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REVIEW

Rutin fatty acid esters: from synthesis to biological health effects and application

JANA VISKUPICOVA – TIBOR MALIAR

Summary
Flavonoids are promising compounds with a significant therapeutic potential. However, rutin efficiency after oral administration is limited due to its poor bioavailability. This paper presents perspectives of rutin fatty acid esters prepared by selective enzyme-mediated synthesis. The process is affected by several factors, such as acyl donor, acyl acceptor, ratios of reactants, solvent, temperature, reaction time and water content. Targeted products retain the antioxidant activity of the parental compound rutin in both hydrophilic and hydrophobic medium but, compared to rutin, the derivatives express increased antioxidant activity in lipophilic models, such as low density lipoprotein oxidation assay, ß-carotene linoleate system or Rancimat test. Moreover, rutin esters have enhanced inhibitory activity toward certain proteases, mainly to serine proteinases of the trypsin-like family. A very interesting finding is the modulatory effect of rutin fatty acid esters on sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA1 isoform) activity. Interestingly, upon oxidation by peroxynitrite (150 μmol·l^{-1}), rutin derivatives exert a dose-response hormetic effect on SERCA1 activity. Moreover, cytotoxic/anti-proliferative and cytogenetic activities of certain rutin fatty acid esters were reported. Based on these findings, rutin fatty acid esters may find application in pharmaceutical, food and cosmetic industrial fields.

Keywords
rutin; fatty acids; acylation; antioxidant activity; proteinase inhibition; Ca^{2+}-ATPase activity modulation; applications

In recent years, there has been a growing demand for efficient bioactive compounds due to increasing incidence of chronic diseases. Structures derived from natural sources play a significant role in the process of drug discovery and may provide selective ligands for disease-related targets. Flavonoids, a group of secondary plant metabolites, have been gaining increasing attention in experimental studies in vitro and in vivo. Based on current knowledge, they appear to represent promising structural groups to be used in prevention and/or treatment of various chronic diseases due to their potent antioxidant, anti-inflammatory, anticancer, antidiabetic, antiviral and neuroprotective properties. There is an inverse correlation between the intake of flavonoid-rich diet and the risk of cardiovascular as well as several chronic diseases [1–6]. These beneficial effects on human health rank flavonoids among the most attractive natural products to support current therapeutic possibilities.

Quercetin is one of the commonly consumed and widely studied flavonoids. It is mainly available in the form of glycosides, such as quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-rhamnoside (quercitrin), quercetin-3-*O*-galactoside (hyperoside), quercetin-3-*O*-glucoside (isoquercetin), quercetin-3-*O*-arabiniside (guajaverin) and quercetin-3-*O*-xyloside [7]. Rutin (rutoside, quercetin-3-*O*-rutinoside) is the glycoside of the flavonol quercetin with the disaccharide rutinose (α-L-rhamnopyranosyl-(1→6))-β-D-glucopyranose). The highest content of rutin was detected in capers (3.33 g·kg^{-1}), black olives

Jana Viskupicova, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dubravska cesta 9, 841 04 Bratislava, Slovakia; Department of Biotechnologies, Faculty of Natural Sciences, University of Ss. Cyril and Methodius, Nam. J. Herdu 2, 917 01 Trnava, Slovakia.
Tibor Maliar, Department of Biotechnologies, Faculty of Natural Sciences, University of Ss. Cyril and Methodius, Nam. J. Herdu 2, 917 01 Trnava, Slovakia.

Correspondence author:
Jana Viskupicova, tel.: +421 232 295 710, e-mail: janaviskupicova@gmail.com

(0.45 g·kg^{-1}), buckwheat (0.36 g·kg^{-1}), black tea (0.20 g·l^{-1}), black raspberry (0.19 g·kg^{-1}) and asparagus (0.23 g·kg^{-1}) [8]. A number of pharmacological activities of rutin have been reviewed in various experimental models, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective effects [9].

Glycosylated flavonoids are in general relatively hydrophilic compounds, thus their application in food matrices, pharmaceutical or cosmetic formulations is limited. Therefore, flavonoid lipophilization, e.g. via lipase-catalysed esterification/transesterification, was introduced in order to increase the solubility and miscibility of glycosylated flavonoids in hydrophobic environments [10]. Flavonoids are unstable due to both the presence of oxygen at the heteroatomic ring C, and due to the presence of many hydroxyl groups in their structures. Thus, they might be degraded, by light, oxygen/oxidizing agents and/or by high temperature, to the related quinones. Acylation of flavonoids with respective fatty acids may represent a solution to increase the stability of flavonoids in lipophilic media [11]. Another drawback is the poor bioavailability of flavonoids. According to pharmacokinetic data, bioavailability of rutin in humans was reported to be rather low (the maximum plasma concentration C_{max} = 0.20 μmol·l^{-1} ± 0.06 μmol·l^{-1}, the mean time to reach the maximum plasma concentration t_{max} = 6.5 h ± 0.7 h) compared to quercetin glucosides (C_{max} = 1.46 μmol·l^{-1} ± 0.45 μmol·l^{-1}, t_{max} = 1.1 h ± 0.3 h) [12]. Selectively acylated rutin derivatives are likely to reach target tissues/cells more readily and thus might be orally more active pharmacological drugs than parent flavonoid molecules. Indeed, rutin transformation to fatty acid esters was reported to improve certain bioactivities, such as antioxidant, anti-proliferative or enzyme inhibitory [13–15].

This review paper provides available information on rutin fatty acid esters synthesized, with their most important biological effects in vitro, as well as on potential practical applications of these hydrophobized rutin derivatives in foods, pharmaceuticals or cosmetics.

Synthesis of rutin esters

Over the past 20 years, there has been a substantial effort to implement acylation strategies into practice. In plants, flavonoid acylation was reported to modulate physiological activity of the resulting flavonoid esters by altering solubility, stability, reactivity and interaction with cellular targets [16].

Presently, enzyme-catalysed flavonoid esterification in organic media is a well-mastered technique for synthesis of selectively acylated flavonoids. The key factors, which influence regioselectivity and the performance of the enzymatic acylation of flavonoids, include type and concentration of enzyme, structure and concentration of substrates (acyl donor, acyl acceptor and their ratio), nature of the reaction media, water content in the media, reaction temperature and nature of the reaction, as reviewed in [10, 17].

Viskupicova et al. [13] previously synthesized rutin derivatives, esterified by fatty acids of chain lengths of C4–C22. Conversion of rutin to a respective rutin fatty acid ester increased with the decreasing length of the carbon chain, with the highest yield achieved when using butyrate as acyl donor. The nuclear magnetic resonance (NMR) analysis of the derivatives prepared showed that the 4'''-OH position at rhamnose moiety was acylated (Fig. 1) [13]. The same acylating position on rutin molecule was identified also by Mellou et al. [14, 18], Razak et al. [19], Katsoura et al. [20] and by Ardhaoui et al. [21–23]. Other research groups reported that rutin acylation may also take place in the 3''-OH position of glucose moiety (Fig. 1) [24, 25]. Alternatively, both the 3''-OH position of glucose and the 4'''-OH position of rhamnose can be acylated, yielding rutin-3'',4'''-*O*-diester (Fig. 1) [26]. The differences in rutin acylation are caused by several factors, most importantly by the solvent type, reaction temperature, acyl donor structure, as well as type and concentration of the enzyme used. Reaction conditions, including enzyme, acyl donor, solvent, temperature, time of incubation and percentage of conversion, employed for the synthesis of rutin fatty acid esters are summarized in Tab. 1. As evident, lipase B from *Candida antarctica*, reaction media such as acetone or 2-methylbutan-2-ol, temperature of 50–60 °C and fatty acids of C4–C22 have been most frequently used. Based on the reaction conditions selected, rutin conversion to appropri-

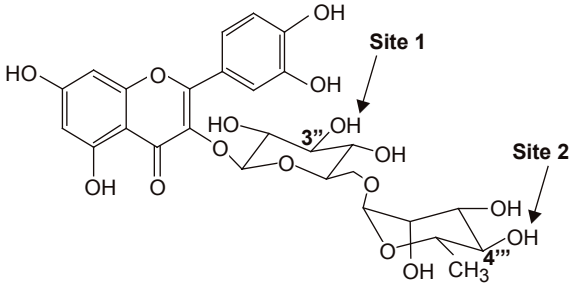

Fig. 1. Acylation sites on rutin molecule.

