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Transformations and antioxidative activities of lignans and stilbenes at high temperatures

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Matairesinol (PubChem CID:119205)
α-Conidenrin (PubChem CID: 457194)
7-Hydroxy-secoisolariciresinol (PubChem CID: 44566585)
Secoisolariciresinol (PubChem CID: 65373)
Lariciresinol (PubChem CID: 332427)
Cyclolariciresinol (PubChem CID: 4177125)
Resveratrol (PubChem CID: 445154)
Pinosylvin (PubChem CID: 5280457)
Pinosylvin Monomethyl Ether (PubChem CID: 5281719)

ABSTRACT

Thermal transformations of polyphenols from the lignan and stilbene families were investigated at temperatures ranging from 200 °C to 250 °C, in polyethylene glycol (PEG-400), dimethylformamide (DMF) and in sunflower oil (SO). The polyphenols showed varying degrees of thermal stabilities and in some cases intramolecular transformations were observed. The formed products were isolated and characterized. Oligomerization of the polyphenols at thermo-oxidative conditions was also investigated. Finally, the antioxidative activity of the polyphenols against thermo-oxidative degradation α-linoleic acid was investigated at 200 °C. The results suggested that the studied substrates retained their antioxidative properties at elevated temperatures, with stilbenes showing most efficient protection against thermo-oxidative degradation of polyunsaturated fatty acids.

1. Introduction

Phytochemical polyphenols such as lignans and stilbenes have attracted much interest in the field of food chemistry as they often exhibit biological activities such as antioxidant and antibacterial properties. Over 8000 phytochemical polyphenols have been identified. They occur in plants both in their free form, as glycosides, or covalently bound to macromolecules (Palermo, Pellegrini, & Fogliano, 2014). Both lignans and stilbenes are phytochemical polyphenols originated from the shikimic acid pathway (Francenia Santos-Sánchez, Salas-Coronado, Hernández-Carlos, & Villanueva-Cañongo, 2019). They are often enantiopure compounds that provide the plant with protection against herbivores and microorganisms (Zarei & Ryan, 2019). Lignans are structurally dimeric compounds bonded with a β,β′-linkage between the monolignol units with varying degrees of oxidation in the side chain and with varying substitution of the aromatic rings. Stilbenes are 1,2-diarylethenes with different substituents at the aromatic ring.

Lignans are found in most plants, and dietary lignans are especially abundant in fiber-rich foods, having a range of potential health benefits (Rodríguez-García, Sánchez-Quesada, Toledo, Delgado-Rodríguez, & Gaforio, 2019; Smeds et al., 2007). Similarly, dietary stilbenes have shown to have a number of health benefits such as antioxidant, anticancer and anti-inflammatory effects (El Khawand, Courtois, Valls, Richard, & Krisa, 2018).

Some of the major dietary lignans in foods are matairesinol (MR), lariciresinol (LARI), secoisolariciresinol (SECO) and the diglucoside derivative of secoisolariciresinol (SDG), sesamin, pinoresinol (PINO), medioresinol and syringaresinol. The health benefits of plant lignans are

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largely due to the antioxidant activities, although the biological activities are partly also related to those of the metabolites enterodiol (END) and enterolactone (ENL). END and ENL are phytoestrogens, formed in mammals by the gastrointestinal microbiota. In contrast to the dietary lignans, the biological activities of END and ENL are mainly due to antiestrogenic effects (Begum et al., 2004; Durazzo et al., 2018; Landete, 2012; Wang, 2002).

The amounts and occurrence of polyphenols vary greatly between plant species, and it has been shown that knotwood in Norway spruce (*Picea abies*) contain high concentrations of the lignan hydroxymatairesinol (HMR) (Fig. 1.)(Willför, Hemming, Reunanen, Eckerman, & Holmbom, 2003). As large quantities of Norway spruce is being cut every day for milling, it is estimated that several thousands of tons of HMR could be isolated annually (Holmbom et al., 2003). Studies have indicated that HMR contributes to a healthy estrogen balance. For this reason, among others, HMR is sold as a food supplement (Cosentino et al., 2007; Giuliano et al., 2020). HMR can also be used as a versatile starting material for the semi-syntheses of other lignans, among others the dietary lignans matairesinol (MR), α-conidendrin (α-Coni), 7-hydroxy-secoisolariciresinol (7-OH-SECO), secoisolariciresinol (SECO), lariciresinol (LARI), and cyclolariciresinol (cLARI) (Fig. 1.)(Eklund, Sillanpää, & Sjöholm, 2002; Eklund, Sundell, Smeds, & Sjöholm, 2004; Ward & Hughes, 2001).

The stilbene resveratrol (Fig. 1.) has been extensively studied for its biological activities (El Khawand et al., 2018; Silva et al., 2017). Resveratrol is readily found in the skin of grapes and tomatoes, and can be found in red wine (Dybkowska, Sadowska, Świderski, Rakowska, & Wysocka, 2018). Pinosylvin (PS) and pinosylvin monomethylether (PSMME) (Fig. 1.) are bioactive stilbenes that are closely related to resveratrol. Both PS and PSMME are readily available from knotwood of scots pine (*Pinus sylvestris*) and can be isolated in large scale (Laavola et al., 2015).

