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Identification, characterisation, and evaluation of bioactive plant-based compounds for the sustainable stabilisation of food packaging materials

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They say one man's trash is another man's treasure.

I beg to differ.

It has always been a treasure. But it is yet to be discovered by someone who recognises its value.

Based on Tina Dinh

Abstract

Typically, plastic packaging materials are produced using additives, like e.g. stabilisers, to introduce specific desired properties into the material or, in case of stabilisers, to prolong the shelf life of such packaging materials. However, those stabilisers are typically fossil-based and can pose risks to both environmental and human health. Therefore, the present study presents more sustainable alternatives based on regional renewable resources which show the relevant antioxidant, antimicrobial and UV absorbing properties to successfully serve as a plastic stabiliser. In the study, all plants are extracted and characterised with regard to not only antioxidant, antimicrobial and UV absorbing effects, but also with regard to additional relevant information like chemical constituents, molar mass distribution, absorbance in the visible range et cetera. The extraction process is furthermore optimised and, where applicable, reasonable opportunities for waste valorisation are explored and analysed. Furthermore, interactions between analysed plant extracts are described and model films based on Poly-Lactic Acid are prepared, incorporating analysed plant extracts. Based on those model films, formulation tests and migration analysis according to EU legislation is conducted.

The well-known aromatic and medicinal plant thyme (*Thymus vulgaris* L.) includes phenolic terpenoids like thymol and carvacrol which have strong antioxidant, antimicrobial and UV absorbing effects. Analyses show that those effects can be used in both lipophilic and hydrophilic surroundings, that the variant *Varico 3* is a more potent cultivar than other analysed thyme variants, and that a passive extraction setup can be used for extract preparation while distillation of the Essential Oils can be a more efficient approach.

Macromolecular antioxidant polyphenols, particularly proanthocyanidins, have been found in the seed coats of the European horse chestnut (*Aesculus hippocastanum* L.) which are regularly discarded in phytopharmaceutical industry. In this study, such effects and compounds have been reported for the first time while a valorisation of waste materials has been analysed successfully. Furthermore, a passive extraction setup for waste materials and whole seeds has been developed.

In extracts of snowdrops, precisely *Galanthus elwesii* HOOK.F., high concentrations of α-tocopherol have been found which promote a particularly high antioxidant capacity in lipophilic surroundings.

Different coniferous woods (*Abies* div., *Picea* div.) which are in use as Christmas trees are extracted after separating the biomass in leafs and wood parts before being analysed regarding extraction optimisation and drought resistance of active substances. Antioxidant and UV absorbing proanthocyanidins are found even in dried biomasses, allowing the circular use of already used Christmas trees as bio-based stabilisers and the production of sustainable paper as a byproduct.

Zusammenfassung

Kunststoffverpackungen werden üblicherweise unter Zusatz von Additiven wie Stabilisatoren produziert, um erwünschte Eigenschaften in das Material einzubringen oder seine Haltbarkeit zu erhöhen. Diese Stabilisatoren sind jedoch in der Regel erdölbasiert und können schädlich für die Umwelt und für die menschliche Gesundheit sein. Diese Thesis präsentiert daher nachhaltigere Alternativen, die aus regionalen nachwachsenden Rohstoffen mit den für Stabilisatoren relevanten antioxidativen, antimikrobiellen und UV-absorbierenden Eigenschaften hergestellt werden. Alle untersuchten Pflanzen werden extrahiert und neben ihrer antioxidativen, UV-absorbierenden und antimikrobiellen Eigenschaften auch auf weitere relevante Parameter wie die chemische Zusammensetzung, die Molmassenverteilung oder ihre Färbung untersucht. Zudem wird der Extraktionsprozess optimiert und eine mögliche Wiederaufwertung von Abfallstoffen analysiert. Weiterhin werden die Interaktionen zwischen den verschiedenen Pflanzenmaterialien beschrieben und pflanzlich additivierte Modell-Verpackungen auf Polymilchsäure-Basis hergestellt, anhand deren auch Formulierungen getestet und das Migrationsverhalten gemäß EU-Vo 10/2011 geprüft werden.

Die bereits gut erforschte Arznei- und Gewürzpflanze Thymian (*Thymus vulgaris* L.) enthält phenolische Terpenoide wie Thymol und Carvacrol, welche starke antioxidative, antimikrobielle und UV-absorbierende Wirkungen aufweisen. Diese Effekte konnten sowohl in lipophilen als auch in hydrophilen Umgebunden beobachtet werden; zudem konnte der Kultivar Varico 3 als potentester untersuchter Kultivar identifiziert und eine passive Extraktionsmethode für Thymian, neben der ggf. effizienteren Destillation ätherischer Öle, etabliert werden.

In den regelmäßig als Abfall der Phytopharmazeutika-Produktion anfallenden Samenschalen der europäischen Rosskastanie (*Aesculus hippocastanum* L.) konnten hochmolekulare antioxidative Polyphenole aus der Gruppe der Proanthocyanidine nachgewiesen werden. Diese Inhaltsstoffe wurden hier erstmals beobachtet und auch eine Aufwertung von Abfällen in diesem Kontext erstmals untersucht. Zudem wurde eine passive Extraktionsmethode für Samenschalen entwickelt.

In Extrakten von Schneeglöckchen, speziell *Galanthus elwesii* HOOK.F., wurden sowohl hohe Gehalte an α-Tocopherol als auch ein besonders hohes lipophiles antioxidatives Potential gefunden.

Verschiedene als Weihnachtsbaum genutzte Nadelhölzer (*Abies* div., *Picea* div.) wurden getrennt nach Nadeln und Holz extrahiert und bzgl. Extraktionsoptmierung und Trocknungsresistenz der Wirkstoffe analysiert. Selbst in getrockneter Biomasse wurden antioxidative und UV-absorbierende Proanthocyanidine gefunden, wodurch die Aufwertung von bereits genutzten Weihnachtsbäumen primär zu Stabilisatoren und sekundär zu nachhaltigeren Papierprodukten ermöglicht wird.

1. Introduction

Since 2005, the member states of the European Union generated a minimum of 14 million tons of plastic packaging waste per year (Eurostat 2021a). In 2018, this abundance peaked to a total of 17.2 million tons with the highest proportion of 3.2 million tons generated by Germany single-handedly (Eurostat 2021b). The majority of these plastic packaging is made of fossil-based plastic materials (Coppola 2019; nova-Institute 2020), typically including fossil-based additives like stabilisers (Maier and Schiller 2016). Particularly those fossil-based stabilisers can pose risks to environmental and human health e.g. when being introduced to the environment which is common despite European countries aiming to prevent such an environmental exposition (Ito et al. 1985; Kahl and Kappus 1993; Lanigan and Yamarik 2002; Coppola 2019; TrashOut 2021). Moreover, using fossil fuels as a base for plastic additive production can pose substantial risks for the environment directly, e.g. via oil spillings (Armenta-Arteaga and Elizalde-González 2003; Buskey et al. 2016), and indirectly, e.g. via long transport routes and energy-intensive production steps (Miller 2014; Fiorentino et al. 2019). It is also expected that the costs of fossil fuels will increase due to ongoing consumption of finite resources and depending on political issues such as trade relations to Arabian countries or possible regulations or taxes on technologies accelerating the climate catastrophe (Eckert 2012; Demirbas et al. 2017; Lincke 2021). Thus, alternative stabilising materials are developed to weaken harmful consequences of improper disposal of plastic materials, to expand the range of applicable additives, and to facilitate the substitution of fossil-based additives with more climate-friendly alternatives by using renewable, CO_2 binding resources and strengthening independent regional production structures in contrast to extensive production routes.

1.1 Scientific background

The following sections present the basic mechanisms underlying the most relevant chemical deterioration processes of plastic materials which are necessary to understand how stabilisers interact with these mechanisms. Furthermore, suitable promising plant species with such stabilising properties are described.

1.1.1 Stabilising properties:

Antioxidant capacity, UV absorbance and antimicrobial effects

When exposed to UV light, thermal or mechanical stress, polymers as organic macromolecules can undergo free radical chain reactions when they have contact to molecular oxygen which can be atmospheric or unintentionally incorporated within the polymer. These reactions lead to the formation of oxidation products and thus promote degradation of the plastic material as well as

deterioration of its properties and quality (López-de-Dicastillo et al. 2012). The altered properties include, but are not limited to the average molecular weight or the molecular mass distribution, affecting the durability or shelf life of the polymer (Wegmann et al. 2016). This reaction process, which is defined as an autocatalytic reaction of organic compounds with molecular oxygen in an oxygen-rich environment and affects both conventional petrol-based polymers and bio-based polymers, is called autoxidation (Bonnet 2016; Márcio Carocho et al. 2018). The autoxidation consists of three major phases. During the Initiation phase, a radical molecule is formed which proceeds to react with other molecules in the Propagation phase, resulting e.g. in chain branching or chain propagation. Finally, during the Termination phase, the reaction ends by forming stable reaction products (Wegmann et al. 2016; Márcio Carocho et al. 2018). This mechanism can have substantial consequences for the properties of the polymer, particularly when the common β-cleavage reaction occurs during the propagation phase. This leads to a break in the macromolecular polymer chain and thus radically affects the molecular mass distribution, resulting in a decreased polymer durability (Wegmann et al. 2016). The described effect of autoxidation can be inhibited by incorporating antioxidants. Such compounds are capable of "catching" radicals right when they occur, preventing propagation and termination phases and thus avoiding a cleavage of the polymer chain. However, as antioxidants are consumed while catching radicals, they are shifting the material deterioration to the point when all antioxidants are consumed instead of prohibiting the deterioration completely (Wegmann et al. 2016).

An effect similar to autoxidation is the so called photooxidation during which a comparable chain reaction of radicals is initiated via UV light. Therefore, comparable impacts as discolouration and embrittlement, which are finally affecting the polymer durability, are observed on polymers exposed to UV light (Feldman 2002; Larché et al. 2012). However, in photooxidation, this effect is caused by high-energy UV light which encounters an organic macromolecular polymer chain and can be absorbed by it. This refers to light of a wavelength of approx. 290 – 400 nm (so-called UV-A and UV-B light) which is found in common sunlight (in contrast to so-called UV-C light with shorter wavelengths which is blocked by ozone the stratosphere at the latest) (Grob et al. 2016; Bundesamt für Strahlenschutz 2021). As the energy of UV-A and UV-B light exceeds the bond energy of most polymeric bonds, this can lead to the disruption of the polymer chain, creating two radicals (Grob et al. 2016). The ongoing radical reactions are similar to the ones described for autoxidation. Therefore, antioxidants can be used to contain the effects of photooxidation as well; however, as antioxidants are consumed when acting against oxidative stress, it is recommended to use so called photostabilisers (which protect the polymer from incident UV light) to limit the formation of radicals as far as possible. This effect is achieved by e.g. absorbing such UV light and transforming

the UV light energy into heat energy instead of chemical energy and thus preventing the polymer chain from breaking (Grob et al. 2016). Generally, photostabilisers are not consumed during absorbing UV light. While they break down eventually, they are much more durable than antioxidants (Grob et al. 2016).

Another typical degradation mechanism of polymers is damage dealt by various microbials, primarily bacteria. Such bacteria can metabolise the polymer or other constituents of the material, e.g. plasticisers or other additives, and thus cause a change in optical and mechanical properties. A bacterial infection of the polymer can cause severe cosmetical changes, odour and deterioration of the polymer. (Ochs 2016) Bacteria are classified with regard to their cell wall structure: the cell wall of Gram positive bacteria consists of a single membrane and a remarkable layer of the peptidoglucan murein while the cell walls of Gram negative bacteria are composed of two membranes enclosing a thin layer of murein with the outer membrane including toxic lipopolysaccharides. This difference in the cell wall structure can be of utmost importance regarding the efficacy of antimicrobials as such compounds typically have to pass the cell wall to take effect. So called antimicrobials, stabilisers impeding a bacterial infection of the polymer, can prevent further spread of bacteria (bacteriostatic effect) or even reduce the already present microbial count (bactericidal effect) (Ochs 2016; Camacho-Cruz et al. 2021). Furthermore, antimicrobial polymers are divided into polymers that preserve the material itself and polymers that aim to affect microbials on its surface as well to create an extended effect for the user, e.g. in medicinal or gardening applications (Ochs 2016; Zhang and Wagner 2017). The application of antimicrobials is relevant for both fossil-based and bio-based polymers. However, bio-based materials are particularly vulnerable as they tend to already have a higher microbial load that needs to be controlled.

As well as for antioxidants and photostabilisers, this can also have positive effects on the packed good as oxidation or microbial infection can cause the spoilage of foods as well (Cirillo et al. 2018; Yusuf 2018). Thus, it is possible to use bioactive additives as components of so-called active packaging which aims at protecting the packed food and prolonging its shelf life (Korte et al. 2021). This approach presents several similarities compared to polymer stabilisation such as mechanisms of action. However, active packaging compounds are supposed to emitted from the packaging to the food in a controlled manner while classic polymer stabilisers are designed to remain in the plastic material (Cirillo et al. 2018). Thus, a systematic difference between assessment of such compounds is present, regarding e.g. incorporation and toxicity. For packaging stabilisers, substances are analysed rigorously to ensure that no relevant amount is migrating into the packed food where stabilisers could e.g. change the sensoric properties of the packed food (e.g. taste or

odour) or endanger human health (European Comission 2004, 2011a). In contrast, active packaging stabilisers themselves are tested intensively, e.g. regarding toxicity, as a migration into packed food is not only possible, but desired (European Comission 2011b). In the present thesis, the key focus is on stabilisers for the stabilisation of packaging materials instead of incorporating them as active packaging compounds. However, a combined use is conceivable for some of the analysed plant species.

1.1.2 Suitable renewable resources

The properties discussed before (antioxidant, antimicrobial, and UV absorbing effects) can be summarised as so-called bioactive effects. Bioactive effects are reported for different chemical groups of compounds, the most important groups are (poly)phenols, tocopherols and carotenoids (Charles 2013b). Plant resources with such constituents are typically reported to have bioactive properties. For the plants analysed in the present study, only fragmentary literature indicators for bioactive effects are present as typically other plant parts or relative species have been analysed by researchers around the globe without analysing the specific resources used for this research devoted to plants that can be grown or ideally already are grown in the region of NRW, Germany. Thus, completing fragmentary information for different local plant resources represents the most important milestones of the present work. For this purpose, common thyme leafs (*Thymus vulgaris* L.; TV), European horse chestnut seeds (*Aesculus hippocastanum* L.; AEH), different snowdrop species (*Galanthus* L.) and different coniferous woods (*Abies* div., *Picea* div.) are thoroughly analysed in the following chapters to determine their bioactive potential. For all four groups of resources, bioactive constituents are assumed due to published analysis of relative species or fragments, including polyphenols for Japanese horse chestnut seeds (*Aesculus turbinata* BLUME) (Ogawa et al. 2008; Oszmiański et al. 2014; Kimura et al. 2017). Furthermore, tocopherols are reported for *Galanthus transcaucasicus* FOMIN (Karimi et al. 2018), which is related to the analysed Galanthus species, while tocopherols, ascorbate and polyphenols are discussed for different coniferous woods (Polle et al. 1990; Hafizoglu and Holmbom 1995; Bağcı and Dığrak 1996; Öncel et al. 2004; Co et al. 2011). For TV, phenolic terpenoid constituents like thymol and carvacrol are reported in literature (Özcan and Chalchat 2004; Gavaric et al. 2015). Finally, optimised extracts of all biomasses are analysed with regard to reciprocal synergistic interactions and introduced into model food packaging films made of Poly-Lactic Acid (PLA) to evaluate practical applications.

1.2 Main contributions

As described before, the present study gains comprehensive knowledge on locally cultivated plants where only fragmentary information were available before to allow a quick and easy implementation of sustainable bio-based bioactive compounds for polymer stabilisation.

The bioactive properties of common thyme extracts and essential oils are known to a large extent. However, not all aspects of those properties are reported before but are part of the present research. For example, the additional benefits are gained by increasing the efficiency via a detailed comparison of cultivars. Furthermore, potential influences during exposition of the plant to varying extents of stress are examined.

For snowdrop extract analysis, information regarding α-tocopherol content are reported in literature. However, this only applies to a species related to the four species analysed in the present study (Karimi et al. 2018); thus, transferability of results can only be assumed. Reliable information on the species analysed was not yet available, the knowledge gained by analysing and comparing snowdrop extracts and their properties is thus new.

In contrast to common thyme, the composition and properties of European horse chestnut seeds are fragmentary only. While the peeled seed is a well-known source for phytopharmaceutical applications, the properties of seed coats are not known at all; assumptions regarding its properties are made based on research on the related Japanese horse chestnut by (Ogawa et al. 2008; Kimura et al. 2017). Therefore, the results obtained during European horse chestnut seed coat analysis are entirely new, including, but not limited to the characterisation of extracts, the optimisation of the passive extraction setup and the evaluation of waste materials as sustainable resources.

For coniferous woods analysis, different indicators for bioactive properties of different species are reported in literature. However, there are several species and fragments where no information or only limited information are available where, e.g., no specific tests on antioxidant effects of extracts were conducted (Rauha et al. 2000; Elmezughi et al. 2013). The information on antioxidant capacities of different coniferous wood extracts in direct comparison and their UV absorbance obtained during the present study, particularly regarding different plant fragments, are widely new. To the best of my knowledge, analysis of sustainable resource utilisation and valorisation, including analysis of dried coniferous woods, extraction optimisation and preparation of paper packaging from already extracted biomass, is an entirely novel approach.

Ultimately, a comprehensive study on formulation and application of model food packaging films made of PLA is presented. So far, packaging stabilisation is underrepresentated in research compared to other applications of bio-based bioactive substances. This particularly applies to the

analysed resources as most of them have not been analysed regarding such applications at all before. In addition to formulation analysis, bioactive effects of films containing stabilising extracts are analysed, and the risk of stabilisers unintentionally migrating into packed food is examined and classified, presenting new and valuable information in the context of extract applications.

Furthermore, conducted synergism analysis allows a highly efficient application of the analysed extracts in combination with each other or isolated compounds. While synergism investigations are already known in literature, it is not examined to an extent appropriate for the application of the presented resources before as synergistic mechanisms are extremely complex, heavily impeding the transferability of results to related analytes, resources, surroundings, or dosages. Therefore, the information on the synergistic interactions of extracts and added compounds at different dosages and in hydrophilic or lipophilic surroundings is new and valuable for the efficient application of said extracts.

The present doctoral thesis successfully provides innovative ideas and comprehensive knowledge needed for their industrial implementation. Thus, the obtained results and their virtually unrestricted publication facilitate the use of plant resources for sustainable (food) packaging stabilisation, promoting a change to a circular economy. Furthermore, various presentations held in the context notably increased the awareness of the problem and may initiate further research and industry involvement.

1.3 Publications

Parts of the present thesis have been pre-published in peer-reviewed book chapters, conference contributions and journal articles:

- **Havelt T**, Schmitz M. Identifizierung und Charakterisierung bioaktiver Inhaltsstoffe in Thymian: 8. Tagung Arznei- und Gewürzpflanzenforschung, 10.-13.09.2018, Bonn (Poster Presentation). *Julius-Kühn-Archiv* **2018**, 112–114, doi:10.5073/JKA.2018.460.030.
- **Havelt T**, Brettschneider S, Do XT, Korte I, Kreyenschmidt J, Schmitz M. Sustainable Extraction and Characterisation of Bioactive Compounds from Horse Chestnut Seed Coats for the Development of Bio-Based Additives. *Resources* **2019**, *8*, 114, doi:10.3390/resources8020114.
    > Re-published in:
    Kusch-Brandt, S (ed) (2020): Underutilised Resources in Urban Environments. MDPI Books, Basel; ISBN: 978-3-03936-018-5 (Print) / 978-3-03936-019-2 (E-Book), DOI: 10.3390/books978-3-03936-019-2
- **Havelt T**, Frase JN, Pude R, Schmitz M. Characterisation of Bioactive Ingredients in Extracts of Fresh and Dried Coniferous Trees for the Development of Sustainable Packaging Materials. *Processes* **2020**, *8*, 1366, doi:10.3390/pr8111366.
- **Havelt T**, Brettschneider S, Korte I, Kreyenschmidt J, Schmitz M. Plant-based Bioactive Compounds for Substitution of Petrol-based Stabilisers in Packaging Materials: 8th International Symposium on Human Health Effects of Fruits and Vegetables, 08.-11-03-2021, virtual (Oral Presentation). *Acta Horticulturae* **2021,** *in press*
- **Havelt T**, Brettschneider S, Schmitz M. Evaluation of practical applicability and synergistic effects of bio-based food packaging materials combined with plant-based stabilisers. *Processes* **2021***, 9,* 1838*,* doi:10.3390/pr9101838.

The following publications have been prepared or contributed to without the publications becoming an integral part of the present thesis:

- Götz B, Hounsou M, Dabadé S, **Havelt T**, Schmitz M, Hounhouigan DJ, Kreyenschmidt J. The Potential of Sustainable Antimicrobial Additives for Food Packaging from Native Plants in Benin: Tropentag, 18.-20.09.**2019**, Kassel (Oral Presentation Götz)
- Götz B, Hounsou M, Dabadé S, **Havelt T**, Schmitz M, Hounhouigan DJ, Kreyenschmidt J. The Potential of a Sustainable Active Packaging Solution to Reduce Food Losses in Benin: Tropentag, 09.-11.09.**2020**, virtual (Oral Presentation Götz)

- Korte I, Kreyenschmidt J, Wensing J, Bröring S, Frase JN, Pude R, Konow C, **Havelt T**, Rumpf J, Schmitz M, Schulze M. Can Sustainable Packaging Help to Reduce Food Waste? A Status Quo Focusing Plant-Derived Polymers and Additives. *Applied Sciences* **2021**, *11*, 5307, doi:10.3390/app11115307.

The following public oral or poster presentations have been performed additionally:

- **Havelt T**, Schmitz M. Identifizierung bioaktiver Inhaltsstoffe in Thymian: Tag der Forschung der Hochschule Bonn-Rhein-Sieg, 08.05.**2019**, Rheinbach (Poster Presentation Havelt, featured as Best Poster Presentation)
- **Havelt T**, Schmitz M, Brettschneider S, Pude R, Maruhn M, Kreyenschmidt J, Korte I. Entwicklung nachhaltiger Stabilisatoren auf pflanzlicher Basis: Kolloquium des Fachbereichs Angewandte Naturwissenschaften der Hochschule Bonn-Rhein-Sieg, 13.06.**2019**, Rheinbach (Oral Presentation Havelt)
- **Havelt T**, Schmitz M. Identifizierung und Charakterisierung bioaktiver Inhaltsstoffe in Thymian zur Entwicklung biobasierter Additive: Informationsveranstaltung Kompetenzzentrum Gartenbau, 10.12.**2019**, Bonn (Poster Presentation Havelt)
- **Havelt T**, Schmitz M, Brettschneider S, Pude R, Maruhn M, Kreyenschmidt J, Korte I. Bio-based stabilisers for the application in food packaging materials: Kolloquium des Fachbereichs Angewandte Naturwissenschaften der Hochschule Bonn-Rhein-Sieg, 10.06.**2021**, virtual (Oral Presentation Havelt)
- **Havelt T**, Schmitz M. Nachwachsende Rohstoffe als Basis für bioaktive Additive in Kunststoffverpackungen: 4. Doktorand:Innenkolloquium „Ressourcen-Wissen“ der Abteilung Ressourcen und Nachhaltigkeit, Promotionskolleg NRW, 03.09.**2021**, virtual (Oral Presentation Havelt, featured as Best Oral Presentation)
- **Havelt T**, Schmitz M. Biobasierte Stabilisierung von Kunststoffprodukten für den Lebensmittelkontakt: Jahrestagung der Gesellschaft für Kunststoffe im Landbau e.V., 12.-13.10.**2021**, virtual (Oral Presentation Havelt)

2. Common thyme (*Thymus vulgaris* L.)

2.1 Identification and characterisation of bioactive compounds in thyme (Havelt and Schmitz 2018)

Abstract

Thyme (*Thymus vulgaris* L.) is a very diverse species that is known and used as a medical plant due to its high amount of therapeutic compounds. Its essential oil contains substances with antioxidative properties as thymol (about 50%). The objective is to take advantage of that potential by incorporating sustainably produced additives based on thyme e.g. in food packaging. Compounds with antioxidative, antimicrobial and UV absorbing effects are of special interest as those substances protect the product from oxidative stress, microbial degradation and loss of quality.

Therefore, six variants of thyme are analysed with regard to different parameters to choose a superior variant to conduct further research on.

The essential oil is extracted by steam distillation and analysed via GC-MS. Additionally, solvent extracts are analysed with regard to total antioxidant capacity (TAC), UV absorbance and chemical composition. The volatile compounds are determined as well. In general, there are little differences in quality but in quantity as one variant's oil contains a considerably higher amount of thymol (about 65 %); the same variant's methanol extract proves to have a high TAC. Thus, a promising variant for further development and optimisation of bio-based, bioactive additives is identified in this study.

Zusammenfassung

Bei Thymian *(Thymus vulgaris L.)* handelt es sich um eine sehr varietätenreiche Art, die aufgrund ihres Gehaltes an therapeutisch wirksamen Inhaltsstoffen als Arzneipflanze monographiert ist. Insbesondere das ätherische Öl mit dem Hauptbestandteil Thymol (ca. 50%) hat eine hohe antioxidative Wirkung. Ziel ist es, dieses Potential als nachhaltig produzierte Additive zu nutzen. Hierfür eignen sich antioxidativ bzw. antimikrobiell wirksame sowie UV-absorbierende Substanzen, die das Produkt bei Zusatz vor oxidativem Stress, mikrobiellem Abbau und Qualitätsverlust schützen.

Hierzu werden zunächst sechs Varianten auf verschiedene Parameter analysiert, um die potenteste Variante auszuwählen. Auf diese Variante wird sich die weitere Forschung konzentrieren.

Daher wird das ätherische Öl durch azeotrope Destillation extrahiert und mittels GCMS analysiert. In Extrakten werden zudem das AP und Absorptionsverhalten bestimmt. Auch die chemische

Zusammensetzung des Extrakts sowie die flüchtigen Stoffe des Thymians werden untersucht. Generell gibt es wenig qualitative, teilweise jedoch quantitative Unterschiede: Eine Variante weist u.a. einen deutlich höheren Thymolgehalt im Öl (ca. 65 %) und ein hohes hydrophiles AP auf. Somit ist eine vielversprechende Variante für die weitere Entwicklung und Optimierung bioaktiver Additive gefunden.

2.1.1 Introduction

Modern society is facing increasing challenges. To handle these challenges, the concept of sustainability has to be the foundation of as many sectors of everyday life as possible. Only a product based on this concept meets the neccessary future-orientated and ethical standards. In a variety of analyses, conducted by scientists of University of Bonn and Bonn-Rhein-Sieg University of Applied Sciences, the development of bio-based packaging and constructing materials is expedited.

Typically, the developed bio-based products cannot be used on their own but are dependent on the addition of stabilisiers (or „additives“) to obtain a higher competetiveness and practicality. Additives appropriate for bio-based products present antioxidant or antimicrobial effects as well as a high UV absorbance. Those claims are met by a variety of secondary plant metabolites. Such stabilisers protect the bio-based product against e.g. oxidative stress and microbial degradation while maintaining the product’s characteristics (Hon et al. 1982; Hon and Chang 1984).

The project aims to present sustainable alternatives to common petrol-based stabilisers which are based on renewable ressources and can be used to substitute the typically used industrial stabilisers. Thus, additives based on secondary plant metabolites that can be produced in an environmentally friendly manner are identified and incorporated into packaging and construction materials, notably increasing their shelf life, quality, and practicality.

One of the most relevant groups of secondary plant metabolites are terpenoids. For instance, the main components of thyme essential oil are the highly antioxidative, phenolic terpenoids thymol and carvacrol (**Fig. 1**) (Cosentino et al. 1999). Thus, thyme has been identified as a potentially relevant resource to obtain bioactive additives. At first, a comparative screening of different thyme variants has been conducted to allow further research to focus on the variant presenting the highest amount of active substances.

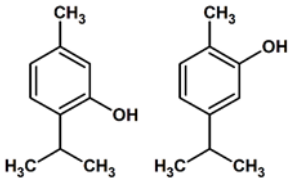


Fig. 1 *Structural formulas of thymol (left) and carvacrol (right)*

2.1.2 Results and Discussion

Six thyme variants, including the cultivars “Varico 2” (Var2) and “Varico 3” (Var3) as well as four different origins of “Deutscher Winter” (DW1-4), have been analysed regarding different parameters to determine the most suitable variant.

Initially, a steam distillation has been conducted for all six variants to obtain the thyme essential oil (EO). The variants DW1-4 show comparable yields while distillation of Var3 results in a yield increased of approx. 80 %. The EO yield of Var2 is slightly increased in comparison to DW1-4, but notably falls short in comparison to Var3.

Analysing the obtained EOs of all variants using gas chromatography coupled with mass spectrometry (GC-MS) proves that all variants have a comparable composition that shows quantitative differences only. In addition to the main component thymol, the EOs include terpinene, cymene, and carvacrol which presents antioxidant effects as well (Aeschbach et al. 1994). The mean EO consists of 59 % thymol and 4 % carvacrol. However, Var3 shows an increased amount of both thymol and carvacrol. In consideration of the increased EO yield observed for Var3 and a particularly low scattering, Varico 3 is the most potent variant regarding EO applications.

Further analyses have been conducted after preparing hexane and methanol extracts of all variants. These extracts have been used to determine the total antioxidant capacity (TAC) and UV absorbance in both surroundings. The TAC determination results in all variants showing a comparably high antioxidant effect which is roughly similar for all six variants. The antioxidant effect is presumably caused by the comparably polar substances thymol and carvacrol which are the main ingredients in methanol extracts as confirmed by GC-MS. The UV absorbance spectra obtained for all variants do not show qualitative differences; however, DW4 and Var3 analysis results in increased UV absorbances.

The obtained polar and unpolar extracts are chemically characterised like the essential oils, including the identification of constituents. For all variants, a similar composition is found, roughly resembling the composition of EOs for hexane extracts. Due to the solvent’s polarity, methanol extracts show a different composition, particularly including phenolic compounds like thymol and

carvacrol while unpolar substances (which are found in hexane extracts and EOs) are not present. The concentrations of all constituents are much lower for all extracts than for EOs.

GC-MS analysis of the gas phase by solid phase microextraction (SPME) results in a chromatogram comparable to the ones obtained for EOs. However, the detected amounts of substances decrease for higher retention times. Such substances, including thymol and other substances with a higher polarity or molar mass, have a higher boiling point and thus, as expected, tend to migrate into the gas phase not at all or in low concentrations only.

2.1.3 Acknowledgements

Particular thanks are due to Maren Maruhn and Prof. Dr. Ralf Pude (INRES, University of Bonn) for providing plant materials and to the European Union for financially supporting this research project as part of the European Regional Development Fund (EFRE.NRW).

2.1.4 Copyright

2.2 Effect of harvest season on bioactive ingredients in *Thymus vulgaris* L.

2.2.1 Introduction

In addition to the analyses described in Chapter **2.1**, a comparative study has been conducted to determine the influence of the time of harvest on the constituents of thyme.

Thymus vulgaris L. or "common thyme" (TV) is a well known aromatic and medical plant used to treat, inter alia, coughs, bronchitis, gastrointestinal disturbances and inflammation of the upper respiratory tract (Charles 2013c). Thus, it is featured in various monographs, including the European pharmacopoeia.

Extracts and essential oils of thyme are known for example for antibacterial, antimicrobial, and antifungal activities (Deans and Ritchie 1987; Tantaoui-Elaraki and Beraoud 1994; Nelson 1997; Smith-Palmer et al. 1998; Tornuk et al. 2011). Additionally, the enormous antioxidative properties of thyme and its essential oil (EO) have been proven several times (Kulisic et al. 2005a; Dandlen et al. 2010; Kulisic et al. 2005b; Lee et al. 2005; Roby et al. 2013). Typically, thyme is under research especially with regard to medicine and food conservation, including active packaging (Zeid et al. 2019; El-Obeid et al. 2018; Dauqan and Abdullah 2017; Mohsin et al. 1989; Javed et al. 2013). However, further applications of thyme are under investigation as well, including the use as a bio-based, bioactive additive to stabilise food packaging itself. By implementing thyme-based additives, it could be avoided to include petrol-based and potentially harmful stabilisers in packaging materials as it is common practice.

To allow ongoing investigations to put the properties of thyme to an effective use by indicating harvest parameters enhancing the potential of thyme leafs, this study explores the differences between various thyme species, origins and harvest seasons. Such deviations, caused by location for instance, are known for other species (Emmons and Peterson 2001). To obtain similar valuable information on thyme, three different TV cultivars Deutscher Winter (DW; including four different origins), Varico 2 (Var2) and Varico 3 (Var3) were cultivated and harvested in spring and summer of 2018 and analysed regarding several relevant parameters to evaluate potential differences in UV absorbance, essential oil content, thymol content and antioxidant activities. Hydrophilic and lipophilic TV leaf extracts as well as TV essential oil were subject to analysis.

2.2.2 Materials and Methods

2.2.2.1 Chemicals and Instrumentation

For determination of UV/Vis spectra and total antioxidant capacities (TAC), a Perkin Elmer Lambda 25 dual-trace spectral photometer was applied. GC-MS analysis was carried out using an Agilent 7890A GC instrument coupled to an Agilent 5975C MSD device.

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2(3)-tert-butyl-4-methoxyphenol (BHA), and dipotassium hydrogen phosphate were purchased from Alfa Aesar (Karlsruhe, Germany), whereas acetic acid, tetrahydrofuran (THF), Trolox, β-carotene, and 2,6-di-tert-butyl-4-methylphenol (BHT) were purchased from Bernd Kraft (Duisburg, Germany), Cayman chemical Company (Ann Arbor, MI, USA), Sigma Aldrich (Darmstadt, Germany), and ThermoFisher (Kandel) GmbH (Karlsruhe, Germany), respectively. Heptane and methanol were obtained from VWR International, Darmstadt, Germany. Dichloromethane, hydrogen peroxide, potassium dihydrogen phosphate and sodium acetate were purchased from Merck KGaA, Darmstadt, Germany, while ß-carotene and linoleic acid were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

2.2.2.2 Sample Preparation and Extraction

The three different cultivars "Deutscher Winter" (analysed with regard to four different origins, thus abbreviated DW1 – DW4), "Varico 2" (Var2) and "Varico 3" (Var3) are cultivated on the premises of University of Bonn, Field Lab Klein-Altendorf. TV leafs are dried at 30 °C until dryness and destemmed afterwards.

Extraction was carried out based on an established method ("Grinding extraction", (Havelt et al. 2019)) with small modifications regarding extraction volume. 200 mg of milled sample material are extracted using 600 µL of heptane or methanol to prepare both lipophilic and hydrophilic extracts which are analysed separately. After centrifuging, the solvent supernatant is pipetted and collected in a 2 mL volumetric flask. Again, 600 µL solvent is given onto the already extracted material, following centrifuging and collecting the supernatant. This step is repeated a third time. Afterwards, the volumetric flask is filled to 2 mL and filtered.

Essential oils are obtained via steam distillation. 15 g of dried TV leafs are distilled for 2 hours using a special distillation apparatus for continuous essential oil extraction. After 2 hours, the distilled oil is collected. Both extracts and essential oils are stored at -20 °C between analyses.

2.2.2.3 Determination of UV absorbance

The UV/Vis spectra of different TV methanol extracts are recorded in the range of 260 – 800 nm in appropriate dilutions to allow a measurement properly using, but not exceeding the linear range of

the photometer. The results are given in relative absorbance units, taking the applied dilutions into account and thus maintaining comparability.

2.2.2.4 Determination of Total Antioxidant Capacity (TAC)

The TAC of lipophilic heptane extracts is determined by applying the β-carotene bleaching assay (lipophilic TAC, liTAC) which utilizes the discolouration mechanism of β-carotene in surroundings with low antioxidant effects (Nickavar and Esbati 2012; Marco 1968). Measurements are conducted at 470 nm as described in literature (Chevolleau et al. 1992; Matthes and Schmitz-Eiberger 2009; Havelt et al. 2021a). As it is considered more suitable for polar analytes (Kschonsek et al. 2018), the TAC of methanol extracts is determined by the 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) Radical Cation Scavenging Capacity Assay (hydrophilic TAC, hyTAC) at 660 nm as described in literature (Erel 2004; Havelt et al. 2019). Results are depicted presenting the mean and standard deviation.

2.2.2.5 Determination of thymol content by Gas Chromatography coupled with Mass Spectrometry (GC-MS)

The extracts and EOs are analysed using an Agilent GC-MSD system. Menthol is used as an internal standard (IStd) for thymol, an appropriate external calibration is prepared. Depending on individual compounds, identification of compounds is conducted by comparing the obtained mass spectra using the NIST database and, if possible, confirming the compound's identity via measurement of standard substances. For analysis, an Agilent DB-5MS UI column (30 m x 248 µm x 0,25 µm) is used, an inlet temperature of 250 °C and a temperature programme (75 °C (1 min), 7 °C min^{-1} to 280 °C (50 min)) are applied. Both extracts and EOs are diluted with IStd solutions before measurement (Extracts: 9 parts extract + 1 part IStd solution; EOs: 2 part EO + 1 part IStd solution + 7 parts solvent). A split ratio of 50 is applied. Results are depicted presenting the mean and standard deviation.

2.2.3 Results and Discussion

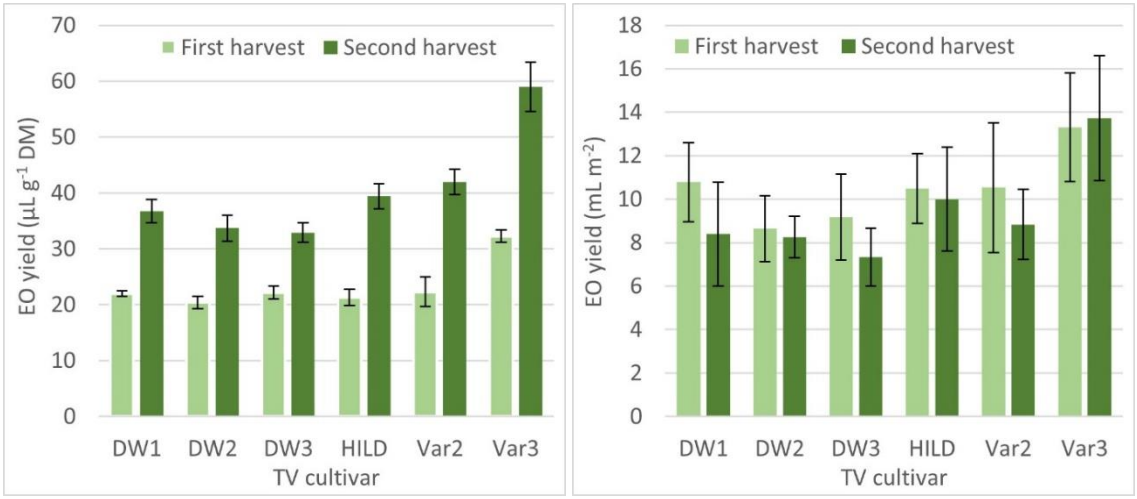
2.2.3.1 Essential Oil Analysis

Quantity

As depicted in **Fig. 2** (**a**), relevant differences regarding the yield of EO per g dried mass have been observed between the first and the second harvest. In general, within each harvest, only small differences between DW1-3, HILD and Var2 occur which is plausible due to the close resemblance of the different origins and cultivars. However, for each TV cultivar, Var3 shows an increased yield of EOs compared to the other cultivars. It is furthermore remarkable that all cultivars show an enhanced yield in the second harvest despite the plants showing a limited growth presumably due

to increased environmental heat stress. The yield is nearly doubled when comparing the second harvest to the first one, resulting in a balancing effect when referring to the needed cultivated area. This increase in secondary metabolites is plausible as a higher production of secondary metabolites, e.g. protecting the plant from UV light or oxidative stress, is observed when such stress is present (Edreva et al. 2008). The second harvest takes place in summer months, thus an increased UV influence is plausible.

As shown in **Fig. 2** (**b**), no significant differences in EO yield per m² cultivated area is detected. This particularly applies to Var3, a cultivar that is of particular interest due to an increased outcome both when referring to biomass yield or cultivated area yield. Thus, the plant seems to balance a limited growth when exposed to heat by producing an appropriate additional amount of secondary metabolites and thus more essential oil. This is plausible as the secondary metabolites have antioxidant and UV absorbing properties and are thus suitable to protect the plant from said environmental stress. Effects of other *Thymus* species showing increased antioxidant effects in summer months are described in literature as well (Galasso et al. 2014).



