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Assessment of Dietary Exposure to Flavouring Substances via Consumption of Flavoured Teas

Anne-Marie Orth

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TABLE OF CONTENTS

ABBREVIATIONS

ANOVA	analysis of variance
APET	Added Portion Exposure Technique
B	brand
CAC	Codex Alimentarius Commission
CSD	control standard
D	Germany
DK	Denmark
E	Spain
EFSA	European Food Safety Authority
ESD	extraction standard
EU	European Union
F	France
FACET	Flavourings, Additives and Food Contact Materials Exposure Task
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavour and Extract Manufacturers Association
FLAVIS	Flavour Information System
FID	flame ionisation detector
FIN	Finland
GC	gas chromatography
H	Hungary
I	Italy
ICPS	International Programme on Chemical Safety
InSD	injection standard
IQR	interquartile range
IRL	Ireland
JECFA	Joint FAO/WHO Expert Committee on Food Additives
ll	loose leaves
LLE	liquid-liquid-extraction
LOD	limit of detection
LOQ	limit of quantification

MDGC	multidimensional gas chromatography
MS	mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	modified Theoretical Added Maximum Daily Intake
n.d.	not detectable (below LOD)
n.q.	not quantifiable (below LOQ)
NUL	normal (added) use level
P	Poland
97.5th perc.	97. 5th percentile
PLB	private label brand
Q1	25th percentile
Q2	50th percentile (median)
Q3	75th percentile
OAV	odour activity value
OTV	odour threshold value
Rf	response factor
RI	retention index
RT	room temperature
SCF	Scientific Committee on Food
SD	internal standard
SPET	Single Portion Exposure Technique
Tb	tea bag
Ts	tea shop
TTC	Threshold of Toxicological Concern
UK	United Kingdom
WHO	World Health Organization

1 INTRODUCTION AND OBJECTIVES

Within the scope of Regulation (EC) No 1334/2008 (EU 2008) a list of flavouring substances authorized for use in or on foods in the European Union has been adopted (EU 2012). The flavouring substances contained in the Union list have been subjected to a comprehensive safety evaluation procedure by the European Food Safety Authority (EFSA), in which estimations of the dietary exposure to flavouring substances via the consumption of flavoured foods played a central role (EU 1996, 2000).

Several calculation techniques estimating the exposure of flavouring substances have been employed during the last decades. They are based either on annual production volumes such as the 'Maximized Survey-derived Daily Intake' (MSDI) or on the application of use levels and portion sizes of food categories such as the 'modified Theoretical Added Maximum Daily Intake' (mTAMDI) developed by EFSA (2004) and the so-called 'Single Portion Exposure Technique' (SPET) developed by the FAO/WHO Joint Expert Committee on Food Additives (JECFA 2006; Leclercq et al. 2009). The application of a similar approach, the 'Added Portion Exposure Technique' (APET) is foreseen in the EFSA guidance for future risk assessments of flavourings to be used in or on foods (EFSA 2010, 2011).

These approaches rely only on rough estimates of standard portions of food and data on the occurrence and concentrations of added flavouring substances in foods provided by industry. One objective of the European Union-funded project 'Flavourings, Additives and Food Contact Materials Exposure Task' (FACET) was to assess uncertainties in the occurrence and concentration levels of flavouring substances through the collection of actual data for flavoured foods (Hearty 2010; Raffo et al. 2011, 2012, 2013). In this context, the aim of this thesis was to experimentally determine distributions of concentrations of added flavouring substances in foods available in the EU.

Bergamot-flavoured, so-called 'Earl Grey' tea, was chosen as an example for a flavoured food product. For this flavoured beverage it is possible to quantitatively cover relevant flavouring substances by analysing only five compounds: Linalyl acetate (15.6-41.4%), linalool (1.7-26.0%) and limonene (10.5-45.8%) are the major constituents of cold pressed bergamot oils; together with β-pinene (0.08-11.0%) and γ-terpinene (3.7-11.4%), they represent over 90% of the volatile fraction (Schenk and Lamparsky 1981; Verzera et al. 1996; Mondello et al. 1998; Melliou et al. 2009; Dugo et al. 2012). Sensory analysis via gas chromatography-olfactometry showed that these substances are not only abundant but also of sensory importance to the aroma of bergamot essential oil (Sawamura et al. 2006).

Data on the contents of flavouring substances in Earl Grey teas were so far limited to quantitative analyses of limonene, linalyl acetate and linalool in a set of twelve samples from

the Swiss market (Neukom et al. 1993). Other information available related solely to the enantiomeric distributions of chiral flavouring substances determined as a basis for authenticity assessments (Neukom et al. 1993; Casabianca and Graff 1994; Casabianca et al. 1995; Aargau 2006; Ravid et al. 2010).

The first objective of this thesis was to extend these data by analysing the amounts of the five main monoterpenes and the enantiomeric distributions of the chiral compounds in 90 Earl Grey teas purchased in 10 member states of the European Union (EU). The data obtained should be subjected to statistical assessments in order to examine possible impacts such as (i) country of purchase, (ii) enantiomeric distributions of chiral substances, (iii) type of packaging, and (iv) source of products. In addition, the effects of storage on the contents of flavouring substances should be investigated.

To finally assess dietary exposure, in the second part of the studies transfer rates of linalyl acetate and linalool from the tea leaves into the tea beverage upon preparation of a hot water infusion were determined. To allow more generalized conclusions regarding the behaviour of monoterpene esters upon hot water infusions, flavouring substances structurally related to linalyl acetate as well as the impact of brewing time were additionally investigated. Considering that in the cold pressed bergamot oil the (*R*)-enantiomers of linalyl acetate and linalool are predominating (Dugo et al. 2001; Costa et al. 2009), the impact of the hot water treatment on the enantiomeric compositions of these flavouring substances should be followed.

To support the data set on flavouring substances in Earl Grey tea, a second flavoured tea, namely ‘Forest Fruit tea’, was analysed via a standard addition method.

The preparation of tea is a simple example of a processing step that may influence the concentrations of flavouring substances in the finally consumed product. Losses and changes of the initially added volatiles may occur during industrial processing of foods (Young et al. 2006) as well as in the course of home-preparation procedures. Therefore, the consideration of the respective correction factors constitutes a key element in the assessment of the actual dietary exposure to flavouring substances.

2 BACKGROUND

2.1 Dietary exposure assessment

Exposure assessment is a key component in the course of risk assessments as defined by the Codex Alimentarius Commission (CAC 2008, 2011). Together with risk management and risk communication it builds the fundamental framework of risk analysis. Exposure assessment is defined as “the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant”. It combines concentrations of chemicals in food with food consumption data (ICPS 2009). In the present work, the term ‘dietary exposure’ is equally used to ‘dietary intake’ or just ‘intake’. The following chapter will deal with dietary exposure assessment in the context of flavouring substances.

2.2 Risk assessment of flavouring substances

The European Union Regulation (EC) No 2232/1996 laid down a four-step community procedure for flavouring substances used or intended for use in or on foodstuffs (EU 1996; SCF 1999). In this context, an inventory of all flavouring substances used at that time in the European Union was notified by all member states summarising all information available like chemical formula, CAS number, IUPAC nomenclature, origin of the substances, and the foods in which the flavourings were mainly used (step 1). After the adoption of this register (step 2) by Commission Decision 1999/217/EC (EU 1999), a programme for the toxicological evaluation of these flavouring substances was adopted (step 3), taking into account the general use criteria given in the Annex of Regulation 2232/1996 such as (i) no risk to health and no misleading of consumers, (ii) appropriate toxicological evaluation, in case of genetically modified origin (GMO), inclusion of environmental safety evaluation, and (iii) constant monitoring and re-evaluation whenever necessary. Step 4 was the adoption of a list of flavouring substances, the use of which is authorised to the exclusion of all others (so-called Union list) (EU 1996; SCF 1999). This list has been adopted by Commission Implementing Regulation (EU) No. 872/2012 of 1 October 2012 (EU 2012).

The approximately 2800 notified flavouring substances in the register were classified into 34 chemical groups and numbered with a FLAVIS number (FL No.: Flavour Information System). The risk assessment was performed on this group-based system since single evaluations were not feasible due to the high number of substances.

The grouping of chemically related substances, e.g. aliphatic alcohols, aldehydes, acids or unsaturated tertiary alcohols, has the advantage that individual toxicological data can be taken into account for the whole group evaluation.

The stepwise approach originally developed by JECFA (Joint FAO/WHO Expert Committee on Food Additives) takes into account “current uses, structure-activity relationships, metabolism and toxicity” (EU 2000). Due to the lack of scientific toxicological data for all flavouring substances, the subdivision of compounds into three classes according to the safety concern plays an important role (EU 2000). These classes of Thresholds of Toxicological Concern (TTC) – as specified by Munro et al. (1999) - are (I): 1800 µg/person/day (“simple chemical structures and efficient modes of metabolism which would suggest a low order of oral toxicity”), (II): 540 µg/person/day (“intermediate" substances which “possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in Class III; substances may contain reactive functional groups”) and (III): 90 µg/person/day (“substances of a chemical structure that permits no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups”). The TTC principle was examined by an expert group of the International Life Sciences Institute (ILSI Europe), which concluded that it is applicable for low concentrations of chemicals in food that lack toxicological data (Cramer et al. 1978; Cadby 1996; Munro et al. 1999; Kroes et al. 2000; Arcella and Leclercq 2005).

As shown in A3 and B3 of Figure 1, the decision-tree indicates clearly the necessity of intake data, because the direction of the decision pathway is dependent on whether the intake exceeds or is lower than the TTC.

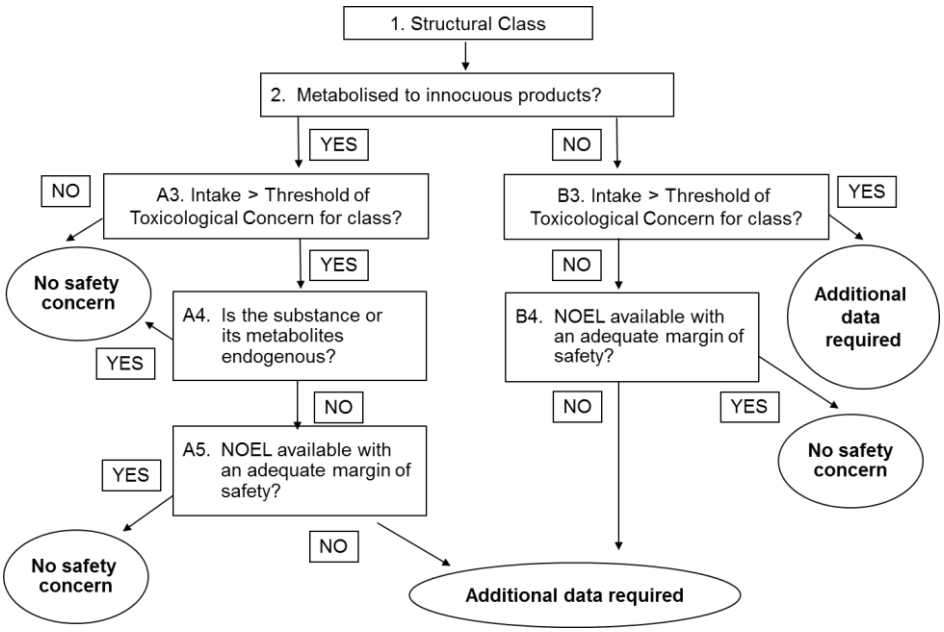


Figure 1. Safety evaluation of flavouring substances via a stepwise approach (decision-tree); after EFSA 2010.

2.2.1 Models for dietary exposure estimations

Several techniques have been employed for the calculation of dietary exposure. These are either based on poundage data (annual production volumes of the chemical) reported by industry such as the 'Maximized Survey-Derived Intake' (MSDI) or on use levels and consumption data for certain food categories such as the 'modified Theoretical Added Maximum Daily Intake' (mTAMDI). The calculations for the MSDI and the mTAMDI are given in equations (1) and (2) (Bergsten et al. 2002):

$$(1)\quad \text{MSDI: Intake [µg/d]} = \frac{(\text{annual volume [kg]}) \times (1 \times 10^9\ [\mu g / kg])}{\text{eaters only} \times 365\ \text{days}}$$

annual volume = *reported annual volume / 0.6*
eaters only = *size of the population x 0.1*

$$(2)\quad \text{mTAMDI: Intake [µg/d]} = \sum (\text{NUL in food category} \times \text{Intake of food category})$$

NUL = *normal use level*
Intake of food category = *foods: 133 g/d*
non-alcoholic beverages: 324 g/d
exceptions:
candy/confectionary: 27 g/d
condiments, seasonings: 20 g/d
alcoholic beverages: 20 g/d
soups, savouries: 20 g/d
others (e.g. chewing gum): 2 g/d

The MSDI method used by JECFA is a per-capita estimate assuming that 10% of the population consume flavoured foods and that only 60% of the volume is reported by the industry. The result is compared to the TTC in the decision-tree approach (Bergsten et al. 2002; JECFA 2006; Figure 1). Uncertainties related to the MSDI technique have been pointed out by Bergsten et al. (2002) and Arcella and Leclercq (2005). The Scientific Committee on Food (SCF) stated that the MDSI may underestimate the intake for certain groups of consumers (SCF 1999). This might be due to the facts that flavouring substances (i) could be present only in very few food categories, (ii) are consumed by only a limited number of persons, or (iii) that the production volumes between different years could vary significantly.

The mTAMDI approach has been employed by EFSA (2004) and is based on normal added use levels (NUL) reported by the applicant in defined food categories. This calculation method often overestimates the intake of flavourings "mainly due to the assumption that all flavoured food and beverages contain the particular flavouring and that the substance is

always present in amounts" (Bergsten et al. 2002) equal to the NUL. In order to improve the use of more specific food groups, the Nordic project group proposed and identified 35 sub-groups of relevance (Bergsten et al. 2002).

The so-called single portion exposure technique (SPET) developed by JECFA combines average added use levels for a flavouring substance with standard portion sizes of flavoured foods (JECFA 2008; Leclercq et al. 2009). The application of a similar approach, the added portion exposure technique (APET), which considers normal occurrence levels of flavouring substances and standard portions for each food category, is foreseen in the EFSA guidance for future risk assessments of flavourings to be used in or on foods (EFSA 2010, 2011). These approaches rely only on rough estimates of standard portions of food and data on the occurrence and concentrations of added flavouring substances in foods provided by industry. They do not consider uncertainties in the occurrence and concentration levels of flavouring substances and the possibility of exceptions of single flavouring substances within one chemical group evaluation. Therefore, the European Commission funded the FACET project as part of its Framework 7 programme (Theme 2 Food, Agriculture and Fisheries, and Biotechnology, KBBE-211686) in order to expand the scientific approach to flavouring substances but also to other food chemicals (additives and migrants from food contact materials).

2.2.2 The FACET Project

The Flavourings, Additives and Food Contact Materials Exposure Task (FACET) was a large-scale collaborative EU-funded research project. It started September 2008 and lasted 48 months (http://www.ucd.ie/facet). It involved 20 collaborative partners from 13 countries across the European Union covering academia, industry, research centres, and small-to-medium enterprises; it was coordinated by Prof. Mike Gibney (University College Dublin). The final output of FACET was the development of a risk management software taking into consideration all data collected in the course of the project (Hearty 2010; Mercea 2010; EC 2011).

One objective of FACET was the assessment of uncertainties in the occurrence and concentration levels of flavouring substances via the collection of actual data for flavoured foods (Hearty 2010). This purpose represents the basis of the present work.

2.3 Tea

Tea is the most popular non-alcoholic beverage in the world after water. The world production increased from 4.2 million tonnes in 2008 to 4.6 million tonnes in 2011 (FAOSTAT 2013). In Germany, tea consumption for males aged 14-80 years amounts to 84 g/d (MRI 2008; MRI 2013). In the UK, where tea is drunk since 350 years, even 411 g/d (mean) and 1271 g/d d (95^{th} percentile) were reported for 19-64 aged men in 2000/2001 (EFSA 2008, Henderson et al. 2002).

2.3.1 Black tea

“Tea derives exclusively from the leaves, buds and tender stems of the species *Camellia sinensis* (L.) O. Kuntze of the tea plant family (Theacea), produced by generally accepted processes” (Leitsätze für Tee 1998, EHIA 2012). The beverage made from tea leaves by a hot water infusion goes back 57 centuries (Bondarovich et al. 1967) which gives rise to the huge number of publications dealing with health benefits (McKay and Blumberg 2002; da Silva Pinto 2013;), trace elements (Sofuoglu and Kavcar 2008; Karak and Bhagat 2010; Chan et al. 2013) and the formation, transformation, and occurrence of volatiles (Bondarovich et al. 1967; Saijo and Kuwabara 1967; Hazarika et al. 1984; Mick and Schreier 1984; Fischer et al. 1987; Owuor 1992; Guth and Grosch 1993; Herrmann 1994; Kawakami et al. 1995; Kumazawa and Masuda 2001; Azin et al. 2006; Alasalvar et al. 2012).

In principle, three kinds of tea exist due to their manufacturing process, although they are all made from the same plant material (*Camellia sinensis*). A distinction is made between green tea (steamed to inactivate polyphenol oxidase, not fermented), oolong tea (partially fermented) and black tea (fermented). In addition, other products like white tea or yellow tea exist (McKay and Blumberg 2002). Approximately 76-78% of the total tea production is consumed as black, 20-22% as green and less than 2% as oolong tea.

2.3.1.1 Manufacturing of black tea

After plucking ‘two leaves and the bud’ the manufacturing of black tea includes the four important steps ‘withering’, ‘rolling’, ‘fermentation’, and ‘firing’. During the withering, the water content is reduced from 70-80% to 62-64%, while the ‘rolling’ implements the damage of cell walls and thereby the reaction of the polyphenol oxidase with its reactants. This step initiates the ‘fermentation’ process (actually an enzymatic oxidation) at 90-95°C and high air humidity, and is stopped by a drying step (‘firing’), reducing the water content again from 50 to 3% (Feldheim 1994; Heiss 2004).

2.3.1.2 Flavouring substances in black tea

Various studies and reviews concerning the flavour of black tea have been published during the last decades (Bondarovich et al. 1967; Yamanishi 1967; Yamanishi et al. 1968; Yamanishi et al. 1972; Sanderson and Graham 1973; Yamanishi 1978; Takeo 1981; Hazarika et al. 1984; Mick and Schreier 1984; Schreier and Mick 1984; Fischer et al. 1987; Owuor 1992; Guth and Grosch 1993; Herrmann 1994; Wang et al. 1994; Hara et al. 1995; Kawakami et al. 1995; Schuh and Schieberle 2006; Kim et al. 2011; Alasalvar et al. 2012).

The focus of most of these investigations was on the analysis of free and bound volatiles in tea leaves of *Camellia sinensis* and their formation upon fermentation. A comprehensive overview on this subject was given by Yamanishi (1978). Despite the high complexity of the volatile fraction of tea (> 300 flavouring substances; Sanderson and Graham 1973), the following section will focus on compounds which are linked to Earl Grey tea. Five flavouring substances account for the main part with > 90% of the volatile fraction of bergamot oil, the ingredient with which Earl Grey tea is flavoured. Linalool makes up the majority of the flavouring substances in black tea leaves as well as in black tea infusions (up to 18 µg/g in Darjeeling tea), followed by geraniol (up to 5 µg/g) as reported by Fischer et al. (1987), Herrmann (1994), Kawakami et al. (1995) and Schuh and Schieberle (2006). Besides, also α-terpineol and limonene were quantified by Fischer et al. (1987) and Herrmann (1994). The hydrocarbons (*Z*)-ocimene, (*E*)-ocimene, γ-terpinene and terpinolene have been determined in smaller quantities in Darjeeling tea in contrast to an Indian broken one, in which only (*E*)-ocimene could be quantified out of this group (Herrmann 1994). In addition to the already mentioned compounds, Yamanishi et al. (1972) identified myrcene, nerol, neryl acetate and geranyl acetate. The only flavouring substance not yet been found in black tea but occurring in bergamot oil is β-pinene.

2.3.2 Flavoured tea

“Flavoured tea is tea to which fragrance and/or flavouring substances are added in order to lend a specific flavour” (Leitsätze für Tee 1998, EHIA 2012).

2.3.2.1 Earl Grey tea

One of the most successful speciality teas, ‘Earl Grey’, refers to a bergamot-flavoured black tea. It tastes lemon-like and pleasantly tart fresh. The tea is flavoured with bergamot oil obtained from the rind of this citrus fruit (see 2.4.2) or with chemically synthesised flavouring substances. Whereas in former times, Chinese black teas have mainly been used for production, Indian teas, especially Darjeeling but also Ceylon teas, are representing the

basic teas today. Recently, also products prepared from green or rooibos tea have been marketed (Gill 1992; Tee-online 2008-2012).

Several legends exist regarding the name of ‘Earl Grey’ tea. One of these reports that during a mission in China, an English diplomat rescued the life of a mandarin, who presented him with gratitude the recipe of the bergamot-flavoured tea for Charles Grey, 2nd Earl Grey then prime minister of England (1830-1834). Another legend describes that a shipment of tea and bergamot oil got scrambled due to a storm on its way from China to England, resulting in a spilling of the oil on the dry tea. Assuming that the tea was spoiled, it was tasted by the Earl. The accidently ‘flavoured’ tea was judged as excellent and shortly marketed (Teaworld 2013; Schöchl 2013).

2.3.2.2 Fruit tea

Fruit teas belong to so-called ‘herbal infusions [German: teeähnliche Erzeugnisse]’ and consist of plants or parts of plants like leaves, fruits, or blossoms that do not originate from the tea plant *Camellia sinensis* (L.) O. Kuntze. However, they are also intended to be used as tea by brewing with boiling water. Fruit teas are named by the kind of plant in combination with ‘tea’ like ‘peppermint’ or ‘strawberry tea’. In case of numerous plant kinds, a collective name like ‘herbal’ or ‘fruit tea’ is used. Additionally, the term ‘flavoured’ has to indicate on the label that the product is flavoured (Leitsätze für Tee 1998; EHIA 2012). Typical ingredients for fruit teas are constituents of apples, rose hips, hibiscus, and peels of citrus fruits; hibiscus is often used for its colouring effect (WKF 2012).

2.4 Bergamot – a unique citrus fruit

2.4.1 Botanical classification, history and application

Citrus bergamia (Risso & Poiteau) (1826) or *Citrus aurantium* L. *subsp. bergamia* (Risso & Poiteau) Wight et Arnott ex. Engler (1896) belongs to the genus of *citrus* and the family of Rutacea (rue family) (Deng et al. 1996; Ashari 1999; Erhardt et al. 2008). In contrast to older literature as reviewed by Federici et al. (2000), recent studies verified the theory of Scora (1988) that bergamot is a hybrid of sour orange (*Citrus aurantium* L.) and citron (*Citrus medica* L.) (Deng et al. 1996; Nicolosi et al. 2000). Another study identified the parentage of bergamot from *Citrus aurantium* L. and *Citrus limetta* Risso (Federici et al. 2000).

Figure 2. Bergamot-Fruit. Illustration from Nürnbergische Hesperides (Volckamer 1704).

Bergamotta (ital.) means 'noble table pear [edle Tafelbirne]' and designates a pear cultivar, which was imported from Asia Minor to Italy, particularly Sicily. The word was affected by the influence of lat. *Pergameus* ('from Pergamon in Mysia'), today the Turkish city Bergama. A theory states that the word bergamot is derived from the Turkish word *beg armūdī* (probably impacted by the city Bergama), meaning 'Pear of the Prince'. In the late 17th century, the name was assigned to the aromatic oil, the fruit and the tree *Citrus bergamia*. The reason of this transfer is not known, but explainable when comparing size, shape and colour of bergamot fruits with big table pears (Genaust 1996; Muller 1966); this is also expressed in the illustration of Volckamer (1704) shown in Figure 2. Another theory assumes that Christopher Columbus brought the plant from the West Indies or Canary islands to the Spanish town Berga from where it was taken to Calabria in Italy, then a Spanish dependency (Ashari 1999).

The geographical origin of bergamot is not exactly known, although Italy, China, the Barbados, Greece, Spain and the Canary Islands have been suggested (Chemat 2010). Probably, it appeared in the middle of the 17th century in Italy (Hodgson 1967; Mazza 1986); the first reported bergamot orchard was planted in 1750 by Nicola Parisi near Reggio Calabria (Consorzio Del Bergamotto 1989).

Bergamot fruits are cultivated almost exclusively to produce essential oils due to the acid and bitter taste of the pulp (Costa et al. 2009; Pernice et al. 2009; Sommella et al. 2013). The first time bergamot oil was registered was 1688 in a pharmacy in Gießen (Hesse); Flückinger (2004) wrote: "Oleum Bergamottae ist mir in keiner älteren Schrift aufgestossen [Oleum Bergamottae did not come up to me in elderly writings]". The oil is an important ingredient in

many cosmetic products such as perfumes, toilet-waters, deodorants, face-powders, blushers and lotions, but also in pharmaceutical products, liquor flavourings and flavoured tea (Chemat 2010). It was also the influence of the famous perfume 'Eau de Cologne', which gave the impetus to the production of bergamot in the last but one century. 'Aqua admirabilis' was invented in Cologne 1676 by the Italian emigrant Paolo Feminis and commercialized since 1709 by his son-in-law Gian Maria Farina who renamed it 'Aqua di Colonia'. Another source speaks of his grandson who provided the perfume in 1818 on the market (Chapot 1962; Hodgson 1967; Muller 1966). Other famous products flavoured with bergamot oil are 'La bergamote de Nancy', a candy from France, and the flavoured black tea - 'Earl Grey', which was invented in England.

Nowadays, bergamots are grown mostly in Reggio Calabria, a province of the region Calabria in Southern Italy. In the early 1990s, the cultivation was limited to approximately 3000 ha in a narrow land stretch about 100 km located at the Calabrian coast between the Ionian and the Tyrrhenian Sea next to Sicily (Figure 3). In total, the world production was 18.000 t of fruits in the 1991/1992 season, yielding about 100 t bergamot oil. Bergamot oil reaches prices of about US$ 125/kg (1997) (Ashari 1999). Italy is the market leader with an amount of 95% (Ashari 1999; Sawamura et al. 1999; Verzera et al. 1999; Verzera et al. 2003; Mandalari et al. 2006). The citrus fruit is also planted in considerable quantities in the Ivory Coast, in Brazil, Argentina and China (Chemat 2010).

Figure 3. Map of the region Calabria of Southern Italy. The Province of Reggio Calabria is highlighted.

The trees of bergamot fruits are only cultivated by grafting; today this is done to a two-year-old plant of sour orange (Verzera et al. 2003). The yield per tree may reach 200-300 kg of fruits, which were usually harvested from December to March (Lamonica et al. 1990; Ashari 1999). Four cultivar groups are known: *Common Bergamot*, *Melarosa*, *Torulosa* and *Piccola*. In practice, three cultivars of *Common Bergamot* are commercially cultivated: *Femminello, Castagnaro and Fantastico*. *Fantastico* (*Inserto*), a hybrid of *Femminello* and *Castagnaro*, has largely replaced the previously primarily grown *Femminello* and *Castagnaro* cultivars (Ashari 1999; Hodgson 1967; Muller 1966).

2.4.2 Production of citrus peel oils

Citrus fruits in general are composed of peel and flesh. The peel consists of the epicarp or flavedo and the albedo. The oil glands located in the flavedo comprise between 0.4-0.6 mm in diameter and release the oil under mechanical damage processes such as pressing or

grinding. The promptness with which the oil is extruded from the glands is influenced by the freshness and ripeness of the fruits. As the oil is rapidly reabsorbed by the surrounding tissues, especially the albedo, which is spongy and able to absorb liquids rapidly, the extraction methods are always performed under strong jets of water to wash away the oil. The second reason for the use of water is that it causes a swelling of the peel due to osmosis. Consequently, the oil squirts out with higher force when the oil glands are injured due to the higher pressure of the surroundings (Di Giacomo 2002; Di Giacomo and Di Giacomo 2002).

Normally, plant essential oils are prepared by steam distillation, whereas citrus peel oils, also called agrumen oils, are usually extracted by mechanical processes based on rupture of the flavedo resulting in a release of the oil from the oil glands.

The traditional technique ‘sfumatura’ (also: ‘Calabrese method’ or ‘slow-folding process’) for the extraction of citrus essential oils is based on manual pressing of the peels with subsequent adsorption of the oil by natural sponges placed in a special container. The first industrial machine, called ‘The Calabrian Machine’, was invented by Nicola Parisi (Di Giacomo and Di Giacomo 2002; Chemat 2010).

The ‘sfumatura’ process belongs to the category of methods by which the citrus oils are extracted after juice extraction. Another principle is the extraction of the essential oil from the whole fruit preceding the juice extraction. The most common method in this category for bergamot oil is the use of a ‘Pelatrice Speciale’ machine (Figure 4), which rasps (‘pelare’ (ital.): to peel / pick / rip off) the surface layer containing the oil glands with screw graters (Figure 5). Showering water carries the peel particles and the oil to several separators. The oils obtained by this technique are named according to the process step as described by Mondello et al. (1998): ‘Pulizia’, ‘Dischi’, ‘Torchiati’, ‘Ricicli’ and ‘Fecci’ oils.

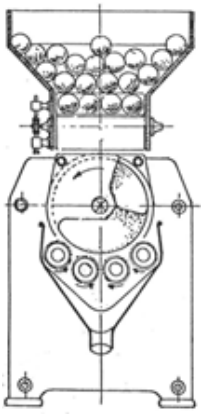


Figure 4. ‘Pelatrice Speciale’ machine (in Di Giacomo and Di Giacomo 2002).

Figure 5. Rolling screw graters in a ‘Pelatrice Speciale’ machine (in Chemat 2010).

Another machine is called ‘Brown extractor’, whereby the peel is punctured with minimal damage to the tissues of the fruit. This technique is mainly used in the United States and in South America.

The third kind of extraction of citrus oils is the simultaneous extraction of both essential oil and juice performed by the FMC-in line process (Food Machinery Corporation). More than 50% of extractors in the United States are of the FMC type; other large producer countries, such as Brazil and Argentina, use these extractors exclusively (Di Giacomo and Di Giacomo 2002; Fischer 2008; Chemat 2010; Kubeczka 2010; Schmidt 2010).

2.4.3 Composition of bergamot essential oil

The appearance of bergamot oil extracted by the Pelatrice method varies from green at the beginning to dark yellow at the end of the harvesting season, which lasts from December to March (Lamonica et al. 1990; Costa et al. 2009).

The oil consists of 93-96% volatiles and 4-7% non-volatiles. Compounds of the non-volatile part are mainly coumarines and psoralenes such as bergapten (5-methoxy-psoralen), citropten (5,7-dimethoxycoumarin), bergamottin (5-geranyloxypsoralen), and 5-geranyloxy-7-methoxycoumarin with photosensitizing activity and therefore not desirable in the commercial oil (Mondello et al. 1993; Poiana et al. 2003).

The volatile part of citrus oils consists mainly of monoterpene hydrocarbons, especially limonene. This hydrocarbon amounted to 86.2% in navel oranges (Usai et al. 1992), peel oil of *Citrus jambhiri* Lush. (rough lemon) exhibited up to 92.2% (Shaw and Wilson, 1976), and sweet oranges contained 88-97% limonene (Ohloff 1994).

Bergamot oil represents an exception with extremely low limonene and extremely high linalool and linalyl acetate contents in contrast to other citrus oils (Shaw 1979). In the late 19th century, linalyl acetate has already been reported as the key component of this special citrus oil (Bornträger 1896). Mai et al. (1903) confirmed the outstanding role of that ester. Another exception beyond citrus oils containing very low amounts of limonene (17%) is *Citrus* subsp. Kiyookadaida, another sour citrus variety, exhibiting > 60% myrcene (Minh-Tu et al. 2003).

Numerous investigations performed in the last three decades deal with the contents of volatiles in bergamot oil (Schenk and Lamparsky 1981; Mazza 1986; Dugo et al. 1991; Verzera et al. 1996; Ferrini et al. 1998; Mondello et al. 1998; Kiwanuka et al. 2000; Kondo et al. 2000; Kirbaslar et al. 2001; Fang et al. 2004; Sawamura et al. 2006; Belsito et al. 2007; Costa et al. 2009; Melliou et al. 2009; Fantin et al. 2010; Dugo et al. 2012). Other studies present analyses of bergamot oils from other regions than Italy: Uruguay (Dellacassa et al.

1997), Africa and Corsica (Huet and Dupuis 1968), North Africa (Schwob 1955), Japan (Sawamura et al. 1999) or French Guinea (Schwob 1953). Mazza (1986) detected a total of 165 volatiles in cold pressed bergamot oil, including 63 alcohols, 39 hydrocarbons, 20 esters, 12 carbonyl compounds, and 19 (ep)oxido compounds.

Ranges of the five main monoterpenes in cold pressed bergamot oil, which represents the most authentic form of the oil, are summarised in Figure 6; linalyl acetate, linalool, limonene, β-pinene and γ-terpinene represent over 90% of the volatile fraction of cold pressed oils.

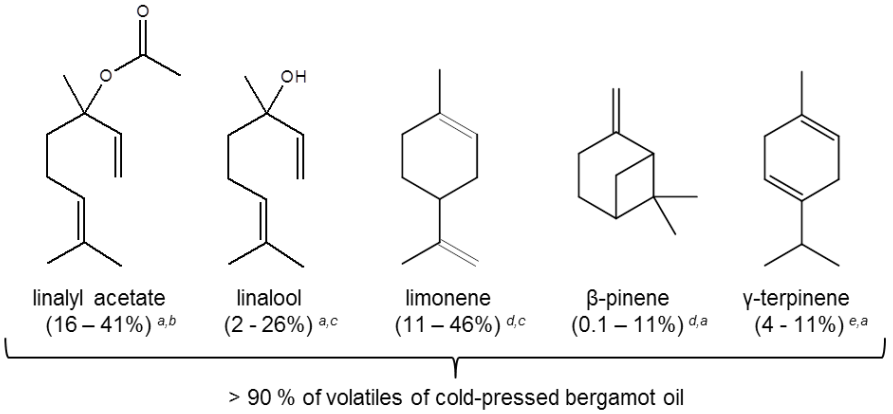


Figure 6. Percentage distribution of the five main monoterpenes in cold pressed bergamot oil; [a] Verzera et al. 1996; [b] Mondello et al. 1998; [c] Dugo et al. 2012; [d] Melliou et al. 2009; [e] Schenk and Lamparsky 1981.

2.4.4 Impact factors on bergamot oil composition

Numerous studies deal with the investigation of the impact of several factors on the composition of volatiles in bergamot oil. Possible impact factors are harvest time, harvest season, origin, latitude, storage, type of processing, and rootstock. The following chapters illustrate the complexity and quantitative variations of flavouring substances of bergamot oil.

2.4.4.1 Harvest time

The impact of the harvest time between December and March was demonstrated by Melliou et al. (2009) in Greek bergamot oil. As shown in Figure 7, the contents of the four main monoterpenes significantly varied during the 2007/2008 season; limonene showed the highest variability ranging between 10.5% and 25.6%. The maximum level of linalool was found in December, whereas linalyl acetate reached its highest content in January (Melliou et al. 2009).

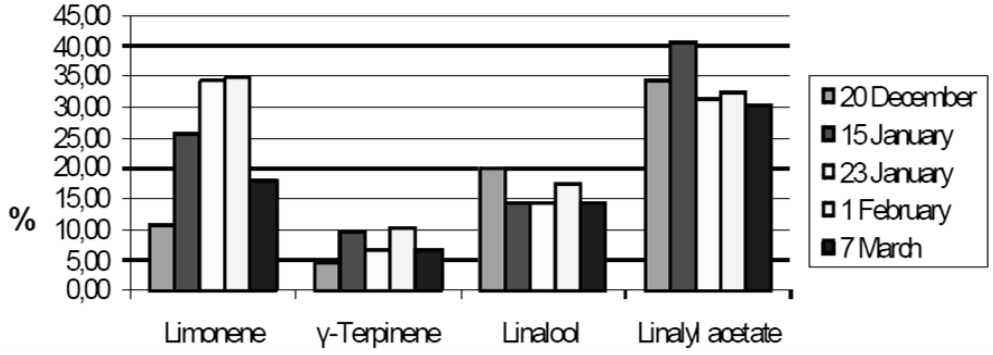


Figure 7. "Variation of the main components concentrations [of bergamot oil from Greece] depending on the harvesting time [in the season of 2007/2008]" (Figure 1 in Melliou et al. 2009).

Bergamot oil from Turkey was analysed by Kirbaslar et al. (2001) during the season of 1998/1999. They chose similar harvesting times like Melliou et al. (2009), but excluded the month March. Whereas the amounts of limonene varied to a lower extent than in the Greek oil, linalool and linalyl acetate showed again large variations. Linalool decreased continuously between December and February, and linalyl acetate showed increasing amounts in this harvest period (Figure 8).

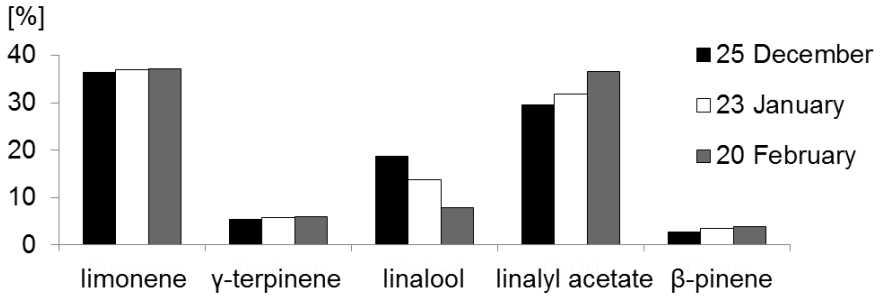


Figure 8. Variation of the concentrations of the main components of bergamot oil from Turkey depending on harvesting time in the season of 1998/1999; according to Kirbaslar et al. 2001.

In agreement with these findings, Dugo et al. (2012) stated that linalool decreased drastically during the season in contrast to hydrocarbons and linalyl acetate, which tended to increase from October to March during every production season (n=3) between 2008 and 2011. Similar results have been reported by Dugo et al. (1991) for seasons between 1984 and 1988. Dellacassa et al. (1997) reported contents of bergamot oil constituents from Uruguayan fruits of the year 1995. They compared values of June and July in contrast to the usual harvesting months in winter. Limonene increased from 27.4 to 41.4%, whereas linalool decreased from 30% to 12.6%. Linalyl acetate was less affected showing values of 30.9 and 28.5%, respectively.

2.4.4.2 Harvest season

An extensive survey including 1082 samples was conducted by Verzera et al. (1996). 829 samples from the years 1984-1988 were compared to 128 samples from 1991/1992 and to 125 samples from 1992/1993. There were large margins between minimum and maximum values for each substance during the harvest period. However, the variability of both, minimum or maximum amounts between the different harvest seasons was low (Table 1).

Regarding the results of Dugo et al. 2012 (Table 2) for the crop years between 2008 and 2011 (3 harvest seasons), some exceptions could be identified: on the one hand, all minimum values were again very similar, whereas the maximum amounts for linalyl acetate and linalool differed more than those of limonene, β-pinene and γ-terpinene.

Table 1. Contents of cold pressed Calabrian bergamot oils (n=1082) during 5 harvest seasons between 1984 and 1993 (after Verzera et al. 1996).

crop year(s)	linalyl acetate	linalool	limonene + β-phellandrene	β-pinene	γ-terpinene
	Minimum values [%]				
1984-1988	15.6	1.7	27.4	4.4	5.7
1991/1992	19.4	2.7	27.2	4.3	5.8
1992/1993	20.2	4.5	25.6	4.8	5.8
	Maximum values [%]				
1984-1988	40.4	20.3	53.2	11.0	11.4
1991/1992	38.4	17.3	49.3	9.2	10.5
1992/1993	40.3	18.6	46.1	10.8	9.6

Table 2. Contents of cold pressed Calabrian bergamot oils during 3 harvest seasons between 2008 and 2011 (according to Dugo et al. 2012).

crop year(s)	linalyl acetate	linalool	limonene	β-pinene	γ-terpinene
	Minimum values [%]				
2008/2009[a]	24.4	4.3	41.8	5.7	7.1
2009/2010[b]	24.2	6.0	39.4	4.9	6.3
2010/2011[c]	27.8	6.0	28.7	4.9	5.9
	Maximum values [%]				
2008/2009[a]	27.6	10.9	45.8	6.4	7.8
2009/2010[b]	30.2	15.4	44.5	7.0	7.5
2010/2011[c]	34.6	26.0	40.5	7.1	7.7

[a] 8 samples each representative of about 1000-2000 kg of oils from November 2008 to March 2009;
[b] 11 samples each representative of about 1000 kg of oils from November 2009 to March 2010;
[a] 23 samples each representative of about 1000 kg of oils from October 2010 to March 2011.

In a previous study, Dugo et al. (1991) postulated the possible influence of meteorological conditions in 1986/1987 and 1987/1988 on the particularly higher values of limonene in bergamot and mandarin oils in contrast to those found in oils of 1984/1985 (higher average temperature, longer periods of sunshine, and less rain).

2.4.4.3 Origin

As previously stated, bergamot oil from Calabria represents 95% of the world production and is said to be of the highest quality. Amounts of the five main monoterpenes in cold pressed Calabrian bergamot oil of eight production seasons (Verzera et al. 1996; Dugo et al. 2012) have been compared to oils originating from Turkey, Greece and Uruguay (Dellacassa et al. 1997; Kirbaslar et al. 2001; Melliou et al. 2009). As shown in Table 3, only the oil from Greece reached amounts of linalyl acetate similar to those from the Italian region. On the other hand, only in Calabria a minimum value of 15.6% was detected for that ester. The amounts of linalool were highest in Uruguay, which could probably be due to the the different hemisphere (Uruguay is located in the southern hemisphere in contrast to the other regions). The harvest time in Uruguay (May to July) corresponds to that in countries on the northern hemisphere (December to March). A very low minimum level for limonene was found in oil from Greece, although the maximum level reached almost that of the Turkey oil. However, the amounts of limonene, representing the most common terpene of citrus peel oils in general (except bergamot oil), were highest in the oil from Calabria. The amounts of β-pinene in the Greek oil were almost negligible. Oils from Turkey and Uruguay contained approximately only half of the amounts of this hydrocarbon compared to the oils produced in the South of Italy. γ-Terpinene of Turkish and Uruguayan oils showed only half of the amounts found in the Italian bergamot oil. The value detected in the Greek product was similar.

Table 3. Contents of monoterpenes in cold pressed bergamot oils of different origins.

	linalyl acetate [%]		linalool [%]		limonene [%]		β-pinene [%]		γ-terpinene [%]	
origin	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Turkey[a]	29.5	36.6	7.9	18.7	36.4	37.2	2.7	3.9	5.4	5.9
Greece[b]	30.3	40.5	14.5	20.2	10.5	34.9	0.1	0.7	4.3	10.3
Uruguay[c]	24.6	30.9	12.6	31.3	27.5[e]	41.3[e]	3.9[f]	5.8[f]	3.8	5.8
Italy[d]	15.6	40.3	1.7	26.0	25.6[g]	46.1[g]	4.3	11.0	5.7	11.4

[a] Kirbaslar et al. 2001, harvest period 1998/1999; [b] Melliou et al. 2009, harvest period 2007/2008 (oils from Kefalonia); [c] Dellacassa et al. 1997, harvest period 1997; [d] Verzera et al. 1996, harvest periods of 1984-1988, 1991/1992 and 1992/1993 and Dugo et al. 2012, harvest periods of 2008/2009, 2009/2010 and 2010/2011 (all oils from Calabria); [e] percentages include β-phellandrene; [f] percentages include sabinene; [g] percentages include β-phellandrene due to coelution.

Sawamura et al. (2006) stated that the linalool content of bergamot oils from other countries in general is higher (10-20%) than in Italian oils. The ratio of linalool to linalyl acetate is an important quality criterion and has been reported to be approximately 0.3 for bergamot oils produced in Reggio Calabria.

2.4.4.4 Cultivars

Peel oils of different bergamot cultivars grown in Sicily were compared by Verzera et al. (2000). The cultivars *Fantastico*, *Castagnaro* and *Feminello* showed very similar contents of all volatiles. However, some exceptions, e.g. linalool, which showed lower amounts in *Castagnaro*, were identified. The results were compared to oils of the same cultivars produced in Reggio Calabria (Verzera et al. 1996). Significant differences were observed between the *Castagnaro* oils from Sicily compared to those from Calabria as lower amounts of alcohols, including linalool, and higher amounts of monoterpenes, including limonene, have been detected.

2.4.4.5 Latitude

A study was performed by Lamonica et al. (1990), also reported by Dugo et al. (1991) and Verzera et al. (1998), regarding the geographical conditions of the bergamot cultivation. They devided Reggio Calabria into ten homogeneous sectors going U-shaped from the Tyrrhenian to the Ionian side of the coast (Figure 9) and compared carbonyl compounds, esters, monoterpenes and alcohols according to this grouping system.

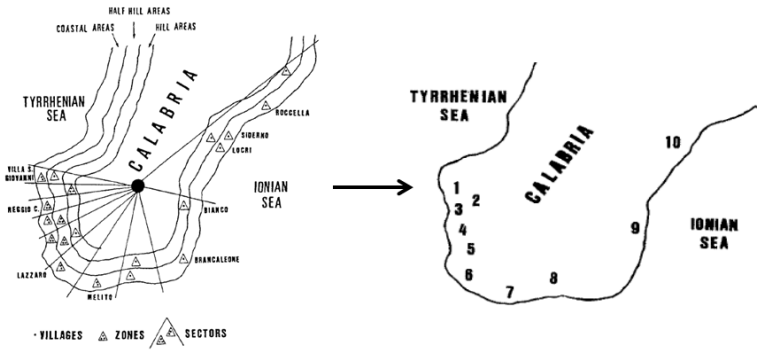


Figure 9. Fragmentation of Reggio Calabria into 10 sectors going from the Tyrrhenian to the Ionian Sea (after Dugo et al. 1991; Lamonica et al. 1990; Verzera et al. 1998.

Curves of amounts of the substance classes plotted against the ten sectors showed either a convex course (monoterpenes) or a concave course (esters and alcohols) according to the shape of the Calabrian Coast. Amounts of carbonyl compounds like neral and geranial were irregular, but had higher values in South Calabria. These courses could be observed in three

harvesting periods (1984/1985, 1986/1987, 1987/1988) and showed clear correlations of volatile amounts in bergamot oil to the latitude. In contrast, the influence of altitude was negligible.

2.4.4.6 Storage

The composition of bergamot oil is influenced by storage (Mazza 1986). The number of substances found in fresh oil decreased from 161 to 126 in a period of two years. The variation of the five main terpenes in oil stored for two years in brown glass bottles at 3°C is illustrated in Figure 10. Limonene decreased by 14%, whereas amounts of γ-terpinene were < 0.1% after storage time. On the other hand, the contents of linalool and linalyl acetate were quite stable.

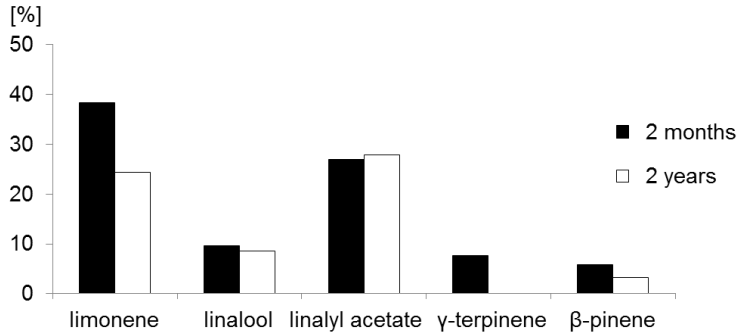


Figure 10. Variation of monoterpene amounts of stored, cold pressed bergamot oil (after Mazza 1986).

