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Rosmarinic acid - From bench to valuable applications in food industry

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ABSTRACT

Background: Rosmarinic acid (RA) is widely distributed in plant species of Lamiaceae and Boraginaceae families, among others. Structurally RA is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid with well explored biosynthetic pathway, physiological functions in plants and (potential) biological activities. Great number of herbal preparations and food supplements, containing RA, are marketed with claims for beneficial health effects. Furthermore, due to the inhibition of lipid peroxidation and bacterial growth, RA is approved for use as natural antioxidant and/or preservative in food industry.

Scope and approach: The present review will explore the contemporary biotechnological approaches for RA mass-production and will attempt to summarize its main biological properties based on recent studies. Future applications in food industry and potential functional food development will be proposed. Implications for technological and chemical modification of RA aiming to improve its bioavailability will be presented.

Key findings and conclusions: The advances in biotechnological production of RA provide effective and "green" approach which worth further implementation to a large-scale application. Numerous *in vitro* and *in vivo* studies confirm many of the claimed health-promoting effects of RA, as well as, its value as food additive. Despite the recent improvements in RA stability and bioavailability, its use in food products should be thoroughly evaluated until a tight balance between safety and efficiency is set.

1. Introduction

Rosmarinic acid (RA) is a valuable secondary metabolite (SM) distributed in many plant families. According to Petersen (2013) RA can be found in 39 plant species, ranging from hornworts to mono- and di-cotyledonous plants. It is exclusively distributed in Lamiaceae and Boraginaceae, but also found in Anthocerotaceae and Blechnaceae families, and in Rosids and Asterids among dicotyledonous plants (Petersen, 2013). In spite of that, still the main sources of RA are the rosemary plants. At the present moment some of the most exploited sources for RA isolation are the plant species *Rosmarinus officinalis* L., *Ocimum basilicum* L. (Celano et al., 2017; Hirondart, Rombaut, Fabiano-Tixier, Bily, & Chemat, 2020; Saad et al., 2021), *Salvia officinalis* L. (Celano et al., 2017), *Salvia miltiorrhiza* Bunge (Deng et al., 2020) and *Mentha spiciata* L. (Yousefian, Lohrasebi, Farhadpour, & Haghbeen, 2020).

Rosmarinic acid is a phenolic molecule with a variety of applications, ranging from food preservatives to cosmetics (Georgiev et al., 2012) and is also a molecule of interest for the pharmaceutical industry (Petersen, 2013). Rosmarinic acid has been reported to possess diverse health promoting properties such as hepatoprotective (Wang et al., 2019), anti-inflammatory (Georgiev et al., 2012; Zhao et al., 2018) and neuroprotective activity (Costa, Sarmento, Gonçalves, & Romano, 2013), just to name a few. Further, its properties to scaverge free radicals, ability to chelate pro-oxidant ions, to prevent lipid peroxidation and to bind to biomacromolecules determined RA as valuable antioxidant with applications in food industry (Ferraro, Madureira, Sarmento, Gomes, & Pintado, 2015; Li, Henning, Zhang, Zerlin, & Li, 2010; Li, Yang, Lee, Huang, & Wang, 2021). Therefore, the increasing demand for RA raises the awareness for its production in large amounts. However, its content in plants is rarely above 1% of the dry weight (DW; Petersen, 2013) and depends on plant physiology, growth and development phases, pre- and

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post-harvest processes, as well as, geographical and environmental factors. Along with that, RA is required with high purity for applications in cosmetic, pharmaceutical and food industry, but when derived from cultivated plants there is a risk for pesticide or herbicide impurities (Açıkgöz, 2020). Hence plant-field harvesting and RA extraction by classical methods is considered environmentally destructive and impractical, since the high cost of the process accompanied with inconsistent and/or low yields of the final product. In this regard, plant *in vitro* systems (calli, cell suspension, shoot cultures and hairy roots) have been considered as an attractive alternative for SMs production, such as RA (Gonçalves, Mansinhos, et al., 2019). Major advantages of plant *in vitro* systems are their totipotency (the potential to biosynthesize the complete spectrum of SMs characteristic for the mother plant) and plasticity (the capability of the cell to adapt its metabolism and growth in accordance to its environment; Marchev, Yordanova, & Georgiev, 2020).

In the present review, we attempted to summarize the bioengineering techniques for enhanced production of RA in plant *in vitro* culture and the pipeline of its "green" biotechnological mass-production. Additionally, selected examples of *in vitro* and *in vivo* studies that explore the RA biological activity and applications in food products have been presented. Further, potential applications in food industry and eventual functional food development were highlighted. Technological and chemical modifications that improve RA stability and bioavailability have been illustrated. For article selection, www.scopus.com was used as a data source. The electronic search strategy was based on evaluation of research publications preferably after 2010 from sources with a minimum impact factor 3.0. Key words for all materials within this review were "rosmarinic acid". Further sorting of the articles was performed by implementation of the following additional terms "metabolic engineering", "bioreactor cultivation", "biological activity", "food application" and "bioavailability". The data from the sorted research publications were reviewed by at least two of the participants independently and then included within the relevant sections.

2. Biosynthetic pathway

Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. It is a naturally derived molecule, distributed in many medicinal plants and herbs (Petersen, 2013). The biosynthesis of RA (Fig. 1) involves phenylpropanoid- and tyrosine-derived branches, as a part of the phenolic acid biosynthesis pathway, using as precursors the amino acids L-phenylalanine and L-tyrosine (Deng et al., 2020). The caffeic acid moiety is derived from L-phenylalanine, while the 3,4-dihydroxyphenyllactic acid is obtained exclusively from L-tyrosine (Zhang et al., 2015). The enzymes involved in the L-phenylalanine conversion to para-coumaroyl-coenzyme A (CoA) precursor are phenylalanine ammonia-lyase (PAL; EC 4.3.1.24), cinnamic acid 4-hydroxylase (C4H; EC 1.14.14.91), and coumaric acid CoA ligase (4CL; EC 6.2.1.12). L-phenylalanine is oxidatively deaminated by PAL, followed by hydroxylation of the produced trans-cinnamic acid to para-coumaric acid by the cytochrome P450-dependent C4H. Further, the para-coumaric acid is activated with the help of CoA by 4CL to para-coumaroyl-CoA, which is the only active form accepted by the ester forming enzyme rosmarinic acid synthase (RAS; EC 2.3.1.140; Deng et al., 2020). Concerning the tyrosine-derived branch, L-tyrosine is first transaminated to 4-hydroxyphenylpyruvic acid (PHPP) by the tyrosine aminotransferase (TAT; EC 2.6.1.5) with 2-oxoglutarate as a co-substrate. Afterwards the enzyme hydroxyphenylpyruvate reductase (HPPR; EC 1.1.1.237) converts PHPP into 4-hydroxyphenyllactic acid (PHPL). Thus, the two intermediates are coupled by ester formation to form 4-coumaroyl-4′-hydroxyphenyllactic acid (4C-PHPL), catalyzed by RAS, which transfers the 4-coumaroyl moiety of 4-coumaroyl-CoA to the aliphatic OH-group of PHPL and release CoA (Fu et al., 2020). In the final step RA is synthesized by the introduction of OH-groups at the 3′ and 3 positions in the aromatic ring by the cytochrome P450-dependent monooxydase 3- and 3′-hydrolase (3-H, 3′-H; CYP98A14; Fu et al., 2020). However, the RA biosynthesis pathway has branching points and common intermediates, such as para-coumaroyl-CoA that are used as substrates from the key enzymes chalcone synthase (CHS; EC 2.3.1.74) for the biosynthesis of flavonols and anthocyanins (Marchev et al., 2020) or stilbene synthase (STS; EC 2.3.1.95) for the formation of stilbenes (Marchev & Georgiev, 2020). Therefore, the identification of the genes responsible for the expression of the biosynthetic enzymes has become an object of intense and in-depth genetic engineering studies, where still a major challenge is to recognize the enzymes that limit the precursor-flow (Deng et al., 2020). In this respect, plant *in vitro* systems offer an excellent platform for basic studies and also to gain profound knowledge of the bottlenecks of plant secondary metabolism.

3. Plant metabolic engineering for rosmarinic acid production

Plant metabolic engineering proposes a set of tools for regulation (overexpression or silencing) of genes that potentially control the carbon flux to the biosynthesis of a target compound. The biosynthetic pathway of the SMs is influenced by its structural genes (encoding the enzymes directly involved in all metabolic reactions) and regulatory genes (encoding the transcriptional factors, TFs) that regulate the structural genes by binding to their promoters; Fu et al., 2020; Hidalgo et al., 2017). The TFs are proteins that regulate the gene expression through direct activation or repression of the binding of RNA polymerase to the promoter regions, thus regulating the gene activities. The regulation of TFs is performed by interacting with *cis*-elements (binding sites) *via* DNA binding domain, therefore one challenge is the identification of the TFs binding sites in the promoters of the target genes. There are three major TFs, namely the myeloblastosis (MYB), Myc (encoding basic helix-loop-helix, bHLH) and WD 40 classes that regulate the phenylpropanoid metabolism. They can play a prominent role in the phenolic acid biosynthesis when all of them form an active WBM complex and further build a transcriptional complex through the promoters of the structural genes. The MYB proteins are classified into four categories 1R, 2R (R2R3), 3R (R1R2R3) and 4R, with the R2R3-MYB TFs being the most abundant class in plants acting as core regulators of the SMs biosynthesis. Even alone they are sufficient to activate the transcription of numerous phenolic molecules structural genes (Wang et al., 2013). However, there is still little known about the regulatory mechanisms or the target genes of the TFs involved in the biosynthesis of phenolic acids (Deng et al., 2020). It is considered that the activity of the TFs is regulated by different elicitors, mainly methyl jasmonate (MeJa; Deng et al., 2020), jasmonic acid (JA; Yang et al., 2017) and abscisic acid (ABA; Jia et al., 2017). The TFs serve as regulatory hubs between the elicitors-induced signaling pathways and regulate the SMs production (Yang et al., 2017). Therefore, a variety of strategies has been employed to study the phenolic acids biosynthesis pathway, such as use of exogenous elicitors and manipulation of structural genes and TFs (Deng et al., 2020).