Tab. 1. Reaction conditions for rutin fatty acid ester synthesis.

Enzyme	Acyl donor (fatty acid)	Solvent	Operating conditions	Conversion yield	Ref.
CAL-B	C4–C18	2-Methylbutan-2-ol	60 °C, 168 h	28–62 %	[13]
CAL-B (Novozym 435)	C18:1, C18:2, C18:3	Acetone	50 °C, 96 h	70 %	[14]
CAL-B	C12	Acetone	20–55 °C, 120 h	20–56 %	[19]
Novozym 435, Lipozyme TLIM, Lipozyme RMIM	C12, C18, C18:1	Ionic liquids, acetone	60 °C, 96 h 50 °C, 96 h	16–51 %	[20]
CAL-B	C6–C18	2-Methylbutan-2-ol	60 °C, 150 h	42–76 %	[21, 22]
CAL-B (Novozym 435)	C2	Pyridine, acetone	45 °C, 45 h	91 %	[26]
	C8, C10, C12	Acetone, THF, 2-Methylbutan-2-ol, solvent-free	45 °C, ns	< 25 %	[27, 28]
Subtilisin	C4	Pyridine	45 °C, 48 h	67 %	[29]
CAL-B (Novozym 435)	C12, C16	Acetone	50 °C, 96 h	70–77 %	[30]
	PUFA concentrate of C18–C22	Acetone	50 °C, 96 h	30 %	[31]
	C18:3 (*n*-3)	Acetone	55 °C, 60 h	92.6 %	[32]
	C16	2-Methylbutan-2-ol	60 °C, 80 h	2 %	[33]
Subtilisin	C4	Pyridine	45 °C, 8 h	90 %	[34]

CAL-B – lipase B (EC 3.1.1.3) from *Candida antarctica*, Novozym 435 – CAL-B lipase (EC 3.1.1.3) immobilized on an acrylic resin, Lipozyme TLIM – lipase (EC 3.1.1.3) from *Thermomyces lanuginosus* immobilized on a non-compressible silica gel carrier, Lipozyme RMIM – lipase (EC 3.1.1.3) from *Rhizomucor miehei* immobilized on a macroporous ion-exchange resin, subtilisin – a serine endopeptidase (EC 3.4.21.62), PUFA – polyunsaturated fatty acids, THF – tetrahydrofuran, ns – not specified.

ate fatty acid ester varied from 2 % to 93 %.

Besides the reaction conditions described in Tab. 1, other factors have also been reported to play an important role in rutin fatty acid ester synthesis. These include water content in reaction media, molar ratio of acyl acceptor/acyl donor (rutin/fatty acids), nature in terms of saturation and molecule length of acyl donors. Water content in reaction media is an important parameter as it alters the thermodynamic equilibrium of the reaction towards hydrolysis or synthesis. Ardhaoui et al. [21] reported the best enzyme activity and the highest rutin conversion when water content in media was kept lower than 200 mg·kg^{-1}. Kontogianni et al. [27, 28] observed the highest conversion yield when water activity of the media was 0.11 or less. The molar ratio of rutin/acyl donor of 1:5 has been preferred for optimal lipase-catalysed acylation performance [13, 14, 28, 29]. Since lipase-catalysed acylation involves the formation of an acyl-enzyme intermediate, the structure of acyl donor has a significant effect on reactivity. To date, acyl donors with chain lengths ranging from short (C2) to long (C30) have been introduced to flavonoid skeleton in acylation reactions [35], with highest yields reached either at short [13, 18] or at medium lengths of fatty acid chain [21, 22, 28]. It seems that the use of aliphatic saturated acyl donors resulted in higher ester yields than if (poly)unsaturated fatty acids were used, probably due to different molecular shape, coiled in the latter case due to stereochemistry of double bonds.

Biological effects of acylated rutin derivatives

Selectively acylated flavonoids with different aliphatic or aromatic acids were reported to have improved physico-chemical properties, such as thermostability, light-resistivity [36], lipophilic solubility and partitioning properties [37]. Aliphatic acylation of flavonoids was described to protect glycosides from enzymatic degradation and to enhance pigment solubility in water [38]. Moreover, these modifications were reported to increase affinity to cell membranes (resulting in better penetration) [34, 39], antioxidant activity [13, 18, 20], enzyme-inhibitory [15, 40], anti-proliferative [14], cytogenetic [34] and antimicrobial [18] properties (reviewed by Viskupicova et al. [41]).

Antioxidant activity

Although rutin is a potent antioxidant, its moderately hydrophilic nature is a critical factor for accessibility to the active sites of oxidative damage in vivo. Esterification of the hydroxyl groups of rutin by fatty acids provides an approach

to increase hydrophobicity and bioavailability, so that lipid oxidation in biological systems can be inhibited more effectively. Increased lipophilicity of rutin fatty acid esters with higher partition coefficient (log *P*) values may lead to its better absorption and distribution. For compounds to have a reasonable probability of being well absorbed, their log *P* values must not be greater than 5.0. In general, the compounds with lower log *P* values have lower extent of absorption, are rapidly eliminated from an organism and, therefore, have a lower systemic effect on physiology. Targeted rutin derivatives acylated by medium chain fatty acids (C6–C14) with log *P* values between 1 and 5 are expected to reach optimal absorption and clearance properties, thus favourable bioavailability and pharmacological response may be achieved. Several research groups reported that medium to long chain fatty acid derivatives of rutin prevented lipid peroxidation more effectively than did rutin or short chain rutin fatty acid esters [13, 14, 37, 42].

The human low density lipoprotein (LDL) oxidation assay is considered to be a biologically relevant in vitro assay for evaluation of antioxidant effects in vivo. Investigations on LDL oxidation performed by Lue et al. [37] revealed that rutin laurate was the most effective compound in inhibiting oxidation by prolonging LDL lag time for an in vitro system, compared to rutin palmitate, parent molecule rutin and synthetic hydrophobic antioxidant butylated hydroxytoluene (BHT), which is frequently used as commercial antioxidant in the food and cosmetic sectors. Acylation of rutin with medium or long chain fatty acids may result in improved antioxidant abilities in more complex systems, including LDL-oxidation assays. Likely reasons may concern improved lipophilic solubility and partitioning properties allowing for better accessibility to the actual site of oxidation [37]. Katsoura et al. [20] investigated the antioxidant potential of rutin derivatives acylated with lauric, stearic and oleic acids on the oxidation of LDL. They found that the introduction of oleic acid (C18:1), the main fatty acid of olive oil, led to improvement of the antioxidant effect towards oxidation of LDL or serum, compared to non-acylated rutin and its saturated acyl derivatives. The authors hypothesized that the lipophilic properties of antioxidants may affect their incorporation into the lipid part of the LDL particle, reaching the site of lipid peroxidation more readily. Apart from an increase in lipophilicity, other mechanisms, related to the structure of the acyl donor (e.g. presence of unsaturated bonds), may also act on the antioxidant properties of flavonoids [20].

