APPLICATION OF HYDROLASES TO THE ENZYMATIC SYNTHESIS OF COSMETIC INGREDIENTS

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ABSTRACT : The cosmetic industry looks for substances able to preserve and take care of the skin. Biotechnology is one of the way to prepare substances able to satisfy the consumers needs. Lipases and glycosidases constitute enzymes families with strong potentialities for the synthesis of many ingredients. These enzymes work in synthesis as well as hydrolysis and catalyze chemio-, regio- and stereo-selectives reactions. Examples could be given such as the modification of antioxidants like vitamin A and C, the synthesis of natural dyes like indigoids, but also the synthesis of biosurfactants like glucamides.

KEYWORDS : enzymatic synthesis, lipases, •-glucosidases, vitamins, dyes, biosurfactants, cosmetic ingredients.

RESUME : L'industrie des cosmétiques recherche des substances capables de préserver et de prendre soin de la peau. La biotechnologie est une des manières de préparer des substances capables de satisfaire les besoins des consommateurs. Les lipases et les glycosidases constituent des familles d'enzymes avec des fortes potentialités pour la synthèse de molécules

d'intérêts. Ces enzymes fonctionnent en synthèse aussi bien qu'en hydrolyse et catalysent des réactions chimio-, régio- et stéréo-sélectives. Nous présentons des exemples de synthèse tel que la modification d'antioxydants comme les vitamines A et C, la synthèse de colorants naturels comme les indigoïdes, mais également la synthèse de biosurfactants comme les glucamides.

MOTS CLE : synthèse enzymatique, lipases, •-glucosidases, vitamines, colorants, biosurfactants, ingrédients pour les cosmétiques.

INTRODUCTION

Cosmetics are commercially available products that are used to improve the appearance of the skin. Since the late 1980s, consumer demand for more effective products that more substantively beautify the appearance has resulted in increased basic science research and product development in the cosmetics industry. The result has been more ingredients that may actually improve not just the appearance of the skin, but the health of the skin as well. We now have products that renew, restore, and rejuvenate not just cleanse, protect, and moisturize.

Among the cosmetics ingredients we find i) actives molecules intended to the care of the skin, such as antioxidants, acidifiers, hydrating ; ii) molecules intended to the esthetic such as the dyes for the make-up; iii) molecules intended to the washing such as surfactants to the shampooing and the soaps.

Antioxidants like vitamin C and A are at present generally considered to be beneficial components from fruit, vegetables and plants. The anti-oxidative properties of these compounds are often claimed to be responsible for the protective effects of these food components against cardiovascular disease, certain forms of cancer and/or photosensitivity diseases. In addition, beneficial health effects in ageing have also been related to antioxidant action [1].

Natural dyes are so called because they are obtained from plants (e.g., alizarin, catechu, indigoids and logwood), from animals (e.g., cochineal, kermes, and purple), and from certain naturally occurring minerals (e.g., ocher and Prussian blue). They have been almost entirely replaced in modern dyeing by synthetic dyes. However, for the cosmetic industry, the natural dyes are always of interest. Indeed it is well known that plants can produces dyes with certain impurities that makes natural dye a more pleasing tinge than synthetic dye [2, 3]. The example of the indigoid biosynthesis will be described in the article.

Soap is an ancient surfactant known since the dawn of civilization. Although the oldest synthetic surfactant, sulfonated castor oil or turkey red oil, was produced over a century ago, synthetic surfactants based upon either fats or petroleum have been developed industrially only during the past four decades. During this period the synthetic gradually replace soap so that at present the latter has practically disappeared from all products used for cleaning, laundering, textile scouring, and so forth. The synthetic surfactants of petrochemical origin gradually attained a dominant position. However, because of the current worldwide emphasis on biodegradable surfactants (the biosurfactants), fatty acid

derived surfactants, assume increasingly greater importance, especially for cosmetic industry. In this class, the glycamide surfactants constitute an extremely important group of non-ionic biosurfactants, stable in alkaline media. Potential toxicological effects of these amides are significantly lower than those of emulsifiers derived from petroleum. They are characterized by their skin tolerance, good biologic degradability and low toxicity [4].

SYNTHESIS OF VITAMIN A DERIVATIVES

Vitamin A and derivatives (retinoids) are widely use in cosmetics and pharmaceuticals, in particular to combat skin disorders such cancer, photo aging, psoriasis, ichtyose or acne [5, 6].