2.4.4.7 Type of processing

As previously stated, citrus fruits are usually processed by cold pressing. Although the application of other methods like steam distillation could result in lower qualities, they are used to obtain higher yields. Additionally, the oil can be treated after the production yielding deterpened or furocoumarin-free oils (see 2.4.4.9).

As shown in Table 4, steam distillation of bergamot oil at different pH conditions and whole fruit distillation (distillation of bergamot fruits cut into pieces with natural pH) caused a decrease of linalyl acetate and an increase of linalool due to hydrolysis reactions of the ester compared to a solvent extraction (Kiwanuka et al. 2000).

Table 4. Contents [%] of main monoterpenes in bergamot oils obtained by different processing methods (after Kiwanuka et al. 2000).

substance [%]	solvent extraction	steam distillation pH 5.3	steam distillation pH 2.5	whole fruit distillation
linalyl acetate	30.5	21.4	15.9	5.4
linalool	5.2	10.7	12.8	13.6
limonene	40.2	41.7	42.2	44.4
β-pinene	4.9	5.0	3.7	5.3
γ-terpinene	7.3	7.7	7.8	10.1
ratio linalyl acetate / linalool	5.8	2.0	0.4	0.4

Similar results were reported by Belsito et al. (2007), who found lower contents of linalyl acetate and linalool after vacuum distillation in contrast to cold pressed bergamot oil. They also showed that the accumulated amounts of limonene and p-cymene increased approximately by 14%.

Additionally, isolations of essential oils from lavender and clary sage via hydrodistillation caused a formation of artefacts (α-terpineol, neryl acetate, geranyl acetate, nerol and geraniol) which could not be observed in oils extracted with a solvent mixture. In turn, the amount of linalyl acetate was 30% lower in the distilled oil of lavender compared to the oil obtained by solvent extraction (Schmaus and Kubeczka 1985).

2.4.4.8 Rootstock

Bergamot trees are only cultivated by grafting. The impact on the peel oil composition of rootstocks other than the normally used sour orange one was analysed by Verzera et al. (2003). They found differences especially in terms of linalool and linalyl acetate contents for trifoliate orange grafting; similar amounts were determined for rootstocks of Alemow and Volkamerian lemon. In addition, bergamot trees with Volkamerian lemon and Alemow as rootstock gave a more fruitful production. Therefore, as stated by the authors, these two cultivars can be considered as substitute rootstocks for sour orange concerning bergamot grafting.

2.4.4.9 Treatment options

Bergamot oil is often deterpened for several reasons like the (i) increase of aroma-active compounds (because of the rather poor odour properties of monoterpene hydrocarbons), (ii) better solubility in aqueous alcoholic solutions, (iii) prevention of oxidation and polymerization of the terpenic fraction due to instability to heat and light, and (iv) lower transport costs because of lower volumes (Russo et al. 2001; Fang et al. 2004).

Terpene-reduced oil can be obtained by distillation, vacuum distillation, solvent extraction or chromatographic separation. Disadvantages of these treatments are changes and degradations in the oil composition due to heat or acidic conditions (Sato et al. 1996; Russo et al. 2001). New gentle methods could be concentrating with solid CO_2 (Russo et al. 2001), host-guest inclusions in e.g. deoxycholic acid (Fantin et al. 2010), ionic liquids (Arce et al. 2006), supercritical CO_2 (Kondo et al. 2000) or a combination of supercritical CO_2 and vacuum distillation. The last option used by Fang et al. (2004) resulted in amounts of limonene of < 1% and a recovery of linalyl acetate and linalool of > 95%. The relative percentages of flavouring substances in the analysed oil were 19-22% linalool (starting concentration: 14.2%) and 40-48% linalyl acetate (starting concentration: 30.7%).

Because of the phototoxic (Zaynoun et al. 1977), mutagenic (Ashwood-Smith et al. 1980) and tumorigenic (Young et al. 1990) properties of coumarines and psoralenes and due to the use of bergamot oil in cosmetics and flavoured foods and beverages, these compounds are often removed from the cold pressed oil. Finsterer (2002) reported a 44 year old man, who experienced muscle cramps, fasciculations, distal paresthesias and eye pressure associated with blurred visions after consuming up to 4 L of Earl Grey tea daily. After five months, all symptoms disappeared within one week after switching back to pure black tea.

Common methods for the removal of these compounds are a distillation of the oil or treatment with NaOH. As already stated these procedures could modify the composition of the product (Verzera et al. 1998). Belsito et al. (2007) used vacuum distillation resulting in a bergapten-free product keeping important properties and contents similar to those of cold pressed oil.

2.4.5 Enantiomeric distributions of flavouring substances in bergamot oil

In cold pressed oil, the (*R*)-enantiomers of linalyl acetate and linalool are predominant (> 99%) (Cotroneo et al. 1992; Juchelka and Mosandl 1996; König et al. 1997; Mosandl and Juchelka 1997; Mondello et al. 1998; Casabianca and Chau 1998; Dugo et al. 2001). The enantiomeric distributions remain stable in genuine oil. Therefore, the determination of the enantiomeric distributions of the chiral main terpenes provides a powerful tool for the authenticity assessment of the oil. On the other hand, bergamot oils produced by other methods than cold pressing show racemisation effects of linalool specifically in acidic media; the percentage of (*R*)-linalyl acetate remains, however, stable (Baigrie et al. 1996; Bernreuther and Schreier 1991; Juchelka and Mosandl 1996; König et al. 1997; Mondello et al. 1998; Kiwanuka et al. 2000; Schmarr and Engel 2012). As shown in Table 5, the

(*R*)-enantiomer of linalool decreased from > 99 to 61.9% by distillation at pH 2.5 (Kiwanuka et al. 2000).

Table 5. Enantiomeric distributions of chiral compounds in bergamot oil before and after processing (after Kiwanuka et al. 2000).

	solvent extraction		distillation pH 5.3		distillation pH 2.5		whole fruit distillation	
	enantiomeric distribution [%]							
substance	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)
linalyl acetate	>99.0	>1.0	>99.0	>1.0	>99.0	>1.0	>99.0	>1.0
linalool	>99.0	>1.0	78.1	21.9	61.9	38.8	56.3	43.7
limonene	98.1	1.9	98.2	1.8	98.5	1.5	98.0	2.0
β-pinene	7.1	92.9	7.3	92.7	8.0	92.0	7.7	92.3

Limonene and β-pinene occur also mainly as almost enantiomerically pure enantiomers; the ranges varied between 97.3 and 98.1% for (*R*)-limonene and between 90.5 and 93.2% for (*S*)-β-pinene during one harvest period (Dugo et al. 2001).

2.4.6 Enantiomeric distributions of linalool from other natural sources

Considering that linalool from natural sources other than bergamot might be used to flavour Earl Grey tea labelled ‘with natural aroma’, a literature research regarding the occurrence and the enantiomeric distributions of linalool has been conducted. Linalool from other natural sources shows enantiomeric distributions from > 99% of the (*R*)-enantiomer in basil (Bernreuther and Schreier 1991; Ravid et al. 1997; Casabianca et al. 1998; Chanotiya and Yadav 2009), > 94% in lavender oil (Bernreuther and Schreier 1991; Schubert and Mosandl 1991; Hener et al. 1992; Casabianca et al. 1998), ~50% in geranium, rose wood, pineapple or red passion fruit (Bernreuther and Schreier 1991; Casabianca et al. 1998; Chanotiya and Yadav 2009) and up to > 80% of the (*S*)-enantiomer for coriander or sweet orange (Bernreuther and Schreier 1991; Casabianca et al. 1998; Chanotiya and Yadav 2009; Özek et al. 2010). Therefore, it is difficult to determine the natural source of added linalool solely via chiral analyses (Hener et al. 1992; Kreis et al. 1993; König et al. 1997; Kiwanuka et al. 2000).

2.4.7 Sensory evaluation of bergamot oil

The odour of bergamot oil has a fresh citrusy top note with a green, woody, sweet flowery, peppery fruity and somewhat balsamic aroma in the background (Roth and Kormann 1997; Ashari 1999; Sawamura et al. 2006). Regarding the quantitatively dominating flavouring substances linalool and linalyl acetate, these olfactory impressions are reflected: (*R*)-linalool

(licareol) has been described as lavender, woody, and flowery, whereas (*R*)-linalyl acetate has been described as bergamot-like, and flowery. The olfactory characteristics of the (*S*)-enantiomer of linalool (coriandrol) have been described as sweet and petit grain like (Ohloff and Klein 1962).

Sawamura et al. (2006) created a bergamot aroma model with a similarity of 7.1 in a nine-point-score sensory test using the determined four bergamot-like aroma compounds ((*Z*)-limonene oxide, decanal, linalyl acetate, and geraniol) together with eight other compounds including all main terpenes quantified in bergamot oil.

2.4.8 Authenticity assessment of bergamot oil

As argued in the preceding chapters, the examination of quantitative contents of flavouring substances in bergamot oils in order to define the qualitative character is ambiguous due to the large variability caused by numerous factors. Also, the analyses of the enantiomeric distributions of chiral flavouring substances, as already discussed in 2.4.5, do not prove the genuineness in every case due to racemisation effects of (*R*)-linalool in the course of some processing methods of bergamot oil. This has been discussed in detail by Hener et al. (1992) and Kreis et al. (1993). The analyses of the $^{2}H/^{1}H$ ratio of linalyl acetate could give information whether the sample comes from natural or synthetic sources; the isotope determination of linalool was not suitable for this conclusion (Hör et al. 2000). Additionally, the measurement of $\delta^{13}C$-values was applicable to prove adulterations for linalyl acetate, linalool, and limonene (Juchelka and Mosandl 1996). Schipilliti et al. (2011) postulated that this technique is able not only to detect adulterations but also to discriminate the bergamot oil samples according to their geographical provenance and the nature of the added adulterants.

Another approach suitable to determine whether linalool is of a natural or synthetic origin is an analysis regarding the occurrence of dihydrolinalool and dehydrolinalool. As shown in Figure 11, the synthesis of linalool (III) is connected with the intermediate product dehydrolinalool (II), and a possible superhydrogenation to dihydrolinalool (IV).

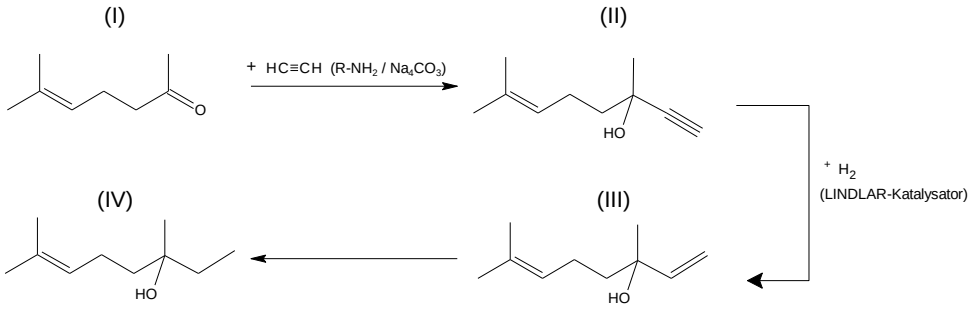


Figure 11. Synthesis of linalool (III) via ethynylation of 6-methyl-5-hepten-2-one (I) to dehydrolinalool (II) and subsequent hydrogenation. The superhydrogenation ends up in dihydrolinalool (IV) (after Agnel and Teisseire 1984 and Breitmaier and Jung 2012).

As postulated by Neukom (1993), synthetic linalool could contain up to 2% dihydrolinalool and the detection of this compound together with the analysis of the enantiomeric distributions of linalool and linalyl acetate can, therefore, serve as verification for the adulteration of natural bergamot oil (Teisseire 1987; Neukom et al. 1993; Ashari 1999; Breitmaier and Jung 2012).

3 MATERIAL AND METHODS

3.1 Materials

3.1.1 Sample material

Black tea samples

Non-flavoured black teas (n=7) were purchased in local stores. The samples were stored in the original packages at room temperature (RT) until analysis. Four samples were obtained in tea bags (tb) and three as loose leaves tea (ll).

Earl Grey tea samples

Ninety 'Earl Grey' teas were purchased in 10 member states of the European Union: Germany (D; n=28), Hungary (H; n=10), Poland (PL; n=10), United Kingdom (UK; n=10), Italy (I; n=6), Spain (E; n=6), Denmark (DK; n=5), Finland (FIN; n=5), France (F; n=5), and Ireland (IRL; n=5). The selected set included samples from international/national brands (B, n=52), private label brands (PLB, n=16) and speciality tea shops (Ts, n=22). Materials in tea bags (tb, n=54) as well as loose teas (ll, n=36) were purchased in local stores of each country. The samples were stored in the original packages at RT until analysis.

A list of all teas including date of purchase, best before date, labelling, number of tea bags, and weights of tea bags is given in the Appendix (Table 37).

Fruit tea samples

A fruit tea labelled as 'Waldbeerentee – fruchtig herb [Forest fruit tea – fruity tart]' was purchased in a local store. According to the list of ingredients, the fruit tea contained "hibiscus, rose hips, apples, flavour (forest fruits), elderberries, strawberries, blackberries, [and] raspberries". The sample was stored in the original package at RT until analysis.

3.1.2 Chemicals

Table 6. Reference substances.

substance	purity[a] (area [%])	column[b]	supplier[c]
benzaldehyde	99.7	DB-WAX	Merck
benzyl acetate	99.9	DB-WAX	FREY & LAU
damascenone, β-	95.8	DB-WAX	FREY & LAU
decanol, 2-	99.6	DB-WAX	Alfa Aesar
eugenol	99.6	DB-WAX	Aldrich
geranyl acetate	99.0	DB-5	Aldrich
heptanol, 2-	99.7	DB-WAX	Aldrich
hexenol, (*Z*)-3-	98.9	DB-WAX	Frey & Lau
hexenyl acetate, (*Z*)-3-	61.2	DB-WAX	Frey & Lau
ionone, α-	90.9	DB-WAX	Frey & Lau
ionone, β-	96.5	DB-WAX	Frey & Lau
limonene, (*R*)-	95.2	DB-5	Fluka
limonene, (*S*)-	95.9	DB-5	Fluka
linalool (racemic)	96.7	DB-5	Frey & Lau
linalool, (*R*)-	96.7	DB-5	Fluka
linalyl acetate (racemic)	>97[d]	DB-5	SAFC
linalyl acetate, (*R*)-	84.3	DB-5	Frey & Lau
linalyl benzoate	96.5	DB-5	SAFC
linalyl butanoate	96.4	DB-5	SAFC
linalyl cinnamate	97.4	DB-5	Frey & Lau
linalyl formate	92.2	DB-5	Frey & Lau
linalyl hexanoate	96.7	DB-5	Penta
linalyl isobutanoate	96.5	DB-5	Frey & Lau
linalyl isovalerate	97.8	DB-5	SAFC
linalyl octanoate	88.7	DB-5	Penta
linalyl phenylacetate	94.4	DB-5	Penta
linalyl propanoate	99.1	DB-5	Frey & Lau
menthyl acetate	97.1	DB-5	Frey & Lau
methyl isoeugenol	89.4	DB-WAX	FREY & LAU
methylbutyl acetate, 3-	96.6	DB-WAX	SAFC
methylpropyl acetate, 2-	98.3	DB-WAX	Merck
myrcene	-[f]	-	Frey & Lau
neryl acetate	99.8	DB-5	Frey & Lau
nonanol, 2-	99.5	DB-WAX	Fluka
ocimene, (*E*)-	-	-	Frey & Lau
ocimene, (*Z*)-	-	-	Frey & Lau
pinene, (1*R*,5*R*)-β-	95.6	DB-5	Fluka
pinene, (1*S*,5*S*)-β-	98.3	DB-5	Fluka
raspberry ketone	99.4	DB-WAX	Frey & Lau

substance	purity[a] (area [%])	column[b]	supplier[c]
terpinene, γ-	97.9	DB-5	Aldrich
terpineol, α-	88.2	DB-5	SAFC
terpinolene	-	-	Frey & Lau
terpinyl acetate, α-	84.9	DB-5	SAFC
undecalactone, γ-	95.9	DB-WAX	FREY & LAU
vanilline	99.5	DB-WAX	FREY & LAU

[a] The purities were determined via capillary gas chromatogaphy on the basis of peak area percentages; the concentrations of the reference substances in n-hexane (HPLC grade) were 0.1 µg/µL, peaks detected in pure n-hexane were omitted; the column temperature was programmed from 40°C (5 min isothermal) to 240°C (20 min isothermal); [b] stationary phase used for the determination of purity; [c] Aldrich (Steinheim, Germany), Alfa Aesar (Karlsruhe, Germany), Fluka (Steinheim, Germany), Frey & Lau (Henstedt-Ulzburg), Merck (Darmstadt, Germany), Penta (Livingston, New Jersey, USA), SAFC (Steinheim, Germany), Silesia (Neuss, Germany), reference substances from Frey & Lau were kindly supplied as gifts from this company; [d] a new sample was bought every three months, the purity was always > 97%; [f] no purities were determined, references were only used for determination of the retention indices (RI).

Purification of (R)-linalyl acetate by column chromatography

(*R*)-linalyl acetate (618 mg, purity 84.3%, 5.9% linalool) was purified for determinations of odour thresholds by column chromatography on silica gel (60, 0.063−0.200 mm, Merck, Germany) with a mixture of *n*-hexane and diethyl ether 20+1, (v+v). The fractions were tested by thin layer chromatography in order to identify those fractions containing linalyl acetate (TLC, ALUGRAM SIL G/UV254, Macherey-Nagel, Germany); plots were visualized by spraying with 10% sulphuric acid and subsequent heating until dryness and identified by comparison with the plot obtained from pure linalyl acetate under the same conditions. The relevant fractions were combined and the solvent was evaporated by rotary evaporator and a subsequent gentle stream of nitrogen. A yield of 76.9% (475.2 mg) was achieved. GC analysis revealed a purity of 93.2% and the absence of linalool.

3.1.3 Synthesis of reference compounds

Synthesis of geranyl and neryl esters

The hexanoates, octanoates, benzoates, phenylacetates, and cinnamates of geraniol and nerol were synthesised according to Staab (1962): 1 mmol of the respective acid was dissolved in 10 mL chloroform containing 1 mmol 1,1'-carbonyldiimidazole in a pear-shaped flask. After the release of the generated carbon dioxide, 1 mmol of the appropriate alcohol was pipetted and the solution was heated smoothly under reflux in an oil bath. The mixture was washed several times with water for the removal of the generated imidazole and dried with anhydrous sodium sulphate (Staab 1962).

Synthesis of (R)-linalyl formate and (R)-linalyl octanoate

For the syntheses of (*R*)-linalyl formate and (*R*)-linalyl octanoate, 1.3 mmol (*R*)-linalool were dissolved in 3 mL dichloromethane containing 1.3 mmol dicyclohexylcarbodiimide and 0.65 mmol 4-dimethylaminopyridine were added. After stirring for 5 min at RT, 1 mmol of formic acid and octanoic acid dissolved in 1 mL dichloromethane, respectively, were added dropwise. After a further 24 h, the coproduct dicyclohexylurea was filtered off and the filter cake was washed with 5 mL dichloromethane. The solvent was removed under reduced pressure and purified on a silica gel column (*n*-hexane: diethyl ether 15:1, v/v).

3.2 Methods

3.2.1 Isolation of flavouring substances

3.2.1.1 Tea leaves

Half of the tea material contained in a tea bag or 1 g of loose tea leaves was placed into a 12 mL glass tube (Pyrex®). After addition of the internal standard (2-decanol; 1 mg in 0.5 mL ethanol (analysis grade)), the sample was homogenized with 2.5 mL saturated aqueous sodium chloride solution using an Ultra-Turrax®-disperser (IKA®, type T25; Staufen, Germany) at 13.500 rpm for 150 sec and at 20.500 rpm for another 30 sec. Remaining tea material was rinsed off the disperser element with 3 mL sodium chloride solution and 2 mL n-hexane (HPLC grade). The tube was closed and placed onto a rotator. After extraction for 2 h (15 rpm) and centrifugation for 60 sec at 3000 rpm (Heraeus Sepatech Biofuge 17S), the organic phase was filtered into a 2.5 mL vial using a micro-filter tipped syringe filled with anhydrous sodium sulphate. Two dilutions (1:5 and 1:50 in *n*-hexane, v/v) were prepared; 0.5 µL of each were subjected to GC-analysis. Every sample was worked-up in triplicate.

3.2.1.2 Tea infusions

Tea infusions were prepared (i) with non-flavoured black tea spiked with reference substances, (ii) with commercially available Earl Grey teas and (iv) with non-flavoured black tea. For (i) 4 mg of a substance in 100 µL ethanol were spiked using a gastight glass syringe (Hamilton®) directly into the tea bag of non-flavoured black tea by puncturing the paper and distributing the solvent evenly onto the tea material. The tea bag was placed in a glass beaker, brewed with 200 mL of distilled, boiling water, covered with a watch glass and dunked 3 times at 0, 1, and 2 min. After 3 min, the tea bag was removed and squeezed by the help of a spoon and winding the thread fixed at the tea bag around it for 2 times. For (ii) and (iii) the procedure was carried out in the same way without spiking the reference material. Samples of loose leaves were filled into a one-way tea bag before the hot water infusion. All tea infusions (i)-(iii) were rapidly cooled in an ice bath until they reached a temperature of 20°C and 500 µL ethanolic standard solution (SD: 2-decanol, 2 mg/mL) were added. For time experiments, the spiked tea bag was removed after the hot water infusion additionally at 0.5, 1.0, 2.0, 5.0 and 10.0 min. The tea bag was dunked at the beginning and at every full minute except the final one (the number of dunking was therefore: 1, 1, 2, 5 and 10 times). For experiments determing the impact of concentration, the spiked amounts of linalool and linalyl acetate were 0.1 mg (minimum) and 20 mg (maximum), respectively. For linalyl acetate, an additional concentration of 0.4 mg was spiked.

The standard tea bag weight was set to 2.0 g since the tea bags´ weight was between 1.5 and 2.5 g (one exception: 4.5 g in tea no. 37). After squeezing and removing of the tea bag, cooling and the addition of the internal standard, an aliquot was extracted with *n*-hexane and used for the GC-analyses. A portion of only 6 mL was sufficient for the analysis of starting and reaction products; the extract of brewed linalool was actually diluted due to its too high concentration for the used GC-column. Furthermore, this also had the advantage of a fast extraction procedure in contrast to that of 200 mL tea, which is very important with respect to possible reaction products in aqueous phase (see 4.2.5.2). The standard was added not at the beginning but after the infusion process, because contents of the final beverage should be analysed. The used GC-FID response factors and recovery rates for quantification are listed in Table 38 (see Appendix); no reaction products were found in the course of the recovery experiments. In contrast to transfer rates, recovery rates represent extraction efficiencies of flavouring substances from cooled infusions of non-flavoured black tea.

a) Fast work-up method

An aliquot of 6 mL of the infusion was transferred into a 12 mL glass centrifuge tube (Pyrex®) and extracted with 2 mL *n*-hexane (HPLC grade) for 2 h on a rotator (Bibby Scientific Limited, type: stuart® Tube Rotator SB3; Staffordshire, United Kingdom). After centrifugation for 2 min at 3000 rpm, the organic phase was filtered into a 2.5 mL vial using a micro-filter tipped syringe filled with anhydrous sodium sulphate. Every sample was worked-up in triplicate.

b) Liquid-Liquid Extraction

The total infusion volume (200 mL) was transferred into a Kutscher-Steudel liquid-liquid extractor (Gattermann et al. 1982). Flavouring substances were extracted for 24 h using 150 mL organic solvent (mixture of n-pentane and diethyl ether, 1:1, v/v). The extract was dried over anhydrous sodium sulphate and concentrated at 38-40°C to a volume of 1 mL using a Vigreux column and subsequently to a final volume of 0.5 mL using a gentle stream of nitrogen. One microliter was subjected to analysis by multidimensional gas chromatography (MDGC, System IIa + b). The described extraction method was only used for the determination of the enantiomeric distribution of linalyl acetate after a hot water infusion of a tea bag spiked with that ester (see 4.2.3.5).

3.2.1.3 Residual tea material

After the infusion step, the squeezed tea bag was emptied and the wet tea material as well as the tea bag paper (cut into pieces) was placed in a 20 mL glass tube. After addition of 500 µL SD solution (2 mg/mL), the material was suspended in 15 mL saturated sodium chloride solution and extracted using 2 mL *n*-hexane. The extraction conditions were the same as described for the tea infusions.

3.2.1.4 Storage

A sample of loose tea leaves (200 g) was split into two parts: One half remained in the paper bag in which the tea had been purchased; the other half was placed into a metal box as it is typically used at home. In a second experiment two samples in tea bags were stored in their original packaging; one of the samples had been with and the other without encapsulated flavouring substances. The samples were kept at RT and analysed via the method described in 3.2.1.1 every four weeks up to a maximum storage time of 16 weeks.

3.2.1.5 Water / tea infusion stability of linalyl acetate

A stock solution of 800 mg linalyl acetate in 10 mL ethanol was prepared. 100 µL of this solution were transferred via a gastight glass syringe (Hamilton®) into a nearly-filled-up 200 mL flask and filled up completely with (i) distilled water and (ii) with a cold tea infusion of non-flavoured black tea. The point of time of addition of the stock solution of linalyl acetate was set as zero point (0 min) resulting in an initial concentration of linalyl acetate of 0.04 mg/mL. After homogenisation via manual shaking for 1 min and sonicating (2-3 sec), 6 mL were sampled into a 12 mL glass centrifuge tube (Pyrex®), 500 µL of SD solution were added (0.1 mg/mL 2-decanol in ethanol) and extracted according to the ‘fast work-up method’ described in 3.2.1.2a) before. Samples were taken at 8, 15, 30, 45, 60, 120, 180, 240, 300, 420 min and at 24, 48 and 144 h. No analysis of linalyl acetate was performed in the tea infusion at 144 h, because of fungal infestation of the solution. Every sample was worked-up in triplicate (3 flasks of (i) and (ii)).

3.2.1.6 ‘Forest fruit’ tea and ‘forest fruit’ tea infusions

A standard addition method was used for the quantification of flavouring substances in ‘forest fruit’ tea material and infusions. The concentrations of the flavouring substances in the ethanolic stock solutions used for spiking were chosen according to the amounts estimated in preliminary analyses of the non-spiked samples; they correspond to approximately 150% and 200%, respectively, of the expected concentrations (Table 7).

Table 7. Concentrations of flavouring substances in the stock solutions used for the standard addition method.

substance	concentrations in stock solutions [µg / 500 µL ethanol]						
	tea material		tea infusion				
	I	II	I[a]	II[a]	III[a]	I(4)[b]	II(4)[b]
raspberry ketone	1949.1	3898.3	121.1	242.2			
vanillin	1029.6	2059.2	44.9	89.8			
3-methylbutyl acetate	39.3	78.3	5.9	11.7			
benzyl acetate	32.8	65.9	1.3	2.6		3.2	1.6
β-ionone	29.8	59.6	1.2	2.4		3.2	1.6
α-ionone	27.7	55.4	1.1	2.2		2.8	1.4
(*Z*)-3-hexenyl acetate	9.6	19.2	1.4	2.9			
β-damascenone	7.2	14.4	0.9	1.8	3.6		
2-methylpropyl acetate	6.7	13.5	0.3	0.5		1.0	0.5
(*Z*)-3-hexenol	5.4	10.9	0.8	1.6			
γ-undecalactone	3.6	7.2	0.4	0.9	1.8		
methyl isoeugenol	1.2	2.3	0.15	0.3	0.6		
benzaldehyde	0.7	1.3	0.1	0.2			
eugenol	0.6	1.2	0.08	0.2	0.3		

[a] stock solutions I-II(III) were each spiked three times to three infusions, respectively; [b] stock solution I(4) and II(4) were each spiked three times to an additional fourth infusion, respectively.

Tea material

Half of the tea material contained in a tea bag (1.25 g) and 0.5 mL of external standard (ESD: 2-heptanol; 0.05 mg/mL in ethanol (analysis grade)) were placed into a 20 mL glass tube (Pyrex®). After addition of 14 mL saturated aqueous sodium chloride solution, the sample was spiked either with 0.5 mL of (i) ethanol, (ii) stock solution I or (iii) stock solution II. Each spiking was done in triplicate. The samples were extracted with 3 mL organic solvent mixture of diethyl ether and *n*-hexane (1:1, v/v) for 2 hours at 15 rpm on a rotator. After centrifugation for 5 min at 3000 rpm, the organic phase was filtered into a 2.5 mL vial using a micro-filter tipped syringe filled with anhydrous sodium sulphate. The extract was concentrated to 0.5 mL using a gentle stream of nitrogen. 100 µL of the internal standard was added (InSD: 2-decanol; 0.5 mg/mL in diethyl ether / n-hexane (1:1, v/v)). A blank test was performed analogously without tea material. For vanillin and raspberry ketone, the concentrations of the standards were 10-fold higher; in turn, the final extracts were diluted 1:10 to prevent an overload of the column.

Tea infusion

One tea bag including the tea bag paper, the thread and the label (gross weight) was weighted. All emptied tea bags used for the tea material analyses (including those of preliminary tests) were weighted before (tare weight = 0.371 ± 0.005 g for n=54 tea bags), and the average was subtracted from this weight, resulting in the net value. 100 µL of a control standard (CSD: 2-nonanol, 1.0 mg/mL in ethanol (analysis grade)) was spiked into the tea bag using a gas-tight syringe puncturing the paper and distributing the solvent evenly onto the tea material. The tea bag was placed in a glass beaker, brewed with 200 mL of distilled, boiling water, covered with a watch glass and dunked 4 times at 0, 2, 4 and 6 min. After 8 min, the tea bag was removed and squeezed by the help of a spoon and winding the thread fixed at the tea bag around it for 2 times. The tea infusions were rapidly cooled in an ice bath until they reached a temperature of 20°C and 500 µL extraction standard solution (ESD: 2-heptanol; 0.05 mg/mL in ethanol (analysis grade)) were added. The tea infusion was transferred quantitatively into a volumetric flask and restocked with water to a volume of 200 mL.

Nine aliquots of 15 mL were filled into 20 mL glass centrifuge tubes (Pyrex®); three of them were spiked with 0.5 mL ethanol (blank stock solution), three with 0.5 mL of stock solution I and another three with 0.5 mL stock solution II. A third stock solution was spiked for some infusions for the purpose of a more precise regression line. The samples were extracted with 3 mL organic solvent mixture of diethyl ether and *n*-hexane (1:1, v/v) for 2 hours at 15 rpm on a rotator. After centrifugation for 5 min at 3000 rpm, the organic phase was filtered into a 2.5 mL vial using a micro-filter tipped syringe filled with anhydrous sodium sulphate. The extract was concentrated to 0.5 mL using a gentle stream of nitrogen. Finally, 100 µL of the internal standard was added (InSD: 2-decanol; 0.05 mg/mL in diethyl ether / *n*-hexane (1:1, v/v)). The concentration of the InSD was lower for tea infusions than for tea material (1:10).

In total, three tea infusions were worked-up as described in this section. For 2-methylpropyl acetate, benzyl acetate, α-ionone and β-ionone a fourth infusion was prepared (stock solutions I(4) and II(4); see Table 7). For vanillin and raspberry ketone, the standard concentrations were 10-fold higher.

3.2.2 Identification of flavouring substances

Volatile substances were analysed by capillary gas chromatography (GC-FID, GC-system I; see 3.2.4.1) and capillary gas chromatography-mass spectrometry (GC-MS, GC-system III; see 3.2.4.3). The identifications were carried out by comparison of mass spectrometric and capillary gas chromatographic data (retention indices) to those of authentic reference compounds.

3.2.2.1 Determination of linear retention indices

Linear retention indices were calculated using retention times of linear alkane series (*n*-alkanes) as standard compounds and reference substances using the following equation (Kováts 1958).

$$(1)\quad RI = 100 \times \left[N + \frac{t_r - t_r(n)}{t_r(n+1) - t_r(n)}\right]$$

RI	=	*linear retention index*
t_r	=	*retention time of unknown substance*
$t_r(n)$	=	*retention time of alkane n*
$t_r(n+1)$	=	*retention time of alkane n+1*
N	=	*number of carbon atoms of atom n*

The determination was performed on GC-system I on a DB-5 column for substances of Part I and II and on a DB-WAX column for Part III. A summary of all linear retention indices is given in the Appendix (Table 39).

3.2.3 Quantifications

3.2.3.1 Tea leaves

Quantifications were carried out on GC-system I (see 3.2.4.1, method I), using 2-decanol as internal standard and taking into account FID response factors (RF). The gradients of the linear equations achieved by FID responses were determined with solutions of authentic references relative to the internal standard (six dilutions ranging from 0.006 to 0.2 mg/mL *n*-hexane). The gradient of a linear equation was calculated by the quotient of the concentration of one reference compound and that of the internal standard plotted against the quotient of area of the reference substance and that of the internal standard. The following FID response factors were calculated: linalool (0.94), α-terpineol (0.89), limonene (0.80), β-pinene (0.80), and γ-terpinene (0.81).

The following equation was used for quantification:

$$(2)\quad C_x = \frac{A_x \times Rf_x \times M_{SD}}{A_{SD} \times M}$$

C_x = *concentration of substance x (mg/g)*
Rf_x = *FID-response factor (Rf) of substance x*
A_x = *peak area of the substance x to be quantified*
A_{SD} = *peak area of the internal standard*
M_{SD} = *weight of added internal standard [mg]*
M = *weight of the tea sample [g]*

3.2.3.2 Tea infusions and residual tea material

Quantifications were carried out on GC-system I (see 3.2.4.1, method II), using 2-decanol as internal standard and taking into account recoveries from tea infusions and residual tea material (Table 38), respectively, and GC-FID RF´s relative to the standard.

The following GC-FID response factors (additional to 3.2.3.1) related to the internal standard 2-decanol were determined (concentrations of 0.01 mg/mL in *n*-hexane): linalyl formate (1.07), linalyl acetate (1.10), linalyl propanoate (0.98), linalyl butanoate (1.01), linalyl isobutanoate (0.97), linalyl isovalerate (1.00), linalyl hexanoate (0.96), linalyl octanoate (0.98), menthyl acetate (0.89), α-terpinyl acetate (0.88), geranyl acetate (0.91), and neryl acetate (0.98). For myrcene, (*Z*)-ocimene, (*E*)-ocimene, terpinolene, nerol and geraniol a response factor of 1.0 was used.

3.2.3.3 Water / tea infusion stability of linalyl acetate

Percentages of remaining linalyl acetate and reaction products were calculated setting the sum of the amounts (as linalyl acetate equivalents in µg) as 100%.

3.2.3.4 Fruit tea material and fruit tea infusions

Standardisation of peak areas

As a first step, all peak areas including those of the extraction and the control standard (ESD and CSD) were normalised in relation to a fixed value of the injection standard (InSD), which was approximately 2000000.

$$(3)\quad a\,(InSD)_{norm} = \frac{FV}{a\,(InSD)} \times a\,(InSD)$$

$$(4)\quad a_{norm} = \frac{a\,(InSD)_{norm}}{a\,(InSD)} \times a$$

a = *peak area*
a_{norm} = *normalized peak area*
FV = *fixed value*
InSD = *injection standard*

Recovery of the extraction standard (ESD)

The recovery of the extraction standard was calculated using the following formula:

$$(5)\quad r\,(ESD) = \frac{Rf\,(ESD) \times m\,(InSD) \times A\,(ESD)_{norm}}{A\,(InSD)_{norm} \times m\,(ESD)}$$

r = *recovery*
ESD = *extraction standard*
Rf = *response factor (relative to InSD)*
m (ESD) = *mass of added extraction standard [µg]*
m (InSD) = *mass of added injection standard [µg]*

Correction of losses

All peak areas were corrected by the recovery rate of the ESD.

$$(6)\quad a_{cor\_1} = \frac{a_{norm.}}{r\,(ESD)}$$

a_{cor_1} = *corrected peak area*

Correction of weight

Normalised and corrected peak areas in the course of quantification of flavouring substances from the tea material were once again corrected by the average weight of tea material.

$$(7)\quad a_{cor\_2} = \frac{a_{cor\_1}}{m \times mean\,(m)}$$

m = *weight of the tea material*
mean (m) = *mean of all tea material weights (1.2564 ± 0.031 g; n=36)*

3.2.3.5 Enantiomeric distributions

Calculation of the enantiomeric distributions was carried out on GC-systems IIa+b (see 3.2.4.2) using peak area percentages. Peak areas below limit of quantification (LOQ) were equalized to the peak area corresponding to the limit of quantification (GDCh 2001).

3.2.3.6 Determination of recovery rates

Recovery rates in tea leaves

Recovery rates were determined in model experiments with non-flavoured black tea sample (no. 101) and authentic reference compounds. The extractions were carried out as described before (3.2.1.1). For concentrations of 0.4, 0.2 and 0.1 mg/g tea, respectively, the following recoveries were determined in triplicate experiments: linalyl acetate (104.8% ± 2.0%), linalool (102.9% ± 2.4%), limonene (100.2% ± 3.1%), β-pinene (98.5% ± 3.5%) and γ-terpinene (97.9% ± 2.7%). At concentrations of 4.0, 2.0 and 1.0 mg/g the following results were obtained: linalyl acetate (104.8% ± 2.4%), linalool (100.5% ± 2.7%), limonene (102.3% ± 2.3%), β-pinene (100.9% ± 2.3%) and γ-terpinene (100.6% ± 2.3%).

Recovery rates in tea infusions and residual tea leaves

Recovery rates were determined (i) for tea infusions and (ii) for the residual (wet) tea bag material. Tea infusions of a non-flavoured black tea and squeezed wet tea bags from these infusions were used as matrices. 500 µL of an ethanolic reference mixture (reference compound and internal standard, each 0.2 mg/mL) were added to (i) a volume of 5.5 mL of tea infusion or to (ii) a squeezed wet tea bag (cut into pieces). The extractions were carried out as described before (3.2.1.2a) and 3.2.1.3). The resulting recovery rates can be taken from the Appendix (Table 38).

3.2.3.7 Determination of limits of detection (LOD) and limits of quantification (LOQ)

Limits of detection (LOD) and quantification (LOQ) were calculated according to previously described procedures (Strohalm 2010; Vogelgesang and Hädrich 1998), using six solutions of reference substances from 0.05 to 0.1 µg/mL in *n*-hexane (GC-system I) for the main terpenes linalyl acetate, linalool, limonene, γ-terpinene, and β-pinene and a series of five dilutions of the chiral reference compounds ranging from 0.2 to 5 µg/mL per enantiomer. Every dilution was injected three times to GC-system II. The peak areas were plotted against the used concentrations. By using the following formulae and the resulting calibration lines, LOD´s and LOQ´s were calculated.

The residual standard deviation s_y is calculated by the calibration line $y = a + bx$.

$$s_y = \sqrt{\frac{\sum_{i=1}^{n} (y_i - (a + bx_i))^2}{n - 2}}$$

n = *number of measured values*
y_i = *peak area of the substance x to be quantified*
a = *y intercept*
x_i = *fortification concentration of sample i*
b = *slope*
i = *index of calibration analyses*

This results in the limit of detection (LOD):

$$LOD = \frac{s_y}{b} \times t_{f;\alpha} \times \sqrt{1 + \frac{1}{n} + \frac{\bar{x}^2}{\sum_{i=1}^{n} (x_i - \bar{x})^2}}$$

and the limit of quantification (LOQ):

$$LOQ = \left(\left(\bar{y} + b\,(ID - \bar{x}) + s_y \times t_{f;\alpha} \times \sqrt{1 + \frac{1}{n} + \frac{(ID - \bar{x})^2}{\sum_{i=1}^{n} (x_i - \bar{x})^2}} \right) - a \right) \div b$$

$t_{f;a}$ = *1.746: quantil of t-distribution for f = n – 2 degrees of freedom and a probability of 95% (error probability α=0.05)*
$\bar{y}$ = *mean value of the signal value of all calibration analyses*
$\bar{x}$ = *mean value of all concentrations*
ID = *identification limit (ID = 2 x LOD)*

The resulting LODs and LOQs were as follows: GC-system I: linalyl acetate (35.8 ng/mL; 119.0 ng/mL), linalool: (34.5 ng/mL; 103.8 ng/mL), limonene (30.9 ng/mL; 94.1 ng/mL), β-pinene (35.8 ng/mL; 109.0 ng/mL), γ-terpinene (28.7 ng/mL; 87.3 ng/mL); GC-system IIb: linalyl acetate (458 ng/mL; 1121 ng/mL), linalool: (506 ng/mL; 1198 ng/mL), limonene (321 ng/mL; 780 ng/mL), β-pinene (371 ng/mL; 917 ng/mL).

The limit values refer to the final concentration resulting from the respective extraction method (tea material, tea infusion, and residual tea bag). LOD and LOQ values expressed as ng/g tea depend on the used tea weight and the individual extraction method. Exemplarily calculated values are listed in the Appendix (Table 41).

3.2.4 Capillary gas chromatographic systems

3.2.4.1 GC-System I – On-column Injection system (GC-FID)

A GC 8000 series gas chromatograph (Fisons Instruments) equipped with an on-column injector and a flame ionization detector (FID; method I and II: 250°C, method III: 235°C) was used. The separations were performed on a DB-5 fused silica column (30 m x 0.25 mm i.d.; film thickness 0.25 µm; J&W Scientific; Agilent Technologies, Böblingen, Germany) for method I and II and on a DB-WAX silica capillary column (60 m x 0.32 mm i.d.; film thickness 0.25 µm; J&W Scientific) for method III. A pre-column (TSP 32045-D10 deactivated with DPTMDS; 1 m x 0.32 mm; BGB Analytik, Boeckten, Switzerland) was used as retention gap and trimmed or changed every week. The carrier gas used was hydrogen at a constant inlet pressure of 80 kPa for method I and II and of 110 kPa for method III. Data acquisition was done via Chromcard software (Thermo Fisher Scientific).

Method I (Tea leaves)

The column temperature was programmed from 40°C (5 min isotherm) to 120°C at a rate of 4°/min and to 240°C at 30°/min (20 min isotherm). A volume of 0.5 µL of tea extract was injected.

Method II (Tea infusions)

The column temperature was programmed from 70°C (10 min isotherm) to 83°C at a rate of 1°/min and to 240°C at 5°/min (20 min isotherm). A volume of 0.5 µL of tea extract was injected.

Method III (Fruit tea)

The column temperature was programmed from 40°C (5 min isotherm) to 240°C at a rate of 4°/min. A volume of 0.5 µL of tea extract was injected.

3.2.4.2 GC-System II - Multidimensional Capillary Gas chromatography (MDGC-FID)

The separations of the enantiomers were performed on two coupled GC 8000 series gas chromatographs (Fisons Instruments) each equipped with an FID. Both systems (IIa + IIb) were connected via a heated transfer line (140°C).

System IIa

System IIa was equipped with a moving column stream switching device (MCSS) (Schmarr et al. 2007; Sulzbach 1996). For pre-separation, an achiral column (pre-column) coated with polyethylene glycol was installed in the first oven (DB-WAX; 60 m x 0.32 mm i.d.; film

thickness 0.25 µm, J&W Scientific). The oven temperature was programmed from 40°C (5 min isotherm) to 140°C at 4°/min and to 240°C at 20°/min (20 min isotherm). Hydrogen was used as carrier gas at a constant pressure of 165 kPa. 1.0 µL of tea extract dilution (1:5, v/v) was injected in the split mode (215°C; split ratio 1:5). The temperature of the FID was set at 230°C. The inlet pressure for the 'dome' of the MCSS was set to 100 kPa, which led to an outlet pressure of 95 kPa (measured via an additional manometer inside), which represents the carrier gas pressure for the chiral column in system IIb.

System IIb

For enantiomeric separations, a chiral column (main column) was installed in the second oven coated with 30% heptakis(2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethyl-silyl)-*β*-cyclodextrin in PS-086 (30 m x 0.25 mm i.d.; film thickness 0.25 µm). The main column was connected to the pre-column in the first oven via a deactivated fused silica transfer capillary (1.5 m x 0.32 mm i.d.) guided through a heated transfer line (140°C) between system IIa and IIb. Syntheses of the cyclodextrin derivative and column preparation were carried out in-house (Schmarr 1992). The temperature of the column was programmed from 37°C (7 min isotherm) to 125°C at 2°/min. The detector was set to 200°C.

The cut intervals were 10.2-10.8 min for β-pinene, 13.9-14.4 min for limonene, 27.6-28.2 min for linalool together with linalyl acetate, 28.9-29.0 min for linalyl formate and 32.3-32.8 min for α-terpineol. Control of the MCSS device and data acquisition was done via Chromcard software (Thermo Fisher Scientific).

3.2.4.3 GC-System III - Mass spectrometry (GC-MS)

A Fisons MD8000^{TOP} mass spectrometer coupled to a GC 8000TOP gas chromatograph (Thermo Fisher Scientific) equipped with a split/splitless injector (220°C, split ratio 1:50) was used. The separations were performed on a DB-WaxEtr fused silica capillary column (30 m x 0.25 mm i.d.; film thickness 0.5 µm; J&W Scientific). The column temperature was programmed from 40°C (5 min isotherm) to 240°C at 4°/min (25 min isotherm). A volume of 1 µL of tea extract was injected. The carrier gas used was helium at a constant inlet pressure of 75 kPa. Ionization was set to 70 eV, source temperature was 200°C, and interface temperature 240°C. Data acquisition was done via *Xcalibur* software, version 1.4 (Thermo Fisher Scientific).

3.2.5 Statistical Analysis

Statistical tests such as Shapiro-Wilk-test (test for normal distribution), Bartlett´s test (test for homogeneity of variances), ANOVA (one-way analysis of variance, parametric test, number of factor-levels > 2) and Kruskal-Wallis test (one-way analysis of variance by ranks, nonparametric test, and number of factor-levels > 2)) were performed with R (version 2.15.2). In all tests, the level of significance was set to α=0.05.

Box-and-whisker plots were created with XLSTAT (version 2008.4.01). The top and the bottom of the box represent the 75th (Q3) and the 25th percentile (Q1), respectively. Accordingly, the box graphically includes 50% of all values. The margin between both is called the interquartile range (IQR = Q3 - Q1). The cross (+) illustrates the mean value, while the median (equally to the 50th percentile=Q2) is represented by the line inside the box. The lengths of the whiskers are limited at most to the 1.5-fold of the IQR; they end at an actual value which just lies in this interval. Outside values (values between Q3 + 1.5 x IQR or Q1 - 1.5 x IQR) are illustrated by triangles (Δ), while X´s represent fare outside values (values between Q3 + 3.0 x IQR or Q1 - 3.0 x IQR).