Mbatia et al. [31] evaluated the antioxidant activities of both rutin and vanillyl esters acylated with a concentrate of *n*-3 polyunsaturated fatty acids (PUFAs), recovered from fish oil, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and thiobarbituric acid reactive substances (TBARS) assays. In the DPPH· assay, rutin esters showed better activity than did vanillyl esters and, on the contrary in lipophilic medium, vanillyl esters were found to be superior to rutin esters. In emulsion, the esters showed better activity than α-tocopherol. Moreover, by attaching to natural phenolics, PUFAs were protected against oxidation and PUFA improved the hydrophobicity of the phenolic, which could enhance its function in lipid systems [31].

Viskupicova et al. [13] also studied the effect of rutin derivatives acylated with C4–C18 on DPPH radical scavenging, inhibition of lipid peroxidation in β-carotene linoleate bleaching model and their potential to act as food antioxidants according to the inhibition of lipid peroxidation in sunflower oil using the Rancimat test. The DPPH radical-scavenging capacity of rutin esters was four to five times higher than that of the comparative antioxidant, BHT. Induction times of esters in the Rancimat test increased with their hydrophobicity, suggesting their potential to increase stability of sunflower oil when added to it. Of the rutin esters tested, rutin stearate exhibited the highest protective effect, which was comparable to that of BHT. Esters of rutin with palmitate, stearate, oleate, linoleate and linolenate were found to be the most efficient antioxidative agents in the inhibition of lipid peroxidation in sunflower oil, with an effect higher than or comparable to that of α-tocopherol. The results suggest that lipophilic rutin esters may be useful agents in protection of oil-based foods against oxidation [13]. The mechanism of the antioxidant effect in lipid oxidation inhibition may influence its activity in different media, which may also have an impact on the relationship between polarity and antioxidant activity [43]. However, antioxidant efficiency depends on many factors, which cannot be fully controlled just by addressing one of them, e.g. hydrophobicity.

Sørensen et al. [42] studied the antioxidant effect of two esters of rutin acylated with C12 and C16 in fish oil-enriched milk emulsions as a model for a complex food system. Rutin laurate showed significantly better antioxidant properties in milk emulsion compared with the parent molecule rutin and with rutin palmitate. According to Sørensen et al. [42], the optimal alkyl chain length for reaching the highest antioxidant capacity of rutin esters is below 16 carbon atoms. When hydrophobicity of

the lipophilized compound increases above a certain level, the lipophilized compound has been suggested to form micelles in the aqueous phase [42].

Findings of Duan et al. [44] also indicate that esterification of the 4'''-OH position in the rhamnose moiety may improve the antioxidant activity of rutin. The antioxidant activities of rutin esters acylated with capric, lauric or stearic acid were assessed using the TBARS assay and based on the inhibitory effects on oxidative modification of lecithin induced by Fe^{2+}. The results showed that all the three rutin esters effectively inhibited oxidation and the effects of rutin-4'''-*O*-laurate or rutin-4'''-*O*-caproate were better than that of rutin [44].

Warnakulasuriya et al. [45] found that fatty acid derivatives of quercetin-3-*O*-glucoside acted as better antioxidants in the oil-in-water emulsion system than the initial molecule. Derivatives acylated with eicosapentaenoic, decosahexaenoic or α-linolenic acid showed significantly higher inhibition of Cu^{2+}- and peroxyl radical-induced LDL oxidation in comparison to non-acylated quercetin-3-*O*-glucoside [45]. The authors also observed that pre-incubation with α-linolenic, eicosapentaenoic or docosahexaenoic acid esters of quercetin-3-*O*-glucoside led to a significantly greater cell viability of both human lung fibroblasts (WI-38) and human primary hepatocytes upon exposure to hydrogen peroxide. Only in human primary hepatocytes, cytoprotection against oxidative stress induced by hydrogen peroxide was observed in the presence of quercetin-3-*O*-glucoside esters acylated by oleic or linoleic acids [46]. Acylation may also enhance the inhibitory activity of glycosylated flavonoids towards the formation of acrylamide in a lipid food system [47].

The results suggest that rutin lipophilization with medium to long chain fatty acids might represent a promising way to increase the antioxidant activity of rutin. However, the antioxidant effect of lipophilic rutin esters tested in different experimental systems (aqueous, lipid or emulsions) may lead to different conclusions.

Enzyme inhibitory properties

Flavonoids were reported to modulate enzyme activities in vitro as well as in vivo through various mechanisms, including covalent association, non-covalent association, allosteric effect, and also via binding to ATP-binding sites on enzymes/receptors, or influencing Ca^{2+} homeostasis within cells [48]. Quercetin was shown to bind into the ATP-binding pocket, thus causing cooperative inhibition of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) [49]. Experimental evidence suggests that acylated flavonoid derivatives may increase enzyme inhibitory activities [15, 50, 51].

Inhibition of sarco/endoplasmic reticulum Ca^{2+}-ATPase activity

Viskupicova et al. [15, 52] studied the effect of rutin fatty acid esters on the sarco/endoplasmic reticulum (SR) calcium transport ATPase (SERCA) protein isolated from rabbit fast-twitch skeletal muscle. This enzyme is responsible for maintaining calcium homeostasis within cells and its dysfunction has been associated with various pathological states. SERCA1 isoform is specific for SR in fast-twitch skeletal muscle.

Viskupicova et al. [15, 52] indicated that selective rutin lipophilization may increase affinity of rutin to membrane proteins such as SERCA1 and thus modulate SERCA1 activity [14, 50]. The authors showed that lipophilic rutin derivatives acylated with fatty acids of chain lengths of 16–22 caused significant concentration-dependent decrease of SERCA1 activity with the half-maximum inhibitory concentrations (IC_{50}) ranging from 23 $\mu mol \cdot l^{-1}$ to 64 $\mu mol \cdot l^{-1}$. Moreover, significant conformational alterations in the transmembrane region of the enzyme were observed. The strongest SERCA1 inhibition was exerted by rutin arachidonate (R20:4) and rutin linoleate (R18:2), with IC_{50} equal to 23 $\mu mol \cdot l^{-1} \pm 6.5$ $\mu mol \cdot l^{-1}$ and 25 $\mu mol \cdot l^{-1} \pm 5.5$ $\mu mol \cdot l^{-1}$, respectively. The inhibition was probably associated with binding of the derivatives to the transmembrane region of the enzyme in the vicinity of calcium binding sites, as supported by in silico studies. Interestingly, upon oxidation by peroxynitrite (150 $\mu mol \cdot l^{-1}$), rutin derivatives exerted a dose-response hormetic effect on SERCA1 activity, i.e. prevention of enzyme activity decrease at low concentrations (< 50 $\mu mol \cdot l^{-1}$) and inhibition of SERCA1 activity at high concentrations (> 50 $\mu mol \cdot l^{-1}$) [15]. Lipophilic rutin derivatives also caused a significant decrease in membrane fluidity in a concentration-dependent manner, as shown by fluorescence anisotropy of erythrocyte membranes [53]. Tsuchiya [54] suggested that structure-dependent membrane interactions of flavonoids, which modify membrane fluidity, may be associated with flavonoid bioactivity in the membrane lipid phase. The ability of flavonoids to interact with membranes at the water-lipid interface seems to contribute to their antioxidant capacity [55].