(a) ***(b)***

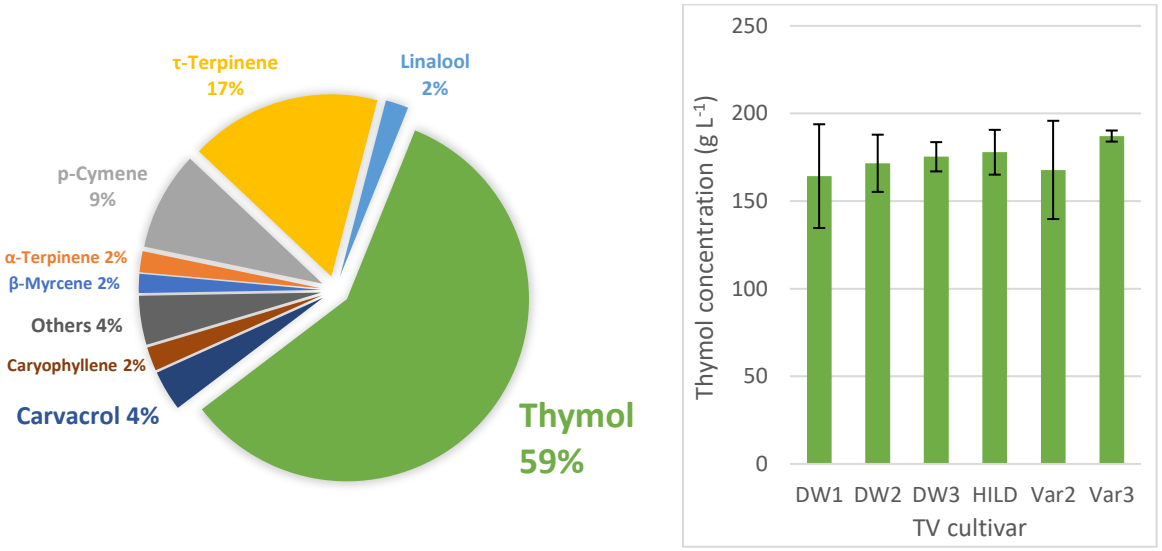
Fig. 2 *Yield of Thymus vulgaris (TV) essential oils (EO) obtained via steam distillation of dried TV leafs. Light green: first harvest; dark green: second harvest. DM: dried mass. Threefold determination, standard deviation indicated by error bars. (**a**): Yield in µL per g dried mass; (**b**): Yield in mL per m² cultivated area*

Chemical Composition and Thymol Content

As shown in **Fig. 3** (**a**), the analysed TV cultivars include a variety of different terpenoids. Aside e.g. terpinenes, cymene, and myrcene, active substances thymol and its isomer carvacrol are found in shares of 59 % and 4 %, respectively. This is a comparable, but higher share of both active substances than reported in literature before where TV EOs include approx. 46.2 % thymol and 2.4 % carvacrol (Shabnum and Wagay 2011); however, chemical composition of TV EO is known to heavily fluctuate depending on surroundings and TV chemotype (Pothier et al. 2001). As discussed in literature and proven in own test measurements, both thymol and carvacrol have antioxidant

properties (Yanishlieva et al. 1999; Havelt and Schmitz 2018; Ruberto and Baratta 2000; Mastelić et al. 2008). Particularly thymol, which represents notably more than 50 % of detected EO compounds, is thus considered the main active components of analysed TV EOs. As depicted in **Fig. 3** (**b**), significant differences in thymol concentration in EOs of different TV cultivars cannot be observed; however, Var3 shows a particularly low scattering. Regarding chemical composition of the oil, no relevant differences between first and second harvest are observed. This is supported by literature in which, regardless of stress, the qualitative composition of TV Var3 volatile compounds does not change substantially (Mahdavi et al. 2020). The composition of an exemplary essential oil as determined via Gas Chromatography coupled with Mass Spectrometry (GC-MS) is shown in **Fig. 4**. This exemplary chromatogram roughly results in the composition of TV EOs discussed for Fig. 3a while giving information on the circumstances on analysis. For example, it is shown that isomers thymol and carvacrol are separated sufficiently using this method.

Overall, this concludes that for EO usage, Varico 3 is the most potent cultivar to use, providing the highest thymol content per cultivated area as no relevant differences between cultivars are observed regarding essential oil properties; however, with Varico 3 it is possible to obtain more of said essential oil per cultivated area. The usage of other cultivars is possible but results in a decreased efficiency.



(a) *(b)*

Fig. 3 *(**a**): Chemical composition of Thymus vulgaris (TV) essential oils (EO). Mean of six cultivars, six-fold determination per variant. Determination by gas chromatography coupled with mass spectrometry (GC-MS), identification via NIST database and standard substances. Data based on first harvest. Others: Compounds < 1%. (**b**): Concentration of thymol in EOs of different TV cultivars. Six-fold determination per cultivar, determination by GC-MS. Identification and quantification via external and internal calibration, standard deviation indicated by error bars. Data based on first harvest.*

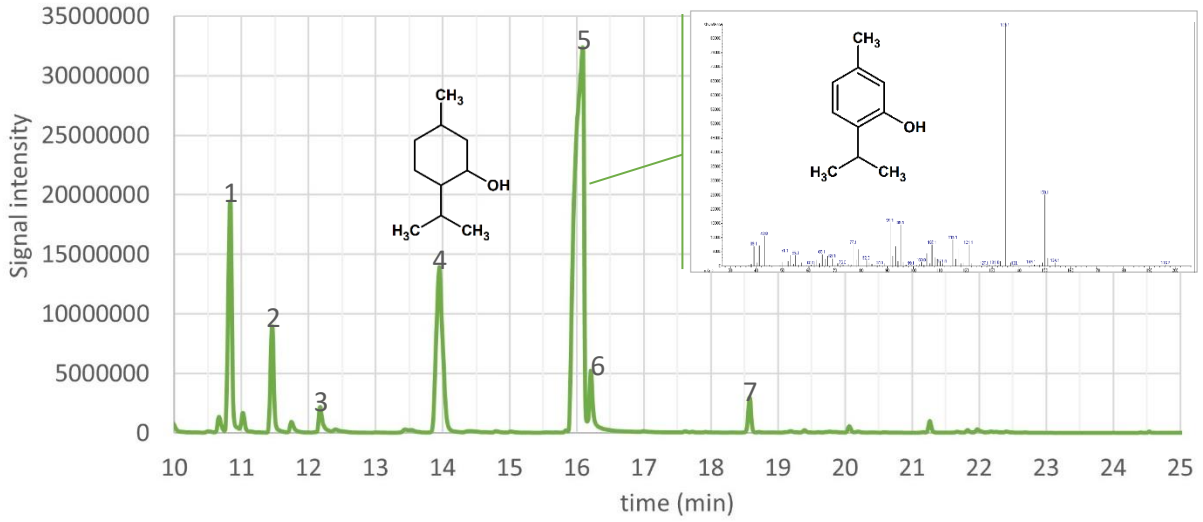


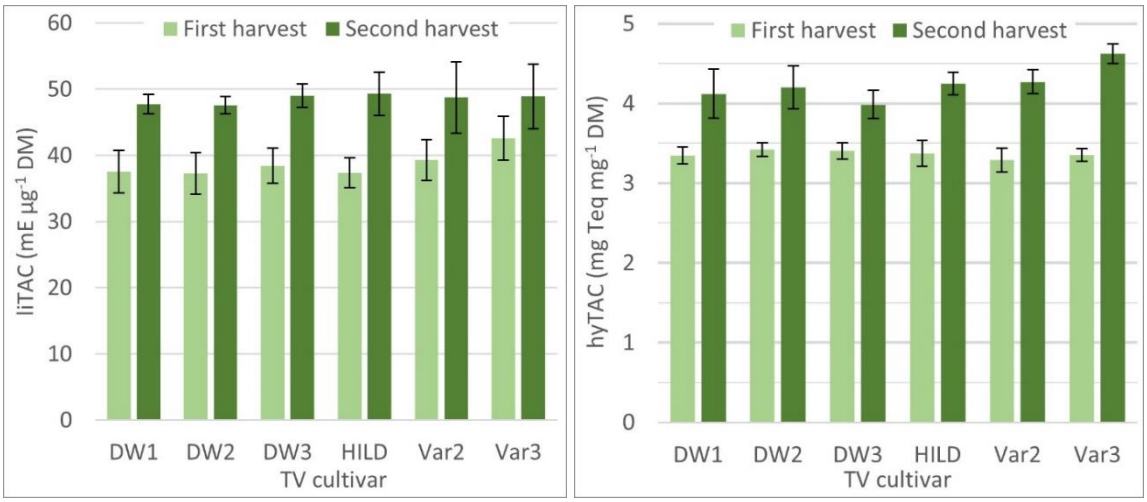
Fig. 4 *Exemplary chromatogram of first harvest TV EO obtained via Gas Chromatography coupled with Mass Spectrometry (GC-MS) and mass spectrum of thymol. Identification via standard substances and NIST database. 1: α-terpinene; 2: τ-terpinene; 3: linalool; 4: menthol (internal standard); 5: thymol; 6: carvacrol; 7: caryophyllene*

2.2.3.2 Extract Analysis

Besides EO distillation, extraction of compounds from TV using chemical solvents is possible. The properties of lipophilic and hydrophilic TV extracts (using heptane and methanol as extractants, respectively) are presented in the following.

Total Antioxidant Capacity

As presented in **Fig. 5**, all TV variants show a considerable antioxidant effect for both lipophilic and hydrophilic extracts. For the hydrophilic determination of Total Antioxidant Capacities (hyTAC), Trolox equivalents (Teq) are used to compare the Total Antioxidant Capacity (TAC) of different compounds with the common antioxidant reference compound Trolox (Erel 2004). The frequently applied β-carotene bleaching assay, which is used for the determination of lipophilic compounds' TACs (liTAC), is evaluated using milli extinction units (mE) (Chevolleau et al. 1992; Matthes and Schmitz-Eiberger 2009; Nickavar and Esbati 2012). Unfortunately, different methods for the determination of antioxidant efficacy cannot be compared directly as they typically utilise different mechanisms which show e.g. differences in dealing with varying compounds. Therefore, data obtained via different assays for the determination of antioxidant capacities are interpreted as complementary information instead of opposing both data sets (Charles 2013a). However, higher values represent a higher antioxidant effect of the particular extract for both liTAC and hyTAC analysis.



*(**a**)* *(**b**)*

Fig. 5 *Total antioxidant capacity (TAC) of extracts of dried leaf extracts of six different Thymus vulgaris (TV) cultivars. Six-fold determination, standard deviation indicated by error bars. Light green: first harvest; dark green: second harvest. DM: dried mass. (**a**): Lipophilic TAC (liTAC) of heptane extracts, determined via β-carotene bleaching assay. mE: milli extinction units. (**b**): Hydrophilic TAC (hyTAC) of methanol extracts, determined via ABTS radical cation scavenging capacity assay. Teq: Trolox equivalents*

Fig. 5 reveals that no significant differences between the examined TV cultivars are shown. The only observed difference is an occasional increase of TAC for the variant Varico 3 which occurs for hyTAC determination of second harvest extracts and liTAC determination of first harvest extracts. This result is supported by the results for EO analysis which again found little variations between the TV cultivars, excluding Varico 3 which appears to be slightly more potent than other cultivars are. Additionally, differences between the first and second harvest are observed: for both lipophilic and hydrophilic extracts, TACs are increased by roughly 25% when comparing the second harvest (late summer) to the first one (early summer). As discussed for EO analysis before, this indicates a plant mechanism to increase secondary metabolites when exposed to environmental stress (Edreva et al. 2008; Galasso et al. 2014).

UV absorbance

As presented in **Fig. 6**, the mean hydrophilic extracts of all TV cultivars show a high UV absorbance in the most relevant areas of the UV range. UV light ranges from a wavelengths approx. 100 nm to approx. 400 nm while UV daylight of wavelengths below 280 nm (UV-C) is blocked from reaching the Earth's surface by the Ozone layer with the remaining light of wavelengths between 280 nm and 400 nm (UV-B and UV-A) being particularly relevant for plastic degradation (Grob et al. 2016; European Commission Scientific Committees; Bundesamt für Strahlenschutz 2021). For wavelengths below approx. 340 nm, all extracts show a considerable UV absorbance of at least 150 relative absorbance units (rAU) which rise to a maximum of approx. 260 rAU. Thus, the extracts are capable to absorb a relevant amount of ultraviolet radiation of a broad range of wavelengths while

showing only limited absorbance in the visible range (> 400 nm). These properties make TV extracts a promising resource for bio-based photostabilisers. Different, but comparable spectra of related Thyme species have been reported in literature as well (Janiak et al. 2017). Again, as observed and discussed for antioxidant effects before, a quantitative difference between the early and late summer harvests is observed with the second harvest UV absorbance surpassing the first harvest UV absorbance by approx. 25 %. Such an increased synthesis of photoprotecting secondary metabolites is observed for various medicinal plants when they are affected by UV stress (Takshak and Agrawal 2019).

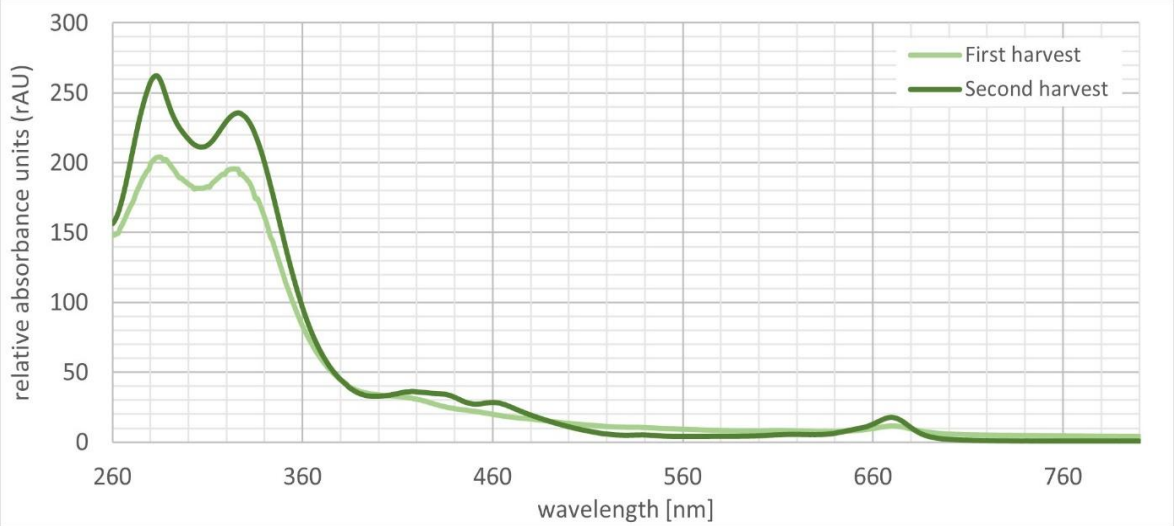
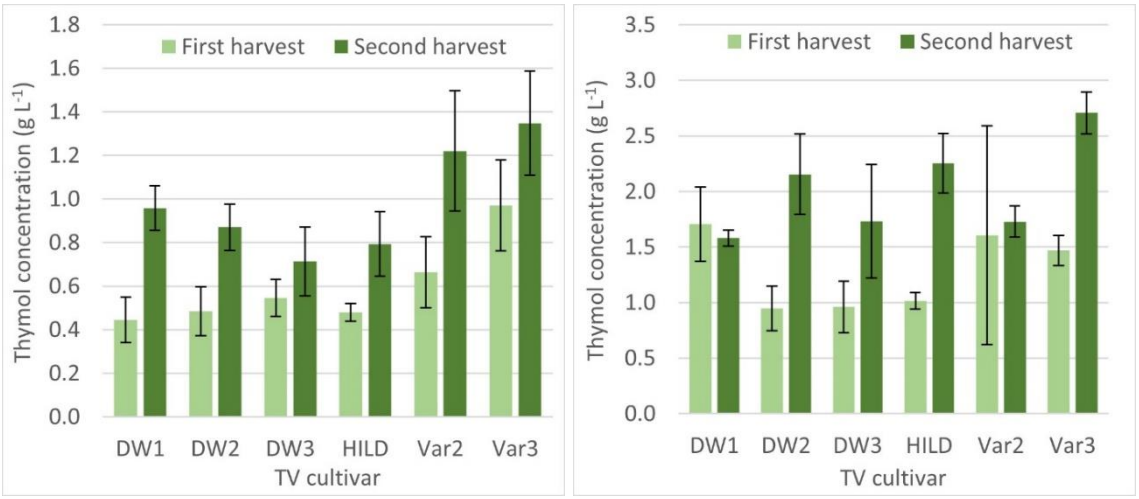


Fig. 6 *Mean UV spectra of hydrophilic methanol extracts of dried leafs of six different TV cultivars (six-fold determination per cultivar) harvested at different seasons. Light green: first harvest; dark green: second harvest. rAU: relative absorbance units, taking different dilutions per measurement into account*

Chemical Composition and Thymol Content

The chemical composition of extracts shows significant differences when comparing hydrophilic and lipophilic extracts to the composition of EOs which generally show a much higher concentration of active compounds. Aside from the difference in concentrations, it is observed that no relevant differences between the qualitative composition of lipophilic extracts and essential oils occur. Thus, the composition strongly resembles the one determined for EOs and presented in Fig. 3 and 4. This is plausible as, due to the lipophilic nature of essential oil components, the same compounds can be extracted using a lipophilic extractant like heptane. However, using the hydrophilic extractant methanol results in extracts containing the most hydrophilic constituents of EOs only, such as thymol, carvacrol and caryophyllene. As those main active substances of TV EO are hydrophilic enough to be extractable via methanol extraction as well, a reduction of overall extracted (passive) compounds does not result in a reduction of observed active effects. This effect is presented in **Fig. 7**: even though a considerable statistical scattering occurs, it is observed that hydrophilic extracts

(Fig. 7 (b)) include a higher concentration of main active compound thymol than lipophilic extracts do (Fig. 7 (a)). Although different TAC determination methods are not comparable with each other, it can thus be assumed that hydrophilic extracts also show a higher TAC than lipophilic extracts. The different observed thymol concentrations can be explained by the comparably polar nature of thymol and carvacrol. Both compounds share a comparably hydrophilic phenolic structure which allows the compounds to solve in both hydrophilic and lipophilic solvents while preferring hydrophilic ones (Zhu et al. 2016). While both extracts are possible and worthwhile (depending on the application), hydrophilic extraction is thus recommended to increase stabilising properties of the extract and to reduce accompanying substances which do not affect the efficacy. Furthermore, evaluation of harvest differences again results in the second harvest extracts showing higher concentrations of thymol.



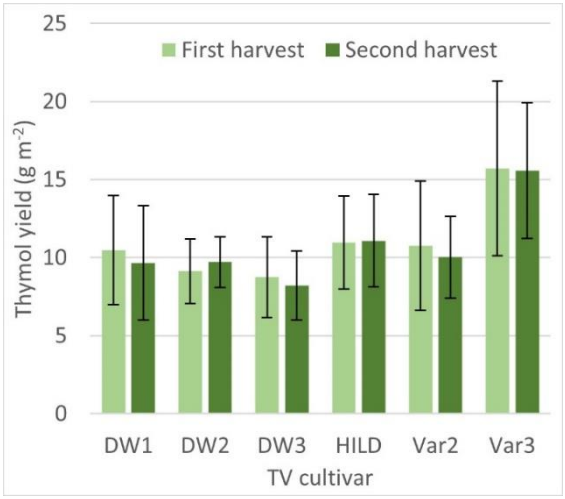
*(**a**)* *(**b**)*

Fig. 7 *Thymol concentration of extracts of dried leaf extracts of six different Thymus vulgaris (TV) cultivars. Six-fold determination, standard deviation indicated by error bars. Light green: first harvest; dark green: second harvest. (**a**): Heptane extracts. (**b**): Methanol extracts*

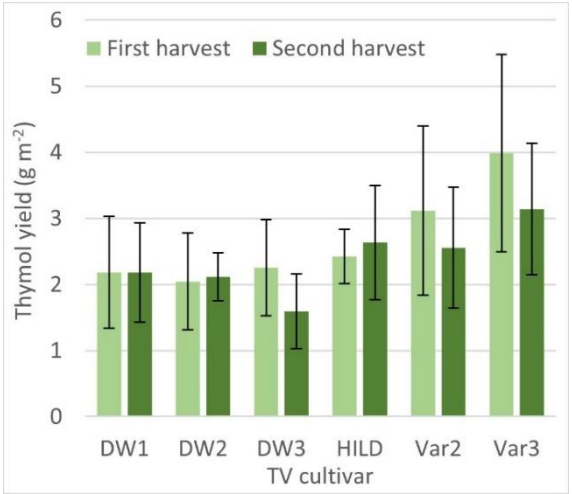
Comparison of Thymol content in Essential Oils and Extracts

To evaluate whether the use of specific cultivars, harvest periods, extracts or essential oils is favourable, thymol yields per m² are compared in **Figure 8**. As described before, the two different harvest periods only have a limited influence when comparing the yield per m² cultivated area in contrast to the yield per g dried mass as the plants not only show a decreased growth when exposed to summer stress (e.g. heat, drought, UV exposure) but also tend to produce more secondary metabolites to meet those stress factors. Those effects roughly balance each other out as discussed before. Generally, previous evaluations are supported by the results presented here as, again, little differences between cultivars are observed with the cultivar Varico 3 showing a slightly higher yield than other cultivars. Comparing the absolute thymol yield of EOs obtained by steam distillation and

extracts obtained by heptane or methanol extraction shows that hydrophilic methanol extracts are more favourable than lipophilic heptane extracts which is plausible due to the hydrophilic properties of thymol discussed before. However, hydrophilic extraction yields seem to be subject to a higher statistical variability. Extraction allows to directly create a higher volume of bioactive agents and a wider applicability compared to EOs while reducing the amount of accompanying substances. However, extracts show a lower concentration of constituents than highly concentrated EOs and thus presumably present a decreased efficiency.



(a)



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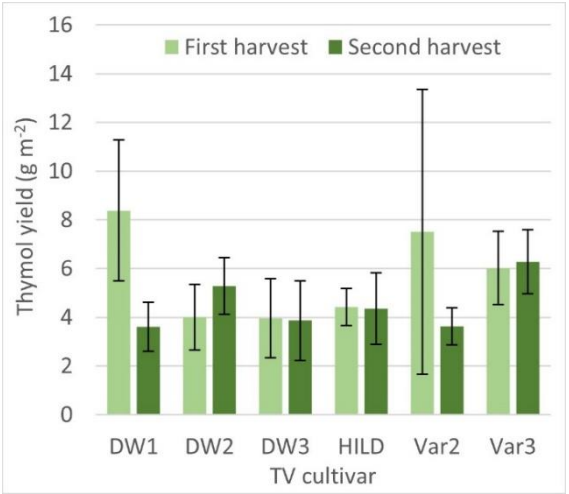


(c)

Fig. 8 *Thymol yield per cultivated area of extracts or essential oil (EO) of dried leaf extracts of six different Thymus vulgaris (TV) cultivars. Six-fold determination, standard deviation indicated by error bars. Light green: first harvest; dark green: second harvest.*
*(**a**): Essential oil. (**b**): Heptane extracts. (**c**): Methanol extracts*

The by far highest thymol yield is obtained by distilling the highly concentrated essential oils which result in a thymol yield of approx. 10 g m^{-2} for cultivars DW1-3, HILD and Varico 2 while Varico 3 results in a maximum thymol yield of ca. 15 g m^{-2}. Thus, it is recommended to use EOs of Varico 3, regardless of the harvest period, to obtain the highest possible thymol yield and thus the best possible stabilising effect.

Essential oils are more difficult to process than extracts due to their comparably small yield volume and liquid properties. However, they allow the use of highly concentrated compounds with for demanding stabilising applications. When the use of stabilising extract is favoured, essential oils could be diluted using organic solvents to obtain the desired extract properties while still benefiting from an increased thymol yield.

2.2.4 Conclusion

Different techniques and parameters of *Thymus vulgaris* (TV) extraction have been evaluated. In general, it is observed that no considerable differences are observed between most TV cultivars except for Varico 3 which shows slightly increased desired effects in several analyses. An influence of different harvest seasons could not be confirmed: while the plant growth is limited in increased stress situations (e.g. for the second harvest in late summer), increased synthesis of secondary metabolites is initiated, roughly balancing the yield of active compounds per cultivated area. While particularly hydrophilic extracts are worthwhile for some applications, steam distillation of essential oils is preferred over extraction as it leads to an increased thymol yield. Furthermore, essential oils introduce a higher flexibility as they could be used without further processing or after dilution with organic solvents to recreate extract properties while maintaining higher active compound concentrations.

2.2.5 Acknowledgements

Thanks are due to Maren Maruhn, Georg Völkering and Prof. Dr. Ralf Pude (INRES, University of Bonn) for providing and processing plant materials and to Bonn-Rhein-Sieg University and the European Union for financially supporting this research project as part of the European Regional Development Fund (EFRE.NRW).

3. European horse chestnut seeds (*Aesculus hippocastanum* L.)

"Sustainable extraction and characterisation of bioactive compounds from horse chestnut seed coats for the development of bio-based additives" (Havelt et al. 2019)

Abstract

Background: To protect renewable packaging materials against autoxidation and decomposition when substituting harmful synthetic stabilizers with bioactive and bio-based compounds, extracts from *Aesculus hippocastanum L.* seeds were evaluated. The study objectives were to determine the antioxidant efficacy of bioactive compounds in horse chestnut seeds with regard to different seed fractions, improve their extraction, and to evaluate waste reuse.

Methods: Different extraction techniques for field samples were evaluated and compared with extracts of industrial waste samples based on total phenolic content and total antioxidant capacity (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS)). The molecular weight distribution and absorbance in ultraviolet range (UV) of seed coat extracts were determined, and the possibility of extracts containing proanthocyanidins was examined.

Results: Seed coat extracts show a remarkable antioxidant activity and a high UV absorbance. Passive extractions are efficient and much less laborious. Applying waste product seed coats leads to a reduced antioxidant activity, total phenolic content, and UV absorbance compared to the field sample counterparts. In contrast to peeled seed extracts, all seed coat extracts contain proanthocyanidins.

Discussion: Seed coats are a potential source of bioactive compounds, particularly regarding sustainable production and waste reuse. With minimum effort, highly bioactive extracts with high potential as additives can be prepared.

3.1 Introduction

A growing population with an apparently even faster growing conscience about environmental issues and sustainability presents new challenges to the food and packaging industries in terms of eco-friendly, safe, and organic packaging systems that will not further contaminate oceans and the environment (German Environment Agency 2017). The demand for bio-products is increasing in agriculture (Moewius et al. 2018), as well as for eco-power and energy-efficient devices (German Environment Agency 2017). Ecological conscience and the necessity of increased sustainability of packaging products made from common plastic or bio-plastic and improved by additives from renewable sources are ubiquitous. However, the instabilities of such materials occur due to

photodegradation and microbial and oxidative stress, which can be mitigated by the application of proper additives. Without incorporation of such, the product undergoes undesired changes in material properties, decreasing their stability and shelf life (Rasselet et al. 2014; Altaf et al. 2018). The global production volume of antioxidant additives in 2007 was 336.9 kt, with the majority of them being synthetic, petrol-based compounds, including the popular but potentially harmful additives butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Ito et al. 1986; Kahl and Kappus 1993; Peltzer et al. 2009; Wegmann et al. 2016). Identifying and preparing bio-based bioactive additives to substitute petrol-based antioxidants is essential for producing packaging systems based on the concept of sustainable production and consumption (Mulder 2007). This study is a precursor to the use of bio-based stabilizers in sustainable food packaging which, to the best of our knowledge, is a new approach.

On the current pharmaceutical market, use of extracts of European horse chestnut, or *Aesculus hippocastanum L.* (AEH) seeds (alias chestnuts), has already been established. Since the phytopharmaceutical industry focuses on ingredients found in the peeled seeds, the seed coats are usually discarded, providing an excellent, unexplored opportunity for by-product valorization. Pre-tests conducted as part of our previous research indicated a significant absorbance of AEH seed extracts in the ultraviolet range (UV). Expected substance groups in the extracts of seed coats are polyphenols, such as flavonols and condensed tannins alias proanthocyanidins (PAs), as both the mentioned structures as well as their glycosides were identified in Japanese horse chestnut seeds (*Aesculus turbinata BLUME* (AET)) and in AEH leaves (Ogawa et al. 2008; Oszmiański et al. 2014; Kimura et al. 2017). Recently published preliminary tests support those findings, suggesting antioxidants, phenols, and a high UV-absorbing activity in the coats of AEH seeds (Makino et al. 2017).

In the context of their possible application as food contact materials, the harmlessness of the additives has to be evaluated. Fast and cost-effective extraction and implementation methods are equally important to create a competitive and attractive product. Therefore, ongoing optimization of extraction and analysis of the local horse chestnut species is essential. In this study, the secondary constituents of AEH seeds were extracted, and their total phenolic content (TPC), UV absorbance, and total antioxidant capacity (TAC) were measured and evaluated. As compounds with a higher molecular weight are more effective and less prone to migration (Hagerman et al. 1998), the molar mass distribution of extracts was determined. Extraction optimization is highly relevant for an efficient method; thus, extraction techniques based on using unprocessed, macroscopic samples were examined. Whereas such extraction methods are uncommon for most applications, they have been previously effective for extraction of PAs from different grape parts

(Sá et al. 2014). Thus, an efficient, simple, less elaborate extraction method was developed, optimized, and evaluated. Differences concerning the secondary ingredients and their amounts in different seed fractions (whole seed, peeled seed, seed coat) were investigated and evaluated. Additionally, seeds collected from the wild (field samples, FS) were compared to waste seed coats of the phytopharmaceutical industry (waste products, WP), and their applicability as additives was evaluated.

3.2 Materials and Methods

3.2.1 Chemicals and Instrumentation

For analysis, a Lambda 25 dual-trace spectral photometer (Perkin Elmer, Waltham, MA, USA) and, for size exclusion chromatography (SEC), a 1260 Infinity system with an 1100 Series column oven were used (Agilent, Santa Clara, CA, USA). The system is supplied with three SEC columns, including a pre-column (particle size: 5 µm) and two main columns (particle size: 5 µm; pore sizes: 1000 Å and 100'000 Å). All columns were produced by Polymer Standard Service (PSS; Mainz, Germany) and are equipped with a modified styrene-divinylbenzene copolymer network (SDV). The polystyrene standard kit used for SEC calibration was obtained from PSS (Mainz, Germany). 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2(3)-tert-butyl-4-methoxyphenol (BHA), and dipotassium hydrogen phosphate were purchased from Alfa Aesar (Karlsruhe, Germany), whereas acetic acid, tetrahydrofuran (THF), Trolox, β-carotene, and 2,6-di-tert-butyl-4-methylphenol (BHT) were purchased from Bernd Kraft (Duisburg, Germany), Carl Roth GmbH + Co. KG (Karlsruhe, Germany), Cayman chemical Company (Ann Arbor, MI, USA), Sigma Aldrich (Darmstadt, Germany), and ThermoFisher (Kandel) GmbH (Karlsruhe, Germany), respectively. Ammonium iron (III) sulfate dodecahydrate, butan-1-ol, concentrated hydrochloric acid, hexane, methanol, acetone, and agar were obtained from VWR International, Darmstadt, Germany. Dichloromethane, Folin-Ciocalteu phenol reagent, hydrogen peroxide, potassium dihydrogen phosphate, sodium hydroxide, sodium acetate, and nutrient broth for microbiology (based on 5 $g \cdot L^{-1}$ peptone from meat, 3 $g \cdot L^{-1}$ meat extract) were purchased from Merck KGaA, Darmstadt, Germany. Physiological saline solution and tryptone were purchased from Blank, Vörstetten, Germany and VWR International, Darmstadt, Germany.

3.2.2 Samples

As sample material, AEH seeds were collected from a single tree in Meckenheim, Germany (field samples (FS); coordinates: 50°36'7.3" N; 7°1'44.9" E) which was identified as AEH by scientific staff of the Faculty of Agriculture (University of Bonn, Germany). For pretests, further samples were

collected and identified in further locations in Germany analogically. The quality of the samples collected from all locations meet the required standards; we focusef on FS from Meckenheim as the seeds were easy to collect and could be obtained in large quantities in one single harvest under similar conditions, promoting a more homogeneous sample material. In the following, the seed fractions are defined as whole seeds (ws) for whole seeds including the seed coat, peeled seeds (ps) for peeled seeds deprived of their coats, and seed coats (sc) for the dark brown seed coat alias seed shell only. The whole seeds were dried at 30 °C for 20 days until dryness (<10% water content). For 50 whole seeds, the average weight was determined, with 15 of those whole seeds being separated into peeled seeds and seed coats afterward to examine the weight ratio. Chopped AEH seed coats as phytopharmaceutical waste products (WP) were kindly provided by Finzelberg, Martin Bauer Group, Andernach, Germany.

Where applicable, results are depicted presenting the mean and standard deviation.

3.2.3 Extraction

Different extraction techniques were applied for analyses. In a pretest, different extractants (water, methanol, water/acetone (1:1 *v/v*), methanol/acetone (1:1 *v/v*)) were used for the extraction of FS peeled seeds and FS seed coats to find a suitable extractant for the following research, evaluated by comparing the TAC. Water/acetone (1:1 *v/v*) was proven to be the most potent extractant regarding the extraction of substances from FS seed coats, whereas water was the most potent extractant for the FS peeled seed (**Figure A1**). Due to the peeled seed showing little antioxidant activity as described later, we focused on the extraction from seed coats. Thus, the extractant used in all further extractions was water/acetone (1:1 *v/v*) if not stated otherwise. Extractions took place at 22 °C.

For internally established extraction (“grinding extraction”), 200 mg ground sample was placed into a centrifuge tube. We pipetted 1 mL of the extractant onto the sample before mixing and centrifuging for 10 min. The supernatant was pipetted into a 5 mL volumetric flask and the previous steps were repeated twice more. All supernatants were combined and finally filled to 5 mL. The grinding extraction was applied to FS (ws, sc, and ps) and WP (sc) samples.

Finally, two variants of passive extraction setups were examined to evaluate a possible facilitation of the sample preparation process in practice.

For passive extraction of chopped seed coats, approx. 5 g of sample material with a size of approx. 5 mm were placed into 20 mL extractant and stored for a specific period in a closed container under exclusion of light. Also, a blank sample was prepared, stored for the maximum time period and subtracted from the results. This extraction was applied both to FS and WP chopped seed coats.

For passive extraction of FS whole seeds, three medium-sized seeds were cleaned with a brush and placed into a closed vessel. This corresponds to approx. 4.86 g seed coat on average. Then the vessel was filled with 67 mL extractant until the seeds were covered and stored for a specific period under exclusion of light. A blank sample was prepared accordingly.

3.2.4 Determination of Antimicrobial Properties

The antimicrobial activity of extracts of ground AEH seed coats was quantitatively analyzed by modifying the JIS Z 2801:2010 test for antimicrobial activity and efficacy (JIS Z 2801). *Staphylococcus aureus* (DSM No. 799) and *Escherichia coli* (DSM No. 1576) were applied as test organisms, and the extractant was used as a reference. The inoculum was prepared by transferring a frozen culture to 10 mL nutrient broth and incubating with the inoculum (37 °C, 24 h). According to the McFarland standard, the inoculum was adjusted in physiological saline solution with tryptone to a concentration of 108 colony forming units (CFU) mL^{-1} before being diluted in physiological saline solution with tryptone to a final concentration of 105 $CFU \cdot mL^{-1}$. 1 mL inoculum was incubated (37 °C, 24 h) in a mixture of 9 mL nutrient broth and 1 mL extract (or solvent reference). Then, the samples were plated on plate-count agar by the drop plate technique. After incubation (37 °C, 24 h), viable counts were determined. A material is considered antimicrobial if the lg reduction calculated by **Equation (1)** is ≥2.0 after incubation (JIS Z 2801):

$$\text{lg reduction} = \text{lg}\,[c_{gew}(\text{reference}) \times (c_{gew}(\text{sample}))^{-1}] \qquad (1)$$

where c_{gew}(reference) is the arithmetic mean of bacterial counts of the reference 24 h after inoculation, and c_{gew}(sample) is the arithmetic mean of bacterial counts of the sample material 24 h after inoculation.

3.2.5 UV/Vis Spectrometry

The UV/Vis spectra of different extracts in appropriate dilutions were determined in the range of 260 to 800 nm. As diluting samples to different extents to obtain measurable and correct spectra was necessary, the results are displayed in relative absorbance units, considering the different dilutions to maintain comparability.

3.2.6 ABTS Radical Cation ($ABTS^{\bullet+}$) Scavenging Capacity Assay

The total antioxidant capacity (TAC) was determined at a wavelength of 660 nm according to the literature (Erel 2004). At least one blank sample per run was prepared. As the assay was calibrated using a Trolox solution, the TAC of the samples is given in mg of Trolox equivalents (Teq) per mg of extracted dried mass (DM) of the sample.

3.2.7 Folin-Ciocalteu Assay

To determine the TPC of the extracts, a modified Folin-Ciocalteu assay was conducted in centrifuge tubes (Singleton et al. 1999; Matthes and Schmitz-Eiberger 2009). First, 0.25 mL deionized water was mixed with the same amount of Folin-Ciocalteu reagent, and 0.25 mL of sample extract was added. At least one blank sample per measuring series was prepared. Then, 30 seconds after the sample was added and carefully mixed, 2.5 mL of 0.1% aqueous sodium hydroxide solution was pipetted into the centrifuge tube. The tube was capped, and the reagents were mixed. After exactly 30 min more, the absorbance of the sample was measured at the wavelength of 720 nm. For evaluation, the assay was calibrated with gallic acid. Therefore, TPC of the samples is given in mg of gallic acid equivalents (GAE) per g of extracted dried mass (DM) of the sample.

3.2.8 Size Exclusion Chromatography

For SEC analysis, samples were prepared by evaporating seed coat extract under a nitrogen stream until complete dryness and subsequent solving in THF/water (20:1 *w/w*). This THF/water mixture is also the mobile phase for SEC measurement as it was used in the literature for polyphenols (Gabetta et al. 2000; Bava et al. 2015). Further parameters were adjusted to a flow rate of 1 $mL \cdot min^{-1}$, a sample injection volume of 100 µL, a measuring time of 30 min, and an isocratic elution at 35 °C. Detection was carried out by applying a UV detector measuring the absorbance at 280 nm. Molar mass calibration was conducted with a polystyrene standard kit.

3.2.9 Further Analyses

The modified Acid Butanol Assay was prepared according to the literature with analysis at a wavelength of 550 nm (Hagerman 2011). The assay is qualitative only as no calibration was prepared. For NMR analysis, the whole seed passive extract with an incubation time of 21 days was diluted with deuterated water and measured using an Avance III 600 NMR device (Bruker Corporation, Billerica, MA, USA).

3.3 Results and Discussion

3.3.1 Pre-Analyses

3.3.1.1 Seed Coat Ratio

Weighing of whole seeds resulted in an average weight of approx. 11.2 g per whole seed (standard deviation (SD): ± 1.6 g; *n* = 50). The average weight of the seed coat was 1.62 g (SD: ± 0.21 g; *n* = 15), and 9.65 g (SD: ± 0.83 g; *n* = 15) for the peeled seed for this average total weight, representing 14% seed coat per whole seed (SD: ± 1.2%; *n* = 15). The seed coat represents a relevant and

potentially worthwhile source of resources. However, reference data concerning the mass ratio of AEH peeled seed and seed coat have not been published yet.

3.3.1.2 Determination of Antimicrobial Properties

Typically, the disc diffusion method is used for the determination of antimicrobial properties. However, to prevent potential issues due to macromolecular analytes that are less prone to diffusion, a modified Japanese Industrial Standard (JIS) method was applied as it is not dependent on the sample molecules successfully migrating into the agar. When determining the antimicrobial properties of AEH seed coat extracts obtained by grinding extraction, the arithmetic mean of bacterial counts of the reference for *S. aureus* is 8.0 lg CFU·mL^{-1} and for *E. coli* is 7.6 lg CFU·mL^{-1} after incubation. The average bacterial counts for *S. aureus* decreased to 1.8 lg CFU·mL^{-1} when applying FS extracts, a reduction of 6.2 lg units. For WP extracts, the average *S. aureus* bacterial counts diminished to 1.6 lg CFU·mL^{-1} (reduction: 6.4 lg units). For *E. coli*, no significant reduction was observed.

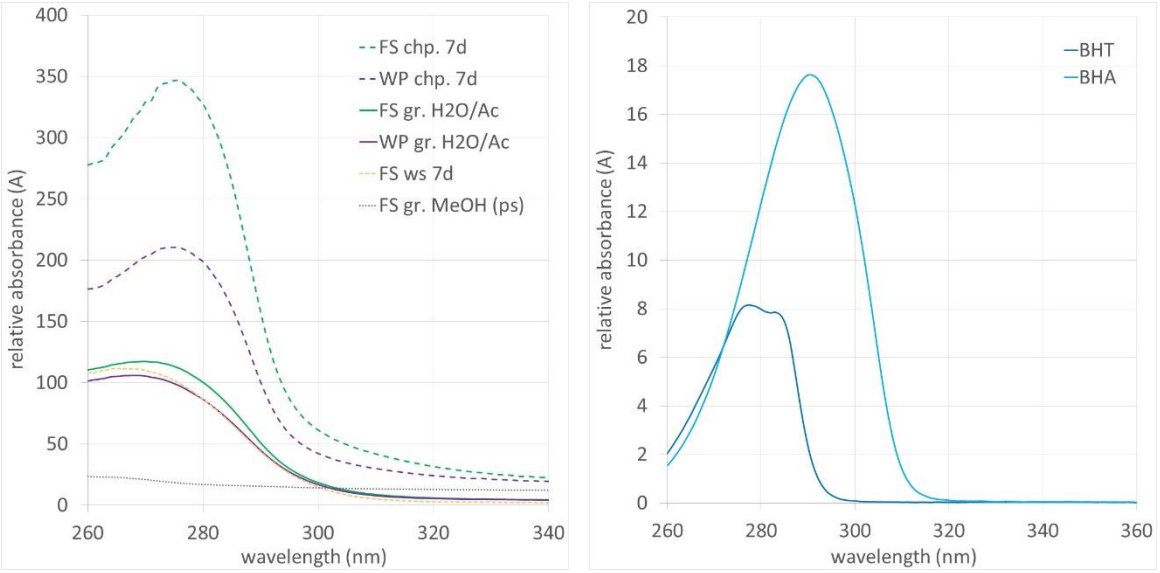
The results show that the gram-positive bacterium *S. aureus* is more sensitive against AEH seed coat extracts than the gram-negative bacterium *E. coli*. This observation of a stronger resistance of gram-negative bacteria against antimicrobial substances of plant origin is confirmed by the literature (Thippeswamy et al. 2013; Rebaya et al. 2016). The effect is caused by differences in the cell wall construction of gram-positive and gram-negative bacteria (Nikaido and Vaara 1985; Smith-Palmer et al. 1998). However, AEH seed coats are a material worthwhile to study for sustainable additive production as a considerable antimicrobial effect of their extracts against *S. aureus* was proven.