3.2.6 Sensory assessment of flavouring substances

3.2.6.1 Determinations of odour thresholds

Odour thresholds in water and tea infusion were determined by an in-house panel which consisted of 9 to 17 participants (average = 14); the sessions lasted no longer than 3 hours per substance. The tea infusion was prepared with one tea bag of non-flavoured black tea per 200 mL boiling water, brewed for 3 min and cooled to 20°C. A 1:1-dilution-step was chosen between two dilution levels. The triangle test with a 'forced-choice-technique' was applied leading the panel members to an answer even in case of non-perception of a difference. The sample tubes made of glass and pear-shaped were labelled with three-digit random numbers. The calculation of odour thresholds of individual participants was done according to the procedure described by Meilgaard et al. (2007).

$$OTV_i = \sqrt{C_r \times C_{r-1}}$$

OTV_i = *individual odour threshold value*
C_r = *concentration of the first recognized sample*
C_{r-1} = *concentration of the previous recognized sample*

The geometric mean was used for the determination of the group odour threshold.

$$\overline{OTV} = \sqrt[n]{\prod_{i=1}^{n} OTV_i}$$

$\overline{OTV}$ = *group odour threshold value*
n = *number of persons*
OTV_i = *individual odour threshold value*
$\prod$ = *product of individual thresholds*

In case of the determination of odour thresholds of linalyl acetate, a concentration correction was performed due to the instability of the substance (see 4.2.5.2 and 4.5.1.1) using the following formulae.

$$c_{r\,corr} = \frac{C_r \times e^{\ln(\text{linalyl acetate}[\%])}}{100}$$

$$c_{r-1\,corr} = \frac{C_{r-1} \times e^{\ln(\text{linalyl acetate}[\%])}}{100}$$

$c_{r\,corr}$ = *corrected concentration of the first recognized sample*
$c_{r-1\,corr}$ = *corrected concentration of the previous recognized sample*

$$\ln(\text{linalyl acetate in water [\%]}) = -0.0265 \times \ln(\text{time [h]})^3 - 0.0414 \ln(\text{time [h]})^2 + 0.0013 \times \ln(\text{time [h]}) + 4.5775$$

$$\ln(\text{linalyl acetate in tea infusion [\%]}) = -0.0354 \times \ln(\text{time [h]})^3 - 0.0313 \ln(\text{time [h]})^2 + 0.04 \times \ln(\text{time [h]}) + 4.5715$$

ln (linalyl acetate [%]) = *logarithmic concentration of linalyl acetate [%]*
ln (time [h]) = *logarithmic value of elapsed time [h]*

4 RESULTS AND DISCUSSION

4.1 Flavouring substances in Earl Grey teas from the EU-market

4.1.1 Introduction

The aim of the first part of the thesis was to determine the amounts of the main flavouring substances linalyl acetate, linalool, limonene, β-pinene and γ-terpinene in Earl Grey teas. The data should cover a broad spectrum of countries within the EU. Therefore, the contents of these flavouring substances were determined in 90 Earl Grey teas purchased in ten member states of the EU (Figure 12).

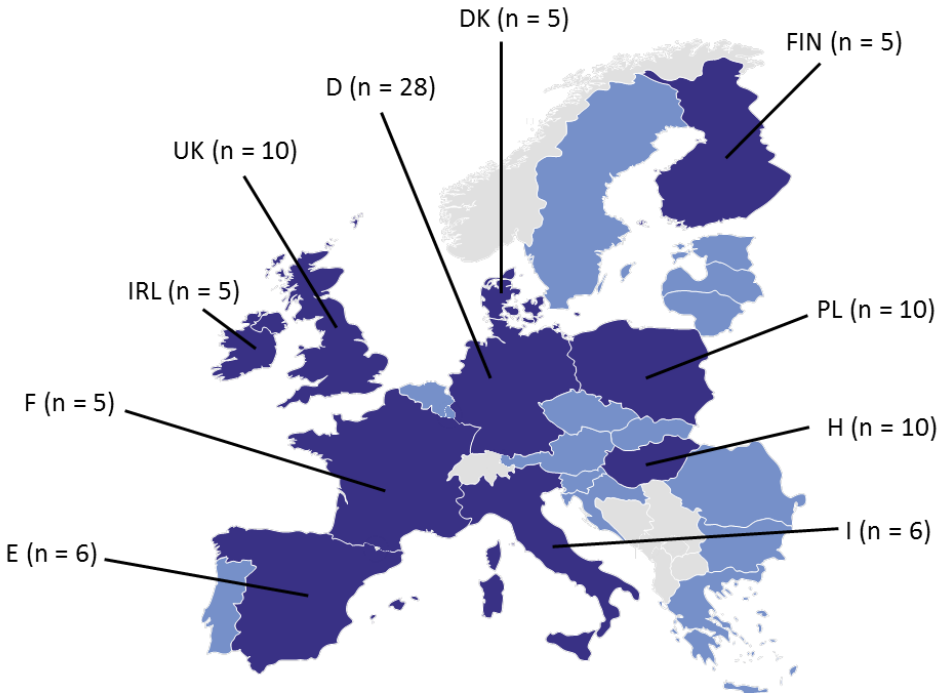


Figure 12. Countries of purchase (dark blue) and number of samples across the European Union (dark and pale blue) of Earl Grey tea samples (n=90). For country abbreviations see 3.1.1.

4.1.2 Development of a fast work-up method

A method was established involving the homogenization of the tea material with saturated aqueous sodium chloride solution, followed by liquid-liquid extraction of the flavouring substances with *n*-hexane on a rotator (Figure 13).

Preliminary experiments showed that flavouring substances encapsulated in granulates (54 teas contained granulate material) were not accessible via direct extraction of the tea leaves with organic solvent as performed by Neukom et al. (1993). With an additional aqueous phase the granules dissolve and the not-longer included flavouring substances can be

extracted into the organic solvent. By using sodium chloride solution, agglutination of the tea leaves was reduced and the suspension of crushed tea material in the two-phase system was improved. The organic phase was dried, filtered and filled into a vial in the course of only one step using a micro-filter tipped single-use syringe filled with anhydrous sodium sulphate. Aliquots of the extract were diluted 1:5 and 1:50, respectively, with *n*-hexane before GC-analysis.

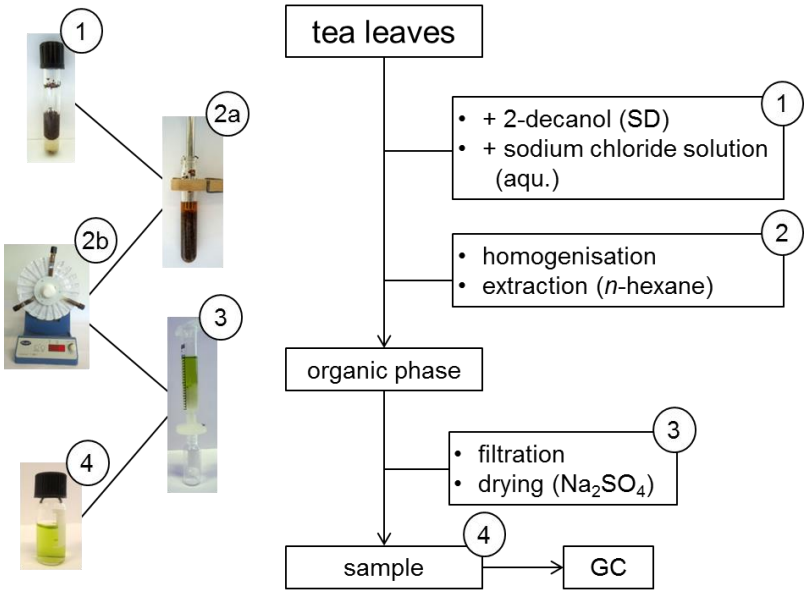


Figure 13: Scheme of the method applied to extract flavouring substances from Earl Grey tea leaves (see 3.2.3.1).

Both solutions were subjected to capillary gas chromatographic analysis using on-column injection (GC-System I). This was necessary since peak areas of β-pinene and γ-terpinene were often smaller than the limits of quantification (LOQ) in the lower concentrated extract dilution. On the other hand, the column was overloaded with linalool, linalyl acetate, and limonene in the higher concentrated solutions. Therefore, the area of the relevant chromatogram combined with the amount of internal standard was used for the calculation of the amount of the respective flavouring; finally, this value was related to the original sample weight.

A typical gas chromatographic separation on a DB-5 stationary phase of the five main terpenes isolated from Earl Grey teas is shown in Figure 14. Although at most 1 g of dry tea material was employed for the extraction of flavouring substances and only a one-time solvent extraction was performed with the small quantity of 2 mL *n*-hexane, the employed on-column injection system (GC-System 1) could detect adequate areas for the main monoterpenes in even highly diluted tea extracts.

Figure 14: Typical capillary gas chromatographic separation of monoterpenes isolated from Earl Grey teas on GC-system I (DB-5, method I); 1: β-pinene, 2: limonene, 3: γ-terpinene, 4: linalool, 5: linalyl acetate, SD: internal standard (2-decanol).

4.1.3 Contents and enantiomeric distributions of flavouring substances

As shown in Figure 14, the spectrum of volatiles was dominated by the monoterpene ester linalyl acetate, the corresponding alcohol linalool, and the monoterpene hydrocarbons limonene, β-pinene and γ-terpinene. The methodology was employed to analyse the contents of these flavouring substances in 90 Earl Grey teas purchased in ten member states of the EU (Figure 12). In addition, the enantiomeric compositions of the chiral volatiles linalyl acetate, linalool, limonene and β-pinene were determined via multidimensional capillary gas chromatographic analysis employing heptakis(2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethyl-silyl)-*β*-cyclodextrin as chiral stationary phase (GC-System IIa + b). Furthermore, the tea extracts were screened for the occurrence of dihydrolinalool as an indicator for the use of synthetic linalool (see 2.4.8). An overview on the data is given in Table 8.

Table 8. Contents and enantiomeric compositions of linalool, linalyl acetate, limonene, β-pinene and γ-terpinene in Earl Grey teas.

samples			linalyl acetate		linalool		limonene		β-pinene		γ-terpinene	dihydro-linalool
no.	type[a]		[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[µg/g]	[%(*R*) : %(*S*)]	[µg/g]	
Germany (n=28)												
1	B1	tb	1.23 ± 0.09	50.5 : 49.5	0.43 ± 0.04	51.9 : 48.1	1.39 ± 0.10	>98.8[b] : n.q[c]	258 ± 19	n.q. : >93.2	171 ± 12	n.q.
2	B1	tb	3.98 ± 0.14	74.7 : 25.3	2.31 ± 0.08	72.8 : 27.2	2.61 ± 0.06	98.1 : 1.9	216 ± 4	6.8 : 93.2	200 ± 5	n.q.
3	B1	ll	5.3 ± 0.4	97.8 : 2.2	1.80 ± 0.11	95.4 : 4.6	1.11 ± 0.08	98.4 : 1.6	102 ± 7	n.q. : >90.8	40 ± 4	n.d.
4	B2	tb	1.02 ± 0.05	94.0 : 6.0	0.95 ± 0.05	95.6 : 4.4	0.96 ± 0.05	98.2 : 1.8	199 ± 9	n.q. : >93.7	161 ± 8	n.d.
5	B2	ll	2.21 ± 0.08	50.0 : 50.0	2.33 ± 0.10	50.4 : 49.6	0.0697 ± 0.0022	n.q. : n.d[d]	2.84 ± 0.04	n.d. : n.d.	1.30 ± 0.16	> LOQ[e]
6	B3	tb	2.12 ± 0.04	49.6 : 50.4	1.169 ± 0.023	50.3 : 49.7	0.624 ± 0.020	>97.8 : n.q.	36.6 ± 0.4	n.d. : n.q.	11.1 ± 0.7	> LOQ
7	B3	ll	3.35 ± 0.04	53.7 : 46.3	1.27 ± 0.04	52.6 : 47.4	0.180 ± 0.010	>89.1 : n.d.	17.4 ± 1.0	n.d. : n.q.	3.82 ± 0.18	n.d.
8	B4	tb	0.824 ± 0.027	97.9 : 2.1	0.681 ± 0.025	95.5 : 4.5	0.242 ± 0.010	>94.6 : n.d.	n.q.	n.d. : n.d.	n.q.	n.d.
9	B4	ll	0.92 ± 0.08	98.0 : 2.0	0.71 ± 0.07	95.2 : 4.8	0.1359 ± 0.0025	>94.5 : n.d.	1.17 ± 0.13	n.d. : n.d.	n.q.	n.d.
10	B5	ll	4.58 ± 0.14	97.4 : 2.6	1.60 ± 0.04	96.5 : 3.5	0.250 ± 0.015	>96.2 : n.d.	20.1 ± 1.3	n.d. : n.q.	8.0 ± 0.9	n.d.
11	B6	tb	1.47 ± 0.19	70.7 : 29.3	0.74 ± 0.11	69.7 : 30.3	1.11 ± 0.17	98.4 : 1.6	45 ± 8	n.d. : n.q.	60 ± 10	n.d.
12	PLB1	tb	1.49 ± 0.19	74.5 : 25.5	0.84 ± 0.11	72.1 : 27.9	0.98 ± 0.13	97.9 : 2.1	75 ± 11	n.d. : n.q.	70 ± 10	n.d.
13	PLB2	tb	1.12 ± 0.06	96.3 : 3.7	1.11 ± 0.08	96.1 : 3.9	1.00 ± 0.06	98.6 : 1.4	239 ± 15	4.8 : 95.2	206 ± 12	n.d.
14	PLB3	tb	1.55 ± 0.12	95.3 : 4.7	1.49 ± 0.15	96.1 : 3.9	1.58 ± 0.18	98.1 : 1.9	328 ± 36	n.q. : >93.2	261 ± 29	n.d.
15	PLB4	tb	1.120 ± 0.014	96.3 : 3.7	0.973 ± 0.018	96.3 : 3.7	0.934 ± 0.010	97.6 : 2.4	192.7 ± 1.4	6.7 : 93.3	151.7 ± 2.1	n.d.
16	PLB5	tb	1.474 ± 0.011	96.0 : 4.0	1.418 ± 0.015	96.5 : 3.5	1.446 ± 0.015	98.2 : 1.8	300 ± 4	5.1 : 94.9	242.8 ± 2.5	n.d.
17	Ts1	ll	3.01 ± 0.20	51.9 : 48.1	1.53 ± 0.11	53.2 : 46.8	0.042 ± 0.004	>82.1 : n.q.	8.14 ± 0.18	n.d. : n.q.	1.09 ± 0.05	> LOQ
18	Ts2	ll	1.17 ± 0.05	52.2 : 47.8	0.607 ± 0.023	53.5 : 46.5	0.0325 ± 0.0013	>73.4 : n.d.	4.18 ± 0.25	n.d. : n.d.	0.54 ± 0.06	> LOQ

Table 8. continued

samples			linalyl acetate		linalool		limonene		β-pinene		γ-terpinene	dihydro-linalool
no.	type [a]		[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[µg/g]	[%(*R*) : %(*S*)]	[µg/g]	
19	Ts3	II	3.33 ± 0.24	53.2 : 46.8	1.41 ± 0.12	53.1 : 46.9	0.247 ± 0.014	>96.8 : n.q.	2.7 ± 0.5	n.d. : n.d.	2.7 ± 0.5	> LOQ
20	Ts4	II	2.43 ± 0.11	49.6 : 50.4	1.45 ± 0.06	50.7 : 49.3	0.278 ± 0.014	>96.5 : n.q.	3.9 ± 0.4	n.d. : n.d.	3.82 ± 0.13	> LOQ
21	Ts5	II	0.0974 ± 0.0025	86.3 : 13.7	0.0609 ± 0.0008	>77.0 : n.q.	0.0063 ± 0.0012	n.q. : n.d.	n.q.	n.d. : n.d.	n.q.	n.d.
22	Ts6	II	2.06 ± 0.12	75.2 : 24.8	0.81 ± 0.04	70.9 : 29.1	0.198 ± 0.023	>95.1 : n.q.	6.4 ± 0.4	n.d. : n.d.	19.1 ± 2.7	> LOQ
23	Ts7	II	2.31 ± 0.15	90.9 : 9.1	0.98 ± 0.07	97.1 : 2.9	1.18 ± 0.07	95.2 : 4.8	164 ± 9	7.2 : 92.8	157 ± 9	n.d.
24	Ts8	II	2.92 ± 0.15	90.8 : 9.2	1.22 ± 0.06	97.3 : 2.7	1.18 ± 0.05	95.3 : 4.7	215 ± 9	6.0 : 94.0	164 ± 6	n.d.
25	Ts9	II	1.97 ± 0.11	74.4 : 25.6	0.81 ± 0.04	69.0 : 31.0	0.71 ± 0.06	98.3 : 1.7	94 ± 8	n.q. : >86.7	38.5 ± 3.0	n.q.
26	Ts10	II	5.2 ± 0.5	98.6 : 1.4	1.50 ± 0.12	97.5 : 2.5	4.9 ± 0.7	98.2 : 1.8	653 ± 104	6.6 : 93.4	793 ± 101	n.d.
27	Ts11	II	10.7 ± 0.8	98.6 : 1.4	9.4 ± 0.7	98.3 : 1.7	5.4 ± 0.4	98.1 : 1.9	810 ± 60	5.7 : 94.3	611 ± 49	n.d.
28	Ts12	II	4.68 ± 0.10	98.6 : 1.4	3.87 ± 0.08	98.3 : 1.7	1.29 ± 0.05	98.4 : 1.6	123 ± 6	n.q. : >89.6	49.8 ± 2.3	n.d.
Hungary (n=10)												
29	B7	tb	2.5 ± 0.5	70.1 : 29.9	1.01 ± 0.18	70.6 : 29.4	0.87 ± 0.15	95.3 : 4.7	82 ± 5	n.d. : >84.9	124 ± 8	n.d.
30	B7	tb	1.26 ± 0.23	98.1 : 1.9	0.74 ± 0.13	95.5 : 4.5	0.48 ± 0.09	>98.3 : n.q.	346 ± 64	3.2 : 96.8	65 ± 12	n.d.
31	B8	tb	4.83 ± 0.24	67.5 : 32.5	1.61 ± 0.08	67.7 : 32.3	2.40 ± 0.11	98.2 : 1.8	497 ± 22	4.1 : 95.9	213 ± 14	n.d.
32	B9	tb	0.85 ± 0.07	51.2 : 48.8	0.50 ± 0.05	59.1 : 40.9	0.90 ± 0.07	98.7 : 1.3	69 ± 5	n.q. : >85.7	45 ± 4	n.d.
33	B10	tb	2.91 ± 0.11	70.0 : 30.0	0.80 ± 0.04	70.5 : 29.5	2.38 ± 0.11	99.2 : 0.8	102 ± 4	n.q. : >90.5	96 ± 6	n.d.
34	B11	tb	1.59 ± 0.22	58.1 : 41.9	0.44 ± 0.06	70.4 : 29.6	1.34 ± 0.20	99.2 : 0.8	58 ± 9	n.d. : n.q.	53 ± 9	n.d.
35	B12	II	1.61 ± 0.04	99.0 : 1.0	4.08 ± 0.12	98.3 : 1.7	0.138 ± 0.003	>92.8 : n.d.	13.0 ± 0.6	n.d. : n.q.	n.q.	n.d.
36	PLB5	tb	1.38 ± 0.07	96.6 : 3.4	1.20 ± 0.05	96.3 : 3.7	1.15 ± 0.07	97.7 : 2.3	242 ± 15	6.2 : 93.8	187 ± 10	n.d.

Table 8. continued

samples			linalyl acetate		linalool		limonene		β-pinene		γ-terpinene	dihydro-linalool
no.	type [a]		[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[µg/g]	[%(*R*) : %(*S*)]	[µg/g]	
37	PLB6	tb	0.24 ± 0.11	57.7 : 42.3	0.13 ± 0.06	54.1 : 45.9	0.25 ± 0.13	>95.9 : n.q.	21 ± 10	n.d. : n.q.	13 ± 7	n.d.
38	Ts13	ll	1.93 ± 0.12	49.5 : 50.5	1.08 ± 0.07	50.1 : 49.9	0.196 ± 0.005	>95.0 : n.q.	7.5 ± 0.4	n.d. : n.q.	17.1 ± 0.8	> LOQ
Poland (n=10)												
39	B7	tb	1.17 ± 0.15	98.0 : 2.0	0.69 ± 0.09	95.3 : 4.7	0.42 ± 0.06	>97.5 : n.q.	294 ± 45	n.q. : >95.9	57 ± 8	n.d.
40	B7	ll	5.7 ± 1.2	49.5 : 50.5	1.9 ± 0.4	50.0 : 50.0	1.17 ± 0.18	98.9 : 1.1	96 ± 15	n.q. : >90.4	58 ± 9	> LOQ
41	B8	tb	4.43 ± 0.28	67.1 : 32.9	1.46 ± 0.10	67.7 : 32.3	2.28 ± 0.17	98.2 : 1.8	468 ± 38	4.1 : 95.9	216 ± 19	n.d.
42	B9	tb	1.31 ± 0.12	51.0 : 49.0	0.76 ± 0.07	59.2 : 40.8	1.40 ± 0.12	98.7 : 1.3	110 ± 11	n.q. : >92.3	75 ± 8	n.d.
43	B13	tb	2.55 ± 0.09	50.5 : 49.5	0.97 ± 0.04	51.4 : 48.6	0.44 ± 0.04	95.6 : 4.4	15.3 ± 3.0	n.d. : n.q.	41 ± 4	n.d.
44	B14	tb	3.37 ± 0.28	50.7 : 49.3	1.44 ± 0.11	51.6 : 48.4	0.121 ± 0.020	93.5 : 6.5	26.3 ± 2.8	n.d. : n.q.	20 ± 5	> LOQ
45	B15	tb	1.98 ± 0.09	49.7 : 50.3	1.91 ± 0.09	50.2 : 49.8	0.910 ± 0.026	97.6 : 2.4	408 ± 15	5.3 : 94.7	195 ± 6	n.d.
46	B16	ll	1.67 ± 0.08	49.5 : 50.5	1.79 ± 0.09	49.8 : 50.2	0.0404 ± 0.0021	n.q. : n.d.	5.6 ± 0.8	n.d. : n.q.	15.13 ± 0.21	> LOQ
47	PLB7	tb	3.23 ± 0.12	49.6 : 50.4	3.36 ± 0.14	49.8 : 50.2	1.88 ± 0.07	99.2 : 0.7	256 ± 9	n.q. : >96.1	180 ± 8	> LOQ
48	Ts14	ll	0.60 ± 0.05	97.9 : 2.1	0.186 ± 0.019	>92.5 : n.q.	0.07902±0.00014	>90.0 : n.d.	13.1 ± 1.9	n.d. : n.q.	9.3 ± 0.5	n.d.
United Kingdom (n=10)												
49	B13	tb	2.86 ± 0.20	50.3 : 49.7	1.04 ± 0.08	51.1 : 48.9	0.46 ± 0.04	95.9 : 4.1	9.8 ± 0.7	n.d. : n.q.	46.6 ± 2.7	> LOQ
50	B13	tb	2.8 ± 0.5	50.3 : 49.7	1.03 ± 0.16	51.1 : 48.9	0.56 ± 0.10	96.0 : 4.0	14.0 ± 1.8	n.d. : n.q.	34 ± 7	> LOQ
51	B17	tb	2.24 ± 0.07	98.3 : 1.7	1.283 ± 0.012	96.8 : 3.2	1.80 ± 0.13	97.7 : 2.3	248 ± 7	7.6 : 92.4	157 ± 13	n.d.
52	B17	tb	3.77 ± 0.27	98.2 : 1.8	2.29 ± 0.25	97.0 : 3.0	1.2 ± 1.1	97.5 : 2.5	138 ± 17	7.7 : 92.3	41 ± 20	> LOQ
53	B18	tb	0.415 ± 0.022	49.6 : 50.4	0.716 ± 0.010	50.3 : 49.7	0.2095 ± 0.0022	97.8 : 2.2	44.9 ± 3.0	n.q. : >89.3	9.5 ± 0.4	> LOQ

Table 8. continued

samples			linalyl acetate		linalool		limonene		β-pinene		γ-terpinene	dihydro-linalool
no.	type [a]		[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[µg/g]	[%(*R*) : %(*S*)]	[µg/g]	
54	B19	tb	3.32 ± 0.26	99.4 : 0.6	2.18 ± 0.26	96.5 : 3.5	1.20 ± 0.14	98.2 : 1.8	101 ± 18	n.q. : >87.6	180 ± 25	n.d.
55	B20	tb	1.19 ± 0.07	97.9 : 2.1	0.324 ± 0.011	96.6 : 3.4	0.0506 ± 0.0011	>90.6 : n.d.	5.9 ± 0.5	n.d. : n.q.	3.03 ± 0.13	n.d.
56	B21	tb	0.603 ± 0.023	96.7 : 3.3	0.459 ± 0.015	>95.9 : n.q.	0.0116 ± 0.0008	n.q. : n.d.	0.76 ± 0.13	n.d. : n.d.	0.32 ± 0.04	n.d.
57	B22	tb	1.8 ± 0.4	97.4 : 2.6	0.67 ± 0.18	96.0 : 4.0	0.17 ± 0.14	>94.8 : n.q.	13 ± 10	n.d. : n.q.	7 ± 5	n.d.
58	B23	tb	1.78 ± 0.21	69.6 : 30.4	0.74 ± 0.08	69.1 : 30.9	1.32 ± 0.20	97.1 : 2.9	66 ± 11	n.q. : >86.8	78 ± 14	> LOQ
Italy (n=6)												
59	B5	tb	2.13 ± 0.04	75.0 : 25.0	1.167 ± 0.018	72.2 : 27.8	1.36 ± 0.04	98.0 : 2.0	104.8 ± 3.0	n.q. : >87.3	98.9 ± 2.6	n.d.
60	B7	tb	0.83 ± 0.07	98.2 : 1.8	0.54 ± 0.05	96.1 : 3.9	0.46 ± 0.04	98.0 : 2.0	260 ± 23	4.3 : 95.7	65 ± 6	n.d.
61	B13	tb	1.63 ± 0.09	50.7 : 49.3	0.62 ± 0.04	50.6 : 49.4	1.09 ± 0.06	98.1 : 1.9	34.3 ± 1.4	n.d. : n.q.	23.4 ± 1.4	> LOQ
62	B24	tb	0.815 ± 0.017	97.7 : 2.3	0.654 ± 0.013	95.4 : 4.6	0.384 ± 0.016	>97.7 : n.d.	1.05 ± 0.06	n.d. : n.d.	n.q.	> LOQ
63	PLB8	tb	3.42 ± 0.18	63.7 : 36.3	1.43 ± 0.06	63.5 : 36.5	0.76 ± 0.06	95.2 : 4.8	74 ± 4	n.q. : >83.0	104 ± 8	> LOQ
64	Ts15	II	0.338 ± 0.005	83.1 : 16.9	0.87 ± 0.04	55.2 : 44.8	0.505 ± 0.009	>98.4 : n.q.	58.6 ± 2.1	n.q. : >86.0	7.21 ± 0.28	> LOQ
Spain (n=6)												
65	B13	tb	2.42 ± 0.15	50.3 : 49.7	0.86 ± 0.05	50.8 : 49.2	0.438 ± 0.029	95.8 : 4.2	10.5 ± 1.3	n.d. : n.q.	28.4 ± 1.4	> LOQ
66	PLB5	tb	1.462 ± 0.022	97.3 : 2.7	1.27 ± 0.04	96.9 : 3.1	1.33 ± 0.04	98.3 : 1.7	287 ± 10	5.0 : 95.0	227 ± 5	n.d.
67	PLB9	tb	2.59 ± 0.09	49.5 : 50.5	1.18 ± 0.04	49.8 : 50.2	0.89 ± 0.07	>98.9 : n.q.	173 ± 16	n.q. : >94.2	4.34 ± 0.14	n.d.
68	Ts16	II	1.88 ± 0.07	57.4 : 42.6	1.23 ± 0.06	53.3 : 46.7	1.91 ± 0.14	92.4 : 7.6	186 ± 18	n.q. : >92.1	138 ± 10	> LOQ
69	Ts17	II	3.2 ± 0.7	64.2 : 35.8	1.8 ± 0.4	65.0 : 35.0	1.6 ± 0.5	98.2 : 1.8	99 ± 31	n.q. : >86.6	118 ± 34	> LOQ
70	Ts18	II	8.7 ± 0.4	57.4 : 42.6	3.20 ± 0.16	60.4 : 39.6	0.814 ± 0.024	97.5 : 2.5	213 ± 6	n.q. : >94.8	96 ± 4	> LOQ

Table 8. continued

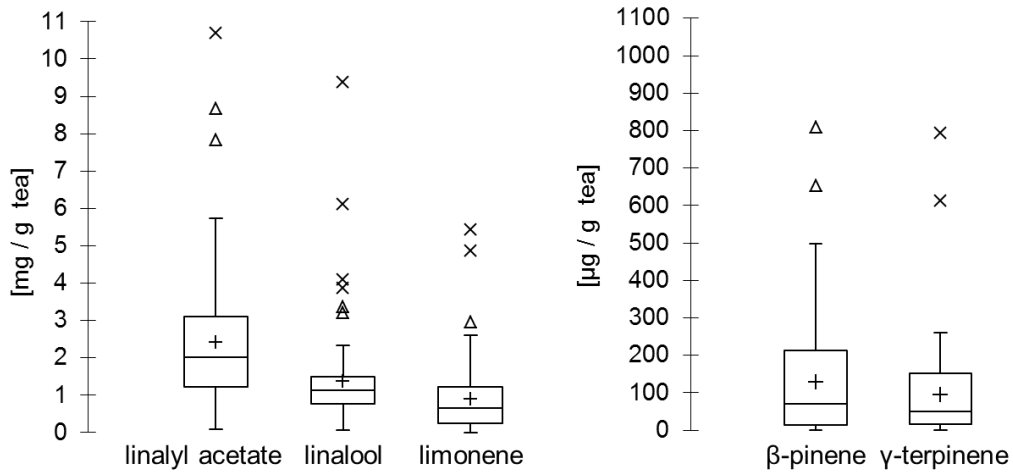
samples			linalyl acetate		linalool		limonene		β-pinene		γ-terpinene	dihydro-linalool
no.	type [a]		[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[µg/g]	[%(*R*) : %(*S*)]	[µg/g]	
Denmark (n=5)												
71	B13	tb	2.5 ± 0.4	97.3 : 2.7	1.09 ± 0.14	94.4 : 5.6	1.59 ± 0.27	97.8 : 2.2	29 ± 4	n.d. : n.q.	45 ± 10	n.d.
72	B25	tb	0.852 ± 0.019	96.7 : 3.3	1.230 ± 0.008	96.4 : 3.6	0.321 ± 0.021	>97.1 : n.q.	89 ± 5	n.q. : n.q.	46 ± 4	n.d.
73	PLB10	II	2.64 ± 0.06	90.3 : 9.7	1.96 ± 0.06	84.8 : 15.2	0.0825 ± 0.0016	91.5 : 8.5	11.97 ± 0.17	n.d. : n.q.	2.11 ± 0.26	n.q.
74	Ts19	II	1.13 ± 0.09	97.9 : 2.1	0.84 ± 0.05	97.6 : 2.4	0.0366 ± 0.0023	n.q. : n.d.	8.1 ± 0.6	n.d. : n.q.	6.8 ± 0.5	n.d.
75	Ts20	II	1.24 ± 0.05	63.6 : 36.4	1.27 ± 0.06	52.1 : 47.9	0.250 ± 0.009	75.4 : 24.6	4.99 ± 0.12	n.d. : n.d.	n.q.	> LOQ
Finland (n=5)												
76	B7	tb	1.6 ± 0.4	98.2 : 1.8	0.98 ± 0.19	95.2 : 4.8	0.58 ± 0.12	98.1 : 1.9	410 ± 77	3.4 : 96.6	76 ± 16	n.d.
77	B7	II	4.85 ± 0.05	49.5 : 50.5	1.818 ± 0.016	50.1 : 49.9	0.974 ± 0.024	>98.7 : n.q.	53.3 ± 1.0	n.d. : n.q.	49.1 ± 1.8	n.d.
78	B13	II	2.80 ± 0.17	49.4 : 50.6	1.26 ± 0.07	50.3 : 49.7	1.45 ± 0.12	98.1 : 1.9	53 ± 5	n.d. : n.q.	42 ± 4	> LOQ
79	PLB5	tb	1.277 ± 0.027	97.4 : 2.6	1.153 ± 0.021	96.9 : 3.1	1.193 ± 0.025	98.3 : 1.7	257 ± 5	4.6 : 95.4	204 ± 4	n.d.
80	PLB11	II	3.68 ± 0.08	51.0 : 49.0	1.82 ± 0.05	51.2 : 48.8	0.0687 ± 0.0011	>85.3 : n.d.	56.4 ± 0.6	n.d. : n.q.	n.q.	> LOQ
France (n = 5)												
81	B7	tb	0.21 ± 0.07	54.6 : 45.4	0.14 ± 0.05	52.2 : 47.8	0.35 ± 0.11	>97.3 : n.d.	11 ± 4	n.d. : n.q.	6.3 ± 2.1	> LOQ
82	B7	II	7.8 ± 0.4	51.2 : 48.8	6.11 ± 0.15	50.7 : 49.3	0.089 ± 0.013	>87.4 : n.d.	16.1 ± 2.7	n.d. : n.q.	12.9 ± 2.0	> LOQ
83	B7	II	0.19 ± 0.12	54.2 : 45.8	0.13 ± 0.08	52.6 : 47.4	0.62 ± 0.12	>98.3 : n.q.	48 ± 34	n.d. : n.q.	18 ± 8	> LOQ
84	B13	tb	2.84 ± 0.15	50.3 : 49.7	1.05 ± 0.06	51.1 : 48.9	0.49 ± 0.04	95.6 : 4.4	n.q.	n.d. : n.q.	46 ± 4	n.q.
85	B26	tb	1.64 ± 0.05	>99.2 : n.d.	1.35 ± 0.09	97.6 : 2.4	0.59 ± 0.08	97.9 : 2.1	50 ± 9	n.q. : >78.4	32 ± 4	n.d.

Table 8. continued

samples			linalyl acetate		linalool		limonene		β-pinene		γ-terpinene	dihydro-linalool
no.	type [a]		[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[mg/g]	[%(*R*) : %(*S*)]	[µg/g]	[%(*R*) : %(*S*)]	[µg/g]	
Ireland (n=5)												
86	B13	tb	2.70 ± 0.20	50.5 : 49.5	0.98 ± 0.08	51.4 : 48.6	0.45 ± 0.04	95.6 : 4.4	9.0 ± 0.4	n.d. : n.q.	45 ± 4	> LOQ
87	PLB5	tb	1.17 ± 0.08	96.0 : 4.0	1.188 ± 0.009	96.3 : 3.7	1.069 ± 0.010	98.7 : 1.3	256.2 ± 2.5	n.q. : >95.2	222.8 ± 1.6	n.d.
88	PLB12	tb	3.83 ± 0.07	95.2 : 4.8	1.662 ± 0.014	98.0 : 2.0	2.95 ± 0.06	97.5 : 2.5	174 ± 4	5.1 : 94.9	112 ± 5	n.d.
89	Ts21	ll	3.14 ± 0.08	57.1 : 42.9	1.08 ± 0.05	58.5 : 41.5	0.0288 ± 0.0011	n.q. : n.d.	5.95 ± 0.11	n.d. : n.q.	3.36 ± 0.08	> LOQ
90	Ts22	ll	3.03 ± 0.08	57.5 : 42.5	0.94 ± 0.04	59.0 : 41.0	0.035 ± 0.004	n.q. : n.d.	10.4 ± 2.1	n.d. : n.q.	3.6 ± 0.4	> LOQ

[a] B: product assignable to specific brands (B1-26); PLB: products assignable to private label brands exclusively used by specific supermarket chains (PLB1-12); Ts: products not assignable to a specific brand / private label brand, but sold in speciality tea-shops (Ts1-22). tb: products offered in tea bags; ll: products offered as loose leaves. [b] Ratios 'greater than (>)' were calculated using the peak area corresponding to the limit of quantification for the second enantiomer (Lebensmittelchemische Gesellschaft (GDCh) 2001). [c] n.q.: below limit of quantification (LOQ) calculated for 1 g extracted tea material and dilution factor 5 (β-pinene: 1.090 µg/g; γ-terpinene: 0.873 µg/g; dihydrolinalool: 1.128 µg/g). [d] n.d.: below limit of determination (LOD) calculated for 1 g extracted tea material and dilution factor 5 (for β-pinene = 0.358 µg/g; for γ-terpinene = 0.287 µg/g; dihydrolinalool: 0.371 µg/g). [e] > LOQ: above limit of quantification calculated for 1 g extracted tea material and dilution factor 5 (dihydrolinalool: 1.128 µg/g). All concentration values were rounded according to DIN 1333 (1992). Data represent means ± standard deviations from triplicate experiments).

The box-and-whisker plots depicted in Figure 15 demonstrate that apart from a few outliers rather narrow content ranges were observed for the major flavouring substances linalyl acetate, linalool, and limonene (order of magnitude: mg/g) as well as for the less abundant β-pinene and γ-terpinene (order of magnitude: µg/g). All five compounds were consistently present in all Earl Grey tea samples; only in seven (γ-terpinene) and two (β-pinene) teas, respectively, the amounts were below the limits of quantification.



	linalyl acetate	linalool	limonene	β-pinene	γ-terpinene
mean	2.4	1.4	0.9	126	91
median	2.0	1.1	0.7	66	47
minimum	0.1	0.06	0.01	n.q.	n.q.
maximum	10.7	9.4	5.4	810	793
2.5 perc	0.2	0.1	0.03	1	1
97.5 perc	7.4	4.0	2.9	493	260

Figure 15. Distributions of the contents of monoterpenes isolated from Earl Grey teas (n=90).

The narrow ranges are also reflected in the frequency distributions shown for linalyl acetate and linalool in Figure 16**.** Except for the 8 highest values for linalyl acetate and the 6 highest for linalool, the contents for both substances were normally distributed (Shapiro-Wilk-test; α=0.05; linalyl acetate (n=82): p=0.07878; linalool (n=84): p=0.3411). The values fit well to those of Neukom et al. (1993) who determined on average 1.7 mg/g tea linalyl acetate and 0.8 mg/g tea linalool in 12 samples of Earl Grey tea of the Swiss market; limonene amounted to 0.2 mg/g.

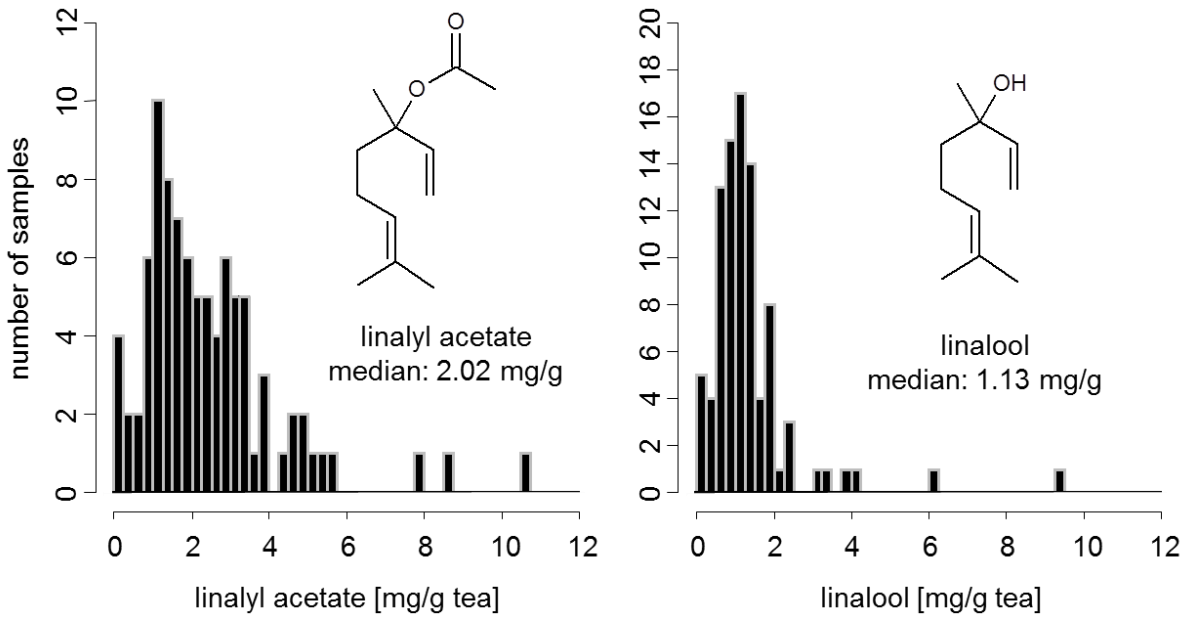


Figure 16: Frequency distributions (concentration steps: 0.25 mg/g) of linalyl acetate and linalool isolated from Earl Grey teas (n=90).

The highest amounts for linalyl acetate (10.7 mg/g), linalool (9.4 mg/g), limonene (5.4 mg/g) and β-pinene (810 µg/g) were found in the same tea (no. 27; D); whereas tea no. 26 (D) showed the highest value for γ-terpinene (793 µg/g). Second and third highest values were found as follows: linalyl acetate (8.7 mg/g, no. 70 (E); 7.8 mg/g, no. 82 (F)), linalool (6.1 mg/g, no. 82 (F); 4.1 mg/g, no. 35 (H)), limonene (4.9 mg/g, no. 26 (D); 2.9 mg/g, no. 88 (IRL)), β-pinene (653 µg/g, no. 26 (D); 497 µg/g, no. 31, (H)), and γ-terpinene (611 µg/g, no. 27 (D); 261 µg/g, no. 14 (D)). It is important to note that these few outliers, shown as triangles and crosses in the box-and-whisker plot diagram, originated not only from one country.

The median of linalyl acetate was nearly two times higher than that of linalool, approximately three times higher than that of limonene and 30 to 40 times higher than of β-pinene and γ-terpinene, respectively. However, ratios of contents of flavouring substances in a single product can differ from these values. This was the case for tea no. 35 where the content of linalool was 2.5 times higher than that of linalyl acetate, or for tea no. 60 where the ratio of linalyl acetate and β-pinene was only 3.

It is notable that the content of limonene in cold pressed (not deterpened) bergamot oils is usually higher than that of linalool (Mazza 1986; Kiwanuka et al. 2000; Belsito et al. 2007; Dugo et al. 2012). In contrast, the median value of limonene in Earl Grey teas was found to be lower than that of linalool. In addition, the contents of β-pinene and γ-terpinene in Earl Grey teas were lower than those reported for bergamot oils. Because of the fact that terpenes are not stable in heat and light, they are often removed from citrus oils (see

2.4.4.9). Fantin et al. (2010), Fang et al. (2004) and Kondo et al. (2000) reported deterpened oils with reduced levels of these compounds. Therefore, it is likely that terpene free oils have been used for some teas.

The ratio of linalool to linalyl acetate is an important quality criterion of bergamot oil. It has been reported to be on average approximately 0.3 for oils produced in Reggio Calabria (Sawamura et al. 2006). Oils produced in Greece, Turkey and Japan revealed a ratio of 0.5 - 0.6, 0.2 - 0.6 and 0.6 respectively (Sawamura et al. 1999; Kirbaslar et al. 2001; Melliou et al. 2009). As shown in Figure 17, the ratios of linalool to linalyl acetate amounts in 90 Earl Grey teas were in a narrow range (0.3 - 1.1); the median was 0.6. Nevertheless there were samples with ratios higher than 1.1 (outliers; tea no. 35 (2.5), 53 (1.7), 64 (2.5), and 72 (1.4)) even in combination with enantiomerically pure linalool and linalyl acetate. In these cases, either linalool from other natural sources or enantiomerically pure synthesized linalool has been added. Another explanation may be the fact that in bergamot oil, extracted by other processing types than cold pressing, partial hydrolysis of linalyl acetate to its alcohol may have occurred (Schmaus and Kubeczka 1985; Kiwanuka et al. 2000).

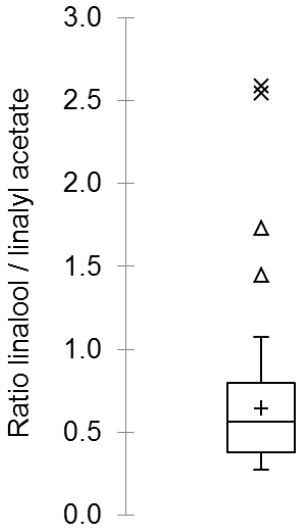


Figure 17: Distribution of the ratios of linalool and linalyl acetate contents [mg/g] in Earl Grey teas (n=90).

Regarding the enantiomeric compositions of chiral flavouring substances, limonene and β-pinene were shown to be consistently present in optically pure form or at least with high enantiomeric excess (Table 8). In contrast, the enantiomeric ratios determined for linalyl acetate and linalool varied considerably. The analysed tea samples could be subdivided into three groups: one third (group I) contained linalyl acetate (n=34) and linalool (n=36) almost exclusively as (*R*)-enantiomers (95-100%); one third (group II) contained these flavouring substances as nearly racemic mixtures (50-55% (*R*); linalyl acetate: n=31, linalool: n=32) and in group III ratios of the enantiomers of 55-95% (*R*) were observed (linalyl acetate: n=25; linalool: n=22). Typical chromatograms showing the separations of enantiomers for representatives of these groups are shown in Figure 18**.**

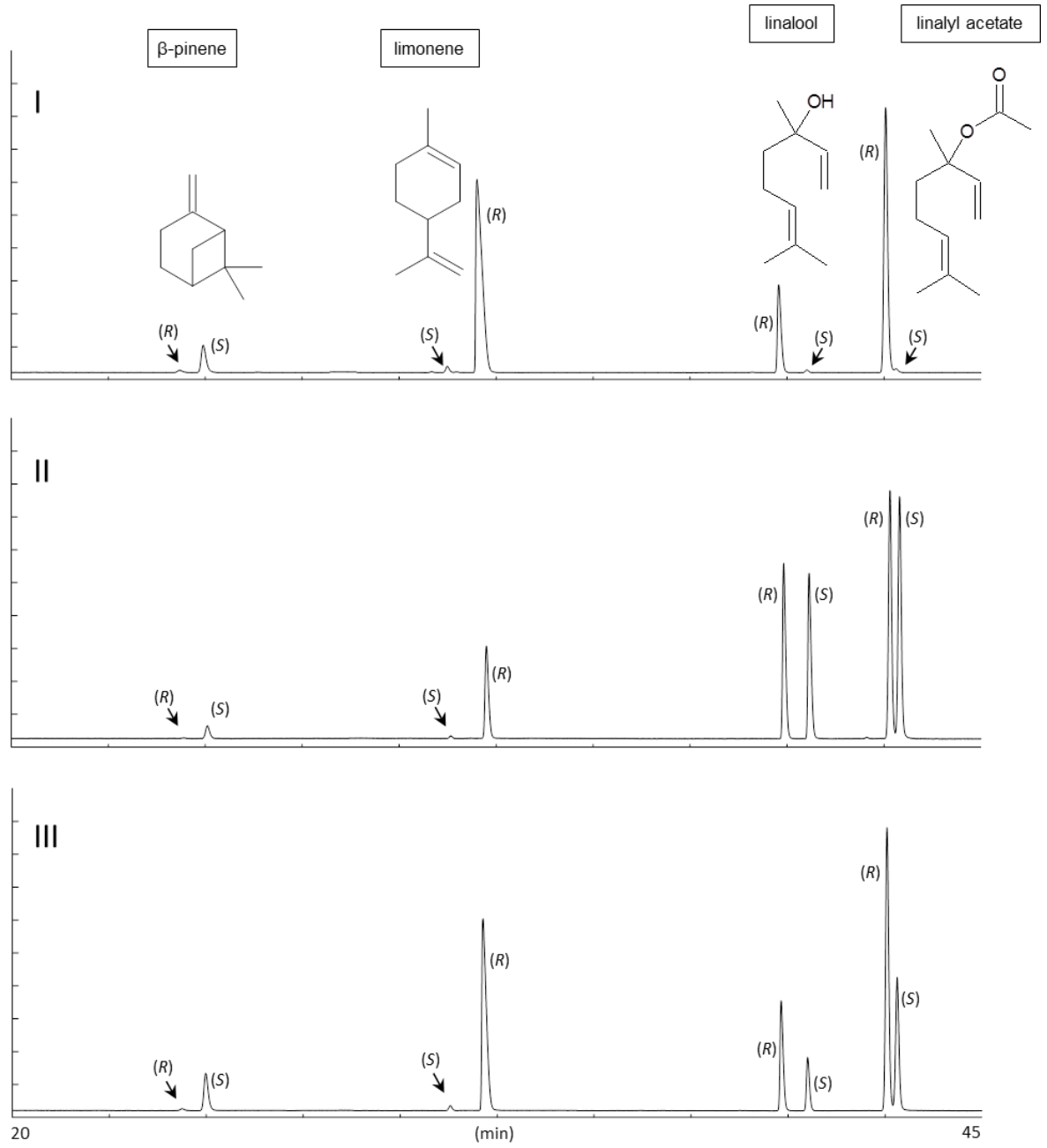


Figure 18: Typical gas chromatographic separations of the enantiomers of chiral monoterpenes isolated from Earl Grey teas using a chiral stationary phase. GC-system IIb. Enantiomeric purities of linalyl acetate and linalool: 95-100% (R) = Group I; 50-55% (R) = Group II; 55-95% (R) = Group III.

