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Enzymatic transformation of flavonoids

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Review

Abstract

There is a growing interest in natural pharmacologically active compounds with either health-protecting or therapeutic properties to be used in medicine, pharmaceutical, cosmetic or food industry. Flavonoids are excellent candidates for this role due to their potent beneficial biological activities. However, many of dietary flavonoids are glycosylated, and therefore their use in certain fields is limited, especially due to their low solubility in lipophilic systems. An effective method of resolving this disadvantage has been introduced, i.e. enzyme-mediated acylation of sugar moieties of flavonoid glycosides with either aliphatic or aromatic molecules. Moreover, specific modifications of a flavonoid skeleton may confer several beneficial characteristics to the parental molecule. Additionally, it can be interesting to increase natural flavonoid diversity and produce potentially bioactive derivatives by enzymatic conversion.

This review summarizes to-date knowledge on flavonoid biotransformations. The key factors affecting enzyme-mediated acylation of flavonoids, such as the type and origin of the biocatalyst, the structure and concentration of the substrates (acyl donor, acyl acceptor and their ratio), the reaction media, and the reaction temperature, are being discussed relating to the reaction rate and the performance of the biocatalytic process.

Keywords: flavonoid transformation, enzymes, acyl donor and acceptor, reaction media

Introduction

Flavonoids are without a doubt an important group among natural plant products, gaining special interest due to their role in reducing the risk of chronic diseases, such as cancer, cardiovascular and neurodegenerative diseases (Li et al., 2007; Naruszewicz et al., 2007; Walle, 2007; Manach et al., 2005; Heo and Lee, 2005; Heo and Lee, 2004; Parillo and Riccardi, 2004; Mojžišová and Kuchta, 2001; Middleton et al., 2000; Willet, 1995). It is established that these beneficial properties of flavonoids are mostly attributed to their ability to scavenge free radicals, chelate metal ions, activate antioxidant enzymes or inhibit certain enzyme systems (Lin and Weng, 2006; Havsteen, 2002; Harborne and Williams, 2000). Due to the above-mentioned beneficial effects, there is a tremendous demand for flavonoids in food, pharmaceutical and cosmetic industry. However, their use is limited due to low solubility and stability in lipophilic preparations. Therefore, the enzyme-catalyzed transformation via esterification of the hydroxyl groups with aliphatic or aromatic molecules has been introduced. This approach enables not only to increase the lipophility of flavonoid glycosides but also to improve their bioactivities and pharmacological properties, as well as their ability to penetrate the cell membrane (Katsoura et al., 2006; Mellou et al., 2006; Mellou et al., 2005; Riva et al., 1998; Kodelia et al., 1994). Moreover, the natural biodiversity of flavonoids can be increased by the means of biotransformation (Azerad, 2006). The enzyme-mediated transformation of flavonoids to relevant esters possesses a high degree of regioselectivity. When optimal conditions (enzyme, temperature, media, and substrates) were applied, the conversion yield was often more than 95% (Chebil et al., 2006).

This paper summarizes available data on enzyme-mediated transformation of flavonoids. The main factors influencing the performance of this biocatalytic process are in the center of the attention.

Enzyme-mediated transformations

The use of enzymes or whole-cell systems to modify physicochemical and biological properties of various organic compounds has been of a great scientific and industrial interest. Biotransformation represents a useful tool for production of valuable commodities in chemical synthesis, pharmaceutical industry, biotechnology and agriculture. The increasing demand of complex chiral compounds and of environmentally clean processes is promoting industrial and academic research in the field of biocatalysis (Brenna, 2005; Serra et al., 2005).

Biocatalysis has several advantages over the conventional chemical approach. Enzymes are highly chemo-, regio- and stero-selective, and act under mild reaction conditions (Collins and Kennedy, 1999; Nagasawa and Yamada, 1995; Leuenberger, 1990). Highly purified products can be obtained (Kang et al., 2005) and purification of the reaction system is usually easier than that of the chemical systems (Faber, 2004). Moreover, enzymes enable the catalysis of environmentally-friendly processes. Several reactions can be done simultaneously due to enzyme compatibility (Faber, 2004). Microbial cells or enzymes can be immobilized and reused for many cycles. In addition, enzymes can be over-expressed to make biocatalytic processes economically efficient, and enzymes with modified activity can be tailor-made (Patel, 2006). To date the use of various enzymes (lipases, esterases, proteases and acyl transferases) or whole cells have been investigated in an effort to find the most potent biocatalyst for the selective flavonoid acylation. These enzymes are often in the immobilized forms which improve enzyme stability, facilitate the product isolation, and enable enzyme reuse (Adamczak and Krishna, 2004).