3.3.1.3 UV Absorbance

Whereas the peeled seed extract only showed a low UV absorbance, the extracts of seed coats demonstrated a significant UV absorbance as shown in **Figure 9** (**a**). All seed coat extracts showed a comparably insignificant absorbance in the visible range while significantly absorbing in the region below 310 nm with maxima at approx. 275 nm. As a high UV absorbance is desired for additives acting against photodegradation (Grob et al. 2016), these results are promising.

The highest absorbance was attained by the FS chopped seed coats with a maximum relative absorbance of approx. 346, followed by the WP chopped seed coats (max. absorbance 210) and the whole seed extract whose max. relative absorbance of approx. 110 was comparable to that of extracts based on grinding extraction. This indicates the applicability efficacy and competitiveness of these easy extraction methods. Furthermore, the WP seed coats absorbed less than their FS counterparts. However, unlike seed coats that were manually collected and separated from the seeds, WP seed coats include a significant amount of peeled seed fragments, which show a marginal

absorbance only as shown in the previous before. Therefore, the lower UV absorbance of the waste seed coats is reasonable. Additionally, the industrial pre-treatment of WP seed coats prior to analysis was unknown. For example, increased contact of the seed coats with extractants during washing steps might have reduced the amount of their ingredients. As known for other plant species, another factor influencing the seeds' properties is the location and climate surrounding of the trees (Emmons and Peterson 2001). The absorbance spectra are qualitatively comparable to those of commonly used stabilizing additives BHT and BHA, plotted in **Figure 9** (**b**).



(a) ***(b)***

Fig. 9 *(**a**) Average relative UV absorbance of Aesculus Hippocastanum L. seed coat and peeled seed extracts. Measurements were recorded in triplicate. No relevant absorbance above 360 nm was measured. FS: Field samples; WP: Phytopharmaceutical waste products; chp.: passive extraction of chopped seed coats; gr.: grinding extraction of seed coats (or peeled seeds (ps), if stated); ws: passive extraction of whole seeds; 7d: Extraction duration of 7 days; H2O/Ac: Extractant water/acetone (1:1 v/v); MeOH: extractant methanol.*
*(**b**) Average relative UV absorbance of BHT and BHA solutions. Measurements in triplicate. No relevant absorbance above 360 nm was measured. BHT: Butylated hydroxytoluene; BHA: Butylated hydroxyanisole. Solvent: methanol; concentration: 1.0 mg mL^{-1}.*

Both BHT and BHA significantly absorb in the UV range below 300 and 320 nm with maxima at approx. 275 nm and 291 nm, respectively. Regarding the absorbance intensity, there is a factor of approx. 43 and 20 from BHT and BHA to FS chopped seed coats, respectively, based on a BHT or BHA solution with a concentration of 1.0 mg·mL^{-1}. Thus, 1 mL of this extract is theoretically capable of substituting approx. 43 mg BHT or 20 mg BHA with regard to UV absorbance. For WP chopped seed coats, the factors decreased to approx. 26 (BHT) and 12 (BHA), whereas the absorbance of the extract obtained by passive extraction of whole seeds resulted in factors of approx. 14 (BHT) and 6.3 (BHA). Therefore, the most potent extracts are based on chopped seed coats (FS, in particular). However, extract sustainability must be considered as using the slightly less potent extracts of the WP seed coats allows reuse of natural resources that otherwise would be lost. Additionally, the

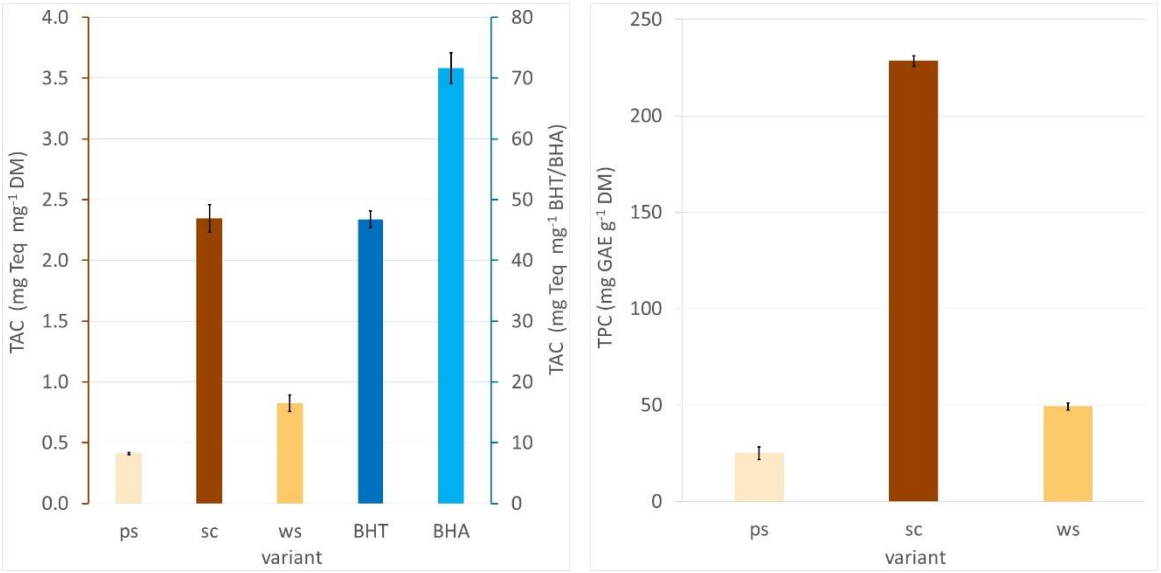
advantage of FS is likely to decrease when peeled industrially and less accurately. The application of chopped WP seed coats passively extracted, for example for seven days, is thus recommended.

3.3.1.4 Total Antioxidant Capacity (TAC) and Total Phenolic Content (TPC)

The comparison of the TAC and TPC of peeled seed and seed coat visualized in **Figure 10** provides insight into the suitability of different plant parts for use as additives. With an average TAC of 1.98 mg Teq·mg^{-1} DM, the seed coat (sc) presented the highest value, followed by the whole seed (ws) with an average of 0.534 mg Teq·mg^{-1} DM and by the peeled seed (ps) with an average TAC of 0.319 mg Teq·mg^{-1} DM. Between the seed coat and whole seed, an approximate factor of 11 was observed, whereas the difference between whole and peeled seed was approximately a factor three. The average TPC of the seed coat extract was 234 mg GAE·g^{-1} DM, of the whole seed was 80 mg GAE·g^{-1} DM and of the peeled seed was 54 mg GAE·g^{-1} DM. The extracts of AEH seeds, in particular their coats, revealed high amounts of phenolic compounds and high antioxidant capacities, whereas the peeled seed extracts showed much lower amounts of phenolics and antioxidants. This also applies to FS seeds that were collected in other locations in Germany, separated in peeled seeds and seed coats and analyzed as a part of the pretests. AEH seed coat extracts in general thus meet the most important requirement for antioxidants.

The findings correspond to the results of Vašková et al. who found phenolics to be one of the main substance groups found in AEH seeds (Vasková et al. 2015). However, further characterization of the ingredients as conducted during this study would be indispensable. Since the substances of interest are prevalent in the seed coats with the peeled seed containing relatively low amounts of antioxidants and phenolic substances, the peeled seed was widely neglected in this study. For the TAC and TPC, a recent short communication reported a mean TAC of 1.78 mg Teq·mg^{-1} DM and a mean TPC of 602 mg GAE·g^{-1} DM for AEH seed coat extracts (Makino et al. 2017). In this study, a higher TAC and a lower TPC were determined. Since the extraction and TAC methods used by Makino et al. differ from the methods applied in this study, the comparability of the results is limited (Makino et al. 2017). However, the results of Makino et al. support the findings presented in this study. Separation of seed and seed coat was conducted by Kimura et al., who also reported a high amount of PAs in AET seed coats with significantly higher amounts in the seed coat than in the peeled seed (Kimura et al. 2017). The measured TAC and TPC reasonably vary from the results of this study, presumably due to biological differences between European and Japanese horse chestnut and methodical deviations in extraction and analysis. The effects of different plant varieties and varying climate properties of different cultivation locations are known, too, most likely promoting differences in the results (Wang and Murphy 1994; Emmons and Peterson 2001). Compared to the TAC of synthetic antioxidants, which are provided in Figure 10 (a), factors of

approx. 20 or 35 between the seed coat and BHT or BHA, respectively, were measured. Therefore, 20 mL or 35 mL of extracts obtained by grinding extraction could substitute 1 mg BHT or BHA, respectively, with regards to antioxidant efficacy.



(a) *(b)*

Fig. 10 *(**a**) Total antioxidative capacity (TAC) of synthetic antioxidants BHT and BHA and Aesculus Hippocastanum L. peeled seeds, seed coats, and whole seeds extracted by grinding extraction. Extracts: primary ordinate, given in shades of brown, measurements in triplicate; BHT/BHA: Secondary ordinate, depicts in shades of blue, six measurements. Standard deviation indicated by error bars. Teq: Trolox equivalents; DM: Dried sample mass; ps: Peeled seed; sc: Seed coat; ws: Whole seed; BHT: Butylated hydroxytoluene; BHA: Butylated hydroxyanisole.*
*(**b**) Total phenolic content (TPC) of Aesculus Hippocastanum L. peeled seeds, seed coats and whole seeds extracted by grinding extraction. Measurements in triplicate, standard deviation indicated by error bars. GAE: gallic acid equivalents; dm: dried sample mass; ps: peeled seed; sc: seed coat; ws: whole seed.*

3.3.1.5 Molar Mass Characterisation of AEH Seed Coat Extracts

The molar mass distribution of AEH seed coat extracts and the corresponding integral curve are plotted in **Figure 11**. The applied detection wavelength of 280 nm is considered characteristic for polyphenols (Kimura et al. 2017). Consequently, we assumed that the sample contained polyphenols in varying molecular sizes that are well displayed in the UV signal at 280 nm. The smallest 10% of the substances in the extract had a molecular weight below 1176 g·mol^{-1}, whereas the biggest 10% had a minimum molar mass of 4862 g·mol^{-1}. The number average molecular weight was 2097 g·mol^{-1}, and the molecular weight at the peak maximum was 2989 g·mol^{-1}. The weight average molecular weight of the compounds extracted from seed coats was determined to be 3095 g·mol^{-1}. This corresponds to approx. 10 condensed catechin molecules, neglecting possible condensations of other compounds. An average molecular weight of 1750 g·mol^{-1} was determined by Czochanska et al. for PAs extracted from ground whole AEH seeds by analyzing the terminal group ratio after thiolysis using ^{13}C NMR (Czochanska et al. 1980). The shift to a higher number

average molecular weight compared to those results is reasonable as they are based on extracting the whole seed, including the inner seed, which is known to contain high amounts of substances with a significantly lower molecular weight than the seed coats' PAs, possibly including smaller polyphenols (Matsuda et al. 1997; Vasková et al. 2015).

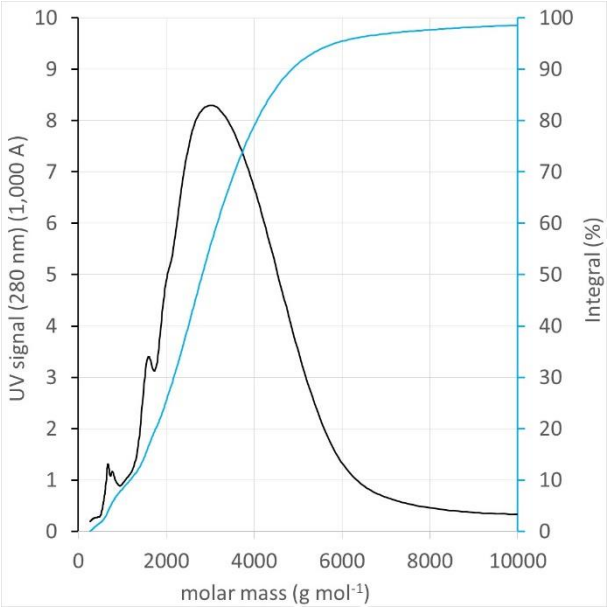


Fig. 11 *Evaluation of Size Exclusion Chromatography (SEC) analysis of Aesculus Hippocastanum L. seed coat extract. Primary ordinate: SEC chromatogram (signal of UV detector (UVD) at 280 nm), given in black and in thousands absorbance units; secondary ordinate: integral of SEC chromatogram, given in blue*

With molar masses ranging from approximately 1100 to 2600 g·mol^{-1}, the masses obtained from AET seed coat extract analysis are lower than the results for the AEH counterparts (Ogawa et al. 2008). However, the dimensions are similar. As a high molecular weight is preferred for substances used in food contact materials due to a reduced migration risk, the SEC results underline the potential of AEH seed coat extracts (European Comission 2011a).

3.3.1.6 Further Analyses

Additional analyses, including ^{1}H-NMR analysis and the Acid Butanol Assay, provided strong hints at different sugars and proanthocyanidins being present in the seed coats, inter alia supported by Kapusta et al. who found sugars in AEH seeds and Kimura et al. and Ogawa et al. proving proanthocyanidins being present in AET seed coats (Kapusta et al. 2007; Ogawa et al. 2008; Kimura et al. 2017). Again, this stresses the potential of AEH seed coats, as proanthocyanidins are classified as food-safe by the European Food Safety Authority (Turck et al. 2017).

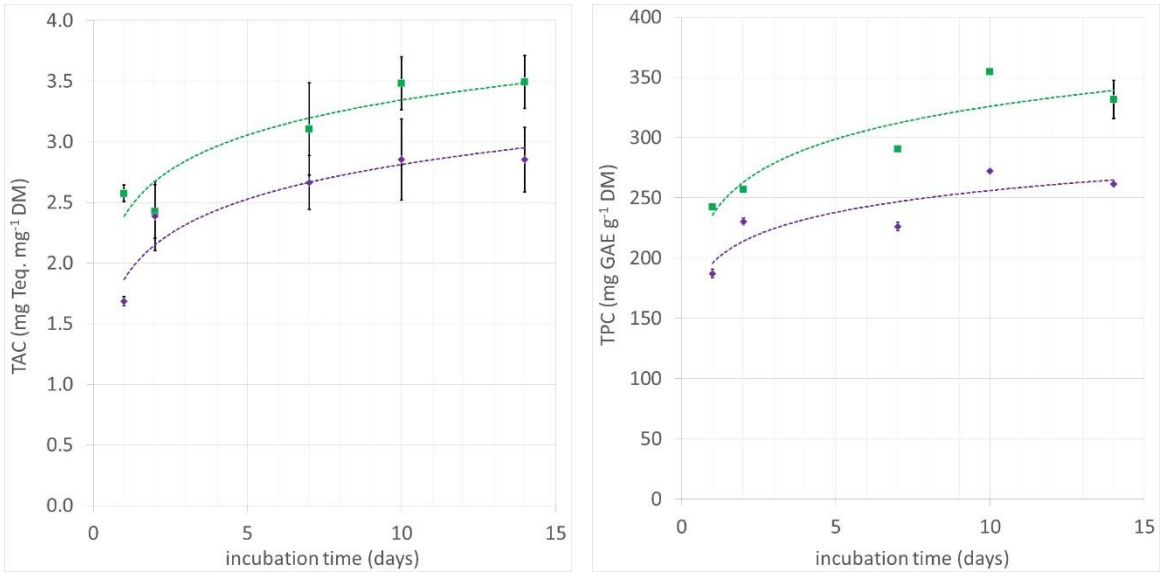
Although the results suggest a separation of the seed fractions to prepare more potent extracts from the seed coat only, the drawbacks of the separation of seed and seed coat cannot be ignored.

The manual separation is a time-consuming difficult task. When done automatically, separation will be less accurate, leading to loss of seed coat material and to incorporating parts of the significantly less potent inner seed. Those issues might be mitigated by passive extraction setups, which are evaluated in the following.

3.3.2 Extraction Evaluation

3.3.2.1 Passive Extraction of Chopped Seed Coats

The passive extraction of seed coats is shown in **Figure 12**. Comparing the curves of FS and WP, a similar curve progression was noticed despite a deviation in the first data point. After two days of incubation, both sample types showed a TAC of approximately 2.4 mg Teq·mg^{-1} DM, following a steep increase. Afterward, the course was less steep, resulting in approximately 3.5 mg Teq·mg^{-1} DM for FS and 2.8 mg Teq·mg^{-1} DM for WP after an incubation time of 10 days with the values for 14 days barely diverging. Over the complete range, FS showed higher TAC values than WP. The corresponding TPCs had a similar progression with FS showing higher values over the complete course. Again, a rapid increase was noticed during the first days of incubation. In the following, a slow increase with a moderate scattering was noticed for both sample types, resulting in a maximum TPC after 10 days of 272 mg GAE·g^{-1} DM for WP and 355 mg GAE·g^{-1} DM for FS.



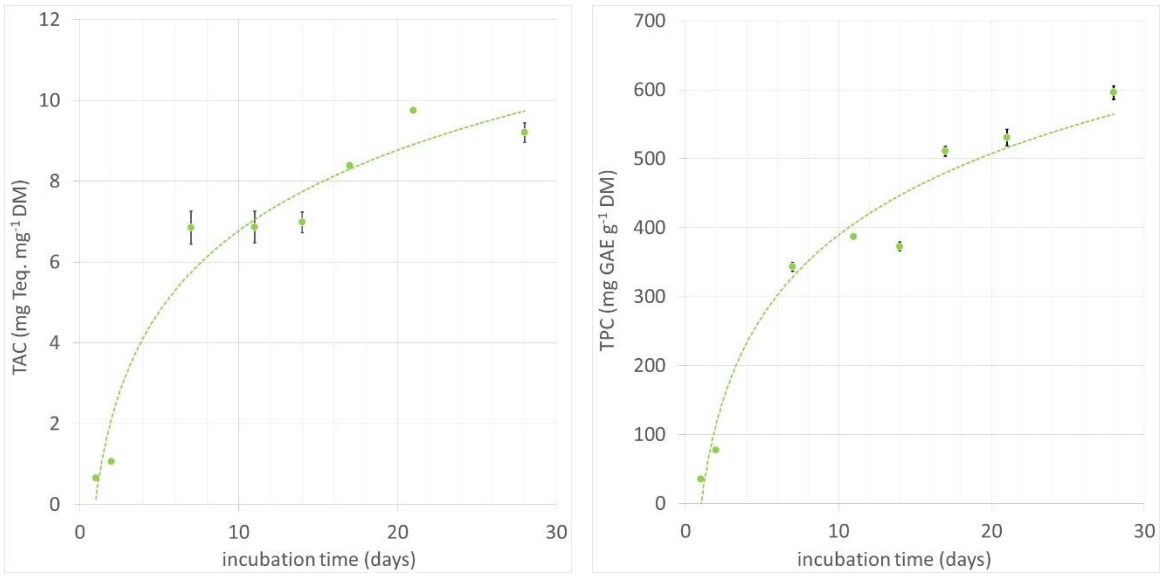
(a) ***(b)***

Fig. 12 *(**a**) TAC of passive extraction from A. Hippocastanum L. seed coats (chopped). Measurements in triplicate, standard deviation indicated by error bars. Teq: Trolox equivalents; DM: Dried seed coat mass. Field samples given in green (squares); waste products given in purple (diamonds).*
*(**b**) TPC of passive extraction from A. Hippocastanum L. seed coats (chopped). Measurements in triplicate, standard deviation indicated by error bars. GAE: Gallic acid equivalents; DM: dried seed coat mass. Field samples given in green (squares); waste products given in purple (diamonds).*

For the reasons discussed above, lower values for WPs are reasonable. However, the time-dependent courses are remarkably similar, so further noticeable deviations between FS and industrial WP seed coats did not occur during passive extraction. The slopes of both TAC and TPC matched the saturation curves. The TAC curves' rising slowed down after approx. seven days for both sample types. With a ratio of sample to solvent of approx. 1:4, this might be a sign of solvent saturation. The ratio is significantly smaller than that applied in the passive extraction of whole seeds (1:12) where no sign of stagnation was observed. This suggests that in the passive extraction setups, a solvent saturation takes place after 7–10 days at ratios between 1:4 and 1:12. Thus, in this setup, longer incubation times appear economically unreasonable. The comparison proves that the examined WP seed coats behave similarly to FS seed coats during extraction, except for the absolute starting concentration of phenolics and antioxidants, presumably for the same reasons as set out before.

3.3.2.2 Passive Extraction of Whole Seeds

In the passive extraction setup using whole, unprocessed seeds, the TAC rapidly increased for the first seven days as shown in **Figure 13** (**a**). After seven days, a TAC of 3.71 mg Teq·mg^{-1} DM was measured. Afterward, the average TAC value increased less steeply, but steadily, until it reached 7.05 mg Teq·mg^{-1} DM after 28 days of incubation. A similar TPC development of the extracts is illustrated in **Figure 13** (**b**). After a rapid increase during the first seven days of incubation, a TPC of 343 mg GAE·g^{-1} DM was obtained. In the later course, a weak, but steady increase occurred up to a TPC of 596 mg GAE·g^{-1} DM after incubation for 28 days. The slopes of the extraction characteristics of whole seeds match a saturation curve that has not yet reached stagnation. As stated before, no sign of stagnation was observed during this experiment with a seed coat to solvent ratio of approx. 1:12, suggesting saturation at a ratio between 1:4 and 1:12.



(a) ***(b)***

Fig. 13 *(**a**) TAC of passive extraction from whole Aesculus Hippocastanum L. seeds. Measurements in triplicate, standard deviation indicated by error bars. Teq: Trolox equivalents; DM: Dried whole seed mass.*
*(**b**) TPC of passive extraction from whole A. Hippocastanum L. seeds. Measurements in triplicate, standard deviation indicated by error bars. GAE: Gallic acid equivalents; DM: dried whole seed mass.*

Again, the rise in the TAC and TPC was steep at the beginning with a flattening after approx. 10 days, making 7–10 days the most efficient incubation time. Further increase in incubation time led to a relatively low extraction yield. For instance, quadrupling the incubation time from 7 to 28 days resulted in an average daily TPC increase of approx. 3.5% (approx. 1.6% for TAC). Those rates are marginal compared to the initial average daily increase rates from day 1 to 7 of 146% in TPC and 158% in TAC. In comparison with the extraction from ground seed coat material, the TAC after seven days' of incubation is higher than the TAC of the grinding extract by a factor of approx. 2.9, whereas the TPC was almost 1.5-fold higher for the passive extract. Referring to Makino et al. again, an extract with a two- to three-fold higher TAC was obtained in this study by passively extracting whole seeds for seven days (Makino et al. 2017). However, the corresponding TPC of the extract was lower by a factor of approximately 1.8. As mentioned earlier, deviations between the extraction and analysis methods can cause variations in the results between the two research groups, enabling only limited comparisons.

3.3.2.3 Comparison of Passive Extractions

The TAC measurements of BHT and BHA allowed a comparison of the antioxidant efficacy of synthetic and bio-based stabilizers. Their values exceeded those of the whole seed passive extracts by factors of approx. 7 or 11, respectively. This means that approx. 7 mL or 11 mL extract, respectively, could substitute 1 mL of a BHT or BHA solution (concentration: 1 mg·mL^{-1}) or 1 mg of BHT or BHA. Therefore, whole seed passive extracts are three times more effective than those

prepared by grinding extraction with regard to TAC. For chopped seed coat passive extractions, the approximate factors for the FS and WP were higher and comparable to grinding extraction (FS: BHT: 15, BHA: 23; WP: BHT: 18, BHA: 27), presumably caused in part by solvent saturation. Although these factors suggest a relatively high amount of AEH seed extract would be needed for substitution, it is important to remember that no resource-consuming synthesis is necessary and preparation efforts can be minimized. Additionally, the chemical structure and molecular size of the extracted compounds suggest that they could be incorporated into the polymeric matrix successfully, allowing a significantly higher amount of extract to be used in the product. An advantage of the passive extraction of whole AEH seeds is that little sample preparation is needed and the produced extracts are more potent. The previously established grinding method requires a much higher amount of sample preparation and active work time. The long sample incubation period during passive extraction can be balanced by the high throughput possible. The application of both passive extraction setups thus has the potential to enable the exploitation of otherwise discarded, sustainable materials.

3.4 Conclusions

Especially by TAC and TPC, the separation of the seed and seed coat of the European horse chestnut AEH was proven to be an essential and powerful tool to increase yields of antioxidants in the extracts. However, to avoid an elaborate sample preparation, a simple yet potent extraction method was developed where solvent is poured over whole seeds. A variation of this method was tested with chopped seed coats in the form of phytopharmaceutical waste products, as they can easily be obtained and used in relatively large amounts, enabling high throughput extraction. For both setups, an incubation period of 7 to 10 days is considered most efficient, yielding in very high amounts of TPC and TAC.

Phytopharmaceutical waste products have been proven to be well-suited as a source of additives. Application of these chopped seed coats is a convenient method of waste reuse, having advantages both from ecological and economical points of view. This also applies to using unused seeds from AEH trees, e.g., in urban environments. This new by-product valorization approach suits the sustainable concept of an environmentally friendly product from regional sources in both cases. Besides extraction optimization and conception, molar mass characterization of the extracted components was conducted in investigating the field samples. All tested seed coat extracts contained macromolecular substances that are likely to be proanthocyanidins, and the peeled seed was found to contain no significant amounts. The weight average molecular weight of the substances in the seed coat extracts was determined to be approx. 3095 $g \cdot mol^{-1}$. The high molecular

weight of PAs diminishes the risk of migration when applied in packaging, potentially making AEH seed coat extracts an excellent additive for food contact materials. The applications of such seed coat extracts will be further examined; compounds of lower molecular weight will be characterized as part of upcoming migration studies.

3.5 Funding

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3.6 Acknowledgements

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3.7 Copyright

4. Snowdrops (*Galanthus* div.)

"Plant-based bioactive compounds for substitution of petrol-based stabilisers in packaging materials" (Havelt et al. 2021b)

Abstract

Urban waste, particularly packaging, is an emerging problem in modern society. Plastic packaging materials typically include stabilising additives to create competitive products. Conventionally, plastic materials and stabilisers are petrol-based, thus having negative impacts on the environment and subsequently on human health. While bio-based alternatives to plastic packaging are already in development, bio-based additives are severely underrepresented in research so far. This study aims at presenting a sustainable alternative to petrol-based stabilisers. For this purpose, a variety of different plant species is analysed and evaluated. Bio-based stabilisers are expected to have a high total antioxidant capacity (TAC), antimicrobial effects or a high UV absorbance. The current study focuses on different snowdrop species (*Galanthus nivalis, G. gracilis, G. elwesii, G. plicatus*), common thyme (*Thymus vulgaris*) and European horse chestnut (*Aesculus hippocastanum*). Extracts of different plant species and fractions were analysed with regard to active substance characterisation, TAC, UV absorbance and possible formulations. This was achieved by GC-MS analysis and photometric methods to determine TAC and UV absorbance. When comparing the snowdrop fractions leaf, petal and bulb, leaf extracts show a higher TAC with G. elwesii leaf extracts significantly exceeding the results of the leaf extracts of the other species. A comparable UV absorbance and α-tocopherol concentration was determined for all four species. Compared to chestnut or thyme extracts, lipophilic snowdrop extracts show a higher TAC, making snowdrops a worthwhile bioresource. The same applies to A. hippocastanum seed coats and T. vulgaris leaves with high molecular weights of active substances, high UV absorbances and antioxidant and antimicrobial properties. While further research on migration, synergistic effects and advanced formulation is still being conducted, the present study already proves that phytochemicals are powerful alternatives to common stabilisers, offering the opportunity to create thorough bio-based products.

4.1 Introduction

The need for ecological alternatives for everyday situations is ubiquitous (German Environment Agency 2017). This includes investigating bio-based materials suitable for substituting current, petrol-based ones. While bio-based plastic packaging materials are already being developed

(Ramos et al. 2018), bio-based alternatives to petrol-based and potentially harmful stabilisers are severely underrepresented in research. For stabilisers used e.g. in packaging materials, properties like total antioxidant capacity (TAC), absorbance in the ultraviolet range (UV) and antimicrobial effects are particularly relevant as they could be used to act against undesired material changes and instabilities resulting from photodegradation and oxidative and microbial stress (Rasselet et al. 2014; Altaf et al. 2018).

A potential bioresource to obtain such substances investigated in the present study is snowdrop (*Galanthus* L.), a common European ornamental plant. A typical secondary metabolite contained in *Galanthus* is α-tocopherol or vitamin E, a compound which is not only safe, but essential for human consumption in moderation (Domke et al. 2005). Furthermore, α-tocopherol is known to have antioxidant effects (Stanciu et al. 2010; Karimi et al. 2018). By preparing tocopherol extracts and using those extracts as packaging stabilisers, a material use of snowdrops could be implemented. When using synthesised α-tocopherol instead of snowdrop extracts, applications for active packaging purposes are already under research (Vasile et al. 2013). Thus, the concept of incorporating α-tocopherol in food packaging is promising. The present study aims at evaluating and optimising the source of α-tocopherol, focusing on sustainability and expanding its possible applications. Other promising plants considered for use as bio-based stabilisers are common thyme (*Thymus vulgaris,* TV) and the European horse chestnut (*Aesculus hippocastanum*, AEH) (Havelt and Schmitz 2018; Havelt et al. 2019). Both resources show a relevant potential as antioxidant stabilisers, presenting thymol and proanthocyanidins (PA) as active substances with further desired properties (Zeid et al. 2019).

The profile of requirements for stabilising additives includes antimicrobial properties discussed in the following. *Galanthus transcaucasicus* extracts are known to be effective against different microorganisms, including *Escherichia coli* and *Staphylococcus aureus* as tested by disc diffusion; thus, it is reasonable to assume similar properties of the *Galanthus* species tested in the present study. Amongst the tested bacteria, the gram-negative bacterium *E. coli* is inhibited at the most, while *S. aureus* is comparatively little affected (Karimi et al. 2018). AEH extract, in contrast, is notably more effective against *S. aureus* than against *E. coli*, presenting a considerable antimicrobial effect against the gram-positive bacterium as tested by the modified JIS Z 2801:2010 test procedure (Havelt et al. 2019; JIS Z 2801). For TV essential oils, similar results are found in literature as they are effective against several bacteria including *S. aureus* and *E. coli* with gram-negative bacteria being more vulnerable (Stahl-Biskup and Venskutonis 2004; Imelouane et al. 2009). In general, the basic requirement of providing antimicrobial effects is met by all three bioresources.

The study at hand presents findings on different snowdrop species with regard to their tocopherol content, UV absorbance and antioxidant capacity to evaluate a possible substitution of petrol-based stabilisers in packaging materials. Thus, the four species *Galanthus plicatus* (G pl), *G. elwesii* (G el), *G. nivalis* (G ni) and *G. gracilis* (G gr) were analysed. For this purpose, leaf extracts of the four species were prepared as both individual pretests and literature indicate that *Galanthus* leaves are the most potent plant fraction for obtaining antioxidant extracts (Bati Ay et al. 2018). While the study focuses on new results on *Galanthus*, it also places the results into context with new and recently published results on *T. vulgaris* and *A. hippocastanum* (Havelt and Schmitz 2018; Havelt et al. 2019).

4.2 Materials and Methods

4.2.1 Chemicals and Instrumentation

For the determination of UV absorbance and TAC, a Lambda 25 dual-beam spectral photometer by Perkin Elmer (Waltham, MA, USA) was used. GC-MS measurements were conducted using an Agilent 8890 GC System, coupled with an Agilent 5977B GC/MSD (Santa Clara, CA, USA). For extraction and analysis, heptane and dichloromethane were provided by Merck KGaA (Darmstadt, Germany) while β-carotene and linoleic acid were provided by Alfa Aesar (Thermo Fisher GmbH, Kandel, Germany) and Acros Organics (Fair Lawn, NJ, USA), respectively. Sigma-Aldrich (St. Louis, MO, USA) supplied both α-tocopherol and α-tocopherol acetate.

4.2.2 Sample Preparation and Extraction

Galanthus nivalis, *G. elwesii*, *G. plicatus* and *G. gracilis* samples were grown and identified by scientific staff of Heinrich-Heine-Universität Düsseldorf in Meerbusch-Ilverich on field (loamy meadow soil). The fresh leaves were frozen using liquid nitrogen directly after collecting. For analysis, the samples were freeze-dried and ground using a ball mill. Afterwards, 100 mg material per sample was gradually extracted by adding 0.6 mL heptane before thoroughly mixing and centrifuging the samples. After collecting the supernatant, the process was repeated two more times, combining the supernatants in a volumetric flask which was brought up to 2 mL with the solvent. After filtration, the extracts were analysed using different methods.

Comparable extraction setups have been used for the extraction of TV leaves and AEH seed coats. Detailed information on TV and AEH sample preparation, extraction and analysis is given in literature (Havelt and Schmitz 2018; Havelt et al. 2019).

4.2.3 Determination of UV Absorbance

The UV/Vis spectra of different extracts were recorded in the range of 260 – 800 nm. The results are given in relative absorbance units, taking the applied dilutions into account.

4.2.4 Determination of Total Antioxidant Capacity (TAC) via β-carotene assay

The TAC is determined by a photometrical assay using the discolouration of β-carotene in absence of further antioxidants as described in literature (Chevolleau et al. 1992; Matthes and Schmitz-Eiberger 2009). However, to improve the detection for the present samples, the amount of sample used for the assay was decreased to 20 µL. This deviation is taken into account for the calculation of results. The discolouration was observed at the wavelength 470 nm; the results are expressed in milli-extinction units per gram of dried mass (mE g^{-1} DM). The statistical significance of results was determined via Tukey test.

4.2.5 Determination of tocopherol content by Gas Chromatography – Mass Spectrometry (GC-MS)

For GC-MS measurements, α-tocopherol acetate was solved in acetone to create an internal standard solution (concentration: approx. 0.25 g L^{-1}) which is diluted by the extract samples (1:10 v/v). The concentration of α-tocopherol in the samples was evaluated on the basis of combined external and internal calibration. For analysis, an HP-5MS UI column was used (30 m x 0.25 mm; 0.25 µm film thickness; Agilent, Santa Clara, CA, USA). 1 µL of sample solution was injected, and the oven was heated from initial 50 °C to 325 °C (10 °C min^{-1}), holding the final temperature for 30 min. The statistical significance of results was determined via Games-Howell test.

4.3 Results

4.3.1 UV absorbance

The UV absorbances of different extracts are given in **Figure 14**. All four extracts showed a significant absorbance in the UV range. Smaller absorbance values were observed in the visible range, particularly in the lower wavelengths adjoining the UV range. Observed maxima were at ca. 338 nm, 435 nm and 673 nm. Below 360 nm, G pl extracts show the strongest absorbance, followed by *G. nivalis*, *G. gracilis* and *G. elwesii* extracts. However, *G. nivalis* extracts showed the highest absorbance in the visible range, followed by *G. elwesii*, *G. gracilis* and *G. plicatus* extracts. Overall, the different extracts show comparable spectra - particularly G el / G ni and G gr / G pl - with relevant deviations. All *Galanthus* extracts showed higher UV absorbances than solutions of synthetic antioxidants BHT and BHA (concentration: 1 mg mL^{-1}). For both substances, relevant

absorbances were observed in a part of the UV range only (up to approx. 320 nm), presenting maxima at 275 nm (BHT) and 292 nm (BHA).

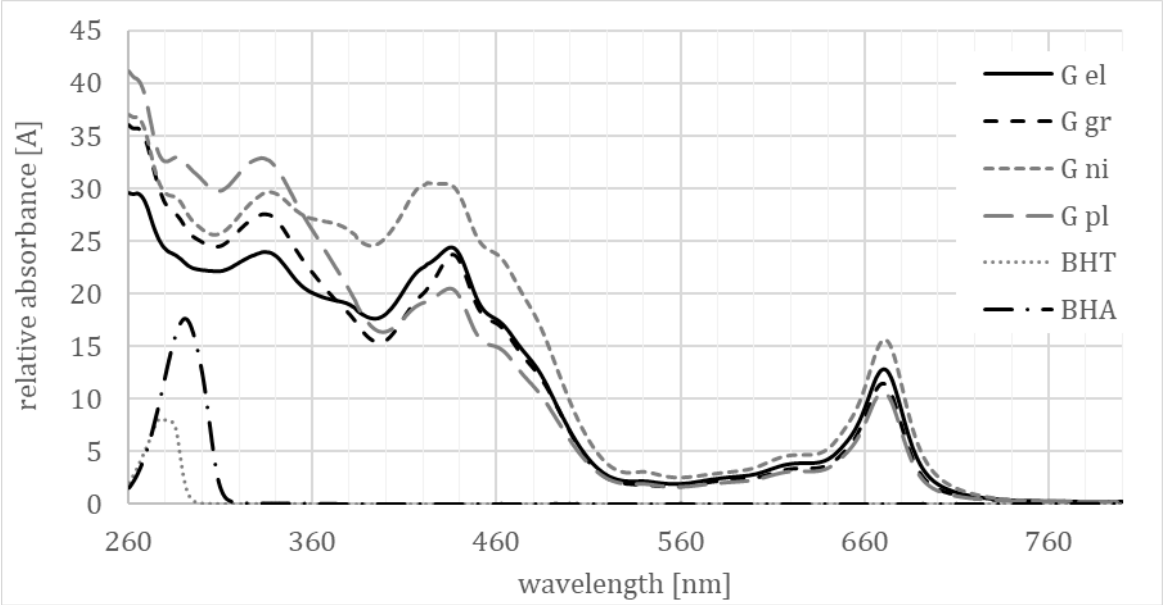

Fig. 14 *UV/Vis absorbance spectra of four different snowdrop leaf extracts (Galanthus plicatus, G. elwesii, G. nivalis, G. gracilis). The absorbance is given in relative absorbance units, taking different dilutions into account. Extractions: n=4, measurements: n=2. G: Galanthus; el: elwesii; gr: gracilis; ni: nivalis; pl: plicatus; BHT: butylated hydroxytoluene; BHA: butylated hydroxyanisole. BHT/BHA: concentration 1 mg mL^{-1}, data reproduced from (Havelt et al. 2019)*

4.3.2 Total antioxidant capacity

As depicted in **Figure 15**, all four snowdrop extracts showed significant antioxidant effects that are traced to lipophilic antioxidants present in the dried leaves. The least antioxidant effects were observed with G pl extracts (83.6 mE g^{-1} DM) while G gr and G ni extracts showed a comparable, but increased TAC of 114.8 mE g^{-1} DM and 123.4 mE g^{-1} DM, respectively. The most effective extract was based on G el leaves, resulting in a TAC of 241.3 mE g^{-1} DM. For TV heptane extracts, the mean TAC was 36.2 mE g^{-1} DM.

4.3.3 Tocopherol content

As shown in **Figure 16**, GC-MS analysis verifies that all four extracts contained α-tocopherol while not containing other vitamin E derivates. G gr, G ni and G pl leaves contained comparable amounts of α-tocopherol (1.52 - 1.65 mg g^{-1} DM). G el leaves presented a significantly increased α-tocopherol content of 2.07 mg g^{-1} DM.