Antiprotease activity

Current knowledge suggests that polyphenolic compounds have the ability of selective inhibition of a wide range of enzymes including proteases

with potential pathological effects [56–59]. Strong inhibitory effects of polyphenols, including flavonoids, on various serine proteinases was reported [56, 60, 61].

VISKUPICOVA et al. [40] investigated the ability of polyphenol fatty acid esters to inhibit the activity of serine proteases trypsin, thrombin, elastase and urokinase. Rutin derivatives esterified with fatty acid of medium to long, mono- and polyunsaturated chains (e.g. laurate, myristate, palmitate, stearate, oleate, linoleate, linolenate, arachidonate and erucate) showed effective serine protease inhibition activities in low micromolar concentration range with the most significant inhibitory effect on thrombin (3–14 μmol·l^{-1}), followed by urokinase (9–50 μmol·l-1) and trypsin (10–130 μmol·l^{-1}). Quantitative structure-activity relationship (QSAR) study of the compounds showed that the most significant parameters for individual inhibition activities were the number of hydrogen bond donors for urokinase, molecular volume for thrombin and solvation energy for elastase. It has been suggested that increased inhibitory activity of acylated polyphenols may be associated with enhanced hydrophobicity of the compounds. Probably the acyl introduced to the polyphenolic skeleton would provide an interaction with the hydrophobic region of serine enzymes, thus providing a higher inhibitory activity. However, nonspecific interactions of the longer acyl chains are also possible and these may result in a decrease in protease activity through aggregation induced by hydrophobic interactions [40] or may act by so called lipophilic cork effect, i.e. by breaking the interaction between ligand molecule and solvation sphere around the enzyme-inhibitor complex.

Cytotoxic or anti-proliferative effects

The cytotoxic effect of rutin derivatives was tested on various cancer cell lines. VISKUPICOVA et al. [62] investigated the ability of rutin esterified with fatty acids of short to long chains (C6–C20) to inhibit proliferation of colon cancer cells CaCO-2 and mouse leukemic cells L1210. The most potent anti-proliferative properties on both CaCO-2 and L1210 cell lines were displayed by rutin caproate (R6), rutin caprate (R10), rutin stearate (R18) and rutin oleate (R18:1) with IC_{50} values lower than 300 μmol·l^{-1} after 72 h of incubation. Based on fluorescent staining, necrosis in CaCO-2 cells evoked by cytotoxic concentrations of R6, R18 and R18:1 were detected after 24 h. Similarly, apoptotic death of L1210 cells was induced by cytotoxic concentrations of R6, R10 and R18 after 24 h. No correlation between fatty acid chain length and cytotoxic effect was observed [62].

The research group of MELLOU et al. [14] studied angiogenic activity of rutin fatty acid esters and reported a significant decrease in the production of vascular endothelial growth factor (VEGF), a major angiogenic factor that plays a key role in tumor growth, in human K562 lymphoblastoma cells. The parental molecules (rutin and unsaturated fatty acids) alone were not effective [14].

Similarly, SALEM et al. [63] observed that fatty acid esters of quercetin-3-*O*-glucoside acylated with C8–C16 exhibited a dose-dependent antiproliferative activity on CaCO-2 cells, indicating their potential as anti-tumor drugs. The authors suggested that the activity of a compound in a biological system does not only depend on its interaction with cell membranes due to its lipophilicity but also on its affinity to specific cell receptors [63].

KODELIA et al. [34] investigated cytological properties of butyryl-rutin ester. The authors found significant differences between rutin and rutin ester in the induced frequency of micronuclei treated cells. At a dose of 100 μg·ml^{-1} of rutin, 3.5% micronuclei was observed, whereas for a similar dose treatment with rutin-ester, a frequency of 8% of micronuclei was observed. The fact that rutin ester caused formation of micronuclei at significantly higher levels than did rutin alone can be considered the manifestation of a stronger action of the agent on the chromosome owing to its easier penetration into the cell after its esterification [34].

Recent analysis of naringin laurate uptake in macrophage cells revealed that the ester bond was partially degraded in the cell membrane and free naringin molecule was translocated to the cytosol [64]. Regarding the structural similarity of naringin and rutin, it may be supposed that the cytotoxic effect of rutin esters may be associated with the action of rutin inside the cells upon crossing the cell membrane and being released from the ester-bound form.

Moreover, flavonoids were reported to act as multidrug resistance modulators due to direct binding to the nucleotide-binding domain (NBD2) of P-glycoprotein [65]. Increased hydrophobicity through the introduction of prenyl or other alkyl groups into the flavonoid structure resulted in enhanced inhibitory effect on P-glycoprotein-based multidrug resistance [66]. A series of flavonoid dimers were found to be efficient P-glycoprotein modulators that increased cytotoxicity of anticancer drugs in vitro and dramatically enhanced their intracellular drug accumulation [67].

Applications of rutin fatty acid esters

Selectively acylated rutin derivatives represent useful active principles applicable in various industrial fields, such as food, dietetics, cosmetics, dermatopharmaceuticals/pharmaceuticals or in agroindustry (Tab. 2). Current patent inventions refer to cosmetic, pharmaceutical formulations and nutritional products comprising acylated rutin derivatives with fatty acids ranging from C2 to C30, and their practical uses.

Lipid oxidation is one of the most important factors for quality preservation in the food or cosmetics industry. Frequently used antioxidants to control lipid oxidation are synthetic, e.g. butylated hydroxyanisole (BHA) or BHT. Recently, lipophilic phenolic compounds were synthesized to provide "natural" and "healthy" alternatives to synthetic antioxidants [75]. This hydrophobization of phenolic molecules can be achieved either by chemical or enzymatic acylation.

Mbatia et al. [31] showed that the attachment of PUFAs to natural (poly)phenols protected PUFAs against oxidation. Moreover, PUFAs improved the hydrophobicity of the phenolic, thus enhancing its solubility and stability in lipid systems. PUFAs are vital for a wide range of biological functions and are implicated in the prevention of cardiovascular diseases.

The invention of Moussou et al. [11] refers to esters of flavonoids such as flavones, flavonols (including rutin), flavanones, flavanols, flavanolols, isoflavones, anthocyanins, proanthocyanidins, chalcones, aurones and hydroxycoumarins conjugated by an ester bond to an ω-substituted C6–C22

Tab. 2. Overview of physico-chemical and biological properties of lipophilic rutin derivatives in respective application fields.

Application field	Physico-chemical and biological properties of lipophilic rutin derivatives	Reference
Food/ dietetics	↑ stability	[11, 31, 68, 69]
	↑ solubility	[11, 31, 69, 70]
	Improved sensory properties/nutritive value	[71]
Pharmaceuticals	↑ bioavailability	[70]
	↑ penetration through cell membrane	[34, 35, 70]
	↑ anticancer effect	[14, 72]
	Suppress multidrug resistance/inhibit P-glycoprotein	[66, 67]
	↑ free radical-scavenging activity	[13, 37, 71]
	↑ antioxidant effect	[42, 63, 70]
	Inhibit development of diabetes	[71]
	↑ vasodilatory activity	[71]
	↑ prophylaxis of oncological, cardiovascular and neurological diseases	[70]
	↑ antiangiogenic effect	[14]
	↓ vascular endothelial growth factor in human leukemic cells K562	[14]
	↑ antiproteinase activity	[40]
Cosmetics/ dermatopharmaceuticals	↑ inhibition of lipid peroxidation/antioxidant efficiency in lipid systems	[18, 37, 42]
	↑ penetration into the skin	[68]
	↑ photo-protective effect	[68]
	↑ skin protection against UV radiation (UV filters)	[11, 71, 73]
	↑ skin-protective effect	[70, 71]
	↑ anti-aging and anti-wrinkling properties	[70, 71]
	↑ antioxidant and transcription-inhibiting activity	[70, 71]
	↑ skin-protective and anti-aging effect	[74]
	↑ anti-inflammatory properties	[74]
	↑ skin elasticity	[35]
	↑ anti-wrinkling and anti-ageing effect	[35]
	↑ strength of blood capillaries	[35]

