de/biooekonomie/international/ (accessed on 24 May 2017).
[3] Langeveld, J.W.A. Results of the JRC-SCAR Bioeconomy Survey; Biomass Research: Wageningen, The Netherlands, 2015. Available online: https://www.scar-swg-sbgb.eu/lw_resource/datapool/_items/item_24/survey_bioeconomy_report1501_full_text.pdf (accessed on 24 May 2017).

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This requires new concepts to realize these international agreements and bring them to action. The circular biobased economic paradigm can be this – it builds on the synergies of the circular economy and bioeconomy concepts. These two concepts have so far been developed in parallel, but now need to be connected to reinforce each other.

In 2017, the European bioeconomy was worth nearly €2 trillion and provided more than 17.5 million jobs to EU citizens[4].

To maintain its competitiveness, Europe will need to ensure sufficient supplies of raw materials, energy, and industrial products under conditions of decreasing fossil carbon resources - oil and liquid gas production is expected to decrease by about 60 % by 2050[5].

Different sources of biobased raw materials have been explored such as:

- Bio-waste (estimated at up to 138 million tons per year in the Union, of which up to 40 % is land-filled) has high potential added value as a feedstock for other productive processes.
- Biological resources and ecosystems could be used in a more sustainable, efficient, and integrated manner.
- Food waste represents another serious concern. An estimated 30 % of all food produced in developed countries is discarded. Major changes are needed to reduce this amount by 50 % in the Union by 2030.

There are a number of hitherto underexploited fractions of biomass which are prime candidates for enzyme-based functional improvements and enhanced sustainable valorisation, yet the current commercial landscape is lacking in suitable candidate enzymes.

[4] European Bioeconomy Alliance.
[5] Bioeconomy | Horizon 2020 (europa.eu)

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3.2 Innovation proposed by EnXylaScope project

ENXYLASCOPE is focused on a clear example of the latter that is Xylan. It is a highly-abundant lignocellulose polymer that, with appropriate modifications, has outstanding physical and chemical properties which make it suitable for incorporation in an array of consumer products, replacing less-sustainable product components and so allowing for greener market options for the consumer.

Enzymes are the most sustainable and selective option for the modification of xylan, through the removal of the polymer's side chains (debranching). This leads to a xylan polymer with unique functional properties (such as reduced water solubility and enhanced viscosity) and is suitable for direct incorporation in consumer products (e.g. everyday skin care) or for further modifications to confer the functional properties for more demanding applications (e.g. speciality skin care, personal care etc).

Suitable enzymes (e.g. laccases and lipases) for the latter modifications (graftings) are well reported and are commercially available. However, the same cannot be said for xylan debranching enzymes as most of them can act only on oligomers and not polymers and the enzymes that can act on polymers have very narrow operating ranges thus making them unsuitable for industrial processing environments and cost-effective technologies for the production of water-insoluble xylan (WIS-xylan).

To date the biobased sector has focused on enzymatic approaches for isolating and upgrading the two other main lignocellulose polymers (cellulose and lignin), in their polymeric form, whilst xylan is still too frequently considered to be a side-stream of comparatively less value, only suitable for hydrolysis.

Partners of ENXYLASCOPE consortium have strong interest in biobased alternatives for synthetic polymers in cosmetics and personal care products (SEPPIC) and in expanding their existing feed and food markets by taking advantage of growing biotechnology (KERRY and FERMEX).

The cosmetics and personal care sectors rarely use enzymes as processing aids or ingredients in the final product with the exception of proteases recently being used as active agents in cosmetics. Occasionally, keratinases are also used.

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EnXylaScope is broadening the range of enzymes by introducing four novel enzymes (xylan debranching) enzymes, not previously used in these sectors, as processing aids to produce polymer ingredients. The new, modified, xylans resulting from the use of these enzymes can then be used as rheology modifiers, thickeners, emulsifiers, and stabilisers in a variety of consumer products and in EnXylaScope they will be demonstrated in products for the cosmetics (CP1 (XylMoist, moisture Cream/lotion) and CP5 (XylSmooth, emollient)) and personal-care (CP4 (XylClean, hand sanitiser) and CP6 (BodiXyl, body wash) sectors.

The EnXylaScope discovered debranching enzymes will also be tested for the functional foods/nutraceutical sector in which the dominant enzymes currently used are proteases, amylases, lipases, and cellulases. In this market xylanases are used in the bakery sector but not much in functional foods, except for xylo-oligos production. EnXylaScope is expanding the use of xylan-debranching enzymes beyond bakery and xylo-oligos, in to producing xylan capable of acting as binder with prebiotic potential, anti-microbial and anti-inflammatory properties which will be of great value in nutraceutical sector or alterantive ingredients market (CP2, HealthXyl and CP3, ModuXyl).

The focus is on nutraceuticals and, particularly, on cosmetics and personal care products due to the urgent need to provide substitutes for the liquid plastics that are currently washed into our water systems through consumers' use. In order to combat this problem, EnXylaScope is demonstrating 2 cosmetics, 2 personal care products and 2 ingredients for nutraceuticals or using innovative enzymes and production technologies to showcase the potential of xylan debranching enzymes and xylans and biotechnology in consumer products sector.