Lipases

Today, lipases stand amongst the most important biocatalysts in industry. Among them, it is the microbial lipases that find the biggest application use. Lipases find use in a variety of biotechnological fields such as pharmaceutical (Gotor-Fernández et al., 2006; Pandey et al., 1999), food, detergent, agrochemical, oleochemical (Houde et al., 2004; Jeager et al., 1999; Pandey et al., 1999; Saxena et al., 1999), leather, pulp and paper industry (Pandey et al., 1999). Lipases can be classified according to sequence alignment into three major groups: mammalian lipases (e.g. porcine pancreatic lipase), fungal lipases (*Candida rugosa* and *Rhizomucor* family) and bacterial lipases (*Staphylococcus* and *Pseudomonas* family) (Hidalgo and Bornscheuer, 2006). More than 50% reported lipases are produced by yeast in the forms of various isozymes (Vakhlu and Kour, 2006). Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) belong to the class of serine hydrolases. They catalyze a wide range of reactions, including hydrolysis, inter-esterification, alcoholysis, acidolysis, esterification and aminolysis (Vakhlu and Kour, 2006). Under natural conditions, they catalyze the hydrolysis of ester bonds at the hydrophilic-hydrophobic interface. At this interface, lipases exhibit a phenomenon termed interfacial activation, which causes a remarkable increase in activity upon contact with a hydrophobic surface. The catalytic process involves a series of differentiated stages: contact with the interface, conformational change, penetration in the

interface, and finally the catalysis itself (Hidalgo and Bornscheuer, 2006). Under certain experimental conditions, such as in the absence of water, they are capable of reversing the reaction. The reverse reaction leads to esterification and formation of glycerides from fatty acids and glycerol (Saxena et al., 1999). This synthetic activity of lipases is being successfully utilized also in flavonoid ester production.

Candida antarctica lipase B (CALB) is one of the most widely used biocatalysts in organic synthesis on both the laboratory and the commercial scale (Anderson et al., 1998; Uppenberg et al., 1995) due to its ability to accept a wide range of substrates, non-aqueous medium tolerance and thermal deactivation resistance (Degn et al., 1999; Anderson et al., 1998; Cordova et al., 1998; Drouin et al., 1997). CALB belongs to α/β hydrolase-fold superfamily with a conserved catalytic triad consisting of Ser105-His224-Asp187 (Uppenberg et al., 1995). It comprises 317 amino acid residues.

The active site contains an oxyanion hole which stabilizes the transition state and the oxyanion in the reaction intermediate (Haeffner et al., 1998). Reaction mechanism of CALB follows the ping-pong bi-bi mechanism which is illustrated in Fig.1. The substrate molecule reacts with serine of the active site forming a tetrahedral intermediate which is stabilized by catalytic residues of His and Asp. In the next step alcohol is released and acyl-enzyme complex is created. A nucleophilic attack (water in hydrolysis, alcohol in transesterification) causes another tetrahydral intermediate formation. In the last step the intermediate is split into product and enzyme is recovered for the next catalytic cycle (Patel, 2006).