The occurrence of dihydrolinalool (Table 8) might be an indicator for the use of synthetic linalool (see 2.4.8). In combination with the analysis of the enantiomeric distributions of linalyl acetate and linalool, which are present as almost optically pure (*R*)-enantiomers in cold pressed bergamot oils, the detection of adulterations could be more strengthened. 34 out of 90 teas contained dihydrolinalool in quantifiable amounts; in five samples the occurrence could be shown qualitatively. Indeed, 27 of these 39 samplings contained racemic linalool. However, in two teas containing dihydrolinalool, linalyl acetate and linalool were almost

enantiomerically pure (no. 52 and 62); these were also labelled either with ‘bergamot oil’ or ‘natural aroma’ although the presence of dihydrolinalool indicates synthetic flavour. In four samples, the declaration said ‘bergamot oil’ although both linalool and linalyl acetate were racemic and the teas contained quantifiable amounts of dihydrolinalool (no. 19, 20, 38, and 68). In these cases, an adulteration is highly probable. Remarkably, all four samples where bought in teashops (Ts).

For samples exhibiting enantiomeric distributions of linalool, which are neither pure nor racemic (group II: 55-95% (R)), a use of synthetic flavourings is not necessarily proven due to possible partial racemisations in bergamot oils isolated by other processing types than cold-pressing (see 2.4.5). This is confirmed by the absence of dihydrolinalool for 12 out of 22 samples with linalool exhibiting enantiomeric ratios of group II. On the other hand, 11 of these 12 teas were labelled with “flavour”. For the remaining 10 samples, dihydrolinalool could be determined in amounts higher than the limit of determination which increases the likelihood of falsification; four were labelled with “flavour” (no. 58, 63, 69 and 73), three did not feature any description (no. 70, 89 and 90), for one the ingredients list exhibited both “natural bergamot oil” and “flavour” (no. 2) and two were denoted as “(natural) bergamot oil” (no. 22 and 25). Because linalyl acetate is not affected by racemisation processes, an adulteration is most probable for 6 samples labelled with “(natural) bergamot oil” containing quantifiable amounts of dihydrolinalool and racemic or partial racemic distributions of linalyl acetate (no. 19, 20, 22, 25, 68, and 75).

4.1.4 Impact factors

In order to reveal factors potentially impacting the contents of linalool and linalyl acetate in Earl Grey teas available on the EU-market, the data were grouped according to different aspects, i.e. (i) country of purchase, (ii) enantiomeric distributions of chiral substances, (iii) type of packaging, and (iv) source of products. In addition, contents of flavourings of different variants of one tea brand or teas of the same variant purchased in different countries were compared. An overview is given in Table 9.

Table 9. Overview on the contents of linalyl acetate and linalool determined in Earl Grey teas depending on various impact factors. (Summary of data from boxplots shown in Figure 19, Figure 20, Figure 21 and Figure 22. For abbreviations see 3.1.1.)

Impact factor	linalyl acetate [mg/g tea]				linalool [mg/g tea]		
	n[a]	median	Q1[b]	Q3[c]	n	median	Q1	Q3
all values (no impact)	90	2.02	1.23	3.11	90	1.13	0.77	1.50
(i) country of purchase								
D	28	2.09	1.21	3.34	28	1.20	0.81	1.51
H	10	1.60	1.29	2.36	10	0.91	0.56	1.17
PL	10	2.27	1.40	3.33	10	1.45	0.81	1.88
UK	6	2.02	1.34	2.85	6	0.89	0.68	1.22
I	6	1.23	0.82	2.01	6	0.76	0.63	1.09
E	6	2.51	2.01	3.07	6	1.25	1.19	1.70
DK	5	1.24	1.13	2.45	5	1.23	1.09	1.27
FIN	5	2.80	1.55	3.68	5	1.26	1.15	1.82
F	5	1.64	0.21	2.84	5	1.05	0.14	1.35
IRL	5	3.03	2.70	3.14	5	1.08	0.98	1.19
(ii) enantiomeric distributions								
I: (*R*) ≥ 95%	34	1.42	1.12	2.40	36	1.17	0.73	1.49
II: (*R*) = 50-55%	31	2.55	1.65	3.12	32	1.21	0.94	1.60
III: (*R*) = 55-95%	25	2.31	1.59	3.14	22	0.89	0.75	1.45
(iii) type of packaging								
tb	54	1.61	1.18	2.67	54	1.02	0.72	1.28
ll	36	2.68	1.65	3.90	36	1.42	0.97	1.82
(iv) source of products								
B	52	2.05	1.22	2.87	52	1.02	0.71	1.50
PLB	16	1.48	1.25	2.79	16	1.24	1.14	1.53
Ts	22	2.37	1.40	3.21	22	1.15	0.85	1.49

[a] number of samples referring to one impact factor; [b] Q1: 25th percentile; [c] Q3: 75th percentile.

4.1.4.1 Country of purchase

As mentioned before, outliers can be found in more than one country. To overcome this, an ANOVA was carried out using just the normally distributed data sets instead of the nonparametric Kruskal-Wallis test including all values because of the stronger explanatory power. Besides normal distribution, homogeneity of variances was tested as a second test-condition via Bartlett´s test (α=0.05; linalyl acetate (n=82): p=0.9244; linalool (n=84): p=0.6431).

Contents of linalyl acetate and linalool according to different countries of purchase are shown in Figure 19. The median values ranged from 1.23 mg/g (I) to 3.03 mg/g (IRL) for linalyl acetate and from 0.76 mg/g (I) to 1.45 mg/g (P) for linalool. However, an analysis of variance (ANOVA, α=0.05) for the normally distributed values (linalyl acetate: n=82; linalool: n=84) showed no statistically significant differences (linalyl acetate: p=0.459; linalool: p=0.347) in the contents depending on the country of purchase.

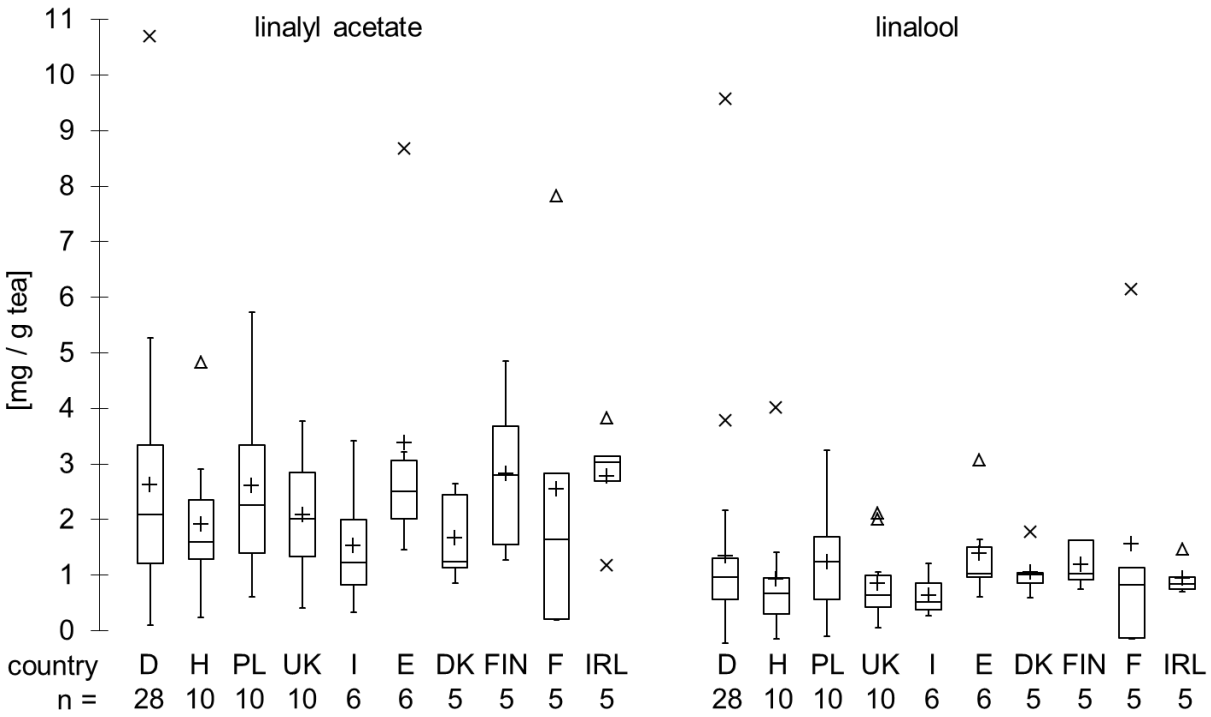


Figure 19: Contents of linalyl acetate and linalool in Earl Grey teas (n=90) depending on the country of purchase. For median, 25th and 75th percentile see Table 9.

4.1.4.2 Enantiomeric purities

Figure 20 shows a comparison of the content distributions of linalyl acetate and linalool in the groups I - III on the basis of the enantiomeric purities of these flavouring substances (Table 8, Figure 18). Group I contained linalyl acetate and linalool almost exclusively as (*R*)-enantiomers (95-100%); group II contained these flavouring substances as nearly racemic mixtures (50-55% (*R*) and in group III ratios of the enantiomers of 55-95% (*R*) were observed.

Statistical assessment via a Kruskal-Wallis-test (α=0.05) revealed that despite some outliers there were no significant differences between the median concentrations of linalyl acetate (p=0.07632) and linalool (p=0.4385) determined in these groups.

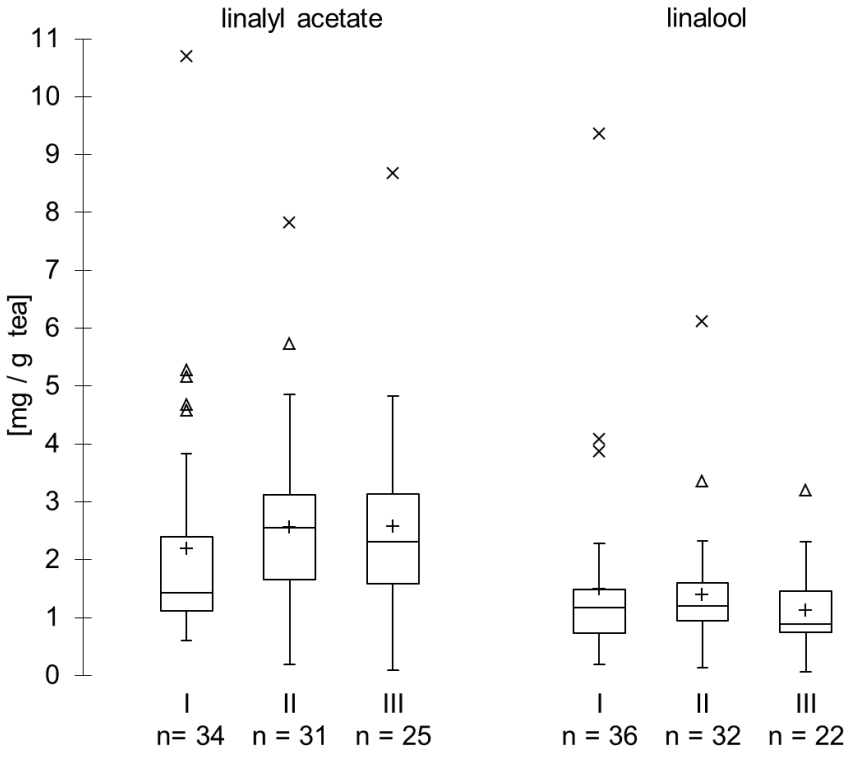


Figure 20: Contents of linalyl acetate and linalool in Earl Grey teas (n=90) depending on the enantiomeric compositions of linalyl acetate and linalool. For explanation of groups I, II and III see Figure 18. For explanations of the symbols see Figure 15. For median, 25th and 75th percentile see Table 9.

4.1.4.3 Type of packaging

The aspect whether the teas have been sold in tea bags (tb, n=54) or as loose leaves (ll, n=36) was examined. Figure 22 shows that the variability in the contents of linalyl acetate and linalool were higher in teas sold as loose leaves than in teas sold in tea bags. A statistical assessment via the Kruskal-Wallis test (α=0.05) demonstrated that the medians of

linalyl acetate (tb=1.6 mg/g, ll=2.7 mg/g) and linalool (tb=1.0 mg/g, ll=1.3 mg/g) were significantly higher in the loose leaves-materials (linalyl acetate: *p*=0.013; linalool: *p*=0.008). A nonparametric test was chosen since the values were not normally distributed. The data show that consumers preferring loose tea material instead of teabags are exposed to higher amounts of linalyl acetate and linalool in Earl Grey teas. More specifically, teas sold as loose leaves contain 67% more linalyl acetate and 39% more linalool than those offered in tea bags (calculated with median amounts). In addition, the number of samples with extremely high contents of linalyl acetate and linalool was higher in the loose leaves-tea. In case of consumers, who prefer only loose leaves-tea, this behaviour or 'loyalty' could lead to higher daily intake levels than consumers preferring teas in tea bags.

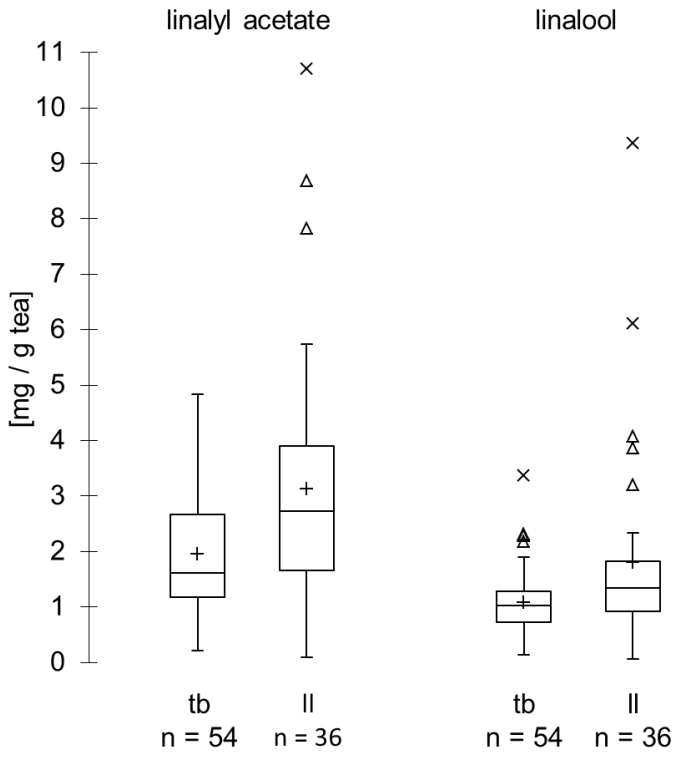


Figure 21: Contents of linalyl acetate and linalool in Earl Grey teas (n=90) depending on type of packaging. tb: products offered in tea bags; ll: teas offered as loose leaves. For explanations of the symbols see Figure 15. For median, 25th and 75th percentile see Table 9.

4.1.4.4 Source of products

The analysed samples were grouped according to the brand of the product. Three groups could be defined: B: products assignable to specific inter-national / national brands (n=52), PLB: products assignable to private label brands exclusively used by specific supermarket chains (n=16), and Ts: products assignable to a specific brand / private label brand, but sold

in speciality tea shops (n=22). All teas from teashops were purchased as loose leaves, whereas brand or private label brand teas have been purchased as loose leave material or in tea bags. Not only because loose leaves-teas contain higher amounts of linalyl acetate and linalool than teas offered in bags (see 4.1.4.3), it was tested whether differences exist depending on the sources of the products.

Box-plots for this grouping system are illustrated in Figure 22. Since there was no normal distribution, a nonparametric Kruskal-Wallis-test (α=0.05) was performed. It demonstrated that there were no significant differences in the contents of linalyl acetate (p=0.5092) and linalool (p=0.2386) between teas from different sources of products.

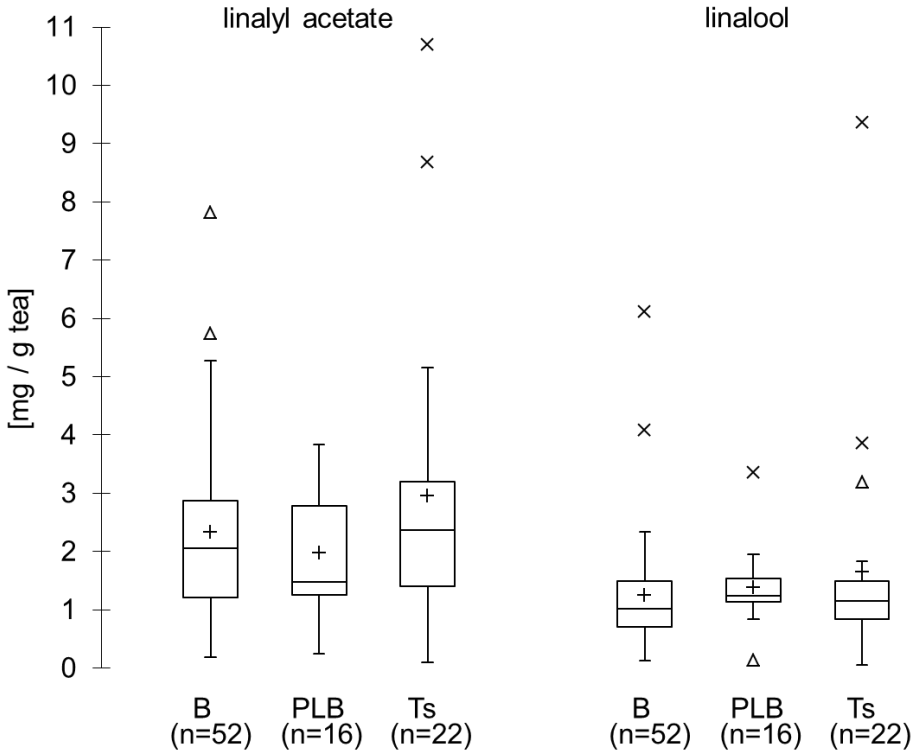


Figure 22: Contents of linalyl acetate and linalool in Earl Grey teas (n=90) depending on the source of product. B: product assignable to specific brands; PLB: products assignable to private label brands exclusively used by specific supermarket chains; Ts: products not assignable to a specific brand / private label brand, but sold in speciality tea shops. For median, 25th and 75th percentile see Table 9.

4.1.4.5 Variability among brands

In order to investigate the variability in the contents of flavouring substances among products from two brand leaders, the amounts of linalyl acetate and linalool were compared in different variants, i.e. teas with different name (e.g. 'Russian Earl Grey' vs. 'Earl Grey Klassik') and/or with different designs (e.g. colour of packaging). For the investigated samples of brand B13, not only the same variant sold in different countries but also different variants purchased in various countries showed very similar contents of linalyl acetate and linalool, except tea no. 61, which exhibited significantly lower contents (Table 10). However, this variant ("Lady Grey Tea") had been explicitly labelled with "light flavour strength". For brand B7, the contents in samples of the same variant offered in different countries were also very similar. However, for different variants the highest and lowest contents of linalyl acetate and linalool differed by factors up to 40 and 47, respectively.

Table 10. Contents of linalyl acetate and linalool from two tea brands (B7 and B13) purchased in different countries of the European Union.

	samples		content [mg/g tea]	
	no.	country	linalyl acetate	linalool
B13: n=9				
Variant 1: n=4	43	PL	2.55 [a]	0.97
	65	E	2.42	0.86
	78	FIN	2.80	1.26
	84	F	2.84	1.05
Variant 2: n=2	49	UK	2.86	1.04
	86	IRL	2.70	0.98
Variant 3: n=1	50	UK	2.82	1.03
Variant 4: n=1	61	I	1.63	0.62
Variant 5: n=1	71	DK	2.45	1.09
mean			**2.56 ± 0.39**	**0.99 ± 0.18**
B7: n=10				
Variant 1: n=3	30	H	1.26	0.74
	39	PL	1.17	0.69
	76	FIN	1.55	0.98
Variant 2 : n=2	81[b]	F	0.21	0.14
	83[c]	F	0.19	0.13
Variant 3: n=1	29	H	2.50	1.01
Variant 4: n=1	40	PL	5.74	1.92
Variant 5: n=1	60	I	0.83	0.54
Variant 6: n=1	77	FIN	4.85	1.82
Variant 7: n=1	82	F	7.82	6.11
mean			**2.61 ± 2.62**	**1.41 ± 1.76**

[a] standard deviations can be taken from Table 8; [c] variant offered in tea bags; [c] variant offered as loose leaves-tea.

4.1.4.6 Storage of tea samples

To investigate the impact of storage on the contents of flavourings substances, one sample of loose leaves-teas was split; one part was stored in a paper bag, the other in a metal box up to 16 weeks. As shown in Figure 23, both storage conditions resulted in significant reductions in the contents of linalyl acetate, linalool and limonene. The losses were more pronounced upon storage in a paper bag (Figure 23A), with a reduction of the content of limonene up to 97% within 16 weeks.

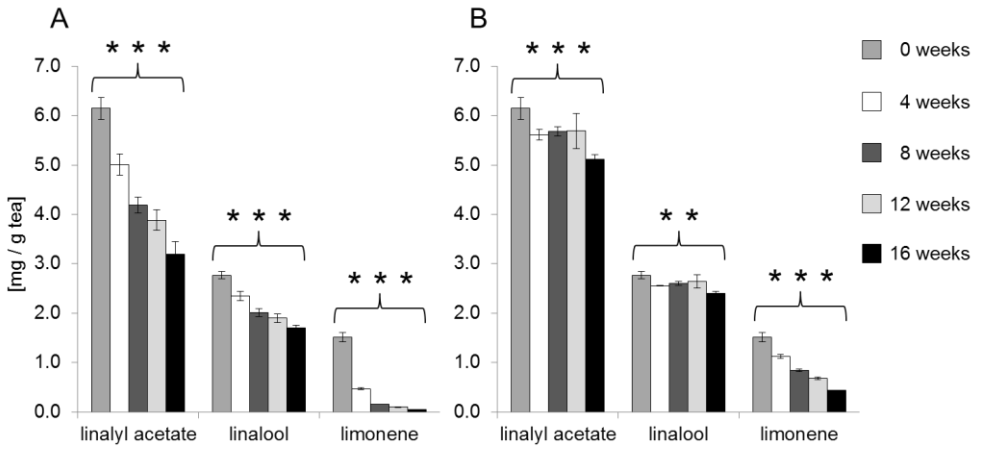


Figure 23. Contents of flavouring substances in an Earl Grey tea (loose leaves tea) stored (A) in a paper bag, and (B) in a metal box, respectively. Correlation analyses were carried out one-sided ('less') with Pearson method. Levels of significance: p=0-0.001: highly significant (***), p= 0.001-0.01: very significant (**), p=0.01-0.05: significant (*).

In a second experiment, Earl Grey teas in tea bags flavoured with encapsulated substances (Figure 24A) and teas flavoured without the use of granulates (Figure 24B) were stored. The contents of linalyl acetate, linalool and limonene in the tea flavoured with the encapsulated substances remained nearly constant, whereas in the tea bags without granulates the contents of the flavouring substances decreased significantly over time.

The differences between teas flavoured with encapsulated and non-encapsulated flavouring substances demonstrate the potential impact of the employed flavouring technology on the contents of flavouring substances actually contained in the product finally consumed. The data are in agreement with the observation that bergamot-flavoured teas stored over 3 months showed better flavour retention in cans than in laminated aluminium foil and metallised polyester, respectively (Dhanaraj et al. 1986).

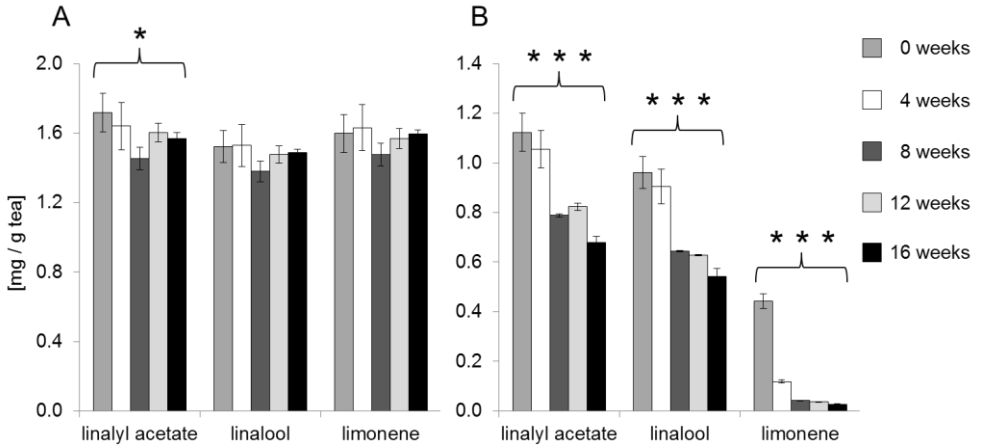


Figure 24. Impact of storage on the contents of flavouring substances in Earl Grey teas; (A): Earl Grey tea (in tea bags) flavoured with encapsulated flavouring substances; (B): Earl Grey tea (in tea bags) flavoured without the use of granulates (B). For levels of significance see Figure 23.

4.1.5 Conclusions

The analyses of Earl Grey teas purchased in 10 member states of the EU revealed that five flavouring substances were consistently present in all 90 samples. The major flavouring substances linalyl acetate, linalool and limonene were found in the mg/g range, the minor monoterpene hydrocarbons γ-terpinene and β-pinene occurred in the µg/g range. These five flavouring substances can be predicted to be consistently present (occurrence probability: 100%) in this specific flavoured food. The results support the use of 'flags' that can be assigned to food items as an additional descriptive element of their flavour so that food consumption databases can be used to assess dietary exposure to flavouring substances, as previously suggested (Crispim et al. 2010). Earl Grey teas are a perfect example of a food subcategory, for which this tool could be used to indicate the occurrence of specific flavouring substances on the basis of the naming of a product.

The contents of the five monoterpenes in the analysed Earl Grey teas were found in narrow ranges; only few extreme values were detected. The median ratio of contents of linalyl acetate to linalool in bergamot oil, which represents an important quality criterion, was 0.6; the value is in agreement with ratios reported in the literature. Only few samples showed higher contents of linalool than of linalyl acetate. This could be due to the use of extraction methods other than 'cold-pressing' or to the addition of single substances within the industrial method of tea material flavouring, which could originate in turn from other sources than bergamot oil.

This variability of commercially employed flavouring techniques is also reflected in the differences of the optical purities of chiral flavouring substances. One third of the samples (group I) showed enantiomeric ratios of at least 95% for (*R*)-linalool and linalyl acetate, which

indicate the use of cold pressed bergamot oil. In group II, linalool and linalyl acetate were present in nearly racemic proportions; this indicates the use of chemically defined substances by the tea manufacturers. For the rest of the samples (group III), various options such as the use of flavouring substances obtained from natural sources 'topped' with chemically synthesised substances or partial racemisations owing to processing conditions, as described for linalool (Kreis et al. 1993; Dugo et al. 2001), may account for the observed ratios of the enantiomers. However, the statistical analyses of these three groups did not show significant differences in the contents of the chiral volatiles (see 4.1.4.2).

Just as this examination, also other grouping comparisons did not show any statistically significant differences. This applies, for example, to the impact factors country of purchase (see 4.1.4.1) and the type of source (international/national brands, private label brands or tea shops, see 4.1.4.4). However, the contents of different variants of two brands illustrate the potential complexity of intake assessments. For one brand, the quantitative distributions of linalyl acetate and linalool were very consistent, whereas the contents of these flavouring substances in the second brand differed by factors up to 40 and 47, respectively. These individual data highlight the fact that despite the observed rather narrow overall frequency distributions observed for linalyl acetate and linalool in the analysed sample set, for consumers whose consumption behaviour is characterised by 'brand loyalty' or even 'variant loyalty' the high contents of these flavouring substances determined in some samples might be of relevance.

This may also be the case for consumers preferring Earl Grey teas offered as loose leaves-tea instead of teas in tea bags, since tea packaged as loose leaves-tea contained 67% more linalyl acetate and 39% more linalool than those offered in tea bags (see 4.1.4.3).

The results have to be put into perspective, when considering the observed reductions in the contents of flavouring substances upon storage (see 4.1.4.6). Whereas the amounts of linalyl acetate, linalool and limonene remaind nearly stable within 16 weeks in teas flavoured with granulates (encapsulated flavouring substances), the contents of these compounds in teas flavoured with non-encapsulated flavouring substances decreased rapidly. This also could affect the intake levels for consumers preferring teas manufactured with the second flavouring technology. On the other hand, only 20% (n=11) of all teas contained in tea bags (n=54) had been flavoured with non-encapsulated flavouring substances, so that the phenomenon of volatilisation and losses upon storage may be of less relevance for this product category. For loose leaves-tea, this observation could play a role since only three of 36 loose leaves samples contained encapsulated flavour. In turn, the higher starting amounts of flavouring substances in this tea category would have to be considered.

The elaborated dataset demonstrates that Earl Grey tea is an example for a flavoured food, for which the occurrence of five flavouring substances can be assumed with a probability of 100%, and for which a rather narrow distribution of the contents of linalyl acetate and linalool has been shown. The lack of significant differences depending on the factors country of purchase, source of the products and enantiomeric compositions demonstrates that the use of median contents of these flavouring substances as the basis for a dietary exposure assessment in the European Union would be connected with a low degree of uncertainty.

4.2 Transfer rates of flavouring substances from tea leaves into tea infusions

4.2.1 Introduction

For the calculation of intakes not only information on the contents of flavouring substances in the tea material but also data on their transfer into the final tea beverage via hot water infusion are required.

Several studys deal with the transfer of flavouring substances occurring naturally in tea leaves into the tea beverage. Schuh and Schieberle (2006), for example, determined 6.6 µg/g linalool in the leaves and 11.8 µg/g tea in the beverage obtained by an infusion of Darjeeling tea. For fennel tea extraction rates of 43% for linalool, 1% for β-myrcene, 16% for (*E*)-anethol, 26% for fenchone, and 2% for limonene have been described by Zeller and Rychlick (2006). These data are in good agreement with those reported by Fehr (1982), who determined transfer rates of fennel tea infusions of approximately 10% for anethol, 20% for fenchone, and 3% for limonene. Ramirez-Rodrigues et al. (2011) compared contents of flavouring substances in hot water infusions of dried hibiscus with those in cold infusions as well as those of hot and cold water infusions of fresh hibiscus; no absolute amounts have been reported.

To the author´s knowledge, no data exist on the transfer of flavouring substances *added* to tea into the final beverage. Therefore, the main flavouring substances found in Earl Grey teas were spiked onto tea bags containing non-flavoured black tea and brewed with boiling water. The suitability of the transfer rates determined in these model experiments to predict the concentrations of flavouring substances in tea beverages was evaluated. Factors potentially impacting the transfer rates, e.g. infusion time or amount of flavouring substance, were investigated.

4.2.2 Analysis of non-flavoured black teas

For the spiking experiments, a black tea should be selected containing no or only low amounts of the major volatile constituents of Earl Grey teas or bergamot oil. In particular, none of the main flavourings linalyl acetate and linalool should be present in levels affecting the determination of transfer rates.

In total, seven black teas were screened as potential candidates for the spiking experiments. In all samples, the content of linalyl acetate was below the limit of determination.

The contents of linalool in the teas, purchased either in tea bags or as loose leaves teas, and in the respective infusions are shown in Table 11.

Table 11. Contents of flavouring substances in black teas and the respective infusions (one tea bag or 2 g loose leaves-tea; 200 mL boiling water; 3 min).

tea	no. [a]	content of linalool [µg/g tea]	
		tea material	tea infusion
tea bag teas			
'Klassik'[b]	(no. 101)	1.85 ± 0.09	n.q
'Darjeeling'[c]	(no. 102)	9.42 ± 0.19	5.04 ± 0.40
'Ostfriesen'[b]	(no. 103)	0.64 ± 0.10	n.d
'pure Ceylon'[c]	(no. 104)	2.19 ± 0.06	0.98 ± 0.08
tea leaves-tea			
'Assam'[c]	(no. 105)	3.41 ± 0.18	2.13 ± 0.08
'Ceylon'[c]	(no. 106)	5.95 ± 0.37	1.97 ± 0.18
'Darjeeling'[c]	(no. 107)	24.05 ± 2.54	8.87 ± 0.16

[a] *numbers correspond to those in Table 37;* [b] *'Klassik', 'Ostfriesen': manufacturers´ labelling;* [c] *'Assam', 'Ceylon', 'Darjeeling': tea species as part of the labelling of the tea sample. Data represent means ± standard deviations from triplicate experiments*

The highest amounts of linalool were found in the two 'Darjeeling' teas; one was offered in tea bags (9.42 µg linalool/g tea) and one as loose leaves-tea (24.05 µg linalool/g tea). These results fit well to those reported by other working groups. Herrmann (1994) reported 18 µg linalool/g in Darjeeling tea compared to only 4 µg/g in Indian Broken tea. Fischer et al. (1987) reported an amount of 15.8 µg/g linalool as the quantitatively predominating, non-bound volatile in Ceylon tea. Alasalvar et al. (2012) found up to 0.133 µg/g linalool in seven grades of black tea.

Schuh and Schieberle (2006) reported linalool amounts of 11.8 µg/g tea in an infusion of Darjeeling tea (recalculated value, original data: 142 µg/L from 12 g tea or 28.4 µg/200 mL from 2.4 g tea). They found less linalool in the tea material than in the infusion (6.6 µg/g tea) and hypothesized that the increase may have been caused by degradation of precursors like glycosides (Schuh 2004). This phenomenon was not observed in the present study.

In the infusions prepared from the two Darjeeling teas available in this study the amounts of linalool were low (tea no. 102: 5.04 µg/g, tea no. 107: 8.87 µg/g tea) compared to the amount intended to be spiked (4 mg to one tea bag of 2 g). However, the other tea samples showed even lower contents of linalool in the prepared infusion.

Among the remaining candidates, tea no. 101 ('Klassik') and no. 103 ('Ostfriesen') showed the lowest quantities of linalool in the tea material; in the infusion the amonuts were below the limits of detection and quantification, respectively.

In conclusion, Darjeeling teas were not considered suitable for spiking experiments. Finally, tea no. 101 was selected for the spiking experiments owing to its better commercial availability.

4.2.3 Transfer of linalool and linalyl acetate

Tea bags containing non-flavoured black tea were spiked with linalool and linalyl acetate, respectively, and subjected to hot water infusion for 3 minutes. Considering the narrow ranges and the nearly normal distributions of contents observed in commercial Earl Grey teas (see 4.1.3), the median content of the major flavouring substance linalyl acetate was chosen as basis for spiking (4045 µg per tea bag of 2 g). Typical capillary gas chromatographic separations of the volatiles isolated from the resulting tea beverages are shown in Figure 25.

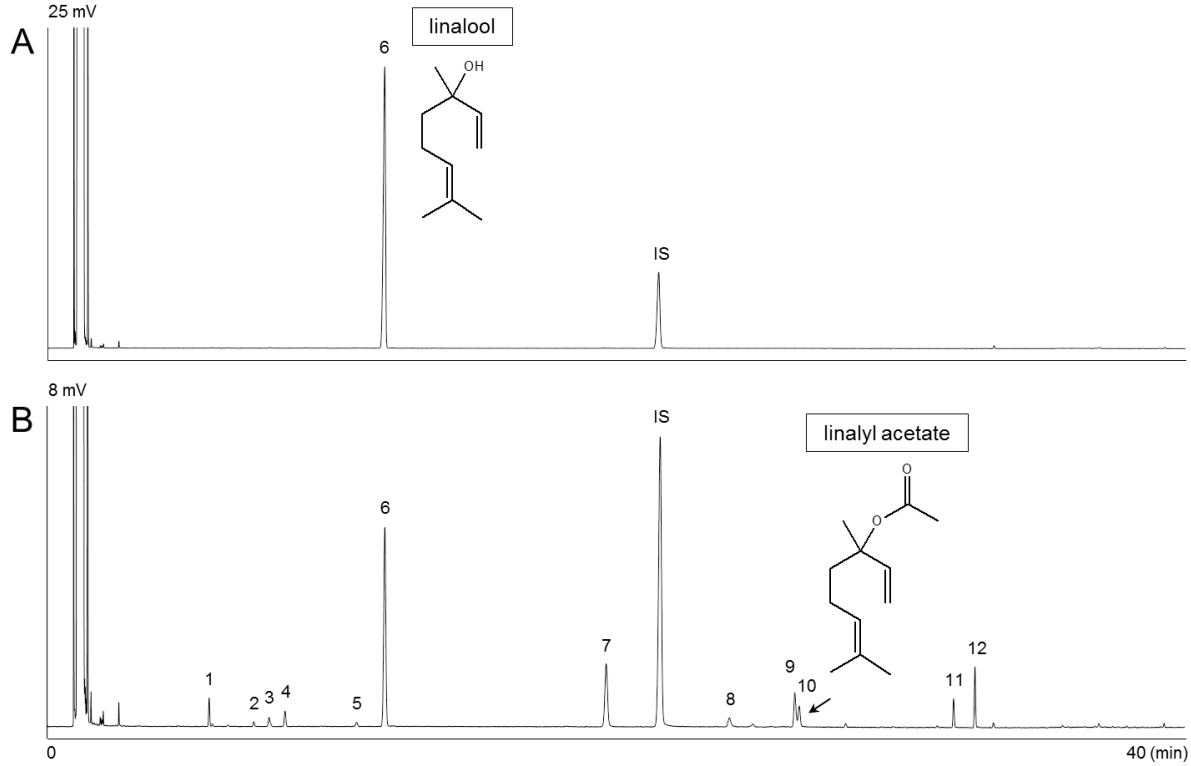


Figure 25. Capillary gas chromatographic separations on GC-system I (DB-5, method II) of volatiles isolated from a 200 mL hot infusion of a tea bag (non-flavoured black tea) spiked with (A) 4 mg linalool and (B) 4 mg linalyl acetate (preparation see 3.2.1.2); 1: myrcene, 2: limonene, 3: (Z)-β-ocimene, 4: (E)-β-ocimene, 5: terpinolene, 6: linalool, 7: α-terpineol, 8: nerol, 9: geraniol, 10: linalyl acetate, 11: neryl acetate, 12: geranyl acetate, IS: internal standard (2-decanol); GC system I (conditions see 3.2.4.1).

4.2.3.1 Transfer of linalool into the tea infusion

A total of 4 mg linalool was spiked onto a tea bag of non flavoured black tea. Triplicate analysis revealed that the average proportion of flavouring substance transferred into the tea beverage amounted to 66.4% ± 2.2% (tea no. 1 in Table 12). The chromatogram in Figure 25 A shows that no other reaction products were found under these experimental conditions.

The spiking experiment was repeated three times (each again in triplicate; see no. 2-4 in Table 12). The transfer rate of linalool was found to vary in a narrow range from 65.4 ± 4.5% to 67.3 ± 5.3%. These results demonstrate good repeatability and therefore the suitability of the developed experimental set-up.

Table 12. Transfer rates of linalool determined in 4 hot water infusions (200 mL) of tea bags (2 g) containing 4000 µg linalool.

	transfer rate into infusion		
no.[a]	linalool [%]		
1	66.4	±	2.2
2	67.3	±	5.3
3	67.2	±	1.4
4	65.4	±	4.5

[a] *number of infusion*

4.2.3.2 Transfer of linalyl acetate into the tea infusion

In contrast to the chromatogram obtained for the infusion prepared from tea spiked with linalool (Figure 25A), the corresponding chromatogram for the tea spiked with linalyl acetate (Figure 25B) revealed a number of additional peaks. They were identified via GC/MS as the monoterpene alcohols α-terpineol, geraniol, and nerol, the monoterpene esters geranyl and neryl acetate, and the monoterpene hydrocarbons myrcene, (*E*)-β-ocimene, (*Z*)-β-ocimene, terpinolene, and limonene (Figure 26).

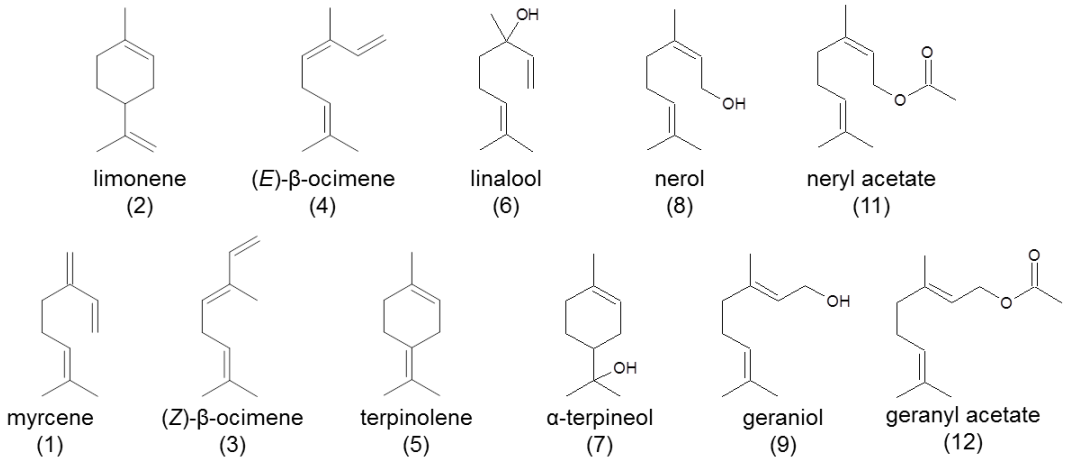


Figure 26. Reaction products identified in the hot water infusion of a tea bag spiked with linalyl acetate. The numbers correspond to those in Figure 25.

The contents of linalyl acetate and of the formed reaction products are listed in Table 13. On average, only 1.9% linalyl acetate was found in the final tea beverage. In turn, its hydrolysis product linalool was determined as main reaction product (17.0%), followed by α-terpineol (8.2%), geraniol (2.8%), and geranyl acetate (2.7%). Percentages were calculated as linalyl acetate equivalents using the molar masses of the substances.

Table 13. Distribution of products obtained by hot water infusion (200 mL) of a tea bag (2 g) containing 4045 µg linalyl acetate (median value obtained for 90 Earl Grey teas).

		content in tea		transfer rate into infusion
no.[a]		[µg]	linalyl acetate equivalent [µg]	linalyl acetate equivalent [%]
10	linalyl acetate	78 ± 16	78 ± 16	1.9 ± 0.4
	hydrolysis product			
6	linalool	541 ± 56	689 ± 71	17.0 ± 1.8
	other reaction products			
7	α-terpineol	260 ± 24	331 ± 30	8.2 ± 0.8
9	geraniol	90 ± 5	114 ± 7	2.8 ± 0.2
8	nerol	31 ± 4	39 ± 5	1.0 ± 0.1
12	geranyl acetate	108 ± 13	108 ± 13	2.7 ± 0.3
11	neryl acetate	58 ± 8	58 ± 8	1.4 ± 0.2
1	myrcene	42 ± 6	61 ± 8	1.5 ± 0.2
3	(*E*)-β-ocimene	24 ± 11	35 ± 16	0.9 ± 0.4
4	(*Z*)-β-ocimene	23 ± 3	33 ± 5	0.8 ± 0.1
5	terpinolene	11 ± 1	15 ± 2	0.4 ± 0.04
2	limonene	7 ± 1	11 ± 2	0.3 ± 0.05
	sum	**1273 ± 117**	**1572 ± 140**	**38.6 ± 3.4**

[a] *Peak numbers according to Figure 25. Data represent means ± standard deviations from triplicate experiments.*

The spiking experiment was repeated three times. As shown in Table 14, the low transfer rate of linalyl acetate, the amount of the hydrolysis product linalool and the sum of other reaction products were confirmed.

Table 14. Distribution of products obtained upon hot water infusions (200 mL) of tea bags (2 g) containing 4045 µg linalyl acetate (median value obtained for 90 Earl Grey teas).

	transfer rates [%]			
no.[a]	linalyl acetate	linalool	reaction products	sum
1	1.9 ± 0.4	17.0 ± 1.8	19.9 ± 1.5	38.9 ± 3.5
2	2.0 ± 0.03	16.5 ± 2.07	20.3 ± 2.6	38.7 ± 4.7
3	2.2 ± 0.3	18.6 ± 0.9	21.6 ± 1.1	42.4 ± 2.2
4	1.9 ± 0.3	18.7 ± 0.2	23.2 ± 0.3	43.8 ± 0.8

[a] *number of infusion*

In order to rule out that the observed reaction products might originate from the non-flavoured black tea used for the spiking experiment, their potential presence in an infusion of the employed basic black tea (‘Klassik’) was analysed. Table 15 shows that the reaction products found in the course of a hot water infusion with linalyl acetate did not occur in an infusion of ‘Klassik’ black tea; only terpinolene and linalool could be detected in negligible amounts below the limits of quantification. This result proves that all reaction products originate from the spiked linalyl acetate.

Table 15. Contents of flavouring substances in the tea material and the tea infusion of ‘Klassik’ black tea (one tea bag, 200 mL boiling water).

	substance	content [µg/g tea]	
		tea material	tea infusion
1	myrcene	n.q.[b]	n.d.
2	limonene	n.d.[c]	n.d.
3	(*E*)-β-ocimene	n.d.	n.d.
4	(*Z*)-β-ocimene	n.d.	n.d.
5	terpinolene	1.69 ± 0.08	n.q.
6	linalool	1.85 ± 0.09	n.q.
7	α-terpineol	3.01 ± 0.17	n.d.
8	nerol	n.d.	n.d.
9	geraniol	n.d.	n.d.
10	linalyl acetate	n.d.	n.d.
11	neryl acetate	n.d.	n.d.
12	geranyl acetate	n.d.	n.d.
	sum	**6.55 ± 0.26**	**-**

[a] *Peak numbers according to Figure 25.* [b] *n.q.: below limit of quantification (LOQ);* [c] *n.q.: below limit of determination (LOD), see 3.2.3.7 and 8.4. Data represent means ± standard deviations from triplicate experiments.*

4.2.3.3 Amounts of linalool and linalyl acetate remaining in the tea bag

In addition to the analysis of linalool and linalyl acetate, respectively, in the infusions, the proportions of the spiked substances remaining in the tea bags were determined.

For linalool, 13.6 ± 0.8% of the spiked material was found to remain in the tea bag; considering the median content determined in 90 Earl Grey teas (2266 µg), this corresponds to 308 ± 19 µg in a tea bag.

Table 16 lists the amounts of linalyl acetate and its hydrolysis/reaction products remaining in the tea material after a hot water infusion. In contrast to the very low contents of linalyl acetate in the tea infusion, 23% were determined in the tea bag. On the other hand, only low amounts of reaction products were found.