Table 1: *Consumer Products developed at Enxylascope.*

ID	Product Name	EnXylaScope Brand Names	Product Category	Xylan Used	Take-up Partner
CP1	Moisture Cream/lotion	XylMoist	Cosmetics	WIS-xylan	SEPPIC
CP2	Nutraceutical binder and prebiotic	HealthXyl	Nutraceuticals/ ingredient	WIS-xylan	FERMEX
CP3	Anti-inflammatory and anti-microbial Supplement	ModuXyl	Nutraceuticals/ ingredient	Xyl-Phe	FERMEX
CP4	Hand Sanitiser	XylClean	Personal Care	Xyl-Phe	SEPPIC
CP5	Emollient	XylSmooth	Cosmetics	Xyl-Phe-FA	SEPPIC
CP6	Body wash	BodiXyl	Personal Care	Xyl-Phe-FA	SEPPIC

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4 Cosmetic and Personal Care products

4.1 Environmental Impact of cosmetics and personal care products

The Top-3 of poorly-biodegradable liquid polymers in cosmetics is led by carbomer, a polyacrylic acid. As much as 24% of the products tested contain this liquid polymer. Cyclopentasiloxane, is the second most frequently used ingredient (19%). Siloxanes are monomeric building blocks of silicones and can also exist in cyclic form. Acrylates copolymer is the third most common liquid polymer, found in 16 percent of the cosmetic products studied.

4.1.1 Polyacrylates (Acrylate polymer)

Polymers used in cosmetics and personal care are multifunctional ingredients and act as thickening, emulsifying, stabilising and texture properties enhancers. The most common polymers are polyacrylates which are versatile and high-performance.

Despite their solubility in water, acrylate compounds are considered non-biodegradable or poorly biodegradable (even if non-acrylic parts of the copolymers can be degraded). Furthermore, similar to microplastics, these liquid polymers can attract environmental pollutants, facilitating the transport of pollutants into organisms — with unknown negative consequences.

On June 10, 2020, Committee for Risk Assessment (RAC) adopted its opinion on European Chemical Agency (ECHA)'s proposal to restrict the use of microplastics that are intentionally added to products on the EU/EEA market in concentrations of more than 0.01 % weight by weight.

ECHA's proposal set out specific test methods and pass criteria for identifying biodegradable polymers, which are excluded from the restriction. Besides that, there is a growing concern on overall impact of liquid plastics, such as acrylate-based polymers, that are poorly degradable in the natural environment.

According to "liquid polymers are everywhere, from sun protection products to nail polishes and all kinds of hair styling products, and yet, they are unattended by the public or regulatory bodies". The report also notes that "every second product in these categories contains at least one of these environmentally-concerning ingredients".

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The researchers found 159 different poorly biodegradable synthetic polymers in more than 50,000 products, spread across 34 product categories, including face masks, skin care products, shampoos and shower gels. Liquid polymers are used in significant amounts in many modern cosmetics and personal care products (e.g. 29% in shampoos and 45% in shower gels).

It would be desirable to reduce these levels greatly. Once in the environment, these liquid plastics are poorly biodegradable, remaining for years in the ecosystem with to-date unknown consequences due to the high diversity of their polymer structures.

Cosmetic and personal care producers are trying to replace these synthetic polymers with natural alternatives but, according to current studies, such natural polymers without any modifications struggle to meet the required physical, textural, and sensory properties.

Polyacrylate (PA) is currently used in large quantities as: a functional polymer (with cross-linked high molecular weights (MWs) used as super adsorbents and low MWs used in detergent applications); dispersants; and as water-treatment chemicals. Water-soluble PAs also enter waste-streams and, while non-toxic and not a cause of eutrophication, are not highly biodegradable and remain in waste-/natural-water systems. Acrylates C/10-30 alkyl cross polymer and carbomers are mainly used as thickening agents with carbomers being high-swelling agents with no water solubility, used for thick gels. The long-term effects of PA/carbomers have not been well studied. Acrylates are stable when stored and handled under recommended conditions. However, there is a risk of explosion with rapid pressure increases. Several case histories are known in which vessels of acrylates exploded due to violent (“runaway”) polymerisation when proper procedures were not followed, indicative of hazards in the production process.

The global warming potential (GWP) of fossil-based Na-PA is 3.8 kg CO_2-eq, with the biobased production of acrylates from side streams (e.g. via sugar fermentation followed by polymerisation) reported to be lower but these are highly dependent on the concentrations of hydrolysis sugars. The manufacture of methacrylic++ and acrylic acids and monomers (raw materials to produce PA and copolymers) yields waste gases and waters that contain various acrylic compounds as impurities, which are toxic to the environment. Reported impurities for carbomer resins include water, benzene, propionic acid, acetic acid, acrylic acid, heavy metals, iron, arsenic, and lead, which are toxic to the environment.

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4.1.2 Silicones

Silicones are an extensive family of compounds used in make up, skin care, and hair care formulas. They are prized for their smoothing, correcting, and mattifying effects, and for their pleasant, silky texture. Synthetic polymers like silicones can also be moulded into solid objects like cookware. However, for cosmetic and personal care use, silicone-based ingredients are commonly liquid polymers. In cosmetics there are more than 300 possible ingredients that are made from or contain silicones. For instance, these polymers may be a purely synthetic form, or a combination of a synthetic polymer connected to a natural substance or substances. For example, dimethicone silicone is a synthetic ingredient used in various skin and health care products such as skin moisturizers, lip care products, anti-aging creams, shampoos, and hair conditioners.