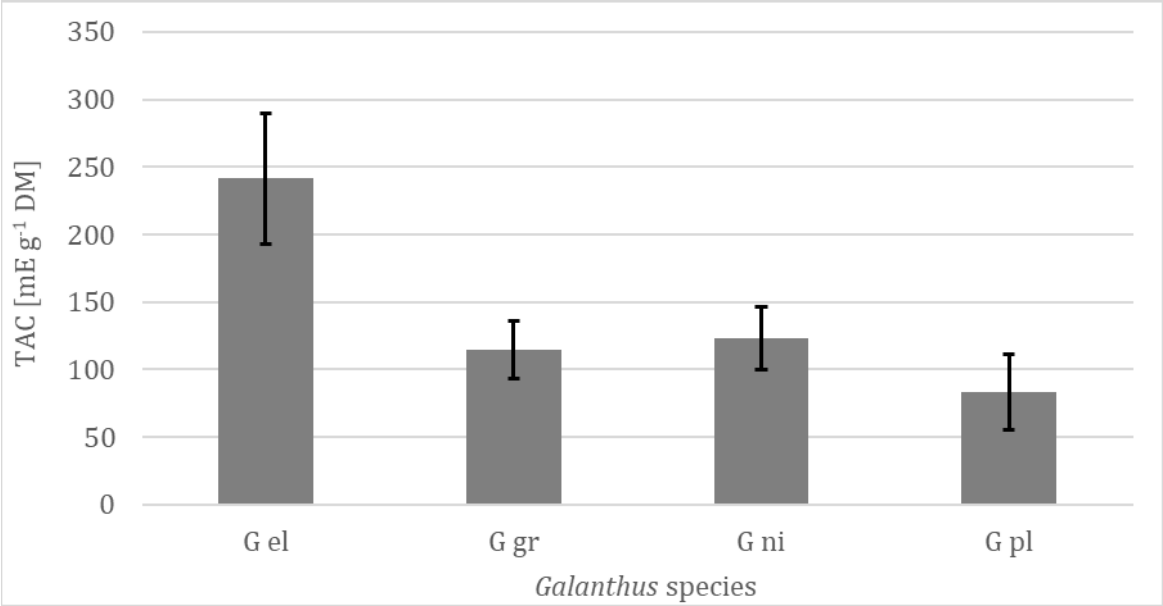

Fig. 15 *Total antioxidant capacity (TAC) of four lipophilic extracts of different freeze-dried snowdrop leaves (Galanthus plicatus, G. elwesii, G. nivalis, G. gracilis). Extractions: n=4, measurements: n=2. Standard deviation indicated by error bars. Significant difference proven for G el according to Tukey test (α < 0.05). mE: milli-extinction units; DM: dried sample mass; G: Galanthus; el: elwesii; gr: gracilis; ni: nivalis; pl: plicatus*

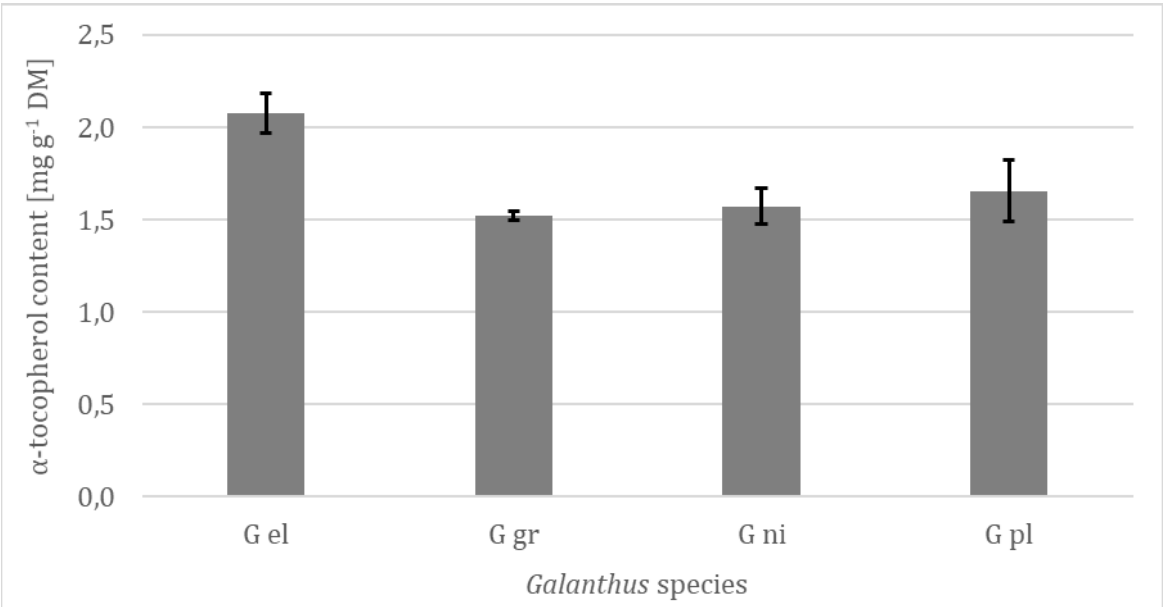

Fig. 16 *α-Tocopherol content of four lipophilic extracts of different freeze-dried snowdrop leaves (Galanthus plicatus, G. elwesii, G. nivalis, G. gracilis). Extractions: n=4, measurements: n=2. Standard deviation indicated by error bars. Significant difference proven for G el according to Games-Howell test (α < 0.05). DM: dried sample mass; G: Galanthus; el: elwesii; gr: gracilis; ni: nivalis; pl: plicatus*

4.4 Discussion

4.4.1 UV absorbance

In general, all *Galanthus* leaf extracts show similar UV/Vis spectra with a relevant absorbance in the UV range. Leaf extracts also show significant absorbances in the visible range in contrast to *Galanthus* flower extracts as described in literature (Stanciu et al. 2010). G pl leaf extracts shows the highest UV absorbance among the tested *Galanthus* species; for higher, but less relevant wavelengths regarding the prevention of photodegradation, G ni shows the highest absorbance. However, *Galanthus* extracts generally show a limited UV absorbance in relation to TV and AEH extracts which exceed G pl extract's maximum UV absorbance by a factor of approx. 3 and 8.5, respectively (Havelt and Schmitz 2018; Havelt et al. 2019). In comparison to common synthetic stabilisers butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) solved in methanol with a concentration of 1.0 mg mL^{-1}, the G pl extract shows an approx. 5- and 2-fold higher UV absorbance for BHT and BHA, respectively. Therefore, about 5 mg BHT or 2 mg BHA could be replaced by 1 mL G pl leaf extract with regard to UV absorbance. For TV and AEH, the theoretical BHT and BHA equivalent is approx. 15 mg BHT / 7 mg BHA per mL TV extract and 43 mg BHT / 20 mg BHA per mL AEH extract (Havelt and Schmitz 2018; Havelt et al. 2019). AEH and TV extracts thus show to be very promising plant-based photostabilising agents. Even the relatively weak *Galanthus* leaf extracts are strong photostabilisers compared to the common stabilisers BHT and BHA.

4.4.2 Total antioxidant capacity

All tested *Galanthus* leaf extracts present a notable TAC in the lipophilic test environment. Within the results, G el extracts stand out by showing a particularly high TAC, roughly doubling the TAC reached by G pl, G ni and G gr extracts. Therefore, G el extracts are distinctly better suited for antioxidant applications in lipophilic environments. However, as indicated by performing an ABTS radical cation scavenging assay as described in literature (Havelt et al. 2019) with a standard solution of the main antioxidant extract component α-tocopherol, the antioxidant potential is not reached in hydrophilic surroundings. The lipophilic nature of α-tocopherol suggests that the antioxidant reaction mechanism of α-tocopherol is impeded when taking place in a polar environment.

Therefore, it is necessary to evaluate the antioxidant potential of extracts in the context of hydro- or lipophilic surroundings, complicating the comparison of *Galanthus*, TV and AEH extracts' TACs. Still, a higher TAC in lipophilic surroundings can be reported for *Galanthus* extracts in contrast to TV extracts: G el extracts reach a lipophilic TAC which is about 6.7 times higher than the one reached by TV extracts while AEH extracts with its polar proanthocyanidins do not show a comparable TAC

in lipophilic surroundings at all. However, TV extracts are still notable hydrophilic antioxidants, exceeding the polar TAC of AEH extracts by a factor of approx. 2 when applying comparable extraction techniques. When applying optimised extraction techniques for AEH, the polar TAC is comparable to the ones of TV extracts or even exceeds them by a factor of approx. 2 (Havelt and Schmitz 2018; Havelt et al. 2019). Thus, *Galanthus* and AEH extracts are deemed to be the best choice for lipophilic and polar surroundings, respectively, while TV extracts with its main antioxidant ingredient thymol are all-rounders which are suitable for both lipophilic and hydrophilic environments. In comparison to synthetic stabilisers, approx. 6 or 10 mL of AEH extracts have to be used to compensate 1 mg BHT or BHA, respectively. For TV extracts, approx. 10 or 16 mL should be used for the same purpose. However, extracts could also be further enriched to obtain greater antioxidant effects per volume.

4.4.3 Tocopherol content

To evaluate the observed antioxidant effects, GC-MS is used to identify the main components of the extracts. One of the major ingredients, α-tocopherol (vitamin E), is a compound known for several positive effects, including its *in vivo* antioxidant efficacy (Fryer 1992). The concentration of α-tocopherol found in the different extracts is given in Figure 3. G el leaves contain a high amount of α-tocopherol, exceeding the amounts in other species' leaves by approx. 30 %. These results match the TAC results and support the assumption of α-tocopherol being the main cause for a high TAC in *Galanthus* extracts. However, the rise of TAC of G el extracts in context with the other extracts is relatively high when compared to the rise of the tocopherol content of G el extracts, again in context with the remaining extracts. This relatively high TAC compared to the increase of α-tocopherol might be based on a different synergistic system in G el leaves. Furthermore, a non-linear correlation of α-tocopherol concentration and antioxidant efficacy could be responsible.

The amount of α-tocopherol in *Galanthus* DM is notably lower than the amount of the main active substance thymol in TV DM (7.5 - 8.5 mg g^{-1} DM). However, the TAC of lipophilic TV extracts are higher for *Galanthus* extracts, showing that Galanthus extracts have a considerably high relative TAC in lipophilic surroundings in comparison with TV extracts, presumably due to its main antioxidant compound α-tocopherol.

4.5 Conclusion

The results discussed before indicate that plants have to be considered a notable resource for sustainable stabilisers. However, the choice of a specific plant highly depends e.g. on its application, including the hydro- or lipophilic character of the packaging material to be stabilised, and the type

of packaged goods. For most applications, combinations of different extracts should be evaluated as a second extract based on another bioresource could overcome the possible deficiencies of the main extract. For instance, the addition of AEH extracts to *Galanthus* extracts could introduce the properties that *Galanthus* extracts lack, including antimicrobial effects on gram-positive bacteria, the UV absorbance and the antioxidant effects in a hydrophilic medium. Such additives could meet a wide majority of demands for bio-based stabilisers, making them a promising potential substitute for petrol-based additives.

In food packaging, such a substitution could result in an improved food safety as petrol-based stabilisers might pose a threat to the environment and to human health when migrating into packed food (Ito et al. 1985; Kahl and Kappus 1993). Bio-based stabilisers based on substances like α-tocopherol, which is allowed to be directly incorporated in food due to its harmlessness (Domke et al. 2005), or proanthocyanidins, which again are safe for use in food and particularly unlikely to migrate (Turck et al. 2017), could provide not only a more sustainable, but also a safer way to achieve desired packaging properties. As TV extracts are prepared from a plant used as a foodstuff itself, they are considered harmless as well. Further work based on this study is in planning, particularly focusing on formulation tests and analysing synergistic effects when combining stabilisers from different bioresources.

4.6 Acknowledgements

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4.7 Copyright

5. Coniferous woods (*Abies* div., *Picea* div.)

"Characterisation of bioactive ingredients in extracts of fresh and dried coniferous trees for the development of sustainable packaging materials" (Havelt et al. 2020)

Abstract

Background: Coniferous woods (*Abies nordmanniana* (STEV.) SPACH, *Abies procera* REHD, *Picea abies* (L.) H.KARST, and *Picea pungens* ENGELM.) could contain useful secondary metabolites to produce sustainable packaging materials, e.g., by substitution of harmful petrol-based additives in plastic packaging. This study aims to characterise the antioxidant and light-absorbing properties and ingredients of different coniferous wood extracts with regard to different plant fragments and drying conditions. Furthermore, the valorisation of used Christmas trees is evaluated.

Methods: Different drying and extraction techniques were applied with the extracts being characterised by determining the total phenolic content (TPC), total antioxidant capacity (TAC), and absorbance in the ultraviolet range (UV). Gas chromatography coupled with mass spectrometry (GC-MS) and an acid–butanol assay (ABA) were used to characterise the extract constituents.

Results: All the extracts show a considerably high UV absorbance while interspecies differences did occur. All the fresh and some of the dried biomass extracts reached utilisable TAC and TPC values. A simplified extraction setup for industrial application is evaluated; comparable TAC results could be reached with modifications.

Conclusion: Coniferous woods are a promising renewable resource for preparation of sustainable antioxidants and photostabilisers. This particularly applies to Christmas trees used for up to 12 days. After extraction, the biomass can be fully valorised by incorporation in paper packaging.

5.1 Introduction

The challenges in modern society are characterised by an urgent need to change towards a more sustainable community. One critical issue is the production and waste management of plastic products. Typically, the formulation of plastic products includes stabilising additives that improve properties like antioxidant or antimicrobial stability or photosensitivity, to promote a reasonable product shelf life and to create competitive products. Globally, 336.9 kt antioxidants were produced in 2007 (Wegmann et al. 2016). Most of those stabilisers are synthetic, petrol-based compounds and potentially harmful to the environment and human health, including the common stabilisers butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Ito et al. 1985; Kahl and Kappus 1993; Peltzer et al. 2009). Antioxidant and antimicrobial properties and absorbance of ultraviolet light (UV) are particularly relevant for stabilisers. Biobased alternatives to such stabilisers are severely underrepresented in research. The development of alternatives based on ecologically favourable biomasses to substitute common plastic packaging is strongly encouraged by the German Environmental Agency (Detzel et al. 2012). In previous studies, the biomasses of Common Thyme (*Thymus vulgaris* L.) and the fruits of the European Horse Chestnut (*Aesculus hippocastanum* L.) have been successfully examined by research groups for such applications (Havelt and Schmitz 2018; Havelt et al. 2019). The present study focuses on the immense potential of different coniferous woods for ecological additive production and furthermore investigates the suitability of both fresh Christmas trees and dried trees after use by individuals or companies.

Depending on the reference, between 23 and 30 million Christmas trees were sold in Germany in 2019 (Hauptverband der Deutschen Holzindustrie e.V. (HDH) 12/22/2019; Schutzgemeinschaft Deutscher Wald 2020). Those trees consist of 75% Nordmann firs (*Abies nordmanniana* (STEV.) SPACH (AN)), 15% blue spruces (*Picea pungens* ENGELM. (PP)), 3% noble firs (*Abies procera* REHD. (AP)), and other species (7%) (Schutzgemeinschaft Deutscher Wald 2020). In addition to these three species, the present study investigates the Norway spruce (*Picea abies* (L.) H. KARST (PA)), too. Typically, after failed sale or after usage as a Christmas tree, the biomass is composted or converted into energy (Agentur für Erneuerbare Energien 1/9/2015). However, direct material use of coniferous trees is promising as some of the critical properties for their use as additives are reported in the literature. In AN leaves, antioxidant compounds like ascorbate and α-tocopherol have been found while the essential oil obtained from the leaves shows considerable antimicrobial effects, including effects against, e.g., different *Bacillus* cultures, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and partly against *Staphylococcus aureus* (Bağcı and Dığrak 1996; Weber et al. 1996; Öncel et al. 2004; Charles 2013b). Substances with antimicrobial effects can also be found in PA and AP leaves with PA leaf extracts showing a slight antimicrobial effect against *S.*

aureus while essential oil prepared from AP leaves shows strong antimicrobial effects against, e.g., *S. aureus* as well as different *Bacillus*, *Streptococcus*, and *Staphylococcus* cultures (Kartnig et al. 1991; Rauha et al. 2000; Elmezughi et al. 2013). Regarding AN, not only the leaves but also the bark includes antioxidant phenolic acids and flavonoids, e.g., catechin isomers and gallic acid (Hafizoglu and Holmbom 1995). For PA, antioxidants like ascorbate are found in the leaf and bark extracts (Polle et al. 1990; Co et al. 2011).

In the present study, extracts of the aerial parts of the four listed coniferous trees are analysed with regard to their total antioxidant capacity (TAC), total phenolic content (TPC), UV absorbance, and chemical composition. These methods allow assessing the suitability of the extracts for application as biobased stabilisers for packaging materials, as described in the literature (Havelt et al. 2019). Where applicable, differences between leaves and wood are explored and characterised. Furthermore, the possibility of waste valorisation by obtaining biobased additives from used or unsold Christmas trees is evaluated. The extraction process is optimised to facilitate possible industrial adaptation.

5.2 Materials and Methods

5.2.1 Chemicals and Instrumentation

A Perkin Elmer Lambda 25 dual-beam spectral photometer was used for all photometrical measurements, including total antioxidant capacity (TAC), total phenolic content (TPC), acid–butanol assay (ABA), and the determination of UV absorbance. For the GC-MS analysis, an Agilent 8890 GC system is used, equipped with an Agilent HP-5MS UI column (30 m × 0.25 mm; 0.25 µm film thickness) and coupled with an Agilent 5977B GC/MSD. A laboratory beater (type “Valley”), a sheet forming unit (type “Rapid Köthen”), a universal testing unit, and a thickness gauge unit provided by the company Frank-PTI, Birkenau, Germany, were used for paper preparation and analysis.

2(3)-tert-butyl-4-methoxyphenol (BHA) and dipotassium hydrogen phosphate were purchased from Alfa Aesar (Karlsruhe, Germany), whereas 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), acetic acid, Trolox, N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), and 2,6-di-tert-butyl-4-methylphenol (BHT) were purchased from AppliChem GmbH (Darmstadt, Germany), Bernd Kraft (Duisburg, Germany), Cayman chemical Company (Ann Arbor, MI, USA), CS-Chromatographie Service GmbH (Langerwehe, Germany), and ThermoFisher (Waltham, MA, USA), respectively. Ammonium iron (III) sulphate dodecahydrate, butan-1-ol, concentrated hydrochloric acid, hexane, methanol, and acetone were obtained from VWR International, Darmstadt, Germany.

Folin–Ciocalteu phenol reagent, hydrogen peroxide, potassium dihydrogen phosphate, sodium hydroxide, and sodium acetate were purchased from Merck KGaA, Darmstadt, Germany.

5.2.2 Sample Preparation and Extraction

To minimise sampling errors, branches from several, randomly chosen trees of all four species were collected from Hof Große Wöstmann, Rinkerode, Germany. The branches of each species were cut into smaller fragments and stored at −20 °C. As acetone has proven to be the most potent extractant in pre-tests, while resulting in the most comparable results for all species (**Figure A2**), it was used to prepare all extracts featured in the study. However, a mixture of acetone and water (1:1 (*v*/*v*)) could be used in suited future applications as it provides similar antioxidant properties. Moreover, more environmentally friendly solvents are applied in this case.

For the in-house established extraction ("grinding extraction"), 600 mg of the sample material was ground using a ball mill applying liquid nitrogen for cooling. Afterwards, the sample material was extracted with 6 mL acetone before centrifuging. The supernatant was collected, filled up to 10 mL, and filtered.

For the passive extraction applied during extraction optimisation, approx. 600 mg of the sample material was chopped and filled into a vessel. The active ingredients were extracted with 15 mL acetone and stored at room temperature under exclusion of light. After varying periods of storing, the supernatant was collected and filtered.

5.2.3 Determination of UV Absorbance

The UV/Vis spectra of the different extracts were recorded in the range of 260–800 nm. For these measurements, it was necessary to dilute the extracts to varying extents to meet the instrument's linear range. The results are given in relative absorbance units (rAU), taking applied dilutions into account. In a previous study, a similar UV/Vis analysis of plant extracts was conducted successfully (Havelt et al. 2019).

5.2.4 Determination of Total Antioxidant Capacity (TAC) via ABTS Radical Cation ($ABTS^{\bullet+}$) Scavenging Capacity Assay (ABTS Assay)

The TAC is determined via a modified ABTS Assay (Erel 2004). The assay is performed in accordance with the literature (Havelt et al. 2019). Discolouration of ABTS radical cations is observed at λ = 660 nm with two blank samples per assay. Results are interpreted with regard to external calibration using Trolox solutions; therefore, the results are given in Trolox equivalents per mg of extracted sample material ($mg \cdot Teq \cdot mg^{-1}$). Where applicable, the type of sample material is specified by FM (fresh mass) or DM (dried mass).

5.2.5 Determination of Total Phenolic Content (TPC) via Folin-Ciocalteu Assay

The total phenolic content (TPC) was determined via the Folin–Ciocalteu assay (Singleton et al. 1999; Matthes and Schmitz-Eiberger 2009) and performed in a modified way as described in the literature with the colour change observed at λ = 720 nm (Havelt et al. 2019). Blank samples are measured at least every 10 samples. For interpretation of the results, an external calibration with gallic acid is prepared; thus, the results are given in gallic acid equivalents (GAE) per mg of extracted sample material (if applicable, specified as FM or DM).

5.2.6 Acid-Butanol Assay (ABA)

The acid–butanol assay (ABA) is based on the literature and specifically proves the presence of proanthocyanidins (Hagerman 2011). The assay was conducted in a modified way, according to the literature, measuring colour changes at λ = 550 nm (Havelt et al. 2019). As no calibration is prepared, the ABA results are interpreted in a semi-quantitative way only.

5.2.7 Gas Chromatography Coupled with Mass Spectrometry (GC-MS)

The GC-MS analysis was conducted to obtain semi-quantitative information on the composition of the coniferous wood extracts. For analysis of polar, non-volatile substances, the extracts were derivatised by mixing 50 µL of extract with 50 µL N-methyl-N-(trimethylsilyl)trifluoracetamide (MSTFA) before incubating the mixture at 80 °C for 15 min, resulting in the hydroxyl groups of the analytes being replaced by trimethylsilyl groups. Then 1 µL of the mixture was injected into the GC-MS apparatus. Afterwards, the oven was heated from an initial 50 °C to 325 °C by applying a heating rate of 10 °C·min^{-1} before holding the final temperature for 30 min.

5.2.8 Preparation and Analysis of the Paper Sheets

For the feasibility analysis of the paper packaging prepared from coniferous wood aerial parts after extraction with acetone, different sheets of paper were produced and their thickness, tensile strength, and elongation at break were analysed. The sample material remaining after passive extraction with acetone for 7 days according to **Section 5.2.3.2** was dried at 60 °C until a constant residual moisture was reached. Afterwards, the sample material was ground using a cutting mill and sieved through a 1 mm sieve. For sheet preparation, the milled sample material was mixed with pinewood pulp in water in the ratio of 1:10 (*w*/*w*). The mixture was transferred to a laboratory beater until 30 Schopper Riegler degrees (°SR) was reached (determination according to (DIN EN ISO 5267-1:2000)). The resulting pulp was used to prepare sheets with different grammages (60, 80, 120, 200, 300, and 400 g·m^{-2}) and a diameter of 200 mm using a sheet forming unit according

to (DIN EN ISO 5269-2:2004). Afterwards, the thickness of the sheets was assessed, and the tensile strength and elongation at break were analysed (ISO 1924-2:2008).

5.2.9 Statistical Interpretation

Statistical evaluation of the appropriate results was conducted by applying the Games–Howell test ($\alpha \leq 0.05$) for comparison of different value groups with regard to significant differences unless otherwise stated. For this purpose, the software IBM SPSS version 26 was used.

5.3 Results and Discussion

5.3.1 General Suitability

5.3.1.1 UV/Vis Absorbance

As depicted in **Figure 17**, all four coniferous wood extracts show an absorbance maximum at approx. 265–270 nm, depending on the wood species. The PP extract shows the highest absorbance with approx. 304 rAU, followed by the AP, PA, and AN extracts with maximum absorbances of 287, 265, and 257 rAU, respectively. The absorbance declines until 350 nm for all four species with all extracts showing little absorbance in the low visible range up to approx. 500 nm and in the range of 655–685 nm. Until a wavelength of 800 nm, no other relevant absorbance is observed and therefore not presented.

All four coniferous wood extracts show a comparable, considerable absorbance in the UV-B and UV-C range, while no relevant absorbance in the visible range is observed. Even the AN extract, which shows a significantly reduced UV absorbance at maximum, is considered a potential worthwhile resource for photostabilisers due to the similar course of all extracts and their comparably minor differences with a factor of approx. 1.2 between the highest and lowest absorbing extracts. Due to the low absorbance in the visible range, the extracts have a limited influence only on the colour of the final product, which is favourable for most applications.

The UV spectra obtained for all wood species is comparable to the ones obtained from *Aesculus hippocastanum* (AEH) seed coats (maximum of AEH extracts: 275 nm) while showing a reduced maximum relative absorbance (coniferous woods: approx. 257–304; AEH seed coats: approx. 350) (Havelt et al. 2019). However, the AEH extraction was improved compared to before with respect to plant fractions and extraction duration. Common antioxidant stabilisers BHT and BHA absorb in the UV-B and UV-C range only, showing maxima at 275 and 291 nm, respectively. Thus, a small shift to higher wavelengths can be observed in comparison to coniferous wood extracts. The extracts surpass the maximum absorbance of the BHT and BHA solutions with a concentration of 1.0 $g \cdot L^{-1}$ by

the factors of approx. 33–40 and 15–18, respectively (depending on the coniferous wood species). Therefore, regarding UV absorbance, 1 mL of extracts obtained from popular AN could substitute approx. 33 mg of BHT or 15 mg of BHA; for the most potent UV absorbing coniferous wood, PP, even 40 mg of BHT or 18 mg of BHA could be substituted.

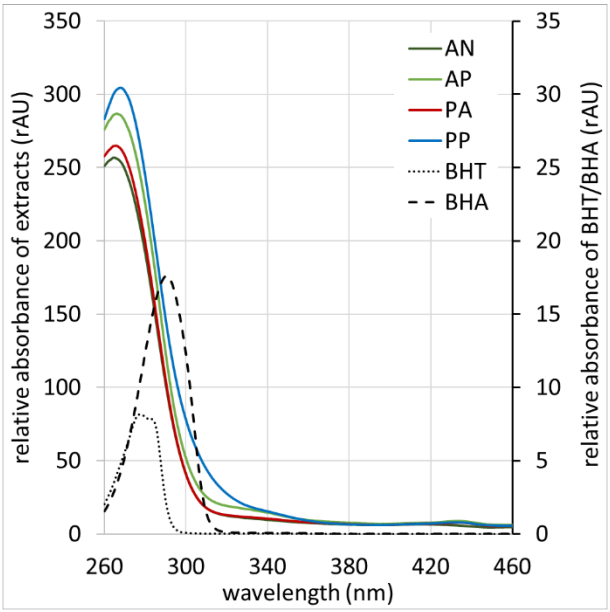
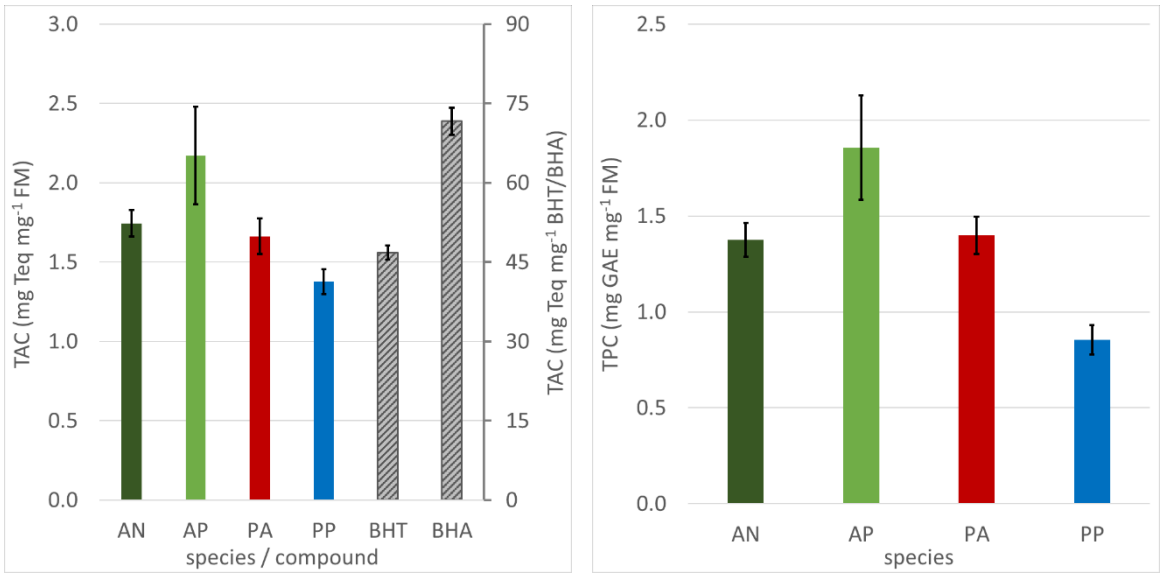


Fig. 17 *Average UV/Vis absorbance of the aerial part extracts of different coniferous woods displayed on the primary ordinate in relative absorbance units (rAU). Extractions in triplicate; measurements in duplicate. Statistical evaluation of the extracts is based on comparison of the absorbance in the respective maximum according to the Games–Howell test ($\alpha \leq 0.05$); significant difference proven for all extract pairs except for AP/PA and AP/PP. Secondary ordinate (black): average UV/Vis absorbance of the BHT and BHA solutions (1.0 g·L^{-1} in methanol, measurement in triplicate). AN: Abies nordmanniana; AP: Abies procera; PA: Picea abies; PP: Picea pungens; BHT: butylated hydroxytoluene; BHA: butylated hydroxyanisole. BHT/BHA data reproduced from (Havelt et al. 2019).*

5.3.1.2 TAC and TPC

As presented in **Figure 18** (**a**), all four coniferous wood extracts show a relevant TAC with AP extracts appearing to be the most potent ones. While no significant differences are proven between the AN, AP, and PA extracts, the PP extracts show a significantly lower TAC of 1.4 mg·Teq·mg^{-1} FM. Generally, those interpretations are applicable to TPC results as well apart from the PA and PP extracts not showing a significant difference (**Figure 18** (**b**)). As the extract constituents indicated by the Folin–Ciocalteu assay and the ABTS assay overlap, the TPC results are expected to roughly confirm the results obtained by the TAC determination. A considerable antioxidant effect of the different extracts is supported by the literature as antioxidant compounds like ascorbate and α-tocopherol have been found in PA and AN leaf extracts (Polle et al. 1990; Öncel et al. 2004).



(a) *(b)*

Fig. 18 *(**a**) Average total antioxidant capacity (TAC) of the synthetic antioxidants BHT and BHA and aerial part extracts of different coniferous woods. Primary ordinate: Extracts, given in colour; extractions in triplicate; measurements in duplicate; secondary ordinate: BHT/BHA 1.0 g·L^{-1} methanol solutions, given in grey, six measurements; data reproduced from (Havelt et al. 2019). Standard deviation indicated by error bars. Statistical evaluation of the extracts according to the Games–Howell test (α ≤ 0.05); significant difference proven for extract pairs AN/PP, AP/PP, and PA/PP. Teq: Trolox equivalents; FM: fresh mass; AN: Abies nordmanniana; AP: Abies procera; PA: Picea abies; PP: Picea pungens; BHT: butylated hydroxytoluene; BHA: butylated hydroxyanisole.*
*(**b**) Average total phenolic content (TPC) of the aerial part extracts of different coniferous woods. Extractions in triplicate; measurements in duplicate; standard deviation indicated by error bars. Statistical evaluation of the extracts according to the Games–Howell test (α ≤ 0.05); significant difference proven for extract pairs AN/PP and AP/PP. GAE: gallic acid equivalents; FM: fresh mass; AN: Abies nordmanniana; AP: Abies procera; PA: Picea abies; PP: Picea pungens.*

In comparison, the synthetic antioxidants BHT and BHA in a concentration of 1 g·L^{-1} show a TAC approx. 30 times higher than the ones of coniferous wood extracts. Thus, approx. 30 mL of extracts could theoretically substitute 1 mg of BHT or BHA for antioxidant stabilisation purposes. However, this comparison is based on extracts prepared to enable analytical comparison instead of optimising the extraction regarding maximum efficiency. For the AEH extracts analysed in a previous study, such an optimisation has been successful while a comparable TAC has been observed for extracts obtained focusing on analytical characterisation (Havelt et al. 2019).

With respect to the UV absorbance results, coniferous wood extracts are thus considered a potentially relevant source of stabilisers for use in plastic products.

5.3.2 Analysis of Waste Valorisation: Utilisation of Christmas Trees after Private Usage

To evaluate the potential of used indoor Christmas trees as raw material for the ecological production of stabilisers, branches of all four species were placed on trays to prevent unwanted loss of sample material, e.g., by leaves falling off. On these trays, the sample branches were dried at room temperature for various periods up to 32 days. That way, realistic surroundings are applied

to the tree branches, resembling a situation of felled Christmas trees set up in a private household without watering. Afterwards, the branches were extracted and analysed.

Due to a considerably reduced outdoor temperature, it is anticipated that the usage of cut outdoor Christmas trees, e.g., set in private yards or in front of public buildings, would result in increased TAC and TPC values, ranging between the ones obtained from the fresh branches extracts and the extracts obtained during this section.

5.3.2.1 UV/Vis Absorbance

As depicted in **Figure 19**, all four species show a decreasing UV absorbance over time, starting at a maximum absorbance of 257–304 rAU as discussed before. However, the decrease rate is varying for the different species. While the AN, PA, and PP extracts show a decrease of approx. 50 rAU during the first 12 days of drying, the AP extracts decrease approx. twice as much. For most species, the decrease rate is high at the beginning while slowing down for longer drying periods, resulting in the UV spectra of 25 d extracts and 32 d extracts not significantly differing. However, for the PP extracts, no approximation of UV spectra with longer drying periods is observed. This is supported by the PP extracts of the 25 d and 32 d samples showing a significant difference in maximum absorbance. Generally, the PP extracts show the highest absorbance values of all four species for each drying period. In contrast to other species' extracts, the PP extracts show a relatively small relative decrease with 57% of the maximum absorbance retained after 32 days of drying (other species' extracts: 48–50%).

Overall, the PP extracts show the highest UV absorbances after drying the biomass for all tested periods of up to 32 days. PP branches are thus seemingly less prone to loss of UV absorbing substances due to drying. AP branches in contrast are particularly sensitive about drying with regard to UV absorbance as the maximum absorbance of the extracts is considerably lower even after the shortest tested drying period of 12 days.

However, all extracts show a significantly higher UV absorbance than solutions of the synthetic stabilisers BHT and BHA (1.0 $g \cdot L^{-1}$; UV spectra plotted in Figure 17). Even the least absorbing extracts of the PA branches dried for 32 days, and the analogously produced extracts from AN and AP thus theoretically substitute circa 7.5 mg BHA or 15.0 mg BHT regarding UV absorbance. Therefore, both fresh and dried coniferous wood could serve as a relevant source of photostabilisers.

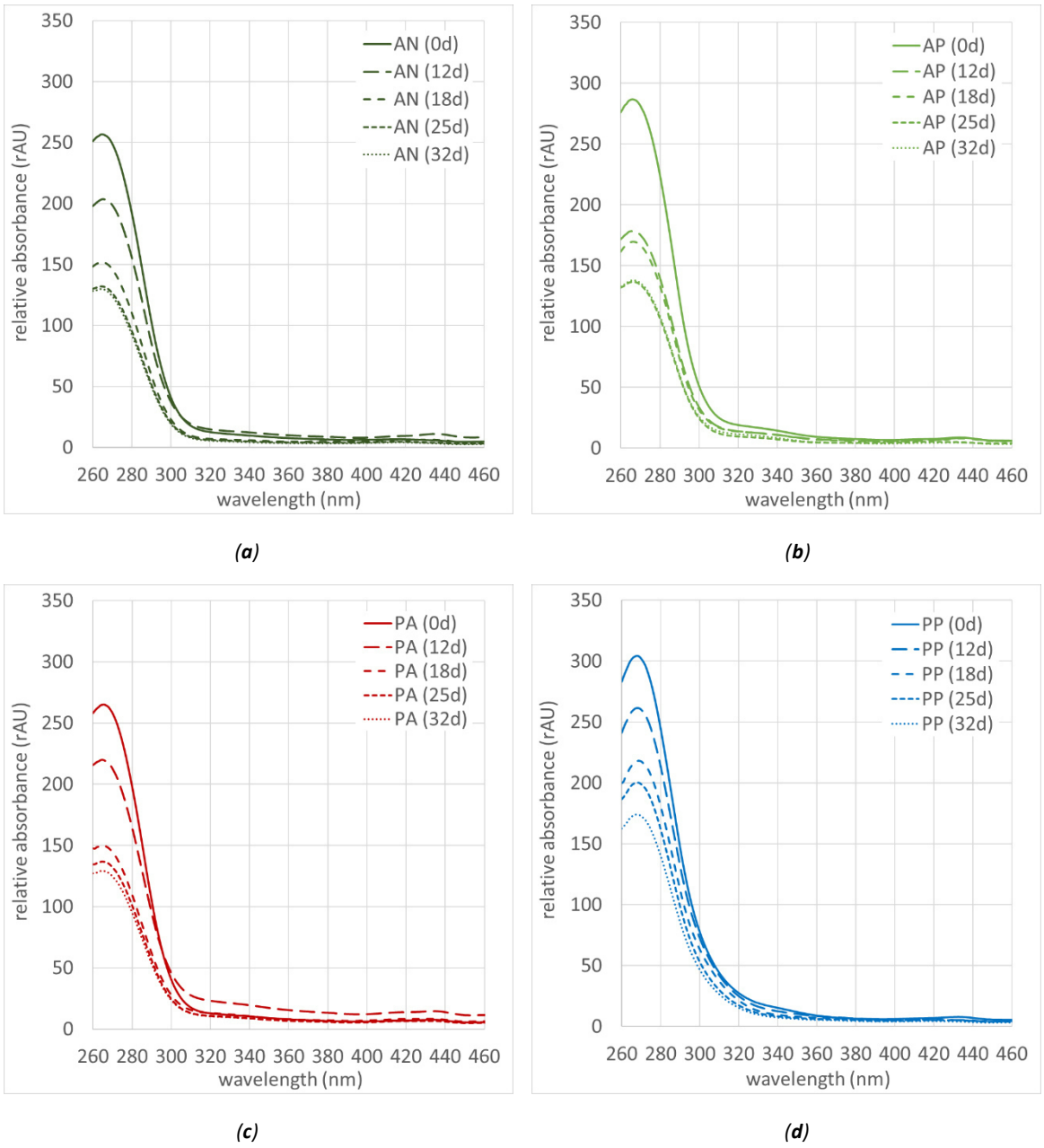


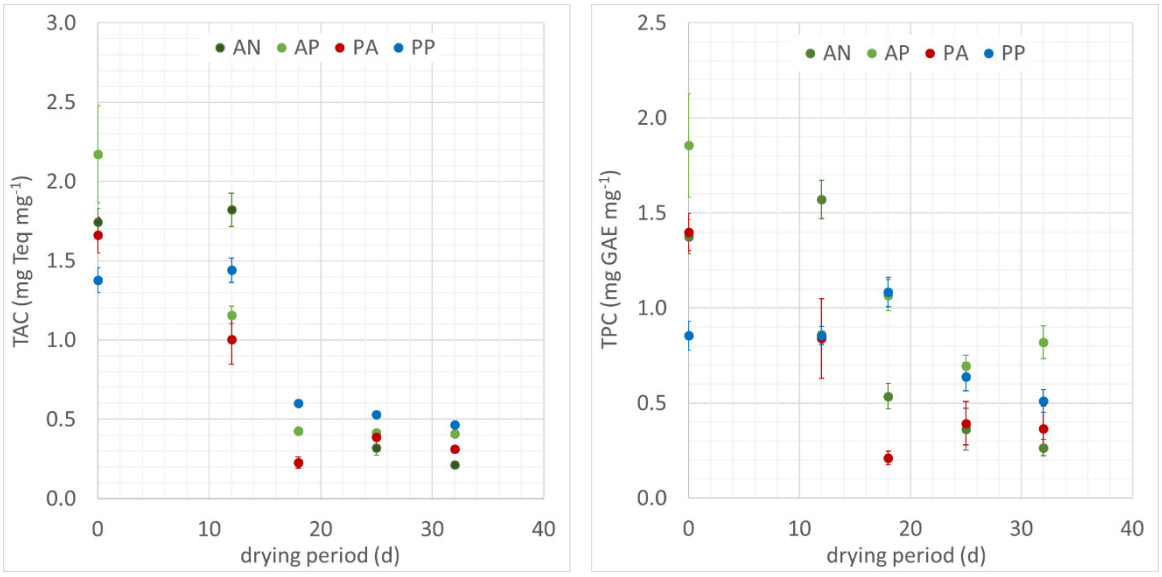
Fig. 19 *Average UV/Vis absorbance of the aerial part extracts of different coniferous woods displayed in relative absorbance units (rAU) after drying for different periods, given in days (d). Results are corrected by weight loss. Extractions in triplicate; measurements in duplicate. Statistical evaluation of the extracts based on comparison of absorbance in the respective maximum according to the Games–Howell test (α ≤ 0.05).*
*(**a**): Abies nordmanniana (AN); significant difference proven for all data pairs except for 18 d/25 d and 25 d/32 d.*
*(**b**): Abies procera (AP); significant difference proven for all data pairs except for 12 d/25 d and 25 d/32 d.*
*(**c**): Picea abies (PA); significant difference proven for all data pairs except for 0 d/18 d, 12 d/18 d, 18 d/25 d, 18 d/32 d, and 25 d/32 d.*
*(**d**): Picea pungens (PP); significant difference proven for data pairs 0 d/25 d, 0 d/32 d, and 25 d/32 d.*

5.3.2.2 TAC and TPC

The total antioxidant capacity remains stable for at least 12 days of drying whole AN and PP branches at room temperature, as shown in **Figure 20** (statistical evaluation according to Tukey test, α ≤ 0.05). For the AP branches, the TAC values significantly decrease during the first 12 days

of drying. The antioxidant capacity drops to a comparably stable minimum of approx. 0.2–0.6 mg·Teq·mg^{-1} after max. 18 days of drying with relatively small interspecies differences. This trend is clearly observable with TAC and approved by determination of the TPC with small deviations; primarily, the TPC values show a slower decrease over time and a higher scattering of values.

Thus, using fresh biomass for secondary metabolite extraction is preferred as expected. However, a storage at room temperature is acceptable for at least 12 days particularly for AN and PP trees as they do not show a significant decrease in antioxidant capacity during this period. After a maximum of 18 days, all species' extracts result in a limited amount of antioxidant capacity only, presumably due to oxidative stress occurring during the long-term drying process (Ramachandra Reddy et al. 2004; Sharma and Dubey 2005). Thus, watering the ornamental branches or Christmas trees used for extraction of antioxidants could expand the acceptable period of usage prior to extraction.