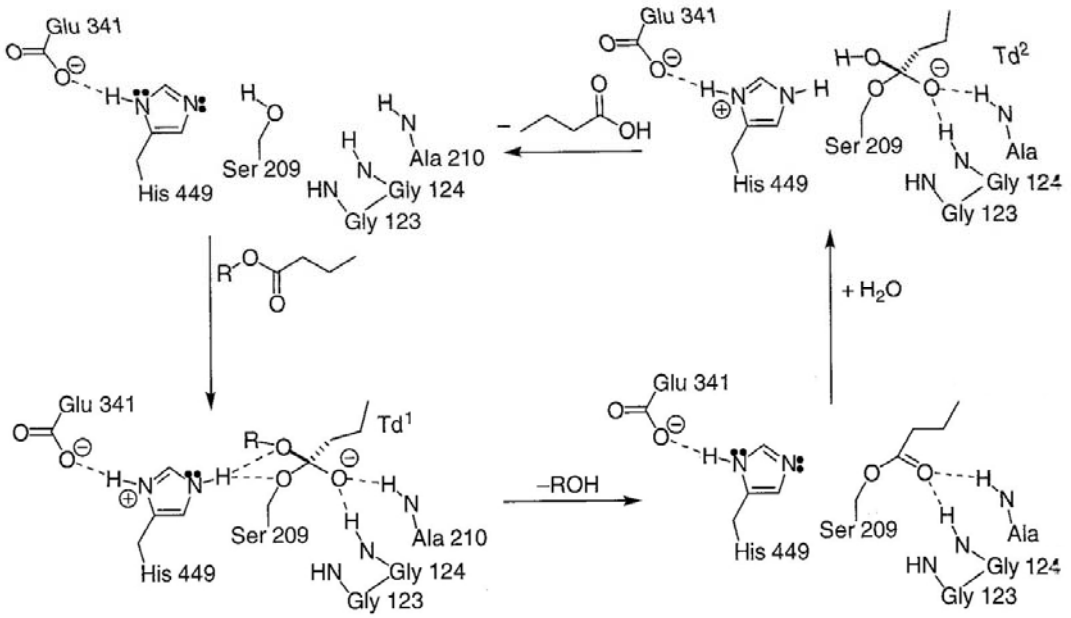
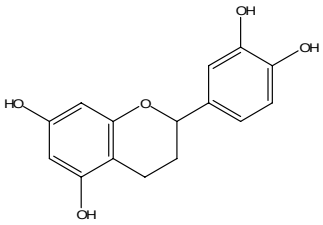
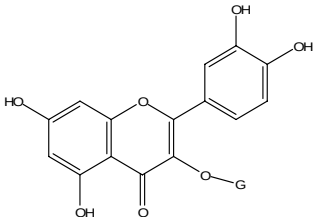
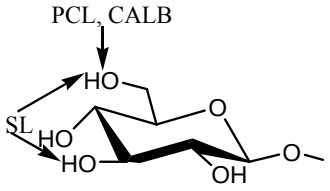
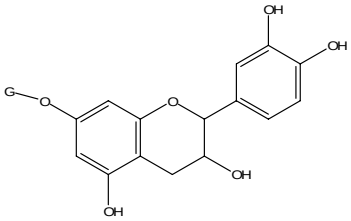
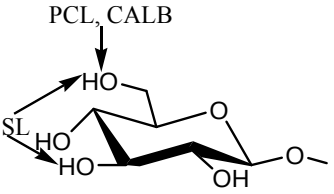
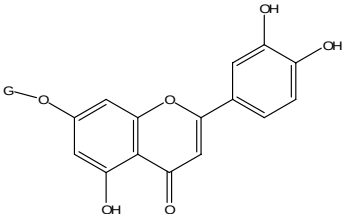
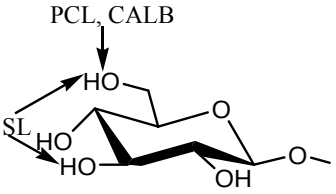
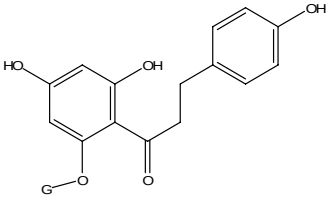
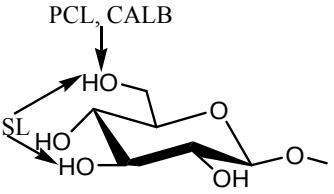
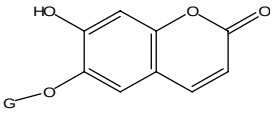
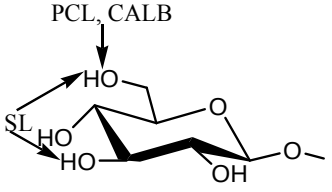


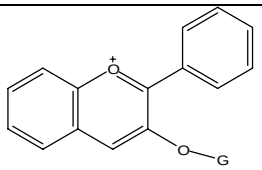
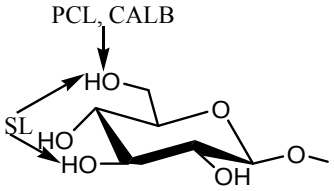
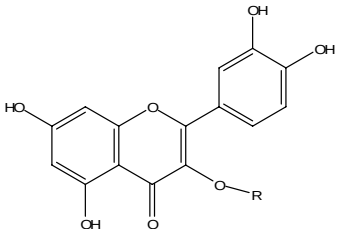
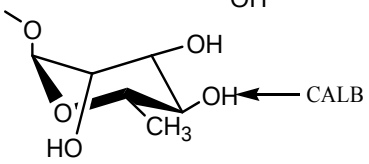
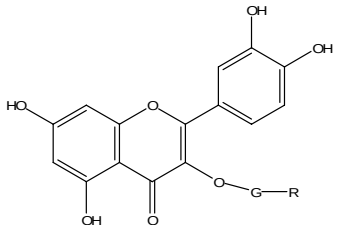
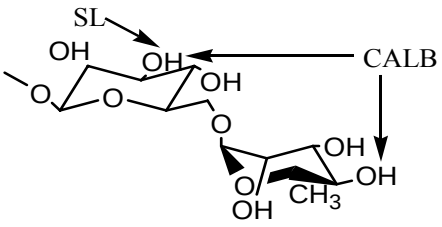
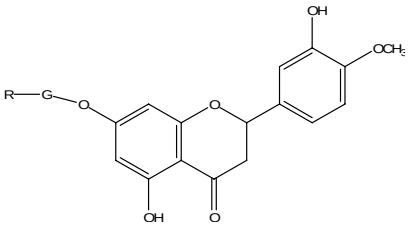
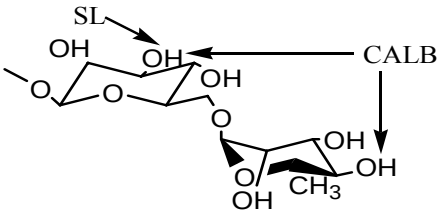
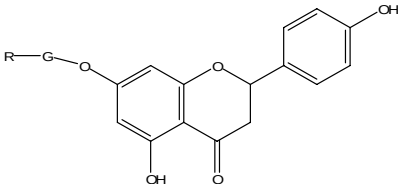
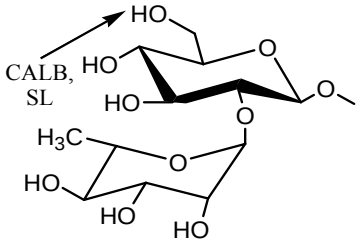
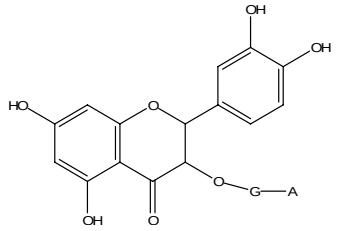
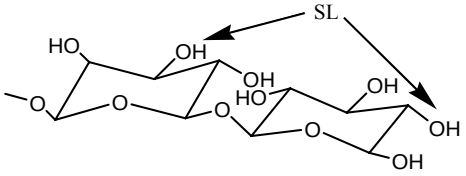
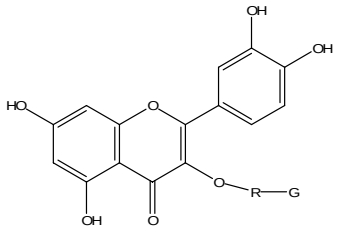
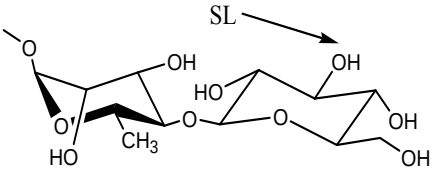
Fig.1 Reaction mechanism catalyzed by *Candida antarctica* lipase (Raza et al., 2001)