Some silicones are readily biodegradable, but others can persist and accumulate in the environment and be toxic, with whether it biodegrades depending on where it ends up. For instance, on land a type of silicone may be broken down more easily but the same may not be true in our waterways or it may not be biodegradable at all. Hence, for cosmetic product usages, silicones have recently raised concerns due to their impact on the aquatic environment, especially since silicones can be found in rinse-off products.

These molecules are chemically inert, very stable, and that raises questions on their biodegradability.

European Chemicals Agency (ECHA)'s Committees for Risk Assessment (RAC) and Socio-Economic Analysis (SEAC) banned octamethylcyclotetrasiloxane (D4) and restricted use of decamethylcyclopentasiloxane (D5) in wash-off personal care products under REACH to concentrations below 0.1%.

The ECHA plans to extend the restriction to leave- on products (those intended to remain on the skin) like creams and make-up. Cyclosiloxanes are a great environment concern and hence linear siloxanes are used in cosmetics yet even these are dangerous to the environment as they can feed into aquatic environments and impact fish and plant life. The quantities of siloxanes entering landfills are estimated to increase by approximately 29% in conjunction with a 5% increase in silicone-containing products over the next 10 years and by 78% over the next 25 years.

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4.2 Health impact of cosmetics and personal care products

4.2.1 Polyacrylates (Acrylate polymer)

Health concerns of acrylate polymers, acrylic and methacrylic++ acids and monomers are extremely dangerous substances causing chronic intoxication, having been found to produce not only systemic toxic, but also embryotoxic effects at low concentrations. Additionally, methyl-acrylate may cause a skin allergy which, if it develops, can cause itching and rashes at very low exposure levels, with prolonged exposure to methyl-acrylate potentially causing liver and kidney damage. In an industry MSDS for acrylates/C10-30 alkyl acrylate cross polymers (as Pemulen TR-1), a 2-year inhalation study was described wherein rats were exposed to a respirable, water-absorbent sodium polyacrylate dust and lung effects (inflammation, hyperplasia, and tumours) were observed.

4.2.2 Silicones

Cyclotetrasiloxane and cylcopentasiloxane (also known as D4 and D5) are toxic, persistent, and have the potential to bioaccumulate in aquatic organisms. The EU classifies D4 as a endocrine disruptor, based on evidence that it interferes with human hormone function, and a possible reproductive toxicant that may impair human fertility. In laboratory experiments, exposure to high doses of D5 has been shown to cause uterine tumours and harm to the reproductive and immune systems. D5 can also influence neurotransmitters in the nervous system.

Though linear siloxanes products such as dimethicone are considered non-toxic, literature indicates that short chain siloxanes trapped in long chains during the synthesis can exhibit toxic effects (e.g. cancerogenicity, genotoxicity, skin irritations, and teratogenicity) and hence the use of low molecular weight silicones should be reduced and the purity of high molecular weight silicones should be monitored.

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4.3 Xylan products as nontoxic alternatives

4.3.1 WIS-xylans as replacement for siloxanes

WIS-xylans have similar properties to siloxanes in terms of inertness and ability to form entanglements of low and high molecular weights, which are of great advantage to modulate the viscosity. WIS-xylan are also suitable to replace silicones due to their inert nature, opaqueness, smooth texture, film forming abilities, and controlled viscosity based on the concentration and average degree of polymerisation. Given that EnXylaScope's WIS-xylan is biomass-derived and enzymatically-produced, it is a significantly greener ingredient compared to produce than silicones and siloxanes. Additionally, WIS-xylan is a biodegradable polysaccharide (taking 12 hrs to get hydrolysed to xylose with enzymes) and its hydrolysis in the environment or in plant or in animal in which it is absorbed will only result in production of xylose monomers, serving as an energy source. Hence, WIS-xylan is an excellent candidate to satisfy a producer- and consumer-need for natural alternatives to dimethicone silicone in cosmetics.

EnXylaScope industry partner SEPPIC recently launched silicone-alternative products such as EMOGREEN which is a C15-19 alkane (plant-based)-coco-caprylate/caprate and continues to search for more products as silicone alternatives since no single chemical can provide exactly the same, feel, texture and function (key qualities in the cosmetics and personal care sectors). Enzymatically-produced WIS-xylan is highly suitable in this regard, especially in aqueous-based formulations as it can be processed in cold conditions and so also reduce formulation energy requirements.

The target is to provide at consumer product with a carbon footprint reduced by at least 50% compared to the silicone-based product. This is possible since WIS-xylan forms a hydrogel in aqueous solutions and has good water-holding capacity, allowing for more functional properties compared to silicone-based products. It is compatible with oil in water emulsions and hence the formulation can be simple (e.g. 4% WIS-xylan, 94% water, 2% coconut milk, tea tree oil and fragrances for CP1).

EnXylaScope's WIS-xylan will be devoid of any toxic chemicals since enzymes are used in its production. This naturally derived polymer, constructed with simple sugar building blocks, is hydrated in an aqueous environment, thereby creating a gel structure (hydrogel or hydrocolloid). This system, where water is immobilized by insoluble polymers, naturally provides a moisturising effect. The gels are highly compatible with biological tissues and are biodegradable according to their natural occurrence classifying them as biopolymer.

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Hence, using WIS-xylan as replacement to silicone will be highly beneficial to consumer health.