The most explored plant *in vitro* system to study the RA biosynthesis is the hairy roots (HRs). The individual overexpression of *C4H*, *TAT* and *HPPR* increased the RA content in *S. miltiorrhiza* HRs. The single expression of *C4H* increased the RA content 3.6-folds in comparison with the control (from 56.1 to 211 mg/L), individually *HPPR* increased the RA content up to 616 mg/L. However, the most significant direction of the flux to RA biosynthesis (906 mg/L) was achieved during the co-expression of *TAT/HPPR* genes and suppression of 4-hydroxyphenylpyruvate dioxygenase (*HPPD*). The latter is encoding the HPPD enzyme (EC 1.13.11.27), which catalyzes the conversion of PHPP into homogentisic acid, a compound involved in a competitive branch of RA biosynthesis (Xiao et al., 2011). The significance of *TAT* in RA biosynthesis was confirmed in the HRs of *Prunella vulgaris* L. The overexpression of this gene increased the RA content approximately 2-folds compared to the control, but the reduction of its activity resulted in decreased content of RA from 36 to 91% in the different transgenic lines (Ru et al., 2017). The

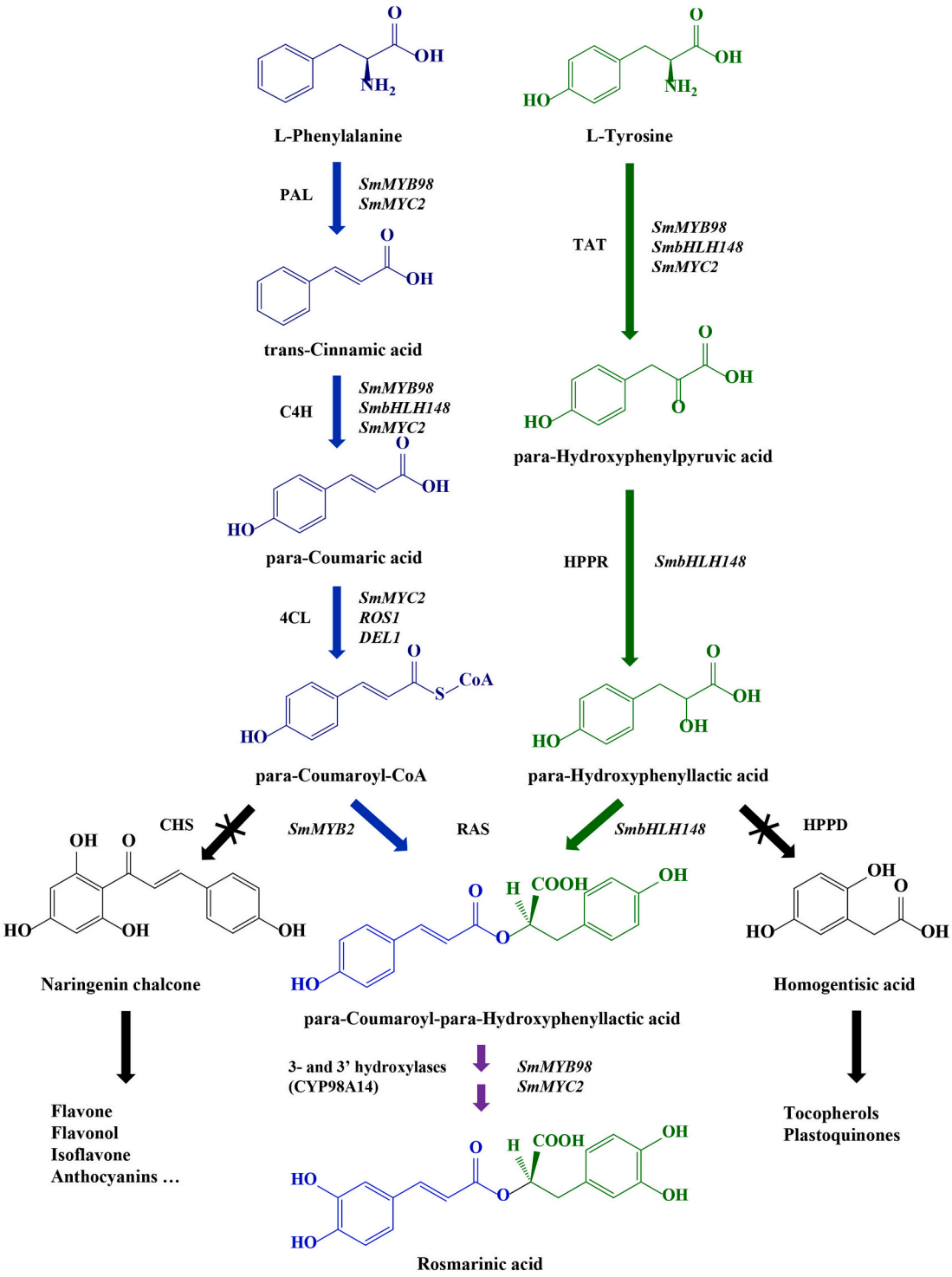

Fig. 1. Schematic overview of the metabolic pathway leading to rosmarinic acid biosynthesis and its regulation through activation or suppression of different transcriptional factors. PAL, phenylalanine ammonia-lyase (EC 4.3.1.24); C4H, cinnamic acid 4-hydroxylase (EC 1.14.14.91); 4CL, coumaric acid CoA ligase (EC 6.2.1.12); TAT, tyrosine aminotransferase (EC 2.6.1.5); HPPR, hydroxyphenylpyruvate reductase (EC 1.1.1.237); RAS, rosmarinic acid synthase (EC 2.3.1.140); CHS, chalcone synthase (EC 2.3.1.74); HPPD, 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27). The overexpression of *SmMYB98*, *SmMYB2*, *SmbHLH148*, *SmMYC2*, *ROS1* and *DEL1* activate the respective enzyme activities in the metabolic pathways, while RNA interference (RNAi) act as suppressors of the CHS and HPPD enzyme activity. The blue arrows indicate the phenylpropanoid pathway, the green arrows indicate the tyrosine-derived pathway, the purple arrows indicate the phenolic acid pathway, while the black arrows indicate the competitive pathways leading to the biosynthesis of anthocyanins, flavones, flavonols, isoflavones, tocopherols and plastoquinones. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

downstream genes *RAS* and *CYP98A14* have been considered as critical and key genes encoding the final enzymatic reactions in the RA biosynthesis. When these genes were introduced and overexpressed in *S. miltiorrhiza* HRs, the RA content increased to 22.57 and 30.35 mg/g DW in *RAS* and *CYP98A14* transgenic lines, respectively, while the RA content in the control was 15.97 mg/g DW. The activation of *RAS* is important since it is the first key enzyme for RA synthesis from branch pathway to the entire pathway (Fu et al., 2020). The overexpression of these genes might be induced by elicitors, e.g. MeJa (0.1 mM) provoked rapid increase of the genes from the phenylpropanoid (*PAL* and *C4H*) and tyrosine-derived (*TAT* and *HPPR*) pathway without changing the expression of *HPPD*. In the HRs of *S. miltiorrhiza* 0.1 mM MeJa also increased the expression levels of *PAL*, 4CL, *C4H*, *TAT*, *HPPR* and *RAS*, with *C4H* having the highest expression (197.6-folds). Thus, the content of RA was increased to 20.3 mg/g DW (1.5-fold of control), while the tanshinone biosynthesis stayed almost unaffected (Xing, Yang, et al., 2018). Methyl jasmonate also triggered the expression of the same structural genes in *M. spicata* HRs increasing the RA content with 11.84 folds (55.44 mg/g DW) than the control (Yousefian et al., 2020).

The overexpression of the R2R3-MYB TF *SmMYB98* in *S. miltiorrhiza* HRs increased the content of RA approximately 2-folds (from 9 to 17 mg/g DW) in comparison to the control roots, which contained an empty vector. Four of the RA biosynthesis pathway genes (*SmC4H1*, *SmPAL1*, *SmTAT1*, *SmCYP98A14*) increased their transcriptional levels more than 2-folds. In the knocked out HR line, the downregulation of *SmCYP98A14* resulted in more than 2-folds lower RA content (Hao et al., 2020). The RA biosynthesis was tightly correlated with the expression of MeJa-responsive *SmMYB2* TF, which strongly upregulated the transcription of key RA biosynthetic genes, such as *CYP98A14* and *RAS*. To a lesser extend were expressed the genes *PAL1*, *HPPR1*, *C4H1*, *4CL1* and *TAT1* (Deng et al., 2020). The sucrose non-fermenting 1 (SNF1)-related protein kinase 2 (SnRK2), which regulates the expression of genes responsive to ABA, through ABA-responsive element (ABRE)-binding proteins positively responded to ABA treatment in *S. miltiorrhiza* HRs. Abscisic acid is an elicitor with a key regulatory role in plant growth and development, including seed germination and fruit ripening and also protects plant from different environmental stressors. The ABA-induced expression of *SmSnRK2.6* and *SmAREB1* increased the RA content with ca. 1.5-folds, due to the increased expression levels of *SmPAL1*, *SmC4H*, *Sm4CL1*, *SmTAT*, *SmHPPR* and *SmRAS*, which had the highest expression level (Jia et al., 2017). Abscisic acid also induced the expression of *SmbHLH1*48 TF leading to the increased content of three phenolic acids, such as caffeic acid, RA and salvianolic acid B with 2.87-, 4.00- and 5.99-folds compared to the control. It was established that this TF binds to the promotor of *PAL1*, *C4H1*, *TAT*, *HPPR*, *RAS* and *CYP98A14* and elevates their transcript levels directly (Xing, Liang, et al., 2018). However, some of the mentioned TFs are not selective, hence they also increased the genes participating in the tanshinone biosynthesis (Hao et al., 2020) and genes, such as *CHS* or chalcone isomerase (*CHI*; EC 5.5.1.6) and flavanone 3-hydroxylase (*F3H*; EC 1.14.11.9) which are key enzymes from the competitive biosynthesis pathway leading to flavonoid and anthocyanins accumulation (Deng et al., 2020; Jia et al., 2017). A bHLH TF that prioritize the RA accumulation is the JA-responsive *SmMYC2* TF. The RA content increased from 2.59 mg/g DW in the control to 6.36 mg/g DW in the transgenic HRs, due to the expression of *SmPAL1*, *SmTAT1*, *SmC4H1*, *Sm4CL* and *SmRAS1*. The first and the rate-limiting enzyme in the phenylpropanoid branch is PAL and the transcription levels of *SmPAL1* in this study increased 367.1-folds. On the other hand, no tanshinone production was observed (Yang et al., 2017). The formation of active MYB/bHLH complex due to the co-expression of *ROS1* (*Rosea1*, a MYB TF) and *DEL1* (*Delila1*, a bHLH TF) increased the content of RA approximately 2-folds due to the increased expression of *Sm4CL* and *SmRAS*, suggesting the synergistic effect of both TFs (Wang et al., 2013).

Post-transcriptional genes silencing is usually triggered by double-stranded RNA formation, referred as RNA interference (RNAi) and is used also as a strategy to increase the RA accumulation by suppressing and/or blocking the competitive pathways in its biosynthesis. The RNAi-mediated silencing of *HPPD* in the HRs of *S. miltiorrhiza* resulted in the production of RA up to 542 mg/L (9.7-folds higher than the control; Xiao et al., 2011). The suppression of *CHS* transcript in *S. miltiorrhiza* HRs through RNAi silencing resulted in 2-fold increase of RA content (21.09 mg/g DW). Along with that, the simultaneous treatment of the silenced HR lines with salicylic acid (50 μM) gave even higher amounts of RA (42.45 mg/g DW). Therefore, this combination of genetic manipulation and elicitor treatment could be applied as an effective approach to direct the substrate flow to RA biosynthesis, since the disruption of the flavonoid pathway (Zhang et al., 2015).

The development of a successful strategy to boost the RA biosynthesis requires in depth knowledge of its metabolic pathway, including not only the rate limiting steps, but also the branching points that can shift the metabolism in a competitive pathway(s). The manipulation of key pathway or regulatory genes to increase the flux of the desired metabolites has gained interest in the resent years. In response, many studies have focused on increasing the RA accumulation by over-expressing or suppressing key enzyme genes within the relevant metabolic pathway, which function is regulated by TFs. However, the activity of the latter is orchestrated by elicitors through the expression of many elicitation-responsive genes. The expression of single pathway gene or TF might be beneficial for RA biosynthesis, however superior results are obtained through the co-expression of multiple pathway and regulatory genes. The co-expression of more than one TF is leading to the establishment of functional regulatory complex, which in turn regulates multiple key steps simultaneously in the metabolic pathway. A complete direction of the substrate flow to RA biosynthesis might be achieved by combining genetic modifications, elicitation treatment and interruption of the competing pathways through RNAi silencing.

4. Biotechnological production and downstream processing of rosmarinic acid

Plant *in vitro* systems are sustainable platforms, which offer a continuous production of valuable SMs with consistency of yield and quality, shorter production cycles compared to the whole plant cultivation, aseptic controlled conditions and independency of geographical and seasonal factors, thus eliminating the possibility of external contaminations (Marchev & Georgiev, 2020). This is especially important in the case of endangered or rare plant species, since plant *in vitro* systems facilitate the mass production without affecting further the plants' natural populations (Gonçalves, Mansinhos, et al., 2019). Furthermore, their potential could be expanded by the utilization of various strategies to enhance their SMs biosynthesis capacity through selection of highly productive lines, nutrient medium optimization, addition of precursors, elicitation, metabolic engineering and selection of appropriate bioreactor system (Açıkgöz, 2020; Marchev et al., 2020). The complete process of RA biotechnological mass-production starting from mother plant species, callus culture initiation through large-scale bioreactor cultivation, isolation and purification is presented in Fig. 2.