The active site of CALB consists of substrate-nonspecific acyl-binding site and substrate-specific alcohol-binding site (Cygler and Schrag, 1997; Uppenberg et al., 1995). It is selective for secondary alcohols (Uppenberg et al., 1995) what is reflected by the geometry of the alcohol-binding site (Lutz, 2004). In contrast to most lipases, CALB has no lid covering the entrance to the active site and shows no interfacial activation (Martinelle et al., 1995).CALB is being frequently used in acylation of various natural compounds such as saccharides, steroids and natural glycosides, including flavonoids (Riva, 2002; Davis and Boyer, 2001).

The proper enzyme selection plays a multiple roles in the flavonoid acylation. The biocatalyst significantly influences the regioselectivity of the reaction. The regioselective flavonoid acylations using lipases, subtilisin and carboxyl-esterase are shown in Tab.1. There is available information mainly on the use of lipases for flavonoid ester synthesis; especially the use of lipase B from *Candida antarctica* which is preferred due to its acceptance of a wide range of substrates, good catalytic activity and a high degree of regioselectivity (Katsoura et al., 2007, 2006; Ghoul et al., 2006; Mellou et al., 2006, 2005; Stevenson et al., 2006; Ardhaoui et al., 2004a, 2004b, 2004c; Passicos et al., 2004; Moussou et al., 2004; Gayot et al., 2003; Ishihara et al., 2003; Kontogianni et al., 2003, 2001; Nakajima et al., 2003, 1999; Ishihara et al., 2002; Gao et al., 2001; Otto et al., 2001; Danieli et al., 1997).

Table 1 The potential acylation sites in flavonoid transformation when *Candida antarctica* lipase (CALB), *Pseudomonas cepacia* lipase (PCL), subtilisin (SL) and carboxyl-esterase (CE) were used as biocatalysts (with modifications from Chebil et al., 2006)

Flavonoid	Structure	Acylation site
Catechin		CE: 3-OH PCL: 5,7-OH
Isoquercitrin		
Catechin-7-*O*-glucoside		
Luteolin-7-*O*-glucoside		
Phloretin-2-*O*-glucoside		
Esculin		

Anthocyanin		
Quercitrin		
Rutin		
Hesperidin		
Naringin		
Peltatoside		
Quercetin derivative		

G = glucose, R = rhamnose, A = arabinose

As for flavonoid aglycons, only two enzymes have been reported to be capable of acylating this skeleton – lipase from *Pseudomonas cepacia* and carboxyl-esterase. Lambusta et al.

(1993) investigated the use of *Pseudomonas cepacia* lipase for catechin modification. They found out that the acylation took place on the C5 and C7 hydroxyls. Sakai et al. (1994) observed that carboxyl esterase showed regio-selectivity towards C3-OH of catechin. Sakai et al. (1994) explored the use of carboxyl esterase from *Streptomyces rochei* and *Aspergillus niger* for the 3-*O*-acylated catechin production.