4.3.2 Xylan-Phe and Xylan-Phe-FA – as replacement for acrylates

Xyl-Phe is a suitable alternative for acrylate cross polymers, while Xyl-Phe-FA is suitable to replace highly hydrophobic acrylate polymers (e.g. carbomers). Both xylans are expected to have high swelling capacity and hydrophobicity, giving unique functionality like polyacrylates, allowing them to be used in medium-viscosity gels like hand sanitiser (CP4, Xyl-Phe) and very high-viscosity creams like emollients (CP5, Xyl-Phe-FA). Xyl-Phe-FA can be used in a body wash (CP6) not only as a thickening agent and viscosity enhancer but also an active molecule carrier due to the lipophilic groups present in the core of the Xyl-Phe-FA polymers. Environment and health benefits Xyl-Phe and Xyl-Phe-FA are biomass derived and enzymatically produced. Due to their structure, they are biodegradable in nature, with the breaking of ester linkages catalysed by laccases and lipases, to be tested in EnXylaScope.

Additionally, these xylans will be slowly fermentable in nature meaning that their entrance in water systems will not cause bacterial or algal blooms. Furthermore, since their production process is much simpler than biobased acrylic acid production, lower GWPs are also expected. This is particularly the case since these xylans are obtained from a biomass fraction (hemicellulose) to date considered a process side stream (e.g. from wheat straw, DDGs, hardwood forestry residues). Our target is to obtain efficient replacement for polyacrylates and carbomers with a GWP that is 50% lower than current synthetic polyacrylate production.

Regarding health benefits, Xyl-Phe and Xyl-Phe-FA are expected to be non-irritants to the skin as they have no free alkyl groups. The phenolic groups present in Xyl-Phe will be mostly in the cross-linked form and hence will not be reactive in the absence of oxidising agents. Very few free phenolic groups will act an ant-oxidant agent for skin and is very suitable property for an emollient. Furthermore, given that the fatty acid linked to the phenolic groups will be lauric acid, additional health benefits may result since lauric acid is frequently used for treating viral infections (including influenza; swine flu; avian flu; the common cold; fever blisters; cold sores; genital herpes caused by herpes simplex virus (HSV); genital warts caused by human papillomavirus (HPV); and HIV/AIDS). Phenolic groups and fatty acid groups provide preservation benefits without the addition of harmful preservants.

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EnXylaScope will test the antioxidant, anti-microbial, and anti-inflammation potentials of Xyl-Phe and Xyl-Phe-FA. Socio-economic benefits Acrylic-acid-based polymers are a workhorse of the industry.

Table 2: *Substitutives from current compounds in Cosmetics and Personal Care products.*

ID	Product Name	Product Category	Current compound	Xylan Used	To be used as
CP1	Moisture Cream/lotion	Cosmetics	Siloxanes (dimethicone)	WIS-xylan	Hydrogel
CP4	Hand Sanitiser	Personal Care	Polyacrylate	Xyl-Phe	High swelling capacity and hydrophobicity
CP5	Emollient	Cosmetics	Polyacrylate	Xyl-Phe-FA	emollients
CP6	Body wash	Personal Care	Polyacrylate	Xyl-Phe-FA	Thickening agent and viscosity enhancer

5 Nutraceutical and well-being enhancing products

With an increase in workload, busy lifestyles, and an increase in the number of working women, the time and means to eat healthy food have reduced. This has led to an increased demand for products that offer balanced nutrition. Thus, the consumer demand for functional foods, nutraceuticals, and supplements has been increasing.

Dietary fibre is the part of plant-based food that mostly passes through the digestive system without breaking down or being digested. There are two types of fibre: soluble and insoluble fibre. Insoluble fibre does not dissolve in water and includes plant cellulose and hemicellulose. Data suggested that arabinoxylan from cereals is not digested and is the important component of the fibre.

In this project extracted xylan, modified xylan (WIS-xylan) and xylan with phenolic groups (Xyl-Phe) will be tested for digestibility and anti-inflammation potential.

The enzymes discovered at Enxylascope will be tested for functional foods and beverages, nutraceuticals, dairy etc and xylans produced will be tested as binders for their prebiotics, anti-microbial, and anti-inflammatory properties. The ability to produce nutraceuticals and functional foods from locally available resources will increase the production tonnage

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possible from a feedstock and a given locale and thus reduce the product price, potentially extending the nutraceuticals market to wider socio-economic classes, fostering an increase in EU health.

EnXylaScope aims at demonstrating the potential of the 4 novel enzymes in the nutraceutical and ingredients for food and feed sector and at least one enzymatically modified xylan as a novel nutraceutical with superior properties to existing fibre based functional foods.

Table 3: *Ingredients from current compounds in Nutraceuticals and Food and Feed products.*

ID	Product Name	Product Category	Current compound	Xylan Used	To be used as
CP2	Nutraceutical binder and prebiotic	Food and Feed products		WIS-xylan	Binders
CP3	Anti-inflammatory and anti-microbial Supplement	Food and Feed products		Xyl-Phe	Anti-inflammatory and anti-microbial

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6 Regulatory Framework

6.1 Cosmetics and Personal Care products

Cosmetics and personal care products are applied to the human body for the purposes of cleaning, beautifying, promoting attractiveness or changing its appearance.