It is well known that natural phenolics are efficient radical scavengers, and the antioxidative properties of most of the substrates in Fig. 1. have been investigated previously (Eklund et al., 2005; Laavola et al., 2015; Stivala et al., 2001; Willför, Ahotupa, et al., 2003). To our knowledge, however, no investigation on the thermal stability and the antioxidative properties at elevated temperatures has been conducted on these substrates. The decrease of phenolic content during thermal food processing has, however, been studied previously (Huang, Xiao, Burton-Freeman, & Edirisinghe, 2016; Palermo et al., 2014), and it seems that polyphenolic compounds are partially degraded. The thermal stability of individual polyphenols and the type of transformation products they result, is however, still unclear.

Most reactions of lignans found in literature have focused on relatively low temperatures (below 120 °C). The aim of this study was to investigate thermal transformations that occur in polyphenols at elevated temperatures, e.g. in thermal food processing and in biorefinery processes. The focus was on the identification of transformation products as well as to study how the structures can affect the thermal stability. The fate of dietary lignans and stilbenes at temperatures ranging between 200 and 250 °C, was studied. These temperatures may be considered high for food processes, but were used to achieve a higher degree of product formation sufficient for isolation. The lignans showed a high stability at temperatures below 200 °C (150–180 °C), and only traces of reaction products were detected, which were insufficient for unambiguous identification and determination of conversion and yield. The trace amounts of products detected, however, were similar to the products identified at higher temperatures, justifying the use of the higher temperatures for this study. This also indicated that, in pure systems and at typical food processing temperatures, these polyphenols are stable and safe.

The antioxidative activities of the polyphenols at elevated temperatures were also investigated by monitoring of the degradation of polyunsaturated fatty acid (PUFA) at high temperature, using α-linoleic acid as model fatty acid. The substrate scope (Fig. 1) was chosen to include different types of functional groups common for phytochemical polyphenols, such as methoxyls, primary and secondary hydroxyls, olefins, and butyrolactones.

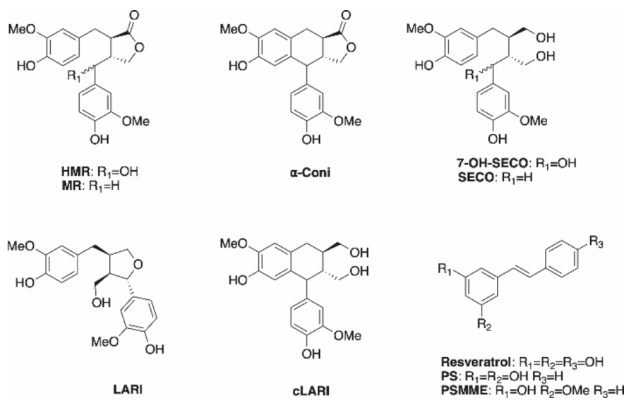


Fig. 1. The substrate scope of this study. Lignans: Hydroxymatairesinol (HMR), Matairesinol (MR), α-Conidenrin (α-Coni), 7-Hydroxy-secoisolariciresinol (7-OH-SECO), Secoisolariciresinol (SECO), Lariciresinol (LARI), Cyclolariciresinol (cLARI). Stilbenes: Resveratrol, Pinosylvin (PS), Pinosylvin monomethyl ether (PSMME).

2. Materials and methods

2.1. Substrates and solvents

All solvents and reagents were purchased from Sigma Aldrich if not stated otherwise and used without further purification. HMR was isolated from dried knotwood material of Norway spruce *(Picea Abies)* by extraction with ethanol, followed by precipitation as a potassium acetate-adduct. To remove the potassium acetate, the adduct was dissolved in acetone/water and extracted with dichloromethane and the solvent was evaporated to give HMR as a white powder. (Holmbom et al., 2003). MR was prepared by catalytic reduction of HMR with Pd/H_2, and α-Coni was prepared by acidic treatment, by dissolving HMR in formic acid. Reduction of MR with LAH (Lithium Aluminum Hydrid) in THF yielded SECO (Ward & Hughes, 2001). 7-OH-SECO was prepared through LAH reduction of HMR, and acid-catalyzed cyclization of 7-OH-SECO yielded LARI and cLARI (Eklund et al., 2002). Stilbenes PS and PSMME were isolated from the knots of Scots pine (*Pinus sylvestris*) by soxhlet extraction first with hexane to remove lipophilic compounds and then with acetone to yield stilbenes which were further purified by column chromatography. (Willför, Hemming, Reunanen, & Holmbom, 2003). Resveratrol was purchased as > 95 % pure *trans*-isomer from abcr GmbH Germany.

2.2. Thermal reactions

80 mg of substrate was dissolved in 2 mL the solvent (DMF or PEG-400 or sunflower oil) in a Duran culture tube. A magnet was added, and the tube was sealed with a PBT screw cap. For an experiment done in oxygen or argon atmosphere, the gas was bubbled through the solution for two minutes prior to sealing the tube. The culture tube was then put on a pre-heated sand bath and was covered with aluminum foil. After stirring for the reported time, the reaction tube was cooled to room temperature followed by work up depending on the solvent.

