Enzymes, key processing aids and additives, essential for enhancing the performance and sustainability of food and feed

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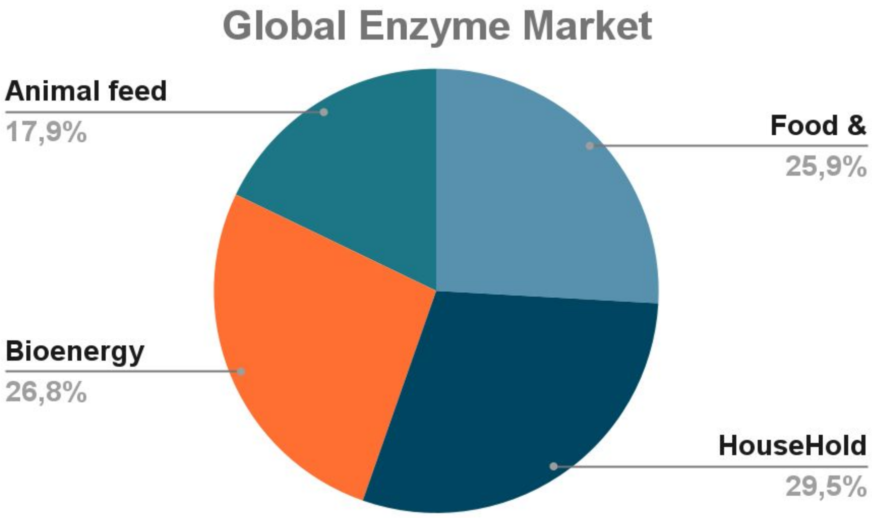
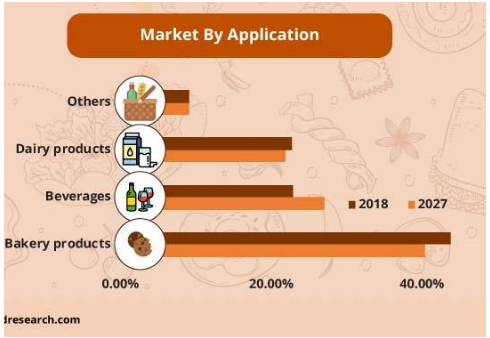
https://www.academia.edu/37748589/Enzymesinindustry_productionapplications?email_work_card=view-paper

Enzyme - crucial ingredients used since decades in food & beverages industries

- 1833: **Diastase** (a mixture of amylases) **first enzyme to be discovered**, quickly followed by other hydrolytic enzymes such as **pepsin and invertase**
- 1911: Purified **proteases clarify beer**
- **1922: First industrial-scale Rapidase factory in Seclin, France - Gist Brocades - DSM**



- **Bakery**
- **Oil & Fats**
- **Dairy Products**
- **Wine, Beer**
- **Fruit extraction**
- **Processing aid**



- **Food Enzymes Market 2,058 M$ (2020)**
- **CAGR 6,1% (2021 - 2032)**

Looking Back: A Short History of the Discovery of Enzymes and How They Became Powerful Chemical Tools (wiley.com)

GRAIN BIOREFINERIES, MOST MATURE APPLICATION IN FOOD

Use of enzyme in grain biorefineries (wheat, corn…)

Enzyme Market for Starch Producers

- Global Production = **70 Million Tons of Starch**
- A **Starch Facility Processes 5,000 Tons of Corn Per Day,** Resulting in 1,500 Tons of Starch. **At a ratio of 2 per 1000,** this yields 2 to 3 Tons of Thermostable Alpha-Amylase Per Day. **Approximately 1 Truckload (20 – 25 Tons) of Thermostable Alpha-Amylase is Used Weekly.**
- Thermostable Alpha-Amylase (pH 5.5, Temperature 105°C, Activity: 1 – 5 IU/g) + Pullulanase (pH 4 – 4.5, Temperature: 60° C, Moisture Content 33%, Activity: 1 IU/g)

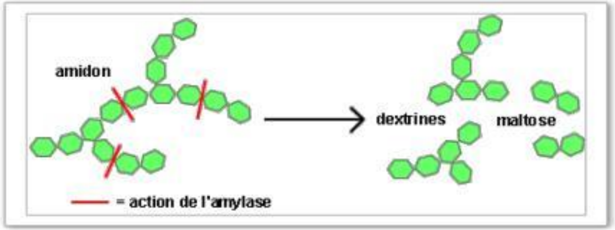
Hydrolysis Products: Maltodextrins (0 – 20 DE), Glucose Syrups (20 – 80 DE), Hydrolysates (DE = 95), Dextrose (DE 100)

- Low DE (Dextrose Equivalent) for Nutritional Applications, Baby Food, Confectionery (Factors: viscosity, binding capability, water activity, anti-crystallizing power, freezing point)
- High DE: Sweetening power, hygroscopicity, browning, flavor carrier, fermentable properties

- **The most common enzymes in biorefineries: Amylases Thermoresistant α-amylases Amyloglucosidases Branching enzymes: pullulanase and isoamylase**

- **Enzymes for specific syrups: β-amylase and “maltogenase”: for syrups rich in maltose Glucose Isomerase (50 to 100 T/y) Detrose x fermentation = D-Xylulose - purification - isometisation - purif/crystallisation => Xylose**

- **Specialty enzymes (self-produced by Roquette) Glucose Oxidase and Catalase Cyclodextrin Glucosyl Transferase (CGTase)"**



Despite the century-long history of enzyme utilization in the food industry

A mature “cash cow” segment

- **Optimization of enzyme blend activity** across the entire spectrum of food processing,
  - multifunctional enzyme blends, customization
  - answering to **customer demand for sustainable food quality product** (healthy, clean label, new sensory…)
  - providing **solutions for food industry to crises challenges** (lack of whey grain, energy crisis, ingredients increasing costs)
- **Biocatalysis paves the way to new compound or alternative sustainable synthesis (not only for chiral molecules)**.
  - prebiotics, low-calorie sweeteners, antioxidants, and pH regulators.
- **Growing market segments:**
  - **Plant based meat and dairy alternatives - Prebiotics- Lipase**

Enzymes and regulatory aspects

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Wide Range of Enzymes in Food and beverage industry

Enzyme	Source	Applications
Amylases	Bacillus and Aspergillus spp.	**Starch liquefaction. baking**, **brewing,** textiles, detergents, etc.
Beta-Glucanases	Bacillus spp.	Brewing and animal feedstuff
Bromelain	Pineapple	**Meat tenderization,** chill-proofing of beer
Cellulases	Trichoderma spp.	Textile biopolishing, pulp and paper, detergents
Chymosin	Calf stomach	**Cheese manufacture**
Ficin	Figs	**Meat** Tenderization
Glucose	Bacillus and Streptomyces spp.	**Glucose isomerization to fructose**
Lipases	Pseudomonas spp.	Detergents, **oils and fats, baking**, leather, paper, etc.

Enzyme	Source	Applications
Papain	Papaya latex	Meat tenderization, brewing
Pectinases	Aspergillus spp.	Pectin hydrolysis in **fruit juice clarification**
Proteases	Bacillus and Aspergillus spp.	Detergents, brewing, Meat tenderization, baking, cleaning, **hydrolyze animals proteins**, functional meat proteins, etc.
Pepsin	Stomach of slaughtered animals	Digestive aid
Transglutaminases	Streptomyces spp.	Protein cross-linking and gelation and meat binding
Trypsin	Stomach of slaughtered animals	Digestive aid.