Ranging from antiperspirants, fragrances, make-up and shampoos, to soaps, sunscreens and toothpastes, cosmetics and personal care products play an essential role in in all stages of our life.

Cosmetics are regulated to ensure safety, governed mainly by the European Union's (EU) Cosmetics Regulation.

The Single Market- the term used to describe the free movement of goods, capital, people and services within the Member States of the European Union - is a cornerstone of the European Union. In order for it to work for a specific product sector, there must be similar legislation in place in all the Member States. For example, if a product is to move freely within the European Union, the same labelling, packaging and safety regulations must apply. In the early 1970's the Member States of the European Economic Community (now called the European Union - EU) decided to harmonise their national cosmetic legislations in order to enable the free circulation of cosmetic products within the Community, on the basis of commonly agreed safety standards. The Cosmetics Directive was adopted in 1976. This Directive was reevaluated in 2009 to enable further harmonization and a EU-wide Cosmetics Products Regulation (Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products) entered into force in July 2013.

The testing ban on animals was already in place since 2009 (but testing for certain complex endpoints could still be done outside the EU) – since 2013 it also became prohibited to market products that contained ingredients which were tested on animals (including for the toxicological endpoints that were exempted in 2009 – as from 2013 the ban is complete both for testing and marketing)

The regulation replaces Directive 76/768/EC, which was adopted in 1976 and had been substantially revised on numerous occasions. It provides a robust, internationally recognised regime, which reinforces product safety while taking into consideration the latest technological developments, including the possible use of nanomaterials. The previous rules on the ban of animal testing were not modified.

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The philosophy of the Cosmetics Regulation is that all products meeting the requirements of the Regulation should have equal and immediate access to the market and should be able to circulate freely throughout the European Union.

In the EU, it is strongly believed that for fast moving consumer products, such as cosmetics, an in-market control system (also known as post-market control) is more effective than pre-market approval procedures.

The key principle of the Cosmetics Regulation is that the person or company who places the cosmetic product on the market is responsible for that product (so called 'Responsible Person'). It is the responsibility of that person or company (usually the manufacturer or the importer) to ensure that the product is safe and meets all the requirements of the Cosmetics Regulation. All stages of the development of the cosmetics product are regulated by the Cosmetics Regulation, from the choice of ingredients to the placing on the market of the product.

Manufacturing according to Good Manufacturing Practices (GMP)
The EU Cosmetics Regulation stipulates that all cosmetics products must be manufactured in accordance with the harmonised standards laid out in GMP, in turn described in the Official Journal of the European Union.

GMP ensures that products are prepared in a clean environment and that the products are not contaminated in production. Microbial contamination can be quite common as many microorganisms live freely in the atmosphere around us, and could lead to degradation and, in severe cases, could cause harm to the consumer.

Before being placed on the European market, all cosmetics products must be listed on a centralised database, the Cosmetic Products Notification Portal (CPNP), managed by the European Commission. When a product has been notified in the CPNP, there is no need for any further notification at national level.

The new Regulation also introduced a single, electronic product notification system (CPNP) that replaced the more than 20 different national systems existing at that time

Chemicals placed on the EU market – be it as such or as part of a finished product, need to be registered with a safety data package and a human and environmental safety assessment. Cosmetic ingredients are covered by this registration obligation. REACH is the most important piece of environmental legislation for the substances used in cosmetics.

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Other applicable regulations are EU main and other applicable legislation, including Guidelines and Amendments to the Cosmetics Regulation and Regulation (EU) 2019/1020 on market surveillance and compliance of products.

The Committee of Ministers is the Council of Europe's statutory decision-making body. It is made up of the Ministers for Foreign Affairs of member States. The Committee meets at ministerial level once a year and at Deputies' level (Permanent Representatives to the Council of Europe) weekly. The conduct of meetings is governed by the Statute and Rules of Procedure. The Ministers' Deputies are assisted by a Bureau, rapporteur groups, thematic coordinators, and ad hoc working parties.

Considering the Council Directive 76/768/EEC and Regulation (EC) No. 1223/2009 the Committee of Experts on Cosmetic Products (P-SC-COS) make the following resolutions that contains recommendation to the governments of States Parties to the Convention. These recommendations shall not prevent governments from maintaining or adopting national measures that implement stricter rules and regulations.

- Resolution CM/ResAP(2012)1 on safety criteria for cosmetic products intended for infants
- Council of Europe Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up
- Council of Europe Resolution ResAP(2006)1 on a vigilance system for undesirable effects of cosmetic products ("cosmetovigilance") in Europe in order to protect public health
- Resolution ResAP(2005)4 on sun protection products to optimise consumer protection

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6.2 Nutraceutical products

Although synthetic bioactive compounds are approved in many countries for food applications, they are becoming less and less welcome by consumers. Therefore, there has been an increasing interest in replacing these synthetic compounds by natural bioactive compounds[6].

These natural compounds can be used as food additives to maintain the food quality, food safety and appeal, and as food supplements or nutraceuticals to correct nutritional deficiencies, maintain a suitable intake of nutrients, or to support physiological functions, respectively.

Recent studies reveal that numerous food wastes, particularly fruit and vegetables byproducts, are a good source of bioactive compounds that can be extracted and reintroduced into the food chain as natural food additives or in food matrices for obtaining nutraceuticals and functional foods.