Indeed, retinol, vitamin A alcohol, is involved in the control of cellular differentiation and proliferation. This vitamin acts as antioxidant with stabilizing free radicals producing DNA mutations [7 - 9]. Retinol, is the most active of retinoids, but its use in cosmetic formulations produces many disadvantages. First, the molecule is unstable: it's readily oxidized by light, air, oxidizing agents or heat [10]. Moreover, excessive doses of the vitamin become irritant for skin. Finally, retinol is insoluble in aqueous solutions.

Considerable effort has been expended to develop synthetic retinol derivatives and more specifically, retinyl esters. The synthesis of retinyl esters by chemical methods now occurs in developed countries, but there are some serious defects [11, 12]. As an alternative, the use of lipases in non-conventional media to catalyze these synthesis reactions has recently become a much more promising method [13]. Lipase-catalyzed reactions are superior to conventional chemical methods owing to mild reaction conditions, high catalytic efficiency and the inherent selectivity of natural catalysts, which results in much purer products [14, 15].

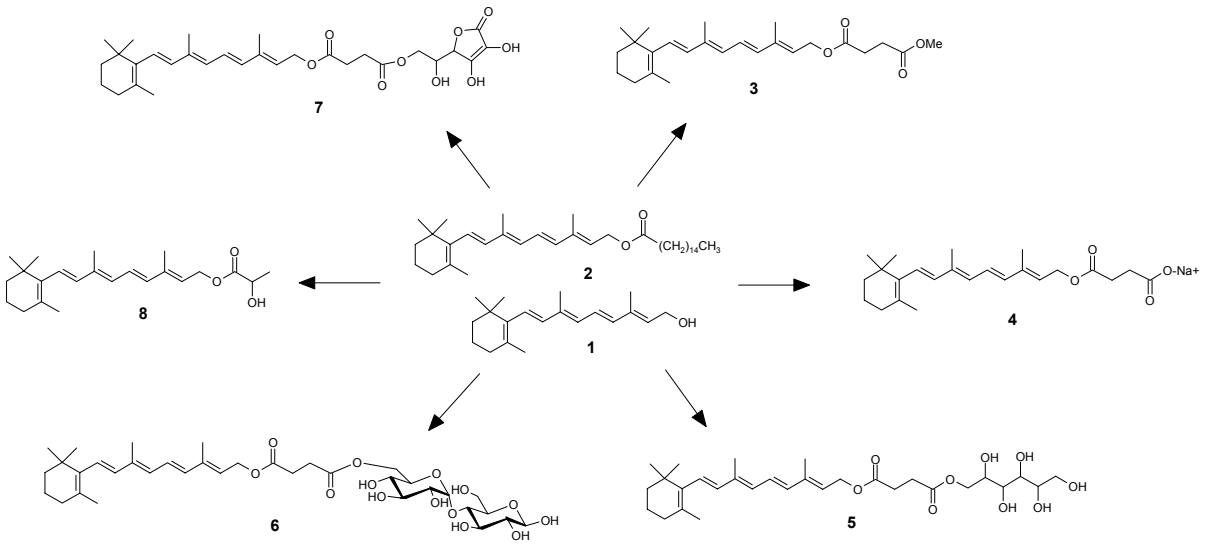
Recently, we have published the enzymatic synthesis of retinol derivatives by reverse hydrolysis, alcoholysis, acidolysis and interseterification. After a wide range of enzymes and solvents were tested, *Candida antarctica* lipase (Novozym 435) and *Rhizomucor miehei* lipase (Lipozyme), were identified to be the most effective catalysts for retinol acylation using dimethyl adipate or adipic acid as the acyl donor. *Rhizomucor miehei* lipase showed a best activity only in apolar solvents such as hexane, whereas *Candida antarctica* lipase showed a best activity in all solvents used (Table 1) [16, 17].

Among the different synthesized compounds, some are water-soluble such as the carbohydrates derivatives (non-ionic water-soluble retinol derivative) and sodium salts of retinyl diacids (ionic water-soluble retinol derivative) (Scheme 1). The water solubility was estimated by the log P (Table 2) [18]. For each reaction, the optimal yield of synthesis is functions of solvent, molar ratio of substrates, concentration of substrates, temperature and water activity [19, 20].