2.2.1. Work up for thermal reactions in DMF or PEG-400

After cooling down to room temperature, the reaction mixture was analyzed by thin layer chromatography (TLC) using chloroform:methanol 9:1 as eluent. To the reaction mixture, 50 mL saturated ammonium chloride solution was added followed by extraction with 3x50 mL ethyl acetate. The combined organic phase was washed with 3x50 mL H_2O,

dried with Na_2SO_4 and concentrated in vacuum at 40 °C. The reaction mixture was analyzed as such, and the products were isolated through column chromatography as described below. Also, the water phases were analyzed, but contained no polyphenols.

2.2.2. Work up for thermal reactions in sunflower oil

The reaction mixture in sunflower oil was diluted in 50 mL methanol and washed with 3x50 mL hexane. The methanol phase, still containing some sunflower oil, was analyzed by TLC, using chloroform:methanol 9:1 as eluent, and by gas chromatography with mass spectroscopy (GC–MS). The hexane phase was also analyzed, showing mostly sunflower oil, but occasionally traces of the same polyphenols as in the methanol-phase, were detected.

2.3. Isolation of monomeric products through column chromatography

The products were isolated through column chromatography using silica gel 60 as stationary phase and chloroform/methanol as eluent. The eluent system started with 100 % chloroform, gradually increasing the ratio of methanol until it reached 10 %. The reported yields are for the isolated products after column chromatography.

2.4. HP-SEC analyses to determine molecular size distribution in reaction mixtures

The liquid high performance size exclusion chromatography (HP-*SEC*) samples were prepared from the reaction mixture after work up (as described above) and concentration in a vacuum oven at 40 °C overnight. The reaction mixtures were dissolved in tetrahydrofuran (THF) containing 1 % acetic acid (AcOH) to a concentration of 1 mg sample /mL, followed by filtration through polytetrafluoroethylene (PTFE) syringe filters.

HP-*SEC* analyses were performed on an Agilent 1100 Series HPLC instrument equipped with a G1315B DAD-detector, 2 × Jordi Gel DVB 500 Å (300 mm × 7.8 mm) columns (Columnex LLC, New York, NY, USA), and a 50 mm × 7.8 mm guard column. The DAD measured UV absorption at 280 nm. 1 % of AcOH in THF served as eluent at a flow rate of 0.8 mL/min.

The HP-*SEC* analyses used in this study has previously been optimized for this purpose and the Mw determinations are based on calibration with standards.

2.5. Characterization of monomeric and degradation products by GC–MS analyses

Samples (~1 mg) containing hydroxyl groups were silylated by three drops hexamethyldisilazane (HMDS) and two drops chlorotrimethylsilane (CTMS) in 0.5 mL pyridine prior to analysis by GC-EIMS. GC–EIMS was done using an Agilent 7890A GC unit with a J&W HP-5MS column (30 m, 0.25 mm, 0.25 μm, fused silica), coupled to a 5975C inert XL EI/CI triple-axis MS-detector. The identification of individual compounds was based on our spectral database of reference compounds (verified with NMR).

2.5.1. Quantification of α-linoleic acid by GC

The quantification of α-linoleic acid was done using the same GC system as described above. After completed reaction, to the reaction mixture (100 μL α-linoleic acid, 180 μL octanoic acid, and polyphenol) was added 3.00 mL pyridine:tetradecane 30:1. The mixture was mixed using vortex for two minutes. A sample of 0.5 mL of the mixture was taken for GC–MS analysis, and the sample was silylated with three drops HMDS and two drops CTMS. The amount of α-linoleic acid was calculated from a calibration curve of tetradecane in the total ion chromatogram. All reactions and analyses were done in duplicates, and the average values and standard deviations are reported.

2.6. NMR analyses of isolated products

5 mg of vacuum dried sample was dissolved in 0.5 mL $CDCl_3$ for nuclear magnetic resonance (NMR) analysis. All the NMR experiments were performed at 298 K on a Bruker AVANCE III spectrometer operating at 500.13 MHz for ^1H and 125.77 MHz for ^{13}C. ^1H and ^{13}C NMR spectra for all isolated products can be found in the Supporting Information.

3. Results and discussion

3.1. Thermal transformations of polyphenols

In this section, the thermal transformations for each substrate are presented. The aim of this study was to obtain sufficient conversions for isolation and characterization of monomeric or oligomeric products and to investigate the major transformation products formed under elevated temperatures. However, the conditions forming large polymeric structures were also avoided, as the polymeric mixtures were difficult to characterize. Following the reactions at different temperatures (by TLC, GC–MS, and HPSEC) revealed that the optimum reaction conditions for most of the substrates were at 220 °C, in closed tubes, and with continuous stirring for 14–15 h. At shorter times only traces of transformation products could be detected. One exception was with the substrate 7-OH-SECO, which was more reactive than the other substrates. The reactions of 7-OH-SECO and the intermediate product, LARI, are discussed in detail later in this section. In Table 1, the most interesting reaction conditions, the isolated yields of the transformation products, and the amounts of recovered starting materials are listed for each substrate. The experiments presented in the table mainly used PEG-400 as solvent, but similar results were also achieved using DMF. Reactions in sunflower oil gave somewhat different results, and the yields were not determined in sunflower oil. In addition to the isolated monomeric products small amounts of oligomers were formed but were not isolated and characterized. The oligomers are discussed in more detail in a later section.