4.1. Selection of highly productive lines

The biosynthesis of RA has been elucidated in many *in vitro* system platforms, including calli and suspension cells from *O. basilicum* (Açıkgöz, 2020), *S. officinalis* (Barberini et al., 2013), *Lavandula vera* DC. (Kovacheva, Georgiev, Pashova, Angelova, & Ilieva, 2006), *S. miltiorrhiza* (Zhang, Xing, et al., 2020), *Agastache rugosa* (Fisch. & C.A. Mey.) Kuntze (Park et al., 2016), *Melissa officinalis* L. (Weitzel & Petersen, 2011), shoot cultures from *Dracocephalum forrestii* L. (Weremczuk-Jeżyna et al., 2016) or HRs mainly from *S. miltiorrhiza* (Fu et al., 2020; Hao et al., 2020; Jia et al., 2017).

Calli and cell suspensions might not be the most suitable biotechnological platform for RA biosynthesis, since their dedifferentiated

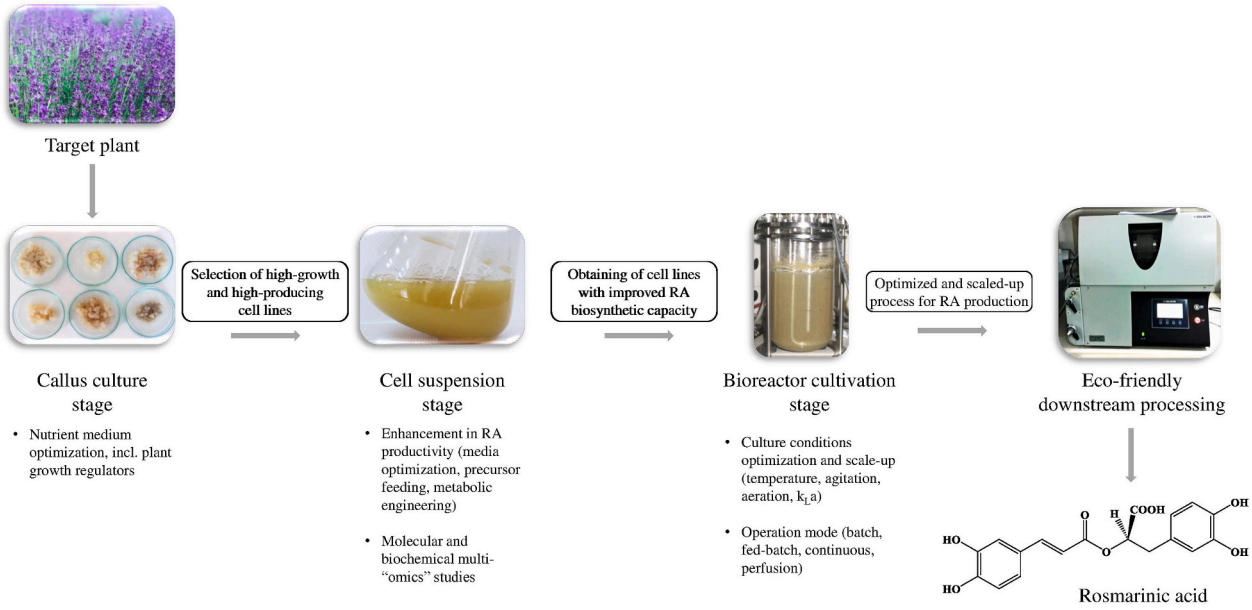

Fig. 2. Proposed scheme and rational improvement approaches for the biotechnological production of RA. For obtaining calli cultures nutrient medium optimization (including plant growth regulators) and appropriate explants type are essential. As a result, selection of high-producing cell lines is performed. After transfer of the callus culture in liquid media at the cell suspension stage several strategies could be applied, *i.e.*, empirical (medium optimization, precursor feeding, elicitation and metabolic engineering) and molecular and biochemical multi-"omics" studies (trascriptomics, proteomics, metabolomics and biological activity assays), which give new insights into RA biosynthesis and regulation, resulting in the development of cell lines with desired biosynthetic capacity. Further, the bioreactor cultivation can simplify and up-scale the bioproduction process and the use of suitable extraction and isolation techniques, such as centrifugal partition chromatography can be used to develop an eco-friendly process.

nature is resulting in large variability in cell growth and inconsistent yield of SMs due to genetic and epigenetic changes (Marchev et al., 2020). A suitable alternative is the utilization of HR cultures obtained through *Agrobacterium rhizogenes*-mediated genetic transformation. They possess fast growth in media without exogenous plant growth regulators and are characterized with genetic and biosynthetic stability (Marchev et al., 2020; Weremczuk-Jeżyna et al., 2016). The RA levels were greatly increased in the HRs from *D. forrestii*. The maximum RA content was 19.97 mg/g DW, which is 4 times higher that the RA content in one-year-old field grown plant (Weremczuk-Jeżyna et al., 2016). The HRs of *Dracocephalum kotschyi* Boiss are also promising biotechnological source for RA biosynthesis, which maximum content was 1.5 mg/g DW (15 times higher than the roots of the intact plant (Fattahi et al., 2013). The HR cultures have been also the preferred model system to study the RA biosynthesis and to apply different strategies to enhance its amount (Fu et al., 2020; Hao et al., 2020; Yousefian et al., 2020). The difference in the growth and biosynthetic potential between the HR lines is due to positional integration effect of the T-DNA from *A. rhizogenes* into the host plant genome (Hidalgo et al., 2017). *Thymus lotocephalus* G. Lopez & R. Morales shoot cultures were also used as a suitable platform for RA production. Under optimal medium conditions of 4% sucrose and elicitation with yeast extract, the RA content increased from 48.61 to 78.57 mg/g extract (Gonçalves, Correa, et al., 2019).

4.2. Nutrient medium optimization

Rosmarinic acid is accumulated intensively in non-differentiated cell cultures, such as calli and cell suspension, frequently in higher amounts than the intact mother plant. The successful calli induction depends on the plant species, explants type, cultural media and plant growth regulators (Wu, Karioti, Rohr, Bilia, & Efferth, 2016). The most frequently used media for induction and maintenance of calli and cell suspensions are full or half strength Murashige and Skoog (MS) medium, Linsmayer and Skoog (LS) medium, Gamborg medium (B5) supplemented with 2–4% sucrose and single or combined use of plant growth regulators, such as α-Naphthaleneacetic acid (NAA), 2.4-Dichlorophenoxyacetic acid (2.4-D) as auxins and kinetin (Kin), 6-Benzylaminopurine (BAP) as cytokines (Açıkgöz, 2020).

Nutrient medium optimization, gamma irradiation and elicitation have been amongst the most frequently used approaches to enhance the RA production in plant *in vitro* systems. For instance, 4% sucrose, added to the CB2 culture medium, doubled the RA content (6%) in comparison with 2% sucrose (3.5% RA) when cell suspension of *M. officinalis* was cultivated for 6 days. The peak in RA biosynthesis coincided with the maximum expression of *RAS* observed on the third day and started to decline after day 6 (Weitzel & Petersen, 2011). The RA biosynthesis appeared dependent on the expression of another key enzyme *HPPR* in cell suspension of *S. officinalis*. The maximum expression of *SmHPPR* was observed at the third day of the cultivation and although its expression stared to decline after the log phase (day 6), its levels were sufficiently enough to maintain stable RA production until the stationary phase, reaching maximum of 34.82 mg/g DW RA at day 9 (Barberini et al., 2013).

4.3. Elicitation

One of the latest trends is the elicitation-based stimulation of *in vitro* SMs biosynthesis. The effectiveness in this treatment depends on the type of elicitor, its concentration, duration of exposure and the age of the cell culture (Açıkgöz, 2020). The addition of yeast extract (biotic elicitor), $CdCl_2$ and $AgNO_3$ (abiotic elicitors) had different impact over the biosynthesis of RA in *O. basilicum* cell suspension. The highest RA content (21.28 mg/g DW) was achieved at day 10 after treatment with 200 mg/L yeast extract, which RA content is 1.25-folds higher than the control. The other elicitors had minor effect and the RA content varied between 1.04 and 12.86 mg/g DW when treated with $CdCl_2$ and 5.43–13.4 mg/g DW after $AgNO_3$ treatment (Açıkgöz, 2020). Following 72 h post-treatment with yeast extract (750 mg/L), the RA content was increased 18.5-folds (4.98 mg/L) compared to the control. This elicitor overexpressed several important genes *PAL*, *C4H* and *RAS* from the

biosynthesis pathway of RA in the cell suspension of *A. rugosa* (Park et al., 2016). The activation of the genes *PAL* and *C4H*, as well as, *TAT* and *HPPR* by MeJa also increased the accumulation of RA (Xiao et al., 2009). Further, it has been established that MYC2 TF controls their up-regulation (Yang et al., 2017).

4.4. Bioreactor cultivation

The final step for sustainable development of RA biosynthetic platform is the bioreactor cultivation. The *O. basilicum* cell suspension was cultivated in a 10-L (7 L working volume) BioFlo 300 stirred-tank bioreactor, equipped with two standard ring spargers and one marine impeller, operating at 100 rpm, 25 °C in dark. The RA content under these conditions was 7.85 mg/g DW and increased almost 2-fold (13.09 mg/g DW) after stimulation with 200 μM MeJa. Along with cell suspension, shoot cultures have been frequently used as a biotechnological source for RA biosynthesis. An option to decrease the operational costs for RA biosynthesis is the use of disposable bioreactors. A suitable cultivation system was found to be the 2-L CellBag, shaken in a Khuhner orbital shaker. The treatment of *S. khuzistanica* cell suspension with 1 μM coronatine resulted in RA content of 3.6 g/L, which is 1.3-folds higher than the elicitor-treated suspension in shake flasks (2.7 g/L; Khojasteh et al., 2019).

The first large-scale bioproduction of RA was established by Ulbrich, Wiesner, and Arens (1985). The process was developed on *C. blumei* cell suspension cultivated on a modified Heller-medium in a 42-L airlift Biostat 30D bioreactor "module spiral stirrer" with a rotation speed of 100 rpm. The RA yield from this batch cultivation process was 5.5 g/L (productivity of 910 mg/L/d, corresponding to 21% RA of the DW) compared to RA 1.1% DW in the mother plant (Ulbrich et al., 1985).

4.5. Downstream processing: extraction, isolation and purification

Another important aspect of RA mass-production is the down-stream processing, including extraction, isolation and purification. Throughout the literature, the most frequently used techniques for RA extraction comprise of conventional solvent extraction (Chen, Wang, Lin, & Yen, 2020), microwave (Liu et al., 2011), ultrasound assisted (Bielecka et al., 2019), supercritical (Quintana, Villanueva-Bermejo, Reglero, García-Risco, & Fornari, 2019), pressurized liquid extraction (Hirondart et al., 2020), extraction with ionic liquids (Liu et al., 2011) or through molecular imprinted polymers (Saad et al., 2021).