(↑/↓) – an increase/decrease in the effect.

fatty acid. These flavonoid derivatives were shown to exhibit excellent skin protecting properties especially against mitochondrial or nuclear DNA damage caused by UV radiation. They showed very good chemical stability and were easily incorporated into cosmetic and pharmaceutical formulations. These ω-substituted fatty acid esters protected skin and scalp against skin aging, oxidative stress and environmental stress (pollutants) or against inflammation. Surprisingly, these esters of flavonoids were found to have the ability to protect the skin cells against damage caused by UV radiation, specifically against UVA and UVB radiation, more effectively than did unesterified flavonoids. Moreover, these esters demonstrated their ability to stimulate the glutathione metabolism of human skin cells after UVA irradiation, i.e. to stimulate their cellular defenses. They have also anti-inflammatory and soothing properties, as demonstrated by the inhibition of released prostaglandin E2 after UVB irradiation. This allows their use as free radical scavengers, anti-oxidants, anti-blotchiness agents, useful for draining treatment, for slimming treatment, for anti-wrinkle treatment, as stimulators of the synthesis of elastin and other extracellular matrix elements, and in toning-up compositions. The compounds may also be used in compositions for applications related to cardiovascular diseases, inflammatory disorders, viral and bacterial infections, venotonic indications, allergies, stabilizing or protecting therapeutic agents [11].

The invention of PERRIER et al. [35] relates to flavonoid esters of at least one flavonoid (including rutin) and an organic monoacid with 3 to 30 carbon atoms. These flavonoid esters may comprise active principles for the manufacture of cosmetic, dermopharmaceutical, pharmaceutical, dietetic or agri-foodstuff compositions. The derivatives can be used for carrying out a tonic treatment for veins, for increasing the strength of blood capillaries, they exhibit an inhibitory effect on blotchiness, an inhibitory effect on chemical, physical or actinic erythema, a treatment for sensitive skin, a decongesting effect, a draining effect, a slimming effect, an anti-wrinkle effect, a stimulating effect on the synthesis of components of the extracellular matrix of the epidermis, a toning effect on the epidermis, an improving effect on the elasticity of the skin and an anti-ageing effect on the skin. Moreover, the compounds of the invention exert lipophilic activity, which provides them with a liposoluble nature and specifically with an affinity to cell membranes and particularly the tissues of the epidermis [35].

CONCLUSIONS

Rutin fatty acid esters possess various biological activities and can interact with multiple cellular or molecular targets. These selectively acylated molecules exert potent antioxidant or anti-radical properties, which are enhanced in lipid/oil matrix compared to the parent molecule rutin. The enzyme inhibitory activity increases with acylation of rutin with fatty acids. This may be associated with a higher degree of penetration through the cell membrane and with enhanced hydrophobicity. Depending on fatty acid chain length, rutin esters might act as possible modulators of calcium signalling pathways via Ca^{2+}-ATPase activity modulation. Selective modification of rutin via lipophilization with fatty acids may represent a new approach to the production of potent low toxic serine protease inhibitors, especially towards thrombin. Moreover, these lipophilic esters express potent cytotoxic or anti-proliferative effects towards various cancer cell lines and may be potentially therapeutically applied for the induction of apoptosis or necrosis in antitumor therapy.

Current research further indicates that amphiphilic or lipophilic antioxidants, such as rutin fatty acid esters, can increase oxidative stability of ω-3 PUFA-enriched food products. The use of efficient antioxidants may lower the amount of antioxidant needed to protect food against lipid oxidation and may, in addition, decrease the costs. These lipophilic compounds may represent food additives that are more “natural” and “healthy” than the existing synthetic antioxidants such as BHT or BHA.

Considering the increased bioavailability or penetration trough the cell membrane of lipophilized rutin derivatives upon oral administration, it is likely that the therapeutic effect, mainly oriented towards oxidative stress-related diseases and cancer, will be enhanced in physiological conditions. However, additional studies are required since there is a lack of in vivo and clinical evaluations and thus the questions of stability, metabolism and in vivo bioactivity must be addressed in order to better understand the action of these prospective compounds.