3.1.1. Thermal treatment of HMR

When HMR was thermally treated, it was transformed into the furanone structure 8,8́-dehydro-matairesinol (dH-MR)(Table 1, entry 1–2). After 15 h at 200 °C the isolated yield of dH-MR was only 10 %, and 70 % HMR was recovered. At 220 °C quantitative thermal transformation was achieved within the same time frame, with an isolated yield of 69 % (dH-MR). A likely mechanism was dehydration of the 7-hydroxyl group followed by isomerization of the double bond to form the unsaturated butanolide ring structure (Scheme 1). At 250 °C, HMR quantitatively reacted through non-selective oligomerization (entry 3).

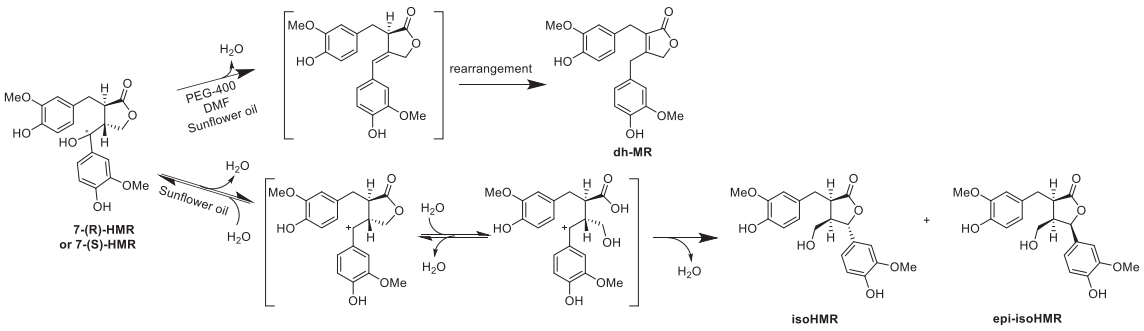
Thermal treatment of HMR in sunflower oil at 220 °C for 20 h resulted in mainly 7-R/S-isomerization of HMR in a 1:1 ratio, comprising around 70 % of the product mixture (observed by GC analysis). Additionally, 5 % dH-MR, 10 % MR, 2 % isohydroxymatairesinol (isoHMR) and 12 % *epi*-isohydroxymatairesinol (*epi*-isoHMR) were observed in the mixture (entry 20). Both the 7-R/S-isomerization of HMR and the formation of isoHMR and *epi*-isoHMR has previously been reported in aqueous alkaline conditions at 50 °C (Eklund et al., 2004). TLC analysis of the reaction mixture in sunflower oil indicated no polymer formation.

The different behavior of HMR in sunflower oil and in PEG-400 or DMF may be due to the water content in PEG-400 and DMF compared to sunflower oil, or the polarity of the medium. However, the formation of the dehydydro product in PEG and DMF as well as the isomerization in Sunflower oil indicated that dehydration is stronger in PEG and DMF and normal conversions known for aqueous media prevail in sunflower oil.

Table 1
Thermal transformations of the studied polyphenols. Reaction conditions, isolated yields, and the recovered starting material are listed. In addition to the isolated products some oligomers were formed.

Entry	Polyphenol	Solvent	Temp [C]	Time [h]	Product(s) (isolated yield)	Total isolated yield	SM recovered
1	HMR	PEG-400	200	15	dH-MR (10 %)	10 %	70 %
2	HMR	PEG-400	220	14	dH-MR (69 %)	69 %	0 %
3	HMR	PEG-400	250	14	Oligomerization	0 %	0 %
4	MR	PEG-400	220	14	NOP	0 %	85 %
5	α-Coni	PEG-400	220	14	β-Coni (81 %)	81 %	10 %
6	7-OH-SECO	PEG-400	220	0.08	LARI (31 %)	31 %	53 %
7	7-OH-SECO	PEG-400	220	0.17	LARI (72 %)	72 %	13 %
8	7-OH-SECO	PEG-400	220	0.25	LARI (90 %)	90 %	0 %
9	7-OH-SECO	PEG-400	250	14	diIMP (24 %) + IMP (34 %) + cIMP (21 %)	79 %	0 %
10	LARI	PEG-400	180	60	diIMP (36 %) + IMP (23 %) + cIMP (trace)	59 %	39 %
11	LARI	PEG-400	220	14	diIMP (55 %) + IMP (41 %) + cIMP (trace)	96 %	0 %
12	LARI	PEG-400	250	14	diIMP (17 %) + IMP (15 %) + cIMP (64 %)	96 %	0 %
13	IMP	PEG-400	250	14	cIMP (31 %)	31 %	0 %
14	SECO	PEG-400	250	15	Anhydrosecoisolariciresinol (59 %)	59 %	21 %
15	SECO	PEG-400	220	15	Anhydrosecoisolariciresinol (44 %)	44 %	49 %
16	cLARI	PEG-400	220	15	NOP	0 %	95 %
17	Resveratrol	PEG-400	220	18	NOP	0 %	98 %
18	PS	PEG-400	220	18	NOP	0 %	99 %
19	PSMME	PEG-400	220	24	NOP	0 %	81 %
20	HMR	SO	220	20	R/S-HMR (1:1) (70 %) + dH-MR (5 %) + MR (10 %) + isoHMR (2 %) + *epi*–isoHMR (12 %)	–	–
21	MR	SO	220	20	NOP	–	–
22	α-Coni	SO	220	20	α-Coni (80 %) + β-Coni (20 %)	–	–
23	7-OH-SECO	SO	220	20	7-OH-SECO(20 %) + LARI (80 %)	–	–
24	LARI	SO	220	20	NOP	–	–
25	Resveratrol	SO	220	20	NOP	–	–
26	PS	SO	220	20	NOP	–	–
27	PSMME	SO	220	20	NOP	–	–