(a) ***(b)***

Fig. 20 *(**a**) Average total antioxidant capacity (TAC) of the aerial part extracts of different coniferous woods after drying for different periods, given in days (d). Results are corrected by weight loss. Extractions in triplicate; measurements in duplicate. Standard deviation indicated by error bars. AN (18 d) covered by AP (18 d). Teq: Trolox equivalents; AN: Abies nordmanniana; AP: Abies procera; PA: Picea abies; PP: Picea pungens.*
*(**b**) Average total phenolic content (TPC) of the aerial part extracts of different coniferous woods after drying for different periods, given in days (d). Results are corrected by weight loss. Extractions in triplicate; measurements in duplicate. Standard deviation indicated by error bars. AP (12 d) covered by PP (12 d). GAE: gallic acid equivalents; AN: Abies nordmanniana; AP: Abies procera; PA: Picea abies; PP: Picea pungens.*

5.3.2.3 Extraction Optimisation

Additionally, a simplified extraction technique was applied to the coniferous wood samples to evaluate a possible application that is easier to adapt in practice. The most promising yet realistic scenarios of Christmas tree purposes are considered (fresh sample mass and sample mass dried at room temperature for 12 days). For this approach, the biomass is roughly chopped and brought

into contact with the solvent for longer time periods of 24 h to 21 days instead of performing the more exact process of conducting analytical cryoextraction on milled samples within several minutes as it is done for prior analyses. To evaluate the extraction outcome, the TAC values were determined in triplicate (**Figure A3**).

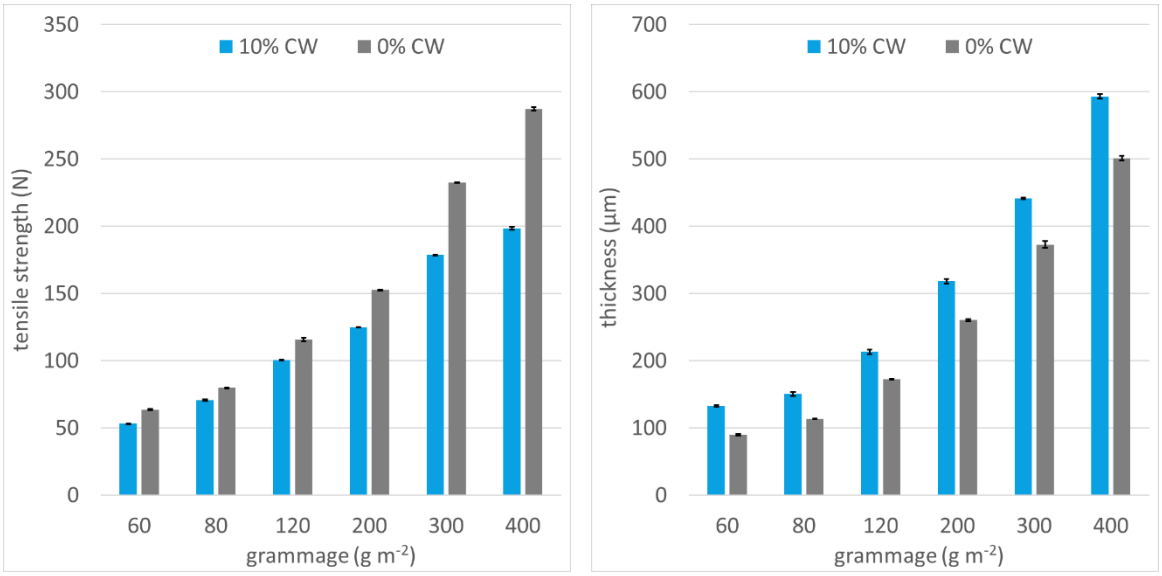
Although the mean values of the individual samples show a scattering that hampers the reasonable interpretation and comparison of individual samples, several trends can be observed. Generally, AP and AN samples show a higher TAC than PA and PP samples when stored for the same period. This applies to both fresh and dried biomass. This general observation is consistent with the findings presented in Figure 18. Especially for species PA and PP, extraction of dried biomass resulted in an increase of approx. 0.3 $mg \cdot Teq \cdot mg^{-1}$ biomass in comparison to fresh biomass samples. A better extractability of the dried biomass in comparison with the fresh biomass has been described in the literature for other plants (Regier et al. 2005; Valadez-Carmona et al. 2017), leading to an enhanced extraction yield. This interpretation is supported by the finding that AP and AN branches lose a higher relative amount of water during the first 12 days of drying than PA and PP branches, resulting in comparatively dry samples. As a general finding for all species, irrespective of the drying conditions, no relevant further increase in TAC is observed after approx. 7–10 days of incubation. These results match the optimum storage period observed for AEH seed coats extracted in a comparable setup (Havelt et al. 2019). However, TAC values only reach approx. half of the maximum value observed in analytical extraction (Figure 18). This is partly caused by reducing the biomass used for the extraction from approx. 60 mg biomass per mL extractant to approx. 47 mg biomass per mL extractant due to the characteristics of the extraction vessel. However, it is also possible that biochemical degradation of secondary metabolites occurs during the long process, given that the extractant does not prevent such reactions (Lewicki 1998). By using extraction vessels with another geometry, condensing the biomass in the reaction vessel, applying the concept to higher amounts of biomass and extractant, or narrowing the extractant after extraction, the biomass extractant ratio could be increased again, compensating the observed loss of TAC and possibly resulting in a lower extract variability. That way, fresh and dried coniferous wood could become a particularly relevant biomass for sustainable additive production while minimising the workload and energy needed for the process with excellent prospects when it comes to transferring the laboratory work to a larger scale.

5.3.2.4 Production of Sustainable Paper Packaging Materials from Extraction Waste Products

To assess the possible application of biomass after extraction for paper production, fresh aerial parts of AN, AP, PA, and PP were roughly chopped, mixed, and extracted with acetone for 7 days based on the method developed in Section 5.3.2.3. After extraction, the biomass was mixed with

pinewood pulp and paper sheets were prepared, resulting in sheets consisting of 10% coniferous wood extraction residuals (10% CW). Additionally, "blank sheets" consisting of 100% pinewood pulp without including any coniferous wood sample biomass were prepared analogically (0% CW).

Both the 10% CW and 0% CW sheets show an elongation at break of approx. 2.4–3.7%. For lower grammages (60–120 g·m^{-2}), both sheet types show a relatively homogeneous elongation of 3.1–3.5% while higher grammage sheets demonstrating a higher scattering with the 10% CW sheets typically showing a lower elongation than the 0% CW sheets. However, these differences appear to be neglectable. The results of the tensile strength and thickness analyses are displayed in **Figure 21**; the elongation at break is presented in **Figure A4**.



(a) ***(b)***

Fig. 21 *(**a**) Average tensile strength of the paper sheets with different grammages and compositions. Three repetitions, with four measurements per repetition. Standard deviation of the mean values per repetition indicated by error bars. CW: share of coniferous wood biomass after passive extraction included in the paper sheet.*
*(**b**) Average thickness of the paper sheets with different grammages and compositions. Three repetitions, with ten measurements per repetition. Standard deviation of the mean values per repetition indicated by error bars. CW: share of coniferous wood biomass after passive extraction included in the paper sheet.*

It can be observed that common cellulose-based 0% CW paper shows a maximum tensile strength of approx. 287 N while the paper prepared with 10% CW shows a decreased maximum tensile strength of approx. 199 N. Generally, 0% CW papers exceed 10% CW papers by approx. 24% on average regarding tensile strength. In contrast to tensile strength, 10% CW papers are considerably thicker than 0% CW papers (approx. 27% on average). Yet, the results of both paper types are comparable as a whole; differences can easily be compensated by choosing another grammage. This application allows a second-grade valorisation of waste materials beyond extraction without major detriments being observed. While the conducted analyses focus on incorporation of 10%

residual extraction materials, higher proportions of coniferous woods after extraction could be successfully incorporated as well.

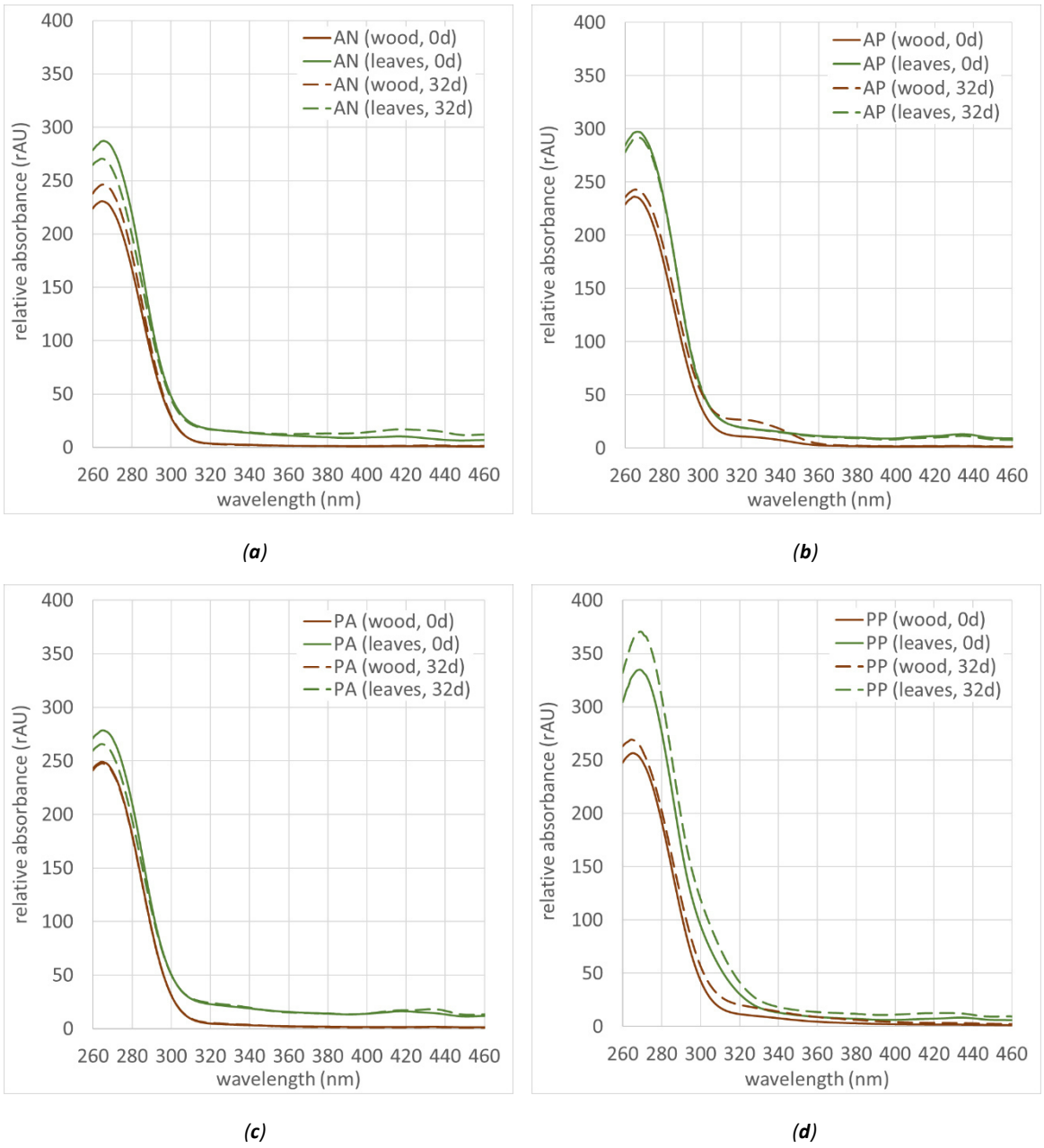
5.3.3 Characterisation of the Extracts of Different Branch Fractions for Fresh and Dried Masses

In the following, the influence of drying on different branch fractions is evaluated. This is particularly relevant for practical application as the necessity of separating the leaves from the wood for further processing is an important factor for extract preparation. Furthermore, the different fractions are chemically characterised to draw conclusions on the chemical differences of the fractions.

Weight loss correction was not performed during this section as determination of such would have required to interfere with the drying setup by drying leaves and wood separately. Thus, the same mass but a higher amount of biomass is used for extraction of the dried biomass in contrast to the fresh samples without correction. Therefore, the values of the dried biomass extracts in this section are anticipated to be reduced to approx. 52% of the given value (AN: 48%; AP: 50%; PA: 51%; PP: 60%; estimation based on whole branch weight development during the drying process; the actual correction factors regarding specific plant fragments might differ).

5.3.3.1 UV/Vis Absorbance

All four species' fragments and degrees of dryness resulted in comparable UV spectra and show limited absorbance in the visible range, which is thus neglected in further interpretations. As depicted in **Figure 22**, all extracts show a similar course of UV absorbance also resembling the UV spectra observed for whole branch extracts in Section 5.3.2.1; however, there are considerable differences in intensities. For all species, a higher absorbance of leaf extracts than of wood extracts can be observed with the intensity scattering around whole branch extracts. This applies to fresh and dried samples. Dried sample extracts seemingly show a UV intensity comparable to fresh biomass extracts without applying a weight loss correction. Thus, a considerable UV absorbance reduction, notably below the UV absorbance values observed for fresh biomass extracts, is anticipated for the dried extracts when performing an appropriate weight correction.



(a) ***(b)***

(c) ***(d)***

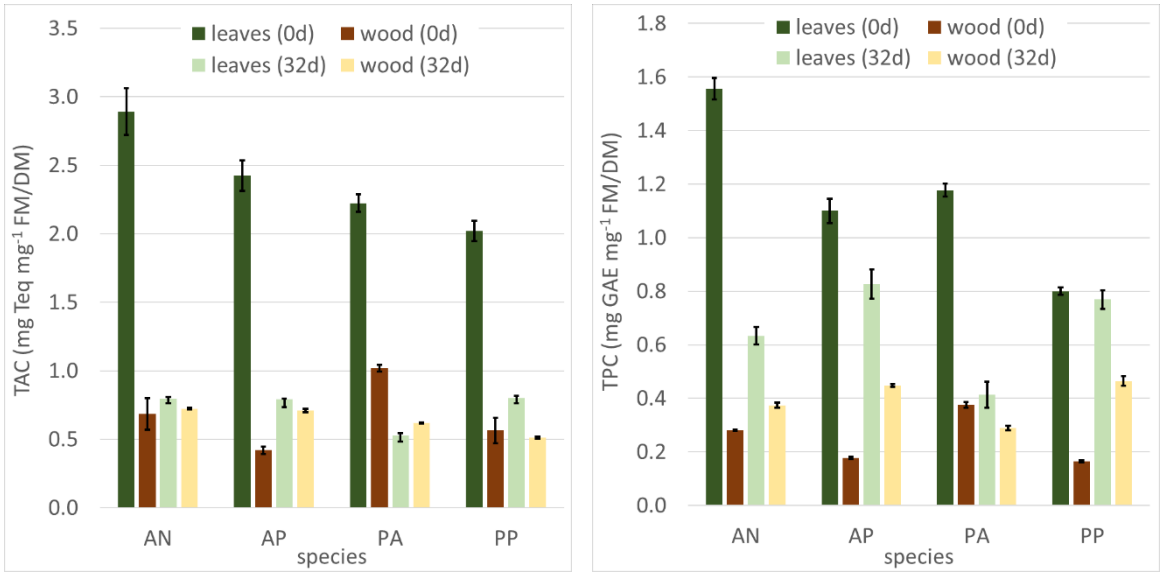
Fig. 22 *Average UV/Vis absorbance of the wood or leaf extracts of the different coniferous woods displayed in relative absorbance units (rAU) after drying for different periods, given in days (d); wavelengths above 460 nm are not shown. Measurements in quadruplicate.*
*(**a**): Abies nordmanniana (AN); (**b**): Abies procera (AP); (**c**): Picea abies (PA); (**d**): Picea pungens (PP).*

While the AN, AP, and PA spectra are roughly comparable, the PP extracts show the highest absorbance values of all four species, particularly for fresh and dried leaves, thus confirming the high UV absorbances observed before. Due to all extracts showing a similar course of UV absorbance, the presence of the similar UV-active ingredients could be assumed. In comparison to the UV absorbance of the whole branch extracts, the absorbance of the fresh mass extracts of the different fractions scatters around the absorbance of the fresh whole branch extracts for all species. Consistently, the leaf extract is showing a higher absorbance, followed by the whole branch and wood extracts. This applies to dried samples as well when applying correction factors obtained by

whole branch drying. Dried sample extracts show a reduced intensity due to loss and degradation of the analyte during drying, as expected. Thus, both fractions seem to be similarly affected by drying with the leaf fraction consistently resulting in a higher UV absorbance than the wood fraction. Following the assumption of the UV absorbance of both fractions being caused by the same ingredient, leaves therefore contain higher amounts of such UV-absorbing compounds.

5.3.3.2 TAC and TPC

As shown in **Figure 23**, significant differences, particularly between fresh leaves and other plant fragments, are observed. While extracts prepared from dried wood, fresh wood, or dried leaves show comparably similar TACs of approx. 0.4–1.0 mg·Teq·mg^{-1} biomass, extracts prepared from fresh leaves result in a significantly higher TAC of approx. 2.0–2.9 mg·Teq·mg^{-1} biomass. The highest antioxidant potential is reached by fresh AN leaves, followed by the AP, PA, and PP leaves. Leaf extracts are also showing notably higher TPC values than the corresponding wood extracts do; however, the difference between fresh and dried biomasses is less distinct.



(a) ***(b)***

Fig. 23 *(**a**) Average total antioxidant capacity (TAC) of the leaf or wood extracts of the different coniferous woods based on fresh or dried mass. Measurements in quadruplicate; standard deviation indicated by error bars. Teq: Trolox equivalents; FM/0d: fresh mass; DM/32d: dried mass after drying for 32 days at 21 °C; AN: Abies nordmanniana; AP: Abies procera; PA: Picea abies; PP: Picea pungens.*
*(**b**) Average total phenolic content (TPC) of the leaf or wood extracts of different coniferous woods based on fresh or dried mass. Measurements in quadruplicate; standard deviation indicated by error bars. GAE: gallic acid equivalents; FM/0d: fresh mass; DM/32d: dried mass after drying for 32 days at 21 °C; AN: Abies nordmanniana; AP: Abies procera; PA: Picea abies; PP: Picea pungens.*

The observation of fresh leaves providing the best bioactive characteristics is confirmed by UV absorbance as well as what was discussed before; however, the difference between the leaf and wood extracts are considerably smaller. Again, the dried biomass extracts are not capable of

reaching a TAC comparable to the fresh leaf extracts; in case of TAC, this also applies to the fresh wood extracts. The decrease of TAC especially in dried leaf extracts could be caused, e.g., by loss or biochemical degradation of bioactive substances during the comparably long drying period, as it is observed for other plants (Lewicki 1998). For the dried fractions, slightly higher values than expected are observed when comparing the extracts of the fractions or the respective whole aerial part extracts. This effect is presumably caused by the higher relative amount of secondary metabolites after the loss of water during drying.

5.3.3.3 Further Analyses

For all samples, the presence of proanthocyanidins (PACs) was evaluated based on the specific acid–butanol assay (ABA). As shown in **Figure A5**, a maximum corrected absorbance of 1.52 is observed for extracts prepared from fresh AN leaves. In general, fresh leaf extracts result in the respective highest absorbance per species, followed by dried leaf extracts for most species excluding PA. Both fresh and dried wood extracts show comparably low absorbances. Excluding particularly low PP fresh and dried wood extracts and AP fresh wood extracts, the obtained absorbances vary between 0.20 and 0.41.

Thus, the presence of PACs is proven at least for the fresh leaves of all four species; the dried AN, AP, and PP leaves are also considered to include a relevant concentration of PACs. Fresh and dried wood as well as PA dried leaves can only be assumed to contain PACs as the observed absorbances are comparably low. It is likely that proof of PACs in the respective fractions could be obtained by preparing extracts with a higher relative sample amount. However, the interpretation of ABA absorbances is limited due to the semi-quantitative characteristics of the assay as absorbance is not only dependent on the concentration of PACs in the sample, but also on the type of PACs contained.

The evaluation of qualitative GC-MS analysis results in several compounds detected for the extracts, including a variety of sugars and other substances (e.g., pinitol, communic acid, and epigallocatechin) of which abietic acid and (+)-catechin have been confirmed by analysing the standard substances in addition to a library comparison (NIST). Abietic acid and dihydroabietic acid are present primarily in fresh and dried leaves and wood of AN and AP, while catechin is present in most biomasses, particularly in leaves. Due to its antioxidant effect (Grzesik et al. 2018), the presence of catechin could be part of the reason for the high TAC observed for leaf extracts. A direct correlation of catechin presence and TAC could not be found; however, TAC values could also be linked to oligomeric PACs, which are determined via ABA and, in the simplest case, based on catechin monomers. This is proved by the results of ABA being comparable to the TAC values of the dried and fresh leaves and wood extracts. With some exceptions, extracts with an estimated higher

amount of PACs show a higher antioxidant capacity. However, TAC results could also be influenced by further extract constituents that are not detected in GC-MS analysis. The general presence of PACs in coniferous wood is reasonable as they have been confirmed in other wood/wood fractions before, including birchbark and AEH seed coats (Karonen et al. 2007; Havelt et al. 2019). The similar course of the UV spectra of the extracts based on AEH seed coats and coniferous woods further supports these findings. PACs, as active compounds in plant extracts, are particularly advantageous for the application of extracts as additives in food packaging as they typically are macromolecular compounds and thus less prone to migration. Additionally, they are considered safe for the application in foods by the European Food Safety Authority (EFSA) (Turck et al. 2017).

5.4 Conclusions

For all the analysed coniferous wood, a general suitability for use as biobased stabilisers is proven as the basic parameters of antioxidant capacity and UV absorbance are satisfactory. As there are differences between the species, separation is recommended, but not mandatory as the species constantly show comparable results. Coniferous woods are a relevant bioresource particularly due to their wide availability, e.g., as used Christmas trees. Highly bioactive extracts can be prepared at least from biomass that has been used as indoor Christmas trees for 12 days; however, as this study applied particularly hard conditions by using un-watered branches to provide a minimum acceptable duration of use, this period might be expendable. Depending on the specific application, the extractant acetone could be substituted by a mixture of water and acetone (1:1 (*v*/*v*)), resulting in comparable antioxidant properties while using more eco-friendly solvents. To exploit the potential of the extracts prepared from such biomasses, further extraction optimisation should be conducted, as described. Additionally, full valorisation of Christmas trees is achieved by incorporating chopped biomass after extraction into paper packaging material, as shown in this pilot trial. Thus, the whole Christmas tree can be utilised to create more sustainable packaging materials by substituting specifically synthesised additives or trees planted for paper production, contributing to the transformation to a circular economy. Upcoming research will include application and migration studies.

5.5 Funding

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5.6 Acknowledgements

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5.7 Copyright

6. Applicational evaluation and analysis of synergistic effects

"Evaluation of practical applicability and synergistic effects of bio-based food packaging materials combined with plant-based stabilisers" (Havelt et al. 2021a)

Abstract

Different analyses and feasibility studies have been conducted on the plant extracts of thyme (*Thymus vulgaris*), European horse chestnut (*Aesculus hippocastanum*), Nordmann fir (*Abies nordmanniana*), and snowdrop (*Galanthus elwesii*) to evaluate bio-based alternatives to common petrol-based stabilisers. For this purpose, in this study, plant extracts were incorporated into poly-lactic acid films (PLA) at different concentrations. The films' UV absorbance and migration into packed food was analysed via photometric assays (ABTS radical cation scavenging capacity assay, β-carotene assay) and GC–MS analysis. Furthermore, the synergistic antioxidant effects of various combinations of extracts and isolated active compounds were determined. This way, antioxidant effects can be increased, allowing for a highly effective use of resources. All extracts were successfully incorporated into PLA films and showed notable photoabsorbing effects, while no migration risk was observed. Depending on extract combinations, high synergistic effects of up to 726% can be utilised to improve the effectiveness of bio-based extracts. This applies particularly to tomato paste and Aesculus hippocastanum extracts, which overall show high synergistic and antioxidant effects in combination with each other and with isolated active compounds. The study shows that it is possible to create safe bio-based antioxidant films which show even improved properties when using highlighted target combinations.

6.1 Introduction

The need for environmentally friendly solutions to everyday problems is ubiquitous (German Environment Agency 2017). This particularly applies to products used in large scales, such as plastic packaging materials (European Parliament 6/5/2019; PlasticsEurope 2020, 2021). Next to the main plastic material, a variety of different active substances, so-called additives, are processed in food packaging to adjust the properties of the material and create a packaging with the specific attributes needed for their application, including, but not limited to, antioxidant, photostabilising, and antimicrobial activities. As well as most plastics themselves, additives are typically fossil-based and can thus constitute a threat to both human and environmental health (Ito et al. 1985; Kahl and Kappus 1993). Thus, approaches were and are made to provide plant-based, bioactive alternatives to those additives. For those alternatives, bioactive properties such as UV-absorbing and antioxidant effects have been reported in the literature (Havelt and Schmitz 2018; Götz et al. 2019; Havelt et al. 2019; Havelt et al. 2020; Havelt et al. 2021b; Korte et al. 2021).

In previous works, we identified different local biomasses presenting a particular potential for the preparation of bio-based additives, including common thyme leaves (*Thymus vulgaris* L.) (Havelt and Schmitz 2018), European horse chestnut seed coats (*Aesculus hippocastanum* L.) (Havelt et al. 2019), snowdrop leaves (*Galanthus elwesii* HOOK.F.) (Havelt et al. 2021b), and aerial parts of Nordmann firs (*Abies nordmanniana* (STEV.) SPACH) (Havelt et al. 2020).

Thymus vulgaris (TV) is an aromatic and medicinal plant, well known for its medical benefits and antimicrobial activities (Deans and Ritchie 1987; Tantaoui-Elaraki and Beraoud 1994; Nelson 1997; Smith-Palmer et al. 1998; Ph. Eur. 2001a, 2001b; Tornuk et al. 2011; Charles 2013b). Furthermore, an enormous antioxidant effect of thyme in both hydrophilic and lipophilic surroundings, caused mainly by the active substance, thymol, has been observed in several studies. This can indeed be used, for example, to extend the shelf life of food products by active packaging (Karabagias et al. 2011; Erkan 2012; Jang et al. 2017; Lee et al. 2017; El-Obeid et al. 2018; Havelt and Schmitz 2018; Zeid et al. 2019). Furthermore, a strong UV-absorbing effect of thyme extracts, based particularly on the main component thymol, has been reported (Havelt and Schmitz 2018; Havelt et al. 2021b).

The European horse chestnut (*Aesculus hippocastanum* (AEH)) is a common ornamental tree in Europe. Furthermore, the inner fragments of AEH seeds are used for the extraction of phytochemicals; usually, the remaining seed coats are discarded. As shown in previous works, these seed coats contain macromolecular proanthocyanidins that can be easily extracted (Havelt et al. 2019). The prepared hydrophilic (water and acetone, 1:1) extracts show strong antioxidant, antimicrobial, and UV-absorbing effects. The possible valorisation of otherwise discarded raw

materials and the macromolecular character of the active substances constitute their special potential for use as an ecological food packaging additive.

Snowdrops are widespread ornamental plants and produce different secondary metabolites, including the lipophilic compound α-tocopherol (or vitamin E), which is not only safe but essential for human health in moderation, and shows a high antioxidant potential (Domke et al. 2005; Stanciu et al. 2010; Karimi et al. 2018). In a previous study, heptane extracts of different snowdrop species were analysed with regard to antioxidant capacity and α-tocopherol content, resulting in *Galanthus elwesii* (GE) showing a significantly higher antioxidant effect (Havelt et al. 2021b). Our study demonstrates the use of extracts prepared from leaf extracts of the same species as bio-based antioxidants in packaging. This approach is highly promising as experiments using synthetic α tocopherol solutions for active packaging applications have already been successful (Vasile et al. 2013).

Nordmann firs (*Abies nordmanniana* (AN)) are the most popular Christmas trees in Germany; in 2019, approximately 23–30 million Christmas trees were sold in Germany, and approximately 75% of those were Nordmann firs, followed by blue spruces and other spruces (Schutzgemeinschaft Deutscher Wald 2020; Hauptverband der Deutschen Holzindustrie e.V. (HDH) 12/22/2019). Nordmann fir leaves contain antioxidant compounds such as ascorbate and α-tocopherol; the essential oil prepared from the AN leaves shows considerable antimicrobial effects against different *Bacillus* cultures, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and partly against *Staphylococcus aureus* (Bağcı and Dığrak 1996; Weber et al. 1996; Öncel et al. 2004; Charles 2013b). Further antioxidant compounds, including flavonoids and phenolic acids, such as catechin isomers and gallic acid, are found in AN bark (Hafizoglu and Holmbom 1995). Substantial antioxidant and UV-absorbing effects have been confirmed in a recent study on different coniferous wood species and the impact of using different fragments and drying setups before extraction by acetone (Havelt et al. 2020). The study showed that even used Christmas trees could be utilised to prepare extracts with the previously mentioned properties, making Nordmann firs a valuable resource for sustainable additive production. This could be a further step towards a circular economy.

In the present study, previously analysed biomasses and developed extraction methods were implemented to evaluate the actual possibility, and indicate the potential benefits, of using bio-based additives as packaging stabilisers. For this purpose, the extracts were incorporated into poly-lactic acid films (PLA) in different concentrations, observing the homogeneity and optical properties of the films. For maximum concentrations of extracts with acceptable characteristics, the antioxidant properties of the films were determined. Furthermore, migration studies, as according

to EU regulation 10/2011 for food contact materials (FCM) (European Comission 2011a), were conducted on those films to ensure the possible application of bio-based additives was in compliance with legislation. To encourage an ideal, efficient use of extracts for specific applications, synergistic effects were analysed. That way, the resulting antioxidant effect could be maximised by combining different extracts or substances that enhance each other's effects when combined, and thereby exceed the expected effect. For synergistic analysis, GE extract was replaced by an extract prepared from tomato paste to evaluate the interaction of the contained lycopene, a carotenoid with a high antioxidant effect (Giovannucci 1999; Rao 2004).

6.2 Materials and Methods

6.2.1 Chemicals and Instrumentation

A Perkin Elmer Lambda 25 double-beam spectral photometer was used to conduct ABTS and β-carotene assays for synergistic examinations and an analysis of migrates. For the determination of UV/Vis absorbance and antioxidant capacity of PLA films, an Agilent Cary 60 dual-beam spectral photometer and a fiber optic probe were used. Migrates were analysed using an Agilent 8890 GC system, coupled with an Agilent 5977B MSD mass spectrometer. Gallic acid, quercetin dihydrate, and dipotassium hydrogen phosphate were purchased from Alfa Aesar (Karlsruhe, Germany), whereas 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), acetic acid, and Trolox were purchased from AppliChem GmbH (Darmstadt, Germany), Bernd Kraft (Duisburg, Germany), and Cayman Chemical Company (Ann Arbor, MI, USA), respectively. Virgin native olive oil and tomato paste were obtained from a local distributor. Thymol, L(+)-ascorbic acid, n-heptane, ethanol, methanol, and acetone were obtained from VWR International, Darmstadt, Germany. Hydrogen peroxide, potassium dihydrogen phosphate, sodium acetate, and dichloromethane were purchased from Merck KGaA, Darmstadt, Germany. Linoleic Acid and β-carotene were obtained from Thermo Fisher Scientific (Waltham, MA, USA), while α-tocopherol, polysorbate, and Tenax porous polymer adsorbent were purchased from Sigma Aldrich (St. Louis, MO, USA). Poly-lactic acid pellets (PLA) were provided by Bio-Fed, Cologne, Germany.

6.2.2 Preparation of Extracts

During the study, different biomasses were extracted. TV and GE biomasses were cultivated and provided by staff of the Faculty of Agriculture, University of Bonn, while AEH seed coats were kindly provided by the company, Finzelberg. AN samples were kindly provided by Hof Große Wöstmann, Rinkerode, Germany, and were approved by staff of the Faculty of Agriculture, University of Bonn.

General extraction methods for AEH (Havelt et al. 2019), GE (Havelt et al. 2021b), and AN (Havelt et al. 2020) extracts are described in literature, but some modifications were made. To ensure better miscibility with other film preparation chemicals, AEH extracts were prepared using methanol instead of a mixture of water and acetone as an extraction solvent. After conducting successful pretests on optimum extraction characteristics, TV extracts were prepared by applying a passive extraction setup inspired by the techniques described by (Havelt et al. 2019; Havelt et al. 2020). This setup allowed for a higher throughput of extracts, thus supporting the introduction of bio-based extracts in industrial processes. Dried TV leaves were ground in a cutting mill, infused with 10 mL methanol per g of dried biomass, and the extraction vessel was shaken for four days under the exclusion of light. Tomato paste (TP) extracts were prepared by applying 2.5 mL acetone to 1 g TP. The extraction vessel was then briefly shaken and centrifuged for 10 min before collecting the supernatant and filling it up to a total volume of 10 mL using acetone.

6.2.3 Preparation of PLA films

The PLA films were prepared following the solvent cast method ((Ahmed et al. 2017), modified). Exactly 0.5 g of PLA pellets were dissolved in 10 mL dichloromethane while stirring. To produce films equipped with plant extracts, such extracts were added to the PLA solution in an appropriate proportion relative to the PLA pellets. Afterwards, the PLA solution was cast on a glass surface, such as petri dishes, and the solvent was fully evaporated at room temperature. This was necessary for the film to gain the desired mechanical properties, and to ensure that no potentially harmful solvent or extractant residues were present.

6.2.4 Evaluation of Homogeneity via Determination of UV/Vis Absorbance

The UV/Vis absorbance and homogeneity of the prepared PLA films was determined using the Agilent Cary 60 dual-beam spectral photometer, equipped with a Xenon light source, which covers both the visual and the UV range. In contrast to typical photometers, which are equipped with two different light sources for UV and visible range, this allowed for the measurement of samples without ambient light interfering with the measurement. This flexibility made it possible to easily measure bigger and more complex samples such as PLA films. Instead of a cuvette, each PLA film was introduced into the sample beam, while a UV/Vis spectrum in the range of 240–800 nm was recorded. Three films were prepared per extract concentration, with every film being measured at three different positions that were equally spread across the film area. This allowed for the determination of a mean UV/Vis spectrum per extract type and concentration and revealed possible turbidities within the films. For the evaluation of homogeneity, the variance (squared standard

deviation) was determined; if the films showed a high inhomogeneity, this would result in a comparatively high variance.

6.2.5 Preparation of Migration Samples and Migration Analysis via Gas Chromatography Coupled with Mass Spectrometry (GC-MS)

For migration analysis, films with different concentrations of extracts, as well as blank films containing no extract, were cast as described above and transferred into adequate migration vessels. Afterwards, an appropriate amount of different food simulants was filled into the vessels (0.6 mL for liquid simulants, 400 mg for Tenax). Migration analysis was conducted, allowing single-sided migration only. In accordance with (European Comission 2011a), a mixture of ethanol and water (1:10, *v*/*v*; simulant A), 3% acetic acid (3:100, *m*/*v*; simulant B), olive oil (simulant D2), and Tenax (simulant E) were used as food simulants. After applying the food simulants to the films and sealing the vessels, the samples were stored for 10 days at 20 °C, 40 °C, and 60 °C, respectively. These conditions allowed for short-period analysis of long-time storage and of different storage conditions that are considered food-safe when the legal limits are met for corresponding accelerated conditions (European Comission 2011a). For example, successful low-migration storage for 10 days at 60 °C enabled storage for more than 6 months at room temperature or when refrigerated or frozen, while successful migration tests at 20 °C allowed for frozen storage only.

Migration was evaluated using GC–MS and the ABTS assay described below. All Tenax samples (E) were transferred into liquid samples by desorbing the migrated compounds into 2.5 mL of the solvent used for the respective biomass extraction. For GC–MS analysis, 1 µL of sample solution was heated to 250 °C and introduced onto an Agilent HP-5MS UI column (30 m × 320 µm, 0.25 µm film thickness). The temperature of the column oven started at 75 °C and, after 1 min, increased to 325 °C at 7 °C min^{-1}, before holding the final temperature for 15 min (30 min for D2 samples). For aqueous samples A and B, injection was performed in pulsed pressure mode, allowing aqueous samples to be rapidly heated without exceeding the liner volume. This GC–MS setup allowed for a sensitive non-target screening of migration samples. Furthermore, chromatograms were interpreted, paying special attention to significant masses associated with expected main compounds included in extracts, to prevent migrate signals being covered by noise.

6.2.6 Photometric Assays

The total antioxidant capacity (TAC) in hydrophilic surroundings (hyTAC) was determined via ABTS radical cation ($ABTS^{\bullet+}$) scavenging capacity assay (ABTS assay, based on (Erel 2004)) for both raw extracts and PLA films by monitoring the decolourisation reaction of ABTS radical cations. For synergism analysis of extracts, the method was performed according to the literature (Havelt et al.

2019; Havelt et al. 2020) while using the wavelength λ = 734 nm and a classic double beam spectral photometer. Depending on the application, the absorbance was also monitored using a fiber optic probe coupled with a dual-beam spectral photometer, allowing for a more flexible measurement protocol.

The method to determine the TAC in lipophilic surroundings (liTAC) for synergism analysis via β-carotene assay (λ = 470 nm) was performed according to the literature (Havelt et al. 2021b). If applicable, the fibre optic probe could be used again to profit from a more flexible experiment design.

Results based on both assays are depicted presenting the mean and standard deviation.

6.2.7 Determination of Synergistic Effects

Synergistic effects are a substantial part of overall antioxidant effects observed for natural samples (Schmitz-Eiberger and Blanke 2012); thus, their analysis is necessary to allow effective applications of plant-based stabilisers. As described in the literature (Tsao 2015), synergistic effects are defined as effects that are higher when combining different active agents in comparison to the sum of the effects of those different agents applied separately; a negative synergistic effect is defined as an antagonistic effect. Thus, the extracts and isolated active compounds (IACs) used in this study were measured separately and in varying concentrations (to take dose–response relationships into account) using hyTAC and liTAC assays. Furthermore, the extracts and/or IACs were measured in the same concentrations but combined with each other to determine whether synergistic or antagonistic effects were observable. To analyse both, synergisms between different extracts (cross-extract synergism, Section 6.3.2.1) and between extracts and IACs (IAC–extract synergism, Section 6.3.2.2) that are well-known for their antioxidant effect were analysed.

The extracts covered in the present synergism study were AEH, TV, AN, and commercial tomato paste (TP) extracts, which include carotenoids such as the highly antioxidant compounds lycopene and β-carotene (Giovannucci 1999; Baysal et al. 2000; Rao 2004). Ascorbic acid (AA), gallic acid (GA), quercetin (Qu), thymol (Th), and α-tocopherol (To) were used for IAC–extract synergism analysis, expanding the analysed range of natural antioxidants. For evaluation, the anticipated value or “base value” is defined as the calculated sum of the effect of two individual active components or extracts. Those base values are corrected by the positive (synergistic) or negative (antagonistic) effects observed via measurement.

6.3 Results and Discussion

6.3.1 Formulation and Analysis of Enriched PLA Films

To evaluate the practical potential of bio-based enrichment of PLA films, exemplar films were produced with different types (TV, AEH, GE, AN) and amounts of extracts before analysing homogeneity, UV/Vis absorbance, and the antioxidant capacity of the films. The five different concentrations applied were 0.2, 0.3, 0.4, 0.6, and 1.0 mL extract per g PLA, corresponding to 1%, 1.5%, 2%, 3%, and 5% (*v*/*v*) during film preparation. To clarify further discussions, the films are described as F1 (1%), F2 (1.5%), F3 (2%), F4 (3%), and F5 (5%). As the density of the used organic extractants was approximately 0.7–0.8 g mL^{-1}, the applied extract concentrations ranged from approximately 0.15% to 0.8% (*w*/*w*). This reflects the proportion of antioxidants of approximately 0.05–1.0% (*w*/*w*) typically incorporated into polymers (Wegmann et al. 2016).

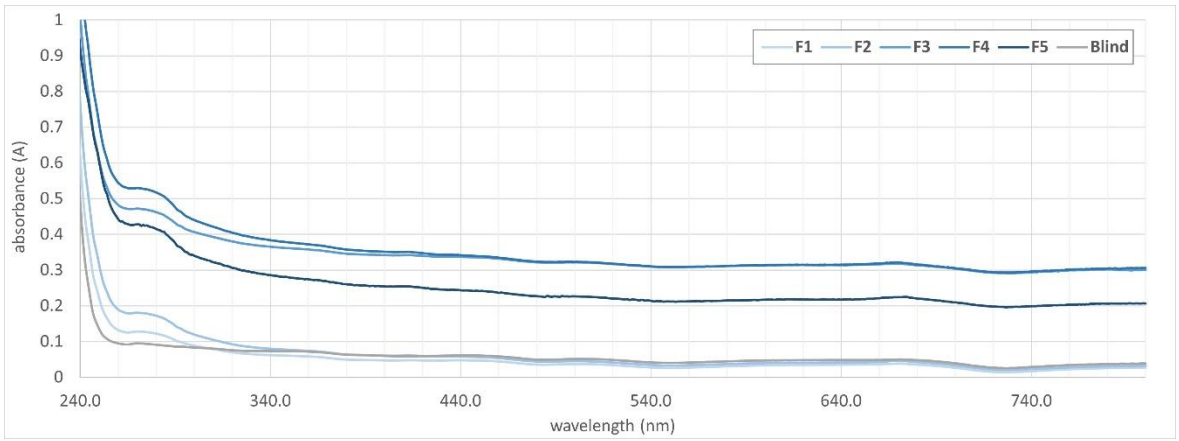
UV/Vis absorbance was used to evaluate whether the UV absorbance determined for pure extracts could still be observed after incorporating those extracts into PLA films, thus pointing out whether a transition of desired properties from the extracts to the films was successful. Additionally, UV/Vis spectra of PLA films include information on whether visible light transmission is generally hindered, such as by turbidities, by showing a high absorbance in the whole visible range. Furthermore, UV/Vis absorbance can be used to determine film homogeneity, which is a key characteristic for chemical and mechanical applications. If stabilisers accumulate in a distinct area of the film, a shortage of stabilisers is thus caused in other film areas. In the areas lacking stabilisers, oxidative stress or UV light can easily cause material deterioration, which then affects the whole film (Rabek 2012; Rasselet et al. 2014; Altaf et al. 2018). Such inhomogeneous films can thus lead to cosmetic changes or even the mechanical failure of plastic components. Homogeneous film preparation was thus considered an important parameter. The homogeneity was determined using the variance of maximum UV absorbances within replicate films to measure whether UV absorbance (caused by the added stabilising extracts) is evenly distributed within the film.