Those bioactive compounds must follow the legal requirements and evaluations to assess the risks for human health and their toxicity must be considered before being launched into the market. To overcome the potential health risk while increasing the biological activity, stability and biodistribution of the supplements' technological alternatives have been studied such as encapsulation of bioactive compounds into micro or nanoparticles or nanoemulsions. This will allow enhancing the stability and release along the gastrointestinal tract in a controlled manner into the specific tissues.

The general food law (GFL), the European framework regulation on foods, has the purpose to assure the maximum protection level of human health and consumer interest while certifying the correct functioning of the internal European market for both food and feed. Furthermore, the GFL is also the founding regulation for EFSA to provide scientific advice and technical support for all European legislation and policies regarding the food and feed safety matters.

[6] Ana A Vilas-Boas, Manuela Pintado, Ana L S Oliveira. DOI: 10.3390/foods10071564

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In Europe, the European Community (EC) **Regulation No. 178/2002**, Article 2 and **Codex Alimentarius** guidelines regulate the use of food waste and by-products as food ingredients or as natural food additives.

Therefore, when food by-products are proposed to be used as natural additives and do not match the current regulations, a proper authorization as novel food, EC Regulation No. 258/97 (1997), is required. The Novel Food Regulation (NFR) had a specific regulation (EU) 2015/2283 that deals with foods and food ingredients that were not used before May 1997 for human consumption. Novel foods must undergo a safety assessment and the request must follow the EC Recommendation No. 97/618.

When a new nutritional substance is not included in the ingredients list from 15 May 1997, it is recommended to be included in the Annexes of the Directives on foods for nutritional uses, of the Directive on food supplements and of the Regulation on fortified foods and should be submitted to the European Commission, Health and Food Safety Directorate-General, Unit E1, Food information and composition, food waste.

In 2009, the European Food Safety Authority (EFSA) published the Guideline entitled "Safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements". This guidance focused on the botanical preparations intended for use in food supplements, but also applicable to other uses in the food area. The safety assessment considers the identification and nature of source material that includes part of the plant (seeds, leaves, and other bioproducts), manufacturing process (extraction method, solvents used) to obtain botanical preparations and chemical characterization of constituents. The list does not specify the use of agri-food waste as raw material for the preparation of such nutraceuticals.

The botanical preparations should not contain toxic substances making required studies on toxicity and toxicokinetic according to the test methods described by Organization for Economic Cooperation and Development (OECD) or in European Commission Directives 87/432/EEC and 67/548/EC—Annex 5 (Authority & Committee, 2009). This two were repelled by the EC Nº 1272/2008.

Bioactive compounds can enter the market as food additives to improve technological and sensory functions, as a supplement or being presented as a functional food. The different applications will imply different regulations to follow.

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The authorization procedure for food additives is laid down in **Regulation (EC) No 1331/2008** and the safety is regulated by the EFSA. Based on this data, EFSA evaluates the level below which the intake of the substance can be considered safe—the so-called acceptable daily intake (ADI). Once accepted, the commission makes a proposal for possible authorization of the additive and present it for voting at the Standing Committee on the Food Chain and Animal Health (SCoFCAH) which is preceded by a presentation to the Council and the European Parliament. However, they can still reject the proposal if does not comply with the EU legislation conditions. The European regulatory framework considers that functional foods must match the general food laws.

Other legislative aspects must be considered when introducing a new compound in the food industry since the development of "eco-extracts" for food application as an additive for instance is governed by various regulations such as No 1333/2008. Another parameter also regulated (**Directive 2009/32/EC**) is the solvent used to obtain that compound which establish maximal solvent residue limits in the final foodstuff.

6.3 Enzymes

6.3.1 Definition of enzymes

Enzymes are regulated in different legislation depending on their use (Enzyme REACH Consortium, 2009, Safety evaluation of technical enzyme products with regards to the REACH legislation).

In the EU, industrial enzymes are defined as chemical substances and are consequently subjected to chemicals legislation; information requirements for notification/reporting are the same as those for chemicals.

A clear distinction between the terms enzyme as active compound, enzyme concentrate and enzyme preparations is essential for the discussion of parameters applicable for a description.

Most parameters used in scientific practice for the description of enzymes focus on the enzyme as the active compound. Enzymes as active compounds could be characterised by their function as well as by their molecular structure. For clearly distinguishing enzymes by function, the information on the catalytic type has to be supplemented by additional

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functional parameters. To unambiguously identify/distinguish enzymes, the primary structure plus information on posttranslational modification has to be specified.

Functional properties of enzymes cannot reliably be deduced from enzyme structure and vice versa. Thus, functional parameters are also relevant for the description of enzymes.

In industrial and regulatory contexts, the enzyme concentrate is relevant, i.e. the active component (i.e. the enzyme) plus any impurities resulting from fermentation and subsequent purification steps. A characterisation of the enzyme concentrate has to be extended to by-products or impurities resulting from fermentation and purification that may comprise 30 to 98% of a final enzyme concentrate. The enzyme concentrate is usually characterised by describing the production process and the production organism. Furthermore, the absence or level of total viable count, known pathogenic microorganisms, known toxins, as well as heavy metals are routinely estimated or verified if required by (some) legislation.

Enzyme preparations, the ready-to-sell products, are described by specifying the intentionally added substances, i.e. protein, carbohydrates, fat, ash, water and diluents as well as stabilisers, standardizers, preservatives, and formulating agents.