Wide Range of Enzymes in Food and beverage industry

- Most uses **focus on hydrolytic reactions for debranching biomass**, improving the solubility, and clarification.
- Biosynthesis of nutritional compounds
- **Demand for NATURAL, NATIVE enzyme** (wild), **tailored enzyme blends for specific process and food applications**

Market by type

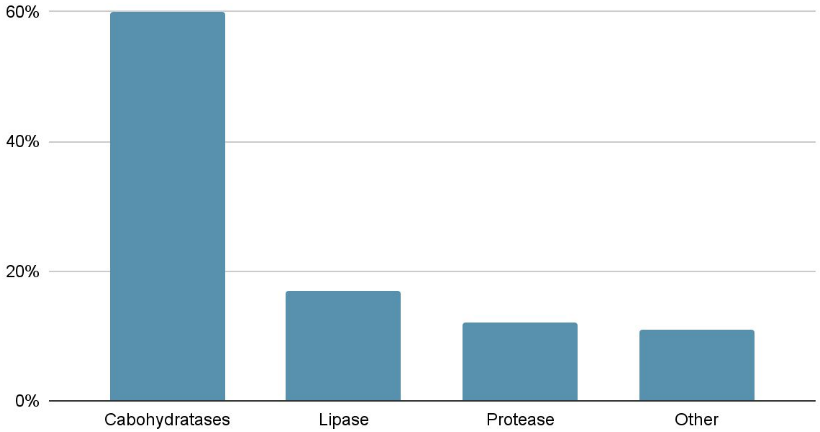
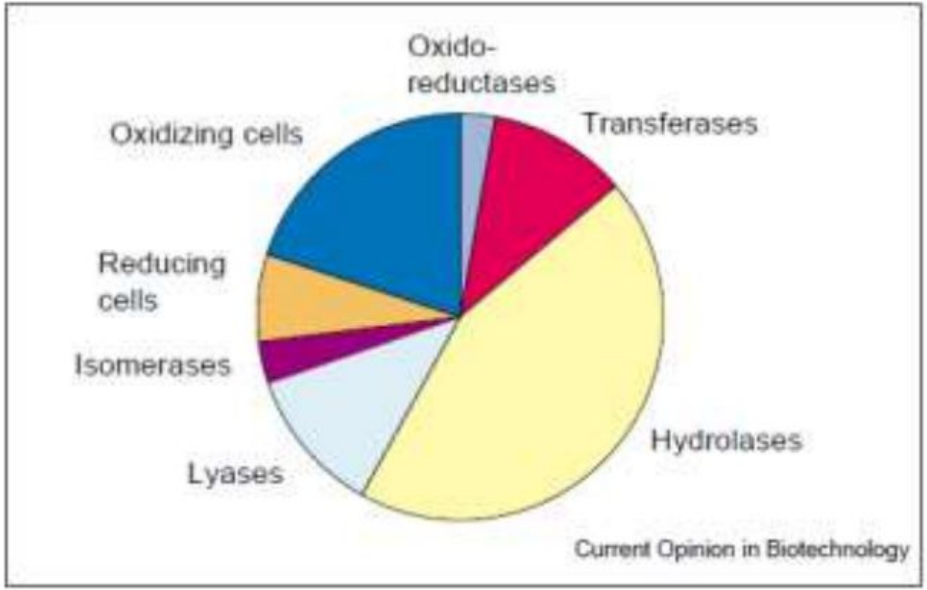


Figure 6



Enzyme types used in industrial biotransformations (based on 134 processes).

EU - Regulatory aspects on food enzymes - EU harmonisation in progress

Enzymes under examination by EFSA

- EU Food Additives Regulation (Regulation (EC) No 1333/2008): **food additive or processing aid,** depending on the intended technological function.
- **Regulation (EC) No 1332/2008** on food enzymes lays down rules for placing on the market and using food enzymes in the EU. It states that **only food enzymes approved in the EU may be sold** as such, and/or may be used to produce food which is sold in the EU.
- Commission has received **over 300 food enzyme applications**

REGISTER OF FOOD ENZYMES TO BE CONSIDERED FOR INCLUSION IN THE UNION LIST

Commission ID	IUBMB[5] number (EC)	Systematic name	Name of the food enzyme as submitted	Name of the production organism as submitted	Name of the production strain as submitted	EFSA Q Number
2013/19	1.1.3.4	β-D-glucose:oxygen 1-oxidoreductase	Glucose oxidase	Aspergillus niger	ZGL	EFSA-Q-2013-01005
2013/22	1.1.3.4	β-D-glucose:oxygen 1-oxidoreductase	Glucose oxidase	Aspergillus niger	not available	EFSA-Q-2013-01018
2015/151	1.1.3.4	β-D-glucose:oxygen 1-oxidoreductase	Glucose oxidase	Aspergillus niger	NZYM-KA	EFSA-Q-2016-00134
2015/179	1.1.3.4	β-D-glucose:oxygen 1-oxidoreductase	Glucose oxidase	Aspergillus niger	DP-Aze23	EFSA-Q-2016-00144
2013/06	1.1.3.4	β-D-glucose:oxygen 1-oxidoreductase	Glucose oxidase	Aspergillus oryzae	NZYM-KP	EFSA-Q-2013-00687

EFSA - oct 2023 - Safety evaluation food enzyme **asparaginase** (GM Aspergillus oryzae strain NZYM-SP **- to prevent acrylamide formation in food)**. Does not give rise **to safety concerns** under the intended conditions of use.. **No match with AA seq allergens - Low likelihood of allergic reactions…Triacylglycerol Lipase, alpha amylase, glucose oxydase (330 abstracts)**

- **France,** approved food enzymes - **list of processing aids,** h: Arrêté du 19 octobre 2006 - **NO WORLD HARMONIZATION**
- **US - Enzymes used in the bakery industry are GRAS food additives** . The FDA regulates their source or origin (food-compatible) and establishes limits to their use (if applicable) based on GMP.5
- **Canada** - List of Permitted Food Enzymes (Lists of Permitted Food Additives) - The Canadian Food Inspection Agency considers e**nzymes to be food additives that must be listed by common nam**e (CFIA Section B.016, Table V).

Regulatory aspects on food enzymes - Winemaking - Oenological CODEX

2013 - International Wine Organisation (OIV) - Enlargement Enzymes authorized

- Application of pectinases (1 ml / hl) **improves yield** for grape pressing by 4-15% with **reduced pressing time**.
- For **clarification** (1-3ml / hl)
- Maceration (pectinase + gases + xylanases + cellulases) - **improvement of flavors, color.**

NEW

Pectinases, cellulases, glucanases, xylanases for several application : **maceration, yield, clarification**
Beta-glucanases for **filtration and for ageing on lees**
Glycosidases for the **release of flavouring compounds (hydrolysis of terpene glycosides)**
Urease for prevention of **ethyl carbamate** (cancer risk)
Lysozyme for prevention of **microbial spoilage**

Sour wine to great vintage

EU Project LACLABW4wine (2021) - use of Laccases from Lactic acid bacteria, **to eliminate undesirable substances** in **wines -** aromatic defects **(off-flavors like volatile phenols (VP),** which are **produced from yeasts, fungi, and bacteria during the winemaking** process **and toxins in wine**