Esterases and proteases

Esterases (carboxyl esterases, EC 3.1.1.1) have the same reaction mechanism as lipases, but differ from them by their substrate specificity, since they prefer short-chain fatty acids whereas lipases usually prefer long-chain fatty acids. Another difference lies in the interfacial activation (Hidalgo and Bornscheuer, 2006). A classification scheme for esterases is based on the specificity of the enzymes for the acid moiety of the substrate, such as the carboxyl esterases, aryl esterases, acetyl esterases, cholin esterases, cholesterol esterases, etc. (Jeager et al., 1994). In contrast to lipases, only a few esterases have practical uses in organic synthesis, because lipases are generally more entantio-selective and resistant to organic solvents. The most widely used esterase is the preparation isolated from pig liver (Hidalgo and Bornscheuer, 2006). The practical applications of esterases in enzymatic transformation of flavonoids is not very attractive because it enables the implementation only the molecule of a short aliphatic chain length such as acetate, propionate and butyrate (Sakai et al., 1994).

Proteases represent the class of enzymes which occupy a pivotal position with respect to their physiological roles as well as their commercial applications. They perform both hydrolytic and synthetic functions. Since they are physiologically necessary for living organisms, proteases occur ubiquitously in a wide diversity of sources such as plants, animals, and microorganisms. They are also classified as serine proteases, aspartic proteases, cysteine proteases, and metalloproteases depending on the nature of the functional group at the active site. Proteases play a critical role in many physiological and pathophysiological processes. Proteases from microbial sources are preferred to the enzymes from plant and animal sources since they possess almost all the characteristics desired for their biotechnological exploration (Rao et al., 1998). Proteases have a large variety of applications, mainly in the detergent and food industries. In view of the recent trend of developing environmentally friendly technologies, proteases are envisaged to have extensive applications in leather treatment and in several bioremediation processes. Proteases are also extensively used in the pharmaceutical industry (Rao et al., 1998). Proteases are the last group of hydrolytic enzymes which have

been used for flavonoid modification. Protease subtilisin was the first enzyme used for flavonoid ester synthesis conducted by Danieli et al. (1990, 1989). Later on, subtilisin was used for the selective rutin acylation in organic solvents (Xiao et al., 2005; Kodelia et al., 1994). However, it has been reported that reactions catalyzed by subtilisin led to low conversion yields and a low degree of regioselectivity was observed (Danieli et al., 1990). These authors reported that the structure of the sugar moiety affected the regioselectivity. For flavonoid transformation, especially serine proteases (subtilisin) are being used in the ester synthesis (Danieli et al., 1989, 1990; Kodelia et al., 1994). Cell extracts from *Ipomoea batatas* and *Perilla frutescens* have also been investigated for selective flavonoid modification with aromatic acids. The natural catalytic activity of acyltransferases was applied (Nakajima et al., 2000; Fujiwara et al., 1998). This approach is slightly inconvenient since it requires either activated acyl donor (ester of coenzyme A) or the presence *in situ* of a system allowing the synthesis of these derivatives.

Reaction conditions for flavonoid esterification

Acyl donor

As acylations catalyzed by lipases take place through the formation of an acyl-enzyme intermediate, the nature of the acyl donor has a notable effect on reactivity. The ideal acyl donor should be inexpensive, fast acylating, and completely non-reactive in the absence of the enzyme (Ballesteros et al., 2006). Many acylating agents have been tested in flavonoid esterifications, such as aromatic or aliphatic organic acids, substituted or not. A special attention has been paid mostly to the fatty acid ester production (Katsoura et al., 2006; Mellou et al., 2006, 2005; Ardhaoui et al., 2004a, 2004b, 2004c; Enaud et al., 2004; Gayot et al., 2003; Kontogianni et al., 2003). This approach enables to improve flavonoid solubility in lipophilic systems. The proper acyl donor selection may significantly influence not only the physico-chemical but also biological properties of the resulting esters.

A simple way to increase the reaction rate and conversion yield in acylations is to use an excess of acyl donor (Patti et al., 2000). Many authors have tried to determine the optimal molar ratio flavonoid to acyl donor in order to achieve the highest possible yields. The molar ratios 1:1 to 1:15 (acyl acceptor/acyl donor) have been tested, whereas the majority agreed on the ratio 1:5 to be the most suitable for the best reaction performance (Mellou et al., 2006; Gayot et al., 2003; Ishihara et al., 2003, 2002; Kontogianni et al., 2001). A better solution is offered by the use of special acyl donors, which ensure a more or less irreversible reaction.

This can be achieved by the introduction of electron-withdrawing substituents (esters), when higher conversion yields and reaction rates can be obtained. The use of vinyl esters allows several times faster reaction progress than other activated esters do (Ballesteros et al., 2006). An example of the flavonoid esterification (a) and transesterification (b) is presented in Fig.2.