Table 16. Distribution of products determined in a squeezed tea bag after a hot water infusion (200 mL) of a tea bag (2 g) containing 4045 µg linalyl acetate (median value obtained for 90 Earl Grey teas).

		original content in tea bag		amount remaining in tea bag after infusion
no.[a]		[µg]	linalyl acetate equivalent [µg]	linalyl acetate equivalent [%]
10	linalyl acetate[b]	933 ± 94	933 ± 94	23.1 ± 2.3
	hydrolysis product			
6	linalool	58 ± 3	74 ± 3	1.8 ± 0.1
	other reaction products			
7	α-terpineol	34 ± 1	43 ± 2	1.1 ± 0.05
9	geraniol	6 ± 0.3	7 ± 0.3	0.2 ± 0.01
8	nerol	4 ± 0.1	39 ± 5	1.0 ± 0.1
12	geranyl acetate	25 ± 4	25 ± 4	0.6 ± 0.1
11	neryl acetate	13 ± 2	13 ± 2	0.3 ± 0.1
1	myrcene	9 ± 1.2	13 ± 2	0.3 ± 0.04
3	(*E*)-β-ocimene	7 ± 0.9	10 ± 1.4	0.2 ± 0.03
4	(*Z*)-β-ocimene	4 ± 0.5	6 ± 0.7	0.1 ± 0.02
5	terpinolene	0.6 ± 0.3	0.9 ± 0.4	0.02 ± 0.01
2	limonene	1.9 ± 0.3	3 ± 0.4	0.1 ± 0.01
	sum	**1094 ± 99**	**1132 ± 100**	**28.0 ± 2.5**

[a] *Peak numbers according to Figure 25. Data represent means ± standard deviations from triplicate experiments.*

Already Schmaus et al. (1985) did not find artefacts in distillates of linalool at pH-values 5 to 8. In contrast, distillation of linalool at pH 2.2 showed substances corresponding to those found in the hot water infusion of linalyl acetate. The acid-related instability of linalool and linalyl acetate has also been described by Baxter et al. (1978), who found α-terpineol and geraniol among others. They reported that after storage of linalyl acetate in 0.025 M citric acid only 0.5% was recovered. The distillation of bergamot oil leads to a degradation of linalyl acetate and a formation of linalool and α-terpineol (Kiwanuka et al. 2000). The distillations of clary sage and linalyl acetate, respectively (Schmaus and Kubeczka 1985), ended up in compounds similar to those observed upon hot water infusion of linalyl acetate in the present thesis. Similar results have been reported by Crabalona (1959), Morin and Richard (1985), Cori et al. (1986), and Clark et al. (1989).

4.2.3.4 Mass balances of the spiking experiments

A combination of the data on the amounts of the spiked flavouring substances linalool and linalyl acetate, respectively, transferred into the tea beverage and the proportions remaining in the tea bag after the infusion allowed the estimation of mass balances.

For linalool, a total of 80% was recovered (66% being transferred into the infusion and 14% remaining in the tea bag); the gap of 20% is expected to be due to evaporation during the hot water processing.

For linalyl acetate, the situation is more complex; a summary is depicted in Figure 27. Only 25% of the spiked material is recovered (2% being transferred into the infusion and 23% remaining in the tea bag). In addition, there is a substantial amount (19%) of linalool being released (17% being transferred into the infusion and 2% remaining in the tea bag) and a similarly high amount (23%) of other reaction products (20% being transferred into the infusion and 3% remaining in the tea bag). The total recoverage of 67% indicates a loss owing to evaporation of 33%.

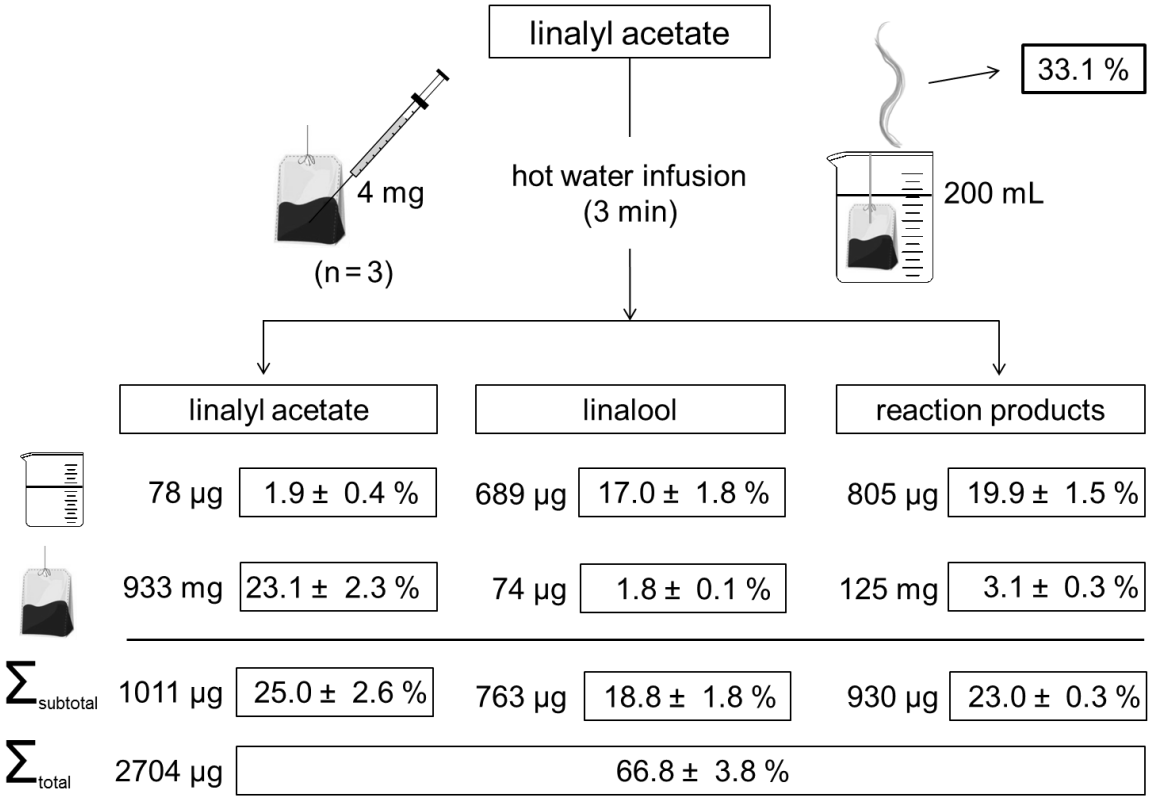


Figure 27. Amounts of linalyl acetate, its hydrolysis product linalool, and other reaction products determined in a hot water infusion (3 min, 200 mL) and in the residual tea material (squeezed tea bag after the infusion) of a tea bag spiked with 4.0448 mg linalyl acetate (median value in Earl Grey teas, n=90). The absolute amounts [µg] are calculated as equivalents of linalyl acetate.

4.2.3.5 Transfer of flavouring substances in samples of commercial Earl Grey teas

To confirm the transfer rates determined for linalyl acetate and linalool in experiments involving spiking of tea bags with the reference substances dissolved in 100 µL ethanol, commercially obtained Earl Grey teas were subjected to hot water infusions. Teas purchased in tea bags, which were either flavoured with encapsulated or non-encapsulated flavouring substances, were investigated. In addition, a loose leaves-tea was infused by using a one-way tea bag.

In order to strengthen the prediction of the final contents of flavouring substances in the tea infusions, transfer rates of other flavouring substances occurring in Earl Grey teas were additionally determined. For some substances, transfer rates of structurally related flavourings were adopted. An overview is given in Table 17.

Table 17. Tansfer rates of flavouring substances spiked onto tea bags containing non-flavoured black tea upon hot water infusion of 3 min.

substance	transfer rate [%]	substances for which the determined transfer rate was adopted
hydrocarbons		
limonene	26.4 ± 4.3	
γ-terpinene	25.7 ± 0.6	β-pinene myrcene (*Z*)-ocimene (*E*)-ocimene terpinolene
monoterpene alcohols		
α- terpineol	69.7 ± 3.6	
linalool	66.4 ± 2.2	nerol geraniol
monoterpene esters		
linalyl acetate	1.9 ± 0.4	
neryl acetate	33.0 ± 1.8	
geranyl acetate	38.0 ± 3.8	

The data expected from the model experiments and the results actually determined for the authentic tea materials are compared in Table 18 to 20.

The data shown in Table 18a and b demonstrate that the predicted contents in the final tea beverage for tea in tea bags with encapsulated flavouring substances (teas no. 91 and 92) were in good agreement with the experimental data.

Also the infusion of the tea in a tea bag without the use of granules (Table 19) showed good results, although experimentally determined amounts of especially minor compounds, like geranyl and neryl acetate but also of limonene differed more from the predicted values.

Whether this phenomenon is due to the missing granulates or to other reasons like the texture of the tea bag or the particle size of the used tea could not be answered.

On the other hand, the transfer rates determined for loose leaves tea did not reflect the actual values as shown in Table 20. The amount predicted for linalool was nearly twice as high as the experimentally determined amount; the predicted sum of the amounts of all other compounds was 3.5 times higher than the actually determined contents in the final tea beverage (predicted: 2097 ± 98%, actual: 591 ± 24%). These deviations might be explained, for example, by differences in surface and absorption capacity between the fine powdery particles packed in tea bags and the loose tea leaves.

In summary, for infusions made from teas in tea bags the contents of the major flavouring substances linalyl acetate and linalool in tea beverages predicted on the basis of the transfer rates determined in spiking experiments were in good agreement with the actual values and thus confirmed the suitability of this approach.

Table 18. Contents of flavouring substances in Earl Grey tea beverages: Predictions based on the transfer rates from spiking experiments vs. data actually determined for Earl Grey tea infusions.

		tea bag	tea beverage	
	substances	[µg / 2 g]	predicted[a,b] [µg / 200 mL]	actual [µg / 200 mL]
a)	*Tea no. 91: tea bags, flavoured with granulates*			
	linalyl acetate	4652 ± 129	90 ± 19	99 ± 12
	linalool	1941 ± 45	1911 ± 77	1549 ± 11
	other reaction products			
	limonene	3095 ± 79	827 ± 134	939 ± 14
	terpinolene	119 ± 3	43 ± 2	56 ± 1
	γ-terpinene	86 ± 4	22 ± 0.5	28 ± 2
	myrcene	70 ± 1	66 ± 7	63 ± 0.8
	β-pinene	38 ± 2	10 ± 0.2	11 ± 0.2
	(*E*)-β-ocimene	n.d.[d]	28 ± 12	36 ± 0.9
	(*Z*)-β-ocimene	n.d.	26 ± 4	21 ± 0.9
	α-terpineol	111 ± 10	377 ± 28	348 ± 3
	geraniol	n.d.	103 ± 6	100 ± 4
	nerol	n.d.	35 ± 4	40 ± 4
	geranyl acetate	n.d.	124 ± 15	109 ± 1
	neryl acetate	n.d.	67 ± 10	59 ± 0.9
	sum others[c]	3520 ± 80	2319 ± 198	1811 ± 7
	sum	10112 ± 253	3729 ± 160	3459 ± 11
b)	*Tea no. 92: tea bags, flavoured with granulates*			
	linalyl acetate	2402 ± 112	46 ± 10	36 ± 6
	linalool	2214 ± 88	1791 ± 60	1809 ± 40
	other reaction products			
	limonene	2398 ± 109	638 ± 104	920 ± 62
	terpinolene	14 ± 1	10 ± 0.7	14 ± 0.9
	γ-terpinene	436 ± 20	112 ± 3	171 ± 11
	myrcene	64 ± 3	41 ± 3	55 ± 3
	β-pinene	483 ± 23	124 ± 3	176 ± 11
	(*E*)-β-ocimene	n.d.	14 ± 6	25 ± 2
	(*Z*)-β-ocimene	n.q.[e]	13 ± 2	18 ± 1
	α-terpineol	169 ± 6	272 ± 15	331 ± 9
	geraniol	52 ± 2	88 ± 3	113 ± 1
	nerol	n.d.	18 ± 2	23 ± 0.5
	geranyl acetate	109 ± 6	105 ± 9	127 ± 7
	neryl acetate	35 ± 4	46 ± 5	54 ± 3
	sum others[c]	3758 ± 171	1484 ± 106	2026 ± 105
	sum	8374 ± 371	3321 ± 122	3870 ± 147

[a] calculated on the basis of reaction rates for linalyl acetate (Table 13) in combination with transfer rates determined in spiking experiments with linalyl acetate (1.9%); linalool (66.4%), also applied to nerol, geraniol; α-terpineol (69.7%); geranyl acetate (38.0%); neryl acetate (33.0%); limonene (26.4%); γ-terpinene (25.7%), also applied to the other hydrocarbons; [b] standard deviations of predicted values were calculated using the Gaussian propagation of uncertainty ($\sigma_{total} = ((\sigma_{1})^2 + (\sigma_{2})^2 + \ldots. (\sigma_{n})^2))^{1/2}$; [c] 'sum others' encompasses the compounds listed as 'other reaction products'; [d] below limit of determination (LOD); [e] below limit of quantification (LOQ), see 3.2.3.7 and 8.4. Data represent means ± standard deviations from triplicate experiments.

Table 19. Contents of flavouring substances in Earl Grey tea beverages: Predictions based on the transfer rates from spiking experiments vs. data actually determined for Earl Grey tea infusions.

substances	tea bag [µg / 2 g]	tea beverage predicted[a,b] [µg / 200 mL]	tea beverage actual [µg / 200 mL]
Tea no. 93: tea bags, flavoured without granulates			
linalyl acetate	1742 ± 21	34 ± 7	25 ± 2
linalool	1394 ± 14	1159 ± 39	876 ± 23
other reaction products			
limonene	506 ± 7	137 ± 22	22 ± 0.2
terpinolene	23 ± 1	11 ± 0.5	14 ± 1
γ-terpinene	n.d.[d]	-	n.d.
myrcene	6 ± 0.2	20 ± 2	6 ± 0.2
β-pinene	2 ± 0.2	0.4 ± 0.01	n.d.
(*E*)-β-ocimene	n.q.[e]	10 ± 5	n.d.
(*Z*)-β-ocimene	n.d.	10 ± 1	n.d.
α-terpineol	1394 ± 14	241 ± 12	206 ± 15
geraniol	28 ± 2	57 ± 2	37 ± 3
nerol	5 ± 0.5	17 ± 2	8 ± 1
geranyl acetate	15 ± 0.3	52 ± 6	18 ± 1
neryl acetate	4 ± 0.2	26 ± 4	8 ± 0.4
sum others[c]	774 ± 9	581 ± 27	319 ± 17
sum	3910 ± 43	1773 ± 48	1219 ± 40

[a] *calculated on the basis of reaction rates for linalyl acetate (Table 13) in combination with transfer rates determined in spiking experiments with linalyl acetate (1.9%); linalool (66.4%), also applied to nerol, geraniol; α-terpineol (69.7%); geranyl acetate (38.0%); neryl acetate (33.0%); limonene (26.4%); γ-terpinene (25.7%), also applied to the other hydrocarbons;* [b] *standard deviations of predicted values were calculated using the Gaussian propagation of uncertainty* ($\sigma_{total} = ((\sigma_{1})^2 + (\sigma_{2})^2 + \ldots. (\sigma_{n})^2))^{1/2}$; [c] *'sum others' encompasses the compounds listed as 'other reaction products';* [d] *below limit of determination (LOD);* [e] *below limit of quantification (LOQ), see 3.2.3.7 and 8.4. Data represent means ± standard deviations from triplicate experiments.*

Table 20. Contents of flavouring substances in Earl Grey tea beverages: Predictions based on the transfer rates from spiking experiments vs. data actually determined for Earl Grey tea infusions.

substances	tea bag [µg / 2 g]	tea beverage predicted[a,b] [µg / 200 mL]	tea beverage actual [µg / 200 mL]
Tea no. 94: loose tea leaves, flavoured without granulates[d]			
linalyl acetate	8087 ± 106	156 ± 32	99 ± 13
linalool	3982 ± 51	3726 ± 143	2057 ± 118
other reaction products			
limonene	1548 ± 22	424 ± 67	41 ± 6
terpinolene	17 ± 3	26 ± 2	8 ± 0.3
γ-terpinene	159 ± 3	41 ± 1	4 ± 0.5
myrcene	36 ± 2	93 ± 12	19 ± 2
β-pinene	312 ± 9	80 ± 2	7 ± 1
(*E*)-β-ocimene	n.d.[e]	49 ± 22	14 ± 2
(*Z*)-β-ocimene	n.q.[f]	45 ± 6	8 ± 1
α-terpineol	14 ± 3	530 ± 48	168 ± 14
geraniol	n.d.	179 ± 11	66 ± 10
nerol	65 ± 4	105 ± 8	58 ± 6
geranyl acetate	8 ± 0.8	219 ± 27	57 ± 6
neryl acetate	103 ± 2	150 ± 17	42 ± 4
sum others[c]	2263 ± 37	1942 ± 43	492 ± 53
sum	14332 ± 191	5824 ± 173	2648 ± 155

[a] *calculated on the basis of reaction rates for linalyl acetate (Table 13) in combination with transfer rates determined in spiking experiments with linalyl acetate (1.9%); linalool (66.4%), also applied to nerol, geraniol; α-terpineol (69.7%); geranyl acetate (38.0%); neryl acetate (33.0%); limonene (26.4%); γ-terpinene (25.7%), also applied to the other hydrocarbons;* [b] *standard deviations of predicted values were calculated using the Gaussian propagation of uncertainty* ($\sigma_{total} = ((\sigma_{1})^2 + (\sigma_{2})^2 + \ldots. (\sigma_{n})^2))^{1/2}$. [c] *'sum others' encompasses the compounds listed as 'other reaction products'.* [d] *tea leaves were weighted into a one-way tea bag for the tea infusion process;* [e] *below limit of determination (LOD);* [f] *below limit of quantification (LOQ), see 3.2.3.7 and 8.4. Data represent means ± standard deviations from triplicate experiments.*

4.2.4 Impact factors on transfer rates of linalool and linalyl acetate

4.2.4.1 Infusion time

The previously described studies on transfer rates of added flavouring substances into Earl Grey tea beverages had been based on the generally recommended infusion time of 3 minutes. In order to take into account potential differences in consumers' behaviour, infusion times ranging from 0.5 to 10 minutes were tested.

The contents of linalool in the final tea beverages obtained from these infusions are shown in Figure 28. The statistical analysis (two-sided *Pearson Test*) showed that the amount of linalool transferred into the tea beverage was not time-dependent (p=0.2789).

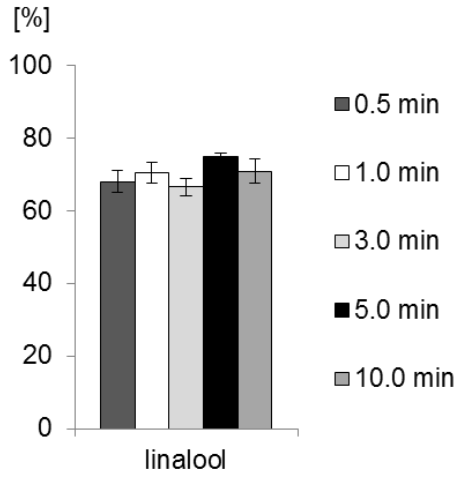


Figure 28. Impact of infusion time on the content of linalool in a tea beverage obtained upon hot water infusion (200 mL) of black tea spiked with 4 mg linalool.

Spiking experiments based on different infusion times for linalyl acetate are depicted in Figure 29. After 0.5 minutes of infusion, the amount of linalyl acetate in the tea beverage was more than 3-fold higher (6.5 ± 1.5%) compared to the standard infusion time (3 min.). Considering the range of infusion times from 0.5 to 10 minutes, the statistical analysis (one-sided Pearson test) showed that the amount of linalyl acetate present in the tea beverage was significantly dependent on time (p=0.01265), i.e. the amounts of linalyl acetate decreased with increasing brewing time. Interestingly, the amounts of transferred linalyl acetate strongly declined from 0.5 to 3 min of infusion time (p=0.00000937); a prolongation of the infusion from 3 to 10 min resulted again in an increase (p=0.002908).

Significant increases of the amounts present in the tea beverage depending on the infusion time were also observed for the released linalool, for α-terpineol and for the other reaction products.

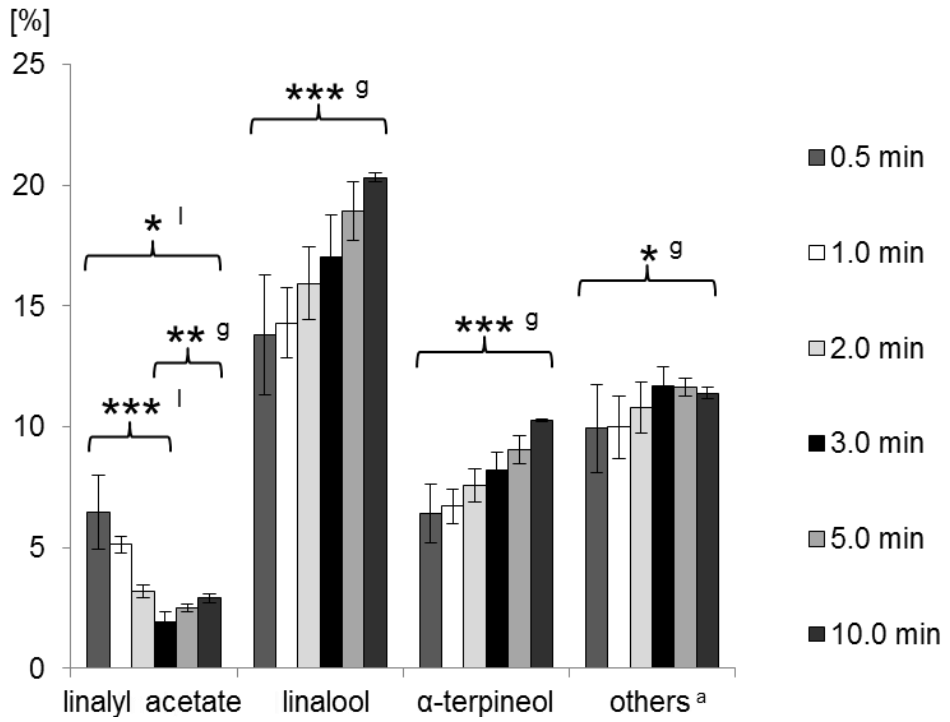


Figure 29. Impact of infusion time on the distribution of flavouring substances in a tea beverage obtained upon hot water infusion (200 mL) of black tea spiked with 4045 µg linalyl acetate; [a] 'others' encompasses the compounds listed as 'reaction products' in Table 13 except α-terpineol. The percentages were calculated using linalyl acetate equivalents. Correlation analyses were carried out one-sided (l: 'less' or g: 'greater') with Pearson method. Levels of significance: p=0-0.001: highly significant (***), p= 0.001-0.01: very significant (**), p=0.01-0.05: significant (*).

The phenomenon of the inverting curve progression might be explained by the fact that on the one hand linalyl acetate diffused consistently from the tea bag into the tea infusion, but was partly converted into linalool or other reaction products. On the other hand, due to lower temperatures over time, the diffusion rate exceeded the converting rate resulting in an increase of the linalyl acetate amounts starting from 3 min of infusion.

The hypothesis that linalyl acetate diffuses consistently from the tea bag into the final beverage is supported by decreasing amounts in the residual tea bag, as shown in Figure 30. The bar chart also shows increasing amounts of linalool, α-terpineol, and other reaction products, but the values did not reach more than 2.9%, 1.3% and 4.9% at 10 minutes, respectively, whereas the starting amount (0.5 min) of linalyl acetate was 34.5%. Linalyl acetate seems to be stabilized by the tea material in contrast to the infusions.

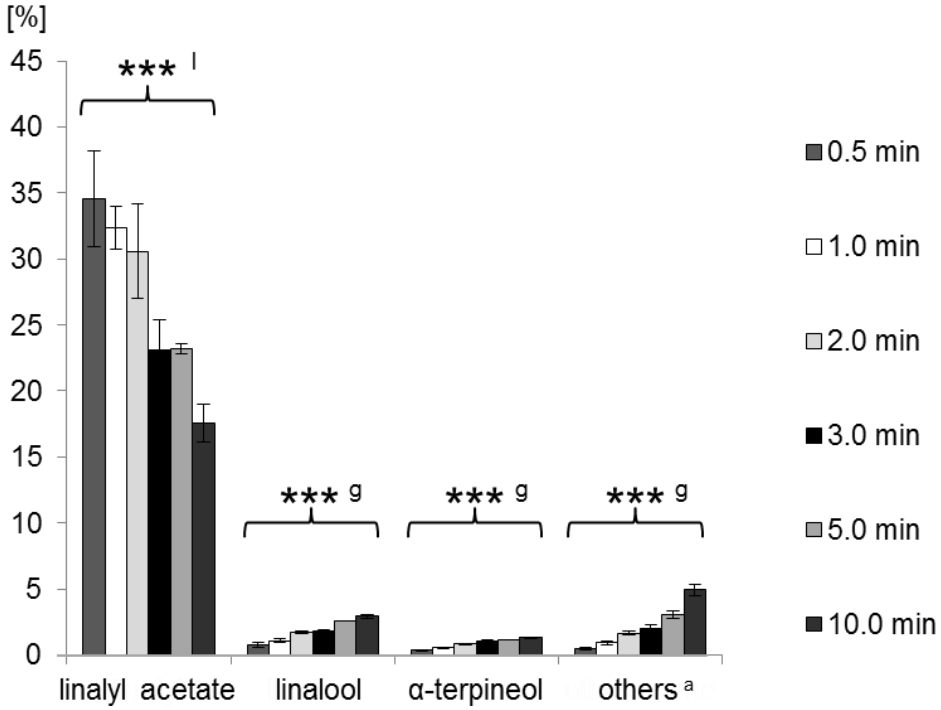


Figure 30. Impact of infusion time on the distribution of flavouring substances remaining in the tea bag obtained after a hot water infusion (200 mL) of black tea spiked with 4045 µg linalyl acetate; [a] 'others' encompasses the compounds listed as 'reaction products' in Table 13 except α-terpineol. The percentages were calculated using linalyl acetate equivalents. Correlation analyses were carried out one-sided (l: 'less' or g: greater) with Pearson method. For levels of significance see Figure 29.

The statistical assessments revealed highly significant dependencies of the amounts of flavouring substances remaining in the tea bag on the duration of the infusion (Figure 30).

The mass balances of all substances in the tea infusion and in the tea bag show that approximately 70% (calculated as linalyl acetate equivalents) were found after each of the employed infusion times (0.5 min: 72.7 ± 2.4%, 1.0 min: 70.9 ± 4.9%, 2.0 min: 72.1 ± 1.9%, 3.0 min: 66.8 ± 3.8%, 5.0 min: 72.1 ± 2.5%, 10.0 min: 71.6 ± 1.4%). This indicates that after an initial loss of flavouring substances due to volatilisation, mainly isomerisation reactions occur.

All individual values and standard deviations of the time experiments are listed in the Appendix (Table 44 and 45).

4.2.4.2 Amount of added flavouring substance

In the previously described studies on transfer rates of added flavouring substances into Earl Grey tea beverages 4045 µg of the flavouring substance had been spiked to black tea; this amount corresponds to the median content of linalyl acetate determined in the investigated commercial Earl Grey teas. In order to reveal a potential impact of the amount of flavouring substance added to the tea material on the transfer rate into the tea beverage upon hot water treatment, the contents of linalool and linalyl acetate in the tea beverage starting from lower and higher amounts, respectively, spiked onto the tea bag were investigated.

The highest contents determined in the investigated 90 Earl Grey teas amounted to 9365 µg linalool/g tea (corresponding to 18730 µg/tea bag of 2 g) and 10691 µg linalyl acetate/g tea (21396 µg/tea bag). The lowest contents of these substances amounted to 61 µg linalool/g tea (122 µg/tea bag) and 97 µg linalyl acetate/g tea (195 µg/tea bag). Taking into account the transfer rate of 2% determined for linalyl acetate, a minimum amount of 400 µg linalyl acetate/tea bag is required to achieve concentrations of the flavouring substance in the final tea beverage above the limit of quantification. An amount of 100 µg linalyl acetate/tea bag is sufficient to generate amounts of the hydrolysis product linalool exceeding the limit of quantification for this substance. Therefore, 100 and 20000 µg linalool and 100, 400 and 20000 µg linalyl acetate, respectively, were spiked onto tea bags containing non-flavoured black tea. The transfer rates determined for linalool and linalyl acetate are summarised in Table 21; additional data (reaction products of linalyl acetate) are given in the Appendix (8.7).

Table 21. Distribution of products in the final tea beverage obtained by hot water infusions (200 mL) of tea bags (2 g) containing different starting amounts of linalool and linalyl acetate, respectively. Percentages were calculated on the basis of linalyl acetate equivalents. Other reaction products formed from linalyl acetate are given in the Appendix (8.7). Data represent means ± standard deviations from triplicate experiments.

	spiked amounts			
	low		median	high
	100 µg	400 µg	4045 µg	20000 µg
substance	transfer rate [%]			
linalool				
linalool	66.9 ± 5.5	-	66.4 ± 2.2	63.8 ± 6.2
linalyl acetate				
linalyl acetate	n.q.[a]	1.5 ± 0.05	1.9 ± 0.4	2.6 ± 0.4
linalool	15.6 ± 0.8	16.6 ± 1.3	17.0 ± 1.8	9.6 ± 1.2

[a] *n.q.: below limit of quantification (LOQ), see 3.2.3.7 and 8.4.*

The data shown in Table 21 demonstrate that the starting amount of linalool did not have an influence on the transfer rate into the final tea beverage.

For linalyl acetate, slightly lower and higher transfer rates, respectively, compared to the median amount have been determined in the tea infusions starting from 400 µg and 20000 µg of spiked flavouring substance. The content of linalool released from linalyl acetate was significantly lower after spiking of the maximum amount of linalyl acetate compared to the experiments with the minimum or median amounts. On the other hand, approximately twice of the amount of linalyl acetate remained after the infusion in the tea bag spiked with 20000 µg compared to those spiked with 100 or 400 µg (Table 22).

Table 22. Distribution of products in the residual tea bag obtained by hot water infusions (200 mL) of tea bags (2 g) containing different starting amounts of linalool and linalyl acetate, respectively. Percentages were calculated on the basis of linalyl acetate equivalents. Other reaction products formed from linalyl acetate are given in the Appendix (8.7). Data represent means ± standard deviations from triplicate experiments.

	spiking amounts			
	low		median	high
	100 µg	400 µg	4045 µg	20000 µg
substance	amount remaining in tea bag after infusion [%]			
linalool				
linalool	17.2 ± 4.7	-	13.6 ± 0.8	24.8 ± 4.7
linalyl acetate				
linalyl acetate	20.8 ± 1.8	27.3 ± 1.3	23.1 ± 2.3	51.9 ± 5.5
linalool	2.5 ± 0.03	2.4 ± 0.2	1.8 ± 0.1	1.5 ± 0.1

A possible explanation might be the limited solubility in the infusion hampering the transfer of the remaining linalyl acetate from the tea bag into the tea infusion. In addition, less linalool is generated; in comparison to median and minimum values spiked onto the non-flavoured tea bag (15.6-17.0%), only 9.6% was found at the maximum spiking level. This was confirmed by an additional experiment with 40000 µg linalyl acetate, where only 6.2% linalool was found in the infusion, but 69.4% linalyl acetate in the residual tea bag.

4.2.5 Studies on the potential reaction mechanism upon hot water infusions of linalool and linalyl acetate

4.2.5.1 Transfer of enantiomerically pure linalool and linalyl acetate

Considering the high excess of the (*R*)-enantiomers of linalool and linalyl acetate (> 97%) in genuine, cold pressed bergamot oil (Cotroneo et al. 1992; Juchelka and Mosandl 1996; Mosandl and Juchelka 1997; Casabianca and Chau 1998; Mondello et al. 1998; Dugo et al. 2001), the impact of the hot water infusion on their enantiomeric compositions should be investigated. The results obtained after infusions of a tea bag spiked with linalool and linalyl acetate exhibiting enantiomeric purities comparable to those present in bergamot oil are shown in Table 23. In addition to the infusion with the recommended brewing time of 3 minutes, the tea bag was removed also after 0.5, 1, 2, 5, and 10 minutes.

The enantiomeric composition of linalool (99.3% (*R*)) remained unchanged in the tea beverage after the hot water treatment for 3 minutes. The distribution of linalyl acetate also only differed slightly from the starting ratio. However, linalool formed as hydrolysis product was already partially racemised after 0.5 minutes of infusion

The enantiomeric composition of linalool from (*R*)-linalyl acetate did not vary much between 0.5 and 10 minutes infusion time. This can be explained by the fact that the degradation of the compound happened very quickly (see time-dependent experiments: 4.2.4.1).

Table 23. Impact of hot water infusions on the enantiomeric compositions of linalool and linalyl acetate.

hot water infusion linalool			hot water infusion linalyl acetate					
infusion time [min]	linalool [%]		linalyl acetate [%]		linalool [%]		α-terpineol [%]	
	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)
0[a]	99.3	0.7	98.3	1.7				
0.5					67.0	33.0	72.5	27.5
1					66.6	33.4	73.5	26.5
3	99.3	0.7	97.0[b]	3.0[b]	66.2	33.8	73.7	26.3
5					64.6	35.4	72.6	27.4
10					65.8	34.2	72.5	27.5

[a] values determined for non-treated reference solutions; [b] values determined in extracts obtained by liquid-liquid extractions of 200 mL tea infusions (see 3.2.1.2b)).

The enantiomeric ratios observed for the hydrolysis product linalool indicate two competing reaction pathways. The first involves the formation of a planar cation with two equally

probable possibilities of the attack of a hydroxyl group resulting in 50% (*R*)- and 50% (*S*)-enantiomer of linalool (Figure 31A). Hydrolysis with retention of the (*R*)-configuration represents the second reaction (Figure 31B).

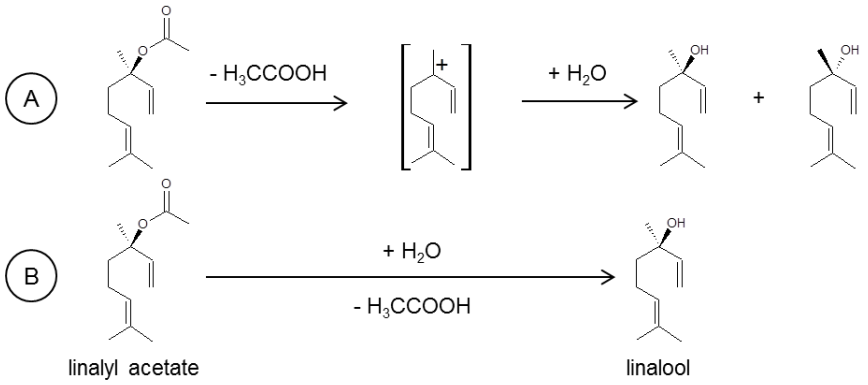


Figure 31. Competing reaction pathways resulting in the generation of linalool from enantiomeric pure (R)-linalyl acetate; (A) racemic linalool via planar cation as transition state; (B) configuration stability via one step hydrolysis.

Racemisation effects of linalool have already been reported by Hener et al. (1992), Kreis et al. (1993), Baigrie et al. (1996), Mosandl and Juchelka (1997), or Fritsch (2001).

α-Terpineol, the second most concentrated reaction product formed during a hot water infusion of linalyl acetate, was also partially racemised. After the recommended infusion time, it was found with a proportion of 73.7% of the (*R*)-enantiomer.

Additionally, α-terpineol was formed with a rather consistent enantiomeric ratio of 73% (*R*) : 27% (*S*). It is well known that the formation of α-terpineol formed from (*R*)-linalool preferably results in the (*R*)-enantiomer due to an S_N2'-reaction of the allylic system (Rittersdorf and Cramer 1968; Godtfredsen et al. 1977) and a cyclisation following the rule of anti-periplanar hydrogen atoms (Seebach and Prelog 1982), respectively. Reaction schemes are shown in Figure 32 and 31. The competitive non-stereoselective reaction could be explained by the formation of a planar α-terpinyl cation (see structure II in Figure 36 and Schmaus and Kubeczka 1985).

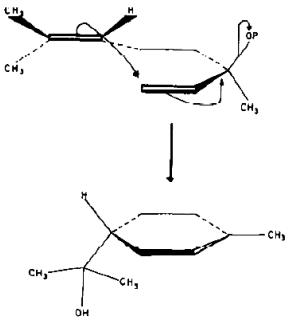


Figure 32. Stereochemical course (S_N2'-reaction in an allylic system) of (R)-linaloyl phosphate to give (R)-α-terpineol (from Rittersdorf and Cramer 1968).

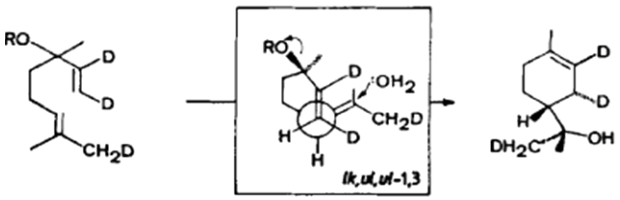


Figure 33. Stereochemical course of the reactions of (R)-linalool to give (R)-α-terpineol following the rule of 'antiperiplanar hydrogen atoms' with respect to both double bonds (from Seebach and Prelog 1982).

4.2.5.2 Stability of linalyl acetate in water and tea infusions

For a better understanding of the reaction pathway underlying the transformation of linalyl acetate, an attempt was made to slow down the reaction by using cold instead of hot water. A stock solution of 0.04 mg/mL linalyl acetate in water was prepared, stored at room temperature (RT, 20°C) and analysed by GC-FID after 0, 15, 30, 45, 60, 120, 180, 240, 300, and 420 minutes as well as after 24, 48, and 144 hours. The starting concentration reflected twice the concentration of an infusion with 4 mg linalyl acetate in case of a transfer of 100%. This amount was chosen to enable a longer quantification, especially of linalyl acetate, which is degraded and partially transferred into the final tea beverage until a leftover of only 1.9%. Additionally, this experiment was repeated with a cold tea infusion of non-flavoured black tea as matrix. An overview on the degradation of the starting substance and the subsequent formation of the main reaction products linalool and α-terpineol is shown in Figure 34.

The amounts of linalyl acetate and its reaction products in water and tea infusion were comparable. This means that the tea infusion matrix has no or only a marginal influence on the stability of linalyl acetate. The standard deviations for triplicate experiments were very small for both matrices; hence, the error bars were omitted in the figures in support of a better clarity. All individual values and standard deviations for water and tea infusion can be found in the Appendix (Table 42 and 46).

The bar chart of Figure 34 shows a rather fast hydrolysis of linalyl acetate even in cold water. The amount of linalool increased from 0.1 to 0.6% just after 15 minutes and to 10.8% after 5 hours. The corresponding values of linalyl acetate were 99.0 and 79.1%, respectively. 0.3% of α-terpineol was formed after 15 minutes; 5.9% after 5 hours. At the end of the study (144 h), only 1.3% of linalyl acetate was left, but the amounts of linalool and α-terpineol increased to 51.3 and 28.4%, respectively.

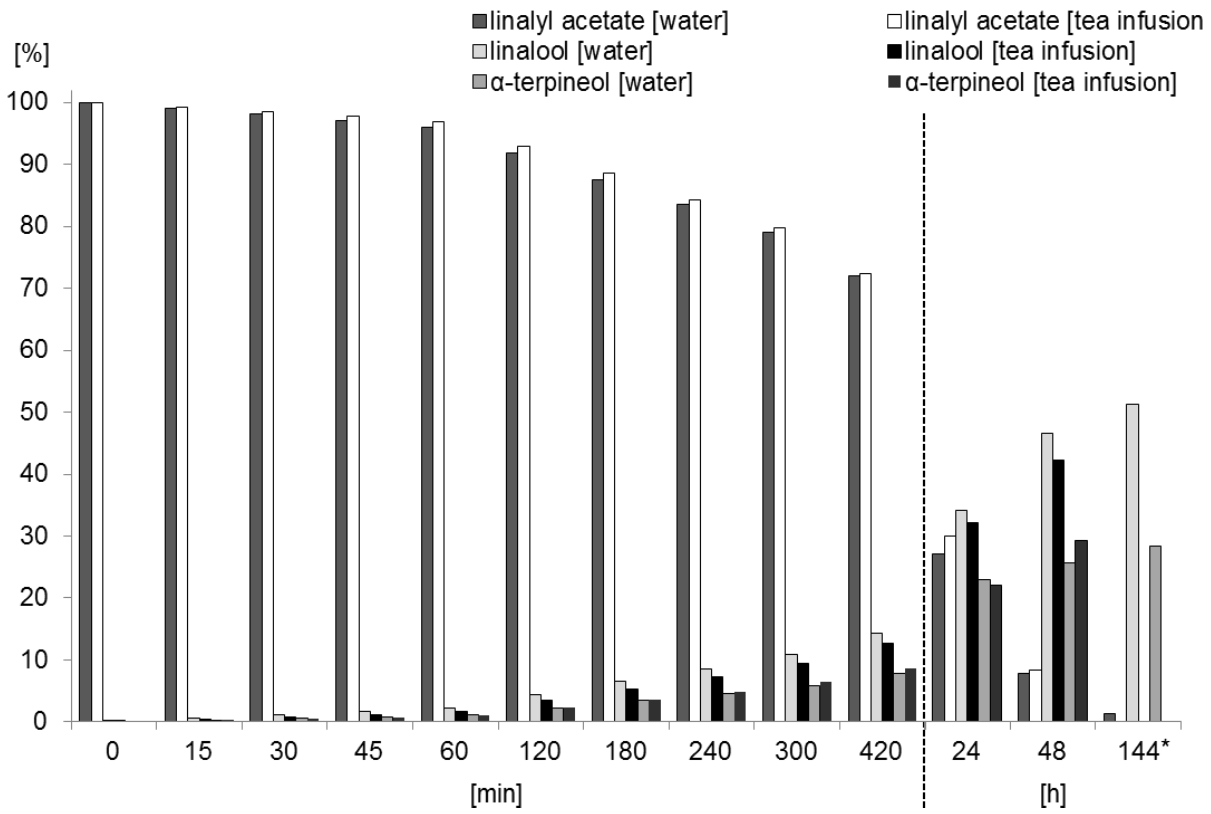


*Figure 34. Degradation of linalyl acetate (starting concentration: 0.04 mg/mL) to linalool and α-terpineol in water and tea infusion stored at RT (20°C) for 48 h and 144 h, respectively. The percentages were calculated with the sum of all measured substances (as linalyl acetate equivalents) set to 100%. Standard deviations determined in triplicate experiments were smaller than 0.4% and not pictured. * no values were collected in the tea infusion for 144 h due to a fungi infestation of the solution.*

Figure 35A and B shows the formation of minor degradation produts from a solution of linalyl acetate in water. Monoterpene alcohols and esters are depicted in Figure 35A. Although the highest amounts at 144 h were found for geraniol (9.0%), geranyl acetate was formed initially just after 15 minutes (0.1%), followed by neryl acetate after 30 minutes (0.1%). Geraniol and nerol were found the first time after 180 minutes (0.20 and 0.18%, respectively). From that point of time until 420 minutes, the proportions of these four substances remained nearly stable. At 24 h, geraniol exceeded the amount of neryl acetate for the first time, increased constantly and represented the highest amount of these four substances at the end of the study. Nerol increased also steadily until 144 h in contrast to geranyl and neryl acetate, which showed a lower amount at 144 h than at 48 h.

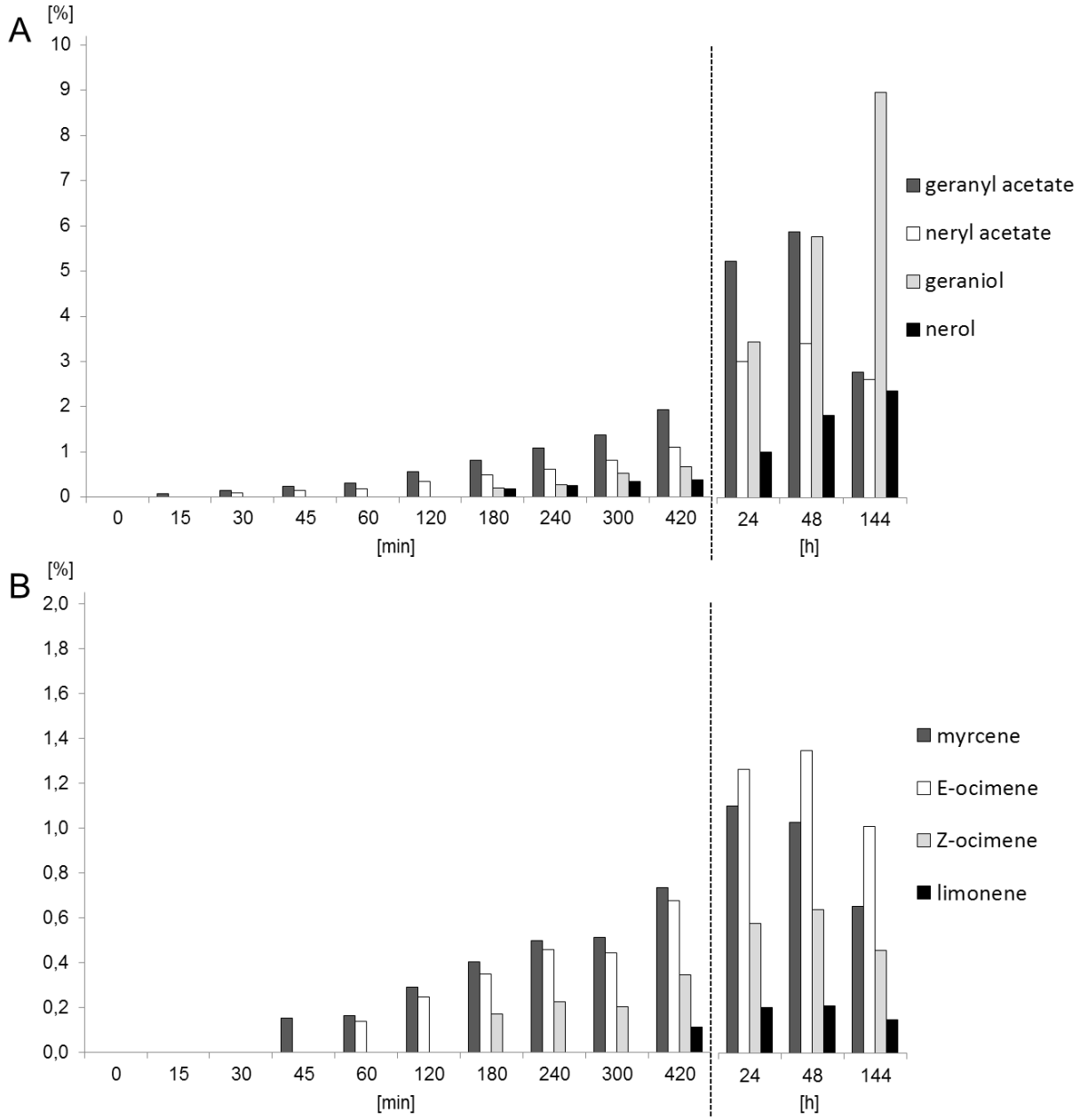


Figure 35. Minor degradation products (A: monoterpene alcohols and esters; B: hydrocarbons) of linalyl acetate (starting concentration: 0.04 mg/mL) in water at ambient RT (20°C) in the course of 144 h. The percentages were calculated with the sum of all measured substances (as linalyl acetate equivalents) set to 100%. Standard deviations determined in triplicate experiments were lower than 0.5% (A) and 0.1% (B) and are not depictured.

Figure 35B illustrates the formation of the hydrocarbons myrcene, (*E*)-ocimene, (*Z*)-ocimene, and limonene. The first substance found was myrcene (45 min, 0.2%), followed by (*E*)-ocimene (60 min, 0.1%), (*Z*)-ocimene (180 min, 0.2%) and limonene (420 min, 0.1%). Interestingly, the amounts of all four substances increased until 24 or 48 h, but decreased thereafter. The proportions remained stable, except for (*E*)-ocimene which exceeded all other

amounts of this group from 24 h on. At the end of the experiment, 0.7% myrcene, 1.0% (*E*)-ocimene, 0.5% (*Z*)-ocimene and 0.2% limonene were found. Terpinene, which was found after a hot water infusion of tea material spiked with linalyl acetate, could not be detected.