NOP: No observed products.



Scheme 1. Thermal transformation of HMR.

3.1.2. Thermal treatment of MR

MR, the structurally simplest of the lignans, remained stable during the heat treatment and no transformations were observed (entry 4). Likewise, thermal treatment (220 °C for 20 h) of MR in sunflower oil led to no transformations. However, traces of oligomers were formed, and the oligomerizations are discussed further in a later section.

3.1.3. Thermal treatment of α-Coni

The aryltetralin type α-Coni, having a higher stability in the core skeleton compared to HMR, did not show any type of structural transformation upon treatment at 220 °C. However, the thermal treatment led to isomerization at the α-position of the lactone (C-8́) to the stereoisomer β-Coni in up to 81 % isolated yield (entry 5). [See Supporting Information for scheme of isomerization].

Likewise, thermal treatment of α-Coni in sunflower oil led to the isomerization to β-Coni. The isomerization rate, however, was slower compared to that in DMF or PEG-400, and after 20 h at 220 °C in sunflower oil the ratio of α-/β-Coni was 4:1.

The isomerization of α-Coni most likely occurred via enolization, as has been suggested previously (Hearon, Lackey, & Moyer, 1951). This mechanism is also supported by the fact that the aryltetralin cLARI, lacking the lactone ring and hence the ability to react through enolization, did not isomerize at C-8́ to the β-derivative, as will be presented in the next section.

3.1.4. Thermal treatment of 7-OH-SECO, SECO, LARI and cLARI

Thermal treatment of 7-OH-SECO led to a chain of reactions (Scheme 2) initiated by the transformation to LARI through a dehydration reaction. Heating for a short time (5–15 min) followed by quick cooling resulted in selective transformation to LARI (entries 6–8). With longer reaction times, LARI reacted further to a mixture of products (entry 9), and the same products were formed when LARI was used as substrate (entries 10–12, Table 1). A likely thermal degradation of LARI to a cation intermediate followed by deformylation formed IMP. IMP was in equilibrium with a dimeric structure (diIMP) through ether bond formation and breakage at the double bond. Also, IMP was partially transformed through intramolecular ring closure to the aryltetralin cycloimperanene (cIMP). The formation of cycloimperanene was accelerated at higher temperatures, with high isolated yields (up to 64 %) at 250 °C.

In sunflower oil, thermal treatment (220 °C for 20 h) of 7-OH-SECO led to partial transformation to LARI, with around 83 % conversion observed by GC (entry 23). No further transformation to the above-mentioned products was observed. Consequently, starting from LARI, no transformations were observed upon thermal treatment at 220 °C in sunflower oil (entry 24).

SECO reacted at 220 °C in PEG-400 through dehydration reaction to Anhydrosecoisolariciresinol (Anhydro-SECO) (entries 14–15). Anhydro-SECO is a dietary lignan found in same foodstuff as SECO, and has shown to have potential anticancer effects (Liggins, Grimwood, & Bingham, 2000). I sunflower oil, however, SECO was stable at 220 °C for 19 h, with minor oligomerization observed.

No thermal transformation or isomerization occurred in the structure of cLARI during treatment at 220 °C for 15 h in PEG-400, nor at 220 °C for 19 h in sunflower oil. However, some oligomerization was observed.

The results indicated that primary hydroxyls (as in SECO and cLARI) did not significantly lower the thermal stabilities of lignans, whereas benzylic hydroxyls (as in HMR and 7-OH-SECO) undergoes thermal cleavage into reactive intermediates.

3.1.5. Thermal treatment of stilbenes (Resveratrol, PS, PSMME)

All studied stilbenes showed a high thermal stability. During thermal treatment at 220 °C in PEG-400 for 18 h or in sunflower oil for 20 h, the stilbenes remained stable and no transformations to monomeric products were observed (GC–MS). Analyses by HP-*SEC* only revealed traces of oligomers.

To further investigate the stability of these stilbenes in sunflower oil, thermal treatment (220 °C for 12 h) was done in open air atmosphere (in culture tubes without caps on). The stilbenes remained mostly intact, although some traces of polymers were detected on TLC and HPSEC.