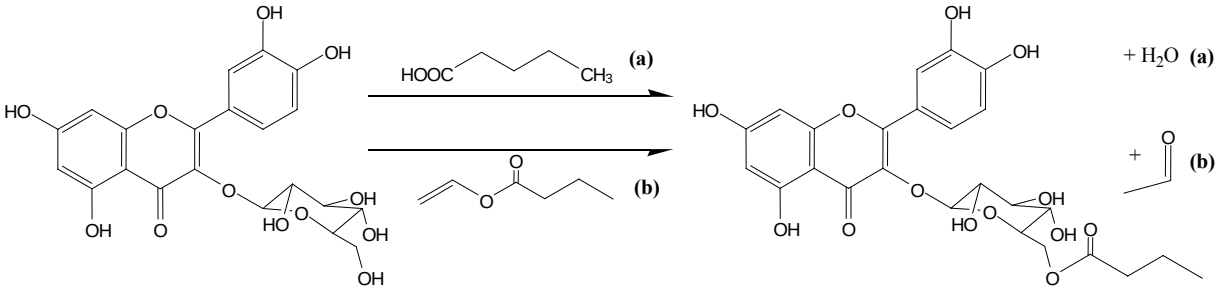


Fig.2 Mechanism of flavonoid esterification (a) and transesterification (b) (Chebil et al., 2006).

Pleiss et al. (1998) studied the acyl binding site of CALB and found out that the enzyme is selective for short and medium fatty acid chain length. This fact may be attributed to the structure of lipase acyl binding pocket which is elliptical, narrow cleft of 9.5 × 4.5 Å. With increasing carbon number of a fatty acid or molecule size, the steric hindrance is involved resulting in low efficiency of the enzymatic reaction (Riva et al., 1988; Wang et al., 1988; Carrea et al., 1989). This fact was experimentally confirmed by Katsoura et al. (2006) and by Viskupičová and Ondrejovič (2007) whose results showed higher performance of the naringin and rutin esterification when fatty acids up to C10 were introduced. This is in contrast with the findings of Ardhaoui et al. (2004b) and Kontogianni et al. (2003) who reported that the fatty acid chain length has no significant effect on conversion yield when fatty acids of a medium and high chain length were used.

Therefore, the effect of fatty acid chain length on the flavonoid acylation still remains a matter of debate. Viskupičová and Ondrejovič (2007) conducted a series of experiments with both saturated and unsaturated fatty acids and found out a correlation between log P of tested acids and conversion yields. It may be interesting to take this parameter into consideration when assessing an influence of acyl donor on the reaction progress.

Only little work has been done in flavonoid esterification with aromatic acids (Stevenson et al., 2006; Enaud et al., 2004; Gao et al., 2001; Nakajima et al., 2000). It has been observed

that the performance of the process mainly depends on the nature of the substitutions, the position of the hydroxyls and the length of the spacers.

Acyl acceptor

The structure of acyl acceptor (flavonoid), especially stereochemistry of glycosidic bonds plays an important role in flavonoid acylation. The majority of work was done on flavonoid glycosides. Naringin and rutin are the most widely used substrates among flavonoids. The structural differences, such as the number and position of hydroxyl groups, the nature of saccharidic moieties, as well as the position of glycosidic bonds, influence the flavonoid solubility, and thus affect overall conversion yield. For naringin molecule, which possesses primary hydroxyl group on glucose, the acylation takes place on the 6"-OH (Katsoura et al., 2006; Konntogianni et al., 2003, 2001; Ishihara et al., 2002; Gao et al., 2001; Otto et al., 2001; Danieli et al., 1990) since the primary hydroxyl is favored by CALB (Fig.3). However, in rutin, which has no primary hydroxyl, either the 3"-OH of glucose (Ishihara et al., 2002; Danieli and Riva, 1994) or the 4"-OH of rhamnose (Fig.3) (Mellou et al., 2006; Ardhaoui et al., 2004a; Ardhaoui et al., 2004c) can be acylated. Whilst Danieli et al. (1997) observed the rutin-3",4"'-*O*-diester formation. When subtilisin was used as biocatalyst, naringin-3"-*O*-ester and rutin-3"-*O*-ester were synthesized (Danieli et al., 1990).

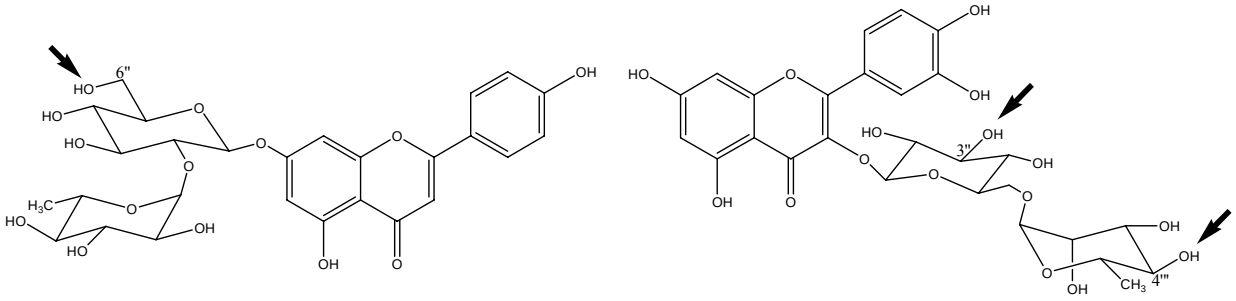


Fig.3 The acylation site of naringin (left) and rutin (right)