For food contact materials, migration studies are substantial as no relevant transition of packaging components, such as stabilisers, into packed foodstuff must be observed. Thus, migration studies according to EU regulations (European Comission 2004, 2011a) were followed to evaluate the suitability of those extracts in PLA systems for food contact applications. For this purpose, the stabilised PLA films were brought into contact with different food simulants as required by law (European Comission 2004, 2011a). As migration is more likely to happen for small volatile compounds (Cataldo 2001; Choi et al. 2009), it was monitored using GC–MS to detect and identify migrating compounds. While the migration of compounds with a higher molecular weight (which

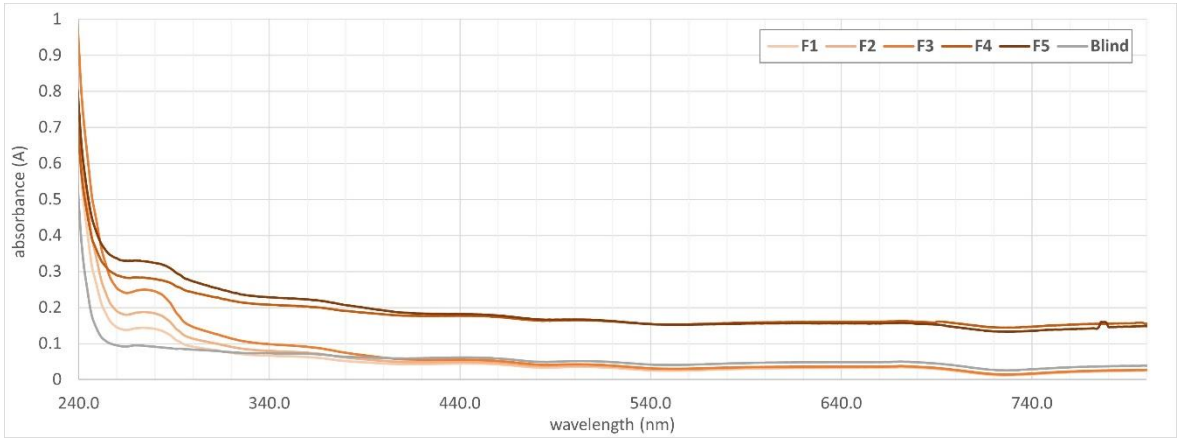
evade detection via GC–MS) was unlikely, hyTAC assays (ABTS radical cation scavenging capacity assays) were further conducted for food simulants to determine the antioxidant potential of food simulants, and thus ensure that no relevant migration of high-molecular active substances, e.g., proanthocyanidins, which are included in AN and AEH extracts (Havelt et al. 2019; Havelt et al. 2020), occurred.

6.3.1.1 UV/Vis Absorbance

As depicted in **Figure 24**, TV, AEH, and AN films show a relevant absorbance in the UV range, while GE films do not show interpretable absorbances, which is supported by the results of previous experiments (Havelt et al. 2021b); thus, GE results are not shown. AN films showed peak absorbances of approximately 0.18 A (F2) and 0.12 A (F1) below circa 280 nm; non-enriched films reached approximately 0.1 A. For higher wavelengths, particularly higher than 340 nm, no difference to non-enriched PLA films was observed. Films with higher AN extract concentrations showed a much higher peak absorbance of up to 0.53 A; however, the absorbance in the visible range increased as well, indicating typically unwanted turbidity. This could also indicate a radical change in material properties, supporting the maximum ideal concentration for F2 found in 3.1.1. For AEH films, a higher peak absorbance of approximately 0.14 A (F1) to 0.25 A (F3) was reached up to approximately 295 nm, with the absorbance decreasing up to a wavelength of 400 nm. No relevant absorbance was observed in the visual range for F1–F3 films. For F4 and F5, a further increase in UV absorbance was detected (up to 0.32 A), accompanied by an absorbance of approximately. 0.15–0.2 A in the whole visible range, again indicating turbidity and possible drastic material changes. As described by (Havelt et al. 2019; Havelt et al. 2020), both AN and AEH extracts include proanthocyanidins, which are macromolecular polyphenols and presumably cause the UV absorbance in both extracts' films surpassing the blind film absorbance. TV films generally showed a broader and higher UV absorbance with approximately 0.23 A (F1)–0.64 A (F5) at its first maximum (280 nm), and approximately 0.18 A (F1)–0.46 A (F5) at its second maximum (335 nm); the determined maximum ideal formulation for F3 showed an absorbance of approximately 0.32 A and 0.23 A at both maxima. The increased absorbance was primarily caused by thymol and carvacrol (John Wiley & Sons, Inc. 2008a, 2008b; Havelt and Schmitz 2018) and, presumably, by other included terpenoids. For all concentrations, absorbance in the visible range was detected, particularly at approximately 670 nm and below approximately 520 nm, while no strict turbidity was observed.



(a)



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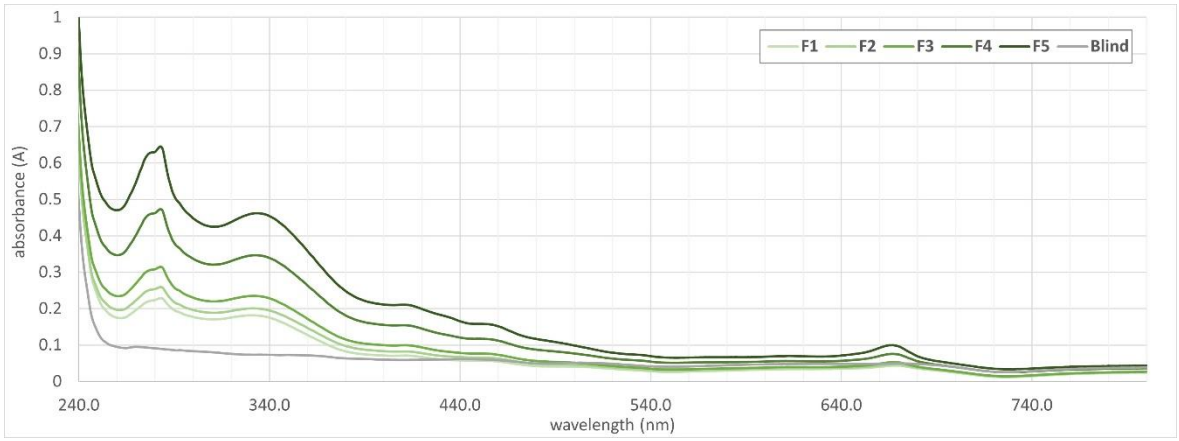


(c)

Fig. 24 *UV/Vis absorbance spectra of PLA films enriched with three different plant-based extracts. Ninefold determination (three films with three measurements conducted at random locations of each film). (**a**): Abies nordmanniana (AN) extract; (**b**): Aesculus hippocastanum (AEH) extract; (**c**): Thymus vulgaris (TV) extract.*

In general, the formation of turbidity can be assumed when the absorbance over the whole analysed range, particularly in the visible range where analytes usually show limited absorbance only, is disproportionately increased when compared to known absorbances, such as in UV range.

This is the case when the polymeric system is disrupted due to interferences caused by high concentrations of foreign substances, in this case, extracts.

The threshold of visible disturbance is dependent on the type of foreign substances; therefore, the observed differences between the different extracts are plausible.

The results suggest maximum ideal formulations of F2 for GE and AN extracts (0.3 mL extract per g PLA), and F3 for TV and AEH extracts (0.4 mL extract per g PLA), as turbidities can occur when applying higher concentrations of extracts for all except TV extracts. Due to only limited turbidites observed for all TV extract concentrations, an application of higher TV extract concentrations might be possible and worthwhile. All determined UV/Vis spectra closely resembled the ones of the sole respective extracts presented in (Havelt and Schmitz 2018; Havelt et al. 2019; Havelt et al. 2020; Havelt et al. 2021b), showing that the incorporation of extracts successfully introduced the property of UV absorbance into PLA films.

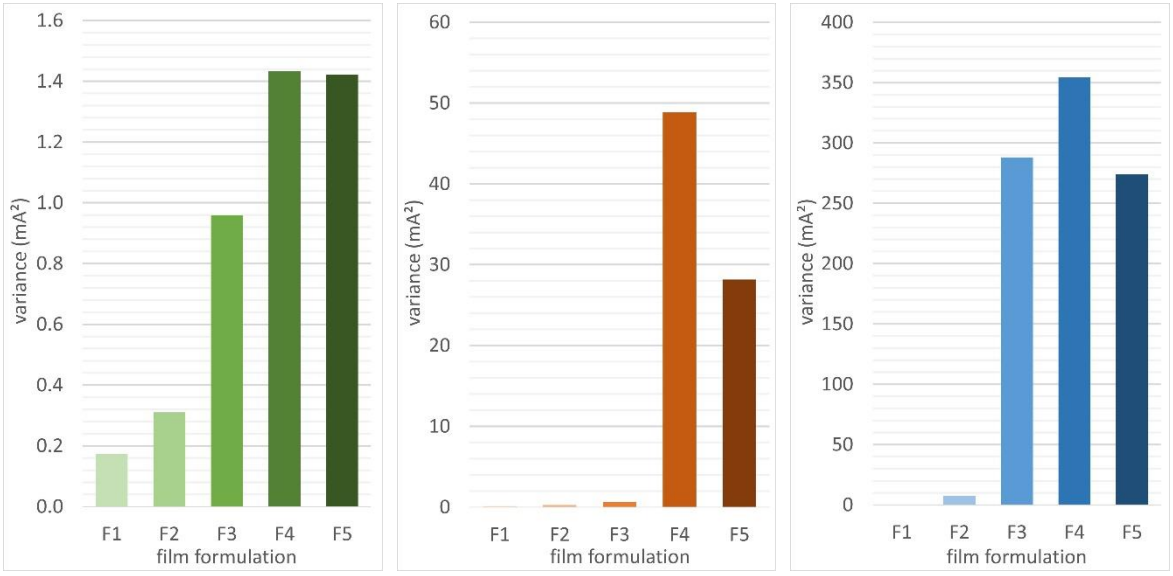
6.3.1.2 Film Homogeneity Analysis

To evaluate the highest possible concentration of extracts while maintaining homogeneous film properties, the produced films were assessed regarding their homogeneity by determining the UV/Vis spectra of three replicate films at three random positions of the film. The variances (squared standard deviation) of those measurements were determined by measuring the maximum UV absorbances of the different samples, as displayed in **Figure 25**. In general, the variance increases alongside the concentration of the incorporated extract. However, the variance of TV films increased until F4, while showing no further increase after F4. In contrast, a low variance was observed for AEH films F1–F3, rapidly increasing for F4 and F5. AN films presented a similar course with F1 and F2 showing a comparably low variation which increases for F3–F5. For GE films, no satisfactory data were obtained as GE films generally show very little UV absorbance. However, F2 was deemed the GE film with the highest extract concentration while maintaining homogeneity based on visual evaluation. Examples of films showing different stages of homogeneity are depicted in **Figure 26**, and photographs for all films are displayed in **Figure A6**.

Following the approach of determining the highest possible extract concentration to conduct further analysis on without considerably affecting homogeneity, F2 films (1.5% *v*/*v* during preparation, 0.3 mL extract per g PLA) were considered the maximum ideal formulation for GE and AN films, while the maximum ideal formulation for films enriched with TV and AEH extracts was deemed F3 (2% *v*/*v* during preparation, 0.4 mL extract per g PLA), as justified by the contextual variance. These results are supported by the results presented in Section 6.3.1.1, in which turbidities were observed for extract concentrations exceeding the determined maximum ideal

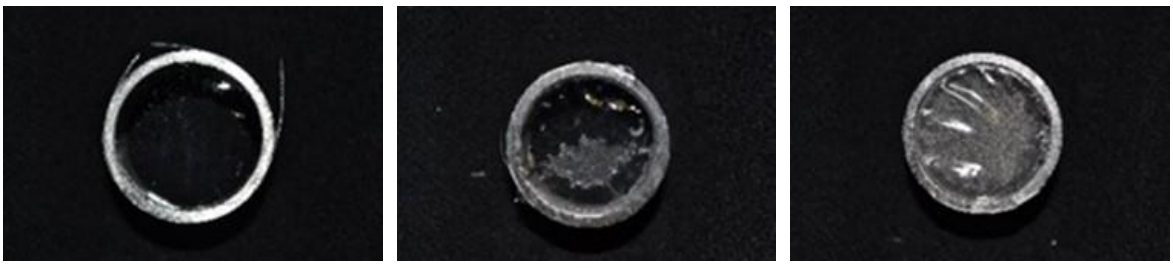
formulations. However, the variance observed for TV films was particularly low in comparison to other extracts, again possibly allowing for an incorporation of higher extract concentrations as well.

The investigated extracts were applied in comparable dimensions with other plant-based extracts discussed in the literature, such as olive leaf extracts applied at approximately 3 g extract per g PLA, or horseradish extract incorporated with approximately 2 g per g PLA (Erdohan et al. 2013; Wegmann et al. 2016; Tesfay and Magwaza 2017).



(a) ***(b)*** ***(c)***

Fig. 25 *Variance within the UV absorbance of three different plant-based extracts at peak maximum. Ninefold determination (three films with three measurements conducted at random locations of each film). mA: milli absorbance units. (**a**): Thymus vulgaris (TV) extract; peak maximum at λ = 283 nm; (**b**): Aesculus hippocastanum (AEH) extract; peak maximum at λ = 274 nm; (**c**): Abies nordmanniana (AN) extract; peak maximum at λ = 275 nm.*



(a) ***(b)*** ***(c)***

Fig. 26 *Examples of cast PLA films including different concentrations of Abies nordmanniana extract, resulting in varying degrees of inhomogeneity and turbidity.*
*(**a**): F1 (0.2 mL extract per g PLA); (**b**): F4 (0.6 mL extract per g PLA); (**c**): F5 (1.0 mL extract per g PLA).*

6.3.1.3 Migration Analysis

In the following section, film analysis following EU regulations 10/2011 and 1935/2004 on food contact materials is evaluated. After selecting the ideal formulation (F2 for GE and AN extracts, F3 for TV and AEH extracts), bringing the dried films into contact with food simulants, and storing the sealed film samples for 10 days at different temperatures, the food simulants were removed from the film samples and analysed via GC–MS to evaluate the possible migration of extract components during storage. GC–MS methods appropriate for analysis of expected compounds were modified to improve higher sensitivity. Furthermore, as GC–MS was not suitable for detecting all extract components including macromolecular AEH and AN constituents (Havelt et al. 2019; Havelt et al. 2020), the samples were analysed by the photometric hyTAC method to determine whether antioxidants migrated into food simulants regardless of molecular weight. This method was capable of quantifying 17 mg Trolox equivalents per L, resembling the minimal contents of AEH or AN extracts. In the case of solid food simulant E, tenax, a solvent desorption method, was developed during pretests. Both analysis methods did not show any signals indicating migration, suggesting that no relevant migration has occurred. This is particularly reasonable for AEH and AN extracts as small molecules in general tend to diffuse more than molecules with a higher molecular weight; thus, a notably low migration is expected for AEH and AN extracts. Depending on their interactions with the PLA matrix, a higher migration rate could have been possible for TV and GE extracts. The findings are supported by the formulation experiments on TV extracts, which show that the limit of TV incorporation (based on optical properties) might not even be reached yet. Low migration of plant-based materials in PLA matrices, excluding highly concentrated essential oils, are observed in the literature as well (Souza et al. 2018; Gavril et al. 2019). Following the obtained results, all extracts are considered safe for food packaging when applied in the given dosage and matrix (European Comission 2011a). This does not only apply to short-term storage or storage at low temperatures, but also covers long-term storage at room temperature, considerably expanding the possible range of applications for bio-based stabilisers. As various studies report the good active packaging properties (the controlled release of active substances into packed foodstuff) of, for example, TV essential oils or GE extracts (Vasile et al. 2013; Sharma et al. 2020; Min et al. 2021), the actions of plant-based stabilisers are highly dependent on the type of incorporation within the material. Typically, extracts or essential oils are not directly incorporated into macroscopic plastic materials but are applied, for example, as parts of nanocomposites, nanofibers, or similar structures, thus promoting a release of the substances into the foodstuff instead of binding them within the packaging material (Vasile et al. 2013; Min et al. 2021). Furthermore, higher proportions

of extracts or highly concentrated essential oils are used (Sharma et al. 2020), again resulting in a higher migration as intended for active packaging applications.

6.3.2 Analysis of Extract Synergism

For practical applications, it is crucial to examine the interactions of stabilisers to avoid antagonistic effects in which the combination of two active substances results in an (partly) inhibition and an effect smaller than anticipated. Ideally, synergistic effects can be observed and utilised in which two active substances or extracts interact positively, resulting in observed effects that surpass the ones anticipated (Tsao 2015). Thus, synergism analysis displays whether the combination of active substances shows the same quantifiable effect as the sum of effects observed for the individual active substances suggests.

Section 6.3.2 focuses on the combination of extracts of different biomasses (cross-extract synergism, Section 6.3.2.1), and on the combination of extracts with different isolated active compounds (IAC) (IAC–extract synergism, Section 6.3.2.2). Each experiment was conducted by applying the different constituents in different relations to include dose–response variations. For evaluation, the anticipated value or "base value" (the calculated sum of the effect of two IACs or extracts) is shown and corrected by the positive (synergistic) or negative (antagonistic) effects observed in measurements.

6.3.2.1 Synergism of Extracts of Different Biomasses (Cross-Extract Synergism)

The combination of different extracts typically results in an altered TAC in both lipophilic and hydrophilic surroundings. **Figure 27** presents the measured liTAC values for different extracts combined in different concentrations while contrasting the results with the theoretical values obtained by mathematically adding up the relevant extracts' results.

For all ratios of the AN/TV combination, antagonistic effects were observed; however, an increasing share of TV extracts lowers the extent of antagonistic effects (−32.5% instead of −57.5%). This effect roughly sets all combinations to a similar TAC of approximately 2–2.5 mE mL^{-1}.

For TV/AEH combinations, both synergistic and antagonistic effects occurred, depending on the ratio of extracts. When AEH extracts overrode TV extracts, a synergistic effect of up to 61.5% was observed. Due to the high liTAC of sole TV extracts, combinations with high shares of TV extracts were still more antioxidant than the ones with low shares, even when being lowered by antagonistic effects of approximately −30.5%.

A comparable situation is observed when interpreting the results for AN/AEH combinations as quite high synergistic effects were measured for high ratios of AEH extracts, but these high synergistic

effects were still exceeded by combinations with high shares of AN extracts despite its base values being lowered by approximately 10%.

The trend of antagonistic effects rising to synergistic effects is continued for TP/AEH extracts. However, for this combination, such high antagonistic and synergistic effects (−67% to 159%) were met that the extracts with the highest base TAC (high TP combinations) were exceeded by high AEH combinations due to the high synergistic effect.

For TP/TV combinations, synergistic effects were observed for all combinations, with the effects increasing for higher shares of TV extracts. However, those combinations also show slightly lower base values, thus showing an aligning effect and resulting in approximately 6.5–7.5 mE mL^{-1} for all ratios.

For TP/AN combinations, synergistic effects were again found for all ratios, ranging from 30% to 98%. This resulted in an aligning effect for all high AN combinations at approximately 4.5-5.0 mE mL^{-1}.

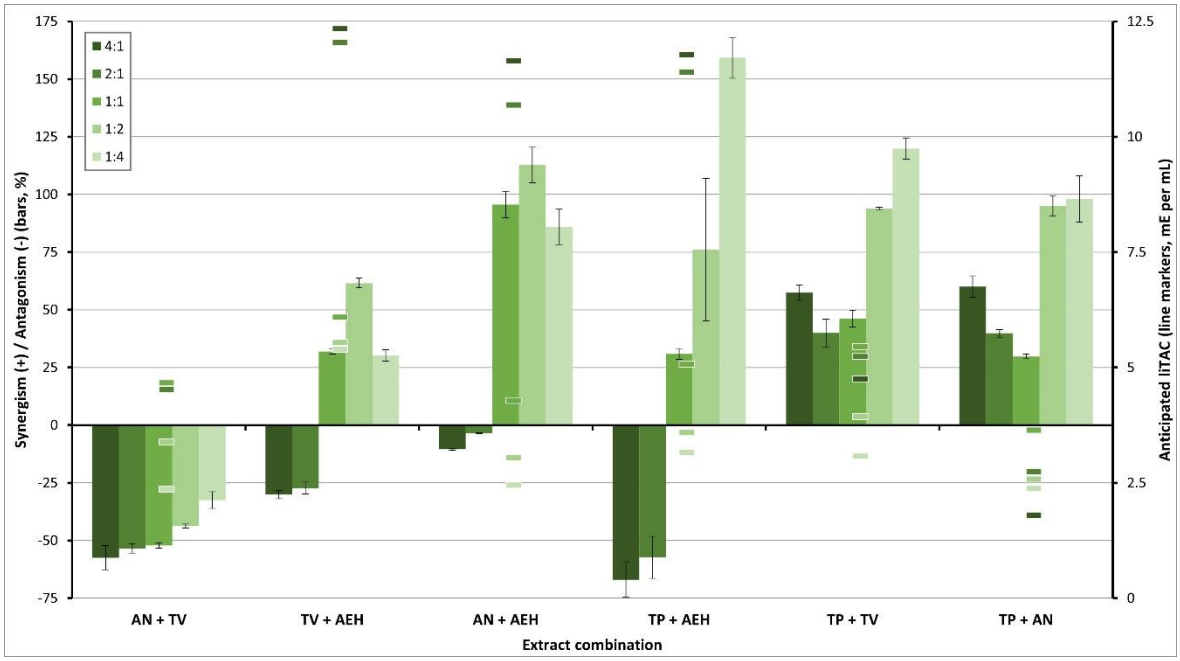


Fig. 27 *Base total antioxidant capacity (TAC) and determined synergistic effects of different plant-based extracts in combination, analysed via liTAC Assay. Primary ordinate (bars): synergistic (+) or antagonistic (−) effect of extract combinations (n = 3; standard deviation indicated via error bars). Secondary ordinate (horizontal markers): absolute base TAC, based on TAC determination of pure extracts (n = 6) and subsequent mathematical accumulation. AN: Abies nordmanniana extract; TV: Thymus vulgaris extract; AEH: Aesculus hippocastanum extract; TP: tomato paste extract; mE: milli extinction units.*

In most cases, so-called aligning effects were observed during which high base values were lowered by antagonistic effects while low base values were raised by synergistic effects, resulting in roughly comparable absolute values after all. Occasionally, however, such as for TP/AEH, synergistic effects changed the base liTAC to an extent that influenced the order of the highest liTAC results, making

combinations more desirable which have not been expected as such. The highest absolute liTAC, including antagonistic and synergistic effects, was reached by high AN combinations of AN/AEH extracts, resulting in approximately 10.3 mE mL^{-1}.

As the main constituents of AN and TV extracts are medium-weight proanthocyanidins and thymol, respectively (Basch et al. 2004; Havelt and Schmitz 2018; Havelt et al. 2020; Kowalczyk et al. 2020), it is plausible to assume that those substances are the main contributors to the observed antagonistic effects. The combination of TV and AEH extracts (which again include proanthocyanidins, but with a much higher molecular mass (Havelt et al. 2019)), however, can result in synergistic effects when AEH extracts are included in even or higher shares. This applies to all AEH combinations. As AEH extracts are the only ones including high-molecular compounds, it is plausible to assume that high-molecular and low-molecular substances tend to produce synergistic effects when combined, as long as high-molecular compounds are added in excess. In other contexts, such synergistic effects of substances with different molecular weights have been discussed in the literature (Coelho et al. 2011).

Furthermore, it was observed that it is possible, or even granted, for most combinations to create desired synergistic effects, particularly for combinations including TP extracts with its main constituents, lycopene and β-carotene (Baysal et al. 2000; Periago et al. 2004; Choksi and Joshi 2007). Generally, lycopene and β-carotene seem to be the most potent low-molecular active compounds observed in this experiment. Thus, it is plausible to see the highest synergistic effect observed at all for TP/AEH extracts where both observed positive relations (presence of lycopene; contrast of high-molecular and low-molecular compounds with high-molecular compounds in excess) are applied.

The synergisms determined in hydrophilic surroundings, as depicted in **Figure 28**, substantially deviate from the ones observed in lipophilic surroundings. However, as the behaviour of antioxidants is dependent on the surroundings, changes in synergism are expected.

For AN/TV, only limited synergistic effects were observed for high-AN combinations, while a 1:1 mixture of both extracts resulted in a comparably high antagonistic effect. High-TV combinations roughly resemble the base antioxidant capacity already anticipated. The highest synergistic effect was thus reached by the combination with the already highest base antioxidant effect, resulting in a maximum observed TAC of approximately 600 µg TEq mL^{-1}. TV/AEH combinations showed a synergistic effect of up to +57% for 1:1 and high-TV combinations, while showing negligible antagonistic effects (approximately −7%) for high-AEH combinations. Including synergistic effects, 1:1 and high-TV mixtures thus showed maximum TAC values of 760 to 807 µg TEq mL^{-1}. AN/AEH combinations showed synergistic effects only, reaching up to +72%. The combination of high

synergistic effects and/or absolute TAC values of single components resulted in maximum TAC values of approximately 680 µg TEq mL^{-1} for high-AN mixtures. Roughly comparable to liTAC results, TP/AEH combinations show a rising synergistic effect with increasing shares of AEH extracts, ranging from −41% to +235%, which is the highest cross-extract synergistic effect in hydrophilic surroundings. Due to comparably low absolute TAC values of those high-AEH combinations, a 1:1 mixture was preferred, resulting in nearly 600 mg TEq mL^{-1}. For TP/TV combinations, only synergistic effects were observed, ranging from +3% to +56% and increasing with a rising share of TV extract. However, as the base TAC values decreased with an increasing amount of included TV extract, an aligning effect was created. The high base TAC value of the combination with the highest share of TP extract was preferred over the synergistic effects of other combinations in this case, resulting in a maximum TAC of approximately 380 µg TEq mL^{-1}. For TP/AN combinations, both neglectable effects (for 1:1 and (TP) 4:1 (AN)) and synergistic effects (for other combinations) of approximately +60% to +73% were observed. Due to comparably low absolute TAC values, only a maximum TAC of 215 µg TEq mL^{-1} was reached by (TP) 2:1 (AN) mixture.

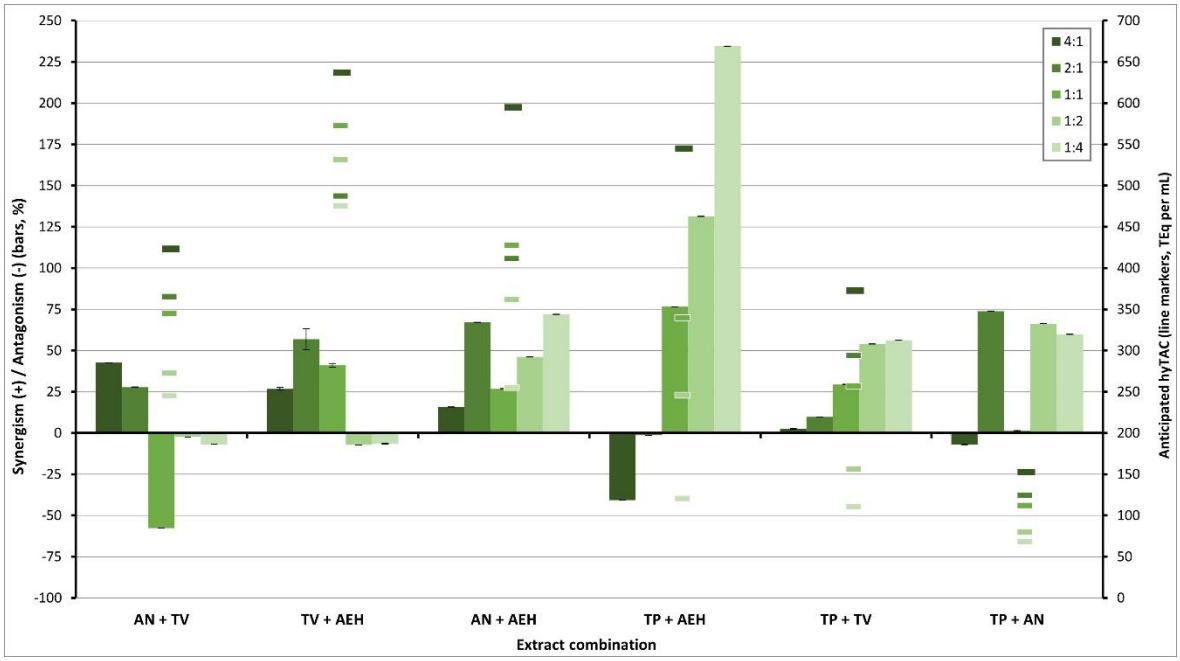


Fig. 28 *Base total antioxidant capacity (TAC) and determined synergistic effects of different plant-based extracts in combination, analysed via hyTAC Assay. Primary ordinate (bars): synergistic (+) or antagonistic (−) effect of extract combinations (n = 3; standard deviation indicated via error bars). Secondary ordinate (line markers): absolute base TAC, based on TAC determination of pure extracts (n = 6) and subsequent mathematical accumulation. AN: Abies nordmanniana extract; TV: Thymus vulgaris extract; AEH: Aesculus hippocastanum extract; TP: tomato paste extract; TEq: Trolox equivalents.*

Generally, high antioxidant capacity values of up to 600–800 µg TEq mL^{-1} were observed, particularly reached by combinations with AEH extracts and the AN/TV combination. In this experiment, even higher synergistic effects have been observed than in lipophilic surroundings;

however, these effects could not always overcome smaller absolute TAC values. Fortunately, antagonistic effects occur at a very low rate.

In both lipophilic and hydrophilic surroundings, maximum synergistic effects were observed for AEH/TP combinations, especially when AEH extracts were included in equal or higher shares than TP extracts. AEH extracts contain hydrophilic active substances (proanthocyanidins) (Havelt et al. 2019), and thus show a much higher antioxidant potential in hydrophilic surroundings, while TP extracts are particularly effective in lipophilic surroundings due to the included lipophilic active compound lycopene, as determined in pretests and as discussed in the literature as well (Giovannucci 1999; Rao 2004). Thus, it can be concluded that synergistic effects are increased or are more likely to occur when active substances with opposite mechanisms of action are combined. Such an assumption is supported by Graßmann, where the synergistic effects, especially of combinations of hydrophilic and lipophilic compounds, are observed (Graßmann 2005). Furthermore, the suggestion of molecular weight having an impact on synergistic effects is plausible as it would again show that different types of antioxidant mechanisms tend to enhance each other. However, there are much more influential factors determining whether a synergistic effect is observed which are partly presented, including, but not limited to, the ratio of extracts and the type of reaction surroundings.

6.3.2.2 Synergism of Extracts Combined with Isolated Active Compounds (IAC-Extract Synergism)

In addition to different bio-based extracts analysed in combination to reveal potential synergistic effects, the same extracts were combined with active substances with antioxidant effects but different chemical properties, as displayed in **Figure 29** and **Figure 30**. In the experiment setup, all four extracts (AEH, TV, AN, and TP) were crossed with the active compounds ascorbic acid (AA), gallic acid (GA), quercetin (Qu), thymol (Th) and α-tocopherol (To) in two different concentrations. Synergistic effects observed in this context could result in new insights on how different groups of chemicals interact in regard to antioxidant properties, they could hint at extract optimisation (particularly in Th and To combinations as both compounds are found in analysed extracts as well), and they could indicate further worthwhile opportunities of bio-based antioxidant extraction. In general, the influence of IACs is small for low concentrations, and naturally increases with adding higher concentrations (without considering synergistic and antagonistic effects). Thus, synergistic effects observed for lower concentrations of IACs are of a higher direct relevance as the base values show a limited distribution. For higher concentrations, the distribution of base values typically increases, resulting in a more complex situation for synergism interpretation.

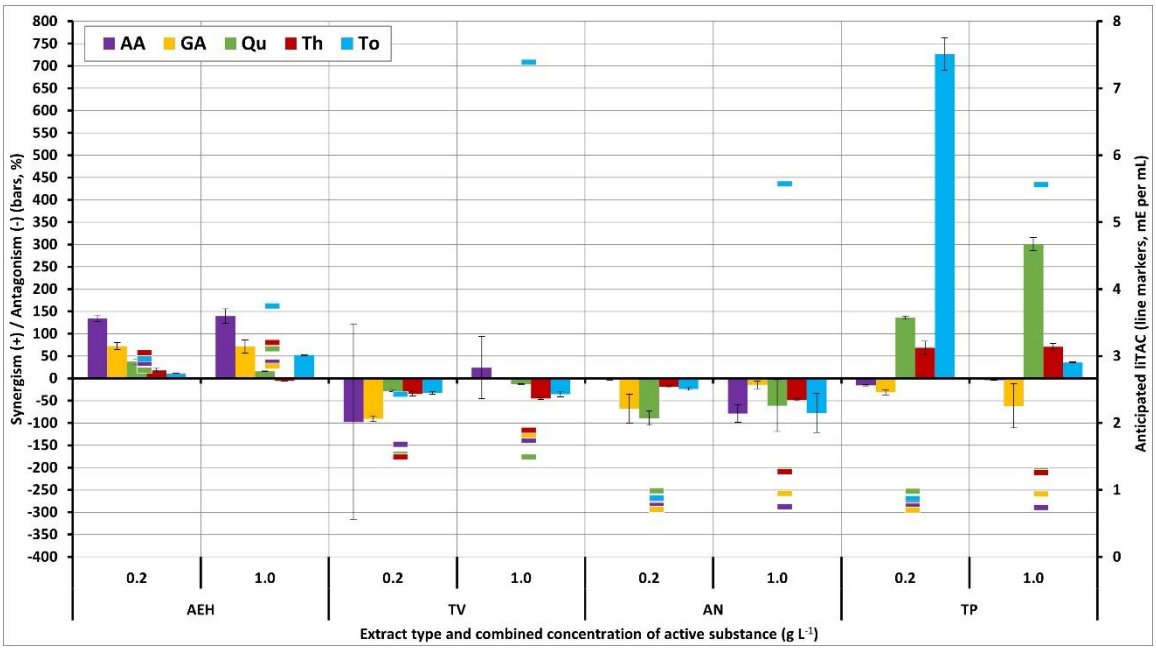


Fig. 29 *Base total antioxidant capacity (TAC) and determined synergistic effects of different plant-based extracts in combination with active standard substances, analysed via liTAC Assay. Primary ordinate (bars): synergistic (+) or antagonistic (−) effect of extract combinations (n = 3; standard deviation indicated via error bars). Secondary ordinate (line markers): absolute base TAC, based on TAC determination of pure extracts/standard solutions (n = 6) and subsequent mathematical accumulation. AA: ascorbic acid; GA: gallic acid; Qu: quercetin; Th: thymol; To: α-tocopherol; AN: Abies nordmanniana extract; TV: Thymus vulgaris extract; AEH: Aesculus hippocastanum extract; TP: tomato paste extract; mE: milli extinction units.*

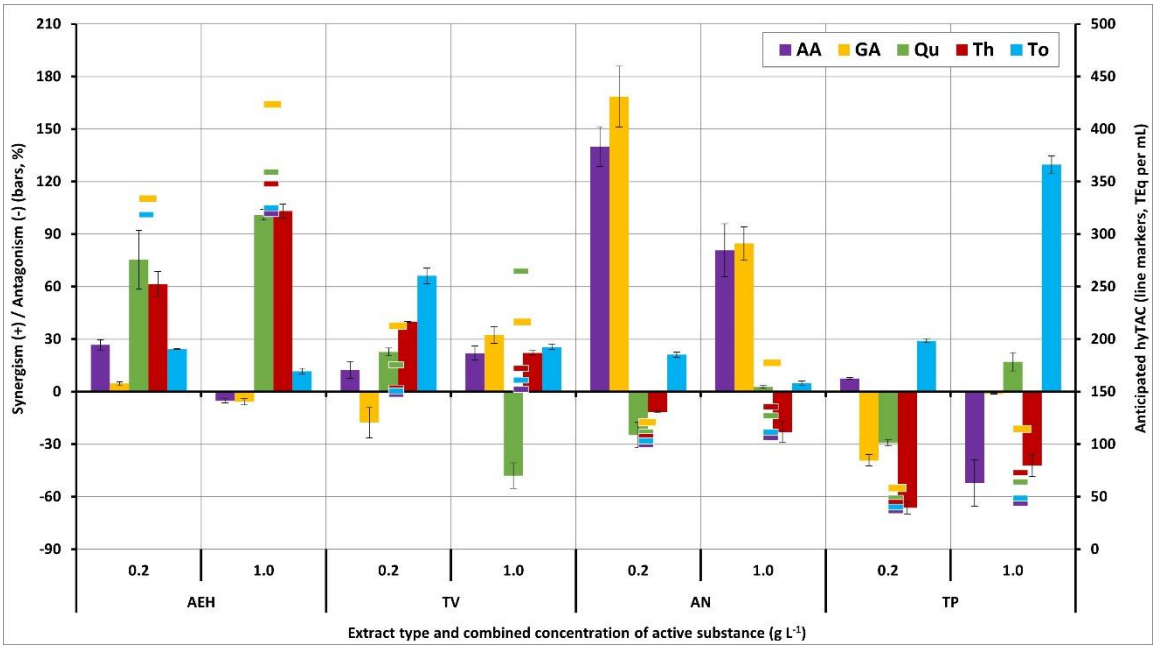


Fig. 30 *Base total antioxidant capacity (TAC) and determined synergistic effects of different plant-based extracts in combination with active standard substances, analysed via hyTAC Assay. Primary ordinate (bars): synergistic (+) or antagonistic (−) effect of extract combinations (n = 3; standard deviation indicated via error bars). Secondary ordinate (line markers): absolute base TAC, based on TAC determination of pure extracts/standard solutions (n = 6) and subsequent mathematical accumulation. AA: ascorbic acid; GA: gallic acid; Qu: quercetin; Th: thymol; To: α-tocopherol; AN: Abies nordmanniana extract; TV: Thymus vulgaris extract; AEH: Aesculus hippocastanum extract; TP: tomato paste extract; TEq: Trolox equivalents.*

Figure 33 shows the observed and anticipated antioxidant effects in lipophilic surroundings. AEH extracts in general did not show relevant antagonistic effects (maximum −6.0% for the high-concentrated (hc) Th combination), while synergistic effects of up to +139.5% were reached for combinations with low concentrations (lc) of AA, GA, and Qu as well as for AA-hc, GA-hc, and To-hc. The synergism observed for To combinations hints at a linear correlation, as the synergistic effect increases by the factor of approximately 5 when increasing the concentration of To by the same factor. For both high and low concentrations, ascorbic acid proved the highest synergistic effect, which is comparable for both concentrations. A maximum liTAC of ca. 6.7 to 6.9 mE mL^{-1} was reached by AEH/AA combinations for both high and low concentrations.

For TV extracts, high and medium antagonistic effects were observed for most combinations. However, TV/AA-hc showed a limited synergistic effect of +23.6%. Due to this synergistic effect, TV/AA-hc reached the maximum TV–liTAC value of approximately 2.2 mE mL^{-1}, while other combinations, especially with low concentrations of AA and GA, should be avoided as they lead to a high-grade reduction in liTAC. However, TV/AA combinations are subject to comparably high scattering.

AN extract combinations show antagonistic effects only. The best effect is obtained by combining AN and AA-lc, resulting in an antagonistic effect of −2.6%. For other combinations, especially for Qu-lc, AA-hc, To-hc, GA-lc, and Qu-hc, antagonistic effects from circa −60% to −88.9% were observed. Thus, the highest lc-liTAC value of approximately 0.75 mE mL^{-1} was reached by AN/AA-lc, with other values being even more neglectable. For high concentrations, the highest liTAC was observed for AN/To due to its high base value of 5.6 mE mL^{-1}; however, this value decreased to approximately 1.3 mE mL^{-1} due to high antagonistic effects.

For TP extracts, most combinations resulted in synergistic effects with relevant antagonistic effects observed for AA and GA only (up to −61.7%). However, the synergistic effects reached remarkably high quantities with +726.4% (To-lc), followed by +300.8% (Qu-hc), +231.4% (GA-lc), and +135.7% (Qu-lc). These extraordinary synergistic effects resulted in record liTAC values of up to 8.1 mE mL^{-1} (TP/To-lc) for low IAC concentrations, while the highest absolute liTAC for high concentrations was reached by TP/To-hc (7.6 mE mL^{-1}), which showed a comparably low synergistic effect of +35.8%, but an already high base value of 5.6 mg mE^{-1}.