The formation of geranyl and neryl acetate is discussed more in detail, as the observed results are not in agreement with known reaction schemes. Because of the fact that both substances were determined before the appearance of their alcohols geraniol and nerol, the reaction scheme via a geranyl and neryl cation, shown in Figure 36, is not probable (Stevens et al. 1972; Schmaus and Kubeczka 1985). The required concentration of acetate (segregated from the initial linalyl acetate), which is needed to form quantifiable amounts of geranyl and neryl acetate, was also not present.

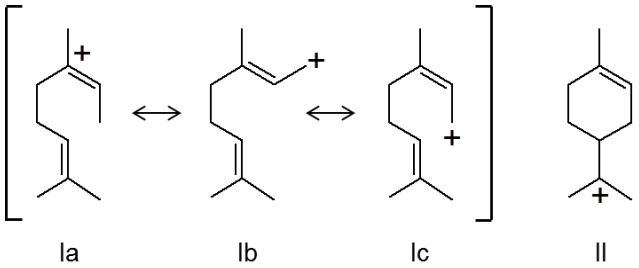


Figure 36. Resonance-stabilised conformers of the intermediate cation: Ia linalyl-, Ib geranyl-, and Ic neryl cation. Cyclization results in the formation of II α-terpinyl cation (after Stevens et al. (1972)).

Therefore, a direct formation from linalyl acetate via an intramolecular isomerisation step is more probable (Figure 37). The proposed reaction pathway could be compared to a typical Claisen rearrangement with an oxygen atom instead of the terminal carbon atom (Figure 38).

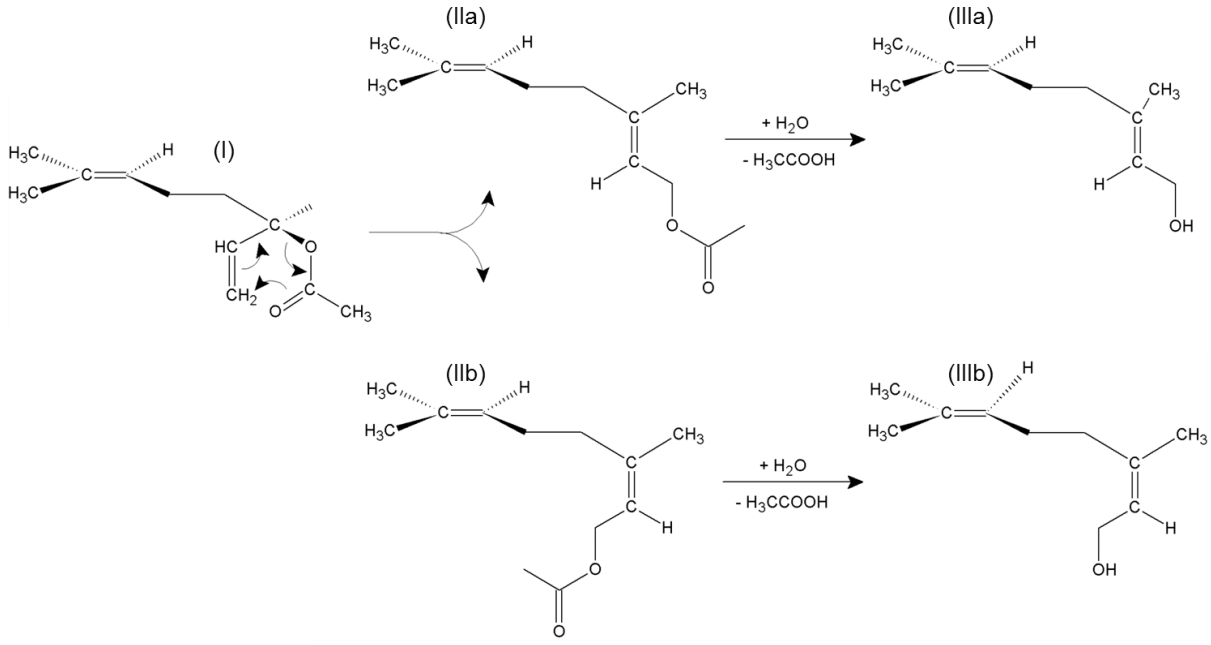


Figure 37. Suggested reaction scheme of isomerisation of (I) linalyl acetate into (IIa) geranyl acetate and (IIb) neryl acetate via intramolecular allylic rearrangement with subsequent hydrolysis to (IIIa) geraniol and (IIIb) nerol.

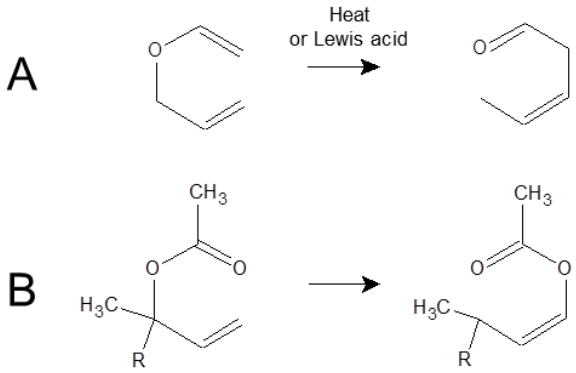


Figure 38. Comparison between (A) Claisen rearrangement (after Marco-Contelles and Soriano 2012) and (B) the suggested reaction scheme of linalyl acetate forming geranyl and neryl acetate (see also Figure 37); R=linalyl group.

The decrease of geranyl and neryl acetate after 144 h indicates further transformation into the other reaction products. In this context, the cyclic products are preferentially formed from the (*Z*)-configurated neryl acetate which explains the higher final amount of geranyl acetate and geraniol in contrast to neryl acetate and nerol. Cyclisation steps were shown in detail by Habermehl et al. 2008.

4.2.6 Transfer of structurally related substances of linalyl esters

Considering the spectrum of reaction products obtained upon hot water infusion of linalyl acetate, the fate of structurally related substances should also be investigated. The structural modifications included variations of the acyl moiety as well as replacement of linalool by other monoterpene alcohols.

Figure 39 shows the homologuos series of aliphatic (chain length C1 to C8) and aromatic linalyl esters which were subjected to hot water infusions.

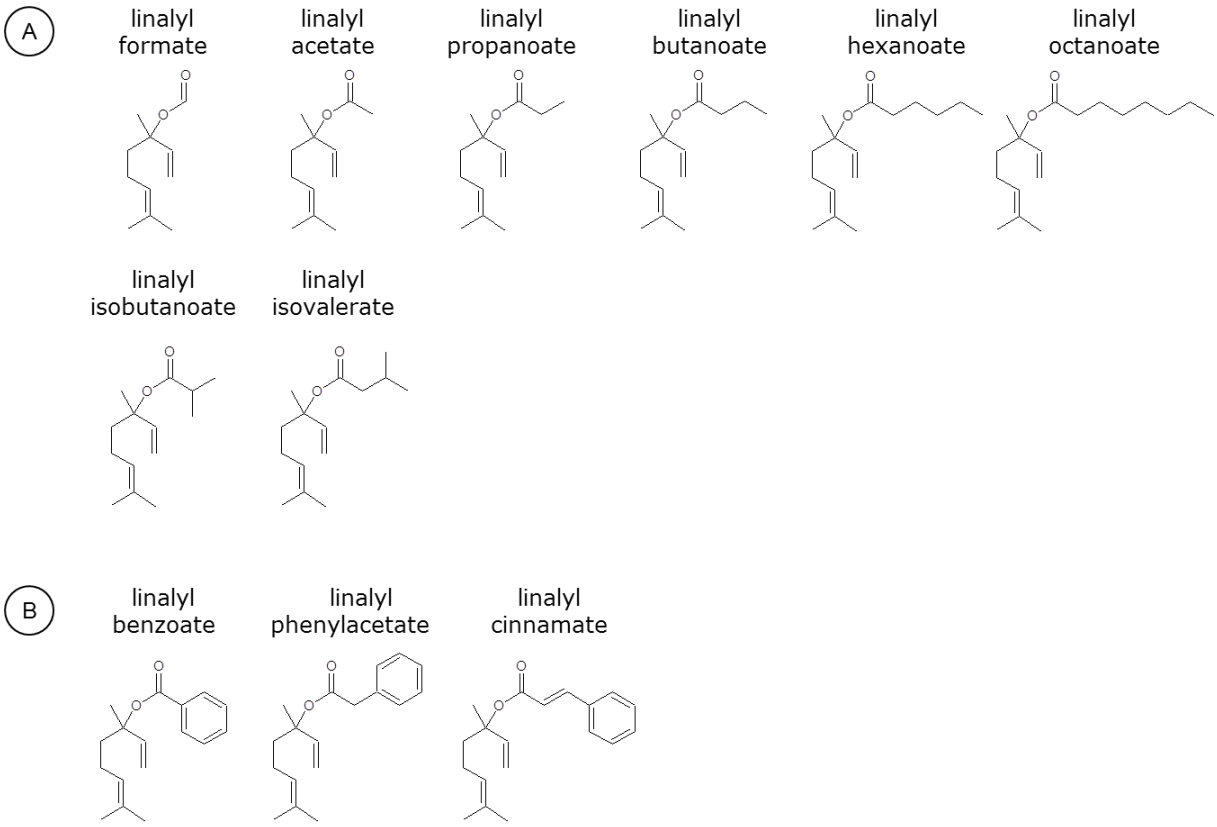


Figure 39. Aliphatic (A) and aromatic (B) linalyl esters subjected to hot water infusions.

The structures of the acetates of other monoterpene alcohols (esters of a primary, a secondary and a tertiary alcohol) employed for the infusion experiments are shown in Figure 40.

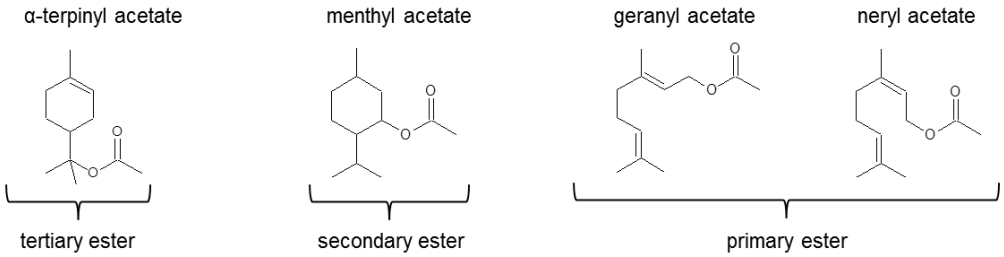


Figure 40. Acetates of monoterpene alcohols subjected to hot water infusions

4.2.6.1 Aliphatic linalyl esters – impact of acyl moiety

As shown in Figure 41, the amount of linalyl ester transferred into the final tea infusion increased with increasing chain length of the acyl moiety. Linalyl formate was not detected (lower than limit of detection: 0.07% (2.6 µg/200 mL tea infusion); on the other hand, 48.7±9.7% of linalyl octanoate was found in the tea infusion. The obviously stabilizing effect of the aliphatic acyl chain on the ester bond was confirmed by the fact that the amount of the hydrolysis product linalool decreased with increasing chain length of the acyl moiety. 23.0±1.0% of linalool (calculated as linalyl ester equivalents) was determined in an infusion of a tea bag spiked with linalyl formate and only 0.3±0.2% in an infusion with linalyl octanoate.

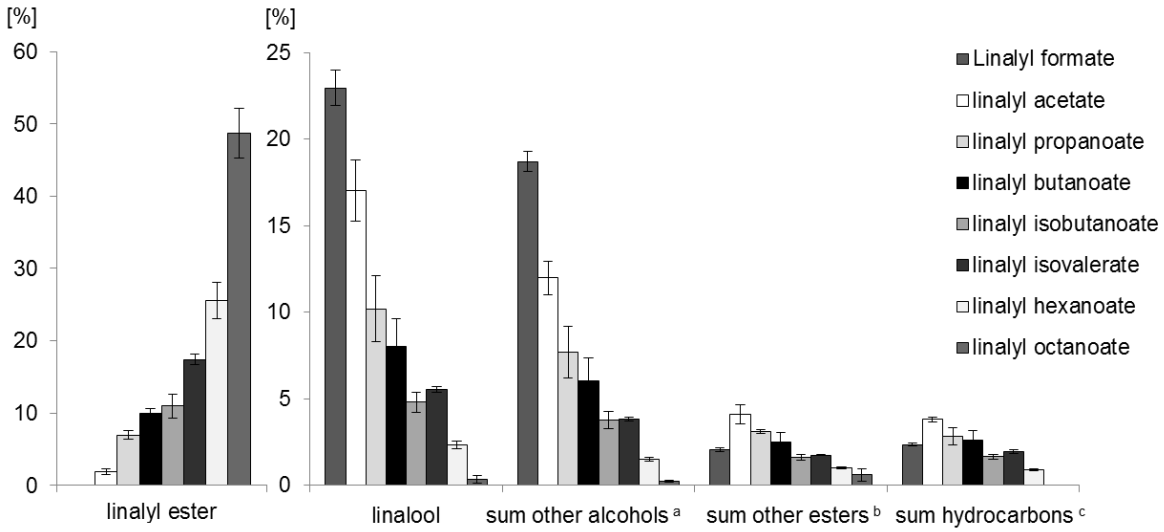


Figure 41. Transfer rates of linalyl esters and amounts of reaction products (calculated as linalyl ester equivalents) determined upon hot water infusions (200 mL) of tea bags (2 g) containing 4 mg of the respective linalyl ester; [a] sum of α-terpineol, geraniol, and nerol; [b] sum of geranyl and neryl ester; [c] sum of myrcene, (E)-β-ocimene, (Z)-β-ocimene, terpinolene, and limonene.

Similar effects were also observed for the sums of other alcohols (α-terpineol, geraniol, and nerol), of other monoterpene esters (geranyl and neryl acetate) and of hydrocarbons

(myrcene, (*E*)-β-ocimene, (*Z*)-β-ocimene, terpinolene, and limonene) formed during the infusion process. The fact that the total amounts of other esters and hydrocarbons formed from linalyl acetate were higher than those formed from linalyl formate may be explained by the volatility of linalyl formate. Probably this ester disappeared before isomerisation processes could have taken place. It should be mentioned that reaction products formed from all linalyl esters were the same, except for neryl and geranyl esters (single values see Appendix, Table 49).

Figure 42 shows the corresponding amounts in the residual tea bag (single values see Appendix, Table 50). In contrast to the infusion, 5.3% ± 0.4% of linalyl formate was found in the residual tea bag; this remaining amount increased up to 44.3 ± 6.4% for linalyl octanoate. The contents of linalool, the sums of other alcohols, other esters, and hydrocarbons were again decreasing with increasing chain length.

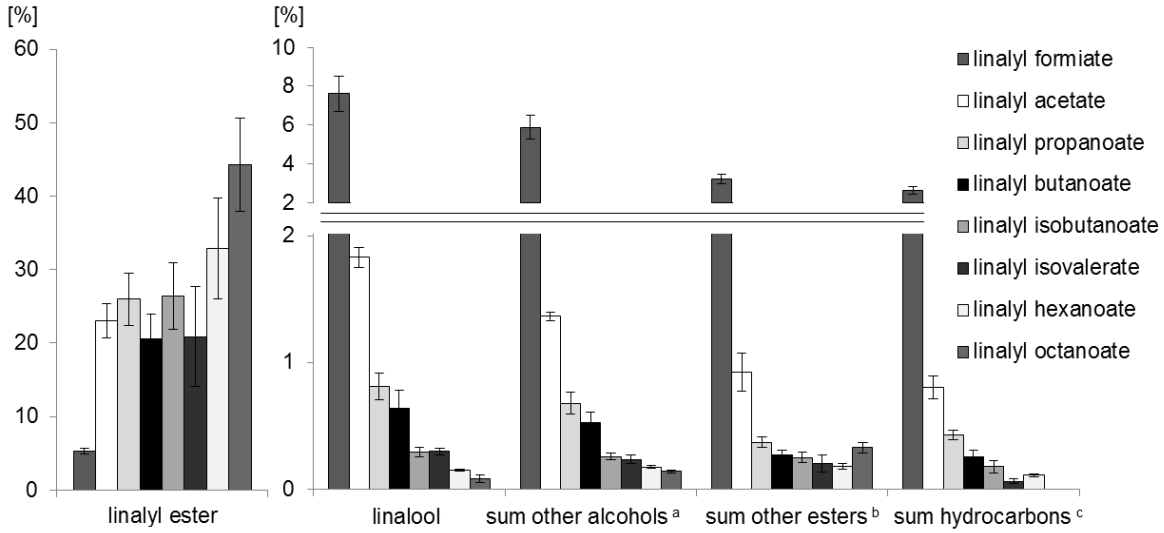


Figure 42. Transfer rates of linalyl esters and amounts of reaction products (calculated as linalyl ester equivalents) determined in residual tea bags of hot water infusions (200 mL) of tea bags (2 g) containing 4 mg of the respective linalyl ester; [a] sum of α-terpineol, geraniol, and nerol; [b] sum of geranyl and neryl esters; [c] sum of myrcene, (E)-β-ocimene, (Z)-β-ocimene, terpinolene, and limonene.

Mass balances of all substances detected in the infusions and the residual tea bags are pictured in Figure 43. The total contents (calculated as linalyl ester equivalents) decreased from *n*-C1 (linalyl formate) to *iso*-C4 (linalyl isobutanoate) and increased again to *n*-C8 (linalyl octanoate).

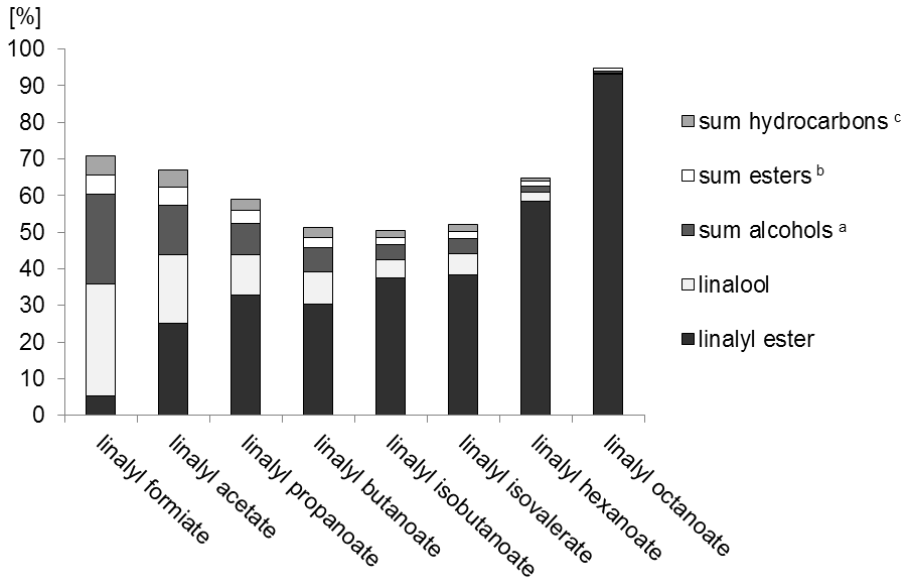


Figure 43. Total amounts of transferred linalyl esters and reaction products (calculated as linalyl ester equivalents) determined in the infusions and in the residual tea bags of hot water infusions (200 mL) of tea bags (2 g) containing 4 mg linalyl ester; [a] sum of α-terpineol, geraniol, and nerol; [b] sum of geranyl and neryl esters; [c] sum of myrcene, (E)-β-ocimene, (Z)-β-ocimene, terpinolene, and limonene.

In summary, the rate of ester cleavage decreased with increasing chain length of the acyl moiety. This may be explained by a lower +I effect (inductive effect) resulting in a reduced probability for the generation of the tetrahedral transition state involved in the hydrolysis of the ester (*Figure 36* III).

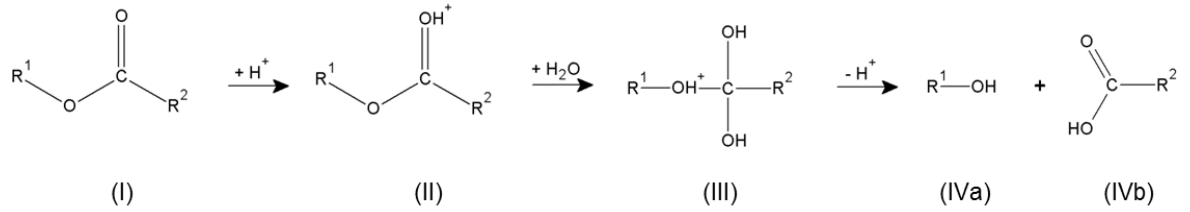


Figure 44. Scheme of the acid catalysed hydrolysis of linalyl esters; (I) linalyl ester; (II) protonation of the ester; (III) tetrahedral transition state by nucleophilic addition of water; separation of (IVa) alcohol and (IV) carboxylic acid; R^1 linalyl group, R^2 acyl chain group.

Enantiomerically pure linalyl esters (C1 and C8)

In order to get more insight into the individual reaction steps concerning the enantiomeric ratio of linalool in the course of the hydrolysis of linalyl esters (see 4.2.3.5), enantiomerically pure linalyl formate and linalyl octanoate, respectively, were synthesized (see 3.1.3) and subjected to a hot water infusion. It was expected that linalool generated from linalyl formate with the better leaving group shows a preference for the reaction pathway via a planar cation as transition state (Figure 31A). For (*R*)-linalyl octanoate, a shifting of the two competing reaction pathways towards a one-step hydrolysis (Figure 31B) was anticipated.

As shown in Table 24, the enantiomeric ratios of the hydrolysis product linalool from linalyl formate and linalyl octanoate, respectively, did not differ significantly. In contrast, the percentage of (*R*)-linalool produced from linalyl acetate was more than 6% higher (see 4.2.3.5). Therefore, the assumption of a shifting to one of the two possible reaction pathways via a shorter or longer acyl chain length could not be proven.

Table 24. Impact of hot water infusions on the enantiomeric compositions of (R)-linalyl formate and (R)-linalyl octanoate (GC-System II).

	linalyl formate [%]	linalyl octanoate [%]
	(*R*) (*S*)	(*R*) (*S*)
reference substance	99.2 : 0.8	-[a]
tea infusion		
linalyl ester	n.d.[b]	-
linalool	57.3 : 42.7	59.5 : 40.5

[a] *enantiomers of (R)-linalyl octanoate could not be separated under the conditions employed (3.2.4.2); however, the optical purity of linalool used for the synthesis was the same as that used for linalyl formate;* [b] *n.d.: below limit of determination of GC-System IIb (linalyl formate: 30.5 µg/200 mL tea infusion determined for linalyl acetate).*

4.2.6.2 Aromatic linalyl esters – impact of the phenyl group

The results obtained by hot water infusions of aromatic linalyl esters are illustrated in Figure 45 (single values are given in Table 49 and 50).

Linalyl benzoate and phenylacetate were found in similar contents in the infusion and in the residual tea bag. Linalyl cinnamate showed approximately 10% higher amounts in the tea beverage. However, with a total sum of about 30% in the infusion as well as in the residual tea bag, no big differences were found in the reactivity of the three compounds. A +M effect (mesomeric effect) of the phenyl group promotes the generation of a quaternary transition

state (Figure 44 III) and hence the hydrolysis of the ester. The elongation of the acyl chain between the linalyl and the phenyl group did not have an effect to the reactivity; the base dissociation constant (P_{Ka}) is nearly the same for all three substances (l. benzoate: 4.20, l. cinnamate: 4.44, l. phenylacetate: 4.31 after Dippy and Lewis 1937, Dippy and Williams 1934, and Duer and Robinson 1971). Only the solubility seems to be different for linalyl cinnamate caused by the additional double bond, although the octanol-water distribution-coefficient ($\log P_{OW}$) for the cinnamic ester is higher (6.06) than those of the benzoic (5.64) or phenylacetic ester (5.51; coefficients were calculated with *molinspiration* software). Experiments with linalyl dihydrocinnamate could give more insight into to this phenomenon.

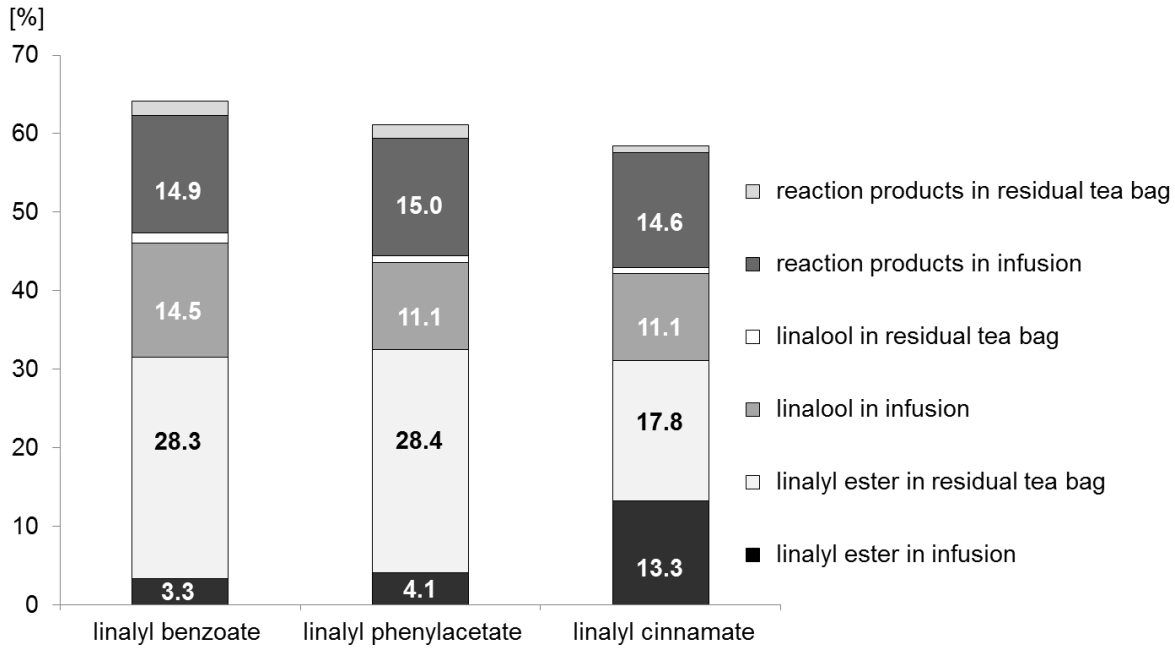


Figure 45. Total amounts of transferred aromatic linalyl esters and reaction products (calculated as linalyl ester equivalents) determined in the infusions and in the residual tea bags of hot water infusions (200 mL) of tea bags (2 g) containing 4 mg linalyl ester; linalool and reaction products in the residual tea bag were lower than 1.9%.

4.2.6.3 Transfer of other acetates - impact of the monoterpene alcohol moiety

The influence of the alcohol moiety on the transfer rate was determined by investigating the recoveries of other monoterpene acetates. Geraniol and nerol, menthol, and α-terpineol were selected as examples for primary, secondary, and tertiary alcohols, and their acetates were subjected to hot water infusions.

As shown in Table 25 the proportions of intact esters transferred into the tea beverage were in the same order of magnitude (29.8 ± 2.3% - 38.0 ± 3.3%) and, thus, significantly higher than the amount of linalyl acetate (1.9 ± 0.4%) transferred upon hot water infusion. The

increased stability of these esters is also reflected by the fact that, except for a minor amount observed for α-terpinyl acetate, no hydrolysis products could be detected. Cori et al. (1986) reported reaction products for the solvolysis not only of linalyl but also of geranyl and neryl trifluoroacetates; limonene, terpinolene, myrcene, ocimene, linalool, nerol, geraniol, and α-terpineol could be determined for the activated forms of the examined molecules.

Thus, these data confirm the outstanding impact of the structure of linalyl esters, i.e. the ester group in allylic position, the double bond at C6, and the existence of a tertiary ester, on the stability of these flavouring substances in the course of a hot water treatment.

Table 25. Contents of reaction products obtained by hot water infusion of a tea bag containing 4 mg monoterpenes esters of the tertiary alcohol α-terpineol, the secondary alcohol menthol or the primary alcohols geraniol and nerol.

remaining substance [%]		hydrolysis product [%][a]		reaction products [%][a]		sum [%]
α-terpinyl acetate	32.5 ± 2.2	α-terpineol	1.2 ± 0.02[b]	terpinolene limonene	0.2 ± 0.02 0.5 ± 0.1	34.4 ± 2.3
menthyl acetate	29.8 ± 2.3	menthol	n.d.[c]	-	-	29.8 ± 2.3
geranyl acetate	38.0 ± 3.3	geraniol	n.d.[c]	linalool	0.9 ± 0.1	38.9 ± 3.5
neryl acetate	33.0 ± 1.8	nerol	n.d.[d]	linalool α-terpineol	0.4 ± 0.05 0.8 ± 0.2	34.2 ± 1.9

[a] Values were calculated as molar equivalent of the starting substance; [b] α-terpineol was found for 0.27% in the reference substance used; [c] n.d.: below limit of detection (determined for linalool: 0.03%); [d] n.q.: below limit of quantification (determined for linalool: 0.09%). Data represent means ± standard deviations from triplicate experiments.

4.2.7 Conclusion

Losses and changes of the initially added volatiles may occur during industrial processing of foods (Young et al. 2006) as well as in the course of home-preparation procedures. Therefore, the consideration of the respective correction factors constitutes a key element in the assessment of the actual dietary exposure to flavouring substances. The rather simple preparation of tea via hot water infusion was shown to be an example of a processing step that may influence the concentrations and the type of flavouring substances in the finally consumed product.

Linalyl acetate and linalool, the two main monoterpenes in Earl Grey tea, show extremely different behaviours upon hot water infusion: 66% of linalool is transferred into the final tea beverage, whereas linalyl acetate shows only a transfer of approximately 2%. However, a spectrum of other monoterpenes appears in the course of the hot water infusion of the ester, among them 17% of the hydrolysis product linalool and several other degradation /

rearrangement products. The comparison of different brewing times between 0.5 and 10 minutes, reflecting potential differences in consumers´ behaviour, showed only dependencies for linalyl acetate but not for linalool. Mass balance calculations, summing up amounts in the infusion and the residual tea bag, showed that approximately 70% was recovered in all experiments. The transfer rate is also not or merely affected by the level of the starting amount of the spiked flavouring substance; solely the amount of linalool as the hydrolysis product at a very high spiking level of linalyl acetate (20 mg) was lower than that determined upon spiking at the median level.

Potential mechanisms underlying the transformation of linalyl acetate into the various reaction products during hot water infusions were investigated by using enantiomerically pure substances and by observing the fate of linalyl acetate under ‘slowed down conditions’ in cold water. Neither racemic nor enantiomerically pure linalool was observed upon infusion of linalyl acetate, indicating the potential involvement of two competing hydrolysis pathways. The investigation of the hydrolysis of linalyl acetate in cold water also gave some indications regarding the reaction steps involved in the formation of geranyl and neryl acetate. Surprisinly, these esters and not the alcohols nerol and geraniol occurred as first products. A Claisen-type rearrangement is proposed as reaction mechanism to explain this phenomenom.

Transfer rates of structurally related substances, i.e. esters of linalool with varying acyl moieties and esters of other monoterpene alcohols, were also investigated. These experiments confirmed the outstanding impact of the structure of linalyl acetate. Only the presence of (i) a tertiary ester combined with (ii) an allylic system and an additional double bond in 6,7-position leads to the instability of this molecule.

The observed significant differences between these structurally related esters are important in the context of the safety evaluations of flavouring substances performed at EU level. The employed strategies are group-based, i.e. for structurally related substances similar chemical and metabolic behaviour is expected. The data elaborated in this thesis underline that for some compound classes caution may be required when applying this principle.

4.3 Dietary exposure to flavouring substances by consumption of Earl Grey teas

4.3.1 Exposure to flavouring substances via consumption of a cup tea

In many cases, the amounts of flavouring substances added to foods can only be estimated on the basis of the use levels reported by the Flavouring Industry. In the case of Early Grey tea, the available data were also limited to use levels provided for linalool and linalyl acetate in the so-called 'Fenaroli´s handbook of flavour ingredients' (Burdock 2005). According to this compilation, the usual and maximum use levels of linalool and linalyl acetate in the broad food category 'non-alcoholic beverages' amount to 3.57 mg/kg (usual) - 6.87 mg/kg (maximum) and 5.53 mg/kg (usual) – 8.46 mg/kg (maximum), respectively.

In order to reduce the uncertainty resulting from this very vague information, in the first part of this study the contents of these flavouring substances have been experimentally determined in a broad spectrum of Earl Grey teas available in the EU. As shown in chapter 4.1, the median and 97.5th percentile contents in 2 g tea leaves (the average amount in a tea bag) amounted to 2.3 and 8.1 mg linalool and to 4.0 and 14.7 mg linalyl acetate, respectively.

In addition, the transfer of flavouring substances from the tea leaves into the tea beverage upon hot water infusion has been determined. As shown in chapter 4.2, approximately 66% of linalool and 2% of linalyl acetate present in a tea bag are extracted into the tea beverage; the hot water treatment of linalyl acetate also results in the formation of approximately 17% linalool and 20% of other reaction products.

These two data sets provide the basis for an assessment of the intake of linalool and linalyl acetate via a cup of Earl Grey tea.

Table 26 shows the results of the intake calculations based (i) on the publicly available use levels for linalool and linalyl acetate in non-alcoholic beverages (Burdock 2005), (ii) on the median contents of linalool and linalyl acetate determined in commercial Early Grey teas, and (iii) on both, the median contents of linalool and linalyl acetate determined in commercial Earl Grey teas and on their transfer rates into the tea beverage in the course of the infusion step.

Table 26. Comparison of the amounts of flavouring substances in a tea cup (200 mL) calculated on the basis of use levels provided by industry vs. experimentally determined median contents and transfer rates.

	calculations based on		
	usual use levels (industry)[a]	median contents (90 Earl Grey teas)[b]	median contents and transfer rates
	[mg / 200 mL tea beverage]		
linalool	0.71	2.3	2.05[c]
linalyl acetate	1.11	4.0	0.08[d]
reaction products	-	-	0.65[d]
	calculations based on		
	maximum use levels (industry)[a]	97.5th perc. contents (90 Earl Grey teas)[b]	97.5th perc. contents and transfer rates
	[mg / 200 mL tea beverage]		
linalool	1.4	8.1	7.3[c]
linalyl acetate	1.7	14.7	0.3[d]
reaction products	-	-	1.2[d]

[a] use levels for non-alcoholic beverages provided by industry in 'Fenaroli's handbook of flavor ingredients' (Burdock 2005). [b] Experimentally determined contents in 90 Earl Grey teas (4.1.3); values refer to 200 mL tea infusion prepared from a 2 g tea bag. [c] Sum of transferred linalool (66.4%) and proportion formed by hydrolysis of linalyl acetate (17.0%). [d] Transfer rates as given in Table 13.

A comparison of the data demonstrates that the sole consideration of the publicly available use levels results in an underestimation of the intake of linalool by a factor of 3 (median) and 5 (high), respectively, and an overestimation of the intake of linalyl acetate by a factor of 14 (median) and 6 (high), respectively. In addition, the exposure to the spectrum of reaction products is completely neglected. The use of the experimentally determined contents without consideration of the transfer rates results in an approximately 50-fold overestimation of the intake of linalyl acetate (median and 97.5th perc.) due to its instability and the low transfer rate of only 1.9%.

4.3.2 Assessment of dietary exposure to flavouring substances

To assess the dietary exposure to linalool and linalyl acetate via consumption of Earl Grey teas, the data calculated for the intake of linalool and linalyl acetate, respectively, via one cup of tea beverage (200 mL) were combined with tea consumption data from the United Kingdom National Diet and Nutrition Survey of 2000/2001 (Henderson et al. 2002). Assuming that all tea consumed would be Early Grey tea, average exposure was calculated using the median contents of the flavouring substances in the tea leaves, the transfer factors and the mean consumption of tea beverage. High exposure was estimated by employing the respective 97.5th percentile values of the contents of the flavouring substances and of the tea consumption.

As shown in Table 27, the intake of linalyl acetate ranges from an average of 0.16 mg/d (assumptions: mean consumption of tea beverage prepared with tea leaves with median content of linalyl acetate) to a high value of 1.8 mg/d (assumptions: 97.5th percentile consumption of tea beverage prepared with tea leaves with 97.5th percentile content of linalyl acetate). The corresponding dietary exposures to linalool are 4.2 mg/d (average) and 15.2 mg/d (high).

It is noteworthy that the dietary exposure to linalool via consumption of the tea beverage is approximately 26 times higher than that of linalyl acetate, although in the flavoured tea leaves the median content of linalyl acetate is approximately 1.8 times higher than that of linalool.

These data refer to male adults aged 19-64 years; the values calculated for female adults were nearly identical.

Table 27. Dietary exposure to linalool and linalyl acetate via consumption of Earl Grey tea based on data consumption data from UK.

tea consumption[a]			*exposure based on median contents*[b]	
	[g / d]	cups (200 mL)	linalyl acetate [mg / d]	linalool [mg / d]
mean	411	~ 2	0.16	4.2
95th perc.	1271	~ 6	0.5	13.0
tea consumption[a]			*exposure based on 97.5th contents*[c]	
	[g / d]	cups (200 mL)	linalyl acetate [mg / d]	linalool [mg / d]
mean	411	~ 2	0.6	15.1
95th perc.	1271	~ 6	1.8	46.6

[a] *United Kingdom National Diet and Nutrition Surveys of 2000/2001 (EFSA 2008, Henderson et al. 2002) for 19-64 aged men including non-consumers.* [b] *Calculation on the basis of median contents of linalool (2.05 mg / 200 mL) and linalyl acetate (0.08 mg / 200 mL), respectively.* [c] *Calculation on the basis of 97.5th percentile contents of linalool (7.33 mg / 200 mL) and linalyl acetate (0.28 mg / 200 mL), respectively.* [d] *97.5th percentiles (x) were calculated using mean (μ) and standard deviation (σ) values given in Henderson et al. (2002) using the following formula* $x_{97.5} = \mu + 1.96 * \sigma$.

In order to take into account consumption behaviours for different age groups, tea consumption data provided in a diet survey of the UK for four age classes between 19-64 years were also considered (Henderson et al. 2002). Table 28 shows the different tea consumptions and the resulting exposures to linalool and linalyl acetate. When considering mean tea consumptions and median contents of flavouring substances, men of 50-64 years have approximately 3-fold higher exposure to both flavouring substances than persons aged 19-24 years. Exposure assessments for the 97.5th percentiles are 3-fold higher than the average data.

Table 28. Dietary exposure to linalool and linalyl acetate via consumption of Earl Grey tea based on consumption data from UK for different age groups including non-consumers (Henderson et al. 2002).

		age			
		19-24	25-34	35-49	50-64
mean tea consumption	[g / d]	201	309	434	542
		exposure based on median content [a]			
linalool	[mg / d]	2.1	3.2	4.4	5.5
linalyl acetate	[mg / d]	0.08	0.12	0.17	0.21
97.5th perc. tea consumption[c]	[g / d]	689	979	1243	1588
		exposure based on 97.5th contents [b]			
linalool	[mg / d]	25.2	35.9	45.5	58.2
linalyl acetate	[mg / d]	1.0	1.4	1.8	2.2

[a] *Calculation on the basis of median contents of linalool (2.05 mg / 200 mL) and linalyl acetate (0.08 mg / 200 mL), respectively.* [b] *Calculation on the basis of 97.5th percentile contents of linalool (7.33 mg / 200 mL) and linalyl acetate (0.28 mg / 200 mL), respectively.* [c] *97.5th percentiles were calculated analogously to Table 27.*

Analogous calculations were performed on the basis of tea consumption data available from Germany. According to the 'Nationale Verzehrsstudie II' (MRI 2013), the mean consumption of black tea in Germany is significantly lower than the consumption in the UK; the mean consumption of males aged 14-80 years amounts to 84 g/d and the 95th percentile to 589 g/d. On the basis of these results, the exposure to linalyl acetate ranged from an average of 0.03 mg/d (assumptions: mean consumption of tea beverage prepared with tea leaves with median content of linalyl acetate) to a high value of 0.8 mg/d (assumptions: 97.5th percentile consumption of tea beverage prepared with tea leaves with 97.5th percentile content of linalyl acetate). The corresponding exposures to linalool were 0.9 mg/d (average) to 21.6 mg/d (high).

Table 29. Dietary exposure to linalool and linalyl acetate via consumption of Earl Grey tea based on consumption data from Germany.

tea consumption[a]			*exposure based on median content*[b]	
	[g / d]	cups (200 mL)	linalyl acetate [mg / d]	linalool [mg / d]
Mean	84	~ 0.4	0.03	0.9
95th perc.	589	~ 2.9	0.23	6.0
tea consumption[a]			*exposure based on 97.5th contents*[b]	
	[g / d]	cups (200 mL)	linalyl acetate [mg / d]	linalool [mg / d]
Mean	84	~ 0.4	0.12	3.1
95th perc.	589	~ 2.9	0.83	21.6

[a] *German National Nutrition Survey II (MRI 2013) for 14-80 aged men including non-consumers.* [b] *Calculation on the basis of median contents of linalool (2.05 mg/200 mL) and linalyl acetate (0.08 mg/200 mL), respectively.* [c] *Calculation on the basis of 97.5th percentile contents of linalool (7.33 mg/200 mL) and linalyl acetate (0.28 mg/200 mL), respectively.*

4.3.3 Conclusion

The calculation of intakes of linalool and linalyl acetate demonstrated the need (i) to consider actual contents of these flavouring substances in commercial Earl Grey teas and (ii) to take into account the individual transfer rates from the tea leaves into the tea beverage upon hot water-treatment. The sole consideration of publicly available use levels results in significant over- and underestimations, respectively, of these flavouring substances via the consumption of Earl Grey tea.

This aspect has to be generally considered in the course of exposure assessments of flavouring substances. There are reports that use levels provided by industry for processed foods such as ‘baked goods’ are on average 72% higher than those of non-processed foods such as ‘non-alcoholic beverages’ (Hall and Ford, 1999). However, the lack of systematic studies on this phenomenon definitely hampers the intake assessment of flavouring substances from processed or, as shown in this study, home-prepared foods.

4.4 Transfer rates of flavouring substances from ‘forest fruit’ tea into a tea infusion

4.4.1 Introduction

In order to extend the data elaborated for Earl Grey teas, contents of flavourings substances and their transfer rates upon hot water infusions were determined in another group of flavoured teas, labelled as ‘forest fruit’ teas.

A selection of 14 flavouring substances was quantitated in tea material as well as in tea infusion. The compounds depicted in Figure 46 were chosen based on their occurrence in four forest fruit teas from different producers analysed in previous experiments (data not shown): 2-methylpropyl acetate, 3-methylbutyl acetate (isoamyl acetate), (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, benzaldehyde, benzyl acetate, β-damascenone, α-ionone, β-ionone, eugenol, methyl isoeugenol, γ-undecalactone, vanillin and raspberry ketone.

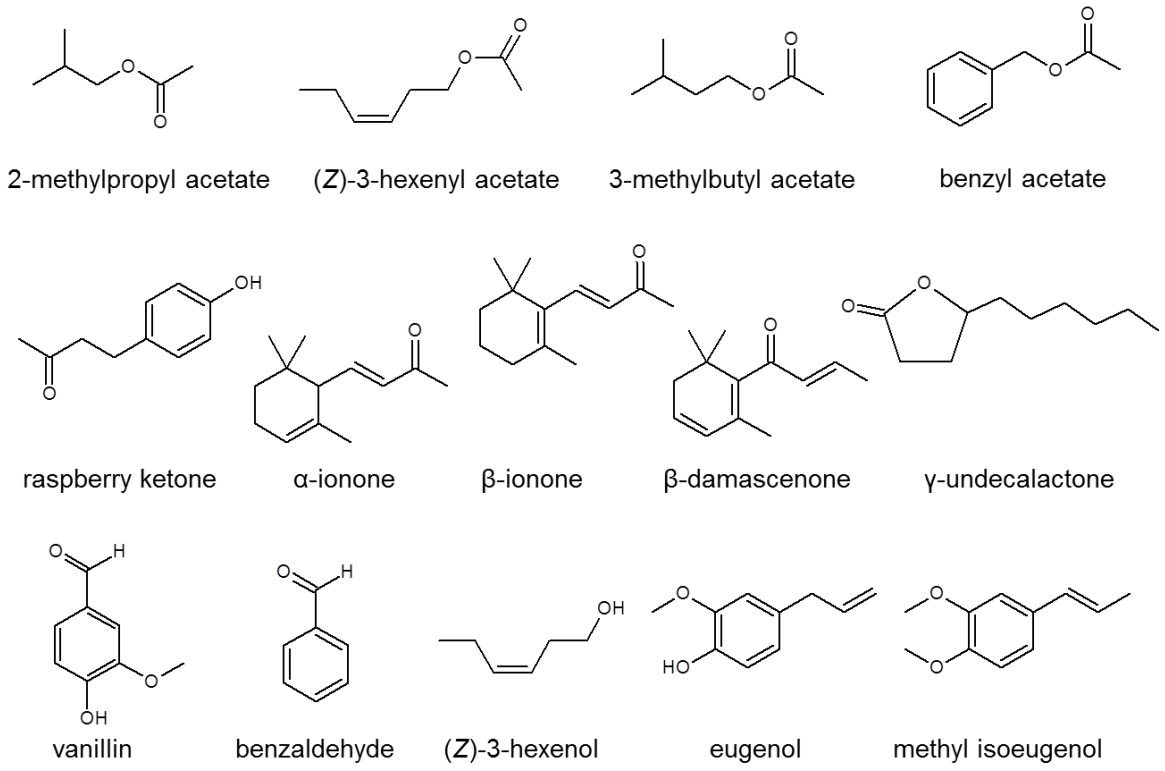


Figure 46. Structures of flavouring substances quantitated in forest fruit tea.

4.4.2 Development of a standard addition method

For forest fruit teas quantifications as performed for Earl Grey teas were not possible because no non-flavoured tea material was available for the determination of the respective recovery rates. Therefore, a standard addition method was established.

A standard addition method is based on the addition of known amounts of the analytes to be quantified to the sample and analysis of the spiked as well as the unspiked samples by the same procedure. It offers a useful alternative to stable isotope dilution assays (Schieberle and Molyneux 2012) and is applied if a matrix-adapted sample is not available (DIN 1998). Commonly, the spiked amount approximately corresponds to the initial amount of the sample (Funk et al. 2005). Instrumental errors as well as disturbing matrix effects are reduced by this methodology. The analyte concentration can be calculated via linear regression.

For the quantifications, three types of standards were used: (i) An extraction standard (ESD; 2-heptanol) was added to the tea material and the cooled-down tea infusion, respectively, before starting the extraction procedure. (ii) An injection standard (InSD; 2-decanol) was spiked to the extracts obtained from tea material and tea infusion, respectively, before injection. (iii) To cover the variability arising from the squeezing of the tea bag, a control standard (CSD; 2- nonanol) was added onto the tea bag before the hot water infusion.

The peak areas normalised on the basis of these standards were plotted against the concentrations resulting from the spiked amounts of the flavouring substances, and a regression line was calculated using Microsoft Office Excel. As example, the principle is shown for an infusion of β-ionone in Figure 47.

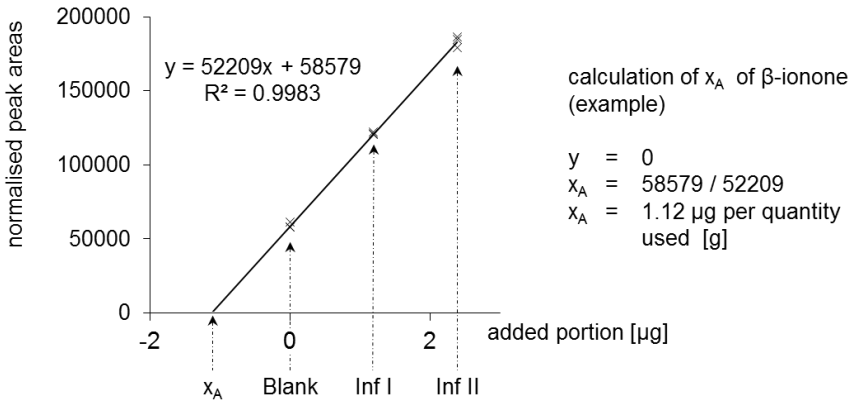


Figure 47. Principle of the standard addition method with normalised areas exemplarily shown for an infusion of β-ionone; x_A: concentration of β-ionone in the tea infusion, Blank: no added stock solution, Inf I/II: stock solutions with concentrations approximately half of and two times higher, respectively, than the blank sample.