Enzymes, key tool for reshaping the way we produce food - Benefits

- **Product quality:** Extending **Shelf Life**, Enhancing **Flavor and Aroma**, **Texture freshness** for Baked goods ….Improving **texture and consistency** of Plant-Based Meat and dairy Alternatives
- **Nutritional Value – Hypoallergenic** Infant Formulas -Adapted for **infants suffering** from proven milk protein **allergies** : **Extensive WPH Whey Protein Hydrolysates** (smaller peptides < 3Kda) -- **Lactose** free - **prebiotics…**
- **Clean Label - lowering the number of ingredients (additives) - Mimic the functionalities of more expensive ingredients:** Phospholipase (emulsfiers) - Statistics Eurostat, Eu **eggs** prices + 30% (Jan 2023 / 2022) => kerry new enzyme system **Biobake EgR for replacing 30% eggs** in recipes, **matching performance (**specific volume muffin or brioche) and **CO_2** emission savings of **up to 14%)**
- **Significant environmental benefits** : reducing energy, food loss and waste - Increasing the **shelf life of baked goods by just two days** with enzymes reduce the V of **wasted items** by **40 %**.
- **Foster Local, sustainable raw materials** : In the brewing industry most common grain used globally is **barley.** exogenous enzymes enables brewers to employ **alternative local grains**, **low-cost cassava potential alternative** source of sugar for syrup extract makers, brewers,
- **Operational efficiencies:** Reduced Processing Time and Costs - improving yield

Challenges and innovation trends in the different Food sectors (baking, dairy, oil & fats, plant based)

Baking Industry - Current progress in formulation

Flour correctors and bread improvers tailored combinations of ingredients and enzymes , **added in low quantity (10 – 100G / t flour)**

FUNCTION	ENZYMES	EFFECTS / MECHANISM
Keep bread fresh for longer	Maltogenic enzymes	• Prevent the bread from staling for up to 1 month • Decrease starch retrogradation, the rigidity of the starch network, and starch/protein interactions
Strengthen bread dough	Phospholipases	• Mimic the effect of traditional emulsifiers: stabilizing air bubbles • Cleave and modify phospholipids, making them act as natural emulsifiers
Improve dough condition	Cellulases Xylanases	• Help the formation of better gluten network in whole wheat flour by releasing water bound by cellulose • Break down arabinoxylans and pentosans, which are indigestible carbohydrates
Alter the gluten network	Glucose-oxidase Proteolytic enzymes	• Catalyze the oxidation of glucose to gluconic acid and hydrogen peroxide • Promote gluten network oxidation and increase disulfide bonds within the gluten network which leads to a stronger, more cross-linked network • Reduce the strength of the gluten network by protein hydrolysis
Improve bread appearance	Amylase	• Release sugars and give bread loaves a colorful crust • Accelerate dough rise by releasing sugars used by yeast for growth
Healthier bread	Asparaginases	• Reduce the amount of acrylamide in bread by up to 95 %

- **Control and improvement of quality**: **New sensory**, better and tastier baked goods with expected texture (crumb, appearance, freshness, moistness, volume…)
- **Extension of shelf life:** decrease the need for chemical preservatives in baked goods
- **Health benefits:** allows the reduction of raw material (sugar, fat…). Allows the development of **fiber-enriched** and/or **gluten-free products.** Allows the enrichment in arabinoxylan oligosaccharides **with prebiotic potential**
- **Compatible with clean label: reduced need for preservatives**, reduce or replace certain raw materials (gluten) and additives **(emulsifiers, antioxidants, ascorbic acid**)
- **Decreased costs**: increased machinability, **reduced need for mixing,** shortened fermentation time, increased water absorption of products…
- **Local and sustainable raw materials:** New local grain replacing wheat

Challenges: right combination of enzymes

- Enzymes **work at all process stages**: mixing, proofing and fermentation, and baking. Enzyme efficiency depends on the enzyme itself and process conditions. With **synergistic effects,** increasing their overall effect.
- Enzymes will be **most effective** in the dough during **mixing** , others will work well during early stages of baking in the **oven**
- Overdose of **enzymes detrimental effects on dough /bread** (sticky).
- Some **amylases specifically tailored to provide yeast fermentables early-on during dough mixing** (deactivated during baking) :**antistaling amylases remain active beyond baking**,

Dairy Industry - Current progress

Dairy: processing cheddar, yogurt, milk, and milk products…

- Enzymes : **Protease** - Rennet (pepsin and chymosin) for **cheese coagulation,** Proteases speeding up **cheese aging,** decrease the **allergic effects** in infant foods, Lipase for cheese **maturing and flavor improvement (sc FFA)**. Lactase for lactose intolerant… catalase removing hydrogen peroxide cheese (instead of pasteurization, **transglutaminase** for **goat milk gel consistency and whey separation**… Many minor enzymes are sulfhydryl oxidase, lactoperoxidase, glucose oxidase, catalase, lysozyme, and superoxide dismutase

Innovations - New commercial solutions

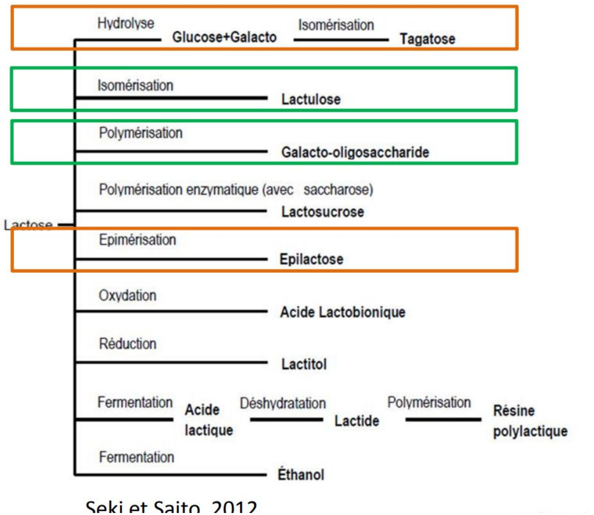
- **Health & Digestibilit**y, DuPont Danisco **Nurica (Nov 2019)** - **new lactase for low sugar (35%) and, high-fibre fermented dairy,** with **prebiotic** dietary fibre **GalactoOligoSaccharides** (stimulate benef Bifidobacteria gut)
- Quality - **boosting protein content dairy yoghurt and fermented beverages :** US Versilk (Dupont) a native enzyme, viscosity-modulating enzyme for high-protein applications, to obtain **a 14% protein** dairy or plant based yoghurt **(/ 9 - 10%) - «ProMilk Yogfluid» Ingredia (12% proteins in drink yoghurt)**
- **Bioactive peptide** (antihypertensive properties)
- **Increasing milk production - Chymostar Dupont (2020), a new milk coagulating preparation for fast flavour development (purity and thermolability - clear of lipase and amylase side activities.)** - New effective **milk clotting enzyme** (B subtilis higher proteolytic activity, **enhance flavour)** - enzymes completely inactivated at normal pasteurization temperatures with very low -
- **Lactose modification, Human Milk Oligosaccharides**

https://www.foodingredientsfirst.com/news/dupont-unveils-new-enzyme-line-for-fermented-dairy-products.html

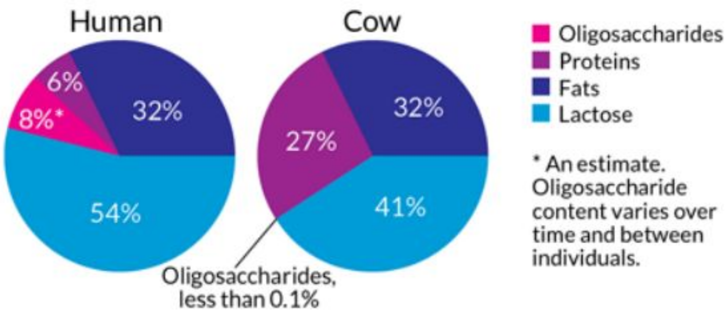
DuPont presents new enzymes for low-sugar, high-fibre, lactose-free dairy - Dairy Industries International https://archrazi.areeo.ac.ir/article_120986.html https://journals.asm.org/doi/10.1128/mmbr.62.3.597-635.1998