Table 1. *Candida antarctica* lipase and *Rhizomucor miehei* lipase catalyzed-acylation of retinol by alcoholysis with dimethyl adipate [21]

Solvent	Log P	Retinol conversion with *C. antarctica* lipase (%, 24 h)[a]	Retinol conversion with *R. miehei* lipase (%, 24 h)[a]
Dioxane	-1.1	47	1
Acetonitrile	-0.33	61	3
Acetone	-0.24	59	0
Tert-butanol	0.73	52	4
Tert-amyl alcohol	1.22	53	5
Toluene	2.5	67	70
Hexane	3.5	64	81

[a]Determined from relative peak areas on HPLC chromatograms.



Scheme 1. *Candida antarctica* lipase catalysed synthesis of methyl retinyl succinate **3**, sodium salt retinyl succinate **4**, sorbityl retinyl succinate **5**, maltosyl retinyl succinate **6**, ascorbyl retinyl succinate **7** and retinyl lactate **8** from retinol **1** or retinyl palmitate **2**

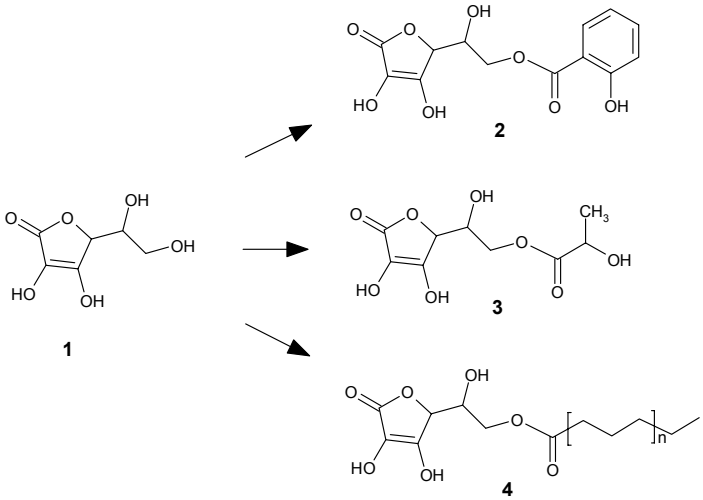
Table 2. Log P of retinol and derivatives

Retinol derivatives	Com-pounds	Log P[a]	Retinol derivatives	Com-pounds	Log P[a]
Retinyl palmitate	2	15.51	Sorbityl retinyl succinate	5	5.69
Methy retinyl adipate		9.64	Retinyl adipate Na^+		5.55
Retinyl adipate		9.36	Retinyl succinate Na^+	4	4.56
Methyl retinyl succinate	3	8.66	Saccharose retinyl adipate		3.94
Retinyl succinate		8.38	Maltose retinyl adipate		2.54
Retinol	1	7.62	Maltose retinyl succinate	6	1.56
Retinyl lactate	8	7.58	Palmitic acid		6.96
Sorbityl retinyl adipate		6.67	Palmitic acid[b]		7.17

[a]Determined from LogKow program methodology [18].
[b]Palmitic acid as reference : Determined by the experiments.