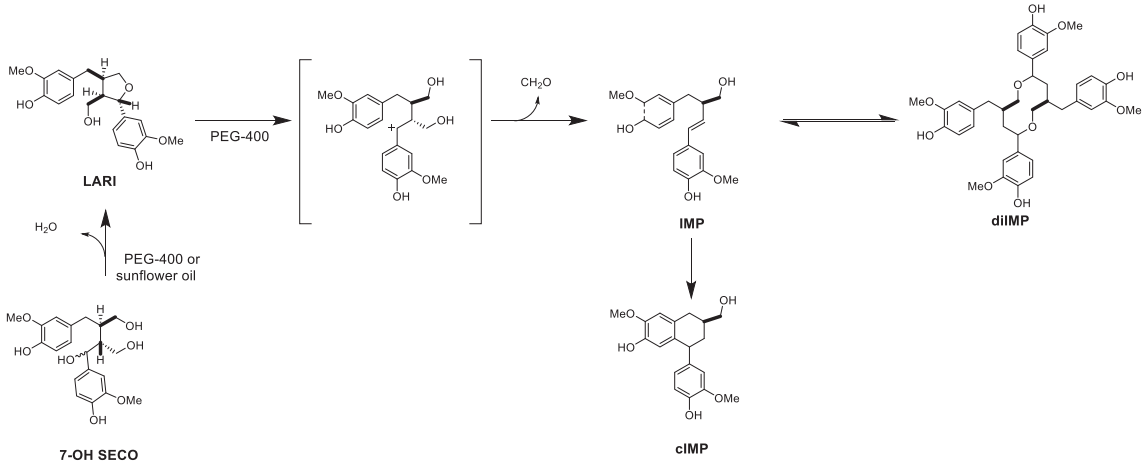
3.2. Effect of oxygen on thermal polymerization of polyphenols

Thermal treatments of the studied substates were compared in oxygen atmosphere and in argon atmosphere. Samples of polyphenols in PEG-400 (40 mg/mL) were bubbled with oxygen or argon for two minutes prior to the reactions, and the reaction tubes were sealed with the studied atmosphere inside. The samples were stirred at 220 °C for 18 h. The temperature and reaction time was optimized to achieve sufficient transformation for comparison between the two reaction environments. The mixtures were analyzed as such by GCMS and TLC. Work up as described in section 2.2.1 was performed prior to analysis by HP-*SEC* (Fig. 2).

The results seen in Fig. 2 showed that only minor degrees of thermal oligomerization took place in argon atmospheres. In O_2-atmospheres the rates of thermal polymerizations increased. The polymerizations may have resulted through oxidation, autoxidation, disproportionation or radical reactions, or more likely a combination of these, as the oxygen atmosphere certainly introduced reactive oxygen species and oxidations. It is worth noting that the UV-absorption spectra in the SEC-chromatograms were not quantitative, and intensities for polymeric signals were weak compared to the monomeric polyphenols.

The polymerizations showed a pattern, where an increase in radical scavenging groups (i.e. phenol hydroxyls) led to more abundant polymerization. Polymers with molecular weights up to 6000 Dalton were formed. Lignans with primary aliphatic hydroxyls (7-OH-SECO, LARI, cLARI) were readily polymerized during thermal treatment in O_2 atmosphere.

During thermal treatment of HMR in O_2 atmosphere partial



Scheme 2. Thermal transformations observed for 7-OH-SECO and LARI.