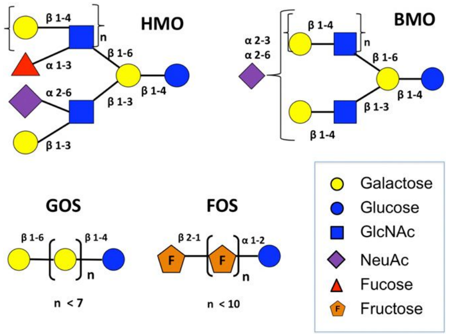
Dairy Industry - Current progress

Lactose transformation



Human Milk Oligosaccharides





New efficient enzymatic production Lactulose

- **Osmotic diholoside - prebiotic, constipation**
- Isomerisation **lactose** Gal-Glc Gal-Fru **β-galactosidase,** Aspergillus oryzae, **cellobiose 2-épimerase**,
- **Less coproducts and investment than the alkaline process (purification steps, water treatment))**

- **HMO,** complex sugars**, stimulating the healthy microflora in baby gut - complex structure / cow - 100 different OS** components in **HM**
- One way : **Supplementation milk powder with OS (FOS, GAS)**
- **Patent EP 2526784A1 -** Milk oligosaccharide-galactooligosaccharide composition for i**nfant formula** containing the soluble oligosaccharide fraction present in milk, and having a low level of monosaccharides, and a process to produce the composition (Nestec Sa) _ **Action of galactosidase on lactose and milk oligosaccharides**
- **SAYENS - Design of an α-L-transfucosidase for the synthesis of fucosylated HMOs**

Recent Advances in Lipases in the Food and Nutraceutical Industry

Oils & fat - modifying physical, nutritional, generating flavors / aromas and increasing compounds stability…

Lipase can be used to **modify fats and oils and synthesize structured lipids or antioxidants** with increased antioxidant power **or modified lipophilicity, flavors, and aromas**

- **hydrolyze** triglycerides to obtain free fatty acids, monoacylglycerols (MAGs), diacylglycerols (DAGs), and glycerol;
- Synthesize new products in organic media by **esterification**, **transesterification**, and aminolysis mechanisms

Bottlenecks of free lipases are the low operational **stability** in synthesis reactions using solvents and substrates such as alcohols and organic acids, the **high cost** of the enzymes, and the need to **reuse the biocatalyst** minimizing product separation.

×

Table 1. Sources of lipases with applications in food and nutraceutical industry.

Source/Commercial Name	Type	Application/Products	Reference
Candida antarctica lipase B (CALB)/Novozym 435/Lipozyme 435	Recombinant	Flavor esters	[32]
Candida rugosa	Wild type	Glycerides, production flavor compounds	[33,34]
Termomyces lanuginosus/Lipozyme TL IM	Engineered	Food formulation, Interesterification of fats and oils	[35,36]
Aspergillus sp.	Wild type	Flavor and fragance	[37]
Aspergillus oryzae	Wild type	Interesterification of fats and oils	[36]
Geotrichum candidum	Wild type	Oil with increased unsaturation	[36]
Rhizomucor miehei/Lipozyme RM IM	Recombinant	Enhancing fruit fragrance	[38]
		Modification of the amount and composition of volatile components in bovine milk	[39]
		Ras Cheese Flavor Concentrate (RCFC)	[40]
Rhizopus oryzae	Wild type	Human Milk Fat Substitutes	[41]
Lactococcus chungangensis	Wild type	Flavoring in milk, cream cheese, yogurt and butter.	[42]
Lactobacillus plantarum	Wild type	Fermented food and cheese	[43,44]
Staphylococcus epidermidis	Wild type	Flavor-compound production	[45]
Ophiostoma piceae	Wild type	Flavor-compound production	[46]
Meyerozyma guilliermondii	Wild type	Feed industry	[47]

Recent Advances in Lipases in the Food and Nutraceutical Industry

Lipases have an impressive number of applications

Dairy products:

- Provide desirable **aromatic** characteristics to **cheddar** conferred by **free short-chain FA** generated in the hydrolysis of fats
- **New alternative for flavoring milk** Recent advances have allowed the biosynthesis of **short-chain ethyl esters with fruity notes in whole milk** by coupling ethanolic fermentation with transesterification using the **commercial lipase Palatase**

Structured LIpids (great importance)

- **Modify FA composition for nutraceutical products (saturated FA, trans FA "natural ingredients")**
- **Human milk fat substitute (HMFS), cocoa butter, low-calorie triacylglycerols**
- **Lc TAG** (**s-n 1,3 Short FA** release and absorbed more rapidely, sn2- Long FA)
- **HMFS enzymatic interesterification of vegetable oils, animal fats, or oil mixtures, using an immobilized regioselective lipase**
- **EU LIPES project "Life Integrated Process for the Enzymatic Splitting of triglyceride" (Oleon) -** New splitting routes of triglycerides - **Zero trans fatty acids** - Replacing current thermal hydrolysis and saponification production routes

Vitamines derivatives - esters - improving stability and physicochemical properties

- **Retinol (Vit A) Esters** - immobilized lipase (Novozym 435) => retinol laurate (less time, and more stability) - **FA Esters of L-Ascorbic Acid (Vit C) - Tocopherols Esters (succinate, ferulate VitE) - /** mixture stereoisomers in the esterified form (all-rac-α-tocopheryl acetate), obtained chemically from soybean byproducts. which include separation strategies

Bakery - Lipases, **alternatives to surfactants** amylose-lipid complexes- Lipases hydrolyze g**alactolipids,** and their presence in the dough improves bread volume

https://www.mdpi.com/2073-4344/12/9/960

Recent Advances in Lipases in the Food and Nutraceutical Industry

Flavors/ fragrances

- **Wide range of flavors/ fragrances by chemical synthesis, - not labeled as natural** according to EL (EC 1334/2008)
- **Biotech widely explored** (**chiral compound, higher yield, selectivity, easier DSP**)
- **Bioflavors: 100–500 $/kg,- > 100 commercialized.** Bioflavor: 0.5 b$ (2019), (+/- 1.5% 28 b$ - CAGR 9.3% 2027).
- **Lipases**, most applied enzyme family to produce flavor and fragrances (**greener route, esterification, trans - inter**)
- **Challenging Short-Chain Fatty Acids and Isoamyl Alcohol Esters** - produced via the esterification of short-chain OH and s-c FA, isoamyl alcohol esters, such as i**soamyl butyrate and acetate (fruity banana and intense banana flavor)** - sc FA (hydrophilic, **lowers pH => enzyme inactivation**, sc OH strip the essential water enzyme dead-end inhibitor, branched structure exerts a higher **steric hindrance**) IAAC
- Ethyl butyrate, are the major component of many fruit flavors, such as pineapple, passion fruit, and strawberry (butyric acid enzyme deactivation, b**iphasic)**

RECENTLY

Improvement Phenolic Antioxidants, antimicrobial, anticarcinogenic, anti-inflammatory, antidiabetic, and antiobesity capacities (low bioavailability - physiological stability, f intermolecular interactions with the macronutrients),

- **Synthesis of lipophilic antioxidants** - cyanidin-3-O-(dodecanoyl6) galactoside - acylating lauric acid with cyanidin-3-O-galactoside extracted from alpine bearberry immobilized enzyme Novozym 435. improved **lipophilicity and thermostability** while retaining its original antioxidant properties.
- **Caffeic esterification** (glyceryl 1 caffeate rom ethyl caffeate and glycerol
- **Umbelliferone esters with high antibacterial power**
- **Bioavailability of a flavonoid, naringin esters with antioxidant power superior to precursor**

Prebiotics and biosurfactants

- **Esterfication of Prebiotic oligosaccharides (**emulsifying), **sugar fatty acid esters (SFAEs) high biodegradability,**

Plant based alternatives to milk and meat - Current progress

26 % millennials are either vegetarian or vegan - 73 % are ready to pay more for less environmental impact products

Consumer are expecting plant based alternatives (ingredients, dairy, meat, culinary) - burger, yoghurt, sausage, drinks, unami experience with clean label, great taste (without off flavor - debittering), good texture, high protein content

Consumers dislike consistency and texture about plant based foods.