SYNTHESIS OF VITAMIN C DERIVATIVES

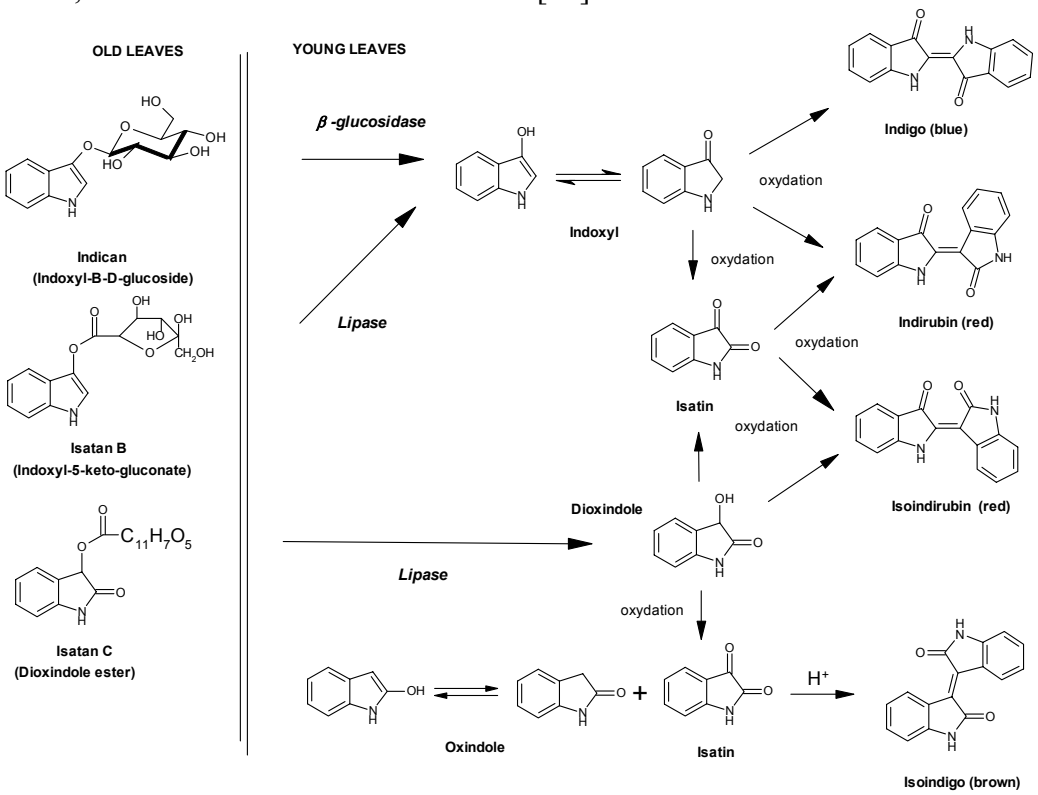
Vitamin C (ascorbic acid) and derivatives are widely used as natural antioxidant and are valuable agents in the treatment of photo aging, skin cancer, and numerous skin disorders. However, its highly hydrophilic behavior prevents its application in cosmetics or in the presence of fats and oils. This can be circumvented by using fatty acid esters of vitamin C, which, due to an amphiphilic structure, not only improve the solubility and miscibility in a more hydrophobic environment but also seem to enhance the radical scavenging performances compared to its free counterpart. Recent studies indicate that lipophilic vitamin C esters are much more effective in the prevention of low density lipoprotein per-oxidation [22]. At present, 6-0-palmitoyl ascorbic acid is produced commercially by chemical means. This is encountered with a number of disadvantages, which reside in the use of less biocompatible chemicals and solvents, the formation of by-products due to the instability of vitamin C and, hence, low yields.
The alternative application of lipase for the synthesis of optically pure compounds, modification of fats and oils and the modification of carbohydrates is well documented in literature [23]. Although the lipase-catalyzed synthesis of vitamin C fatty acid esters, such as 6-0-ascorbyl palmitate and 6-0-ascorbyl oleate has been already described with good yieds [24, 25].
Recently in my group, we have describe the synthesis of a new type of vitamin C esters, the hydroxy acids esters of vitamin C (Scheme 2)[17, 26]. The ascorbyl lactate and the ascorbyl salicylate produced may be considered as an excellent carrier of lactic and salicylic acid. Indeed, in the presence of esterase-type epidermal enzymes, they can undergo hydrolysis, thus releasing lactic and salicylic acids progressively. Derived from fruit and dairy products, hydroxy acids are widely included in cosmetics as exfoliants. The most commonly used are lactic acid, glycolic acid and salicylic acid, all three seem to exert slight but significant effects in reducing skin discolorations and roughness when applied in a cream [27, 28]. Significant irritation is often associated with the use of hydroxy acids alone. Esters of vitamin C and hydroxy acids are unusually effective as skin conditioners, with significant reductions in the irritation problems characteristic of vitamin C and hydroxy acids in nonesterified form.