Regulatory practice for describing technical, feed, food and other enzymes largely makes use of the parameters described above, thereby focusing on parameters for enzyme identification via its catalytic activity, information required on the production organism, requirements for the production process, and additives and other ingredients used.

Enzyme products must meet strict specifications with regard to toxicity and other safety aspects.

Quality of production of therapeutic enzymes is controlled through pharmaceutical GMP (good manufacturing practice) with strict demands on hygienic standards during all stages of production, process validation and documentation. Production of food and feed enzymes has to comply with Food GMP regulations with a clear focus on sanitary processing. However, MetGen Oy operates with the production of technical enzymes which are not subject to such regulations.

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6.3.2 REACH and enzymes

REACH is a regulation of the European Union, adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the hazard assessment of substances in order to reduce the number of tests on animals.

Industrial enzymes have a low toxicity in humans and most enzymes are classified as GRAS (Generally Recognized As Safe) by food safety experts and FDA. Enzyme concentrates present no concern for endpoints like acute toxicity, genotoxicity, sub-acute and repeated dose toxicity, reproductive toxicity and carcinogenicity.

Enzymes (as with any protein) have the potential for sensitization of the respiratory tract which may cause respiratory allergy in individuals exposed continuously to sufficiently high airborne concentrations of enzyme dust or aerosols. The typical symptoms for this type of respiratory allergy include hay fever or asthma. The risk of sensitization correlates to the concentration of inhalable enzyme particles, thus the formulation of the enzyme product and how the enzyme product is handled is important.

Liquid enzyme products can be handled with low release of inhalable enzyme particles, but improper handling processes or inappropriate equipment may create significant levels of airborne enzyme particle in aerosols.

Granulated enzyme products must be handled very carefully to ensure the integrity of granulates as broken granulates could results in airborne exposure levels that may lead to the development of sensitization and allergy.

There are no indications that enzymes are skin sensitizers but enzyme preparations containing proteolytic enzymes can irritate eye, skin and mucous membranes due to catalytic activity. Other enzyme classes, including amylases, lipases and cellulases are essentially free from any irritating effects to both eye and skin.

Within REACH, there is a requirement to define acceptable exposure limits in order to ensure safe application of enzymes. The derived minimal effect level (DMEL) for the occupational exposure limit is 60 ng/m^3 whereas for consumers a DMEL of 15 ng/m^3 for

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pure enzyme protein can be adopted as the starting point for new and existing enzymes for which there are no other data to indicate that a different value may be more appropriate.

REACH requires all EU-based manufacturers and importers of chemicals to register chemicals that are manufactured or imported in yearly tonnages exceeding a certain level: 1000, 100, 10 and 1 ton(s)/year.

The incentive behind REACH is to improve the protection of human health and the environment from the potential risk of chemicals. Identification of the intrinsic properties of chemical substances is done by the four processes of REACH, namely the registration, evaluation, authorization, and restriction of chemicals.

The European Chemicals Authority (ECHA) supervises the registration process. The manufacturer or importer should register its substance immediately before placing the product on the EU market.

The process of registering novel enzymes in REACH will be considered if the anticipated tonnage will exceed 1 ton/year after the project.

6.3.3 EU´s Biodiversity Strategy and enzymes

The EU's biodiversity strategy for 2030 is a comprehensive, ambitious, and long-term plan to protect nature and reverse the degradation of ecosystems. The strategy aims to put Europe's biodiversity on a path to recovery by 2030 and contains specific actions and commitments.

It is the proposal for the EU's contribution to the upcoming international negotiations on the global post-2020 biodiversity framework. A core part of the European Green Deal, it will also support a green recovery following the Covid-19 pandemic.

To report on progress to the EU 2020 biodiversity strategy, the European Commission extracted relevant information from the EU Member States' 5th national reports to the CBD (Convention on Biological Diversity). Each Member State had the opportunity to review the synthesis of its report and to provide additional input.

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In addition to the country synthesis which is shown below, Denmark provided information on the cross-linkages between their national strategy and the European and global biodiversity targets.

Resources for global biodiversity conservation

Through its national contribution to IUCN, Denmark has provided financial support to the international TEEB programme (The Economics of Ecosystems and Biodiversity), which has helped create the scientific and practical foundation to be able to appreciate the value of natural resources with the aim of compiling green accounts and other measures. Financial support has been granted for the development of green national accounts in collaboration with the World Bank. Consideration for biological diversity and combating poverty is an integrated element in Danish foreign aid policies.

Action 20: Access to genetic resources and sharing of benefits

Denmark has been one of the leading proponents for ratification of the Nagoya Protocol within the EU and internationally. We will continue to strive nationally, within the EU and globally to ensure the goal can be reached. Parliament passed a new law in December 2012 on regulation of the use of genetic resources from abroad by domestic consumers (businesses and scientists). The law is designed to protect developing countries from exploitation of their genetic resources from rainforests, coral reefs etc. for the development of medicines, enzymes, cosmetics, food products etc. without their prior consent or agreement on benefit-sharing.

The government has supported African countries in negotiations on the Nagoya Protocol and continues to support focus on the development of good governance in this area in developing countries, with a subsidy of DKK 15 million for the period of 2013-2015. The subsidy is managed by the Ministry of Foreign Affairs in the form of co-financing of an ABS Capacity Development Initiative.