The conventional solvent extraction is following the principles of solid-liquid extraction in which the solvent diffuses into plant cells, dissolves the metabolites and hence diffuses them out of the cell. The most frequent solvents used are water, methanol, ethanol (Hadi et al., 2017; Nourozi, Hosseini, Maleki, & Mandoulakani, 2019) or their aqueous solutions (Chen et al., 2020). The aqueous-organic solutions are more efficient for RA extraction, since water increases its diffusion from plant cells to the solvent. The highest RA extraction efficacy from *Trichodesma khasianum* C. B. Clarke was achieved with 60% aqueous ethanol (19.3%), while the 20 and 95% ethanol extracted 14.67 and 5.93% RA (Chen et al., 2020). The efficiency of the classical solvent extraction could be increased by applying reflux of the solvent, ultrasound treatment or microwave heating. The RA content in 90% ethanolic rosemary extract obtained by maceration (40 °C and 30 min) was 10%, the ultrasound assisted extraction at the same conditions resulted in 13.1% RA, while the reflux and microwave assisted extraction (150 °C, 30 min and 300 W microwave power) gave 19 and 25.2% RA, respectively (Jacotet-Navarro et al., 2015).

Beyond the classical methods for RA extraction, several rapid, environmentally friendly and clean extraction techniques, such as supercritical carbon dioxide extraction ($SCCO_2$; Quintana et al., 2019), pressurized liquid extraction (Hirondart et al., 2020), ionic liquids based extraction (Liu et al., 2011) and molecular imprinted polymers selective extraction have gained the research interest during the latest years (Saad et al., 2021). The pressurized liquid extraction of rosemary with 96% ethanol gave a RA content of 10 mg/g DW and 1.5-fold higher total extract yield compared to the Soxhlet extraction (Hirondart et al., 2020). In the $SCCO_2$ extraction, the solute is precipitated from a solution which is expanded through a nozzle while mixed with $SCCO_2$ and further followed by nucleation and crystal growth. The performance of supercritical antisolvent precipitation performed at 20 MPa, 313.15 K, and 50 g/min $SCCO_2$ flow resulted in RA enriched fraction, which contained 3-fold more RA (45.4 mg/g), compared to the crude ethanolic extract (14.6 mg/g; Quintana et al., 2019). Liu et al. (2011) investigated the effect of ionic liquids supported with microwave assisted extraction on RA extraction from rosemary. Ionic liquids are composed of bulky organic cations and inorganic or organic anions, liquid at room temperature, with good solubility and extractability for various organic compounds and efficiently absorb and transfer microwave energy. The results showed that the ionic liquids containing BF_4^-, and Br^- were the most efficient solvent systems for RA extraction, which means that its extraction efficacy is anion-dependent. Along with that, the increase of alkyl chain length of cation in ionic liquids was found to increase the RA yield. At optimal conditions ([C8mim]Br at 1.0 M concentration; solid-liquid ratio 1:12, microwave power 700 W and treatment for 15 min) gave the highest yield of RA 3.97 mg/g DW (Liu et al., 2011). Molecularly imprinted solid phase extraction is using molecularly imprinted polymers as selective sorbents for rapid and cost effective extraction, which is consuming significantly small volume of organic solvents. This technique involves the polymerization of solution containing template, functional monomer, cross-linker and initiator. The use of methacrylic acid as functional monomer resulted in the extraction of 49.11 mg/g DW RA with 80% purity from *R. officinalis* (Saad et al., 2021). Column chromatography is also an efficient method for separation and purification of RA. The combined column chromatography over macroporous and polyamide resins based fractionation of 50% ethanolic extract of *Perilla frutescens* L. increased the content of RA from 0.27% in the original extract to 16.58% in the 50% ethanol fraction. During further purification 90.23% pure RA was obtained in the 70% ethanol fraction. Finally, after one crystallization in methanol to remove the phytochromes, the purity increased to 95% (Tang, Sun, & Zhao, 2014). Ion-exchange centrifugal partition chromatography (CPC) is also used to purify many molecules, such as hydroxycinnamic acids. The application of ionic liquid benzalkonium chloride (strong anion-exchanger) and iodide (displacer), using the ternary biphasic system CHCl3: n-BuOH: water 4.5:1:4.5 v/v/v in the ascending mode resulted in high purity RA (90%) with high yield (3.4% of dry extract), isolated from *L. vera* MM cell suspension extract (Georgiev, Kovacheva, Marcheva, & Ilieva, 2006; Maciuk et al., 2005).

At large-scale production of RA from *C. blumei*, the following downstream process has been applied. After cultivation, the cell biomass was separated by the production medium through centrifugation, followed by hot water extraction (pH 3, 80 °C) and additional centrifugation. The filtrate was poured onto an Amberlite XAD-2 column to absorb the metabolite, which after washing the column with water was eluted with 40% aqueous methanol. Further, the eluate was evaporated to dryness and redissolved in highly purified water for several hours at 4 °C. Finally, the obtained crystals were washed with ice cold water and freeze dried. The obtained RA had a purity of 95% and 65% yield (Ulbrich et al., 1985).

4.6. Analytical methods for identification and quantification

Some of the most frequently used analytical platforms for identification and quantification of RA include high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry and nuclear magnetic resonance (NMR). For HPLC analysis is usually used HPLC system with a reverse phase column, UV/VIS or diode array detector (DAD) and water and acetonitrile acidified with 0.1% formic acid at 280 nm wavelength (Celano et al., 2017; Fattahi et al., 2013).

Regarding the liquid chromatography equipped with OrbiTrap (Celano et al., 2017), electrospray ionization (Saad et al., 2021) or Quadrupole time-of-flight mass spectrometer (Bielecka et al., 2019), the RA is recognized by its base peak at m/z 359 [M–H] and 719 [2M–H] (Wu et al., 2016). The characteristic feature in the proton NMR spectrum of RA is the two doublets at ca. δ 7.39 and 6.11 ppm with J = 16.0 Hz assigned as a pair of trans olefinic protons, suggesting that the compound is a transcaffeoyl ester of 3.4-dihydroxyphenyllactic acid. Further, the double doublet signals at δ 2.92 (dd, J = 14.3, 9.5), 3.08 (dd, J = 14.3, 3.3) and 5.07 (dd, J = 9.5, 4.0) are showing the presence of dihydroxyphenyllactic acid unit (Achamlale, Rezzonico, & Grignon-Dubois, 2009).

The establishment of sustainable biotechnological process for biosynthesis of valuable molecules, such as RA based on green cell factories opens the avenues for its production on an industrial scale. The successful implementation of this process requires the union utilization of both empirical and modern multi-"omics" technologies. Using this approach could be achieved selection of biosustainable source for RA biosynthesis based on plant *in vitro* systems, cultivated in optimal growth and biosynthesis conditions. Metabolic engineering along with "omics" technologies could contribute to the establishment of supreme RA-productive cell lines through activation/suppression of the metabolic pathways and regulation of these processes at transcriptional or post-transcriptional level. The transfer of the process from benchtop to large-scale production raises the awareness for the correct selection of bioreactor design and cultivation parameters. In addition, the use of elicitors and permeabilizing agents could be applied in plant cell factories to simultaneously increase the RA production and its release in the culture medium, simplifying the downstream processes. Regarding the homogeneous growth pattern and its similarities with microorganisms, the most used source for biotechnological production from lab-to large-scale level are the cell suspension cultures. The application of single use bioreactors is frequently preferred compared to the conventional stainless steel bioreactors to make the process cost competitive.

In this section, we describe a rational process for the biotechnological production of RA, starting from the initiation of the plant *in vitro* systems, selection of highly productive cell lines and going through empirical and multi-"omics" strategies to increase cell growth and product accumulation before the process transfer in large-scale volume. Finally, an eco-friendly downstream process is proposed, combining the advantages of ionic liquid extraction and CPC. In this way is achieved one-step purification process of RA from crude extract in high yield and purity. It could be stated that ionic liquids-based extraction could be one of the most suitable procedures for RA extraction. The main advantage is that the ionic liquids are "green solvents", which are preferred in front of the traditional toxic, flammable and volatile organic solvents. They have negligible vapour pressure, good thermal stability and miscibility with water and organic solvents, therefore suitable for extraction of wide range of compounds with variable polarity. The application of ionic liquids require less solvent volumes and shorter time for extraction and have been used in solid-liquid, liquid-liquid or two-phase extraction systems. The proposed combination of liquid ions and CPC is also leading to reduction of steps (one-step process) required to obtain the final product, compared to supercritical extraction process, requiring two steps of separation. However, some limitations could be the cost for preparing the ionic liquids and that in the case of water-soluble ionic liquids, water may be present in trace amounts.

5. Rosmarinic acid utilization in food industry

Development of a high-throughput biotechnological approach for RA production is provoked by its utilization as a valuable compound of medical importance, as well as, preferred natural antioxidant and additive in food and cosmetic products (Petersen, 2013). Detailed analysis of the RA bioactivity and its pharmaceutical value is beyond the scope of the present review and could be found within the articles of Nunes et al. (2017) and Hitl, Kladar, Gavaric, and Bozin (2020). However, selected examples for its most prominent bioactivities will be mentioned in order to provide evidence about the RA potential future development into functional food products.

5.1. Biological activity

Health benefits resulting from RA administration are most commonly determined as neuroprotective, especially with respect to prevention of Alzheimer's disease (Costa et al., 2013), hepatoprotective (Wang et al., 2019) and anti-inflammatory effects (Georgiev et al., 2012; Zhao et al., 2018), among others.

Rosmarinic acid being a potent free radical scavenger has attracted attention in the experimental treatment of various neurological pathologies. The neuroprotective role of RA investigated *in silico* using molecular docking simulations revealed that RA forms stable complex with amyloid beta (Aβ) peptide, which inhibits further aggregation and is considered as a possible mechanism in AD therapy and prophylaxis. Structure-activity relationship analysis has defined the phenolic hydroxyl group in the molecule as the essential structural characteristics responsible for the observed activity of RA. Additional, suggested mechanisms of RA activity in AD involved inhibition of intracellular reactive oxygen species accumulation (Costa et al., 2013).

Activation of energy expenditure, reduction of lipid accumulation and activation of transcription factors responsible for cellular redox homeostasis such as AMP-activated protein kinase (AMPK) are among the beneficial effects upon RA treatment in non-fatty liver disease model (Wang et al., 2019). Correspondingly, data from a recent study of ours indicate that RA inhibits white adipogenic differentiation, decrease lipid accumulation and induce adipocyte basal lipolysis in human adipocytes through modulation of key adipogenic transcription factors and decrease in inflammatory cytokines expression (Vasileva, Savova, Tews, Wabitsch, & Georgiev, 2021).

An *in vivo* study investigating the anti-inflammatory effect of RA in dextran sulfate sodium-induced colitis model confirmed its beneficial properties represented by decreased nitric oxide, inducible nitric oxide synthase, myeloperoxidase, and cyclooxiganase 2 (Zhao et al., 2018). Correspondingly, in another model of genetically induced model of inflammatory colitis, RA is used as a reference compound due to its anti-inflammatory activity mediated by selective inhibition of complement component 3 (C3) convertase (Park et al., 2021).

Additionally, RA exerted potential for prevention and management of inflammatory skin conditions. Primary human keratinocytes stimulated with interferon gamma reacted with decreased production of inflammatory factors, such as monocyte chemoattractant protein 1 and interferon gamma induced protein 10 upon treatment with RA in concentrations of 10 and 50 μM (Georgiev et al., 2012).

Regardless of the numerous research studies, the data on the bioavailability and pharmacokineteic profile of RA is inconclusive. Furthermore, the available pharmacokinetic parameters of RA are concerning predominantly rodents that could differ at least at metabolism and elimination phases from the actual fate of RA in humans. For example, the gastrointestinal stability of RA containing extract of *R. officinalis* was tested *in vitro* and *in vivo* in rats. The presented results suggest substantial decrease in the RA content resulting from the action of the digestive enzymes (Gonçalves, Correa, et al., 2019). Another study evaluating single dose oral administration of RA in rats with liver injury model indicates that all pharmacokinetic parameters are changed compared to controls, hence, the bioavailability and actual bioactivity of RA could be hardly predicted (Min et al., 2018). Further, tissue distribution of RA is related to its ability to bind albumin that is a key drug-delivery protein. Clarifying the binding affinity and mode of interaction of RA and human serum albumin shed light into the predicted tissues of distribution (Peng et al., 2015; Peng, Wang, Qi, Su, & He, 2016). Considering the neuroprotective action of RA especially regarding its implementation in AD management, the potential of this

small molecule to cross the highly selective blood-brain barrier is of great importance. Overall, development of brain-targeted drug-delivery systems is needed to achieve effective concentrations within the brain (Bhatt, Singh, Prakash, & Mishra, 2015). However, such studies in humans are scarce which leave the question of the actual oral bioavailability of RA open (Hitl et al., 2020). More detailed information on RA pharmacokinetics has been systematically reviewed by Nunes et al. (2017) and recently updated by Hitl et al. (2020).