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REFERENCES

1. Tang, Z. – Li, M. – Zhang, X. – Hou, W.: Dietary flavonoid intake and the risk of stroke: a dose-response meta-analysis of prospective cohort studies. BMJ Open, *6*, 2016, pp. 1–8. DOI: 10.1136/bmjopen-2015-008680.
2. Feliciano, R. P. – Pritzel, S. – Heiss, C. – Rodriguez-Mateos, A.: Flavonoid intake and cardiovascular disease risk. Current Opinion in Food Science, *2*, 2015, pp. 92–99. DOI: 10.1016/j.cofs.2015.02.006.
3. Ponzo, V. – Goitre, I. – Fadda, M. – Gambino, R. – De Francesco, A. – Soldati, L. – Gentile, L. – Magistroni, P. – Cassader, M. – Bo, S.: Dietary flavonoid intake and cardiovascular risk: a population-based cohort study. Journal of Translational Medicine, BioMed Central, *218*, 2015, pp. 1–13. DOI: 10.1186/s12967-015-0573-2.
4. Liu, Y.-J. – Zhan, J. – Liu, X.-L. – Wang, Y. – Ji, J. – He, Q.-Q.: Dietary flavonoids intake and risk of type 2 diabetes: a meta-analysis of prospective cohort studies. Clinical Nutrition, *33*, 2014, pp. 59–63. DOI: 10.1016/j.clnu.2013.03.011.
5. Woo, H. D. – Kim, J.: Dietary flavonoid intake and smoking-related cancer risk: a meta-analysis. PLoS ONE, *8*, 2013, Article ID e75604. DOI: 10.1371/journal.pone.0075604.
6. Knekt, P. – Kumpulainen, J. – Järvinen, R. – Rissanen, H. – Heliövaara, M. – Reunanen, A. – Hakulinen, T. – Aromaa, A.: Flavonoid intake and risk of chronic diseases. The American Journal of Clinical Nutrition, *76*, 2002, pp. 560–568. ISSN: 0002-9165 (print), 1938-3207 (online). <http://ajcn.nutrition.org/content/76/3/560.full.pdf+html>
7. Majewska, M. – Skrzycki, M. – Podsiad, M. – Czeczot, H.: Evaluation of antioxidant potential of flavonoids: an *in vitro* study. Acta Poloniae Pharmaceutica, *68*, 2011, pp. 611–615. ISSN: 0001-6837. <http://www.ptfarm.pl/pub/File/Acta_Poloniae/2011/4/611.pdf>
8. Phenol-Explorer: Database on polyphenol content in foods [online]. Version 3.6. Paris : Institut national de la recherche agronomique, released June 2015 [cit. 6 June 2017]. <http://phenol-explorer.eu/>.
9. Ganeshpurkar, A. – Saluja, A. K.: The pharmacological potential of rutin. Saudi Pharmaceutical Journal, *25*, 2017, pp. 149–164. DOI: 10.1016/j.jsps.2016.04.025.
10. Chebil, L. – Humeau, C. – Falcimaigne, A. – Engasser, J.-M. – Ghoul, M.: Enzymatic acylation of flavonoids. Process Biochemistry, *41*, 2006, pp. 2237–2251. DOI: 10.1016/j.procbio.2006.05.027.
11. Cognis France, S. A. S. (FR). Esters of flavonoids with ω-substituted C6-C22 fatty acids. Inventors: Moussou, P. – Falcimaigne, A. – Ghoul, M. – Danous, L. – Pauly, G. PCT/EP2004/006281. FR Patent Application WO2005000831 A1. 6 January 2005.
12. Manach, C. – Williamson, G. – Morand, C. – Scalbert, A. – Rémésy, C.: Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. The American Journal of Clinical Nutrition, *81*, 2005, pp. 230S–242S. ISSN: 0002-9165 (print), 1938-3207 (online). <http://ajcn.nutrition.org/content/81/1/230S.full.pdf+html>
13. Viskupicova, J. – Danihelova, M. – Ondrejovic, M. – Liptaj, T. – Sturdik, E.: Lipophilic rutin derivatives for antioxidant protection of oil-based foods. Food Chemistry, *123*, 2010, pp. 45–50. DOI: 10.1016/j.foodchem.2010.03.125.
14. Mellou, F. – Loutrari, H. – Stamatis, H. – Roussos, C. – Kolisis, F. N.: Enzymatic esterification of flavonoids with unsaturated fatty acids: Effect of the novel esters on vascular endothelial growth factor release from K562 cells. Process Biochemistry, *41*, 2006, pp. 2029–2034. DOI: 10.1016/j.procbio.2006.05.002.
15. Viskupicova, J. – Majekova, M. – Horakova, L.: Inhibition of the sarco/endoplasmic reticulum Ca2+-ATPase (SERCA1) by rutin derivatives. Journal of Muscle Research and Cell Motility, *36*, 2015, pp. 183–194. DOI: 10.1007/s10974-014-9402-0.
16. Ferrer, J.-L. – Austin, M. B. – Stewart, C. – Noel, J. P.: Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. Plant Physiology and Biochemistry, *46*, 2008, pp. 356–370. DOI: 10.1016/j.plaphy.2007.12.009.
17. Chebil, L. – Anthoni, J. – Humeau, C. – Gerardin, C. – Engasser, J.-M. – Ghoul, M.: Enzymatic acylation of flavonoids: effect of the nature of the substrate, origin of lipase, and operating conditions on conversion yield and regioselectivity. Journal of Agricultural and Food Chemistry, *55*, 2007, pp. 9496–9502. DOI: 10.1021/jf071943j.
18. Mellou, F. – Lazari, D. – Skaltsa, H. – Tselepis, A. D. – Kolisis, F. N. – Stamatis, H.: Biocatalytic preparation of acylated derivatives of flavonoid glycosides enhances their antioxidant and antimicrobial activity. Journal of Biotechnology, *116*, 2005, pp. 295–304. DOI: 10.1016/j.jbiotec.2004.12.002.
19. Razak, N. N. A. – Annuar, M. S. M.: Enzymatic synthesis of flavonoid ester: elucidation of its kinetic mechanism and equilibrium thermodynamic behavior. Industrial and Engineering Chemistry Research, *54*, 2015, pp. 5604–5612. DOI: 10.1021/acs.iecr.5b00996.
20. Katsoura, M. H. – Polydera, A. C. – Tsironis, L. – Tselepis, A. D. – Stamatis, H.: Use of ionic liquids as media for the biocatalytic preparation of flavonoid derivatives with antioxidant potency. Journal of Biotechnology, *123*, 2006, pp. 491–503. DOI: 10.1016/j.jbiotec.2005.12.022.
21. Ardhaoui, M. – Falcimaigne, A. – Ognier, S. – Engasser, J. M. – Moussou, P. – Pauly, G. – Ghoul, M.: Effect of acyl donor chain length and substitutions pattern on the enzymatic acylation of flavonoids. Journal of Biotechnology, *110*, 2004, pp. 265–272. DOI: 10.1016/j.jbiotec.2004.03.003.
22. Ardhaoui, M. – Falcimaigne, A. – Engasser, J. M. – Moussou, P. – Pauly, G. – Ghoul, M.: Acylation of