The concentration of the flavonoid also affects the performance of the acylation reaction. The conversion yield and the initial rate rise with the increase of flavonoid concentration. However, the amount of flavonoid is limited by its solubility in a reaction medium (Chebil et al., 2007, 2006).

Reaction media

Reaction media play an important role in enzymatic biotransformations. Methodologies for enzymatic flavonoid acylation have focused on searching a reaction medium which allows appropriate solubility of polar acyl acceptor (flavonoid glycoside) and nonpolar acyl donor as well as the highest possible enzymatic activity. Moreover, the medium has often been required to be nontoxic and harmless to biocatalyst. In order to meet the above-mentioned requirements, more scientific teams have dealt with the proper medium selection (Mellou et al., 2005; Kontogianni et al., 2003, 2001; Gao et al., 2001; Nakajima et al., 1999; Danieli et al., 1997; Viskupičová et al., 2006).

Solvents play crucial roles in the synthesis of organic compounds. Non-aqueous biocatalysis has several advantages over conventional aqueous catalysis: the suppression of hydrolytic activity of the biocatalyst which is favored in water (Fossati and Riva, 2006), the enhanced solubility of hydrophobic substrates, the improvement of enzyme enantioselectivity, the exclusion of unwanted side reactions, the easy removal of some products, the enhanced enzyme thermostability and the elimination of an microbial contamination (Rubin-Pitel and Zhao, 2006; Torres and Castro, 2004). Laane (1987) pointed out that log P, as a solvent parameter, correlates best with enzyme activity. Zaks and Klibanov (1988) reported that the activity of lipases was higher in hydrophobic solvents than in hydrophilic ones. Narayan and Klibanov (1993) claimed that it is hydrophobicity and not polarity or water miscibility which is important, whereas the log P parameter can be called a measure of solvent hydrophobicity. Trodler and Pleiss (2008), using multiple molecular dynamics simulations, showed that the structure of CALB possesses a high stability in solvents. In contrast to structure, the flexibility is solvent-dependent; a lower dielectric constant led to a decreased protein flexibility. This reduced flexibility of CALB in non-polar solvents is not only a consequence of the interaction between organic solvent molecules and the protein, but also due to the interaction with the enzyme-bound water and its exchange on the surface (Trodler and Pleiss, 2008). It has been suggested in organic solvents the surface area is reduced which leads to improved packing and increased stability of the enzyme (Toba and Merz, 1997).

Polar aprotic solvents such as dimethyl sulfoxid (DMSO), dimethylformamide (DMF), tetrahydrofuran (THF) and pyridine were first investigated (Nakajima et al., 1999; Danieli et al., 1997). However, it was observed that enzyme activity was readily deactivated in these solvents. To date enzymatic acylation of flavonoids have been successfully carried out in various organic solvents (Tab.2), whereas the most frequently used are 2-methylbutan-2-ol

and acetone because of their low toxicity, its polarity allows the proper solubilization of both substrates, and also the high conversion yields can be achieved.

Table 2 The organic solvents used in flavonoid acylation

Solvent	Reference
2-Methylbutan-2-ol	Ghoul et al., 2006; Ardhaoui et al., 2004a, 2004b, 2004c; Passicos et al., 2004; Gayot et al., 2003
Acetone	Ghoul et al., 2006; Mellou et al., 2006, 2005; Kontogianni et al., 2003, 2001; Ishihara et al., 2003, 2002; Nakajima et al., 2003, 1999; Danieli et al., 1997
Acetonitrile	Ghoul et al., 2006; Ishihara et al., 2003; Nakajima et al., 1999, 1997
2-Methylpropan-2-ol	Ghoul et al., 2006; Stevenson et al., 2006; Mellou et al., 2005; Moussou et al., 2004; Kontogianni et al., 2003, 2001; Otto et al., 2001
Dioxane	Ghoul et al., 2006; Danieli et al., 1997
Pyridine	Danieli et al., 1997, 1990
THF, DMSO, DMF	Kontogianni et al., 2003, 2001; Danieli et al., 1997
Binaric mixtures of solvents	Ghoul et al., 2006; Gao et al., 2001; Nakajima et al., 1999; Danieli et al., 1997

The effect of solvent on conversion yield depends on both the nature of acyl donor and the flavonoid (Chebil et al., 2006). Despite much has been done in this area, it is quite difficult to deduce any general conclusion on solvent choice because the available data are controversial and sometimes even contrary.