Rosmarinic acid has proven its potential as anti-inflammatory agent with promising profile for further drug-development. Despite the substantial number of *in vitro* and *in vivo* studies available, translation to clinical settings is still insufficient. However, the use of RA in food industry is extensive, hence, combined data from the safety doses used in food products and reported data from bioactivity studies could accelerate RA implementation in the development of innovative functional food products. Collectively, these data on RA beneficial health promoting effects are highlighting its potential implementation in the development of innovative functional food products.

5.2. Application as antioxidant

Over the last decades, many scientific-based reports evidence that lipid oxidation during food processing (especially deep-frying processes) results in the formation of potentially toxic products, including oxidized fats, trans fatty acids, sterol derivatives, acrylamide and heterocyclic compounds (Li et al., 2010, 2021). This might result not only in deterioration and negative quality changes in the food products, but the initiation of hazardous materials as a result, one of which being the malondialdehyde (MDA), could provoke many inflammatory processes in humans, even carcinogenesis (Li et al., 2010, 2021). The oxidation process involves a free radical chain reaction initiated by exposure of the food or its ingredients (fatty acids, proteins, etc.) to heat, light, ionization radiation, metalloprotein catalysis or metal ions (Choi et al., 2019). Therefore, various approaches have been explored to extend the shelf-life of food, delay its oxidation and to retard the formation of undesirable components, among which the addition of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) or tertiarybutyl hydroxyquinone (TBHQ) is the most direct and frequently used way (Guitard, Paul, Nardello-Rataj, & Aubry, 2016). Nowadays, consumers are highly concerned about the safety of synthetic antioxidants, which has led to increased use of natural polyphenols as such. Rosmarinic acid and RA-containing extracts, *e.g.* rosemary extract are the most widely used natural antioxidants incorporated in food products (Choi et al., 2019). Rosemary extract has been legally permitted to a maximum concentration of 300–700 mg/L in China and 30–250 mg/L in European Union, depending on the food type and the antioxidant capacities of the extract were mainly contributed by the presence of non-polar phenolic diterpenes such as carnosic acid, carnosol and rosmanol (Choi et al., 2019; Li et al., 2021). In parallel, the use of TBHQ in China has been limited to 0.2 g/kg, being gradually prohibited in Japan, Canada and Europe (Li et al., 2021).

Regarding this, RA and rosemary extract (which contains 4–4.5% RA typically) have been investigated in many models and real foods, such as edible oils (Guitard et al., 2016), processed meat products (Kovatcheva-Apostolova et al., 2008; Li et al., 2010), dairy products (Ferraro et al., 2015) and beverages (Klisurova et al., 2019; Zhang, Wang, Zhou, Li, & Han, 2020), evaluating their capacity as antioxidants and co-pigments in prevention of food oxidation and storage, as well as, their stability in the investigated food system (Ferraro et al., 2015). Along with that formation of undesired products even originating from the degradation of the RA and adduct formation with amino acids, which leads to deterioration of meat quality and risk the human health have been considered as well (Ferraro et al., 2015).

Omega-3 essential fatty acids have drawn the attention of the scientists due to their ability to improve many biological parameters in humans, including triglyceride level, blood pressure and to promote cancer prevention. In a model system containing palmitic acid methyl ester (C16:0), stearic acid methyl ester (C18:0), oleic acid methyl ester (C18:1), linoleic acid methyl ester (C18:2) and linolenic acid methyl ester (C18:3), among the 22 tested polyphenols, RA, myricetin and carnosic acids were the most efficient in prevention of omega-3 oils oxidation. This high antioxidant activity was attributed to the catechol-type ring moiety, conjugated double bonds and alkyl substituents on the phenol rings in the mentioned polyphenols. These structural peculiarities contribute to low bond dissociation enthalpies, which is corresponding to the electron-donating capacity of the antioxidant and allowed the entrapment of 4 radicals per 1 molecule of RA. This turns RA as one of the best natural alternatives possessing higher antioxidant capacity than α-tocopherol, BHA, BHT and TBHQ (Guitard et al., 2016). During the preparation of deep frying foods (155–190 °C), such as French fries, RA and RA-based antioxidants showed better efficacy than TBHQ in oxidation prevention of soybean oil, evaluated through color stability and sensory analysis (Li et al., 2021). A spice blend, used as a source of RA was used to prevent lipid oxidation and MDA formation during hamburger meat cooking. The spice blend at concentration of 11.3 g/burger reduced the MDA formation with 71% compared to the burgers without spice blend. Furthermore, the urinary MDA values decreased with 49% in the subjects consuming the burgers with spice (Li et al., 2010). Reduction in lipid oxidation and preservation of tocopherol content in cooked meat during storage for 10 days was detected upon addition of *L. vera* extract with high RA content (95.3 mg/g dry extract) which further support the utilization of RA as natural antioxidant and preservative in food products (Kovatcheva-Apostolova et al., 2008).

Rosmarinic acid has been frequently investigated not only as an antioxidant, but also in terms of co-pigment stabilizing/intensifying the color of beverages (juices and wines), containing unstable natural colorants, such as anthocyanins and carotenes (Klisurova et al., 2019; Mesnier, Gregory, Fança-Berthon, Boukobza, & Bily, 2014). In most cases, the co-pigment/pigment ratio is important to obtain the desired effect. The best stabilizing effect and magnification of color intensity in aronia anthocyanins model system was achieved at molar ratio pigment: co-pigment 1:50 when using RA. Although this ration cannot be achieved in plant matter, the RA resulted in the biggest hyperchromic effect of 51% and batochromic effect of 10 nm shift, which is indicative for the color stabilization and intensification (Klisurova et al., 2019). Rosmarinic acid had better color stabilizing properties of wine than other phenols, such as caffeic acid and danshensu. The RA hyperchromic affects and batochromic effect in ratios between 1:10 and 1:60 varied between 2 and 161% and 7–19 nm. In the same molar concentrations, these values for the caffeic acid were 2–82% concerning the hyperchromic affects. Along with that at the highest molar ration of RA was obtained the highest color intensity leading to darker and more vivid bluish colors (Zhang, Wang, Zhou, Li, & Han, 2020). In a carotene emulsion containing 1% natural carotene, the addition of 150 ppm rosemary extract increased the color stability of the emulsion from 31 to 51 days, heat and light stability at 500 ppm from 20 to 55 h (Mesnier et al., 2014).

Polyphenols, including RA are prone to oxidation into quinones, which further can continue to react with nucleophilic group thiols and amines, leading to the formation of different adducts with amino acids and proteins, decreasing the bioavailability of the polyphenols, as well as, the food quality in milk and meat products (Ferraro et al., 2015; Li et al., 2020). Such adducts were observed in model system of milk between RA and α-lactalbumin, β-lactoglobulin and lactoferrin. Although the antioxidant activity of RA decreased in the presence of the milk proteins in comparison with the free RA, during the time the antioxidant activity of the bounded RA decreased more slowly (Ferraro et al., 2015). The formation of RA-adducts has been determined in model systems containing amino acids (Li et al., 2020) and meat proteins in a gel model (Tang, Zhang, Wang, Xing, & Xu, 2016). Most of the adducts have been formed with the amino acids L-lysine, L-cysteine (Li et al., 2020), or L-lysine, L-cysteine and L-arginine (Tang et al., 2016). It has been

concluded that the adduct regulation can be done by the addition of amino acids in the meat product, which can react with the RA first and avoid the formation of proteins adducts and hence prevent the meat quality (Tang et al., 2016).

The use of RA or RA-containing extracts appears an interesting alternative for prevention the oxidation of food ingredients and to extend its shelf-life in both, microbiological and sensorial way. This would prevent not only the economic losses for the food industry but also bring eventually beneficial health effect on humans.

6. Contemporary approaches for improvement of RA stability and bioavailability

Rosmarinic acid is natural compound with valuable applications in food and nutrition, characterized with low lipophilicity, which reflects in a negative way to its transmembrane penetration and oral availability (Filipe et al., 2018). Along with that, the acidic environment and enzymes in the human digestive tract or in many food products, the presence of excipients in pharmaceutical and cosmetic products, impair the stability of RA, resulting in low bioavailability and significant reduction of its biological activities (Sahiner et al., 2019).

Regarding the above mentioned, alternative approaches are needed to increase the stability, permeability and hence the bioavailability of RA through incorporation in nano- or microformulations by including RA into cyclodextrin (Aksamija, Polidori, Plasson, Dangles, & Tomao, 2016), chitosan (Madureira, Pereira, Castro, & Pintado, 2015), lipid nanoparticles (Chaiyana et al., 2020) or through chemical modifications (Cardullo et al., 2019). The use of these approaches reflects in improved shelf-life, stability, biocompatibility, prolonged biological effect and minimization of the eventual toxic effect of RA for humans and the gut microbiome (Madureira, Campos, Gullon, et al., 2016; Sahiner et al., 2019). Selected examples for the encapsulation and inclusion of RA into complexes with carrier molecules aiming to improve its stability, permeability and oral bioavailability are presented in Table 1.

6.1. Polymer-based formulations

The formation of polyRA particles through emulsion crosslinking decreased not only the RA toxicity for the mammalian cells with 66%, but also revealed their potent application in treatment of diabetes, since α-glycosidase was 100% inhibited by RA at concentration of 1.2 mg/L (Sahiner et al., 2019). The potential application of RA as a functional food was revealed by the fact that it exhibited positive effect on gut microbiome growth when included in solid lipid nanoparticles (Madureira, Campos, Gullon, et al., 2016). The value of RA might be elevated by increasing its stability, water resistance and mechanical properties through formation of RA-gelatin edible films, which improve its long-term antioxidant and antibacterial activities (Ge et al., 2018). The oral availability of RA was increased by its incorporation in RA-phospholipid complex. This complex reduces the contact of RA with the gastrointestinal fluids and increases its ability to cross the membrane barrier through the uptake of phospholipids (Huang, Chen, Rogers, & Wettig, 2019).

The encapsulation of RA in cyclodextrins (CD) revealed a good possibility for the application of RA in both medicine and food industry, because of the formation of water-soluble complex with improved stability, solubility, bioavailability and biological activities (Fateminasab, Bordbar, Shityakov, & Saboury, 2020). The incorporation of RA into β-cyclodextrin resulted in stable complex of acidic pH, which preserved its biological activity (Aksamija et al., 2016).

Chitosan-based inclusion of RA or RA-containing extracts from *S. officinalis* and *Satureja montana* are also promising delivery systems for ophthalmic preparations. They posses increased retention time on the ocular mucosa, as well as, prolonged antioxidant activity (da Silva, Ferreira, Pintado, & Sarmento, 2016). Along with that chitosan nanoparticles could be an appropriate system for delivering polyphenols with antimicrobial and antioxidant activity. In view of possible use as an antimicrobial agent for the prevention or control of intestinal disorders provoked by bacterial pathogens, the production of chitosan nanoparticles with polyphenols such as RA has been optimized and

Table 1
Selected approaches towards improvement of rosmarinic acid stability and bioavailability.