natural flavonoids using lipase of *Candida antarctica* as biocatalyst. Journal of Molecular Catalysis B: Enzymatic, *29*, 2004, pp. 63–67. DOI: 10.1016/j.molcatb.2004.02.013.
23. Ardhaoui, M. – Falcimaigne, A. – Engasser, J. M. – Moussou, P. – Pauly, G. – Ghoul, M.: Enzymatic synthesis of new aromatic and aliphatic esters of flavonoids using *Candida antarctica* lipase as biocatalyst. Biocatalysis and Biotransformation, *22*, 2004, pp. 253–259. DOI: 10.1080/10242420400005796.
24. Ishihara, K. – Nishimura, Y. – Kubo, T. – Okada, C. – Hamada, H. – Nakajima, N.: Enzyme-catalyzed acylation of plant polyphenols for interpretation of their functions. Plant Biotechnology, *19*, 2002, pp. 211–214. DOI: 10.5511/plantbiotechnology.19.211.
25. Danieli, B. – Riva, S.: Enzyme-mediated regioselective acylation of polyhydroxylated natural products. Pure and Applied Chemistry, *66,* 1994, pp. 2215–2218. DOI: 10.1351/pac199466102215.
26. Danieli, B. – Luisetti, M. – Sampognaro, G. – Carrea, G. – Riva, S.: Regioselective acylation of polyhydroxylated natural compounds catalyzed by *Candida antarctica* lipase B (Novozym 435) in organic solvents. Journal of Molecular Catalysis B: Enzymatic, *3*, 1997, pp. 193–201. DOI: 10.1016/S1381-1177(96)00055-0.
27. Kontogianni, A. – Skouridou, V. – Sereti, V. – Stamatis, H. – Kolisis, F. N.: Regioselective acylation of flavonoids catalyzed by lipase in low toxicity media. European Journal of Lipid Science and Technology, *103*, 2001, pp. 655–660. DOI: 10.1002/1438-9312(200110)103:10<655::AID-EJLT655>3.0.CO;2-X.
28. Kontogianni, A. – Skouridou, V. – Sereti, V. – Stamatis, H. – Kolisis, F.: Lipase-catalyzed esterification of rutin and naringin with fatty acids of medium carbon chain. Journal of Molecular Catalysis B: Enzymatic, *21*, 2003, pp. 59–62. DOI: 10.1016/S1381-1177(02)00139-X.
29. Danieli, B. – De Bellis, P. – Carrea, G. – Riva, S.: Enzyme-mediated regioselective acylations of flavonoid disaccharide monoglycosides. Helvetica Chimica Acta, *73*, 1990, pp. 1837–1844. DOI: 10.1002/hlca.19900730705.
30. Lue, B.-M. – Guo, Z. – Glasius, M. – Xu, X.: Scalable preparation of high purity rutin fatty acid esters. Journal of the American Oil Chemists' Society, *87*, 2010, pp. 55–61. DOI: 10.1007/s11746-009-1471-7.
31. Mbatia, B. – Kaki, S. S. – Mattiasson, B. – Mulaa, F. – Adlercreutz, P.: Enzymatic synthesis of lipophilic rutin and vanillyl esters from fish byproducts. Journal of Agricultural and Food Chemistry, *59*, 2011, pp. 7021–7027. DOI: 10.1021/jf200867r.
32. Wang, L. – Zheng, M. – Huang, Q. – Guo, P. – Zheng, C. – Huang, F.: Biocatalysis synthesis of rutin esters of α-linolenic acid. Chinese Journal of Oil Crop Sciences, *35*, 2013, pp. 717–722. ISSN: 1007-9084. In Chinese.
33. Passicos, E. – Santarelli, X. – Coulon, D.: Regioselective acylation of flavonoids catalyzed by immobilized *Candida antarctica* lipase under reduced pressure. Biotechnology Letters, *26*, 2004, pp. 1073–1076. DOI: 10.1023/B:BILE.0000032967.23282.15.
34. Kodelia, G. – Athanasiou, K. – Kolisis, F. N.: Enzymatic synthesis of butyryl-rutin ester in organic solvents and its cytogenetic effects in mammalian cells in culture. Applied Biochemistry and Biotechnology, *44*, 1994, pp. 205–212. DOI: 10.1007/BF02779657.
35. Coletica. Flavonoide esters and their use notably in cosmetics. Inventors: Perrier, E. – Mariotte, A.-M. – Boumendjel, A. – Bresson-Rival, D. US 09/113,158. U.S. Patent Application US6235294 B1. 22 May 2001.
36. Ishihara, K. – Nakajima, N.: Structural aspects of acylated plant pigments: stabilization of flavonoid glucosides and interpretation of their functions. Journal of Molecular Catalysis B: Enzymatic, *23*, 2003, pp. 411–417. DOI: 10.1016/S1381-1177(03)00106-1.
37. Lue, B.-M. – Nielsen, N. S. – Jacobsen, C. – Hellgren, L. – Guo, Z. – Xu, X.: Antioxidant properties of modified rutin esters by DPPH, reducing power, iron chelation and human low density lipoprotein assays. Food Chemistry, *123*, 2010, pp. 221–230. DOI: 10.1016/j.foodchem.2010.04.009.
38. Nakayama, T. – Suzuki, H. – Nishino, T.: Anthocyanin acyltransferases: specificities, mechanism, phylogenetics, and applications. Journal of Molecular Catalysis B: Enzymatic, *23*, 2003, pp. 117–132. DOI: 10.1016/S1381-1177(03)00078-X.
39. Suda, I. – Oki, T. – Masuda, M. – Nishiba, Y. – Furuta, S. – Matsugano, K. – Sugita, K. – Terahara, N.: Direct absorption of acylated anthocyanin in purple-fleshed sweet potato into rats. Journal of Agricultural and Food Chemistry, *50*, 2002, pp. 1672–1676. DOI: 10.1021/jf011162x.
40. Viskupicova, J. – Danihelova, M. – Majekova, M. – Liptaj, T. – Sturdik, E.: Polyphenol fatty acid esters as serine protease inhibitors: a quantum-chemical QSAR analysis. Journal of Enzyme Inhibition and Medicinal Chemistry, *27*, 2012, pp. 800–809. DOI: 10.3109/14756366.2010.616860.
41. Viskupicova, J. – Ondrejovic, M. – Maliar, T.: Enzyme-mediated preparation of flavonoid esters and their applications. In: Ekinci, D. (Ed.): Biochemistry. Rijeka : InTech, 2012, pp. 263–286. ISBN: 978-953-51-0076-8. DOI: 10.5772/34174.
42. Sørensen, A. D. M. – Petersen, L. K. – de Diego, S. – Nielsen, N. S. – Lue, B.-M. – Yang, Z. – Xu, X. – Jacobsen, C.: The antioxidative effect of lipophilized rutin and dihydrocaffeic acid in fish oil enriched milk. European Journal of Lipid Science and Technology, *114*, 2012, pp. 434–445. DOI: 10.1002/ejlt.201100354.
43. Shahidi, F. – Zhong, Y.: Revisiting the polar paradox theory: a critical overview. Journal of Agricultural and Food Chemistry, *59*, 2011, pp. 3499–3504. DOI: 10.1021/jf104750m.
44. Duan, Y. – Du, Z.-L. – Wu, D. C.: Inhibitory effects of three new rutin esters on oxidative modification of lecithin induced by Fe^{2+}. Natural Product Research and Development, *21*, 2009, pp. 586–588. ISSN: 1001-6880.
45. Warnakulasuriya, S. N. – Ziaullah – Rupasinghe, H. P. V.: Long chain fatty acid acylated