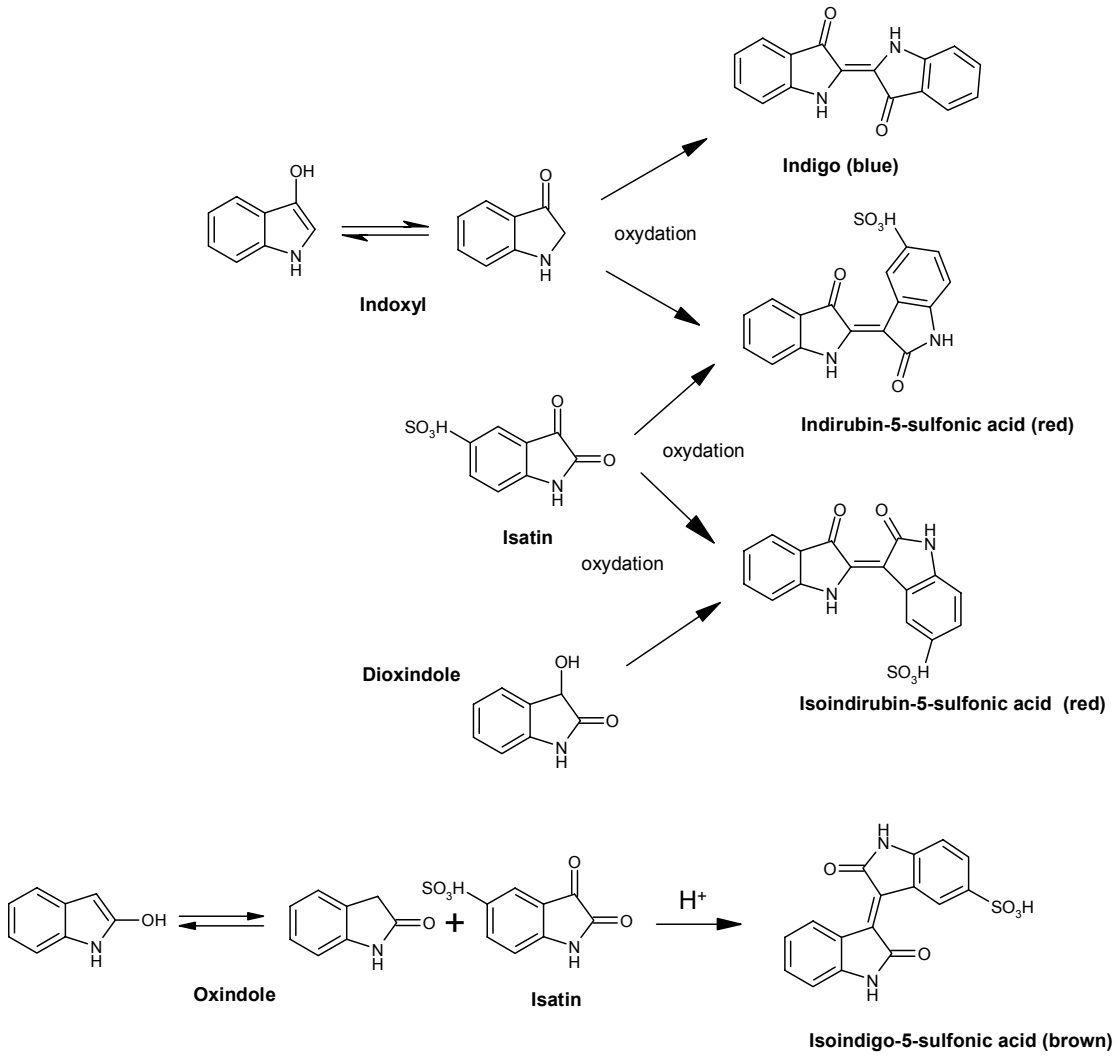
Scheme 2. *Candida antarctica* lipase catalysed synthesis of ascorbyl salicylate **2**, ascorbyl lactate **3** and ascorbyl fatty acid ester **4** from ascorbic acid **1**