Type of carrier	Composition	Particles size (nm)	Key findings	Reference
Polymer-based carries	TMPGDE	925 ± 37	The stability and bioavailability of RA is enhanced upon crosslinking into microparticles	Sahiner et al. (2019)
	Cyclodextrin	N/A	Encapsulation of RA into a cyclodextrin complex improved RA stability especially providing improved stability in food applications	Aksamija et al. (2016)
	Cyclodextrin	N/A	Enhancement of RA stability, solubility, bioavailability and improvement of its antioxidant capacity upon encapsulation into cyclodextrins	Fateminasab et al. (2020)
	LMWC HMWC	355.9 ± 21.6 604.2 ± 30.6	Best encapsulation performance and improved antibacterial activity is obtained for the particles with RA and LMWC. Potential application as food preservative	Madureira et al. (2015)
	Chitosan-based	280 ± 16	Improved mucoadhesion, epithelial and retinal permeability of the chitosan-RA nanoparticles which propose their application in cosmetics and pharmaceutical industry	da Silva et al. (2016)
	Chitosan modified chitosan	800 100	Improved availability of RA for topical applications. Modified chitosan microparticles permit modified release of RA in both oil- and water-based formulation	Casanova et al. (2016)
	Gelatin-based	N/A	Gelatin-based edibles films containing RA could be used in food packaging as they have good water and UV resistance and strong mechanical stability, as well as, preserved RA antioxidant and antibacterial properties	Ge et al. (2018)
Lipid-based carriers	WSLN CSLN	Range of 310–950 Range of 300–1000	Both lipid nanoparticles formulations improved the RA bioavailability and preserve the normal growth of the gut microflora, hence are safe to be used in RA-containing food products	Madureira, Campos, Gullon, et al. (2016)
	WSLN CSLN	903 ± 15 874 ± 67.9	Lack of genotoxic or cytotoxic effect suggests that both lipid nanoparticles can be considered as safe carries for RA with potential nutraceutical applications	Madureira, Campos, Oliveira, et al. (2016)
	WSLN	411 ± 1 at day 0 821 ± 48 at day 28	The obtained lipid nanoparticles improve thermal stability and RA oral availability	Campos et al. (2014)
	NLC, containing lecithin and cholesterol	464 ± 50.1	Incorporation of RA and RA-containing extract in liposomes provide higher skin retention and permeabilization and is suitable delivery system for cosmetic products	Chaiyana et al. (2020)

Abbreviations: CSLN, solid lipid nanoparticles based on carnauba wax; HMWC, high molecular weight chitosan; LMWC, low molecular weight chitosan; N/A, not applicable; NLC, nanostructured lipid carriers; TMPGDE, trimethylolpropane triglycidyl ether; WSLN, solid lipid nanoparticles based on Witepsol wax.

characterized. The encapsulated RA showed higher inhibitory activity on *Bacillus cereus*, *Escherichia coli* O157, *Listeria innocua*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Yersinia enterocolitica*, indicating its use as a functional food (Madureira et al., 2015). Chitosan and modified chitosan particles containing RA increased its water solubility and facilitate its incorporation in oily and aqueous cosmetic formulation, while protecting the RA beneficial properties. Chitosan particles have a slower release in oily than in water formulations, while modified chitosan particles show faster release in both formulations (Casanova, Estevinho, & Santos, 2016).

6.2. Solid-lipid nanoparticles

Lipid nanoparticles have been used to develop safe dermal nano-delivery system for RA as an anti-aging molecule. The nanostructured lipid carriers containing RA-rich extract from *O. santicum* have been proposed as useful approach in the development of topical anti-aging cosmetic products. These lipid carriers not only improved the delivery of RA with significant skin retention amount, but also did not cause any skin irritation (Chaiyana et al., 2020). The solid lipid nanoparticles improve the thermal stability, protect from digestive processes and increase the oral availability of RA (Campos, Madureira, Gomes, Sarmento, & Pintado, 2014). As a result, these RA complexes support the growth of the human gut microflora and further prevent an eventual toxic effect for humans through avoidance of its passage in the bloodstream (Campos et al., 2014; Madureira, Campos, Gullon, et al., 2016). The solid lipid nanoparticles are suitable matrices for oral delivery and adsorption of RA, since they have a controlled release (40–60%) of RA, while still maintaining its biological activity (Madureira, Campos, Oliveira, et al., 2016). The RA-oil-based structured lipid particles could be used to replace the synthetic antioxidants, such as butylated hydroxytoluene (BHT). These complexes appeared to exhibit higher antioxidant activity and stability than BHT (Zhang, Willett, Hyatt, Martini, & Akoh, 2021).

6.3. Structural modifications

The synthesis of structurally related molecules to RA with improved free radical scavenging or anti-diabetic activities is another alternative to enhance the biological activity of RA (Cardullo et al., 2019). The RA methyl ester revealed improved melanogenesis inhibition mechanism *in vitro* through reduction of the expressions of the melanocortin-1 receptor, microphthalmia-associated transcription factor, tyrosinase and tyrosinase-related proteins in murine melanoma B16 cells. Moreover, its depigmentation effect was confirmed *in vivo* in mice model (Ding, Chou, & Liang, 2010).

The presented approaches for inclusion of RA in different formulations, as well as, the modification of its chemical structure are improving its stability, preventing eventual toxic adducts formation and increasing its bioavailability. Despite that, such modifications do not change the actual bioactivity of RA, the enhanced bioavailability would asure a delivery of RA at effective concentrations to induce health beneficial effects.

7. Conclusions and future perspectives

Rosmarinic acid is widely distributed within the plant kingdom, exhibiting simultaneously protective functions in plants and large variety of beneficial properties for humans. This intensive utilization of RA is raising the demand for its large-scale production. Its content in plants is usually less than 1% of the DW and field harvesting is eventually destructive for the natural plant populations and in most case impractical due to the constantly processing. For that reason, plant *in vitro* systems (calli, cell suspension, shoot cultures and HRs) have been considered as a major alternative for biotechnological production of RA at lab- and large-scale level. In the present review, we have proposed a scheme for biotechnological production and rational improvement approaches of the biosynthetic RA-capacity of green cell factories and eco-friendly downstream processing. These approaches include both, empirical and modern multi-"omics" technologies, resulting in the selection of supreme RA-producing cell lines in optimal growth and biosynthesis environment. The most effective RA production could be achieved by the simultaneous application of metabolic engineering, including TFs overexpression, elicitation, permeabilization and selective silencing of the competitive metabolic pathways. However, the industrial production of RA highly depends on the right selection of cultivation parameters and the bioreactor type and its operational mode.

The discussed *in vitro* and *in vivo* models reveal that the most pronounced biological activities with well-documented molecular studies involve neuroprotective, hepatoprotective and anti-inflammatory, besides abundant anti-cancer properties. Regarding the instability and quick degradation of RA various approaches, such as incorporation in nano- or microformulations by including RA into cyclodextrin, chitosan, lipid nanoparticles or chemical modifications have been applied. The use of these approaches reflects in improved shelf-life, stability, biocompatibility, prolonged biological effect and minimization of the eventual toxic effect of RA for humans. As a result, RA could be successfully applied in food, cosmetic industry and pharmacy, incorporated in oils, milk, meat products and beverages, cosmetic emulsions, or functional food products.

Despite the biotechnological challenges that remain open and gaps concerning RA bioavailability that need further improvements, the promising bioactivity of this natural molecule highlights its utilization in functional food products development.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Achamlale, S., Rezzonico, B., & Grignon-Dubois, M. (2009). Rosmarinic acid from beach waste: Isolation and HPLC quantification in *Zostera detritus* from Arcachon lagoon. *Food Chemistry, 113*(4), 878–883. https://doi.org/10.1016/j.foodchem.2008.07.040

Aksamija, A., Polidori, A., Plasson, R., Dangles, O., & Tomao, V. (2016). The inclusion complex of rosmarinic acid into beta-cyclodextrin: A thermodynamic and structural analysis by NMR and capillary electrophoresis. *Food Chemistry, 108*, 258–263. https://doi.org/10.1016/j.foodchem.2016.04.008

Açıkgöz, M. (2020). Establishment of cell suspension cultures of *Ocimum basilicum* L. and enhanced production of pharmaceutical active ingredients. *Industrial Crops and Products, 148*, Article e112278. https://doi.org/10.1016/j.indcrop.2020.112278

Barberini, S., Savona, M., Raffi, D., Leonardi, M., Pistelli, L., Stochmal, A., et al. (2013). Molecular cloning of *SoHPPR* encoding a hydroxyphenylpyruvate reductase, and its expression in cell suspension cultures of *Salvia officinalis*. *Plant Cell, Tissue and Organ Culture, 114*, 131–138. https://doi.org/10.1007/s11240-013-0300-8

Bhatt, R., Singh, D., Prakash, A., & Mishra, N. (2015). Development, characterization and nasal delivery of rosmarinic acid-loaded solid lipid nanoparticles for the effective management of Huntington's disease. *Drug Development, 22*(7), 931–939. https://doi.org/10.3109/10717544.2014.880860

Bielecka, M., Zielińska, S., Pencakowski, B., Stafiniak, M., Ślusarczyk, S., Prescha, A., et al. (2019). Age-related variation of polyphenol content and expression of phenylpropanoid biosynthetic genes in *Agastache rugosa*. *Industrial Crops and Products, 141*, Article e111743. https://doi.org/10.1016/j.indcrop.2019.111743

Campos, D., Madureira, A., Gomes, A., Sarmento, B., & Pintado, M. (2014). Optimization of the production of solid Witepsol nanoparticles loaded with rosmarinic acid. *Colloids and Surfaces B: Biointerfaces, 115*, 109–117. https://doi.org/10.1016/j.colsurfb.2013.10.035

Cardullo, N., Catinella, G., Floresta, G., Muccilli, V., Rosselli, S., Rescifina, A., et al. (2019). Synthesis of rosmarinic acid amides as antioxidative and hypoglycemic agents. *Journal of Natural Products, 82*(3), 573–582. https://doi.org/10.1021/acs.jnatprod.8b01002

Casanova, F., Estevinho, B. N., & Santos, L. (2016). Preliminary studies of rosmarinic acid microencapsulation with chitosan and modified chitosan for topical delivery. *Powder Technology, 297*, 44–49. https://doi.org/10.1016/j.powtec.2016.04.014

Celano, R., Piccinelli, A., Pagano, I., Roscigno, G., Campone, L., Falco, E., et al. (2017). Oil distillation wastewaters from aromatic herbs as new natural source of antioxidant compounds. *Food Research International, 99*(1), 298–307. https://doi.org/10.1016/j.foodres.2017.05.036

Chaiyana, W., Anuchapreeda, S., Somwongin, S., Marsup, P., Lee, K.-H., Lin, W.-C., et al. (2020). Dermal delivery enhancement of natural anti-ageing compounds from *Ocimum sanctum* Linn. extract by nanostructured lipid carriers. *Pharmaceutics, 12*(4), e309. https://doi.org/10.3390/pharmaceutics12040309

Chen, S.-Y., Wang, G.-Y., Lin, J.-H., & Yen, G.-C. (2020). Antioxidant and anti-inflammatory activities and bioactive compounds ofthe leaves of *Trichodesma khasianum* Clarke. *Industrial Crops and Products, 151*, Article e112447. https://doi.org/10.1016/j.indcrop.2020.112447

Choi, S.-H., Jang, G.-W., Choi, S.-I., Jung, T.-D., Cho, B.-Y., Sim, W.-S., et al. (2019). Development and validation of an analytical method for carnosol, carnosic acid and rosmarinic acid in food matrices and evaluation of the antioxidant activity of rosemary extract as a food additive. *Antioxidants, 8*(3), e76. https://doi.org/10.3390/antiox8030076