- **28,4% do not enjoy the taste of plant meat**
- **54%** dislike the **Hidden unhealthy ingredients (hydrocolloides)**
- **3rd** reason for not consuming - **dairy providing better nutrition**
- **5th** for not consuming **- Perception that is is more processed than dairy**

This **requires new enzymes** that can provide plant-based protein sources with the **functional attributes** of animal-derived protein while improving the **taste and texture** of final products. **with great taste, texture and nutritional profile - sweetness - higher solubility, less grittiness…**

- ***Cellulases, hemicellulases, and proteases*** *(extraction, reduce viscosity, prevent suspensions)*
- ***Amylase, xylanase and glucanase*** *increase sweetness, reduce sugar addition and improve fermentation process for beverages*
- ***Lipase*** *flavor aroma and emulsifications*
- ***Transglutaminases , texture, firmness and elasticity***

https://www.novozymes.com/en/solutions/plant-based-foods/dairy-alternatives

The Role of Enzymes in Food – Kerry Health And Nutrition Institute

Plant based alternatives to milk and meat - Current progress

Challenges: modify the physicochemical and functional properties of plant proteins by catalyzing reactions such as **hydrolysis, cross-linking, and deamidation.**

- **Hydrolysed plant protein (HPP) -** commonly **produced via the enzymatic hydrolysis of a plant protein** source such as **soy, wheat, rice, sunflower, potato** and alternative pulse proteins, and are used in a wide variety of food applications such as **protein fortified bars and beverages.** HPP products are synonymous with **bitter,** unpleasant tastes often **attributed to a high concentration of hydrophobic free amino-acids, smaller peptides and volatile compounds in the HPP mixture. Enzymatic hydrolysis, both pre- and post-hydrolysis** can help to significantly improve these undesirable sensory properties of HPP (**exoproteases fro debittering, phenylalanine ammonia lyase fpr patients with phenylketonuria**)
- **Oat drinks growing 30% a year (cereal tastes or no sweetness in north europe) - adjusting sensory profile with tailored enzyme blend** (alpha amylase, glucoamylase) **to meet requirements for viscosity and mouthfeel** during liquefaction step - increasing **yield up 10 % dry solids content**
- **High Protein Vegurts -** based on pulses and legumes like pea, soy or lentil,delivering up to 9% protein content - specific protease to get desired and delicious smooth texture.
- **Soy yogurt -** reducing grittiness
- **Plant based meat alternatives -** great taste and **high protein content (50%)** in **breaking peptide bonds - cross linking Lys - Glu in plant proteins to create texture thus illuminating hydrocolloids ,** carrageenan, agar, konja, gellan gum - reducing ingredient lists.
- Oat or rice milk can - **emulsion stability (**meaning products might separate out over their shelf life instead of remaining a consistent mixture)

The Role of Enzymes in Food – Kerry Health And Nutrition Institute The Role of Enzymes in Food – Kerry Health And Nutrition Institute https://www.novozymes.com/en/solutions/plant-based-foods/dairy-alternatives

RECENT PATENTS ON BIOCATALYSIS - FOOD SECTOR

Objective: To identify **targeted molecules, new bioprocess - future industrial applications.**

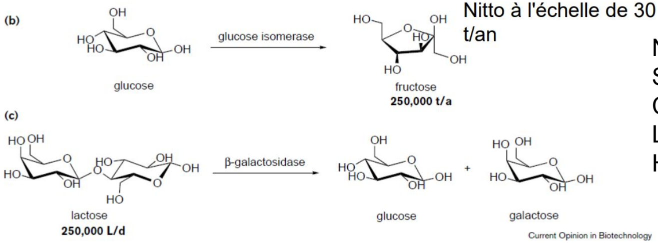
Exemples de composés produits à l'échelle industrielle par biocatalyse

Compagnie	Production	Tonnes/an
Ajinomoto Co.	dihydroxyphénylalanine	250
AMINO GmbH	acide malique	2 000
BASF	(R)-phényléthylamine	> 100
Chemferm	pénicillines hémisynthétiques	2 000
Degussa-Hüls AG	L-aminoacides	200
Dr Vig Medicaments	7-ADCA	300
DSM	aspartame	> 2 000
DuPont	5-cyanovaléramide	10
Gist-Brocades/Novo-Nordisk	fructose	> 250 000
Hoechst Marion	acide 7 amino céphalosporanique	200
Kanegafuchi	D-p-hydroxyphénylglycine	200
Krebs Biochemicals Ltd.	phénylacétylcarbinol	120
Lonza AG	vitamine B3	3 000
Monsanto	acides aminés	> 10
Nitto Chemical Industry	acrylamide	> 30 000
Tanabe Seiyaku Co.	acide malique	450
Toray Industries	lysine	4 000
Zeneca	acide (S)-2-chloropropionique	2 000

> 100 compounds -industrial levels at big scale
> T/year- AA,, Vit precursors, Lc sweeteners

Table 1. Green Chemistry and Biocatalysis

	green chemistry principle	biocatalysis
1	waste prevention	significantly reduced waste
2	atom economy	more atom- and step-economical
3	less hazardous syntheses	generally low toxicity
4	design for safer products	not relevant (product not process)
5	safer solvents and auxiliaries	usually performed in water
6	energy efficiency	mild conditions/energy-efficient
7	renewable feedstocks	enzymes are renewable
8	reduced derivatization	avoids protection/deprotection steps
9	catalysis	enzymes are catalysts
10	design for degradation	not relevant (product not process)
11	real-time analysis	applicability to biocatalytic processes
12	inherently safer processes	mild and safe conditions



Nitto à l'échelle de 30 t/an

Not toxic catalysts
Soft reaction coiditions (T< 100 ° C)
Low thermal degradation risk
High specificity and selectivity

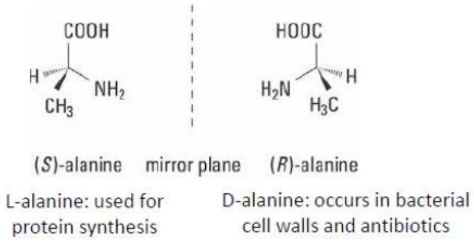
RECENT PATENTS ON BIOCATALYSIS - FOOD SECTOR

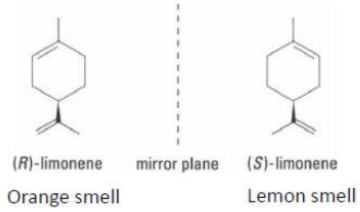
Objective: To identify **targeted molecules, new bioprocess - future industrial applications..**

What will be the next industrial compounds made through biocatalysis ?