SYNTHESIS OF NATURAL INDIGOIDS

Indigo is considered to be the oldest dye, with uses known in ancient times [29]. The dye was generally extracted from various species of plants such as *Indigofera* (tropical species, Africa, Asia, East Indies and South America), *Polygonum tinctorium* (Far East, China, Korea) and *Isatis tinctoria*, woad (Europe). At the end of the 19th century synthetic indigo almost completely replaced natural indigo. The actual annual production of synthetic indigo is estimated as 22000 tons of dyestuff [30]. More recently, due to the importance of natural indigo, considerable research has been performed to replace chemical synthesis of indigo by an application of biotechnological methods [31 - 35]. This is especially so in the cosmetic and textile industries where regulatory pressure has encouraged the development and marketing of natural compounds. Indeed, plants contain in addition to *trans*-indigo (indigotin, blue) and *trans*-indirubin (isoindigotin, red), certain impurities such as *cis*-indigo (blue), *cis*-indirubin (isoindirubin, red), indigo brown (isoindigo), indigo gluten, indigo yellow and traces of flavonoids, that makes natural indigo a more pleasing tinge than synthetic indigo [36, 37]. Indigo is an artifact of secondary metabolism. *Indigofera tinctoria* and *Polygonum tinctorium* contain indican (indoxyl-β-D-glucoside) which serves as starting material for indigo production, whereas *Isatis tinctoria* (woad), contains isatan B (indoxyl-5-ketogluconate), as the major indigo precursor and indican, as the minor indigo precursor. In recent experiments we have shown for the first time that young leaves of *Isatis tinctoria*, harvested in June, contained isatan C, a novel indigo precursor, in addition to isatan B and indican [38].



Scheme 3. *Rhizomucor miehei* Lipase and *Aspergillus niger* •-glucosidase catalysed synthesis of natural indigoids dyes



Scheme 4. Synthesis of sulfated indigoids dyes

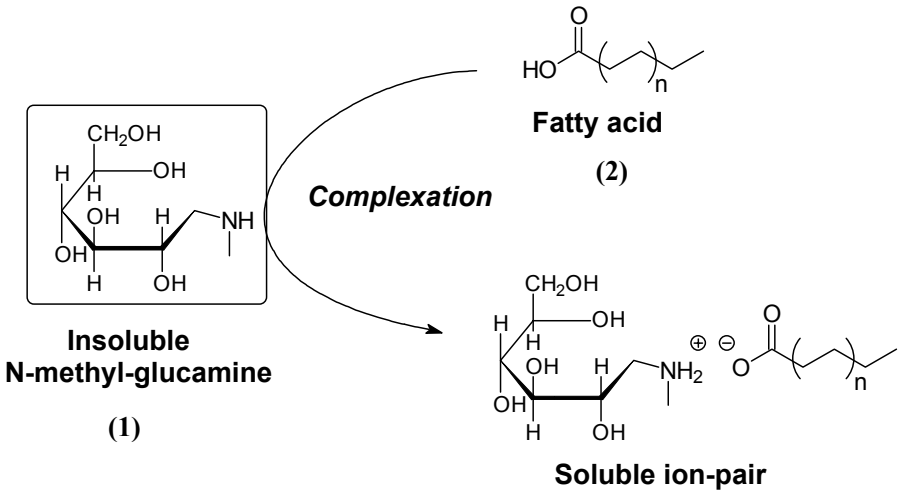
We have shown that indigo precursors after extraction can be broken down by hydrolases. *Rhizomucor miehei* Lipase hydrolyzes isatan B and isatan C, whereas *Aspergillus niger* •-glucosidase hydrolyzes indican. Indoxyl liberated from isatan B, isatan C and indican can be oxidized by air yielding indigo. Isatin is generated from indoxyl and/or dioxindole in an oxygen-rich environment as a side reaction, and dioxindole is generated from isatan C. The condensation of indoxyl with isatin produces indirubin, whereas the condensation of dioxindole with isatine yields isoindirubin and indirubin, which are by-products of indigo biosynthesis (Scheme 3) [39].
Furthermore, it is possible to replace isatin by an isatin derivative (as isatin 5-sulfonic acid) in order to produce a sulfated indigoids (as indirubin 5-sulfonic acid), which is a water soluble dyes of interest for the cosmetics but also a cycline-depended kinases (CDKs) inhibitor of interest for the pharmaceuticals (Scheme 4) [40].

SYNTHESIS OF BIOSURFACTANTS CONTAINING AMIDE BOND

Glycamide surfactants are non-ionic biosurfactants in which the hydrophilic moiety (an amino-alditol derivative) and the hydrophobic moiety (a fatty acid) are linked via an amide bond [4]. Biosurfactants containing amide bonds are a particularly attractive class of compounds that are potential substitutes for emulsifiers derived from petroleum. Potential toxicological effects of these amides are significantly lower than those of emulsifiers derived from petroleum. They are characterized by their skin tolerance, good biologic degradability and low toxicity. This results in a chemical linkage that is highly stable under alkaline conditions, which is of key interest for many surfactant applications [41]. Such sugar fatty amide surfactants can be obtained by chemical synthesis, using for example the Schotten-Baumann reaction between an amino-alditol and a fatty acid chloride in aqueous alkaline medium. An important drawback of this approach is the formation of salts by neutralization.