Costa, P., Sarmento, B., Gonçalves, S., & Romano, A. (2013). Protective effects of *Lavandula viridis* L'Hér extracts and rosmarinic acid against H_2O_2-induced oxidative damage in A172 human astrocyte cell line. *Industrial Crops and Products, 50*, 361–365. https://doi.org/10.1016/j.indcrop.2013.07.054

Deng, C., Wang, Y., Huang, F., Lu, S., Zhao, L., Ma, X., et al. (2020). *SmMYB2* promotes salvianolic acid biosynthesis in the medicinal herb *Salvia miltiorrhiza*. *Journal of Integrative Plant Biology*, 1–15. https://doi.org/10.1111/jipb.12943

Ding, H.-Y., Chou, T.-H., & Liang, C.-H. (2010). Antioxidant and antimelanogenic properties of rosmarinic acid methyl ester from *Origanum vulgare*. *Food Chemistry, 123*(2), 254–262. https://doi.org/10.1016/j.foodchem.2010.04.025

Fateminasab, F., Bordbar, A., Shityakov, S., & Saboury, A. (2020). Molecular insights into inclusion complex formation between β- and γ-cyclodextrins and rosmarinic acid. *Journal of Molecular Liquids, 314*, Article e113802. https://doi.org/10.1016/j.molliq.2020.113802

Fattahi, M., Nazeri, V., Torras-Claveria, L., Sefidkon, F., Cusido, R., Zamani, Z., et al. (2013). A new biotechnological source of rosmarinic acid and surface flavonoids: Hairy root cultures of *Dracocephalum kotschyi* Boiss. *Industrial Crops and Products, 50*, 256–263. https://doi.org/10.1016/j.indcrop.2013.07.029

Ferraro, V., Madureira, A., Sarmento, B., Gomes, A., & Pintado, M. (2015). Study of the interactions between rosmarinic acid and bovine milk whey protein α-lactalbumin, β-lactoglobulin and lactoferrin. *Food Research International, 77*(3), 450–459. https://doi.org/10.1016/j.foodres.2015.08.024

Filipe, H., Sousa, C., Marquês, J., Vila-Viçosa, D., De Granada-Flor, A., Viana, A., et al. (2018). Differential targeting of membrane lipid domains by caffeic acid and its ester derivatives. *Free Radical Biology and Medicine, 115*, 232–245. https://doi.org/10.1016/j.freeradbiomed.2017.12.002

Fu, R., Shi, M., Deng, C., Zhang, Y., Zhang, X., Wang, Y., et al. (2020). Improved phenolic acid content and bioactivities of *Salvia miltiorrhiza* hairy roots by genetic manipulation of *RAS* and *CYP98A14*. *Food Chemistry, 331*. https://doi.org/10.1016/j.foodchem.2020.127365

Georgiev, M., Kovacheva, E., Marcheva, N., & Ilieva, M. (2006). Purification of rosmarinic acid extracts from *Lavandula vera* MM cell biomass. *Food Chemistry, 94*(1), 111–114. https://doi.org/10.1016/j.foodchem.2004.10.055

Georgiev, M., Pastore, S., Lulli, D., Alipieva, K., Kostyuk, V., Potapovich, A., et al. (2012). *Verbascum xanthophoeniceum*-derived phenylethanoid glycosides are potent inhibitors of inflammatory chemokines in dormant and interferon-gamma-stimulated human keratinocytes. *Journal of Ethnopharmacology, 144*(3), 754–760. https://doi.org/10.1016/j.jep.2012.10.035

Ge, L., Zhu, M., Li, X., Xu, Y., Ma, X., Shi, R., et al. (2018). Development of active rosmarinic acid-gelatin biodegradable films with antioxidant and long-term antibacterial activities. *Food Hydrocolloids, 83*, 308–316. https://doi.org/10.1016/j.foodhyd.2018.04.052

Gonçalves, G. A., Correa, R. C. G., Barros, L., Dias, M. I., Calhelha, R. C., Correa, V. G., et al. (2019a). Effects of *in vitro* gastrointestinal digestion and colonic fermentation on a rosmary (*Rosmarinus officinalis* L.) extract rich in rosmarinic acid. *Food Chemistry, 271*, 393–400. https://doi.org/10.1016/j.foodchem.2018.07.132

Gonçalves, S., Mansinhos, I., Rodríguez-Solana, R., Pérez-Santín, E., Coelho, N., & Romano, A. (2019b). Elicitation improves rosmarinic acid content and antioxidant activity in *Thymus lotocephalus* shoot cultures. *Industrial Crops and Products, 137*, 214–220. https://doi.org/10.1016/j.indcrop.2019.04.071

Guitard, R., Paul, J.-F., Nardello-Rataj, V., & Aubry, J.-M. (2016). Myricetin, rosmarinic and carnosic acids as superior natural antioxidant alternatives to α-tocopherol for the preservation of omega-3 oils. *Food Chemistry, 213*, 284–295. https://doi.org/10.1016/j.foodchem.2016.06.038

Hadi, N., Sefidkon, F., Shojaeiyan, A., Šiler, B., Jafari, A.-A., Aničić, N., et al. (2017). Phenolics' composition in four endemic *Nepeta* species from Iran cultivated under experimental field conditions: The possibility of the exploitation of *Nepeta* germplasm. *Industrial Crops and Products, 95*, 475–484. https://doi.org/10.1016/j.indcrop.2016.10.059

Hao, X., Pu, Z., Cao, G., You, D., Zhou, Y., Deng, C., et al. (2020). Tanshinone and salvianolic acid biosynthesis are regulated by*SmMYB98*in *Salvia miltiorrhiza* hairy roots. *Journal of Advanced Research, 23*, 1–12. https://doi.org/10.1016/j.jare.2020.01.012

Hidalgo, D., Georgiev, M., Marchev, A., Bru-Martínez, R., Cusido, R., Corchete, P., et al. (2017). Tailoring tobacco hairy root metabolism for the production of stilbenes. *Scientific Reports, 7*, Article e17976. https://doi.org/10.1038/s41598-017-18330-w

Hirondart, M., Rombaut, N., Fabiano-Tixier, A., Bily, A., & Chemat, F. (2020). Comparison between pressurized liquid extraction and conventional soxhlet extraction for rosemary antioxidants, yield, composition, and environmental footprint. *Foods, 9*(5), e584. https://doi.org/10.3390/foods9050584

Hitl, M., Kladar, N., Gavaric, N., & Bozin, B. (2020). Rosmarinic acid – human pharmacokinetics and health benefits. *Planta Medica*. https://doi.org/10.1055/a-1301-8648 (in press).

Huang, J., Chen, P., Rogers, M., & Wettig, S. (2019). Investigating the phospholipid effect on the bioaccessibility of rosmarinic acid-phospholipid complex through a dynamic gastrointestinal *in vitro* model. *Pharmaceutics, 11*(4), e156. https://doi.org/10.3390/pharmaceutics11040156

Jacotet-Navarro, M., Rombaut, N., Fabiano-Tixier, A.-S., Danguien, M., Bily, A., & Chemat, F. (2015). Ultrasound versus microwave as green processes for extraction of rosmarinic, carnosic and ursolic acids from rosemary. *Ultrasonics Sonochemistry, 27*, 102–109. https://doi.org/10.1016/j.ultsonch.2015.05.006

Jia, Y., Bai, Z., Pei, T., Ding, K., Liang, Z., & Gong, Y. (2017). The protein kinase *SmSnRK2.6* positively regulates phenolic acid biosynthesis in *Salvia miltiorrhiza* by interacting with *SmAREB1*. *Frontiers of Plant Science, 8*, e1384. https://doi.org/10.3389/fpls.2017.01384

Khojasteh, A., Metón, I., Camino, S., Cusido, R., Eibl, R., & Palazon, J. (2019). *In vitro* study of the anticancer effects of biotechnological extracts of the endangered plant species *Satureja khuzistanica*. *International Journal of Molecular Sciences, 20*(10), Article e2400. https://doi.org/10.3390/ijms20102400

Klisurova, D., Petrova, I., Ognyanov, M., Georgiev, Y., Kratchanova, M., & Denev, P. (2019). Co-pigmentation of black chokeberry (*Aronia melanocarpa*) anthocyanins with phenolic co-pigments and herbal extracts. *Food Chemistry, 279*, 162–170. https://doi.org/10.1016/j.foodchem.2018.11.125

Kovatcheva-Apostolova, E. G., Georgeiv, M. I., Ilieva, M. P., Skidsted, L. H., Rødtjer, A., & Andersen, M. L. (2008). Extracts of plant cell cultures of *Lavandula vera* and *Rosa damascena* as sources of phenolic antioxidants for use in foods. *European Food Research and Technology, 227*, 1243–1249. https://doi.org/10.1007/s00217-008-0842-x

Kovacheva, E., Georgiev, M. I., Pashova, S., Angelova, M., & Ilieva, M. (2006). Radical quenching by rosmarinic acid from *Lavandula vera* MM cell culture. *Zeitschrift für Naturforschung C, 61*, 517–520.

Li, Z., Henning, S., Zhang, Y., Zerlin, A., Li, L., Gao, K., et al. (2010). Antioxidant-rich spice added to hamburger meat during cooking results in reduced meat, plasma, and urine malondialdehyde concentrations. *American Journal of Clinical Nutrition, 91*(5), 1180–1184. https://doi.org/10.3945/ajcn.2009.28526

Li, Y., Qi, H., Fan, M., Zhu, Z., Zhan, S., Li, L., et al. (2020). Quantifying the efficiency of o-benzoquinones reaction with amino acids and related nucleophiles by cyclic voltammetry. *Food Chemistry, 317*, Article e126454. https://doi.org/10.1016/j.foodchem.2020.126454

Liu, T., Sui, X., Zhang, R., Yang, L., Zu, Y., Zhang, L., et al. (2011). Application of ionic liquids based microwave-assisted simultaneous extraction of carnosic acid, rosmarinic acid and essential oil from *Rosmarinus officinalis*. *Journal of Chromatography A, 1218*(47), 8480–8489. https://doi.org/10.1016/j.chroma.2011.09.073

Li, P., Yang, X., Lee, W., Huang, F., Wang, Y., & Li, Y. (2021). Comparison between synthetic and rosemary-based antioxidants for the deep frying of French fries in refined soybean oils evaluated by chemical and non-destructive rapid methods. *Food Chemistry, 335*, Article e127638. https://doi.org/10.1016/j.foodchem.2020.127638

Maciuk, A., Toribio, A., Zeches-Hanrot, M., Nuzillard, J.-M., Renault, J.-H., Georgiev, M. I., et al. (2005). Purification of rosmarinic acid by strong ion-exchange centrifugal partition chromatography. *Journal of Liquid Chromatography & Related Technologies, 28*, 12–13. https://doi.org/10.1081/JLC-200063599

Madureira, A. R., Campos, D., Gullon, B., Marques, C., Rodríguez-Alcalá, L. M., Calhau, C., et al. (2016). Fermentation of bioactive solid lipid nanoparticles by human gut microflora. *Food and Function, 7*(2016), 516–529. https://doi.org/10.1016/j.jfoodeng.2015.06.010

Madureira, A. R., Campos, D. A., Oliveira, A., Sarmento, B., Pintado, M. M., & Gomes, A. M. (2016). Insights into the protective role of solid lipid nanoparticles on rosmarinic acid bioactivity during exposure to simulated gastrointestinal conditions. *Colloids and Surfaces B: Biointerfaces, 139*(2016), 277–284. https://doi.org/10.1016/j.colsurfb.2015.11.039

Madureira, A. R., Pereira, A., Castro, P., & Pintado, M. (2015). Production of antimicrobial chitosan nanoparticles against food pathogens. *Journal of Food Engineering, 167*(B), 210–216. https://doi.org/10.1016/j.jfoodeng.2015.06.010

Marchev, A., & Georgiev, M. (2020). Plant *in vitro* systems as a sustainable source of active ingredients for cosmeceutical application. *Molecules, 25*(9). https://doi.org/10.3390/molecules25092006