In general, TP and AEH extracts are thus preferred in lipophilic surroundings, while TV and AN extracts show a high risk of showing unintended antagonistic effects. When applying targeted combinations of, for example, TP extracts and α-tocopherol or AEH extracts and ascorbic acid, both exceptionally high synergistic effects and absolute antioxidant potentials can be reached. High synergistic effects in combinations of α-tocopherol and lycopene, which is found in tomato paste

(Giovannucci 1999; Rao 2004), are confirmed by (Shi et al. 2004; Zanfini et al. 2010). Furthermore, β-carotene (which is also a constituent of tomato paste (Baysal et al. 2000)) is known to show synergistic effects when combined with α-tocopherol in lipophilic surroundings and thus contributing to the observed synergism (Palozza and Krinsky 1992). The determined synergistic effect of tomato extracts and quercetin increasing with rising quercetin concentrations is supported by Graßmann as well (Graßmann 2005). The observed antagonistic effects cannot be explained in detail, but are supported by the literature, for example, Hras et al. and Yin et al. reported antagonistic effects for combinations of α-tocopherol and some (poly)phenols (as included in TV, AN and AEH extracts) (Hras et al. 2000; Yin et al. 2012). However, a comprehensive explanation for the antagonistic effect of some, but not all, polyphenols has not yet been found despite both synergistic and antagonistic effects of combined plant extracts being observed frequently, as disclosing the underlying mechanisms is particularly challenging (Wang et al. 2011a).

Figure 34 shows the synergistic and antagonistic effects observed for extract/IAC combinations in hydrophilic surroundings. As in lipophilic surroundings, synergistic effects are predominant for AEH combinations as antagonistic effects were observed at −5.8% at maximum (To-hc), while synergistic effects reached from +101.0 to +103.0% for Qu-hc and Th-hc combinations; both combinations showed comparably high synergistic effects in low concentrations as well (+75.4%; +61.4%), which resulted in a maximum hyTAC value of approximately 556 µg TEq mL^{-1} (Qu-lc). For high concentrations, both quercetin and thymol showed comparably high hyTAC values of approximately 721 and 706 µg TEq mL^{-1}.

TV extract combinations show lower synergistic, and increased, but still low, antagonistic effects. The synergistic effects still clearly dominate the results, with up to +66.2% (To-lc), while GA-lc and Qu-hc combinations resulted in antagonistic effects from −17.6 to −48.0%. Overall, a slight aligning effect was observed. Due to its comparably high synergistic effect, TV/To-lc resulted in the maximum hyTAC of approximately 250 µg TEq mL^{-1} for low IAC concentrations, while high concentration combinations resulted in maximum 286 µg TEq mL^{-1} for TV/GA-hc.

For AN extract combinations, low synergistic and antagonistic effects between −24.8 and +21.1% were observed for quercetin, thymol, and α-tocopherol in both concentrations. For ascorbic acid and gallic acid, high synergistic effects were measured in both concentrations with lc-synergisms reaching the highest observed values observed in the hyTAC-experiment (AA-lc: +140.0%; GA-lc: +168.6%; AA-hc: +80.7%; GA-hc: +84.6%). Thus, the maximum hyTAC-values reached by AN/GA combinations for both concentrations were similar, resulting in approximately 325 µg TEq mL^{-1} for low and approximately 328 µg TEq mL^{-1} for high concentrations.

TP extract combinations showed a prevalence for strong antagonistic effects of up to −66.3% (Th-lc). As in lipophilic surroundings, the highest synergistic effects for TP combinations were reached by introducing α-tocopherol, resulting in +29.0% (lc) and +129.7% (hc). The maximum hyTAC reached for low concentrations was approximately 52 µg TEq mL^{-1} (To-lc), while hc combinations obtained up to approximately 113 µg TEq mL^{-1} by applying GA without utilising relevant synergistic effects or approximately 112 µg TEq mL^{-1} by taking advantage of high synergistic effects of α-tocopherol.

Overall hyTAC evaluation showed that individual AN extract combinations show the highest synergistic effects, followed by TP and AEH extract combinations. It is remarkable that AN and AEH extracts, which have comparable ingredients, both consistently and highly constructively interact with two IACs each when both pairs of IACs are different. However, when evaluating the overall hyTAC results, AEH extract combinations show the highest hyTAC values by far, approximately doubling the second-best values reached by AN and TV extracts. Outstandingly, α-tocopherol interacts with the extracts and results in positive synergistic effects for all tested combinations in hydrophilic surroundings, making it a promising all-rounder to add to the formulation. This is supported by the literature in which, in the context of in vivo antioxidant efficacy, the combined use of vitamins and phenolic acids is recommended by (Wang et al. 2011b). Comparable antagonistic effects have been reported by (Becker et al. 2007) who observed antagonistic effects for combinations of quercetin and astaxanthin, a carotenoid with structural similarities to lycopene and β-carotene, which are included in TP extracts (Giovannucci 1999; Baysal et al. 2000; Rao 2004).

It is also notable that the results seem to be highly dependent on the properties of the surroundings as the IAC/extract synergism results highly deviate from each other when changing from lipophilic to hydrophilic surroundings; this effect is less striking for cross-extract synergism. However, depending on the added IAC, AEH extracts are a potent base for combinations in both surroundings. The observed synergistic effects of AEH and AN extracts in combination with AA are supported by the literature, as synergistic effects for combinations of different polyphenols and ascorbic acid are described by (Murakami et al. 2003). It is also observed that the addition of quercetin (Qu) can lead to synergistic effects, as observed by (Heo et al. 2007). In agreement with previous proposals, the effect is observed particularly in combination with AEH extract (combining high-molecular and low molecular compounds) or with TP extract (combining different polarities of active compounds).

6.4 Conclusions

In general, plant materials are complex samples that are subject to fluctuations resulting from, for example, different environmental influences on individual plants, causing altered chemical interactions within the plants. Nevertheless, this study successfully demonstrated the feasibility of producing bio-based alternatives to common petrol-based antioxidants and photostabilisers, and effectively incorporating them into plastic packaging. The tests also showed that the stabilisers are still effective when added to their required surroundings. All four analysed plant extracts can be incorporated into PLA packaging films at different concentrations with the obtained films showing different stabilising effects. Furthermore, concerns regarding health and safety of the films are rebutted as migration studies did not show any anomalies. It is nevertheless recommended for further studies to evaluate the use of food-safe solvents and extractants, such as water and ethanol instead of methanol and heptane, to fully eliminate a possible health risk. Furthermore, the conducted synergism analyses successfully highlighted several possibilities to increase antioxidant effects, some of which are already showing high synergistic effects for cross-extract synergism or IAC/extract synergism, with only low concentrations of active substances added. This allows for a further optimisation of environmentally friendly solutions for biobased stabilisers. However, the entire underlying mechanisms of synergisms still need to be uncovered. In addition to the findings already discussed, it is worth noting that an addition of thymol to thyme extracts can synergistically improve the antioxidant effects, suggesting that an upconcentration of thyme extracts could be a worthwhile approach to disproportionately increase the antioxidant effect. However, this approach needs to be verified, as cross-extract synergism analysis shows divergent results, thus suggesting that thyme extracts act notably different than the IAC thymol, regardless of thymol being the main ingredient (Havelt and Schmitz 2018). Furthermore, synergism analysis clearly indicates that tomato paste extracts could be a promising renewable resource for antioxidants as TP combinations show remarkable overall synergistic effects. Based on the results of TP combined with α-tocopherol, the combination of TP and GE extracts could be of special interest as GE extracts serve as a renewable source of α-tocopherol (Havelt et al. 2021b). The present study successfully provides important impulses for proceeding works, especially for those pursuing comparative industrial implementation and synergism mechanism break down. For this purpose, promising combinations should be analysed in various concentrations to receive a more detailed view.

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6.7 Copyright

7. Conclusions

The previous chapters showed how plant biomasses can be used to create sustainable plastic stabilisers. The different analyses on Common Thyme, European Horse Chestnut, Coniferous Woods and Snowdrops and their application in Poly-Lactic Acid (PLA) films for food contact prove that the bio-based stabilisation of plastic materials is possible. However, this is naturally dependend on the application and the type of biomass used for stabilisation.

The most potent snowdrop species *Galanthus elwesii*, for example, shows an extraordinarily high antioxidant capacity in lipophilic extracts, making it an appropriate stabiliser for proper applications. Thyme extracts, specifically the ones prepared from *Thymus vulgaris* 'Varico 3', show good, but not outstandingly good results in all analysed aspects like UV absorbance, antimicrobial activity and antioxidant capacity in both hydrophilic and lipophilic surroundings, making Varico 3 extracts an all-rounder for most applications. The use of Thyme also allows the use of high-concentrated Essential Oils instead of extracts to further increase the observed effects. Extracts from European Horse Chestnut Seed Coat show a remarkable UV absorbance and antioxidant capacity in hydrophilic extracts, a particularly high average molar mass (reducing the risk of migration into packed goods), and provide the opportunity to use biological waste materials which – in contrast to Thyme and Snowdrops – have not to be grown specifically for the use as bio-based stabiliser resources, promoting a circular economy. The properties of Coniferous Wood extracts, prepared e.g. from Nordmann firs, roughly resemble the ones observed for Horse Chestnut Seed Coats as both biomasses include macromolecular proanthocyanidins. However, Coniferous Wood extracts, particularly extracts from used Christmas trees, provide an extensive opportunity for a comprehensive valorisation of waste materials.

Overall, the different properties of all analysed plant resources allow the formulation to be adjusted specifically to the respective application while no migration risks are expected. Furthermore, the efficiency of extracts can be increased by exploiting synergistic effects via combinations of extracts and/or isolated compounds as described before. Utilising the different coherent techniques, innovative approaches and novel results presented allows the substitution of petrol-based stabilisers by stabilisers made of plants and, partially, plant waste and thus successfully expedites the process of transformation into a circular economy.

8. References

Aeschbach, R.; Löliger, J.; Scott, B. C.; Murcia, A.; Butler, J.; Halliwell, B.; Aruoma, O. I. (1994): Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. In *Food and Chemical Toxicology* 32 (1), pp. 31–36. DOI: 10.1016/0278-6915(84)90033-4.

Agentur für Erneuerbare Energien (1/9/2015): Weihnachtsbäume liefern grüne Energie. Alexander Knebel. Available online at https://www.unendlich-viel-energie.de/presse/pressemitteilungen/weihnachtsbaeume-liefern-gruene-energie, checked on 1/22/2020.

Ahmed, Jasim; Mulla, Mehrajfatema; Arfat, Yasir Ali (2017): Application of high-pressure processing and polylactide/cinnamon oil packaging on chicken sample for inactivation and inhibition of Listeria monocytogenes and Salmonella Typhimurium, and post-processing film properties. In *Food Control* 78 (1), pp. 160–168. DOI: 10.1016/j.foodcont.2017.02.023.

Altaf, U.; Kanojia, V.; Rouf, A. (2018): Novel packaging technology for food industry. In *Journal of Pharmoacognosy and Phytochemistry* (7(1)), pp. 1618–1625, checked on 24.04.18.

JIS Z 2801, 2010: Antibacterial products - Test for antibacterial activity and efficacy.

Armenta-Arteaga, Gustavo; Elizalde-González, María P. (2003): Contamination by PAHs, PCBs, PCPs and Heavy Metals in the Mecoacán Lake Estuarine Water and Sediments after Oil Spilling. In *Journal of Soils and Sediments* 3 (1), pp. 35–40. DOI: 10.1065/jss2003.02.067.

Bağcı, Eyüp; Dığrak, Metin (1996): Antimicrobial Activity of Essential Oils of some Abies (Fir) Species from Turkey. In *Flavour and Fragrance Journal* 11, pp. 251–256.

Basch, Ethan; Ulbricht, Catherine; Hammerness, Paul; Bevins, Anja; Sollars, David (2004): Thyme (Thymus vulgaris L.), Thymol. In *Journal of Herbal Pharmacotherapy* 4 (1), pp. 49–67. DOI: 10.1080/J157v04n01_07.

Bati Ay, Ebru; Gul, Melek; Acikgoz, M. Akif; Yarilgac, Tarik; Kara, S. Metin (2018): Assessment of Antioxidant Activity of Giant Snowdrop (Galanthus elwesii Hook) Extracts with Their Total Phenol and Flavonoid Contents. In *IJPER* 52 (4s), s128-s132. DOI: 10.5530/ijper.52.4s.88.

Bava, Martina; Arnoldi, Sebastiano; Dell'Acqua, Lucia; Fontana, Sergio; La Forgia, Flavia; Mustich, Giuseppe et al. (2015): Quali-Quantitative Analysis by LC/DAD and GPC of the Polyphenols of “Uva Di Troia Canosina” Grape Seeds for the Development of an Industrial Nutraceutical Product. In *J Chromatogr Sep Tech* 06 (03). DOI: 10.4172/2157-7064.1000266.

Baysal, T.; Ersus, S.; Starmans, D. A. (2000): Supercritical CO2 extraction of beta-carotene and lycopene from tomato paste waste. In *Journal of agricultural and food chemistry* 48 (11), pp. 5507–5511. DOI: 10.1021/jf000311t.

Becker, Eleonora Miquel; Ntouma, Georgia; Skibsted, Leif H. (2007): Synergism and antagonism between quercetin and other chain-breaking antioxidants in lipid systems of increasing structural organisation. In *Food chemistry* 103 (4), pp. 1288–1296. DOI: 10.1016/j.foodchem.2006.10.034.

Bonnet, M. (2016): Kunststofftechnik. Grundlagen, Verarbeitung, Werkstoffauswahl und Fallbeispiele. 3., überarb. u. erw. Aufl. 2016. Wiesbaden: Springer Fachmedien Wiesbaden GmbH; Springer Vieweg (Lehrbuch).

Bundesamt für Strahlenschutz (2021): Was ist UV-Strahlung? Edited by Bundesamt für Strahlenschutz. Available online at https://www.bfs.de/DE/themen/opt/uv/einfuehrung/einfuehrung_node.html, updated on 7/8/2021, checked on 10/21/2021.

Buskey, Edward J.; White, Helen K.; Esbaugh, Andrew J. (2016): Impact of Oil Spills on Marine Life in the Gulf of Mexico. Effects on Plankton, Nekton, and Deep-Sea Benthos. In *Oceanography* 29 (3), pp. 174–181. Available online at https://www.jstor.org/stable/24862719.

Camacho-Cruz, L. A.; Velazco-Medel, M. A.; Cruz-Gómez, A.; Bucio, E. (2021): Antimicrobial Polymers. In Inamuddin, Mohd Imran Ahamed, Ram Prasad (Eds.): Advanced Antimicrobial Materials and Applications. Singapore: Springer Singapore, pp. 1–42.

Cataldo, Franco (2001): On the ozone protection of polymers having non-conjugated unsaturation. In *Polymer Degradation and Stability* 72, pp. 287–296.

Charles, Denys J. (2013a): Antioxidant Assays. In Denys J. Charles (Ed.): Antioxidant Properties of Spices, Herbs and Other Sources. New York, NY: Springer New York, pp. 9–38.

Charles, Denys J. (2013b): Natural Antioxidants. In Denys J. Charles (Ed.): Antioxidant Properties of Spices, Herbs and Other Sources. New York, NY: Springer, pp. 39–64.

Charles, Denys J. (2013c): Thyme. In Denys J. Charles (Ed.): Antioxidant Properties of Spices, Herbs and Other Sources. New York, NY: Springer New York, pp. 553–561.

Chevolleau, S.; Debal, A.; Ucciani, E. (1992): Determination of the antioxidant activity of plant extracts. In *Rev. Fr. Corps* 39, pp. 120–126.

Choi, Sung-Seen; Im, Song-Hee; Park, Je-Hwan; Kim, Jae Seong (2009): Analysis of wax solubility of rubber vulcanizates using wax solution in toluene and molten wax. In *Polymer Testing* 28 (7), pp. 696–701. DOI: 10.1016/j.polymertesting.2009.05.012.

Choksi, Pratik M.; Joshi, Vishal Y. (2007): A Review on Lycopene — Extraction, Purification, Stability and Applications. In *International Journal of Food Properties* 10 (2), pp. 289–298. DOI: 10.1080/10942910601052699.

Cirillo, Giuseppe; Curcio, Manuela; Spataro, Tania; Picci, Nevio; Restuccia, Donatella; Iemma, Francesca; Spizzirri, Umile G. (2018): Antioxidant Polymers for Food Packaging. In Alexandru Mihai Grumezescu, Alina Maria Holban (Eds.): Food packaging and preservation. London: AP Academic Press an imprint of Elsevier (Handbook of Food Bioengineering, Volume 9), pp. 213–238.

Co, Michelle; Fagerlund, Amelie; Engman, Lars; Sunnerheim, Kerstin; Sjöberg, Per J. R.; Turner, Charlotta (2011): Extraction of antioxidants from spruce (Picea abies) bark using eco-friendly solvents. In *Phytochemical analysis : PCA* 23 (1), pp. 1–11. DOI: 10.1002/pca.1316.

Coelho, Elisabete; Reis, Ana; Domingues, M. Rosário M.; Rocha, Sílvia M.; Coimbra, Manuel A. (2011): Synergistic effect of high and low molecular weight molecules in the foamability and foam stability of sparkling wines. In *Journal of agricultural and food chemistry* 59 (7), pp. 3168–3179. DOI: 10.1021/jf104033c.

Coppola, Daniela (2019): Plastic Waste in Europe. A Statista Dossierplus on Waste, Recycling, and the Future of Plastic Material in Europe. Edited by Statista. Statista. Available online at https://www.statista.com/study/67448/plastic-waste-in-europe/, checked on 10/22/2021.

Cosentino, S.; Tuberoso, C. I. G.; Pisano, B.; Satta, M.; Mascia, V.; Arzedi, E.; Palmas, F. (1999): In-vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. In *Lett Appl Microbiol* 29 (2), pp. 130–135. DOI: 10.1046/j.1472-765X.1999.00605.x.

Czochanska, Z.; Foo, L. Y.; Newman, R. H.; Porter, L. J. (1980): Polymeric proanthocyanidins. Stereochemistry, structural units, and molecular weight. In *J. Chem. Soc., Perkin Trans. 1*, pp. 2278–2286. DOI: 10.1039/P19800002278.

Dandlen, S. Anahi; Lima, A. Sofia; Mendes, Marta D.; Miguel, M. Graça; Faleiro, M. Leonor; Sousa, M. João et al. (2010): Antioxidant activity of six Portuguese thyme species essential oils. In *Flavour and Fragrance Journal* 25 (3), pp. 150–155. DOI: 10.1002/ffj.1972.

Dauqan, Eqbal M. A.; Abdullah, Aminah (2017): Medicinal and Functional Values of Thyme (Thymus vulgaris L.) Herb. In *Journal of Applied Biology & Biotechnology* 5 (2). DOI: 10.7324/JABB.2017.50203.

Deans, S. G.; Ritchie, G. (1987): Antibacterial properties of plant essential oils. In *International Journal of Food Microbiology*, pp. 165–180.

Demirbas, Ayhan; Omar Al-Sasi, Basil; Nizami, Abdul-Sattar (2017): Recent volatility in the price of crude oil. In *Energy Sources, Part B: Economics, Planning, and Policy* 12 (5), pp. 408–414. DOI: 10.1080/15567249.2016.1153751.

Detzel, Andreas; Kauertz, Benedikt; Derreza-Greeven, Cassandra; Reinhardt, Joachim; Kunze, Sybille; Krüger, Martina et al. (2012): Endbericht Untersuchung der Umweltwirkungen von Verpackungen aus biologisch abbaubaren Kunststoffen. Edited by Federal Environmental Agency of Germany. Federal Environmental Agency of Germany. Dessau-Roßlau. Available online at http://www.uba.de/uba-info-medien/3986.html, checked on 4/15/2020.

Domke, A.; Großklaus, R.; Niemann, B.; Przyrembel, H.; Richter, K.; Schmidt, E. et al. (2005): Use of vitamins in foods. Toxicological and nutritional-physiological aspects - Part 1. Berlin: Bundesinstitut für Risikobewertung (BfR-Wissenschaft, 2005,4).

Eckert, Werner (2012): Wenn der Treibstoff der Welt versiegt, Teil I. Öl - bald ein Luxusgut? In *Tagesschau*, 7/3/2012. Available online at https://www.tagesschau.de/wirtschaft/oelknappheit100.html, checked on 10/22/2012.

Edreva, A.; Velikova, V.; Tsonev, T.; Dagnon, S.; Gürel, A.; Aktas, L.; Gesheva, E. (2008): Stress-protective Role of Secondary Metabolites: Diversity of Functions and Mechanisms. In *Gen Appl Plant Physiology* 34 (1-2), pp. 67–78.

Elmezughi, Jamal; Shittu, Hafsat; Clements, Carol; Edrada-Ebel, Ru Angelie; Seidel, Veronique; Gray, Alexander (2013): Bioactive natural compounds from Prosopis africana and Abies nobili. In *J. App. Pharm. Sci.* DOI: 10.7324/JAPS.2013.30308.

El-Obeid, Tahra; Yehia, Hany M.; Sakkas, Hercules; Lambrianidi, Louisa; Tsiraki, Maria I.; Savvaidis, Ioannis N. (2018): Shelf-life of smoked eel fillets treated with chitosan or thyme oil. In *International journal of biological macromolecules* 114, pp. 578–583. DOI: 10.1016/j.ijbiomac.2018.03.125.

Emmons, Cheryld L.; Peterson, David M. (2001): Antioxidant Activity and Phenolic Content of Oat as Affected by Cultivar and Location. In *Crop Science* 41 (6), p. 1676. DOI: 10.2135/cropsci2001.1676.

Erdohan, Zekiye Özge; Çam, Belgizar; Turhan, Kamile Nazan (2013): Characterization of antimicrobial polylactic acid based films. In *Journal of Food Engineering* 119 (2), pp. 308–315. DOI: 10.1016/j.jfoodeng.2013.05.043.

Erel, Ozcan (2004): A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. In *Clinical biochemistry* 37 (4), pp. 277–285. DOI: 10.1016/j.clinbiochem.2003.11.015.

Erkan, Nuray (2012): The Effect of Thyme and Garlic Oil on the Preservation of Vacuum-Packaged Hot Smoked Rainbow Trout (Oncorhynchus mykiss). In *Food Bioprocess Technol* 5 (4), pp. 1246–1254. DOI: 10.1007/s11947-010-0412-7.

European Comission (2004): Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. In *Official Journal of the European Union*. Available online at https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R1935&from=DE, checked on 7/6/2020.

European Comission (2011a): COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. In *Official Journal of the European Union* 12 (1). Available online at https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0010&from=EN, checked on 7/31/2018.

European Comission (2011b): EU Guidance to the Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. Available online at https://ec.europa.eu/food/sites/food/files/safety/docs/cs_fcm_legis_active-intelligent_guidance.pdf, checked on 7/21/2018.

European Commission Scientific Committees: UV-Strahlung. Available online at https://ec.europa.eu/health/scientific_committees/opinions_layman/de/glossar/tuv/uv-strahlung.htm, checked on 10/21/2021.

European Parliament (6/5/2019): Directive (EU) 2019/904 on the reduction of the impact of certain plastic products on the environment. EU 2019/904. In *Official Journal of the European Union*.

Eurostat (2021a): Generation of plastic packaging waste in the European Union (EU-28) from 2005 to 2018 (in million metric tons) [Graph]. Edited by Statista. Available online at https://www.statista.com/statistics/881996/plastic-packaging-waste-generated-eu/, checked on 10/22/2021.

Eurostat (2021b): Generation of plastic packaging waste in the European Union (EU-28) in 2018, by country (in kilograms per capita) [Graph]. Edited by Statista, checked on 10/22/2021.

Feldman, D. (2002): Polymer Weathering: Photo-Oxidation. In *Journal of Polymers and the Environment* 10 (4), pp. 163–173. Available online at https://link.springer.com/content/pdf/10.1023/A:1021148205366.pdf, checked on 10/25/2021.

Fiorentino, Gabriella; Zucaro, Amalia; Ulgiati, Sergio (2019): Towards an energy efficient chemistry. Switching from fossil to bio-based products in a life cycle perspective. In *Energy* 170, pp. 720–729. DOI: 10.1016/j.energy.2018.12.206.

Fryer, M. J. (1992): The antioxidant effects of thylakoid Vitamin E (α-tocopherol). In *Plant, Cell and Environment* 15, pp. 381–392. Available online at https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-3040.1992.tb00988.x, checked on 3/10/2020.

Gabetta, B.; Fuzzati, N.; Griffini, A.; Lolla, E.; Pace, R.; Ruffilli, T.; Peterlongo, F. (2000): Characterization of proanthocyanidins from grape seeds. In *Fitoterapia* 71 (2), pp. 162–175.

Galasso, Silvia; Pacifico, Severina; Kretschmer, Nadine; Pan, San-Po; Marciano, Sabina; Piccolella, Simona et al. (2014): Influence of seasonal variation on Thymus longicaulis C. Presl chemical composition and its antioxidant and anti-inflammatory properties. In *Phytochemistry* 107, pp. 80–90. DOI: 10.1016/j.phytochem.2014.08.015.

Gavaric, Neda; Mozina, Sonja Smole; Kladar, Nebojša; Bozin, Biljana (2015): Chemical Profile, Antioxidant and Antibacterial Activity of Thyme and Oregano Essential Oils, Thymol and Carvacrol and Their Possible Synergism. In *Journal of Essential Oil Bearing Plants* 18 (4), pp. 1013–1021. DOI: 10.1080/0972060X.2014.971069.

Gavril, Georgiana-Luminita; Wrona, Magdalena; Bertella, Anis; Świeca, Michał; Râpă, Maria; Salafranca, Jesús; Nerín, Cristina (2019): Influence of medicinal and aromatic plants into risk assessment of a new bioactive packaging based on polylactic acid (PLA). In *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 132, p. 110662. DOI: 10.1016/j.fct.2019.110662.

German Environment Agency (2017): Umweltbewusstsein und Umweltverhalten. Available online at https://www.umweltbundesamt.de/daten/private-haushalte-konsum/umweltbewusstsein-umweltverhalten#textpart-7, updated on April 2017, checked on 5/24/2019.

Giovannucci, E. (1999): Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. In *Journal of the National Cancer Institute* 91 (4), pp. 317–331. DOI: 10.1093/jnci/91.4.317.

Götz, Barbara; Hounsou, Mathias; Dabadé, Sylvain; Havelt, Thomas; Schmitz, Michaela; Hounhouigan, Djidjoho Joseph; Kreyenschmidt, Judith (2019): The Potential of Sustainable Antimicrobial Additives for Food Packaging from Native Plants in Benin. In : Tropentag 2019. Filling gaps and removing traps for sustainable resource management. Tropentag. Kassel, 18.-20.09.2019. University of Kassel; University of Göttingen.

Graßmann, J. (2005): Terpenoids as Plant Antioxidants. In : Plant Hormones, vol. 72: Elsevier (Vitamins & Hormones), pp. 505–535.

Grob, Markus; Huber, Gregor; Herbst, Heinz; Le Gal, André: Müller, Daniel; Priest, Howard; Tartarini, Cinzia et al. (2016): Lichtschutzmittel. In Ralph-Dieter Maier, Michael Schiller (Eds.): Handbuch Kunststoff-Additive. 4th ed. München: Hanser, pp. 156–467.

Grzesik, Michalina; Naparło, Katarzyna; Bartosz, Grzegorz; Sadowska-Bartosz, Izabela (2018): Antioxidant properties of catechins: Comparison with other antioxidants. In *Food chemistry* 241, pp. 480–492. DOI: 10.1016/j.foodchem.2017.08.117.

Hafizoglu, H.; Holmbom, B. (1995): Chemical composition of extractives from Abies nordmanniana. In *Holz als Roh- und Werkstoff* 53, pp. 273–275.

Hagerman, A. E. (2011): Tannin Chemistry. Available online at https://www.users.miamioh.edu/hagermae/, updated on 2011, checked on 2/5/2018.

Hagerman, Ann E.; Riedl, Ken M.; Jones, G. Alexander; Sovik, Kara N.; Ritchard, Nicole T.; Hartzfeld, Paul W.; Riechel, Thomas L. (1998): High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants. In *Journal of agricultural and food chemistry* 46 (5), pp. 1887–1892.

Hauptverband der Deutschen Holzindustrie e.V. (HDH) (12/22/2019): Deutsche kaufen in diesem Jahr 29,8 Millionen Weihnachtsbäume. Weihnachtsbaum 2019: Stabile Stückzahlen, stabile Preise. Bad Honnef. Geismann, Ursula; Tews, Fabian, u.geismann@holzindustrie.de; f.tews@holzindustrie.de. Available online at https://www.holzindustrie.de/pressemitteilungen/2926/deutsche-kaufen-in-diesem-jahr-29-8-millionen-weihnachtsbaeume.html, checked on 1/21/2020.

Havelt, Thomas; Brettschneider, Sarah; Do, Xuan Tung; Korte, Imke; Kreyenschmidt, Judith; Schmitz, Michaela (2019): Sustainable Extraction and Characterisation of Bioactive Compounds from Horse Chestnut Seed Coats for the Development of Bio-Based Additives. In *Resources* 8 (2), p. 114. DOI: 10.3390/resources8020114.

Havelt, Thomas; Brettschneider, Sarah; Schmitz, Michaela (2021a): Evaluation of Practical Applicability and Synergistic Effects of Bio-Based Food Packaging Materials Combined with Plant-Based Stabilisers. In *Processes* 9 (10), p. 1838. DOI: 10.3390/pr9101838.

Havelt, Thomas; Brettschneider, Sarah; Schmitz, Michaela (2021b): Plant-based Bioactive Compounds for Substitution of Petrol-based Stabilisers in Packaging Materials. 8th International Symposium on Human Health Effects of Fruits and Vegetables (Oral Presentation). In *Acta Horticulturae* in press.

Havelt, Thomas; Frase, Jan Niklas; Pude, Ralf; Schmitz, Michaela (2020): Characterisation of Bioactive Ingredients in Extracts of Fresh and Dried Coniferous Trees for the Development of Sustainable Packaging Materials. In *Processes* 8 (11), p. 1366. DOI: 10.3390/pr8111366.

Havelt, Thomas; Schmitz, Michaela (2018): Identifizierung und Charakterisierung bioaktiver Inhaltsstoffe in Thymian. 8. Tagung Arznei- und Gewürzpflanzenforschung, Bonn (Poster Presentation). In *Julius-Kühn-Archiv* (460), pp. 112–114. DOI: 10.5073/JKA.2018.460.030.

Heo, Ho Jin; Kim, Young Jun; Chung, Donghwa; Kim, Dae-Ok (2007): Antioxidant capacities of individual and combined phenolics in a model system. In *Food chemistry* 104 (1), pp. 87–92. DOI: 10.1016/j.foodchem.2006.11.002.

Hon, David N.-S.; Chang, Shang-Tzen (1984): Surface degradation of wood by ultraviolet light. In *J. Polym. Sci. Polym. Chem. Ed.* 22 (9), pp. 2227–2241. DOI: 10.1002/pol.1984.170220923.

Hon, David N.-S.; Chang, Shang-Tzen; Feist, W. C. (1982): Participation of singlet oxygen in the photodegradation of wood surfaces. In *Wood Science and Technology* 16, pp. 193–201.

Hras, Andreja Rizner; Hadolin, Majda; Knez, Zeljko; Bauman, Davorin (2000): Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. In *Food chemistry* 71, pp. 229–233.

Imelouane, B.; Amhamdi, Hassan.; Wathelet, J. P.; Ankit, M.; Khedid, Khadija; El Bachiri, Ali (2009): Chemical Composition and Antimicrobial Activity of Essential Oil of Thyme (Thymus vulgaris) from Eastern Morocco. In *International Journal of Agriculture & Biology* 11, pp. 205–208. Available online at https://www.researchgate.net/profile/Amhamdi_Hassan/publication/266048756_Chemical_Composition_and_Antimicrobial_Activity_of_Essential_Oil_of_Thyme_Thymus_vulgaris_from_Eastern_Morocco/links/562e770208ae518e34837d2d/Chemical-Composition-and-Antimicrobial-Activity-of-Essential-Oil-of-Thyme-Thymus-vulgaris-from-Eastern-Morocco.pdf, checked on 3/10/2020.

Ito, N.; Fukushima, S.; Tsuda, H. (1985): Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. In *Critical reviews in toxicology* 15 (2), pp. 109–150. DOI: 10.3109/10408448509029322.

Ito, N.; Hirose, M.; Fukushima, S.; Tsuda, H.; Shirai, T.; Tatematsu, M. (1986): Studies on antioxidants: Their carcinogenic and modifying effects on chemical carcinogenesis. In *Food and Chemical Toxicology* 24 (10-11), pp. 1071–1082. DOI: 10.1016/0278-6915(86)90291-7.

Jang, Si-Hoon; Jang, So-Ri; Lee, Gyeong-Min; Ryu, Jee-Hoon; Park, Su-Il; Park, No-Hyung (2017): Halloysite Nanocapsules Containing Thyme Essential Oil: Preparation, Characterization, and Application in Packaging Materials. In *Journal of food science* 82 (9), pp. 2113–2120. DOI: 10.1111/1750-3841.13835.

Janiak, Michał; Slavova-Kazakova, Adriana; Kancheva, Vessela; Ivanova, Milena; Tsrunchev, Tsvetelin; Karamać, Magdalena (2017): Effects of γ-Irradiation of Wild Thyme (Thymus serpyllum L.) on the Phenolic Compounds Profile of Its Ethanolic Extract. In *Pol. J. Food Nutr. Sci.* 67 (4), pp. 309–315. DOI: 10.1515/pjfns-2017-0020.

Javed, Hina; Erum, Shazia; Tabassum, Sobia; Ameen, Farhana (2013): An Overview on Medicinal Importance of Thymus vulgaris. In *Journal of Asian Scientific Research* 10 (3), pp. 974–982.

John Wiley & Sons, Inc. (2008a): UV Spectrum of Carvacrol. Available online at https://spectrabase.com/spectrum/1zFrwZ5GI2Z, checked on 10/11/2021.

John Wiley & Sons, Inc. (2008b): UV Spectrum of Thymol. Available online at https://spectrabase.com/spectrum/32Hmd4OFCpI, checked on 10/11/2021.

Kahl, Regine; Kappus, Hermann (1993): Toxikologie der synthetischen Antioxidantien BHA und BHT im Vergleich mit dem natürlichen Antioxidans Vitamin E. In *Z Lebensm Unters Forsch* 196, pp. 329–338.

Kapusta, Ireneusz; Janda, Bogdan; Szajwaj, Barbara; Stochmal, Anna; Piacente, Sonia; Pizza, Cosimo et al. (2007): Flavonoids in horse chestnut (Aesculus hippocastanum) seeds and powdered waste water byproducts. In *Journal of agricultural and food chemistry* 55 (21), pp. 8485–8490. DOI: 10.1021/jf071709t.

Karabagias, I.; Badeka, A.; Kontominas, M. G. (2011): Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. In *Meat science* 88 (1), pp. 109–116. DOI: 10.1016/j.meatsci.2010.12.010.

Karimi, Ehsan; Mehrabanjoubani, Pooyan; Homayouni-Tabrizi, Masoud; Abdolzadeh, Ahmad; Soltani, Mozhgan (2018): Phytochemical evaluation, antioxidant properties and antibacterial activity of Iranian medicinal herb Galanthus transcaucasicus Fomin. In *Food Measure* 12 (1), pp. 433–440. DOI: 10.1007/s11694-017-9656-5.

Karonen, Maarit; Leikas, Anne; Loponen, Jyrki; Sinkkonen, Jari; Ossipov, Vladimir; Pihlaja, Kalevi (2007): Reversed-phase HPLC-ESI/MS analysis of birch leaf proanthocyanidins after their acidic degradation in the presence of nucleophiles. In *Phytochemical analysis : PCA* 18 (5), pp. 378–386. DOI: 10.1002/pca.992.

Kartnig, Theodor; Still, Franz; Reinthaler, Franz (1991): Antimicrobial activity of the essential oil of young pine shoots (Picea abies L.). In *Journal of Ethnopharmacology* 35, pp. 155–157.

Kimura, Hideto; Ogawa, Satoshi; Ishihara, Tomoe; Maruoka, Mahoko; Tokuyama-Nakai, Shota; Jisaka, Mitsuo; Yokota, Kazushige (2017): Antioxidant activities and structural characterization of flavonol O-glycosides from seeds of Japanese horse chestnut (Aesculus turbinata BLUME). In *Food chemistry* 228, pp. 348–355. DOI: 10.1016/j.foodchem.2017.01.084.

Korte, Imke; Kreyenschmidt, Judith; Wensing, Joana; Bröring, Stefanie; Frase, Jan Niklas; Pude, Ralf et al. (2021): Can Sustainable Packaging Help to Reduce Food Waste? A Status Quo Focusing Plant-Derived Polymers and Additives. In *Applied Sciences* 11 (11), p. 5307. DOI: 10.3390/app11115307.

Kowalczyk, Adam; Przychodna, Martyna; Sopata, Sylwia; Bodalska, Agnieszka; Fecka, Izabela (2020): Thymol and Thyme Essential Oil-New Insights into Selected Therapeutic Applications. In *Molecules (Basel, Switzerland)* 25 (18). DOI: 10.3390/molecules25184125.

Kschonsek, Josephine; Wolfram, Theresa; Stöckl, Annette; Böhm, Volker (2018): Polyphenolic Compounds Analysis of Old and New Apple Cultivars and Contribution of Polyphenolic Profile to the In Vitro Antioxidant Capacity. In *Antioxidants* 7 (1). DOI: 10.3390/antiox7010020.

Kulisic, T.; Radonic, A.; Milos, M. (2005a): Antioxidant Properties of Thyme (Thymus vulgaris L.) and Wild Thyme (Thymus Serpyllium L.) Essential Oils. In *Italian Journal of Food Science* 17 (3), pp. 1–10.

Kulisic, Tea; Dragovic-Uzelac, Verica; Milos, Mladen (2005b): Antioxidant Activity of Aqueous Tea Infusions Prepared from Oregano, Thyme and Wild Thyme. In *Food Technology & Biotechnology* 44 (4), pp. 485–492.

Lanigan, Rebecca S.; Yamarik, Torill A. (2002): Final Report on the Safety Assessment of BHT. In *International Journal of Toxicology*, pp. 19–94.

Larché, J.-F.; Bussière, P.-O.; Thérias, S.; Gardette, J.-L. (2012): Photooxidation of polymers: Relating material properties to chemical changes. In *Polymer Degradation and Stability* 97 (1), pp. 25–34. DOI: 10.1016/j.polymdegradstab.2011.10.020.

Lee, Min Hyeock; Seo, Hyun-Sun; Park, Hyun Jin (2017): Thyme Oil Encapsulated in Halloysite Nanotubes for Antimicrobial Packaging System. In *Journal of food science* 82 (4), pp. 922–932. DOI: 10.1111/1750-3841.13675.

Lee, Seung-Joo; Umano, Katumi; Shibamoto, Takayuki; Lee, Kwang-Geun (2005): Identification of volatile components in basil (Ocimum basilicum L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. In *Food chemistry* 91 (1), pp. 131–137. DOI: 10.1016/j.foodchem.2004.05.056.

Lewicki, Piotr P. (1998): Effect of pre-drying treatment, drying and rehydration on plant tissue properties: A review. In *International Journal of Food Properties* 1 (1), pp. 1–22. DOI: 10.1080/10942919809524561.

Lincke, Felix (2021): Klimaschutz. Wie die neue CO2-Abgabe wirkt. In *Tagesschau*, 10/11/2021. Available online at https://www.tagesschau.de/wirtschaft/verbraucher/co2-preis-107.html, checked on 10/22/2012.

López-de-Dicastillo, C.; Pezo, D.; Nerín, C.; López-Carballo, G.; Catalá, R.; Gavara, R.; Hernández-Muñoz, P. (2012): Reducing Oxidation of Foods Through Antioxidant Active Packaging Based on Ethyl Vinyl Alcohol and Natural Flavonoids. In *Packag. Technol. Sci.* 25 (8), pp. 457–466. DOI: 10.1002/pts.992.

Mahdavi, Atiyeh; Moradi, Parviz; Mastinu, Andrea (2020): Variation in Terpene Profiles of Thymus vulgaris in Water Deficit Stress Response. In *Molecules* 25 (5). DOI: 10.3390/molecules25051091.

Maier, Ralph-Dieter; Schiller, Michael (2016): Vorwort. In Ralph-Dieter Maier, Michael Schiller (Eds.): Handbuch Kunststoff-Additive. 4th ed. München: Hanser, pp. V–VIII.

Makino, M.; Katsube, T.; Ohta, Y.; Schmidt, W.; Yoshino, K. (2017): Preliminary study on antioxidant properties, phenolic contents, and effects of Aesculus hippocastanum (horse chestnut) seed shell extract on in vitro cyclobutane pyrimidine dimer repair. In *J Intercult Ethnopharmacol* 6 (4), p. 1. DOI: 10.5455/jice.20170814074812.

Márcio Carocho; Patricia Morales; Isabel C.F.R. Ferreira (2018): Antioxidants_ Reviewing the chemistry, food applications, legislation and role as preservatives. In *Trends in Food Science and Technology* (71), pp. 107–120. DOI: 10.1016/j.tifs.2017.11.008.

Marco, Gino J. (1968): A Rapid Method for Evlauation of Antioxidants. In *The Journal of the American Oil Chemists' Society* 45, pp. 594–598.