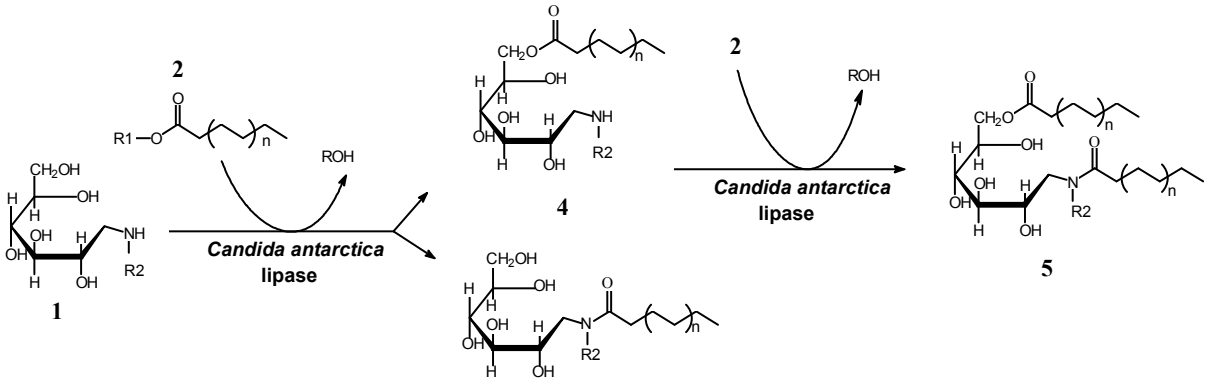
An alternative approach consists in using an enzymatic synthesis route, which avoids the formation of by-products and salts. The synthesis of amides can be catalyzed by proteases, using reverse hydrolysis. However, proteases are generally highly specific for the given amino acid and sensitive to organic solvents [42]. Besides proteases, lipases have proven to catalyze the synthesis of amides in non-conventional media [43] and involved in the obtention of peptides [44, 45], fatty amides [46 - 48], N-acyl-amino acids [48 - 51] and acyl-amino-propanol [52]. However, the yields reported were too low to allow any industrial development.

Thus, we have studied the possibility of catalyzing the amidification of a widely available amino-alditol derivative, N-methyl-glucamine, by fatty acids, using commercially available immobilized lipase preparations as catalysts. N-methyl glucamine is easily obtained by reductive amination of D-glucose with methylamine. The efficient coupling of N-methyl-glucamine to fatty acid can be catalyzed with *Rhizomucor miehei* Lipase in hexane or with *Candida antarctica* lipase in *tert*-amyl alcohol as solvents. In hexane, the formation of a salt complex between the fatty acid and N-methyl-glucamine allows the efficient acylation of the amine. Fatty acid conversion is limited to 50%, due to salt formation (Scheme 5) [53].



Scheme 5. Ion-pair formation between a fatty acid and N-methyl-glucamine

In *tert*-amyl alcohol conversion yield of the fatty acid up to 100% can be obtained by removing the water co-product under reduced pressure [54]. Acido-basic conditions allow the control of the reaction chemo-selectivity [55]. This reaction can also be completed with using various amines and by using triglycerides or fatty acid esters as acyl donors, which opens the way to the valorization of plant oils for surfactant synthesis (Scheme 6) [56].



R1 : H ou CH_3
R2 : CH_3 ou C_8H_{17}

2a : oleoyl
2b : stearoyl
3c : palmitoyl
3d : lauroyl
3e : decanoyl
3f : adipoyl
3g : lactoyl

Scheme 6. Enzymatic synthesis of amide, ester and amide-ester derivatives

CONCLUSION

In this article, we showed that lipases and glycosidases constitute enzymes families with strong potentialities for the synthesis of many cosmetic ingredients. We produced vitamins A and C derivatives like antioxydants, dyes of the indigoids family and finally glucamides like biosurfactants. Some of the synthesized compounds showed a real potential for the cosmetics industry and are currently under development [57].

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