Marchev, A., Yordanova, Z., & Georgiev, M. (2020). Green (cell) factories for advanced production of plant secondary metabolites. *Critical Reviews in Biotechnology, 40*(4), 443–458. https://doi.org/10.1080/07388551.2020.1731414

Mesnier, X., Gregory, C., Fança-Berthon, P., Boukobza, F., & Bily, A. (2014). Heat and light colour stability of beverages coloured with a natural carotene emulsion: Effect of synthetic versus natural water soluble antioxidants. *Food Research International, 65* (B), 149–155. https://doi.org/10.1016/j.foodres.2014.06.025

Min, J., Chen, H., Gong, Z., Liu, X., Wu, T., Li, W., et al. (2018). Pharmacokinetic and pharmacodynamic properties of rosmarinic acid in rat cholestatic liver injury. *Molecules, 23*, 2287. https://doi.org/10.3390/molecules23092287

Nourozi, E., Hosseini, B., Maleki, R., & Mandoulakani, B. (2019). Pharmaceutical important phenolic compounds overproduction and gene expression analysis in *Dracocephalum kotschyi* hairy roots elicited by SiO_2 nanoparticles. *Industrial Crops and Products, 133*, 435–446. https://doi.org/10.1016/j.indcrop.2019.03.053

Nunes, S., Madureira, A. R., Campos, D., Sarmento, B., Gomes, A. M., Pintado, M., et al. (2017). Therapeutic and nutraceutical potential of rosmarinic acid—Cytoprotective properties and pharmacokinetic profile. *Critical Reviews in Food Science and Nutrition, 57*(9), 1799–1806. https://doi.org/10.1080/10408398.2015.1006768

Park, W., Arasu, M., Al-Dhabi, N., Yeo, S., Jeon, J., Park, J., et al. (2016). Yeast extract and silver nitrate induce the expression of phenylpropanoid biosynthetic genes and induce the accumulation of rosmarinic acid in *Agastache rugosa* cell culture. *Molecules, 21*(4), e426. https://doi.org/10.3390/molecules21040426

Park, J. W., Kim, J. E., Choi, Y. J., Kang, M. J., Choi, H. J., Bae, S. J., et al. (2021). Deficiency of complement component 3 may be linked to the development of constipation in FVB/N-C3^{em1Hlee}/Korl mice. *The FASEB Journal, 35*, Article e21221. https://doi.org/10.1096/fj.202000376R

Peng, X., Wang, X., Qi, W., Huang, R., Su, R., & He, Z. (2015). Deciphering the binding patterns and conformation changes upon the bovine serum albumin-rosmarinic acid complex. *Food and Function, 6*, 2712–2726. https://doi.org/10.1039/C5FO00597C

Peng, X., Wang, X., Qi, W., Su, R., & He, Z. (2016). Affinity of rosmarinic acid to human serum albumin and its effect on protein conformation stability. *Food Chemistry, 192*, 178–187. https://doi.org/10.1016/j.foodchem.2015.06.109

Petersen, M. (2013). Rosmarinic acid: New aspects. *Phytochemistry Reviews, 12*, 207–227. https://doi.org/10.1007/s11101-013-9282-8

Quintana, S., Villanueva-Bermejo, D., Reglero, G., García-Risco, M., & Fornari, T. (2019). Supercritical antisolvent particle precipitation and fractionation of rosemary (*Rosmarinus officinalis* L.) extracts. *Journal of CO2 Utilization, 34*, 479–489. https://doi.org/10.1016/j.jcou.2019.07.032

Ru, M., Wang, K., Bai, Z., Peng, L., He, S., Wang, Y., et al. (2017). A tyrosine aminotransferase involved in rosmarinic acid biosynthesis in *Prunella vulgaris L. Scientific Reports, 7*, Article e4892. https://doi.org/10.1038/s41598-017-05290-4

Saad, E., El Gohary, N., Abdel-Halim, M., Handoussa, H., El Nashar, R., & Mizaikoff, B. (2021). Molecularly imprinted polymers for selective extraction of rosmarinic acid from *Rosmarinus officinalis* L. *Food Chemistry, 335*(15), Article e127644. https://doi.org/10.1016/j.foodchem.2020.127644

Sahiner, M., Blake, D., Fullerton, M., Suner, S., Sunol, A., & Sahiner, N. (2019). Enhancement of biocompatibility and carbohydrate absorption control potential of rosmarinic acid through crosslinking into microparticles. *International Journal of Biological Macromolecules, 137*, 836–843. https://doi.org/10.1016/j.ijbiomac.2019.07.032

da Silva, S., Ferreira, D., Pintado, M., & Sarmento, B. (2016). Chitosan-based nanoparticles for rosmarinic acid ocular delivery-*in vitro* tests. *International Journal of Biological Macromolecules, 84*, 112–120. https://doi.org/10.1016/j.ijbiomac.2015.11.070

Tang, W., Sun, B., & Zhao, Y. (2014). Preparative separation and purification of rosmarinic acid from perilla seed meal *via* combined column chromatography. *Journal of Chromatography B, 947–948*, 41–48. https://doi.org/10.1016/j.jchromb.2013.12.007

Tang, C.-B., Zhang, W.-G., Wang, Y.-S., Xing, L.-J., Xu, X.-L., & Zhou, G.-H. (2016). Identification of rosmarinic acid-adducted sites in meat proteins in a gel model under oxidative stress by triple TOF MS/MS. *Journal of Agricultural and Food Chemistry, 64* (33), 6466–6476. https://doi.org/10.1021/acs.jafc.6b02438

Ulbrich, B., Wiesner, W., & Arens, H. (1985). Large-scale production of rosmarinic acid from plant cell cultures of *Coleus blumei* Benth. In K. H. Neumann, W. Barz, & E. Reinhard (Eds.), *Primary and secondary metabolism of plant cell cultures* (pp. 293–303). Berlin: Springer (Heidelberg).

Vasileva, L. V., Savova, M. S., Tews, D., Wabitsch, M., & Georgiev, M. I. (2021). Rosmarinic acid attenuates obesity and obesity-ralated inflammation in human adipocytes. *Food and Chemical Toxicology, 149*, Article 112002. https://doi.org/10.1016/j.fct.2021.112002

Wang, S.-J., Chen, Q., Liu, M.-Y., Yu, H.-Y., Xu, J.-Q., Wu, J.-Q., et al. (2019). Regulation effects of rosemary (*Rosmarinus officinalis* Linn.) on hepatic lipid metabolism in OA induced NAFLD rats. *Food and Function, 10*, 7356–7365. https://doi.org/10.1039/c9fo01677e

Wang, D., Song, Y., Chen, Y., Yao, W., Li, Z., Liu, W., et al. (2013). Metabolic pools of phenolic acids in *Salvia miltiorrhiza* are enhanced by co-expression of *Antirrhinum majus Delila* and *Rosea1* transcription factors. *Biochemical Engineering Journal, 74*, 115–120. https://doi.org/10.1016/j.bej.2013.02.014

Weitzel, C., & Petersen, M. (2011). Cloning and characterisation of rosmarinic acid synthase from *Melissa officinalis* L. *Phytochemistry, 72*(7), 572–578. https://doi.org/10.1016/j.phytochem.2011.01.039

Weremczuk-Jeżyna, I., Skała, E., Olszewska, M., Kiss, A., Balcerczak, E., Wysokińska, H., et al. (2016). The identification and quantitative determination of rosmarinic acid and salvianolic acid B in hairy root cultures of *Dracocephalum forrestii* W.W. Smith. *Industrial Crops and Products, 91*, 125–131. https://doi.org/10.1016/j.indcrop.2016.07.002

Wu, C.-F., Karioti, A., Rohr, D., Bilia, A., & Efferth, T. (2016). Production of rosmarinic acid and salvianolic acid B from callus culture of *Salvia miltiorrhiza* with cytotoxicity towards acute lymphoblastic leukemia cells. *Food Chemistry, 201*, 292–297. https://doi.org/10.1016/j.foodchem.2016.01.054

Xiao, Y., Gao, S., Di, P., Chen, J., Chen, W., & Zhang, L. (2009). Methyl jasmonate dramatically enhances the accumulation of phenolic acids in *Salvia miltiorrhiza* hairy root cultures. *Physiologia Plantarum, 137*, 1–9.

Xiao, Y., Zhang, L., Gao, S., Saechao, S., Di, P., Chen, J., et al. (2011). The *c4h, tat, hppr* and *hppd* genes prompted engineering of rosmarinic acid biosynthetic pathway in *Salvia miltiorrhiza* hairy root cultures. *PloS One, 6*(12), Article e29713. https://doi.org/10.1371/journal.pone.0029713

Xing, B., Liang, L., Liu, L., Hou, Z., Yang, D., Yan, K., et al. (2018). Overexpression of *SmbHLH148* induced biosynthesis of tanshinones as well as phenolic acids in *Salvia miltiorrhiza* hairy roots. *Plant Cell Reports, 37*, 1681–1692. https://doi.org/10.1007/s00299-018-2339-9

Xing, B., Yang, D., Liu, L., Han, R., Sun, Y., & Liang, Z. (2018). Phenolic acid production is more effectively enhanced than tanshinone production by methyl jasmonate in *Salvia miltiorrhiza* hairy roots. *Plant Cell, Tissue and Organ Culture, 134*, 119–129. https://doi.org/10.1007/s11240-018-1405-x

Yang, N., Zhou, W., Su, J., Wang, X., Li, L., Wang, L., et al. (2017). Overexpression of *SmMYC2* increases the production of phenolic acids in *Salvia miltiorrhiza*. *Frontiers of Plant Science, 8*, Article e1804. https://doi.org/10.3389/fpls.2017.01804

Yousefian, S., Lohrasebi, T., Farhadpour, M., & Haghbeen, K. (2020). Effect of methyl jasmonate on phenolic acids accumulation and the expression profile of their biosynthesis-related genes in *Mentha spicata* hairy root cultures. *Plant Cell, Tissue and Organ Culture, 142*, 285–297. https://doi.org/10.1007/s11240-020-01856-9

Zhang, S., Li, H., Liang, X., Yan, Y., Xia, P., Jia, Y., et al. (2015). Enhanced production of phenolic acids in *Salvia miltiorrhiza* hairy root cultures by combing the RNAi-mediated silencing of chalcone synthase gene with salicylic acid treatment. *Biochemical Engineering Journal, 103*, 185–192. https://doi.org/10.1016/j.bej.2015.07.019

Zhang, B., Wang, Q., Zhou, P.-P., Li, N.-N., & Han, S.-Y. (2020). Copigmentation evidence of oenin with phenolic compounds: A comparative study of spectrographic, thermodynamic and theoretical data. *Food Chemistry, 313*, Article e126163. https://doi.org/10.1016/j.foodchem.2020.126163

Zhang, S., Willett, S., Hyatt, J., Martini, S., & Akoh, S. (2021). Phenolic compounds as antioxidants to improve oxidative stability of menhaden oil-based structured lipid as butterfat analog. *Food Chemistry, 334*, Article e127584. https://doi.org/10.1016/j.foodchem.2020.127584

Zhang, C., Xing, B., Yang, D., Ren, M., Guo, H., Yang, S., et al. (2020). SmbHLH3 acts as a transcription repressor for both phenolic acids and tanshinone biosynthesis in *Salvia miltiorrhiza* hairy roots. *Phytochemistry, 169*, Article e112183. https://doi.org/10.1016/j.phytochem.2019.112183

Zhao, L., Zhang, Y., Liu, G., Hao, S., Wang, C., & Wang, Y. (2018). Black rice anthocyanin-rich extract and rosmarinic acid, alone and in combination, protect against DSS-induced colitis in mice. *Food and Function, 9*, 2796–2808. https://doi.org/10.1039/c7fo01490b