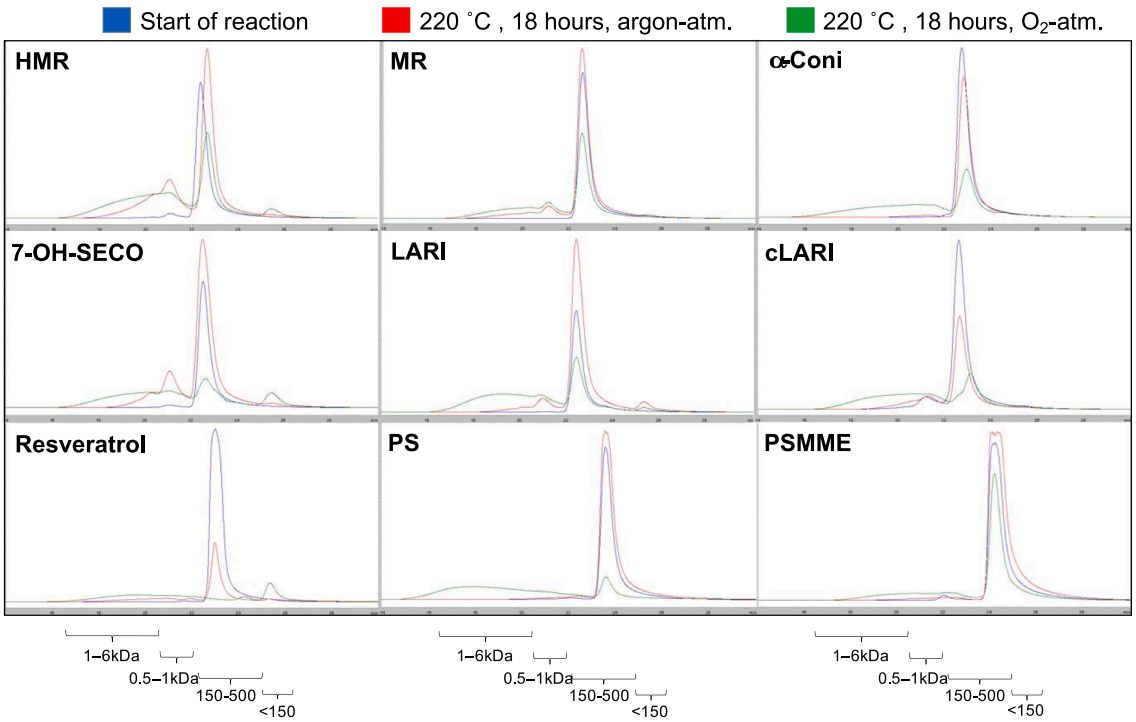


Fig. 2. Size-exclusion chromatograms of polyphenols before reactions (shown in blue); after thermal treatment (220 °C for 18 h in PEG-400) in argon atmosphere (shown in red); and in O_2 atmosphere (shown in green). The molecular weights (in Dalton) for different retention time regions are shown below. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

polymerization was observed. Additionally, formation of dH-MR from HMR was observed. From the SEC-chromatogram in Fig. 2, dH-MR can be seen as the signal shifted slightly to the right of HMR.

MR had the highest thermal and aerobic stability of the studied lignans, although some oligo- and polymers were observed even in argon atmosphere. This was expected due to the stability of the structure and the lack of aliphatic or benzylic hydroxyls.

The lignan *α*-Coni remained stable in argon-atmosphere. In O_2-atmosphere, *α*-Coni was readily polymerized. Traces of the naphthalene derivative didehydroconidendrin was observed by GC–MS. Didehydroconidendrin (also called dehydroconidendrin) has previously been prepared from *α*-Coni by DDQ oxidation (Ramdayal, Kiemle, & LaLonde, 1999).

The stilbenes also remained mostly stable in argon atmosphere, with no observed monomeric products and resveratrol showing traces of polymers. In O_2, the rate of polymerization was in accordance with the degree of radical scavenging groups (phenolic hydroxyls), where resveratrol was completely polymerized, PS was partially polymerized, and PSMME remained mostly stable. Analyses by GCMS after the thermal treatment (in O_2 atmosphere) of PS revealed traces of signals for cross-coupled PEG and PS. It is known that PEG undergoes random chain scission degradation at thermo-oxidative conditions, forming peroxides and radical intermediates (Lai & Liau, 2003; Wang, He, & Miao, 2009). As PEG-400 is not UV-active it was not detected by Diod-Array Detection used in the size exclusion chromatography, and analysis of a reference PEG-400 sample after thermal oxidation detected no signal either. As a conclusion the polymeric signals observed by HPSEC analyses correspond to polymerized polyphenols, although they may include products of cross-coupled PEG-polyphenols.

3.3. Antioxidative properties of polyphenols against thermal autoxidation of PUFAs

Thermal degradation reactions of polyunsaturated fatty acids (PUFAs) follows an autoxidation-process involving free radicals, primarily reacting with the PUFA double bonds to peroxides followed by subsequent reactions to mixtures of secondary products (Choe & Min, 2006; Moya Moreno, Mendoza Olivares, Amézquita López, Gimeno Adelantado, & Bosch Reig, 1999). The PUFA used in the study was the *α*-linoleic acid, an omega-6 essential fatty acid found in food products (Whelan & Fritsche, 2013). The thermal stability of isolated α-linoleic acid has been reported (Hădărugă et al., 2006). Already at 100 °C α-linoleic acid reacted through degradation to aldehydes, epoxides and vicinal dihydroxy acids. After heating at 150 °C for two hours, degradation of 45 % of α-linoleic acid was observed.

The antioxidative effects of the polyphenols used as substrates have been studied previously, including mechanistical studies, but not at elevated temperature (P. C. Eklund et al., 2005; Laavola et al., 2015; Stivala et al., 2001; Willför, Ahotupa, et al., 2003). In this study, the degree of autoxidation of α-linoleic acid was measured after two hours at 200 °C, and compared to the oxidation in the presence of the studied polyphenols. Samples containing 28 vol% α-linoleic acid in caprylic acid (used as diluent), and 9 wt% of polyphenol were vigorously stirred at 200 °C in open air atmosphere for two hours. The temperature, reaction time, and dilution of α-linoleic acid were optimized to get a sufficient degradation rate of α-linoleic acid in order to accurately monitor the process. The amounts of unreacted α-linoleic acid were quantified by GC using an internal standard (tetradecane). The results are shown in Fig. 3. [A more detailed procedure is described in section 2.5.].

Thermal autoxidation treatment of the reference sample showed a decrease of 84 % of α-linoleic acid, leaving only 16 % intact. Analysis by GC showed only traces (<1 %) of small molecular compounds formed, suggesting that α-linoleic acid mostly reacted through oligomerization. As the degradation products of α-linoleic acid has been well studied and are outside the scope of this study, no further characterization of the formed oligomer was done.