Recently, ionic liquids have received growing attention as an alternative to organic solvents used for the enzymatic transformation of various compounds (Katsoura et al., 2006; Kragl et al., 2006; Jain et al., 2005; Lozano et al., 2004; Reetz et al., 2003; Van Rantwick et al., 2003). The potential of these "green solvents" lies in unique physicochemical properties such as non-volatility, nonflammability, thermal stability and good solubility for many polar and less polar organic compounds (Jain et al., 2005; Wilkes, 2004; Itoh et al., 2003; Van Rantwick et al., 2003). Probably the most promising advantage of the use of ionic liquids is their potential application in food, pharmaceutical and cosmetic preparations due to their

reduced toxicity (Jarstoff et al., 2003). Due to the many above-mentioned advantages of the ionic liquids for the enzyme-mediated transformations, several flavonoid esters have been recently prepared in such media (Katsoura et al., 2007; Kragl et al., 2006; Katsoura et al., 2006). The biocatalytic process showed significantly higher reaction rates, regioselectivity and yield conversions compared to those achieved in organic solvents. Thus ionic liquid use seems to be challenging approach to the conventional solvent catalysis.

Solvent-free approach for the elimination of the co-solvent of the reaction has been recently introduced as an alternative for conventional solvents (Enaud et al., 2004; Kontogianni et al., 2003, 2001). It is based on the use of one reactant in the role of the solvent. The authors reported rapid reaction rates; however, the conversion yields were slightly decreased. In spite of the attractiveness, the use of solvent-free systems is characterized by a serious drawback due to the necessity of the elimination of the excess of the acyl donor for the recovery of the synthesized products (Chebil et al., 2006).

Water content is a crucial parameter in lipase-catalyzed synthesis as it alters the thermodynamic equilibrium of the reaction towards hydrolysis or synthesis. Moreover, it is involved in noncovalent interactions which keep the right conformation of an enzyme catalytic site (Foresti et al., 2007). The amount of water required for the catalytic process depends on the enzyme, its form (native or immobilized), the enzyme support, and on the solvent nature (Arroyo et al., 1999; Zaks and Klibanov, 1988). In general the water amount which is considered to be optimal for esterifications in organic solvents is 0.2 – 3 % (Rocha et al., 1999; Yadav and Piyush, 2003; Iso et al., 2001). The enzymatic esterification of flavonoids in non-aqueous media is greatly influenced by the water content of the reaction system (Ardhaoui et al., 2004b; Gayot et al., 2003; Kontogianni et al., 2003). Ardhaoui et al. (2004b) observed the best enzyme activity when water content was maintained at 200 ppm. Gayot et al. (2003) found out that the optimal value of water in an organic reaction medium equals 0.05% (v/v). Kontogianni et al. (2003) reported that highest flavonoid conversion was reached when initial water activity was 0.11 or less.

Temperature

Temperature represents a significant operating factor in enzyme-catalyzed reactions. It affects viscosity of the reaction medium, enzyme stability and substrate and product solubility. Since lipase from *C. antarctica* belongs to thermostable enzymes, the improved catalytic activity at higher temperatures has been observed (Arroyo et al., 1999). To date, flavonoid

transformation has been carried out in the temperature range 30 – 100 °C (Ghoul et al., 2006; Katsoura et al., 2006; Stevenson et al., 2006; Mellou et al., 2005; Ardhaoui et al., 2004a, 2004b, 2004c; Moussou et al., 2004; Passicos et al., 2004; Enaud et al., 2004; Gayot et al., 2003; Kontogianni et al., 2003; Ishihara et al., 2002; Gao et al., 2001; Otto et al., 2001; Nakajima et al., 1999; Danieli et al., 1990). The choice of temperature depends on the enzyme and solvent used. The majority of authors have chosen for flavonoid acylations 60 °C because the best enzyme activity and highest yields of resulting esters were allowed (Ghoul et al., 2006; Katsoura et al., 2006; Stevenson et al., 2006;; Ardhaoui et al., 2004a, 2004b, 2004c; Moussou et al., 2004; Passicos et al., 2004; Enaud et al., 2004; Gayot et al., 2003; Otto et al., 2001; Viskupičová et al., 2006).

Conclusion

Current results on enzymatic esterification of flavonoids with fatty acids suggest that this method is useful tool for the implementation of these compounds into lipophilic systems. Moreover, many studies have shown that by selecting appropriate acyl donors, an improvement of bioavailability and biological properties of parental molecules is possible to be achieved. The performance and regioselectivity of the enzyme-catalyzed flavonoid transformation is affected by several factors, including the type of enzyme, the nature of reaction medium, operating conditions, water content in the media, the structure and concentration of substrates and their molar ratio. By varying of these factors, significant changes in ester production and regioselectivity can be achieved.

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