The latest Danish report to the Biodiversity Convention Secretariat on resource mobilization for nature preservation purposes states and annual contribution of DKK 2.3 billion, equivalent to USD 390 million p.a. (average for 2006-2010). This amount corresponds to approx. DKK 410 per person per year, or 0.13% of GNAs such, Denmark's contribution per capita is one of the very highest compared to other developed nations. In addition, ratification and implementation of the Nagoya Protocol plays a vital role, as implementation will facilitate benefit-sharing with the developing countries supplying the genetic resources for new medicines, enzymes, cosmetics etc.

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6.3.4 Standardization

6.3.4.1 Environmental Standards

Enzyme concentrates have low toxicity to aquatic systems and seem unlikely to be dangerous to the aquatic environment due to their ready biodegradability and the low effects on aquatic organisms observed. Enzymes denature and biodegrade in the environment and the potential for bioaccumulation is virtually nonexistent as proteins are readily metabolized in living systems.

However, enzymes derived from new technologies might have increased stability (e.g. with higher stability to temperature or pH), therefore, the actual biodegradability of such enzymes should be tested. Enzyme preparations usually contain surfactant, detergents and heavy metals which could put additional burden to environment.

Use of recombinant microorganisms for enzyme production puts focus on the prospects of the release into the environment. It needs to be ensured that they do not survive in the general environment.

Environmental issues have special focus in case of bulk enzymes because of relatively larger production scale, hence reduction in the environmental impact form processing is done according to ISO14000 standards.

6.3.4.2 Quality Standards

For all types of enzymes, quality and efficient production are ensured by conforming to ISO9000 standards. As bulk enzyme products undergo a relative crude purification process, they contain all components of the fermentation broth in small or large quantities. It is therefore necessary to ensure that no toxic products are formed at any stage of the process, e.g. by the metabolism of enzyme generating organism. This is normally controlled during the strain development stage.

During fermentation process contamination tests are regularly performed, and contaminated batches discarded. Regular controls of the microbial standard of the finished product are also essential.

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7 Other policy impacts

7.1 Green Deal

Climate change and environmental degradation are an existential threat to Europe and the world. To overcome these challenges, the European Green Deal will transform the EU into a modern, resource-efficient and competitive economy, ensuring:

- *no net emissions of greenhouse gases by 2050*
- *economic growth decoupled from resource use*
- *no person and no place left behind*

The European Green Deal is also our lifeline out of the COVID-19 pandemic. One third of the 1.8 trillion euro investments from the NextGeneration EU Recovery Plan, and the EU's seven-year budget will finance the European Green Deal.

Some of the beneficts of the European Green Deal will be reached thanks to enzymes, as will be helping to re-used materials, with cutting-edge clean technological innovation and that will help to reach a globally competitive and resilent industry.

7.2 2018 EU bioeconomy strategy

The EU bioeconomy strategy focuses on sustainable biobased products development to help develop EU, national, regional, and local-level bioeconomies.

The results delivered in EnXylaScope's reports will cover the methodologies used, processes developed, results, achievements, gaps, focus points and call for action. The reports will link product performance with sustainability and in-depth life-cycle assessment of the products.

The report will also cover product toxicity studies and consumer knowledge on biobased products, with indications on necessary actions required to help consumers make right choices for responsible consumption of products.

7.3 Circular Economy Action Plan

The European Commission adopted the new circular economy action plan (CEAP) in March 2020.

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It is one of the main building blocks of the European Green Deal, Europe's new agenda for sustainable growth. The EU's transition to a circular economy will reduce pressure on natural resources and will create sustainable growth and jobs. It is also a prerequisite to achieve the EU's 2050 climate neutrality target and to halt biodiversity loss.

The new action plan announces initiatives along the entire life cycle of products. It targets how products are designed, promotes circular economy processes, encourages sustainable consumption, and aims to ensure that waste is prevented and the resources used are kept in the EU economy for as long as possible.

Enzymes help to design sustainable products, facilitate the reuse of biomass, thereby supporting the transition from a linear to a circular economy. Moreover, these enzymes reduce energy, water, and chemical usage in industry processes.

7.4 Sustainable Development Goals

EnXylaScope is closely aligned with **Sustainable Development Goals** (SDGs) thus contributing to global sustainability. The project aligns directly with SDG:

Goal 9: Build resilient infrastructure, promote sustainable industrialization and foster innovation. Inclusive and sustainable industrialization, together with innovation and infrastructure, can unleash dynamic and competitive economic forces that generate employment and income. They play a key role in introducing and promoting new technologies, facilitating international trade and enabling the efficient use of resources.
Goal 12: Ensure sustainable consumption and production patterns. Sustainable consumption and production is about doing more and better with less. It is also about decoupling economic growth from environmental degradation, increasing resource efficiency and promoting sustainable lifestyles.
Goal 13: Take urgent action to combat climate change and its impacts. Carbon dioxide (CO_2) levels and other greenhouse gases in the atmosphere rose to new records in 2019. Climate change is affecting every country on every continent. It is disrupting national economies and affecting lives. Weather patterns are changing, sea levels are rising, and weather events are becoming more extreme.

EnXylaScope indirectly aligns with 11 other SDGS (01, 02, 03, 05, 06, 08, 10, 11, 14, 15, 17).

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EnXylaScope

Unleashing Xylan's Potential with Enzymes
for a Scope of Consumer Products

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