Lignans added at 9 wt% decreased the degradation to 61–75 %. Of the lignans, MR and 7-OH-SECO showed to be most effective, leaving 39

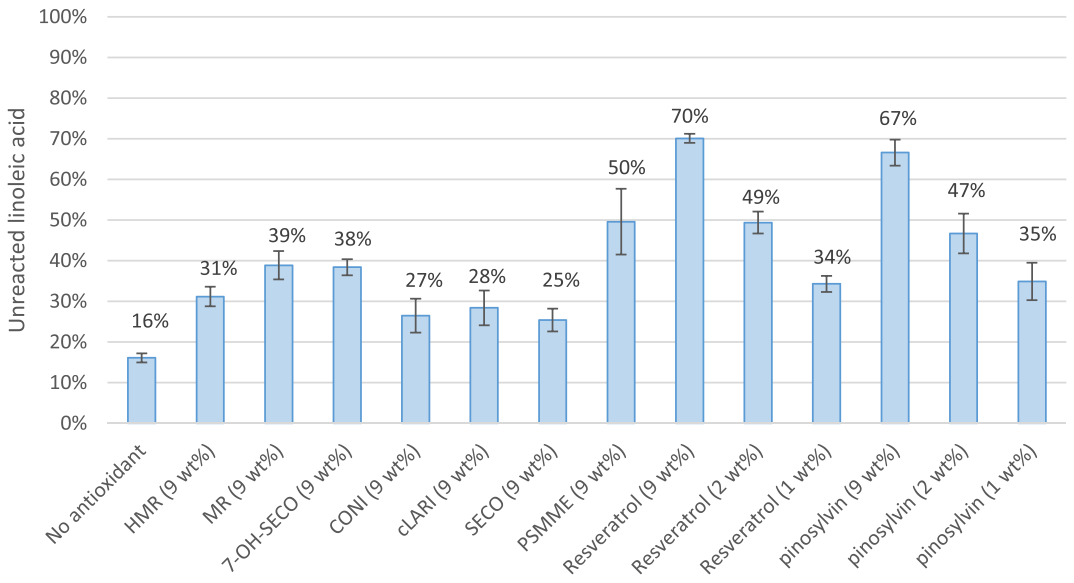


Fig. 3. Thermal autoxidation of α-linoleic acid in presence of polyphenolic antioxidants. The samples (70 μL α-linoleic acid + 180 μL caprylic acid + polyphenol) were stirred at 200 °C for 120 min in open air. The reported percentages of unreacted α-linoleic acid are mean values of two parallel samples.

% and 38 % respectively of α-linoleic acid intact. The stilbenes showed even higher antioxidant activities, at 9 wt% only 30–50 % of α-linoleic acid was reacted. Most efficient were resveratrol and PS, which were further investigated at lower concentrations. At 1 wt% of either resveratrol or PS, around 34 % α-linoleic acid remained unreacted after thermal treatment, more than doubled the amount compared to the samples with no antioxidant.

As a conclusion, the polyphenolic substrates used in this study seem to be relatively stable at elevated temperatures and mainly interconversion (by ionic reaction mechanisms) between structures of different oxidation degrees were identified, but formation of other or toxic products was not observed. A small difference regarding transformation product can be seen in different media and temperatures. The polarity and the water content of the solvent may influence on the specific transformations observed, which was indicated by some similarities with known transformations in aqueous media. In the presence of oxygen and at higher temperatures, oxidation leading to oligomerizations was observed, especially on substrates with phenolic groups in combination with aliphatic or benzylic hydroxyls. In oxygen atmosphere it is expected that reactive species are formed leading to phenoxyl radical which is known to polymerize lignans mainly by 5–5-radical couplings. The most stable substrate against oxidative oligomerization were the lignan MR and the stilbene PSMME. The antioxidant activities of the studied substrates also remained at 200 °C and in air atmosphere, which suggest that these compounds could be used as antioxidants in high-temp applications. All substrates showed to decrease the rate of α-linoleic acid degradation, stilbenes being the most effective ones. Although the degradation of α-linoleic acid is not a direct evaluation of antioxidant activity and antioxidant mechanisms, the results showed a clear antioxidant effect. As stilbenes can be readily extracted from biorefineries, they could be interesting to utilize in big scale food processes. The information gathered in this study can be useful for future studies aiming at increasing the quality of foods and feeds, as well as to understand transformational changes that may occur in other polyphenols at elevated temperatures. However, more specific and in detailed studies are still needed before concluding if dietary lignans and stilbenes are safe to use in high-temperature food processing, or if they are able to withstand high-temperature biorefinery processes.

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CRediT authorship contribution statement

Patrik Runeberg: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft. **Dmitry Ryabukhin:** Investigation, Methodology, Supervision, Validation, Writing – original draft. **Lucas Lagerquist:** Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Jani Rahkila:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Patrik Eklund:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Patrik Runeberg reports financial support was provided by Raisio Research Foundation. Patrik Eklund reports financial support was provided by Academy of Finland. Patrik Runeberg reports financial support was provided by Alfred Kordelin Fundation.

The remaining 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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134641.

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