derivatives of quercetin-3-*O*-glucoside as antioxidants to prevent lipid oxidation. Biomolecules, *4*, 2014, pp. 980–993. DOI: 10.3390/biom4040980.
46. Warnakulasuriya, S. N. – Ziaullah – Rupasinghe, H. P. V.: Long chain fatty acid esters of quercetin-3-*O*-glucoside attenuate H_2O_2-induced acute cytotoxicity in human lung fibroblasts and primary hepatocytes. Molecules, *21*, 2016, pp. 452–471. DOI: 10.3390/molecules21040452.
47. Ma, X. – Wang, E. – Lu, Y. – Wang, Y. – Ou, S. – Yan, R.: Acylation of antioxidant of bamboo leaves with fatty acids by lipase and the acylated derivatives' efficiency in the inhibition of acrylamide formation in fried potato crisps. PloS One, *10*, 2015, Article ID e0130680. DOI: 10.1371/journal.pone.0130680.
48. Spencer, J. P. E.: The interactions of flavonoids within neuronal signalling pathways. Genes and Nutrition, *2*, 2007, pp. 257–273. DOI: 10.1007/s12263-007-0056-z.
49. Ogunbayo, O. A. – Michelangeli, F.: Related flavonoids cause cooperative inhibition of the sarcoplasmic reticulum Ca^{2+} ATPase by multimode mechanisms. The FEBS Journal, *281*, 2014, pp. 766–777. DOI: 10.1111/febs.12621.
50. Salem, J. H. – Chevalot, I. – Harscoat-Schiavo, C. – Paris, C. – Fick, M. – Humeau, C.: Biological activities of flavonoids from *Nitraria retusa* (Forssk.) Asch. and their acylated derivatives. Food Chemistry, *124*, 2011, pp. 486–494. DOI: 10.1016/j.foodchem.2010.06.059.
51. Lin, S. F. – Lin, Y.-H. – Lin, M. – Kao, Y.-F. – Wang, R.-W. – Teng, L.-W. – Chuang, S.-H. – Chang, J.-M. – Yuan, T.-T. – Fu, K. C. – Huang, K. P. – Lee, Y.-S. – Chiang, C.-C. – Yang, S.-C. – Lai, C.-L. – Liao, C.-B. – Chen, P. – Lin, Y.-S. – Lai, K. T. – Huang, H. J. – Yang, J. Y. – Liu, C. W. – Wei, W. Y. – Chen, C. K. – Hiipakka, R. A. – Liao, S. – Huang, J. J.: Synthesis and structure-activity relationship of 3-*O*-acylated (–)-epigallocatechins as 5α-reductase inhibitors. European Journal of Medicinal Chemistry, *45*, 2010, pp. 6068–6076. DOI: 10.1016/j.ejmech.2010.10.011.
52. Viskupicova, J. – Strosova, M. – Sturdik, E. – Horakova, L.: Modulating effect of flavonoids and their derivatives on sarcoplasmic reticulum Ca^{2+}-ATPase oxidized by hypochloric acid and peroxynitrite. Neuroendocrinology Letters, *30*, 2009, Supplement 1, pp. 148–151. ISSN: 0172-780X.
53. Augustyniak, A. – Bartosz, G. – Cipak, A. – Duburs, G. – Horakova, L. – Luczaj, W. – Majekova, M. – Odysseos, A. D. – Rackova, L. – Skrzydlewska, E. – Stefek, M. – Strosova, M. – Tirzitis, G. – Venskutonis, P. R. – Viskupicova, J. – Vraka, P. S. – Zarkovic, N.: Natural and synthetic antioxidants: an updated overview. Free Radical Research, *44*, 2010, pp. 1216–1262. DOI: 10.3109/10715762.2010.508495.
54. Tsuchiya, H.: Structure-dependent membrane interaction of flavonoids associated with their bioactivity. Food Chemistry, *120*, 2010, pp. 1089–1096. DOI: 10.1016/j.foodchem.2009.11.057.
55. Erlejman, A. G. – Verstraeten, S. V. – Fraga, C. G. – Oteiza, P. I.: The interaction of flavonoids with membranes: potential determinant of flavonoid antioxidant effects. Free Radical Research, *38*, 2004, pp. 1311–1320. DOI: 10.1080/10715760400016105.
56. Maliar, T. – Jedinák, A. – Kadrabová, J. – Šturdík, E.: Structural aspects of flavonoids as trypsin inhibitors. European Journal of Medicinal Chemistry, *39*, 2004, pp. 241–248. DOI: 10.1016/j.ejmech.2003.12.003.
57. Maliar, T. – Baláž, S. – Tandlich, R. – Šturdík, E.: Viral proteinases - possible targets of antiviral drugs. Acta Virologica, *46*, 2002, pp. 131–140. ISSN: 001-723X (print), 1336-2305 (online). <http://www.aepress.sk/acta/acta302.htm#VIRAL>
58. Mantle, D. – Falkous, G. – Perry, E. K.: Effect of flavonoids on protease activities in human skeletal muscle tissue *in vitro*. Clinica Chimica Acta, *285*, 1999, pp. 13–20. DOI: 10.1016/S0009-8981(99)00050-9.
59. Puri, R. N. – Colman, R. W.: Thrombin- and cathepsin G-induced platelet aggregation: effect of protein kinase C inhibitors. Analytical Biochemistry, *210*, 1993, pp. 50–57. DOI: 10.1006/abio.1993.1149.
60. Mozzicafreddo, M. – Cuccioloni, M. – Eleuteri, A. M. – Fioretti, E. – Angeletti, M.: Flavonoids inhibit the amidolytic activity of human thrombin. Biochimie, *88*, 2006, pp. 1297–1306. DOI: 10.1016/j.biochi.2006.04.007.
61. Parellada, J. – Guinea, M.: Flavonoid inhibitors of trypsin and leucine aminopeptidase: a proposed mathematical model for IC_{50} estimation. Journal of Natural Products, *58*, 1995, pp. 823–829. DOI: 10.1021/np50120a001.
62. Viskupičová, J. – Píšová, P. – Jantová, S.: Cytotoxic effect of rutin fatty acid esters on L1210 and Caco-2 cell lines. In: Nemeček, P. – Ondrejovič, M. (Eds.): International Conference Applied Natural Sciences 2011. Trnava : University SS. Cyril and Methodius, 2011, pp. 336–340. ISBN: 978-80-8105-266-8.
63. Salem, J. H. – Humeau, C. – Chevalot, I. – Harscoat-Schiavo, C. – Vanderesse, R. – Blanchard, F. – Fick, M.: Effect of acyl donor chain length on isoquercitrin acylation and biological activities of corresponding esters. Process Biochemistry, *45*, 2010, pp. 382–389. DOI: 10.1016/j.procbio.2009.10.012.
64. Hattori, H. – Tsutsuki, H. – Nakazawa, M. – Ueda, M. – Ihara, H. – Sakamoto, T.: Naringin lauroyl ester inhibits lipopolysaccharide-induced activation of nuclear factor κB signaling in macrophages. Bioscience, Biotechnology and Biochemistry, *80*, 2016, pp. 1403–1409. DOI: 10.1080/09168451.2016.1156477.
65. Di Pietro, A. – Conseil, G. – Pérez-Victoria, J. M. – Dayan, G. – Baubichon-Cortay, H. – Trompier, D. – Steinfels, E. – Jault, J. M. – de Wet, H. – Maitrejean, M. – Comte, G. – Boumendjel, A. – Mariotte, A. M. – Dumontet, C. – McIntosh, D. B. – Goffeau, A. – Castanys, S. – Gamarro, F. – Barron, D.: Modulation by flavonoids of cell multidrug resistance mediated by P-glycoprotein and related ABC transporters. Cellular and Molecular Life Sciences, *59*, 2002, pp. 307–322. DOI: 10.1007/s00018-002-8424-8.
66. Chan, K.-F. – Zhao, Y. – Burkett, B. A. – Wong, I. L. K – Chow, L. M. C. – Chan, T. H.: Flavonoid dimers as bivalent modulators for P-glycoprotein-based multidrug resistance: synthetic apigenin homodimers

linked with defined-length poly(ethylene glycol) spacers increase drug retention and enhance chemosensitivity in resistant cancer cells. Journal of Medicinal Chemistry, *49*, 2006, pp. 6742–6759. DOI: 10.1021/jm060593+.

67. The Hong Kong Polytechnic University, Mcgill University. Flavonoid dimers and methods of making and using such. Inventors: Chan, T.-H. – Chow, L. M.-C. PCT/IB2007/051730. U.S. Patent Application US8710097 B2. 29 April 2014.
68. Abbott GmbH & Co. KG. Nanokristalle zur verwendung in topischen kosmetischen formulierungen und herstellungsverfahren dafür. (Nanocrystals for use in topical cosmetic formulations and method of production thereof). Inventor: Petersen, R. PCT/EP2007/009943. European Patent Application EP2099420 A1. 16 September 2009.
69. Cognis France S. A. Enzymatic production of acyl flavonoid derivatives. Inventors: Ghoul, M. – Engasser, J.-M. – Moussou, P. – Pauly, G. – Ardhaoui, M. – Falcimaigne, A. PCT/EP2003/013143. U.S. Patent Application US20060115880 A1. 1 June 2006.
70. The Rowett Research Institute. Flavonoid compounds as therapeutic antioxidants. Inventors: Caldwell, S. – Bennett, C. – Hartley, R. – McPhail, D. – Duthie, G. PCT/GB2003/003054. European Patent Application EP1534698 B1. 19 September 2007.
71. Geers, B. – Otto, R. – Weiss, A. – Petersohn, D. – Schroeder, K. R. – Schlotmann, K.: Novel flavone glycoside derivatives for use in cosmetics, pharmaceuticals and nutrition. Inventors: Geers, B. – Otto, R. – Weiss, A. – Petersohn, D. – Schroeder, K. R. – Schlotmann, K. US 12/057,425. U.S. Patent Application US20080176811 A1. 24 July 2008.
72. Purdue Research Foundation. Chemotherapeutic flavonoids, and syntheses thereof. Inventors: Cushman, M. S. – Pezzuto, J. M. – Maiti, A. PCT/US2007/087283. U.S. Patent Application US8946287 B2. 3 February 2015.
73. Wirth, C. – Buchholz, H. – Carola, C.: Cosmetic formulation comprising flavonoid derivatives. Inventors: Wirth, C. – Buchholz, H. – Carola, C. US 12/107,526. U.S. Patent Application US20080199414 A1. 21 August 2008.
74. Merck Patent GmbH. Preparation containing oxidized flavonoid derivatives. Inventors: Carola, C. – Huber, S. – Buchholz, H. PCT/EP2005/011687. U.S. Patent Application US7867993 B2. 11 January 2011.
75. Liu, L. – Jin, C. – Zhang, Y.: Lipophilic phenolic compounds (Lipo-PCs): emerging antioxidants applied in lipid systems. RSC Advances, *4*, 2014, pp. 2879–2891. DOI: 10.1039/C3RA44792H.

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