The suitability of the established standard addition method was confirmed by analysing a blank solution according to the procedure applied to the tea material. Four flavouring substances were spiked to the blank solution at two levels (Table 7). The resulting peak areas were normalised (see 3.2.3.4) and plotted against the spiked concentrations (Figure 48). For all four compounds the concentrations calculated in the blank solution amounted to nearly zero (β-ionone: 0.09 µg/g tea, α-ionone: 0.09 µg/g tea, 2-methylpropyl acetate: 0.05 µg/g tea, and benzyl acetate: 0.12 µg/g tea).

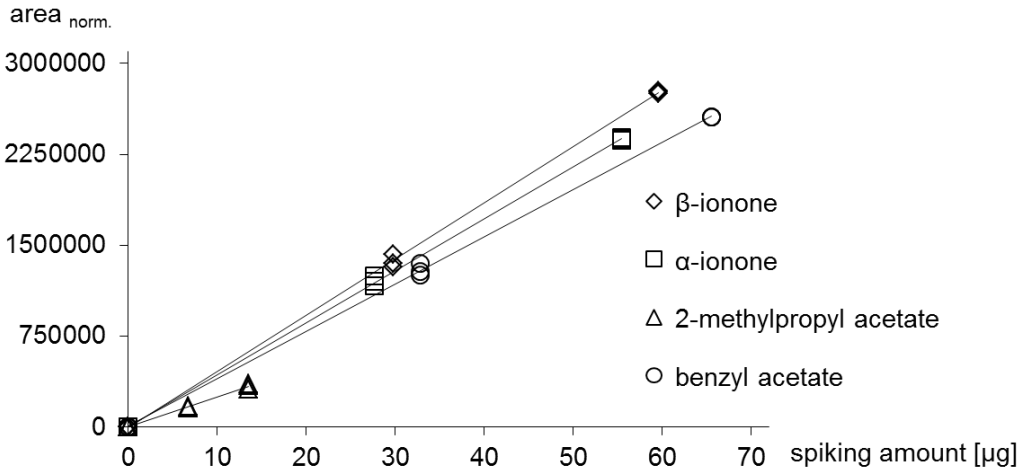


Figure 48. Normalised peak areas determined after spiking a blank solution with β-ionone, α-ionone, 2-methylpropyl acetate and benzyl acetate, respectively, at two levels (Table 7) and analysis according to the procedure applied to tea material.

The repeatability of the procedure, in particular of the squeezing step, was confirmed by determining the recovery of the control standard spiked to the tea bag before the hot water infusion in 11 experiments. As shown in Table 30, the recovery of the control standard ranged from 51.9% to 63.3%, with a total standard deviation of 3.7%.

Table 30. Recovery rates of the control standard 2-nonanol determined in 11 tea infusions.

flavour group	infusion no.	recovery of CSD [%]
fg 1[a] (n=9)	1	53.3 ± 1.8
	2	59.0 ± 1.9
	3	63.3 ± 1.2
	4[e]	53.5 ± 0.4
fg 2[b] (n=12)	5	59.7 ± 1.9
	6	51.9 ± 1.3
	7	56.9 ± 0.8
fg 3[c] (n=9)	8	53.5 ± 0.4
	9	58.5 ± 0.3
	10	61.4 ± 0.3
fg 4[d] (n=9)	9	58.5 ± 0.3
	10	61.4 ± 0.3
	11	58.2 ± 0.5
total mean		**57.1 ± 3.7**

[a] *flavour group 1: 2-methylpropyl acetate, benzyl acetate, α-ionone, β-ionone;* [b] *flavour group 2: β-damascenone, eugenol, methyl isoeugenol, γ-undecalactone;* [c] *flavour group 3: vanillin, raspberry ketone;* [d] *flavour group 4: 3-methylbutyl acetate, (Z)-3-hexenyl acetate, (Z)-3-hexenol, benzaldehyde¸* [e] *for fg 1 a fourth infusion was prepared in order to confirm the results (see Table 7).*

4.4.3 Contents of flavouring substances in ‘forest fruit’ tea material and infusions

The contents of fourteen flavouring substances were determined via the established standard addition method in ‘forest fruit’ tea material and the respective hot water infusions; chromatograms are shown in Figure 49. Triacetin, a substance typically used as carrier for flavourings substances added to foods and the fatty acid hexanoic acid were not quantified.

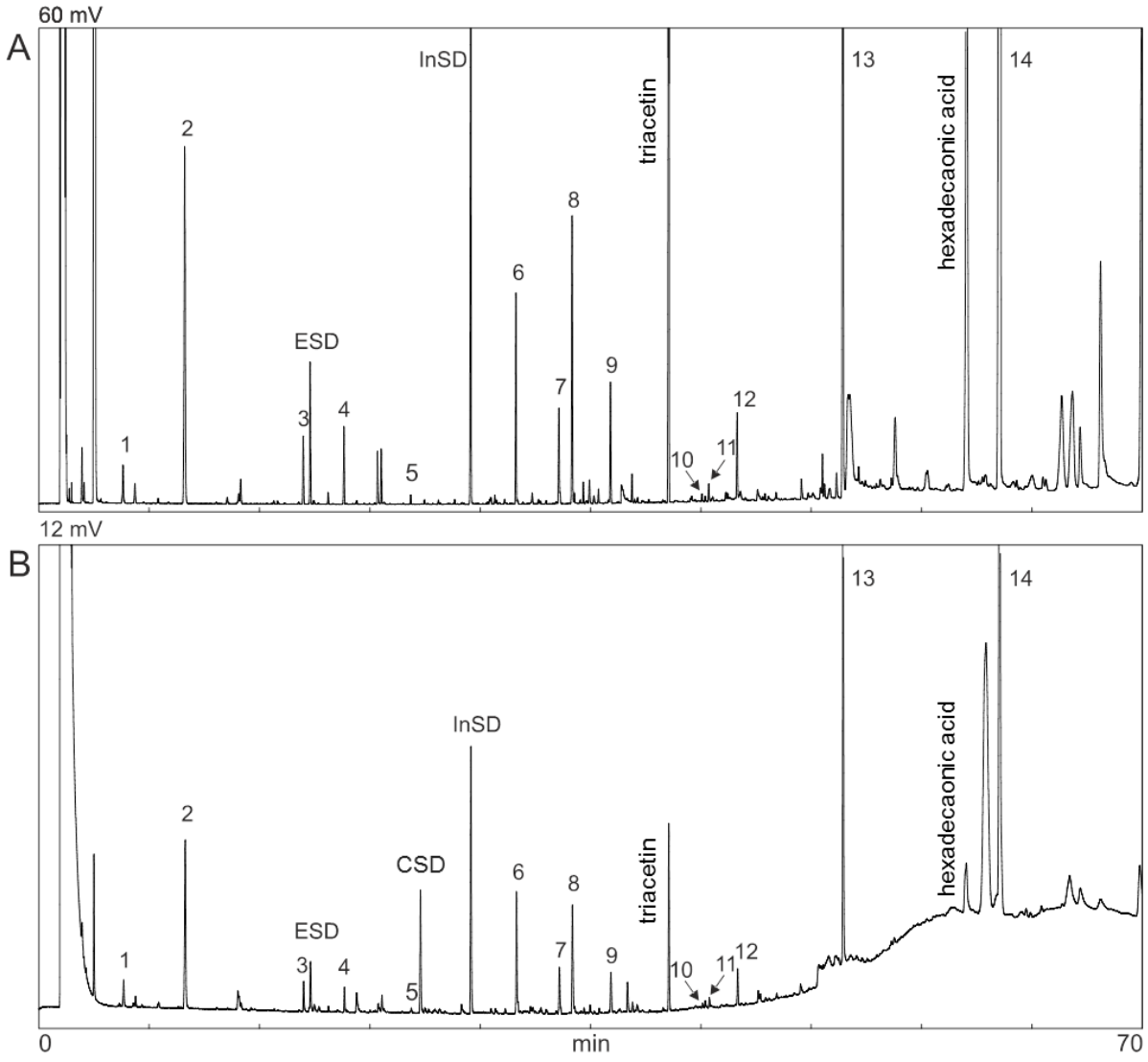


Figure 49. Capillary gas chromatographic separation on GC-system I (DB-WAX, method III) of volatiles isolated from (A) tea material and (B) an tea infusion of forest fruit teas: 1: 2-methylpropyl acetate, 2: 3-methylbutyl acetate, 3: (Z)-3-hexenyl acetate, 4: (Z)-3-hexenol, 5: benzaldehyd, 6: benzyl acetate, 7: β-damascenone, 8: α-ionone, 9: β-ionone, 10: eugenol, 11: methyl isoeugenol, 12: γ-undecalactone, 13: vanillin, 14: raspberry ketone, ESD: extraction standard, CSD: control standard, InSD: injection standard.

As shown in Table 31, raspberry ketone and vanillin were by far the major constituents in the tea material as well as in the tea beverage. As expected, the transfer rates of these flavouring substances were quite high because of their hydrophilic properties. On the other hand, the transfer rates of less polar substances like α- and β-ionone were significantly lower (50% and 39%).

The transfer rates are not only affected by the polarities but also by the boiling points of the substances. Benzyl acetate, for example, is less polar than (*Z*)-3-hexenyl acetate, but 81% was recovered in the beverage. (*Z*)-3-Hexenyl acetate in turn, has a lower boiling point than benzyl acetate but only 51% was transferred from the tea material into the tea beverage.

A third possible parameter, as suggested by Mui et al. (2002), could be the molecular weight. In case of ketones, the transfer rates decreased with increasing molecular mass; on the other hand the values for alcohols and esters increased.

Table 31. Transfer rates of 14 flavouring substances occurring in 'forest fruit tea' from tea material (tea bag weight 2.5 g) into the tea beverage upon hot water infusion (200 mL, 8 min).

	log p_{OW}[a]	boiling point [°C]	mol. mass	content		transfer rate [%][c]
				tea material [µg / 2.5 g]	tea infusion [µg / 200 mL][b]	
esters						
benzyl acetate	1.98	214	150	58.2 ± 8.6	47.1 ± 6.5	81 ± 16
2-methylpropyl acetate	1.63	118	116	26.2 ± 4.4	19.9 ± 3.3	76 ± 18
(Z)-3-hexenyl acetate	1.80	174	142	52.8 ± 10.4	26.8 ± 3.5	51 ± 12
3-methylbutyl acetate	2.04	142	130	234 ± 50	103 ± 16	44 ± 12
ketones / lactones						
raspberry ketone	1.70	292	164	3508 ± 584	2703 ± 210	77 ± 14
γ-undecalactone	3.72	286	187	24.2 ± 3.9	14.1 ± 1.3	58 ± 11
β-damascenone	3.19	276	190	30.5 ± 6.7	17.5 ± 1.0	57 ± 13
α-ionone	3.13	258	192	82.1 ± 15.7	40.7 ± 2.5	50 ± 10
β-ionone	3.45	255	192	34.0 ± 6.9	13.4 ± 1.1	39 ± 9
aldehydes						
benzaldehyde	1.83	178	106	4.2 ± 0.6	3.4 ± 1.0	82 ± 27
vanillin	1.07	285	152	2615 ± 459	1929 ± 285	74 ± 17
alcohols						
eugenol	2.10	253	164	2.4 ± 0.1	2.3 ± 0.5	97 ± 20
(*Z*)-3-hexenol	1.35	157	100	31.6 ± 5.5	22.6 ± 3.8	72 ± 17
other						
methyl isoeugenol	2.69	255	128	6.4 ± 0.6	4.2 ± 0.5	66 ± 10

[a] *octanol / water partition coefficients, calculated by molinspiration software (www.molinspiration.com/ cgi-bin/properties);* [b] *standard deviations of the tea material were calculated using the standard deviation of the peak areas for non-spiked samples (blank), the concentration of each substance and the rule of three (Δtm = concentration × Δ (area) / mean (area)),* [c] *standard deviations of transfer rates were calculated via error propagation using the following terms:* $\Delta tr = \sqrt{\left(\frac{\partial tr}{\partial tm} \times \Delta tm\right)^2 + \left(\frac{\partial tr}{\partial ti} * \Delta ti\right)^2}$ *and* $f(tr\,[\%]) = \frac{ti \times 100}{tm}$ *(tr: transfer rate, tm: tea material, ti: tea infusion).*

4.4.4 Assessment of dietary exposure to flavouring substances of 'forest fruit' tea

4.4.4.1 Exposure to flavouring substances via consumption of a cup tea

Table 32 summarises the calculation of exposure to flavouring substances occurring in 'forest fruit' tea by drinking one cup of tea beverage (200 mL) based on (i) available use levels from 'Fenaroli's handbook of flavor ingredients' (Burdock 2005), (ii) the median contents determined in the 'fruit tea' material, and (iii) the median contents determined in the 'fruit tea' material and the transfer rates into the hot water-infusion. The comparison shows that the sole consideration of use levels reported by the industry (FEMA) mostly ends up in by far too high assumptions for exposure (factors 4 for vanillin – 1728 for benzaldehyde); on the other hand, the exposure to raspberry ketone is underestimated by factor 5. The differences between the values calculated on the basis of the experimentally determined median contents either with or without consideration of the transfer rates are not as striking as those observed for Earl Grey tea. However, they are in an order of magnitude that they cannot be neglected in the course of exposure calculations as part of a safety assessment.

Table 32. Comparison of the amounts of flavouring substances in a tea cup (200 mL) calculated on the basis of use levels provided by industry vs. experimentally determined median contents and transfer rates.

	calculations based on		
	usual use levels[a]	median contents [µg / 2.5 g][b]	median contents and transfer rates [µg/200 mL][c]
	[µg / 200 mL tea beverage]		
esters			
3-methylbutyl acetate	10178	234	103
benzyl acetate	988	58.2	47.1
(*Z*)-3-hexenyl acetate	460	52.8	26.8
2-methylpropyl acetate	1108	26.25	19.9
ketones / lactones			
raspberry ketone	552	3508	2708
α-ionone	1246	82.1	40.7
β-damascenone	-[d]	30.5	17.5
γ-undecalactone	650	24.2	14.1
β-ionone	310	34.0	13.4
aldehydes			
vanillin	7876	2615	1929
benzaldehyde	5874	4.2	3.4
alcohols			
(*Z*)-3-hexenol	1170	31.6	22.6
eugenol	266	2.4	2.3
other			
methyl isoeugenol	490	6.4	4.2

[a] *Normal use levels for non-alcoholic beverages provided by industry in Fenaroli's handbook of flavor ingredients (Burdock 2005);* [b] *experimentally determined contents in 'forest fruit' tea material;* [c] *values refer to 200 mL tea infusion prepared from a 2.5 g tea bag;* [d] *not available.*

4.4.4.2 Assessment of dietary exposure to flavouring substances

To assess the dietary exposure to flavouring substances via consumption of ‘forest fruit’ tea, the data sets underlying Table 32 were combined with tea (herbal and fruit tea) consumption data from the German National Nutrition Survey of 2005/2006 (NVS II: MRI 2008). Assuming that all consumed tea would be ‘forest fruit’ tea, the average exposure was calculated using the median contents of the flavouring substances in the tea material, the transfer factors, and the mean consumption of tea beverage. High exposures could not be calculated because high consumption data such as the 97.5th perc. were not available. The data shown in Table 33 refer to consumers aged from 14-80 years. In contrast to data for black tea, the consumptions of this type of tea differed between genders; women drank more than twice than males (318 and 149 g/d), resulting in two-fold higher dietary exposures to all flavouring substances.

An exposure calculation via the mTAMDI revealed similar values as those for female consumers of the NVS II due to the similar consumption amount for the food category ‘non-alcoholic beverages’ of 324 g/d (see 2.2.1).

Table 33. Dietary exposure of flavouring substances occurring in forest fruit tea based on consumption data of the ‘German National Nutrition Study II’ (MRI 2008) for herbal- and fruit tea (male: 149 g/d; female: 318 g/d).

substance	dietary exposure [µg/d]	
	male	female
esters		
3-methylbutyl acetate	76	163
benzyl acetate	35	75
(*Z*)-3-hexenyl acetate	20	43
2-methylpropyl acetate	15	32
ketones / lactones		
raspberry ketone	2014	4298
α-ionone	30	65
β-damascenone	13	28
γ-undecalactone	11	22
β-ionone	10	21
aldehydes		
vanillin	1437	3067
benzaldehyde	3	5
alcohols		
(*Z*)-3-hexenol	17	36
eugenol	3	7
other		
methyl isoeugenol	2	4

4.4.5 Conclusion

Quantification of 14 flavouring substances occurring in ‘forest fruit’ tea was performed via a standard addition method. The contents of flavouring substances varied in a broad range from 1.0 µg/g tea for eugenol to 1046 µg/g tea for vanillin (Table 31).

Similar to the results obtained for Earl Grey tea, the calculation of intake of these flavouring substances via consumption of one cup of tea only on the basis of publicly available use levels for the broad food category ‘non-alcoholic beverages’ resulted in drastic overestimations (e.g. up to factor 1728 for benzaldehyde) or underestimations (e.g. factor 5 for raspberry ketone).

The transfer rates of the flavouring substances from the tea material into the tea beverage were influenced by parameters such as polarity and boiling point. The observed discriminations upon the hot water-infusion step were not as pronounced as for linalyl acetate. However, the example of a ‘fruit tea’ confirmed the need to take into account transfer rates and the respective correction factors when performing intake assessments of flavouring substances from processed or prepared foods.

4.5 Sensory evaluations

4.5.1 Sensory evaluation of Earl Grey teas

As shown before, the actual amounts of flavouring substances in the final tea beverage did not reflect the initial amounts occurring in an Earl Grey tea bag. Particularly, the content of linalyl acetate, the quantitatively dominating flavouring compound in the tea bag, is reduced by 98%; linalool showed a transfer rate of 66%. In addition, the enantiomeric distribution of linalool, the hydrolysis product formed from linalyl acetate, was nearly racemic (66.2% (*R*) : 33.8% (*S*)). The aim of the final part of the thesis was therefore to investigate the importance of these effects regarding the sensory qualities of the final tea beverage. The experiments should clarify the impact of linalyl acetate, playing a major role in the aroma of bergamot oil (Sawamura et al. 2006), on the sensory characteristics of an Earl Grey tea infusion.

4.5.1.1 Time-dependent correction of the concentration of linalyl acetate

As demonstrated in chapter 4.2.5.2, linalyl acetate is not only unstable in the course of a hot water infusion but also in a cold (20°C) aqueous solution. This has to be taken into consideration when determining odour threshold values (OTV) of linalyl acetate in water. To minimize the effect of degradation, the panellists were asked to participate in the sensory evaluation within 3 hours. After this maximum permitted time, 87.4% (in water) and 88.6% (in tea infusion), respectively, of the initial amounts of linalyl acetate were left. Therefore, the concentrations were corrected depending on the individual time-point of the sensory evaluation for each panellist. Based on the kinetics observed for linalyl acetate in cold water and in a cold tea infusion (section 4.2.5.2), polynomial equations of the logarithmic values of time plotted against the logarithmic concentration values of linalyl acetate were derived (Figure 50). The underlying time-dependent concentrations of linalyl acetate are compiled in Table 35 and 36.

These regressions allowed a time-dependent correction of the actual concentration of linalyl acetate perceived by the panellist; they do, of course, not consider the potential contributions of the formed reaction and degradation products.

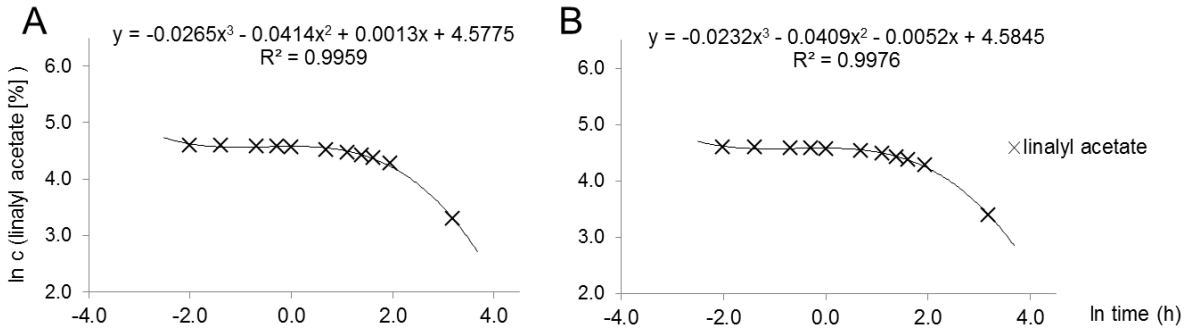


Figure 50. Degradation of linalyl acetate in (A) water and (B) tea infusion stored at 20°C depending on time (8 min - 24 h). Double logarithmic scaled (values see Appendix Table 52 and 53).

4.5.1.2 Odour thresholds of flavouring substances in Earl Grey tea

Odour threshold of both racemic as well as enantiomerically pure reference compounds of linalool and linalyl acetate were determined. Additionally, a mixture of linalool enantiomers of 66.2% (*R*) and 33.8% (*S*), reflecting the enantiomeric distribution obtained after hydrolysis of linalyl acetate in the course of a hot water infusion (see 4.2.3.2), was evaluated. Furthermore, odour thresholds were determined for (*R*)-limonene, (*S*)-β-pinene and γ-terpinene. All evaluations were performed in water as well as in a tea infusion matrix.

As shown in Table 34, OTVs in water were generally lower (4-180 times) than those in tea infusion. The difference was least pronounced for enantiomerically pure (*R*)-limonene and most for (*R*)-linalool. (*R*)-linalool had a 17-fold higher odour threshold than racemic linalool in water and a two-fold higher value in tea infusion. The slight increase of the proportion of (*R*)-linalool from 50% to 66% had a distinct effect on the odour threshold in water (from 3.4 to 0.6 µg/L); the influence in tea infusion was less pronounced. The OTVs of racemic linalyl acetate were 7 (water) and 4 (tea infusion) times higher than those of racemic linalool; for (*R*)-linalyl acetate a 100 and 8 times higher OTV was determined than for (*R*)-linalool. The differences between the OTVs of racemic and (*R*)-linalyl acetate in both matrices were negligible. However, the impact of the tea infusion in contrast to water was again distinctive. For monoterpene hydrocarbons, the determined odour thresholds were considerably higher than those of the oxygenated flavouring substances linalool and linalyl acetate.

The quite high standard deviations illustrate the differences of inter-individual odour thresholds and the importance of the calculation of group thresholds via the geometric mean and not the harmonic one (average), thus avoiding the shift of the overall value up or down towards the outlier.

The experimentally determined OTVs in water for (*R*)-linalool, limonene and γ-terpinene fitted into the range of values cited in the literature (Belitz and Grosch 1992; Padrayuttawat et al. 1997; Boonbumrung et al. 2001; Czerny et al. 2008; Miyazawa et al. 2010); for linalyl

acetate, (*S*)-β-pinene and racemic linalool even smaller values were observed. For the first time, the odour threshold in water for (*R*)-linalyl acetate was determined (the enantiomeric distribution of linalyl acetate used by Tateo et al. 2000 is not clear), as well as OTVs in tea infusion for racemic linalool, (*R*)-linalool, racemic linalyl acetate, (*R*)-linalyl acetate, (*R*)-limonene, (*S*)-β-pinene and γ-terpinene.

Table 34. Odour thresholds of flavouring substances in water and in tea infusion of non-flavoured black tea.

substance	enantiomeric proportion [%][a]	OTV [µg/L]			
		in-house panel			literature
		water	tea infusion	factor[b]	water
linalool	98.9 (*R*)	0.2 ± 0.9	36 ± 97	180	0.087[c] - 0.8[d]
linalool	66.2 (*R*)	0.6 ± 0.8	55 ± 514	92	-
linalool	50.0 (*R*)	3.4 ± 4.4	66 ± 72	19	6[e,f]
linalyl acetate	98.3 (*R*)	20 ± 67	273 ± 70	14	50[g]
linalyl acetate	49.6 (*R*)	24 ± 20	274 ± 311	11	
limonene	>98.8 (*R*)	319 ± 298	1336 ± 1730	4	200[h]-1200[d,i]
β-pinene	98.9 (*S*)	651 ± 1052	5648 ± 9327	9	4160[d,i]
γ-terpinene	-	227 ± 897	3041 ± 1629	13	260[i,j]

[a] enantiomeric purities relate to volatile compounds used by the in-house panel, determined on heptakis(2,3-di-O-ethyl-6-O-tert-butyldimethyl-silyl)-β-cyclodextrin (GC-system II); [b] factor = odour threshold in tea infusion [µg/L] : odour threshold in water [µg/L]; [c] Czerny et al. 2008; [d] Padrayuttawat et al. 1997; [e] Buttery et al. 1971; [f] Guadagni et al. 1966; [g] Tateo et al. 2000, enantiomeric distribution not specified; [h] Belitz and Grosch 1992; [i] Boonbumrung et al. 2001; [j] Miyazawa et al. 2010.

4.5.1.3 Odour activity values in Earl Grey tea

The odour activity value (OAV) is a measure for the characterisation of the sensory impact of single substances to the odour of a complex aroma sample; synergistic as well as antagonistic impacts are not considered (Rothe and Thomas 1963). It is calculated as the ratio between the concentration of a volatile in a sample and the OTV of this substance. Volatile substances exhibiting OAV´s ≥ 1 are considered to be odour-active. It is assumed that substances having higher odour activity values contribute more to the overall aroma.

Among the investigated substances, linalool showed the highest impact on the aroma of Earl Grey tea infusions prepared with a tea bag of 2 g containing median values of 90 Earl Grey teas purchased in the European Union (see 4.1.3), independent of the enantiomeric distribution (Table 35). Also β-pinene and γ-terpinene showed OAVs that were significantly higher than the critical value 1.

Table 35. Odour activity values (OAV´s) calculated for median contents in a 200 mL tea infusion prepared with 2 g tea (tea bag) of Earl Grey tea.

substance	enantiomeric percentage [%][a]	median contents in Earl Grey tea		OTV[c] [µg/200 mL]	OAV
		in 2 g dry tea [µg]	in 200 mL infusion [µg][b]		
linalool	98.9 (*R*)	2266	2046	7	287
linalool	66.2 (*R*)	2266	2046	11	185
linalool	50.0 (*R*)	2266	2046	13	155
linalyl acetate	98.3 (*R*)	4045	78	55	1.4
linalyl acetate	49.6 (*R*)	4045	78	55	1.4
limonene	>98.8 (*R*)	1329	359	267	1.3
β-pinene	98.9 (*S*)	135165	34766	1130	31
γ-terpinene	-	95675	24609	608	40

[a] enantiomeric purities relate to volatile compounds used by the in-house panel, they were determined on heptakis(2,3-di-O-ethyl-6-O-tert-butyldimethyl-silyl)-β-cyclodextrin (GC-system II); [b] On the basis of reaction rates (Table 13) in combination of transfer rates determined in spiking experiments with linalyl acetate (1.9%); linalool (66.4%), limonene (26.5%); γ-terpinene (25.7%), also applied to limonene and β-pinene. [c] OTV: odour threshold values calculated for 200 mL tea (Table 34).

On the other hand, for linalyl acetate, which is a prominent aroma-contributing compound in bergamot essential oil (Sawamura et al. 2006), the amount transferred from flavoured Earl Grey tea into the beverage results only in a rather low odour activity value of 1.4. Therefore, the contribution of linaly acetate to the overall aroma of the final tea beverage seems negligible.

However, further studies would be needed to investigate the contributions of the degradation products originating from linalyl acetate in the course of the hot water-infusion to the overall aroma of the final tea beverage.

4.5.2 Sensory evaluation of 'forest fruit' tea infusions

Fourteen flavouring substances were quantified in fruit tea material and infusions. An assessment of their impact on the overall aroma of infusions was performed by determining odour activity values. Table 36 shows the concentrations of the 14 selected flavouring substances determined in a forest fruit tea and the OAVs calculated on the basis of OTVs reported in literature (Sega et al. 1967; Keith and Powers 1968; Buttery et al. 1971; Schmidlin-Mészáros 1971; Pyysalo et al. 1977; Buttery et al. 1990; Semmelroch et al. 1995; Masanetz and Grosch 1998; Gladisch 2007; Pino and Quijano 2012; Hempfling et al. 2013). Although raspberry ketone and vanillin showed by far the highest concentrations in tea infusions, their OAVs are lower than those of minor substances like β-damascenone and β-ionone. The first listed ingredients on the label (except 'flavour') were hibiscus, rose hips, apples, and elderberries. β-Damascenone has been described in apples (Kato et al. 2003) and elderberries (Kaack 2008), ionones are typically used for synthetic aroma compositions of red berry fruits (Pickenhagen and Smith 1979). γ-Undecalactone was found in blackberries (Du et al. 2010), which were also mentioned as ingredient on the tea label. Eugenol, reported to occur in the main ingredient hibiscus (Ramirez-Rodrigues et al. 2011)), only showed an OAV of 1.6. Other flavouring substances, such as benzaldehyde, a typical flavour compound in cherries (Schmid and Grosch 1986), benzyl acetate and methyl isoeugenol, were not odour-active (OAV < 1).

Table 36. Concentrations and odour activity values of flavouring substances in ´forest fruit´ tea.

substance	content [µg/L][a]	OTV [µg/L][b]	OAV[c]
β-damascenone	87.4	0.002[d]	43715
β-ionone	67.1	0.007[d]	9581
α-ionone	203.4	0.4[e]	508
vanillin	7715.0	25[f]	309
raspberry ketone	10813.2	100[g]	108
γ-undecalactone	70.6	1.82[h]	39
3-methylbutyl acetate	512.8	19[i]	27
(*Z*)-3-hexenyl acetate	134.2	8[j]	17
(*Z*)-3-hexenol	113.1	39[j]	3
eugenol	113.1	70[d]	1.6
benzyl acetate	235.3	270[k]	<1
2-methylpropyl acetate	99.3	441[i]	<1
benzaldehyde	17.2	350[m]	<1
methyl isoeugenol	21.1	770[n]	<1

[a] calculated for a tea bag of 2.5 g brewed with 200 mL water; [b] odour threshold values according to literature; [c] odour activity value; [d] Buttery et al. 1990; [e] Keith and Powers 1968; [f] Semmelroch et al. 1995; [g] Schmidlin-Mészáros 1971; [h] Gladisch 2007; [i] Sega et al. 1967; [j] Masanetz and Grosch 1998; [k] Pyysalo et al. 1977; [l] Hempfling et al. 2013; [m] Buttery et al. 1971; [n] Pino and Quijano 2012.

4.5.3 Conclusion

The sensory evaluation demonstrated that the odour threshold values (OTVs) of linalool and linalyl acetate, the two major flavouring substances of Earl Grey teas, differed significantly in water and in tea infusion. OTVs in water for (*R*)-linalool were 180-fold lower than those in tea infusion; for (*R*)-linalyl acetate, a 14-fold factor was determined. For the non-oxygenated flavouring substances limonene, β-pinene and γ-terpinene, the differences in OTVs depending on the matrix were less pronounced.

The determination of odour activity values (OAV) demonstrated that linalool is the major aroma-contributing compound in the Earl Grey tea beverage. The hydrocarbons ß-pinene and γ-terpinene also exhibited OAV > 1. In contrast, owing to the loss of linalyl acetate in the course of the infusion step, the contribution of this flavouring substance to the final tea beverage is negligible (OAV: 1.4).

Further studies would be needed to determine whether it might be possible to leave out linalyl acetate when flavouring an Earl Grey tea with a chemically synthesized substance or whether the hydrolysis/degradation products resulting form this flavouring substance in the course of the hot water treatment are essential for the aroma of the tea beverage.

5 SUMMARY

Intake estimation plays a central role in the course of the safety evaluation of flavouring substances. To assess uncertainties, actual data regarding the occurrence and concentration levels of flavouring substances were elaborated in commercially available flavoured foods, using so-called ‘Earl Grey’ tea as example. The contents of the consistently occurring monoterpenes linalyl acetate, linalool, limonene, β-pinene, and γ-terpinene were investigated in 90 tea samples purchased in 10 member states of the European Union.

Rather narrow content ranges were observed for the major compounds linalyl acetate and linalool. The median contents amounted to 2.0 mg/g tea for linalyl acetate and to 1.1 mg/g tea for linalool. The statistical analysis of the data set revealed that factors such as the country of purchase, the type of source (international/national brands, private label brands or speciality tea shops) or the enantiomeric distributions of linalyl acetate and linalool, had no significant impact on the contents of the flavouring substances. Only in teas sold as loose leaves the median contents of linalyl acetate and linalool were higher (66% and 39%, respectively) than in teas offered in tea bags. Significant losses of flavouring substances were observed upon storage of teas, indicating an impact of the type of packaging and the flavouring technology on the contents of the flavouring substances.

For calculation of intakes not only information on the contents of flavouring substances in the tea material but also data on their transfer into the final tea beverage via hot water infusion are required. Spiking experiments revealed a transfer rate of 66% for linalool. In contrast, the transfer rate of linalyl acetate was only 1.9%; in turn, the hydrolysis product linalool (17.0 %) and a spectrum (20%) of degradation and rearrangement products (monoterpene alcohols, esters and hydrocarbons) were present in the tea beverage. The transfer rates were followed depending on the length of the infusion and the amount of flavouring substance added to the tea material. The impact of the hot water-treatment on the enantiomeric compositions of linalool and linalyl acetate was determined, and structure-dependent experiments were performed by variation of the acyl and the alcohol moiety of the monoterpene ester. The data revealed that the presence of a tertiary ester combined with an allylic system and an additional double bond in 6,7-position are the key structural features responsible for the instability of linalyl acetate in the course of the tea infusion step.

On the basis of the elaborated data, comparative intake assessments were performed (i) using publicly available use levels for linalool and linalyl acetate in the food category ‘non-alcoholic beverages’, (ii) using the median contents of linalool and linalyl acetate determined in commercial Earl Grey teas, and (iii) using both, the determined median contents of these flavouring substances in commercial products and their transfer rates into the tea beverage in the course of the tea infusion step. The sole consideration of the publicly available use

levels resulted in an underestimation of the intake of linalool by a factor of approximately 3 (median) and 5 (97.5^{th}) perc., respectively, and an overestimation of the intake of linalyl acetate by a factor of 14 (median) and 6 (high), respectively. If the actual transfer rates into the tea beverage are not taken into account, the intake of linalyl acetate is overestimated by a factor of approximately 50.

Based on tea consumption data from the National Diet and Nutrition Survey of the United Kingdom, the exposure to linalyl acetate ranges from 0.2 mg/day (average) to 1.8 mg/day (95^{th} percentile). The corresponding values for linalool are 4.2 mg/day (average) and 46.6 mg/day (high). The exposure to linalool via consumption of the tea beverage is approximately 26 times higher than that of linalyl acetate, although in the flavoured tea leaves the median content of linalyl acetate is approximately 1.8 times higher than that of linalool.

In order to extend the data elaborated for Earl Grey teas, contents of flavouring substances and their transfer rates upon hot water infusions were also determined in another type of flavoured teas, labelled as ‘forest fruit’ teas. Fourteen flavouring substances were quantified in the tea material and in the tea beverage using a standard addition method. The transfer rates were shown to be mainly dependent on polarity and boiling point. The data confirmed that similar to Earl Grey tea, the calculation of the intake of flavouring substances via consumption of one cup of ‘forest fruit’ tea solely on the basis of publicly available use levels for the broad food category ‘non-alcoholic beverages’ resulted in overestimations (e.g. factor 1728 for benzyladehyde) or in underestimations (e.g. factor 5 for raspberry ketone).

Considering that the actual amounts of flavouring substances in the final tea beverage did not reflect the initial amounts occurring in the flavoured tea material, the final part of the studies was devoted to a preliminary investigation of the impact of this phenomenon on the sensory properties of the tea beverage. The determination of odour activity values (OAV) demonstrated that linalool is the major aroma-contributing compound in the Earl Grey tea beverage. The hydrocarbons ß-pinene and γ-terpinene also exhibited OAV > 1. In contrast, owing to the loss of linalyl acetate in the course of the infusion step, the contribution of this flavouring substance to the final tea beverage seems negligible (OAV: 1.4).

6 ZUSAMMENFASSUNG

Für die Sicherheitsbewertung von Aromastoffen spielt die Abschätzung deren Aufnahmemengen eine entscheidende Rolle. Um Unwägbarkeiten einzuschätzen, wurden kommerziell erhältliche aromatisierte Lebensmittel auf deren tatsächliche Gehalte an Aromastoffen untersucht. Als Beispiel wurde sogenannter ‘Earl Grey’ Tee gewählt. In 90 Teeproben aus 10 Mitgliedsstaaten der Europäischen Union wurden die Gehalte der Monoterpene Linalylacetat, Linalool, Limonen, β-Pinen und γ-Terpinen, die durchgängig enthalten waren, bestimmt.

Die Gehalte der Hauptkomponenten Linalylacetat und Linalool schwankten nur in relativ engen Grenzen. Der Median für Linalylacetat lag bei 2.0 mg/g Tee und bei 1.1 mg/g Tee für Linalool. Statistische Auswertungen des Datensatzes zeigten, dass Faktoren wie Erwerbsland, Art der Quelle (internationale/nationale Marken, Handels-/ Eigenmarken oder Teespezialitätenläden) oder die Enantiomerenverteilungen von Linalylacetat und Linalool die Gehalte dieser Aromastoffe nicht signifikant beeinflussten. Einzig als lose Tees verkaufte Proben wiesen signifikant höhere Gehalte an Linalylacetat und Linalool auf als solche, die in Teebeuteln angeboten wurden (66% bzw. 39% bezogen auf den Median). Es zeigten sich darüber hinaus signifikante Verluste der Aromastoffe im Zuge der Lagerung, welche auf die Verpackungsart und die Aromatisierungstechnologie zurückgeführt werden konnten.

Um letztendlich die Aufnahmemenge berechnen zu können, werden neben den Gehalten im Teematerial auch die Transferraten in das verzehrfertige Teegetränk benötigt. Versuche, bei denen die Aromastoffe zugesetzt wurden, zeigten für Linalool eine Transferrate von 66%, für Linalylacetat jedoch lediglich von 1.9%. Im Gegenzug traten im Teegetränk das Hydrolyseprodukt Linalool (19.0%) und weitere Abbau- und Umlagerungsprodukte (20%) (Monoterpenalkohole, -ester und –kohlenwasserstoffe) auf. Die Transferraten wurden in Abhängigkeit von der Infusionszeit und der dem Teematerial zugesetzten Menge an Aromastoffen verfolgt. Des Weiteren umfassten die Untersuchungen den Einfluss der Heißwasserinfusion auf die Enantiomerenverteilungen von Linalool und Linalylacetat, sowie strukturabhängige Experimente, bei denen entweder der Acyl- oder der Alkoholrest des Monoterpenesters variiert wurde. Das Vorliegen eines tertiären Esters kombiniert mit einem allylischen System und einer zusätzlichen Doppelbindung an der 6,7-Position erwies sich als das für die Instabilität von Linalylacetat im Zuge des Heißwasseraufgusses verantwortliche Schlüsselmerkmal.

Auf der Basis dieser Daten konnte eine Aufnahmeabschätzung vorgenommen werden. Dabei wurden (i) öffentlich zugängliche Verwendungsmengen für Linalool und Linalylacetat in der Lebensmittelkategorie ‘Nicht-alkoholische Getränke’, (ii) Mediangehalte von Linalool und Linalylacetat in kommerziellen Earl Grey Teeproben und (iii) sowohl diese

Mediangehalte der untersuchten Aromastoffe also auch deren Transferraten im Zuge der Teeinfusion in das verzehrfertige Getränk berücksichtigt. Die alleinige Berücksichtigung der öffentlich zugänglichen Verwendungsmengen führte dazu, dass die Aufnahmemenge von Linalool um den Faktor von ca. 3 (Median) bzw. 5 (97.5th Perzentile) unterschätzt, die von Linalylacetat dagegen um den Faktor 14 (Median) bzw. 6 (97.5th Perzentile) überschätzt wurde. Wenn die tatsächliche Übergangsrate von Linalylacetat in das Getränk nicht berücksichtigt wird, resultiert sogar eine Überschätzung um einen Faktor von ca. 50.

Legt man Verzehrdaten der Nationalen Ernährungsstudie des Vereinigten Königsreichs (UK) zugrunde, reichte die Exposition von Linalylacetat von 0.2 mg/Tag (Mittelwert) bis 1.8 mg/Tag (95th Perzentile). Die entsprechende Berechnung für Linalool ergab Werte von 4.2 mg/Tag (Mittelwert) und 46.6 mg/Tag (95th Perzentile). Die Aufnahmemenge über den Verzehr eines Teegetränks von Linalool ist damit ca. 26-mal höher als die von Linalylacetat, obwohl der Mediangehalt von Linalylacetat in den Teeblättern um den Faktor 1.8 über dem von Linalool liegt.

Um den für Earl Grey Tee erarbeiteten Datensatz zu erweitern, wurden Gehalte an Aromastoffen und die entsprechenden Transferraten für einen weiteren aromatisierten Tee untersucht, welcher als ‘Waldfruchttee’ deklariert war. Mit Hilfe einer Standardadditions-methode wurden vierzehn Aromastoffe sowohl im Teematerial als auch im verzehrfertigen Getränk quantifiziert. Die Transferraten erwiesen sich als hauptsächlich von der Polarität und dem Siedepunkt der Substanzen abhängig. Die Untersuchungen bestätigten, dass – ähnlich wie bei Earl Grey Tee – die Berechnung der Aufnahmemengen von Aromastoffen über eine Tasse ‘Waldfruchttee’ bei lediglicher Einbeziehung öffentlich zugänglicher Verwendungsmengen in ‘Nicht-alkoholischen Getränken’ in einer Überschätzung (z.B. um den Faktor 1728 für Benzaldehyd) bzw. in einer Unterschätzung (z.B. Faktor 5 für Himbeerketon) resultierten.

Die Tatsache berücksichtigend, dass die Gehalte an Aromastoffen im verzehrfertigen Teegetränk nicht die des ursprünglichen aromatisierten Teematerials widerspiegeln, konzentrierte sich der letzte Teil der Studien darauf, welche Einflüsse dieses Phänomen auf die sensorischen Eigenschaften des Teegetränks ausübt. Die Bestimmung der Aromawerte zeigte, dass Linalool den größten Beitrag zum Aroma in Earl Grey Tee leistet. Die Kohlenwasserstoffe ß-Pinen und γ-Terpinene weisen ebenfalls Aromawerte > 1 auf. Im Gegensatz dazu scheint der Beitrag von Linalylacetat durch die deutliche Verringerung dieser Substanz im Zuge des Heißwasseraufgusses mit einem Aromawert von 1.4 vernachlässigbar.

7 REFERENCES

Aargau. **2006**. *Authentizität von Bergamotte-Aroma bei „Earl Grey“ Schwarztee. In: Amt für Verbraucherschutz Kanton Aargau. Jahresbericht 2006. Lebensmittelkontrolle, Veterinärdienst, Chemiesicherheit [Authenticity of bergamot flavor in "Earl Grey" black tea. In: Office of Consumer Protection of the canton of Aargau. Annual Report 2006. Food testing, veterinary services, chemical safety]*. Aargau (Schweiz). p. 67-68

Adams RP. **1995**. *Identification of essential oil components by gas chromatography and mass spectroscopy*. Carole Stream: Allured Publ. Corp.

Agnel R, Teisseire P. **1984**. *Essential oil of French lavender - its composition and its adulteration*. Perfum. Flavor. 9(5):53–6.

Alasalvar C, Topal B, Serpen A, Bahar B, Pelvan E, Gökmen V. **2012**. *Flavor Characteristics of Seven Grades of Black Tea Grown in Turkey*. J. Agric. Food Chem.60:6323–32.

Arce A, Marchiaro A, Rodriguez O, Soto A. **2006**. Essential oil terpenless by extraction using organic solvents or ionic liquids. Aiche J. 52(6):2089–97.

Arcella D, Leclercq C. **2005**. Assessment of dietary intake of flavouring substances within the procedure for their safety evaluation: advantages and limitations of estimates obtained by means of a per capita method. Food Chem. Toxicol. 43(1):105–16.

Ashari S. **1999**. *Citrus bergamia Risso & Poiteau*. In: Oyen LPA, Dung NX, editors. Plant Resources of South-East Asia 19: Essential-oil plants. Leiden, The Netherlands: Backhuys Publishers. p. 83–6.

Ashwood-Smith MJ, Poulton GA, Barker M, Mildenberger M. **1980**. 5-Methoxypsoralen, an ingredient in several suntan preparations, has lethal, mutagenic and clastogenic properties. Nature 285(5764):407–9.

Azin NZ, Parviz AA, Kambiz L, Shiva RH. **2006**. *Comparison of volatile compounds of Iranian black teas during process*. Asian J. Chem. 18(3):2249–60.

Baigrie BD, Chisholm MG, Mottram DS. **1996**. *The effect of processing on chiral aroma compounds in fruits and essential oils*. In: Flavour science: Recent developments. Cambridge, UK: Royal Society of Chemistry. p. 151–7.

Baxter RL, Laurie WA, McHale D. **1978**. T*ransformations of monoterpenoids in aqueous acids. The reactions of linalool, geraniol, nerol and their acetates in aqueous citric acid.* Tetrahedron 34(14):2195–9.

Belitz HD, Grosch W. 1992. *Lehrbuch der Lebensmittelchemie*. 4th ed. Berlin, Germany: Springer. pp 340.

Belsito EL, Carbone C, Di Gioia ML, Leggio A, Liguori A, Perri F, Siciliano C, Viscomi MC. **2007**. *Comparison of the Volatile Constituents in Cold-Pressed Bergamot Oil and a Volatile Oil Isolated by Vacuum Distillation*. J. Agric. Food Chem. 55(19):7847–51.

Bergsten C, Andersen NL, Gry J. **2002**. *Methods for calculating the intake of flavourings*: TemaNord 2002:550. Copenhagen: Nordic Council of Ministers.

Bernreuther A, Schreier P. **1991**. M*ultidimensional gas chromatography/mass spectrometry: A powerful tool for the direct chiral evaluation of aroma compounds in plant tissues. II. Linalool in essential oils and fruits*. Phytochem. Anal. 2(4):167–70.

Bondarovich HA, Giammarino AS, Renner JA, Shephard FW, Shingler AJ, Gianturco MA. **1967**. *Some Aspects of the Chemistry of Tea. A Contribution to the Knowledge of the Volatile Constituents*. J. Agric. Food Chem. 15(1):36–47.

Boonbumrung S, Tamura H, Mookdasanit J, Nakamoto H, Ishihara M, Yoshizawa T, Varanyanond W. **2001**. *Characteristic Aroma Components of the Volatile Oil of Yellow Keaw Mango Fruits Determined by Limited Odor Unit Method*. Food Sci. Technol. Res. 7(3):200–6.

Bornträger A. **1896**. *Zur Prüfung des Bergamottöles auf Reinheit* [Examination of oil of bergamot]. Zeit. anal. Chem. 35:35–8.

Breitmaier E, Jung G. **2012**. *Organische Chemie: Grundlagen, Verbindungsklassen, Reaktionen, Konzepte, Molekülstruktur, Naturstoffe, Syntheseplanung, Nachhaltigkeit*. 7th ed. Stuttgart, New York, NY: Thieme.

Brown DGW, Clapperton JF, Meilgaard MC, Moll M. **1978**. *Flavor thresholds of added substances*. J. Am. Soc. Brew. Chem. 36:73–80.