- **Rare Sugar and low calorie natural sweeteners** : Herperitin dihydrochalcone with **high stereo-selectivity**, REBAUDIOSIDE (stevioside glycosylation), monatin M D-Tagatose
- Health: L Fucose and HMO, myo-inositol (anti-cholesterolemia)
- **Aroma & Flavors:** isopulegol (terpene cyclisation)**,** alkyl pyrazine (barbecue)
- **Amino Acids and derivatives:** PhosphatidylSerine (benefits in cognitive health) (L-asp, L Tert Leucine) ,
- **Vitamins** derivatives
- **Antioxydants :** gallates propyl
- **Prebiotics :** alphaglucan (thickening agents, stabilizers, or emulsifier), alpha glucan in Juice, FOS in situ (Food)

Natural - Green route
The mastery of chirality is necessary in the nutraceutical, flavors sectors





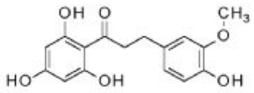
LENS : class_cpc.symbol:C12P* AND (title:(enzym*) OR abstract:(enzym*) OR claim:(enzym*)) filtre 2018 10 01 - granted patent -

BIOCATALYTIC PATENT ON SWEETENERS, RARE SUGAR

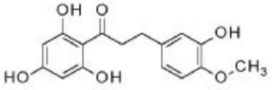
N°Patent	Pub date.	Title	Applicant	Abstract - Revendication
US 10494654 B2	2019	Production of Stereoisomers of Monatin via D-Tryptophan	Cargill	• Monatin is a **high-intensity sweetener**l combination of monatin stereoisomers, (e.g., a composition including only the R,R and S,S, stereoisomers of monatin), as well as a single isomeric form. cocktail enzymes : r**acemases L-aminotransferases…**
US 10428361 B2	2019	**Biocatalytic production of l-fucose**	**BASF**	**L-fucose is found, inter alia, in human breast milk.** (human milk oligosaccharides (HMO)) A method for producing L-fucose, comprising the following steps: (a) providing L-fucitol, a galactose oxidase of the enzyme class EC 1.1.3.9, a peroxidase and a catalase,(b) combining **L-fucitol, the galactose oxidase, the peroxidase, and the catalase to form a mixture,**(c) incubating the resulting mixture under conditions permitting the biocatalytic oxidation of L-fucitol to L-fucose, and(d) optionally isolating the synthesized L-fucose.
US 10745720 B2	2020	**Production method for tagatose**	**Cj Cheiljedang Corporation**	A method for producing **tagatose** from fructose - performing **epimerization of fructose using hexuronate C4-epimerase** -enzyme derived from Thermotoga maritima **sweetener** (alternative to lactose / gal pathway)
US 10752888 B2	2020	**Method for enzymatically preparing highly concentrated myo-inositol**	**Cj Cheiljedang Corporation**	**using myo-inositol monophosphate synthase - myo-inositol** is known to have an important role in the metabolism of cholesterol and fat, and is reported to be effective in **preventing or treating hypercholesterolemia,** skin functions such as moisture maintenance, sebum control, anti-aging via regulation of an antioxidant activity, etc. converting glucose-6-phosphate to myo-inositol monophosphate

BIOCATALYTIC PATENT ON Low calorie SWEETENERS, RARE SUGAR

homoeriodictyol dihydrochalcone

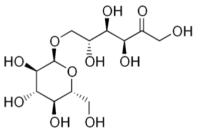


Hesperetin dihydrochalcone (2)



WO2021058115 - PROCÉDÉS DE FABRICATION BIOCATALYTIQUE DE DIHYDROCHALCONES - SYMRISE AG (2021) [2]

NATURAL Sweetener and sweeteness enhancer - sweet impression or to mask **bittering substances of foodstuffs, pharmaceuticals, beverages** or similar finished goods which **can be classified** as produced by a fully **natural** manufacturing method. n a two-step process from **phloretin and/or its glycosides** using at least one **oxidase**, at least one **reductase** and at least one ***methyltransferase***. A **high stereo-selectivity can be achieved only by using enzymes, which is a major advantage over a chemical process**.

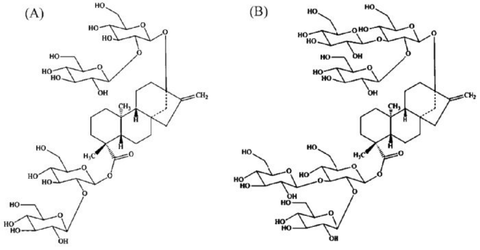


Isomaltulose

WO2019175634 - NOUVEAU BIOCATALYSEUR À CELLULES ENTIÈRES POUR LA PRODUCTION D'ISOMALTULOSE

Naturally present in honey in very low quantities,
isomaltulose shows physical and organoleptic characteristics that are very similar to sucrose

CN109750071 - BIOCATALYTIC METHOD FOR SYNTHESIS OF REBAUDIOSIDE M



REBAUDIOSIDE M

Rebaudioside M, reported in 2009 for the first time is extracted from leaves of a **new variety of stevia rebaudiana, whose sweetness is 400 times that of sucrose, which is superior to that of rebaudioside A commercially available in the market**.

Firstly, **rebaudioside E is synthesized by a glycosylation reaction of stevioside** with UDP-glycosyltransferase from tomatoes and sucrose synthetase from potatoes; then, **rebaudioside M is synthesized by a glycosylation reaction of rebaudioside E with UDP-glycosyltransferase** from stevia rebaudiana and **sucrose synthetase** from potatoes. The method uses a molecular cloning technique, escherichia coli genetically engineered bacteria for **heterologous expression of UDP-glycosyltransferase and sucrose synthetase** are obtained,after fermentation and enzyme production, crude extract of cells is directly used for a catalytic reaction, additionally added saccharose is decomposed with sucrose synthetase to obtain UDP-glucose,UDP in the crude extract and the UDP-glucose serve as raw materials for the glycosylation reaction, a **double-enzyme cycle reaction** system is established, and the rebaudioside M is produced by effectively catalyzing stevioside. The cost of raw materials is lower, the processing steps are simple, and the method has an important application value

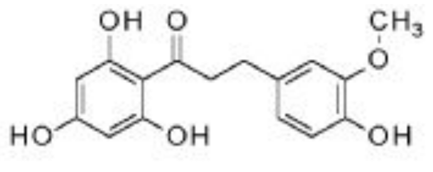
BIOCATALYTIC PATENT ON SWEETENERS ENHANCERS

WO2021058115 - PROCÉDÉS DE FABRICATION BIOCATALYTIQUE DE DIHYDROCHALCONES - SYMRISE AG (2021)

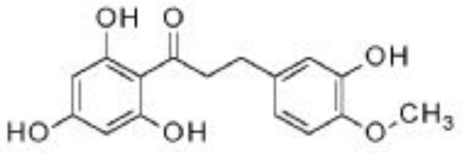
- Methods for the biocatalytical manufacturing of **dihydrochalcones** (homoeriodictyol dihydrochalcone and/or hesperetin dihydrochalcone)
- Dihydrochalcones are compounds with an increased **Sweetener** and sweeteness enhancer - sweet impression or to **mask bittering** substances of foodstuffs, pharmaceuticals, beverages or similar finished goods
- **Manufacturing biocatalytical method for mixture Natural homoeriodictyol dihydrochalcone and hesperetin dihydrochalcone**
- In a two-step process from **phloretin** and/or its glycosides using at least one ***oxidase***, at least one ***reductase*** and at least one ***methyltransferase***.
- A **high stereo-selectivity can be achieved only by using enzymes, which is a major advantage over a chemical process**.