Mastelić, Josip; Jerković, Igor; Blazević, Ivica; Poljak-Blazi, Marija; Borović, Suzana; Ivancić-Baće, Ivana et al. (2008): Comparative study on the antioxidant and biological activities of carvacrol, thymol, and eugenol derivatives. In *Journal of agricultural and food chemistry* 56 (11), pp. 3989–3996. DOI: 10.1021/jf073272v.

Matsuda, H.; Li, Y.; Murakami, T.; Ninomiya, K.; Yamahara, J.; Yoshikawa, M. (1997): Effects of Escins Ia, Ib, IIa, and IIb from Horse Chestnut, the Seeds of Aesculus hippocastanum L., on Acute Inflammation in Animals. In *Biological and Pharmaceutical Bulletin* 20 (10), pp. 1092–1095. DOI: 10.1248/bpb.20.1092.

Matthes, Anne; Schmitz-Eiberger, Michaela (2009): Polyphenol content and antioxidant capacity of apple fruit: effect of cultivar and storage conditions. In *Journal of Applied Botany and Food Quality* 82, pp. 152–157.

Miller, Stephen A. (2014): Sustainable polymers: replacing polymers derived from fossil fuels. In *Polym. Chem.* 5 (9), p. 3117. DOI: 10.1039/c4py90017k.

Min, Tiantian; Sun, Xiaoli; Yuan, Zhipeng; Zhou, Liping; Jiao, Xiangyu; Zha, Junwei et al. (2021): Novel antimicrobial packaging film based on porous poly(lactic acid) nanofiber and polymeric coating for humidity-controlled release of thyme essential oil. In *LWT - Food Science and Technology* 135. Available online at https://www.sciencedirect.com/science/article/abs/pii/S0023643820310239.

Moewius, J.; Röhrig, R.; Schaack, D.; Rampold, C.; Brzukalla, H.; Gottwald, F. et al. (2018): Die Bio-Branche 2018. Zahlen, Daten, Fakten. Edited by Bund Ökologische Lebensmittelwirtschaft e. V. (BÖLW). Bund Ökologische Lebensmittelwirtschaft e. V. (BÖLW). Available online at https://www.boelw.de/fileadmin/user_upload/Dokumente/Zahlen_und_Fakten/Brosch%C3%BCre_2018/ZDF_2018_Inhalt_Web_Einzelseiten_kleiner.pdf, updated on 2018, checked on 2/5/2019.

Mohsin, A.; Shah, A. H.; Al-Yahya, M. A.; Tariq, M.; Tanira, M. O. M.; Ageel, A. M. (1989): Analgesic, antipyretic activity and phytochemical screening of some plants used in traditional Arab system of medicine. In *Fitoterapia* 60 (2), pp. 174–177.

Mulder, Karel F. (2007): Innovation for sustainable development: from environmental design to transition management. In *Sustain Sci* 2 (2), pp. 253–263. DOI: 10.1007/s11625-007-0036-7.

Murakami, M.; Yamaguchi, T.; Takamura, H.; Matoba, T. (2003): Effects of Ascorbic Acid and α-Tocopherol on Antioxidant Activity of Polyphenolic Compounds. In *Journal of food science* 68 (5), pp. 1622–1625.

Nelson, R. R. S. (1997): In-vitro activities of five plant essential oils against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecium. In *Journal of Antimicrobial Chemotherapy* 40 (2), pp. 305–306. DOI: 10.1093/jac/40.2.305.

Nickavar, Bahman; Esbati, Naser (2012): Evaluation of the antioxidant capacity and phenolic content of three Thymus species. In *Journal of acupuncture and meridian studies* 5 (3), pp. 119–125. DOI: 10.1016/j.jams.2012.03.003.

Nikaido, H.; Vaara, M. (1985): Molecular basis of bacterial outer membrane permeability. In *Microbiological reviews* 49 (1), pp. 1–32.

nova-Institute (2020): Plastic production worldwide in 2018 by feedstock type (in million metric tons) [Graph]. Edited by Statista. Available online at https://www.statista.com/statistics/1114289/global-cumulative-plastic-production-by-type/, checked on 10/22/2021.

Ochs, Dietmar (2016): Biozide Wirkstoffe. In Ralph D. Maier, Michael Schiller (Eds.): Handbuch Kunststoff Additive. 4th ed. München: Carl Hanser Verlag GmbH & Co. KG, pp. 1139–1188.

Ogawa, S.; Kimura, H.; Niimi, A.; Katsube, T.; Jisaka, M.; Yokota, K. (2008): Fractionation and structural characterization of polyphenolic antioxidants from seed shells of Japanese horse chestnut (Aesculus turbinata BLUME). In *Journal of agricultural and food chemistry* 56 (24), pp. 12046–12051. DOI: 10.1021/jf802506r.

Öncel, Işıl; Yurdakulol, Ender; Keleş, Yüksel; Kurt, Latif; Yıldız, Atilla (2004): Role of antioxidant defense system and biochemical adaptation on stress tolerance of high mountain and steppe plants. In *Acta Oecologica* 26 (3), pp. 211–218. DOI: 10.1016/j.actao.2004.04.004.

Oszmiański, Jan; Kalisz, Stanisław; Aneta, Wojdyło (2014): The content of phenolic compounds in leaf tissues of white (Aesculus hippocastanum L.) and red horse chestnut (Aesculus carea H.) colonized by the horse chestnut leaf miner (Cameraria ohridella Deschka & Dimić). In *Molecules (Basel, Switzerland)* 19 (9), pp. 14625–14636. DOI: 10.3390/molecules190914625.

Özcan, Musa; Chalchat, Jean-Claude (2004): Arome profile of Thymus vulgaris L. growing wild in Turkey. In *Bulgarian Journal of Plant Physiology* 30, pp. 68–73.

Palozza, Paola; Krinsky, Norman I. (1992): Beta-Carotene and Alpha-Tocopherol Are Synergistic Antioxidants. In *Archives of Biochemistry and Biophysics* 297 (1), pp. 184–187.

ISO 1924-2:2008: Paper and board - Determination of tensile properties - Part 2: Constant rate of elongation method (20 mm/min).

Peltzer, Mercedes Ana.; Wagner, Jorge; Jiménez, Alfonso (2009): Migration study of carvacrol as natural antioxidant in High Density Polyethylene for active packaging. In *Food Additives and Contaminants* 26 (6), pp. 938–946, checked on 5/2/2018.

Periago, María Jesús; Rincón, Francisco; Agüera, Maria Dolores; Ros, Gaspar (2004): Mixture approach for optimizing lycopene extraction from tomato and tomato products. In *Journal of agricultural and food chemistry* 52 (19), pp. 5796–5802. DOI: 10.1021/jf049345h.

Ph. Eur. (2001a): Thyme (Thymi herba). Edited by European Directorate for the Quality of Medicines and Healthcare. Pharmacopoeia Europaea. Strasbourg.

Ph. Eur. (2001b): Thyme oil (Thymi aetheroleum). Edited by European Directorate for the Quality of Medicines and Healthcare. Pharmacopoeia Europaea. Strasbourg.

PlasticsEurope (2020): Anteile an der Verwendung von Kunststoff in Europa nach Einsatzgebieten in den Jahren 2017 bis 2019. Edited by Statista. Available online at https://de.statista.com/statistik/daten/studie/206528/umfrage/verwendung-von-kunststoff-in-europa-nach-einsatzgebieten/, checked on 5/4/2018.

PlasticsEurope (2021): Anteile der Verwendung von Kunststoff nach Einsatzgebieten in Deutschland im Jahr 2019. Edited by Statista. Available online at https://de.statista.com/statistik/daten/studie/226759/umfrage/verwendung-von-kunststoff-in-deutschland-nach-einsatzgebieten/, checked on 5/4/2018.

Polle, Andrea; Chakrabarti, Krisanu; Schürmann, Wolfgang; Rennenberg, Heinz (1990): Composition and Properties of Hydrogen Peroxide Decomposing Systems in Extracellular and Total Extracts from Needles of Norway Spruce (Picea abies L., Karst.). In *Plant Physiology* 94, pp. 312–319.

Pothier, Jacques; Galand, Nicole; El Ouali, Mohamed; Viel, Claude (2001): Comparison of planar chromatographic methods (TLC, OPLC, AMD) applied to essential oils of wild thyme and seven chemotypes of thyme. In *Il Farmaco* 56 (5-7), pp. 505–511. DOI: 10.1016/S0014-827X(01)01085-0.

DIN EN ISO 5267-1:2000: Pulps - Determination of drainability - Part 1: Schopper-Riegler method.

DIN EN ISO 5269-2:2004: Pulps - Preparation of laboratory sheets for physical testing - Part 2: Rapid-Köthen method.

Rabek, Jan. F. (2012): Physical Aspects of the Photodegradation of Polymers. In Jan. F. Rabek (Ed.): Polymer Photodegradation. Mechanisms and experimental methods. Dordrecht: Springer Netherlands, pp. 1–23.

Ramachandra Reddy, Attipalli; Chaitanya, Kolluru Viswanatha; Vivekanandan, Munusamy (2004): Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. In *Journal of plant physiology* 161 (11), pp. 1189–1202. DOI: 10.1016/j.jplph.2004.01.013.

Ramos, Óscar L.; Pereira, Ricardo N.; Cerqueira, Miguel A.; Martins, Joana R.; Teixeira, José A.; Malcata, F. Xavier; Vicente, António A. (2018): Bio-Based Nanocomposites for Food Packaging and Their Effect in Food Quality and Safety. In : Food Packaging and Preservation: Elsevier, pp. 271–306.

Rao, A. Venket (2004): Processed tomato products as a source of dietary lycopene: bioavailability and antioxidant properties. In *Canadian journal of dietetic practice and research : a publication of Dietitians of Canada = Revue canadienne de la pratique et de la recherche en dietetique : une publication des Dietetistes du Canada* 65 (4), pp. 161–165. DOI: 10.3148/65.4.2004.161.

Rasselet, D.; Ruellan, A.; Guinault, A.; Miquelard-Garnier, G.; Sollogoub, C.; Fayolle, B. (2014): Oxidative degradation of polylactide (PLA) and its effects on physical and mechanical properties. In *European Polymer Journal* 50, pp. 109–116. DOI: 10.1016/j.eurpolymj.2013.10.011.

Rauha, Jussi-Pekka; Remes, Susanna; Heinonen, Marina; Hopia, Anu; Kähkönen, Marja; Kujala, Tytti et al. (2000): Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. In *International Journal of Food Microbiology* 56, pp. 3–12.

Rebaya, Ahlem; Belghith, Souad Igueld; Hammrouni, Safa; Maaroufi, Abderrazak; Ayadi, Malika Trabelsi; Chérif, Jamila Kalthoum (2016): Antibacterial and Antifungal Activities of Ethanol Extracts of *Halimium halimifolium*, *Cistus salviifolius* and *Cistus monspeliensis*. In *International Journal of Pharmaceutical and Clinical Research* 8 (4), pp. 243–247. Available online at http://impactfactor.org/PDF/IJPCR/8/IJPCR,Vol8,Issue4,Article7.pdf.

Regier, Marc; Mayer-Miebach, E.; Behsnilian, D.; Neff, E.; Schuchmann, H. P. (2005): Influences of Drying and Storage of Lycopene-Rich Carrots on the Carotenoid Content. In *Drying Tech.* 23 (4), pp. 989–998. DOI: 10.1081/DRT-200054255.

Roby, Mohamed Hussein Hamdy; Sarhan, Mohamed Atef; Selim, Khaled Abdel-Hamed; Khalel, Khalel Ibrahim (2013): Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. In *Industrial Crops and Products* 43, pp. 827–831. DOI: 10.1016/j.indcrop.2012.08.029.

Ruberto, Giuseppe; Baratta, Maria T. (2000): Antioxidant activity of selected essential oil components in two lipid model systems. In *Food chemistry* 69 (2), pp. 167–174. DOI: 10.1016/S0308-8146(99)00247-2.

Sá, M. de; Justino, V.; Spranger, M. I.; Zhao, Y. Q.; Han, L.; Sun, B. S. (2014): Extraction yields and anti-oxidant activity of proanthocyanidins from different parts of grape pomace: effect of mechanical treatments. In *Phytochemical analysis : PCA* 25 (2), pp. 134–140. DOI: 10.1002/pca.2479.

Schmitz-Eiberger, Michaela A.; Blanke, Michael M. (2012): Bioactive components in forced sweet cherry fruit (Prunus avium L.), antioxidative capacity and allergenic potential as dependent on cultivation under cover. In *LWT - Food Science and Technology* 46 (2), pp. 388–392. DOI: 10.1016/j.lwt.2011.12.015.

Schutzgemeinschaft Deutscher Wald (2020): Weihnachtsbaum in Zahlen und Fakten. Daten zum Weihnachtsbaum 2019. Schutzgemeinschaft Deutscher Wald. Available online at https://www.sdw.de/waldwissen/weihnachtsbaum/, updated on 12/16/2019, checked on 1/22/2020.

Shabnum, Shazia; Wagay, Muzafar G. (2011): Essential Oil Composition of Thymus Vulgaris L. and their Uses. In *Journal or Research & Development* 11, pp. 83–94.

Sharma, Pallavi; Dubey, Rama Shanker (2005): Drought Induces Oxidative Stress and Enhances the Activities of Antioxidant Enzymes in Growing Rice Seedlings. In *Plant Growth Regulation* 46 (3), pp. 209–221. DOI: 10.1007/s10725-005-0002-2.

Sharma, Shubham; Barkauskaite, Sandra; Duffy, Brendan; Jaiswal, Amit K.; Jaiswal, Swarna (2020): Characterization and Antimicrobial Activity of Biodegradable Active Packaging Enriched with Clove and Thyme Essential Oil for Food Packaging Application. In *Foods (Basel, Switzerland)* 9 (8). DOI: 10.3390/foods9081117.

Shi, John; Kakuda, Yukio; Yeung, David (2004): Antioxidative Properties of Lycopene and Other Carotenoids from Tomatoes: Synergistic Effects. In *BioFactors* 21 (1-4), pp. 203–210.

Singleton, V.; Orthofer, R.; Lamuela-Raventós, R. (1999): Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. In *METHODS IN ENZYMOLOGY* (299).

Smith-Palmer, A.; Stewart, J.; Fyfe, L. (1998): Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. In *Letters in Applied Microbiology* 26, pp. 118–122. DOI: 10.1046/j.1472-765X.1998.00303.x.

Souza, Victor Gomes Lauriano; Rodrigues, Patrícia Freitas; Duarte, Maria Paula; Fernando, Ana Luísa (2018): Antioxidant Migration Studies in Chitosan Films Incorporated with Plant Extracts. In *j renew mater*. DOI: 10.7569/JRM.2018.634104.

Stahl-Biskup, E.; Venskutonis, R. P. (2004): Thyme. In K. V. Peter (Ed.): Handbook of herbs and spices. Volume 2. 2nd ed. Cambridge, England: Woodhead Publishing Limited.

Stanciu, Gabriela; Chirila, Elisabeta; Dobrinas, Simona; Negreanu-Pirjol, Ticuta (2010): Studies Regarding the Determination of Antioxidant Properties of New Plant Extracts for Cosmetic Purposes. In *Revista de Chimie - Bucharest- original Edition*, pp. 41–44. Available online at https://www.researchgate.net/publication/234120183_Studies_Regarding_the_Determination_of_Antioxidant_Properties_of_New_Plant_Extracts_for_Cosmetic_Purposes?enrichId=rgreq-6eede86f894aeba5192c8830d3c4297e-XXX&enrichSource=Y292ZXJQYWdlOzIzNDEyMDE4MztBUzoxMDMyMTIwMjc3NDQyNzFAMTQwMTYxOTA3Mzc1Nw%3D%3D&el=1_x_2&_esc=publicationCoverPdf, checked on 5/15/2018.

Takshak, Swabha; Agrawal, S. B. (2019): Defense potential of secondary metabolites in medicinal plants under UV-B stress. In *Journal of Photochemistry and Photobiology B: Biology* 193, pp. 51–88. Available online at https://www.sciencedirect.com/science/article/abs/pii/S1011134418314301.

Tantaoui-Elaraki, A.; Beraoud, L. (1994): Inhibition of growth and aflatoxin production in Aspergillus parasiticus by essential oils of selected plant materials. In *Journal of Environmental Pathology, Toxicology and Oncology : Official Organ of the International Society for Environmental Toxicology and Cancer* 13 (1), pp. 67–72.

Tesfay, Samson Zeray; Magwaza, Lembe Samukelo (2017): Evaluating the efficacy of moringa leaf extract, chitosan and carboxymethyl cellulose as edible coatings for enhancing quality and extending postharvest life of avocado (Persea americana Mill.) fruit. In *Food Packaging and Shelf Life* 11 (11), pp. 40–48. DOI: 10.1016/j.fpsl.2016.12.001.

Thippeswamy, N. B.; Naidu, K. Akhilender; Achur, Rajeshwara N. (2013): Antioxidant and antibacterial properties of phenolic extract from *Carum carvi L*. In *Journal of Pharmacy Research* 7 (4), pp. 352–357. DOI: 10.1016/j.jopr.2013.03.028.

Tornuk, Fatih; Cankurt, Hasan; Ozturk, Ismet; Sagdic, Osman; Bayram, Okan; Yetim, Hasan (2011): Efficacy of various plant hydrosols as natural food sanitizers in reducing Escherichia coli O157:H7 and Salmonella Typhimurium on fresh cut carrots and apples. In *International Journal of Food Microbiology* 148 (1), pp. 30–35. DOI: 10.1016/j.ijfoodmicro.2011.04.022.

TrashOut (2021): Estimated number of illegal dumps containing plastic waste in Europe as of 2021, by selected country [Graph]. Edited by Statista. Available online at https://www.statista.com/statistics/990554/estimated-illegal-dumps-containing-plastic-waste-in-europe/, checked on 10/22/2021.

Tsao, Rong (2015): Synergistic interactions between antioxidants used in food preservation. In Fereidoon Shahidi (Ed.): Handbook of Antioxidants for Food Preservation: Woodhead Publishing (Food Science, Technology and Nutrition, 276), pp. 335–347.

Turck, D.; Bresson, J.-L.; Burlingame, B.; Dean, T.; Fairweather-Tait, S.; Heinonen, M. et al. (2017): Safety of cranberry extract powder as a novel food ingredient pursuant to Regulation (EC) No 258/97. In *EFS2* 15 (5), p. 1731. DOI: 10.2903/j.efsa.2017.4777.

Valadez-Carmona, Lourdes; Plazola-Jacinto, Carla Patricia; Hernández-Ortega, Marcela; Hernández-Navarro, María Dolores; Villarreal, Francisco; Necoechea-Mondragón, Hugo et al. (2017): Effects of microwaves, hot air and freeze-drying on the phenolic compounds, antioxidant capacity, enzyme activity and microstructure of cacao pod husks (Theobroma cacao L.). In *Innovative Food Science & Emerging Technologies* 41, pp. 378–386. DOI: 10.1016/j.ifset.2017.04.012.

Vasile, Cornelia; Darie, Raluca Nicoleta; Cheaburu-Yilmaz, Catalina Natalia; Pricope, Gina-Mihaela; Bračič, Matej; Pamfil, Daniela et al. (2013): Low density polyethylene – Chitosan composites. In *Composites Part B: Engineering* 55, pp. 314–323. DOI: 10.1016/j.compositesb.2013.06.008.

Vasková, J.; Fejercáková, A.; Mojzisová, G.; Vasko, L.; Patlevic, P. (2015): Antioxidant potential of Aesculus hippocastanum extract and escin against reactive oxygen and nitrogen species. In *European Review for Medical and Pharmacological Sciences* 19, pp. 879–886.

Wang, Huei-ju; Murphy, Patricia A. (1994): Isoflavone Composition of American and Japanese Soybeans in Iowa. Effects of Variety, Crop Year, and Location. In *J. Agric. Food Chem.* 42 (8), pp. 1674–1677.

Wang, Sunan; Meckling, Kelly A.; Marcone, Massimo F.; Kakuda, Yukio; Tsao, Rong (2011a): Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. In *Journal of agricultural and food chemistry* 59 (3), pp. 960–968. DOI: 10.1021/jf1040977.

Wang, Sunan; Melnyk, John P.; Tsao, Rong; Marcone, Massimo F. (2011b): How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health. In *Food Research International* 44 (1), pp. 14–22. DOI: 10.1016/j.foodres.2010.09.028.

Weber, P.; Bendich, A.; Schalch, W. (1996): Vitamin C and human health--a review of recent data relevant to human requirements. In *Int J Vitam Nutr Res* 66 (1), pp. 19–30.

Wegmann, A.; Le Gal, A.; Müller, D. (2016): Antioxidantien. In Ralph-Dieter Maier, Michael Schiller (Eds.): Handbuch Kunststoff-Additive. 4th ed. München: Hanser, pp. 1–153, checked on 7/8/2018.

Yanishlieva, Nedyalka V.; Marinova, Emma M.; Gordon, Michael H.; Raneva, Violeta G. (1999): Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. In *Food chemistry* 64 (1), pp. 59–66. DOI: 10.1016/S0308-8146(98)00086-7.

Yin, Jie; Becker, Eleonora Miquel; Andersen, Mogens L.; Skibsted, Leif H. (2012): Green tea extract as food antioxidant. Synergism and antagonism with α-tocopherol in vegetable oils and their colloidal systems. In *Food chemistry* 135 (4), pp. 2195–2202. DOI: 10.1016/j.foodchem.2012.07.025.

Yusuf, Mohd (2018): Natural Antimicrobial Agents for Food Biopreservation. In Alexandru Mihai Grumezescu, Alina Maria Holban (Eds.): Food packaging and preservation. London: AP Academic Press an imprint of Elsevier (Handbook of Food Bioengineering, Volume 9), pp. 409–438.

Zanfini, Assunta; Corbini, Gianfranco; La Rosa, Caterina; Dreassi, Elena (2010): Antioxidant activity of tomato lipophilic extracts and interactions between carotenoids and α-tocopherol in synthetic mixtures. In *LWT - Food Science and Technology* 43 (1), pp. 67–72. DOI: 10.1016/j.lwt.2009.06.011.

Zeid, Amr; Karabagias, Ioannis K.; Nassif, Madonna; Kontominas, Michael G. (2019): Preparation and evaluation of antioxidant packaging films made of polylactic acid containing thyme, rosemary, and oregano essential oils. In *J Food Process Preserv* 83 (5), p. 1299. DOI: 10.1111/jfpp.14102.

Zhang, Zheng; Wagner, Victoria E. (2017): Antimicrobial Coatings and Modifications on Medical Devices. Cham: Springer International Publishing.

Zhu, Peipei; Chen, Yanxin; Fang, Jing; Wang, Zelin; Xie, Chuang; Hou, Baohong et al. (2016): Solubility and solution thermodynamics of thymol in six pure organic solvents. In *The Journal of Chemical Thermodynamics* 92, pp. 198–206. DOI: 10.1016/j.jct.2015.09.010.

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Appendix

Supplementary Information and Figures

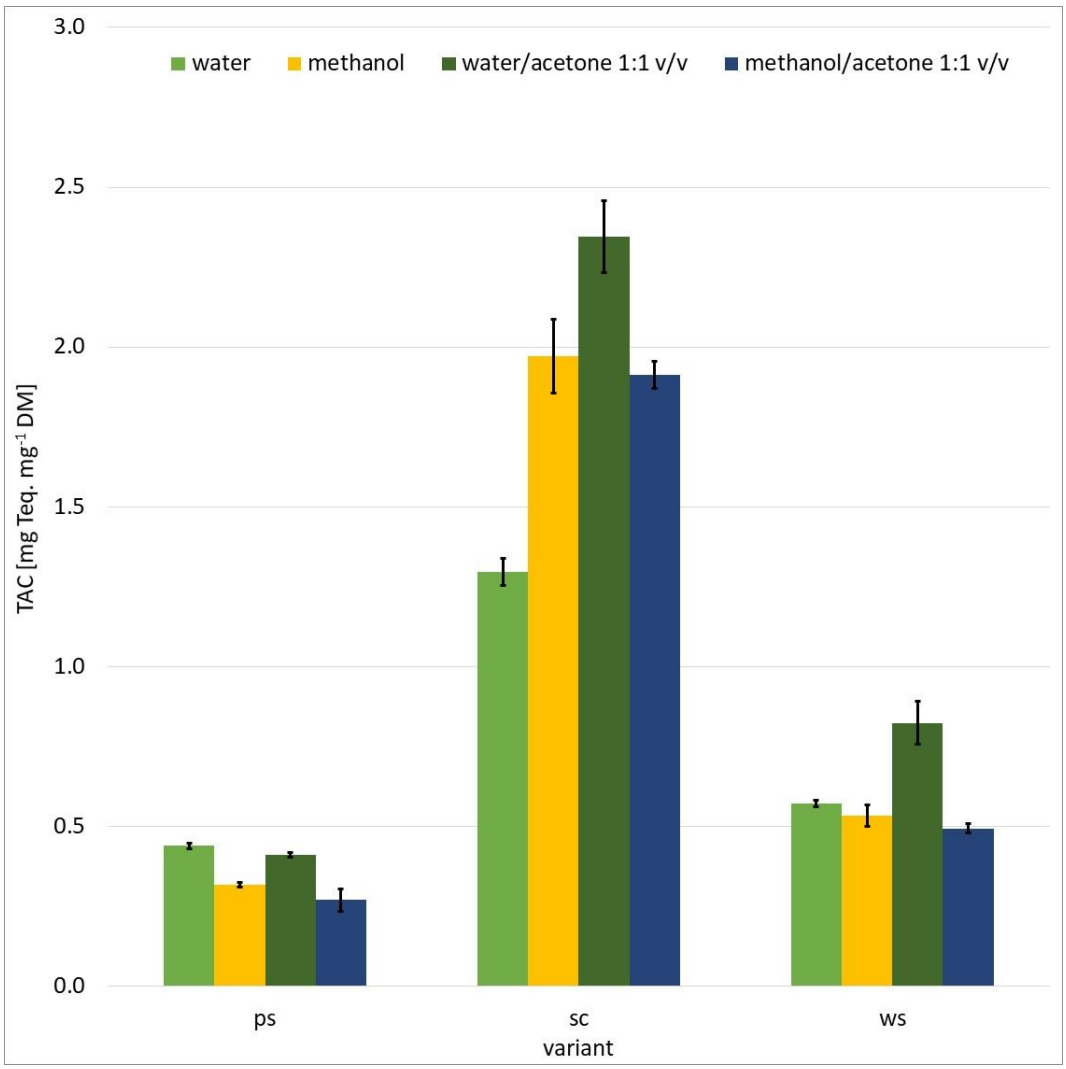


Fig. A1 (referring to Section 3.2.3 / Page 32) *Solvent-dependent total antioxidative capacity (TAC) of peeled seeds (ps), seed coats (sc) and whole seeds (ws) from Aesculus hippocastanum L. extracted by grinding extraction. Measurements in triplicate, standard deviation indicated by error bars. Teq.: Trolox equivalents; DM: dried sample mass.*

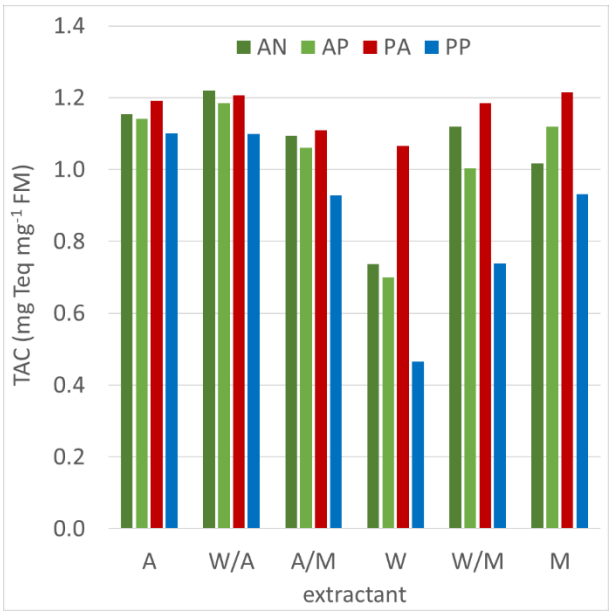
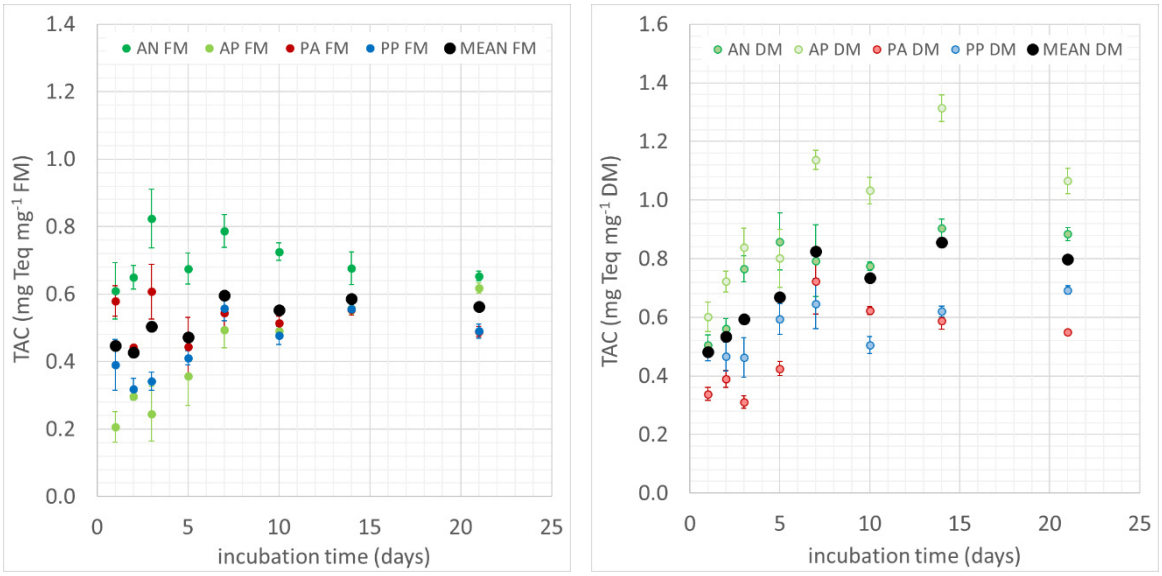


Fig. A2 (referring to Section 5.2.2 / Page 58) *Total antioxidant capacity (TAC) of aerial part extracts of different coniferous woods using different extractants. Single determination (pretest). Teq: Trolox equivalents, FM: fresh mass, AN: Abies nordmanniana, AP: Abies procera, PA: Picea abies, PP: Picea pungens. Extractants: A: Acetone, W: Water, M: Methanol, mixtures: 1:1 (v/v).*



(a) ***(b)***

Fig. A3 (referring to Section 5.3.2.3 / Page 66) *(**a**) Average total antioxidant capacity (TAC) of aerial part extracts (passive extraction) of different coniferous woods in different drying conditions. Results are corrected by weight loss. Measurements in quadruplicate, standard deviations indicated by error bars. Teq: Trolox equivalents, AN: Abies nordmanniana, AP: Abies procera, PA: Picea abies, PP: Picea pungens. (**a**): fresh biomass; (**b**): dried biomass (dried at room temperature for 12 days).*

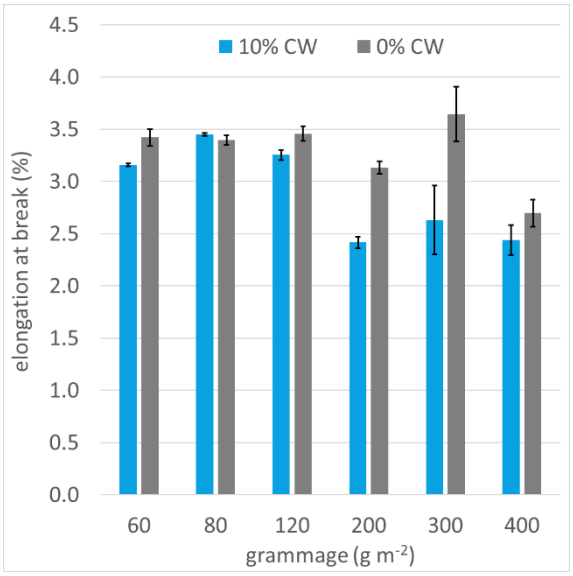


Fig. A4 (referring to Section 5.3.2.4 / Page 67) *Average elongation at break of paper sheets with different grammages and compositions. Three repetitions, four measurements per repetition. Standard deviation of mean values per repetition indicated by error bars. CW: share of coniferous wood biomass after passive extraction included in paper sheet.*

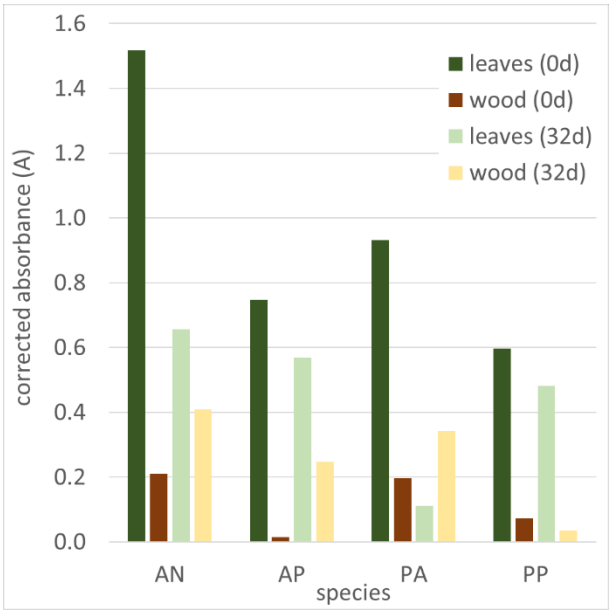


Fig. A5 (referring to Section 5.3.3.3 / Page 71) *Corrected absorbance of different coniferous wood samples (different species, plant fractions, drying conditions) after conduction of acid butanol assay. Single determination. 0d: fresh mass, dried for 0 days, 32d: dried mass, dried for 32 days at room temperature, AN: Abies nordmanniana, AP: Abies procera, PA: Picea abies, PP: Picea pungens.*

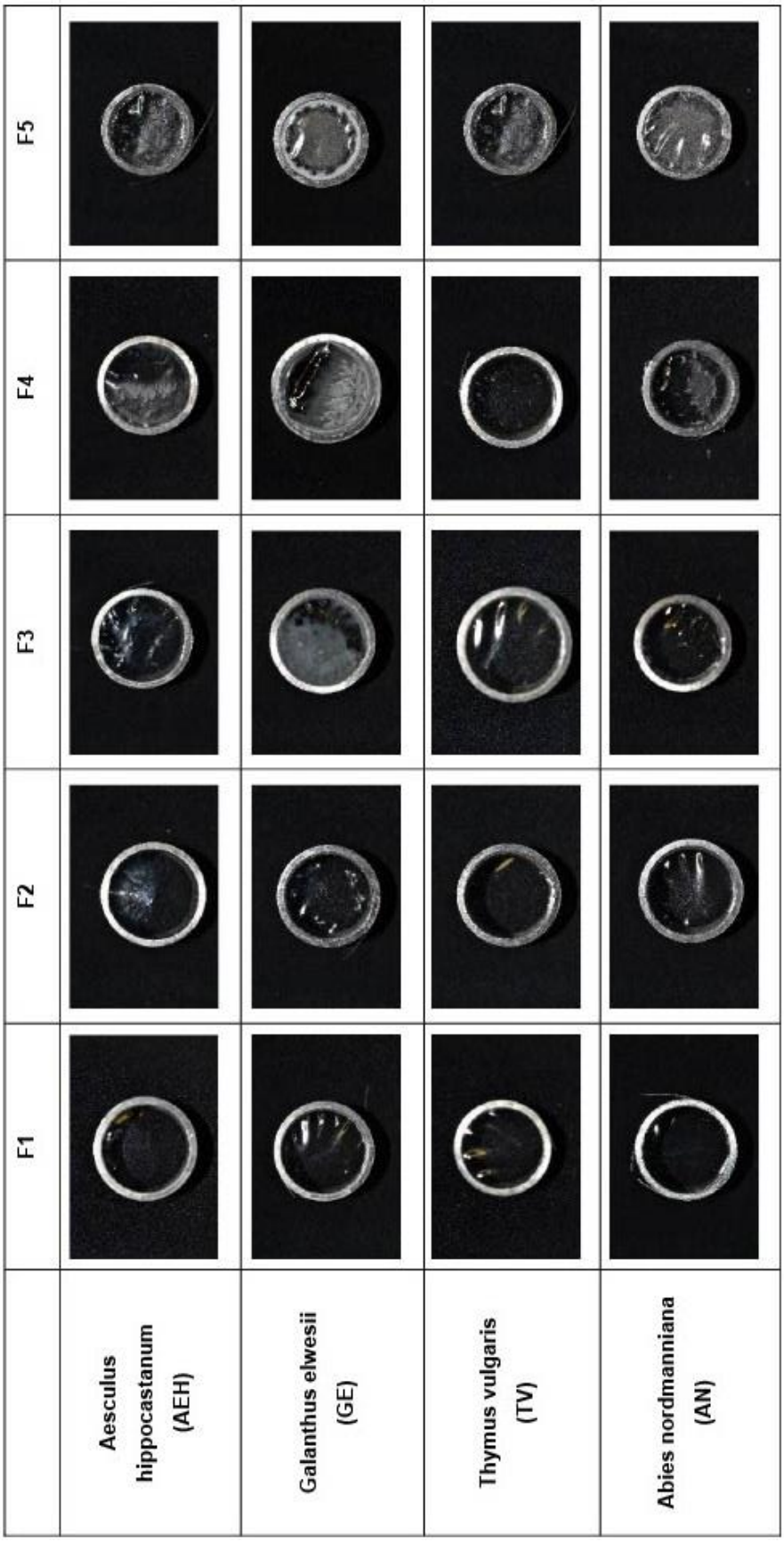


Fig. A6 (referring to Section 6.3.1.2 / Page 85) *Photographs of different PLA films including up to 0.8 % (w/w) antioxidant plant extracts.*

Table of Figures

Abbreviations: *TV – Thymus vulgaris; GC-MS: Gas Chromatography coupled with Mass Spectrometry; TAC: Total antioxidant capacity; UV: Ultraviolet light range; UV/Vis: Ultraviolet and Visible light range; AEH: Aesculus hippocastanum; TPC: Total Phenolic Content; GE: Galanthus elwesii; CW: Coniferous Woods; AN: Abies nordmanniana*

- Table of Figures (continued) -

Abbreviations: *TV – Thymus vulgaris; GC-MS: Gas Chromatography coupled with Mass Spectrometry; TAC: Total antioxidant capacity; UV: Ultraviolet light range; UV/Vis: Ultraviolet and Visible light range; AEH: Aesculus hippocastanum; TPC: Total Phenolic Content; GE: Galanthus elwesii; CW: Coniferous Woods; AN: Abies nordmanniana;*

Table of Abbreviations

Abbreviation	Description
A	Absorbance units
AA	Ascorbic Acid
ABA	Acid Butanol Assay
ABTS/ABTS$^{\bullet+}$	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (radical cation)
AEH	*Aesculus hippocastanum* L.
AN	*Abies nordmanniana* (STEV.) SPACH
AP	*Abies procera* REHDER
BHA	Butylated hydroxyanisole, 2(3)-tert-butyl-4-methoxyphenol
BHT	Butylated hydroxytoluene, 2,6-di-tert-butyl-4-methylphenol
CFU	Colony Forming Units
chp.	chopped
CW	Coniferous Woods
d	Day(s)
div.	Used in botanical names to describe different species in one specific genus
DM	Dried mass
DW1/2/3	*Thymus vulgaris* L. cultivar Deutscher Winter, origin no. 1 / 2 / 3
FM	Fresh mass
FS	Field Samples
G.	*Galanthus*
G el	*Galanthus elwesii* HOOK.F.
G gr	*Galanthus gracilis* CELAK.
G ni	*Galanthus nivalis* L.
G pl	*Galanthus elwesii* M.BIEB.
GA	Gallic Acid
GAE	Gallic Acid Equivalents
GC-MS	Gas Chromatography coupled with Mass Spectrometry
GE	*Galanthus elwesii* HOOK.F.

- Table of Abbreviations (continued) -

Abbreviation	**Description**
gr.	ground
hc	High concentration
HILD	*Thymus vulgaris* L. cultivar Deutscher Winter, origin HILD
hyTAC	Determination of the Total Antioxidant Capacity using the hydrophilic ABTS Radical Cation Scavenging Capacity Assay
IAC	Isolated Active Compound
lc	Low concentration
liTAC	Determination of the Total Antioxidant Capacity using the lipophilic β-carotene bleaching assay
mE	Milli Extinction Units
MSTFA	N-Methyl-N-(trimethylsilyl)trifluoroacetamide
n	Number of replicates for statistical matters
NMR	Nuclear Magnetic Resonance Spectroscopy
PA/PAC	Proanthocyanidins
PA	*Picea abies* (L.) H. KARST. (in chapter 5)
PP	*Picea pungens* ENGELM.
ps	Peeled seed
Qu	Quercetin
rAU	Relative Absorbance Units
sc	Seed Coat
SEC	Size Exclusion Chromatography
SPME	Solid Phase Micro Extraction
TAC	Total Antioxidant Capacity
TEq	Trolox Equivalents
Th	Thymol
To	α-tocopherol
TPC	Total Phenolic Content
TV	*Thymus vulgaris* L.

- Table of Abbreviations (continued) -

Abbreviation	**Description**
UV	Ultraviolet light range
UV/Vis	Ultraviolet and Visible light range
Var2/3	*Thymus vulgaris* L. cultivars Varico 2 / 3
WP	Waste Products
ws	Whole Seed