sweetness enhancers and/or flavouring agents

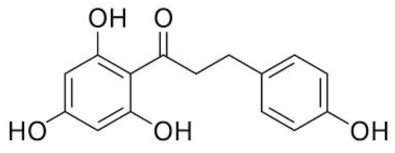
homoeriodictyol dihydrochalcone



hesperetin dihydrochalcone

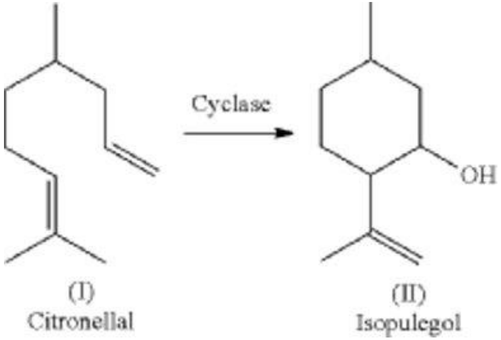


Phloretein



BIOCATALYTIC PATENT ON AROMAS AND FLAVORS

ISOPULEGOL

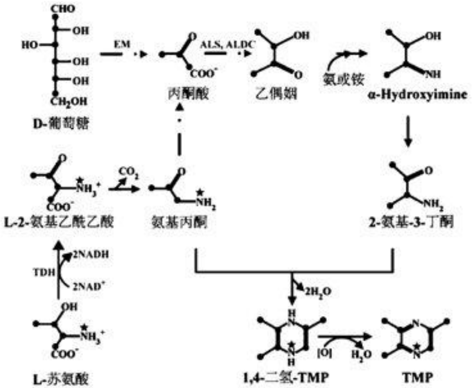


US20190119665 - METHOD FOR THE BIOCATALYTIC **CYCLIZATION OF TERPENES** AND CYCLASE MUTANTS EMPLOYABLE THEREIN **– BASF – 25/04/2019**

- Terpene that is used as an aroma compound, to **generate "flower notes"**. it is an intermediate in the synthesis of **menthol from citral.**
- Enzyme having the activity of **citronellal-isopulegol cyclase**

Alkyl pyrazine

CN110205347 - METHOD FOR BIOCATALYTIC SYNTHESIS OF ALKYLPYRAZINE CONTAINING MONOMETHYL SEMI RING



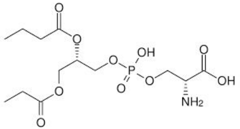
- As an important flavor substance, primarily contributing **nut flavor, barbecue flavor, and baked bread flavor in the food product.**
- although research has been explored for an alkyl pyrazine microbial source for a long period of time, it is also very limited for the cognition of its synthesis mechanism
- Synthetisis with L **threonine substrate using threonine dehydrogenase (TDH)**

BIOCATALYTIC PATENT ON HEALTH COMPOUND - PREBIOTIC

N°Patent	Pub date .	Title	Applicant	Abstract - Revendication
US 10808 269 B2	20 20	**Synthesis of S glucan comprising alpha-1,3 glycosidic linkages with phosphorylase enzymes**	Danisco	• **Alpha glucan polymer** alpha-1,3 glycosidic linkage (G1P - phosphorylase)) glucan **phosphorylase** reaction) (do not rely on glucansucrase enzyme activity) • **Thickening agents, stabilizers, or emulsifier prebiotics,**
EP 19518 85 B1		**In Situ Fructooligosaccharide Production and Sucrose Reduction**	Danisco	Producing **fructooligosaccharides** in a food product. he Aspergillus japonicus **fructosyltransferase** to enzymatically convert **sucrose in the food product to fructooligosaccharides (FOSs)**
EP 31047 17 B1		**Sucrose Reduction and Generation of Insoluble Fiber in Juices**	Danisco	**method of making a lower calorie, higher insoluble fiber beverage comprising; treating a sucrose-containing beverage with a glucosyltransferase** to convert **sucrose to alpha (1-3) glucan to make the lower calorie, higher insoluble fiber beverage**

APPLICATIONS BIOMOLECULES – FOOD ADDITIVES (TARGETS !)

PhosphatidylSerine

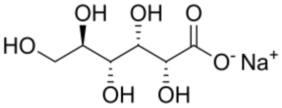


- **Phosphatidylserine is the main phospholipid present in the brain**
- Applications in food and pharmaceutical industries**. Benefits in cognitive health**
- **Global Phosphatidylserine Market is valued at 92 million USD in 2020 is expected to reach 183.6 million USD by the end of 2026,**

CN111778295 - METHOD FOR SYNTHESIZING PHOSPHATIDYLSERINE BY USING IMMOBILIZED BIOCATALYST - NANTONG HOUYUAN BIOTECHNOLOGY CO., LTD.

- **Immobilizing free phospholipase D** with a ZnO nanowire/mesoporous silica, temperature and pH stability improved - phospholipase (EC 3.1.4.4),
- **L-serine + phosphatidylcholine**

GLUCONATE



Food additive, ph regulating, edible salts

Medicine, chemical industry, construction

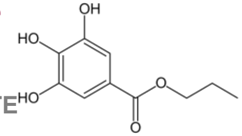
CN110904164 - BIOCATALYSIS METHOD FOR PREPARING GLUCONATE - WUHAN SUNHY BIOLOGICAL CO., LTD.

- S1, taking dry **starch**, adding water to stir to obtain starch slurry, adding **alpha-amylase liquefaction treatment** in the starch slurry, then adding **glucoamylase saccharification treatment**, liquefying and saccharifying the starch slurry to generate glucose slurry;
- S2, **glucose oxidase and catalase** are added to the generated glucose slurry, glucose slurry is catalytically oxidized into **gluconic acid and hydrogen peroxide** by glucose oxidase in an oxygen environment atmosphere;
- S3, continuing to flow an alkaline solution **to neutralize and react with gluconic acid to generate gluconate;**
- S4, **hydrogen peroxide** is hydrolyzed under the catalytic action of catalase to generate water and oxygen, and oxygen is supplemented to S2.

Gallate de propyle

Antioxydant E310

Food oil , medicines, cosmetics, feeds



CN111850058 - METHOD FOR SYNTHESIZING PROPYL GALLATE THROUGH TANNIC ACID BIOCATALYSIS

- Synthesizing propyl gallate through **tannic acid biocatalysis.**
- Tannic acid is used as a reactionmonomer, sodium carboxymethyl cellulose **immobilized tannase** is used as a catalyst, catalytic reaction is performed in **an organic solvent** by cooperating with **microwaves**, and then reduced pressure distillation, cooling crystallization and vacuum drying are performed to obtain propyl gallate.

The food industry continues to explore and invest in enzymes and faces challenges

- **Cost:** The cost of enzymes can be high, which can limit their use in the food industry.
- **Temperature and pH Sensitivity-** Many **food processes involve conditions that are not optimal** for the enzyme activity
- **Stability:** Enzymes can be unstable and can lose their activity under certain conditions such as high temperature, pH, or pressure. This can **limit** their use in some food processing applications.
- **Regulatory approval:** Enzymes used in the food industry must be approved by regulatory agencies such as the FDA. The approval process can be **time-consuming and expensive.**
- **Limited substrate specificity:** Some enzymes have limited substrate specificity, which can limit their use in certain food processing applications.**Developing enzymes with broader substrate specificity** or engineering enzymes to enhance their catalytic activity can help overcome this limitation.
- **Production scale-up**:particularly for complex enzymes that are difficult to produce in high quantities
- **Process Integration:** may r**equire modifications to equipment and processes,** which can be costly and time-consuming.
- **Quality Control**: Ensuring that the enzyme **performs as expected in every batch**
- **Safety concerns:** Enzymes used in the food industry must be safe for human consumption. Some enzymes may have allergenic properties or may produce toxic byproducts during food processing
- **Consumer perception -** perceive the use of enzymes in food processing as unnatural or potentially harmful
- **Limited knowledge**: There is still limited knowledge about the mechanisms of action of some enzymes, which can limit their use in the food industry
- ….

Annex

EUROPEAN PROJECT ANALYSIS - FEW PROJECTS APPLIED TO FOOD

Targeted reactions: **glycosylation (synthesis of glycosides), starch functionalization, synthesis of rare sugars and custom oligosaccharides, glycosylation of flavonoids**, hydrocolloid production, and **modification of alginate, carrageenan, chitosan, glycosaminoglycan, pectin, and xanthan gum….**

Project SMARTBOX (2019 - 2023) develop an advanced computational engineering platform specifically for oxidative enzyme - Producing vanillin for the flavour and fragrance market from softwood (lignin)

Project BIOphiCS (2021) - Bio- & Photo-Catalytic Methods for the Construction of Enantiomerically Pure C-S Bonds in Thiols and SulphidesNovel methods for the **synthesis of chiral sulfur compounds - flavours and fragrances - light photocatalysis and oxidoreductase enzymes photo-biocatalytic synthesis of enantiomerically pure sulfur compounds**

Project ROBOX (2015 - 2019) Expanding the industrial use of Robust Oxidative Biocatalysts for the conversion and production of alcohols - applied robust Alcohol DeHydrogenase (ADH), Alcohol OXidase (AOX) and Baeyer-Villiger MonoOxygenase (BVMO) enzymes

Project AROMAs-FLOW (2018 - 2020) Biocatalytic **flow reactors** using **extremophilic enzymes for a greener generation of aroma-compounds**

Project **ENZYOX (BBI 2016 - 2019**) - NEW ENZYMATIC **OXIDATION/OXYFUNCTIONALIZATION TECHNOLOGIES FOR ADDED VALUE BIO-BASED PRODUCTS LIPIDS** the enzyme from Marasmius rotula catalyzes unique reactions on fatty acids **including terminal/subterminal hydroxylation and chain shortening** For the enzymatic production of lipid "sensu lato" added value compounds, the action of UPOs on different compound types has been investigated - a patent has been deposited for the controlled one-carbon shortening of fatty acids .**TERPENES - Challenging selective oxyfunctionalization of four model terpenes - selective synthesis of 4-hydroxyisophorone and 4-ketoisophorone, of interest for both pharmaceutical and flavour & fragrance sectors**

Project SUSY (2013 - 2017) - Sucrose Synthase as Cost-Effective Mediator of **Glycosylation Reactions - Glycosyl transferases (GT) low operational stability and by the high cost of their glycosyl donor nucleotide sugars**

Project VegProteins (2017 - 2019): a novel source of next generation functional hydrolysates - superior tasting vegetable protein hydrolysates - investigating the efficacy of a range of commercially available proteases in producing HVPs under controlled enzymatic conditions using a variety of vegetable substrates…Degree of hydrolysis, protein recovery, chemical composition and techno-functional properties of HVPs

Project LIPES - "Life Integrated Process for the Enzymatic **Splitting of triglyceride"** (Oleon) - New splitting routes of triglycerides - **Zero trans fatty acids -** Replacing current **thermal hydrolysis and saponification production routes**

Project LACALB4wine LAB laccase enzymes to eliminate aromatic defects and toxins in wine

RECENT PATENTS ON BIOCATALYSIS - FOOD SECTOR

Commercialized bioprocesses.

Company	Strategy	Product(s)	Substrate(s)	Catalyst(s)	Remarks	References
Avecia (ICI)	Kinetic resolution	(*S*)-2-Chloro-propionic acid	Racemic 2-chloro-propionic acid	Whole cells, (*S*)-specific dehalogenase	Knock-out of (*R*)-specific dehalogenase. Scale: several 1000 tons/year	[11]
BASF	Fermentation	L-Lysine	Glucose	*C. glutamicum*	Scale: >100 tons/year	[37•]
		(*R*)-Isobutyl lactate	Glucose	Microorganism	Precursor for (*S*)-chloropropionic acid. Scale: several 100 tons/year	[38•]
	Kinetic resolution	Enantiopure alcohols	Racemic alcohols	Lipases	Enantiospecific acylation, ChiPros™ Scale: several 100 tons/year	[37•]
		Chiral amines	Racemic *sec*-amines	Lipases	Enantiospecific acylation, ChiPros™ Scale: up to 1000 tons/year	[37•]
	Dynamic resolution	e.g. (*R*)-mandelic acid	Racemic cyanohydrins	Nitrilase	Racemization via pre-equilibrium ChiPros™. Scale: several tons/year	[37•]
	Oxidation	(*R*)-2-(4′-Hydroxy-phenoxy) propionic acid	(*R*)-2-Phenoxy-propionic acid	Whole cells, oxidase	Broad substrate range of the biocatalyst	[38,39]
Chirotech	Kinetic resolution	Various α-amino acids	Lactams, *N*-protected racemic α-amino acid esters	Lactamases	Complementary stereoisomers via complementary lactamases Scale: kg to tons/year	[40–42]
		Various D-amino acids	Racemic *N*-acylated amino acids	D-Aminoacylase		[40]
		Various L-amino acids	Racemic *N*-acetyl amino acids	*N*-Acetyl-L-amino-acid amidohydrolase (aminoacylase)	Immobilized enzyme in packed-bed reactor, Scale: up to several kg on demand	[43]
		4-Endo-hydroxy-2-oxabicyclo[3.3.0]-oct-7-en-3-one	4-Hydroxy-2-oxabicyclo[3.3.0]-oct-7-en-3-one butyrate ester	Triacylglycerol acylhydrolase	Scale: up to multi-kg on demand	[42]
	Dynamic resolution	Various (*S*)-ester amides	Racemic aralactones	Immobilized triacylglycerol acylhydrolase (triacylglycerol lipase)	The reaction is performed in organic media, spontaneous racemization of the substrate	[44]
Degussa	Fermentation	L-Threonine	Glucose	Whole cells	REXIM (subsidiary in France). Scale: multi 1000 tons/year	[14•,45]
	Dynamic resolution	Enantiopure L-amino acids	Racemic *N*-acetyl amino acids	L-Acylases	*In vitro* in an EMR, chemical or enzymatic racemization. Scale: 100 tons/year, D-acylase process under preparation	[14•,45]
		Enantiopure D-amino acids	Racemic hydantoins	Hydantoinases, decarbamylases Racemase	*In vivo* process, three enzymes cloned in *E. coli*. Process for L-amino acids under development	[14•,45]
	Enantio-selective production	L-*tert*-Leucine	Trimethyl pyruvic acid	Leucine dehydrogenase	NADH-regeneration with formate dehydrogenase in an EMR. Scale: tons/year	[14•,46]

The use of enzymes in the chemical industry in Europe
Schmid et al. 36

Commercialized bioprocesses

A series of processes operate on the **multi-hundred or thousand tons/year** scale, which **illustrates the technological feasibility and increasing acceptance of biocatalysis for industrial organic synthesis**