Burdock GA. **2005**. *Fenaroli's handbook of flavor ingredients*. 5th ed. Boca Raton, FL, USA: CRC Press (Taylor & Francis Group).

Buttery RG, Seifert RM, Guadagni DG, Ling LC. **1971**. *Characterization of additional volatile components of tomato*. J. Agric. Food Chem. 19(3):524–9.

Buttery RG, Teranishi R, Ling LC, Turnbaugh JG. **1990**. *Quantitative and sensory studies on tomato paste volatiles*. J. Agric. Food Chem. 38(1):336–40.

CAC (Codex Alimentarius Commission). **2008**. *Procedural manual: Eighteenth edition. Codex alimentarius*. Rome: *Joint FAO/WHO Food Standards Programme*. Available from: ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_18e.pdf; cited 2013 Sep 18.

CAC (Codex Alimentarius Commission). **2011**. *Procedural manual: Twentieth edition. Rome: Joint FAO/WHO Food Standards Programme*. Available from: ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_20e.pdf; cited 2013 Sep 18.

Cadby P. **1996**. *Estimating intakes of flavoring substances*. Food Addit. Contam. 13(4):453–60.

Casabianca H, Graff J. **1994**. *Separation of linalyl acetate enantiomers: Application to the authentication of bergamot food products*. J. High. Resolut. Chromatogr. 17(3):184–6.

Casabianca H, Graff J, Jame P, Perrucchietti C, Chastrette M. **1995**. *Application of hyphenated techniques to the chromatographic authentication of flavors in food products and perfumes*. J. High. Resolut. Chromatogr. 18(5):279–85.

Casabianca H, Chau C. **1998**. *Chiral analysis of the main components of bergamot oils*. Riv. Ital. EPPOS (Spec. Num.):46–58.

Casabianca H, Graff J, Faugier V, Fleig F, Grenier C. **1998**. *Enantiomeric distribution studies of linalool and linalyl acetate. A powerful tool for authenticity control of essential oils*. J. High. Resolut. Chromatogr. 21(2):107–12.

Chan L, Mehra A, Saikat S, Lynch P. **2013**. *Human exposure assessment of fluoride from tea (Camellia sinensis L.): A UK based issue?* Food Res. Int. 51(2):564–70.

Chanotiya CS, Yadav A. **2009**. *Enantiomeric composition of (3R)-(-)- and (3S)-(+)-linalool in various essential oils of Indian origin by enantioselective capillary gas chromatography-flame ionization and mass spectrometry detection methods*. Nat. Prod. Commun. 4(4):563–6.

Chapot H. **1962**. *Le bergamotier [The bergamot tree]*. Al Awamia 5:1–27.

Chemat F. **2010**. *Techniques for Oil Extraction. In: Sawamura M, editor. Citrus essential oils: Flavor and fragrance. Hoboken*, N.J: Wiley. pp 9–36

Clark BC JR., Chamblee TS, Iacobucci GA. **1989**. *Micellar-induced selectivity and rate enhancement in the acid-catalyzed cyclization and rearrangement of monoterpenes. The solvolysis of linalyl and geranyl acetates*. J. Org. Chem. 54(5):1032–6.

Consorzio Del Bergamotto. **1989**. *Il Bergamotto di Reggio Calabria*. Reggio Calabria: Domenico Laruffa, editor.

Cori O, Chayet L, Perez LM, Bunton CA, Hachey D. **1986**. *Rearrangement of linalool, geraniol, nerol and their derivatives*. J. Org. Chem. 51(8):1310–6.

Costa R, Dugo P, Navarra M, Raymo V, Dugo G, Mondello L. **2009**. *Study on the chemical composition variability of some processed bergamot (Citrus bergamia) essential oils*. Flavour Frag. J. 25(1):4–12.

Cotroneo A, Stagno dI, Trozzi A. **1992**. *On the genuineness of citrus essential oils. Part XXXIV. Detection of added reconstituted bergamot oil in genuine bergamot essential oil by high resolution gas chromatography with chiral capillary columns*. Flavour Frag. J. 7(1):15–7.

Crabalona LL. **1959**. *Évolution de l´acétate de linalyle et du linalol au cours de l´hydrodistillation extractive de certaines huiles essentielles. I. action des acides organiques sur l´acétate de linalyle et sur le linalol en présence d´eau. [Formation of linalyl acetate and linalool during extractive distillation of essential oils. I. Action of organic acids on linalyl acetate and linalool in the presence of water].* Fr. Ses Parfums 2(12):28–36.

Cramer GM, Ford RA, Hall RL. **1978**. *Estimation of toxic hazard - a decision tree approach*. Food Cosmet. Toxicol. 16(3):255–76.

Crispim S, Geelen A, Le Donne C, Vries J de, Sette S, Raffo A, Siebelink E, Ocke M, van't Veer P, Leclercq C, on behalf of the European Food Cons. **2010**. *Dietary exposure to flavouring substances: from screening methods to detailed assessments using food consumption data collected with EPIC-Soft software*. Food Addit. Contam.: Part A 27(4):433–46.

Czerny M, Christlbauer M, Christlbauer M, Fischer A, Granvogl M, Hammer M, Hartl C, Hernandez NM, Schieberle P. **2008***. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions*. Eur. Food Res. Technol. 228(2):265–73.

da Silva Pinto M. **2013**. *Tea: A new perspective on health benefits*. Food Res. Int. 53(2):558–67.

Davis RG. **1978**. *Increased bitter taste detection thresholds in Yucatan inhabitants related to coffee as a dietary source of niacin*. Chem. Senses 3(4):423–9.

Dellacassa E, Lorenzo D, Moyna P, Verzera A, Cavazza A. **1997**. *Uruguayan Essential Oils. Part V. Composition of Bergamot Oil*. J. Essent. Oil Res. 9(4):419–26.

Deng ZN, Gentile A, Nicolosi E, Continella G, Tribulato E. **1996**. *Parentage determination of some citrus hybrids by molecular markers*. Proc. Int. Soc. Citricul. 8:849–54.

Dhanaraj N, Nataraj CP, Seshadri R. **1986**. *Studies of incorporation and stability of model flavouring compounds on black tea*. J. Sci. Food Agric. 37(2):185–93.

Di Giacomo A. **2002**. *5. Development of the citrus industry: Historical note*. In: Dugo G, Di Giacomo A, editors. Citrus: The genus citrus. London; New York: Taylor & Francis. pp. 63–70.

Di Giacomo A, Di Giacomo G. **2002**. 8. *Essential oil production*. In: Dugo G, Di Giacomo A, editors. Citrus: The genus citrus. London; New York: Taylor & Francis. pp. 114–47.

DIN Deutsches Institut für Normung e. V. **1992**. *Zahlenangaben [Presentation of numerical data] (1333)*. Berlin: Beuth Verlag GmbH.

DIN Deutsches Institut für Normung e. V. **1998**. *Chemische Analytik - Verfahren der Standardaddition - Verfahren, Auswertung [Chemical analysis - Methods of Standard addition - Procedure, evaluation] (32633).* Berlin: Beuth Verlag GmbH.

Dippy JFJ, Williams FR. **1934**. *Chemical constitution and the dissociation constants of monocarboxylic acids. Part II*. J. Chem. Soc.:1888–92.

Dippy JFJ, Lewis RH. **1937**. *Chemical constitution and the dissociation constants of monocarboxylic acids. Part VI. (a) The polarity of vinyl and phenyl groups. (b) An apparent linear relation between dissociation constants and dipole moments*. J. Chem. Soc.:1008-13.

Dorries KM, Schmidt HJ, Beauchamp GK, Wysocki CJ. **1989**. *Changes in sensitivity to the odor of androstenone during adolescence*. Dev. Psychobiol. 22(5):423–35.

Du X, Kurnianta A, McDaniel M, Finn C, Qian M. **2010**. *Flavour profiling of 'Marion' and thornless blackberries by instrumental and sensory analysis*. Food Chem. 121(4):1080–8.

Duer WC, Robinson RA. **1971**. *Dissociation constants of phenylacetic acid and three substituted phenylacetic acids*. J. Chem. Soc. B 12:2375.

Dugo G, Cotroneo A, Verzera A, Donato MG, Del Duce R, Licandro G, Crispo F. **1991**. *Genuineness characters of Calabrian bergamot essential oil*. Flavour Frag. J. 6(1):39–56.

Dugo G, Mondello L, Cotroneo A, Bonaccorsi I, Lamonica G. **2001**. *Enantiomeric distribution of volatile components of citrus oils by MDGC*. Perfum. Flavor 26(1):20, 22-26, 28-35.

Dugo G, Bonaccorsi I, Sciarrone D, Schipilliti L, Russo M, Cotroneo A, Dugo P, Mondello L, Raymo V. **2012**. *Characterization of cold-pressed and processed bergamot oils by using GC-FID, GC-MS, GC-C-IRMS, enantio-GC, MDGC, HPLC and HPLC-MS-IT-TOF.* J. Essent. Oil Res. 24(2):93–117.

EC (European Commission). **2011**. *European research on environment and health funded by the Seventh Framework Programme: Volume 1*. Luxembourg: Publications Office of the European Union. Available from: ec.europa.eu/research/environment/pdf/fp7_catalogue_eh.pdf; cited 2013 Sep 24.

EFSA (European Food Safety Authority). **2004**. *Minutes of the 7th Plenary meeting on the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 2004 Jul 12–13. Annex*. Brussels, 2004 Sep 28. Available from: www.efsa.europa.eu/en/events/.../afc040712-m.pdf; cited 2013 Sep 23.

EFSA (European Food Safety Authority). **2008**. *Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies on a request from the European Commission on the safety of leaves from Morinda citrifolia L*. The EFSA Journal (2008) 769, 1-17. Available from: http://www.efsa.europa.eu/de/efsajournal/doc/769.pdf; cited 2013 Sep 23.

EFSA (European Food Safety Authority). **2010**. *Guidance on the data required for the risk assessment of flavourings to be used in or on foods*. EFSA J. 8:1623, 38 pp. Available from: www.efsa.europa.eu/en/scdocs/doc/1623.pdf; cited 2013 Sep 23.

EFSA (European Food Safety Authority). **2011**. *Scientific report of EFSA, overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances*. EFSA J. 9:2490, 33 pp. Available from: www.efsa.europa.eu/de/efsajournal/ doc/2490.pdf; cited 2013 Sep 23.

EHIA [European Herbal Infusions Association]. **2012**. *Compendium of Guidelines for Herbal and Fruit Infusions*. Available from: http://www.ehia-online.org/fileadmin/EHIA/PDF/EHIA_COMPENDIUM_OF_GUIDELINES_FOR_HERBAL_AND_FRUIT_INFUSIONS.pdf; cited 2013 Sep 17.

Erhardt W, Erich G, Bödeker N, Seybold S. **2008**. *Der große Zander: Enzyklopädie der Pflanzennamen. Band 2: Arten und Sorten*. Stuttgart (Hohenheim): Eugen Ulmer KG.

EU (European Union). **1996**. *Regulation (EC) No 2232/1996 laying down a Community procedure for flavouring substances used or intended for use in or on foodstuffs*. OJEU. L 299:1–4.

EU (European Union). **1999**. *Commission Decision of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996.* OJEU. L 84:1–137.

EU (European Union). **2000**. *Commission regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of regulation (EC) no 2232/96 of the European parliament and of the council*. Official Journal of the European Union. L 180:8–16.

EU (European Union). **2008**. *Regulation (EC) No 1334/2008 of the European parliament and of the council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending council regulation (EEC) No 1601/91, regulations (EC) No 2232/96 and (EC) No 110/2008 and directive 2000/13/EC.* OJEU. L 243:34–50.

EU (European Union). **2012**. *Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) no 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217(EC)*. OJEU. L 267:1–161.

Fang T, Goto M, Sasaki M, Hirose T. **2004**. *Combination of supercritical CO_2 and vacuum distillation for the fractionation of bergamot oil*. J. Agric. Food Chem. 52(16):5162–7.

Fantin G, Fogagnolo M, Maietti S, Rossetti S. **2010**. *Selective Removal of Monoterpenes from Bergamot Oil by Inclusion in Deoxycholic Acid*. J. Agric. Food Chem. 58(9):5438–43.

FAOSTAT (Food and Agriculture Organization of the United Nations); Available from: http://faostat.fao.org; cited 2013 Sept 18.

Federici CT, Roose ML, Scora RW. 2000. *RFLP analysis of the origin of Citrus bergamia, Citrus jambhiri, and Citrus limonia*. In: Goren R, Goldschmidt EE, editors. Proceedings of the First International Symposium on Citrus Biotechnology, held in Eilat, Israel, November 29-December 3, 1998. Leuven, Belgium: Secretariat of ISHS. Acta horticulturae. 535:55-62.

Fehr D. **1982**. *Bestimmung flüchtiger Inhaltsstoffe in Teezubereitungen. 1. Mitteilung: Freisetzung des ätherischen Öls aus Fenchelfrüchten [Determination of volatile constituents in tea preparations. 1. Liberation of volatile oils from fennel fruits]*. Pharm. Ztg. 127(46): 2520-2.

Feldheim W. **1994**. *Tee und Tee-Erzeugnisse*. Berlin: Blackwell-Wissenschafts-Verlag.

Ferrini AM, Mannoni V, Hodzic S, Salvatore G, Aureli P. **1998**. *Antimicrobial activity of bergamot oil in relation to chemical composition and different origin*. Riv. Ital. EPPOS (Spec. Num):139–53.

Finsterer J. **2002**. *Earl Grey tea intoxication*. The Lancet 359(9316):1484-1484.

Fischer AS. **2008**. *Characterisation of the odour-active compounds in peel oils of Jeruk Pontianak orange (Citrus nobilis Lour. var. microcarpa Hassk.) and Brazilian green mandarin (Citrus reticulata)* [Thesis]. Garching b. München, Germany: Technische Universität München.

Fischer N, Nitz S, Drawert F. **1987**. *Über gebundene Aromastoffe in Pflanzen: 2. Mitteilung: Freie und gebundene Aromastoffe in grünem und schwarzem Tee (Camellia sinensis) [Bound flavor compounds in plants. Part 2. Free and bound flavor compounds in green and black tea (Camellia sinensis)]*. Z Lebensm Unters Forsch 185(3):195–201.

Flückiger FA. **2004**. *Dokumente zur Geschichte der Pharmazie*. Düsseldorf, Germany: VDM-Verlag Müller: p. 73.

Fritsch HT, Schieberle P. **2005**. *Identification based on quantitative measurements and aroma recombination of the character impact odorants in a Bavarian Pilsner-type beer*. J. Agric. Food Chem. 53:7544–51.

Fritsch HT. **2001**. *Einfluss des Hopfens auf wertgebende Aromastoffe in Pilsener-Bieren sowie in Zwischenstufen des Brauprozesses* [Thesis]. Germany: Technische Universität München.

Funk W, Dammann V, Donnevert G. **2005**. *Qualitätssicherung in der Analytischen Chemie.* 2nd ed. Weinheim: Wiley-VCH.

Gattermann L, Wieland T, Sucrow W. **1982**. *Die Praxis des organischen Chemikers: Allgemeine Arbeitsanweisungen*. 43rd ed. New York: Walter de Gruyter; pp. 64-65.

GDCh (Lebensmittelchemische Gesellschaft) **2001**. *Enantioselektive Analyse zur Natürlichkeitsbewertung von Aromastoffen [Enantioselective analysis for the naturalness valuation of aroma substances]*. Lebensmittelchemie 55:24–6.

Genaust H. **1996**. *Etymologisches Wörterbuch der botanischen Pflanzennamen*. 3rd ed. Basel, Schweiz: Birkhäuser.

Gill M. **1992**. *Speciality and herbal teas*. In: Willson KC, Clifford MN, editors. Tea: Cultivation to consumption, 1st ed. London: Chapman & Hall. p. 513–34.

Gladisch T. **2007**. *Aromastoffe in frischen und gelagerten Schokoladenkeksen* [Thesis]. Garching b. München, Germany: Technische Universität München.

Godtfredsen S, Obrecht JP, Arigoni D. **1977**. *The Cyclization of Linalool to α-Terpineol: Stereochemical Course of the Reaction*. Chimia 31(2):62–3.

Gomez E, Ledbetter CA, Hartsell PL. **1993**. *Volatile compounds in apricot, plum, and their interspecific hybrids*. J. Agric. Food Chem. 41(10):1669–76.

Goodner K. **2008**. *Practical retention index models of OV-101, DB-1, DB-5, and DB-Wax for flavor and fragrance compounds*. LWT Food Sci. Technol. 41(6):951–8.

Guadagni DG, Buttery RG, Harris J. **1966**. *Odour intensities of hop oil components*. J. Sci. Food Agric. 17(3):142–4.

Guth H, Grosch W. **1993**. *Identification of potent odorants in static headspace samples of green and black tea powders on the basis of aroma extract dilution analysis (AEDA)*. Flavour Frag. J. 8(4):173–8.

Habermehl G, Hammann P, Krebs H, Ternes W. **2008**. *Naturstoffchemie: Eine Einführung*. Eine Einführung. 3rd ed. Berlin, Germany: Springer.

Hall RL, Ford RA. **1999**. *Comparison of two methods to assess the intake of flavouring substances*. Food Addit. Contam. 16(11):481–95.

Hara Y, Luo S, Wickremasinghe RL, Yamanishi T. **1995**. *Flavor of tea*. Food Rev. Int. 11(3):477–525.

Hazarika M, Mahanta PK, Takeo T. **1984**. *Studies on some volatile flavor constituents in orthodox black teas of various clones and flushes in north-east India*. J. Sci. Food Agric. 35(11):1201–7.

Hearty Á. **2010**. *FACET: providing an integrated and holistic approach to the exposure assessment of food chemicals*. Nutrition Bulletin 35:340–7.

Heiss R. **2004**. *Lebensmitteltechnologie*. 6th ed. Berlin, Germany: Springer.

Hempfling K, Fastowski O, Celik J, Engel K. **2013**. *Analysis and Sensory Evaluation of Jostaberry (Ribes x nidigrolaria Bauer) Volatiles*. J. Agric. Food Chem. 61(38):9067–75.

Henderson L, Gregory J, Swan G. **2002**. *National Diet and Nutrition Survey: Adults Aged 19 to 64 Years: Volume 1: Types and quantities of foods consumed*. London: TSO.

Hener U, Braunsdorf R, Kreis P, Dietrich A, Mass B, Euler E, Schlag B, Mosandl A. **1992**. *Chiral compounds of essential oils: X. The role of linalool in the origin evaluation of essential oils*. Chem. Mikrobiol. Technol. Lebensm. 14(5-6):129–33.

Herrmann K. **1994**. *Die zum Flavour des schwarzen Tees beitragenden flüchtigen Verbindungen: Aromastoffe [Flavor of black tea volatile compounds: Aroma substances]*. Gordian 94(4):54–7.

Hodgson WR. **1967**. *Horticultural Varieties of Citrus. In: Reuther W, Webber HJ, Batchelor LD, editors. The citrus industry: Vol. 1 History, world distribution, botany, and varieties*. Riverside, CA: University of California Press. p. 431–588.

Hör K, Ruff C, Weckerle B, König T, Schreier P. **2000**. *Flavor Authenticity Studies by $^{2}H/^{1}H$ Ratio Determination Using On-line Gas Chromatography Pyrolysis Isotope Ratio Mass Spectrometry*. J. Agric. Food Chem. 49(1):21–25.

Högnadóttir Á, Rouseff RL. **2003**. *Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry*. J. Chromatogr. A 998(1-2):201–11.

Huet R, Dupuis C. **1968**. *L´huile essentielle de bergamote en Afrique et en Corse [Bergamot essential oil in Africa and Corsica*]. Fruits 23(6):301–11.

ICPS (International Programme on Chemical Safety). **2009**. *Principles and Methods for the Risk Assessment of Chemicals in Food: Dietary Exposure Assessment of Chemicals in Food*. Geneva, Switzerland; Available from: http://whqlibdoc.who.int/ehc/WHO_EHC_240_9_eng_Chapter6.pdf; cited 2013 Nov 12.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). **2006**. *Sixty-seventh meeting. Rome, 20-29 June 2006. Summary and conclusions: Issued 7 July 2006*. Available from: ftp://ftp.fao.org/ag/agn/jecfa/jecfa67_final.pdf; cited 2013 Sep 23.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). **2008**. *Sixty-ninth meeting, Rome, Italy, 17-26 June 2008. Summary and conclusions: Issued 4 July 2008*. Available from: ftp://ftp.fao.org/ag/agn/jecfa/jecfa67_final.pdf; cited 2013 Sep 23.

Jellinek G. 1981. *Sensorische Lebensmittelprüfung: Lehrbuch für die Praxis*: Pattensen, Germany: Verlag Doris und Peter Siegfried. pp. 10-17.

Jirovetz L, Buchbauer G, Stoyanova AS, Georgiev EV, Damianova ST. **2003**. *Composition, Quality Control, and Antimicrobial Activity of the Essential Oil of Long-Time Stored Dill (Anethum graveolens L.) Seeds from Bulgaria*. J. Agric. Food Chem. 51(13):3854–7.

Jordán MJ, Margaría CA, Shaw PE, Goodner KL. **2002**. *Aroma Active Components in Aqueous Kiwi Fruit Essence and Kiwi Fruit Puree by GC-MS and Multidimensional GC/GC-O*. J. Agric. Food Chem. 50(19):5386–90.

Juchelka D, Mosandl A. **1996**. *Authenticity profiles of bergamot oil*. Pharmazie 51(6):417–22.

Kaack K. **2008**. *Aroma composition and sensory quality of fruit juices processed from cultivars of elderberry (Sambucus nigra L.)*. Eur. Food Res. Technol. 227(1):45–56.

Karak T, Bhagat RM. **2010**. *Trace elements in tea leaves, made tea and tea infusion: A review*. Food Res. Int. 43(9):2234–52.

Kato T, Shimoda M, Suzuki J, Kawaraya A, Igura N, Hayakawa I. **2003**. *Changes in the odors of squeezed apple juice during thermal processing*. Food Res. Int. 36(8):777–85.

Kawakami M, Ganguly SN, Banerjee J, Kobayashi A. **1995**. *Aroma Composition of Oolong Tea and Black Tea by Brewed Extraction Method and Characterizing Compounds of Darjeeling Tea Aroma*. J. Agric. Food Chem. 43(1):200–7.

Keith ES, Powers JJ. **1968**. *Determination of Flavor Threshold Levels and Sub-Threshold, Additive, and Concentration Effects*. J Food Sci. 33(2):213–8.

Kim Y, Goodner KL, Park J, Choi J, Talcott ST. **2011**. *Changes in antioxidant phytochemicals and volatile composition of Camellia sinensis by oxidation during tea fermentation*. Food Chem. 129(4):1331–42.

Kirbaslar FG, Kirbaslar SI, Dramur U. **2001**. *The compositions of Turkish bergamot oils produced by cold-pressing and steam distillation*. J. Essent. Oil Res. 13(6):411–5.

Kiwanuka P, Mottram DS, Baigrie BD. **2000**. *The effects of processing on the constituents and enantiomeric composition of bergamot essential oil*. Proc. Phytochem. Soc. Eur. 46(Flavour and Fragrance Chemistry):67–75.

Kondo M, Goto M, Kodama A, Hirose T. **2000**. *Fractional Extraction by Supercritical Carbon Dioxide for the Deterpenation of Bergamot Oil*. Ind. Eng. Chem. Res. 39(12):4745–8.

König WA, Fricke C, Saritas Y, Momeni B, Hohenfeld G. **1997**. *Adulteration or natural variability? Enantioselective gas chromatography in purity control of essential oils*. J. High. Resolut. Chromatogr. 20(2):55–61.

Kováts E. **1958**. *Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone [Gas chromatographic characterization of organic compounds. I. Retention indexes of aliphatic halides, alcohols, aldehydes, and ketones]*. Helvetica Chimica Acta 41(7):1915–32.

Kreis P, Braunsdorf R, Dietrich A, Hener U, Maas B, Mosandl A. **1993**. *Enantioselective analysis of linalool - scope and limitations*. In: Schreier P, Winterhalter P, editors. Proceedings of the International Conference. Progress in flavour precursor studies: Analysis, generation, biotechnology; 1992 Sep 30 - Oct 2; Würzburg (Germany). pp. 77-82.

Kroes R, Galli C, Munro I, Schilter B, Tran L, Walker R, Wurtzen G. **2000**. *Threshold of toxicological concern for chemical substances present in the diet: A practical tool for assessing the need for toxicity testing*. Food Chem. Toxicol. 38(2/3):255–312.

Kubeczka KH. **2010**. *History and sources of essential oil research*. In: Başer KHC, Buchbauer G, editors. Handbook of essential oils: Science, technology, and applications. Boca Raton, FL, USA: CRC Press (Taylor & Francis Group). p. 3–38.

Kumazawa K, Masuda H. **2001**. *Change in the Flavor of Black Tea Drink during Heat Processing*. J. Agric. Food Chem. 49(7):3304–9.

Lamonica G, D'Alcontres IS, Donato MG, Merenda I. **1990**. *On the Calabrian bergamot essential oil*. Chim. Oggi 8(5):59–63.

Le Magnen J. **1952**. *Les phenomenes olfacto-sexuels chez l´homme [Olfactory-sexual phenomena in man]*. Arch Sci Physiol (Paris) 6(2):125–60.

Leclercq C, Charrondiere UR, Di NM, Baines J, Leblanc JC, Verger P, Renwick A, Abbott P, Sipes G. **2009**. *Dietary exposure assessment of flavouring agents: incorporation of the single portion exposure technique (SPET) into the procedure for the safety evaluation of flavouring agents*. WHO Food Addit. Ser. 60 (Safety Evaluation of Certain Food Additives):267–89.

Leitsätze für Tee, teeähnliche Erzeugnisse, deren Extrakte und Zubereitungen in der Fassung vom 2. Dezember **1998** [Revised Version Guidelines for Tea, Herbal infusions, Extracts thereof and Preparations]. BAnz (Bundesanzeiger). Nr. 66a vom 9. April 1998, GMBI (Gemeinsames Ministerialblatt). Nr. 11 p. 228.

Mai C, Rammul A, Oelker A. **1903**. *Gebrauchsgegenstände*. Zeitschr. f. Untersuchung d. Nahr. u. Genußmittel 6(11):503–17

Mandalari G, Bennett RN, Bisignano G, Saija A, Dugo G, Lo Curto RB, Faulds CB, Waldron KW. **2006**. *Characterization of flavonoids and pectins from bergamot (Citrus bergamia Risso)* peel, a major byproduct of essential oil Extraction. J. Agric. Food Chem. 54(1):197–203.

Marco-Contelles J, Soriano E. **2012**. 8. *Rearrangements in Natural Product Synthesis*. In: Cossy J, Arseniyadis S, editors. Modern tools for the synthesis of complex bioactive molecules. Hoboken, New Jersey: John Wiley & Sons Inc., p 252.

Masanetz C, Grosch W. **1998**. *Key odorants of parsley leaves (Petroselinum crispum [Mill.] Nym. ssp. crispum) by odor-activity values*. Flavour Frag. J. 13(2):115–24.

Mazza G. **1986**. *Gas chromatographic and mass spectrometric study of the aroma composition of bergamot (Citrus aurantium subsp. bergamia Risso and Poiteau Engler) essential oil*. J. Chromatogr. 362(1):87–99.

McKay DL, Blumberg JB. **2002**. *The role of tea in human health: An update*. J. Am. Coll. Nutr. 21(1):1–13.

Meilgaard MC, Civille GV, Carr BT. **2007**. *Sensory evaluation techniques.* 4th ed. Boca Raton, FL, USA: CRC Press (Taylor & Francis Group).

Melliou E, Michaelakis A, Koliopoulos G, Skaltsounis A, Magiatis P. **2009**. *High quality bergamot oil from Greece: Chemical analysis using chiral gas chromatography and larvicidal activity against the West Nile virus vector*. Molecules 14(2):839–49.

Mercea P. **2010**. *EU-Project „FACET“*. Presentation at the seminar "The use of diffusion modelling to predict migration" held in JRC Ispra on 15-16 April 2010. Available from: http://ihcp.jrc.ec.europa.eu/our_labs/eurl_food_c_m/training/seminar2010/doc/L4_modellimo_multilyers_Mercea.pdf; cited 2013 Sep 24.

Mick W, Schreier P. **1984**. *Additional volatiles of black tea aroma*. J. Agric. Food Chem. 32(4):924–9.

Minh-Tu NT, Onishi Y, Choi HS, Kondo Y, Ukeda H, Sawamura M. **2003**. *Characteristic odour components of Citrus sp. (Kiyookadaidai) cold-pressed peel oil*. Flavour Fragr. J. 18(6):515–20.

Miyazawa M, Kawauchi Y, Utsumi Y, Takahashi T. **2010**. *Character Impact Odorants of Wild Edible Plant-Cacalia hastata L. var. orientalis-Used in Japanese Traditional Food*. J. Oleo Sci. 59(10):527–33.

Mondello L, Stagno d'Alcontres I, Del Duce R, Crispo F. **1993**. *On the genuineness of citrus essential oils. Part XL. The composition of the coumarins and psoralens of Calabrian bergamot essential oil (Citrus bergamia Risso)*. Flavour Fragr. J. 8(1):17–24.

Mondello L, Bartle KD, Dugo P, Gans P. **1994**. *Automated LC-GC: a powerful method for essential oils analysis. Part IV. Coupled LC-GC-MS (ITD) for bergamot oil analysis*. J. Micro. Sep. 6(3):237–44.

Mondello L, Verzera A, Previti P, Crispo F, Dugo G. **1998**. *Multidimensional Capillary GC-GC for the Analysis of Complex Samples. 5. Enantiomeric Distribution of Monoterpene Hydrocarbons, Monoterpene Alcohols, and Linalyl Acetate of Bergamot (Citrus bergamia Risso et Poiteau) Oils*. J. Agric. Food Chem. 46(10):4275–82.

Morin P, Richard H. **1985**. *Thermal degradation of linalyl acetate during steam distillation*. Dev. Food Sci. 10(Prog. Flavour Res):563–76.

Mosandl A, Juchelka D. **1997**. *Advances in the authenticity assessment of citrus oils*. J. Essent. Oil Res. 9(1):5–12.

MRI (Max Ruber-Institut). **2008**. *Nationale Verzehrsstudie II - Teil 2: Ergebnisbericht [Report of the National Nutrition Survey II - Part 2: report of results]*. Karlsruhe (Germany).

MRI (Max Rubner-Institut). **2013**. *Trinkmengen von Schwarztee in Deutschland [Consumption rates of black tea in Germany]* (Nationale Verzehrsstudie, NVS II), Karlsruhe, unpublished data.

Mui WWY, Durance TD, Scaman CH. **2002**. *Flavor and Texture of Banana Chips Dried by Combinations of Hot Air, Vacuum, and Microwave Processing*. J. Agric. Food Chem. 50(7):1883–9.

Muller PA. **1966**. *Bergamot and bergamot oil*. Perfum. Essent. Oil Rec. 57(1):18–25.

Munro IC, Kennepohl E, Kroes R. **1999**. *A procedure for the safety evaluation of flavouring substances. Joint FAO/WHO Expert Committee on Food Additives*. Food Chem. Toxicol. 37(2-3):207–32.

Neukom HP, Meier DJ, Blum D. **1993**. *Nachweis von natürlichem oder rekonstituiertem Bergamottöl in Earl Grey Tees anhand der Enantiomerentrennung von Linalool und Dihydrolinalool [Detection of natural and reconstituted bergamot oil in Earl Grey teas by separation of the enantiomers of linalool and dihydrolinalool]*. Mitt. Geb. Lebensmittelunters. Hyg. 84(5):537–44.

Nicolosi E, Deng ZN, Gentile A, La Malfa S, Continella G, Tribulato E. **2000**. *Citrus phylogeny and genetic origin of important species as investigated by molecular markers*. Theor Appl Genet 100(8):1155–66.

Ohloff G. **1994**. *Scent and fragrances: The fascination of odors and their chemical perspectives. 8.1 Citrus oils*. Berlin Heidelberg, Germany: Springer.

Ohloff G, Klein E. **1962**. *Absolute configuration of linalool by relation to the pinane system*. Tetrahedron 18:37–42.

Owuor PO. **1992**. *Comparison of gas chromatographic volatile profiling methods for assessing the flavor quality of Kenyan black teas*. J. Sci. Food Agric. 59(2):189–97.

Özek T, Tabanca N, Demirci F, Wedge DE, Baser KHC. **2010**. *Enantiomeric Distribution of Some Linalool Containing Essential Oils and Their Biological Activities*. Rec. Nat. Prod. 4(4):180–92.

Padrayuttawat A, Yoshizawa T, Tamura H, Tokunaga T. **1997**. *Optical Isomers and Odor Thresholds of Volatile Constituents in Citrus sudachi*. Food Sci. Technol. Int. Tokyo. 3(4):402–8.

Pernice R, Borriello G, Ferracane R, Borrelli RC, Cennamo F, Ritieni A. **2009**. *Bergamot: A source of natural antioxidants for functionalized fruit juices*. Food Chem. 112(3):545–50.

Pickenhagen W, Smith AY. **1979**. *Aromakomposition [Aroma composition]* (DE2924031A1).

Pino JA, Quijano CE. **2012**. *Study of the volatile compounds from plum (Prunus domestica L. cv. Horvin) and estimation of their contribution to the fruit aroma*. Ciênc. Tecnol. Aliment. 32(1):76–83.

Poiana M, Mincione A, Gionfriddo F, Castaldo D. **2003**. *Supercritical carbon dioxide separation of bergamot essential oil by a countercurrent process*. Flavour Frag. J. 18(5):429–35.

Pokorny J, Pudil F, Ulmannova K, Ficova E. **1995**. *Effect of oxidation products of scented tea aroma compounds on flavor of tea infusion*. Dev. Food Sci. 37A:815–25.

Pyysalo T, Suihko M, Honkanen E. **1977**. *Odor thresholds of the major volatiles identified in cloudberry (Rubus chamaemorus L.) and arctic bramble (Rubus arcticus L.)*. Lebensm.-Wiss. Technol. 10(1):36–9.

Raffo A, Nicoli S, Leclercq C. **2011**. *Quantification of estragole in fennel herbal teas: Implications on the assessment of dietary exposure to estragole.* Food and Chemical Toxicology 49(2):370–5.

Raffo A, D'Aloise A, Magrì A, Leclercq C. **2012**. *Quantification of allyl hexanoate in pineapple beverages and yogurts as a case study to characterise a source of uncertainty in dietary exposure assessment to flavouring substances*. Food Addit. Contam.: Part A 29(1):43-53.

Raffo A, D'Aloise A, Magrì AL, Leclercq C. **2013**. *Quantitation of tr-cinnamaldehyde, safrole and myristicin in cola-flavoured soft drinks to improve the assessment of their dietary exposure*. Food and Chemical Toxicology 59:626–35.

Ramirez-Rodrigues MM, Balaban MO, Marshall MR, Rouseff RL. **2011**. *Hot and cold water infusion aroma profiles of Hibiscus sabdariffa: fresh compared with dried*. J. Food Sci. 76(2):C212-C217.

Ravid U, Putievsky E, Katzir I, Lewinsohn E. **1997**. *Enantiomeric composition of linalol in the essential oils of Ocimum species and in commercial basil oils*. Flavour Frag. J. 12(4):293-6.

Ravid U, Elkabetz M, Zamir C, Cohen K, Larkov O, Aly R. **2010**. *Authenticity assessment of natural fruit flavour compounds in foods and beverages by auto-HS-SPME stereoselective GC-MS*. Flavour Frag. J. 25(1):20-7.

Rittersdorf W, Cramer F. **1968**. *Cyclization of nerol and linalool on solvolysis of their phosphate esters*. Tetrahedron 24(1):43–52.

Rothe M, Thomas B. **1963**. *Aromastoffe des Brotes. Versuch einer Auswertung chemischer Geschmacksanalysen mit Hilfe des Schwellenwertes [Aroma of bread. Evaluation of chemical taste analysis with the aid of threshold value]*. Z Lebensm Unters Forsch 119(4):302–10.

Rothe M. **1972**. *Zur Terminologie der Grundbegriffe der Aromaforschung*. Nahrung 16(5):473–81.

Roth L, Kormann K. **1997**. *Duftpflanzen, Pflanzendüfte*. Landsberg, Germany: ecomed verlagsgesellschaft AG & Co. KG. p 186.

Russo M, Antonelli A, Carnacini A. **2001**. *Experiences with Solid $C0_2$ Concentration of Bergamot Cold-Pressed Essential Oil (Citrus bergamia Risso)*. J. Essent. Oil Res. 13(4):247–9.

Rychlik M, Schieberle P, Grosch W. **1998**. *Compilation of odor thresholds, odor qualities and retention indices of key food odorants*. München, Germany: Deutsche Forschungsanstalt für Lebensmittelchemie der Technischen Universität München.

Saijo R, Kuwabara Y. **1967**. *Volatile flavor of black tea. I. Formation of volatile components during black tea manufacture*. Agr. Biol. Chem. 31(4):389–96.

Sanderson GW, Graham HN. **1973**. *Formation of black tea aroma*. J. Agric. Food Chem. 21(4):576–85.

Sato M, Goto M, Hirose T. **1996**. *Supercritical Fluid Extraction on Semibatch Mode for the Removal of Terpene in Citrus Oil*. Ind. Eng. Chem. Res. 35(6):1906–11.

Sawamura M, Onishi Y, Ikemoto J, Tu NTM, Phi NTL. **2006**. *Characteristic odour components of bergamot (Citrus bergamia Risso) essential oil*. Flavour Frag. J. 21(4):609–15.

Sawamura M, Poiana M, Kawamura A, Itoh T, Song HS, Ukeda H, Mincione B. **1999**. *Volatile components of peel oils of Italian and Japanese lemon and bergamot*. Ital. J. Food Sci. 11(2):121–30.

SCF (Scientific Committee on Food). **1999**. *Opinion on a Programme for the Evaluation of Flavouring Substances, SCF/CS/FLAV/TASKF/11 Final*. European Commission, Health & Consumer Protection Directorate-General, Brussels. Available from: ec.europa.eu/food/fs/sc/scf/out45_en.pdf; cited 2013 Sep 23.

Schenk HP, Lamparsky D. **1981**. *Analysis of citrus oils, especially bergamot oil*. Seifen, Oele, Fette, Wachse 107(13):363–9.

Schieberle P, Molyneux RJ. **2012**. *Quantitation of Sensory-Active and Bioactive Constituents of Food: A Journal of Agricultural and Food Chemistry Perspective*. J. Agric. Food Chem. 60(10):2404–8.

Schipilliti L, Dugo G, Santi L, Dugo P, Mondello L. **2011**. *Authentication of bergamot essential oil by gas chromatography-combustion-isotope ratio mass spectrometer (GC-C-IRMS)*. J. Essent. Oil Res. 23(2):60–71.

Schmarr HG. **1992**. *Beiträge zur On-line LC-GC Kopplung und modifizierte Cyclodextrine als chirale stationäre Phasen in der Kapillar-GC* [Thesis]. Frankfurt, Germany: Johann Wolfgang Goethe-Universität.

Schmarr HG, Ganß S, Sang W, Potouridis T. **2007**. *Analysis of 2-aminoacetophenone in wine using a stable isotope dilution assay and multidimensional gas chromatography-mass spectrometry*. J. Chromatogr. A 1150(1-2):78–84.

Schmarr HG, Engel KH. **2012**. *Analysis and stereodifferentiation of linalool in Theobroma cacao and cocoa products using enantioselective multidimensional gas chromatography*. Eur. Food Res. Technol. 235(5):827–34.

Schmaus G, Kubeczka KH. **1985**. *The influence of isolation conditions on the composition of essential oils containing linalool and linalyl acetate*. In: Baerheim Svendsen A, Scheffer JJC, editors. Proceedings of the 15. International Symposium on Essential Oils; 1984 Jul 19-21; Noordwijkerhout, The Netherlands. pp. 127–34.

Schmidlin-Mészáros J. **1971**. *Probleme im Gebiet der Lebensmittelzusatzstoffe: Ermittlung des p-Hydroxyphenylbutanon-(3)s, des Himbeerketons*. Alimenta 10:39–48.

Schmid W, Grosch W. **1986**. *Quantitative analysis of the volatile flavor compounds having high aroma values from sour (Prunus cerasus L.) and sweet (Prunus avium L.) cherry juices and jams*. Z. Lebensm. Unters. Forsch. 183(1):39–44.

Schmidt E. **2010**. *Production of Essential Oils*. In: Başer KHC, Buchbauer G, editors. Handbook of essential oils: Science, technology, and applications. Boca Raton, FL, USA: CRC Press (Taylor & Francis Group). pp. 83–120.

Schöchl J. **2013**. *Lebensmittelgeschichten: Schmackhaft - wissenswert - kurios. Earl Grey Tee*. Salzburg, Austria: Pustet. p. 41.

Schreier P, Mick W. **1984**. *Analytische Differenzierung von zwei Qualitäten schwarzen Tees mittels Kapillargaschromatographie-Massenspektrometrie [Analytical differentiation of two black tea qualities by capillary gas chromatography-mass spectrometry]*. Chem. Mikrobiol. Technol. Lebensm. 8(4):97–104.

Schubert V, Mosandl A. **1991**. *Chiral compounds of essential oils. VIII*: Stereodifferentiation of linalool using multidimensional gas chromatography. Phytochem. Anal. 2(4):171-4.

Schuh C. **2004**. *Wichtige Aromastoffe in schwarzem und grünem Tee (Camellia sinensis)* [Thesis]. Germany: Technische Universität München

Schuh C, Schieberle P. **2006**. *Characterization of the Key Aroma Compounds in the Beverage Prepared from Darjeeling Black Tea: Quantitative Differences between Tea Leaves and Infusion*. J. Agric. Food Chem. 54(3):916–24.

Schwob R. **1953**. *Oil of bergamot from French Guinea*. Industrie Parfum. 8:369-373,406-409.

Schwob R. **1955**. *Bergamot oil from North Africa*. Industrie Parfum. 10:46–50.

Scora RW. **1988**. *Biochemistry, taxonomy and evolution of modern cultivated citrus*. In: Goren R, Mendel K, editors. Citriculture. Proceedings of the Sixth International Citrus Congress Middle-East: Volume 1. Introduction, Genetics, Breeding and Propagation, Modern Breeding and Taxonomy, Plant Physiology and Ecology, held in Tel Aviv, Israel, March 6 - 11, 1988, Vol. 1. p. 277–89.

Seebach D, Prelog V. **1982**. *Specification of the steric course of asymmetric syntheses*. Angew. Chem. 94(9):696–702.

Sega GM, Lewis MJ, Woskow MH. **1967**. *Evaluation of beer flavor compounds*. Amer. Soc. Brew. Chem. Proc.:156–64.

Semmelroch P, Laskawy G, Blank I, Grosch W. **1995**. *Determination of potent odourants in roasted coffee by stable isotope dilution assays*. Flavour Fragr. J. 10(1):1–7.

Shaw PE, Wilson CW III. **1976**. *Comparison of extracted peel oil composition and juice flavor for rough lemon, Persian lime, and a lemon-lime cross*. J. Agric. Food Chem. 24(3):664-6.

Shaw PE. **1979**. *Review of quantitative analyses of citrus essential oils*. J. Agric. Food Chem. 27(2):246–57.

Sofuoglu SC, Kavcar P. **2008**. *An exposure and risk assessment for fluoride and trace metals in black tea*. J. Hazard. Mater. 158. (2-3):392–400.

Sommella E, Pepe G, Pagano F, Tenore GC, Dugo P, Manfra M, Campiglia P. **2013**. *Ultra high performance liquid chromatography with ion-trap TOF-MS for the fast characterization of flavonoids in Citrus bergamia juice*. J. Sep. Sci. 36(20):3351–5.

Staab HA. **1962**. *Syntheses with heterocyclic amides (azolides)*. Section 4a. Angew. Chem. 74:407–23.

Stevens KL, Jurd L, Manners G. 1**972**. *Transformations of geraniol in aqueous acid solutions*. Tetrahedron 28(7):1939–44.

Strohalm H. **2010**. *Passionsfrucht-typische 2-Alkylester: Analytik, enzymkatalysierte Gewinnung und sensorische Bewertung [2-Alkyl esters from passion fruits: analysis, enzyme-catalyzed preparation and sensory evaluation]* [Thesis]. Freising, Germany: Technische Universität München.

Sulzbach H. **1996**. *Multidimensionale Kapillarsäulen-Gaschromatographie*. GIT Fachz. Lab. 40:131.

Takeo T. **1981**. *Production of linalol and geraniol by hydrolytic breakdown of bound forms in disrupted tea shoots*. Phytochemistry 20(9):2145–7.

Takeo T. **1982**. *Variations in aroma compound content in nonfermented and semifermented tea*. Nippon Nogei Kagaku Kaishi 56(9):799–801.

Tateo F, Bononi M, Lubian E. **2000**. *Enantiomeric distribution and sensory evaluation of linalol and linalyl acetate in flavourings*. Ital. J. Food Sci. 12(3):371–5.

Tee-online. **2008-2012**. *Earl Grey Tee - Der alte Engländer [Earl Grey tea – The old English]*. Available from: http://www.tee-online.info/earl-grey-tee.php; cited 2013 Sep 17.

Teeworld. *Earl Grey*. Available from: https://www.teaworld.de/tee/earl-grey-tee.html; cited 2013 Sep 17.

Teisseire P. **1987**. *7. Industrial quality control of essential oils by capillary GC*. In: Sandra P, Bicchi C, editors. Capillary gas chromatography in essential oil analysis. Heidelberg: Huethig. pp. 215–87.

Usai M, Arras G, Fronteddu F. **1992**. *Effects of cold storage on essential oils of peel of Thompson navel oranges*. J. Agric. Food Chem. 40(2):271–5.

Venstrom D, Amoore JE. **1968**. *Olfactory Threshold, in Relation to Age, Sex or Smoking*. J Food Science 33(3):264–5.

Verzera A, Lamonica G, Mondello L, Trozzi A, Dugo G. **1996**. *The composition of bergamot oil*. Perfum. Flavor 21(6):19–34.

Verzera A, Trozzi A, Stagno dI, Mondello L, Dugo G, Sebastiani E. **1998**. *The composition of the volatile fraction of Calabrian bergamot essential oil*. Riv. Ital. EPPOS 25:17–38.

Verzera A, Dugo P, Mondello L, Trozzi A, Cotroneo A. **1999**. *Extraction technology and lemon oil composition*. Ital. J. Food Sci. 11(4):361–70.

Verzera A, La Rosa G, Zappala M, Cotroneo A. **2000**. *Essential oil composition of different cultivars of bergamot grown in Sicily*. Ital. J. Food Sci. 12(4):493–501.

Verzera A, Trozzi A, Gazea F, Cicciarello G, Cotroneo A. **2003**. *Effects of Rootstock on the Composition of Bergamot (Citrus bergamia Risso et Poiteau) Essential Oil*. J. Agric. Food Chem. 51(1):206–10.

Vogelgesang J, Hädrich J. **1998**. *Limits of detection, identification and determination: a statistical approach for practitioners*. Accredit. Qual. Assur. 3(6):242–55.

Volckamer JC. **1704**. *Nürnbergische Hesperides oder Gründliche Beschreibung der edlen Citronat-, Citronen-, und Pomerantzen-Früchte*. Zirndorf, Germany: Leipold.

Wang D, Ando K, Morita K, Kubota K, Kobayashi A. **1994**. *Optical isomers of linalool and linalool oxides in tea aroma*. Biosci. Biotech. Biochem. 58(11):2050–3.

WKF [Wirtschaftsvereinigung Kräuter- und Früchtetee e.V.]. **2012**. Hagebutte - Hagebutten-Tee - rot und gut [Rose hip - Rose hips tea - red and good]. Available from: http://www.wkf.de/Details.1781.0.html?&L=0&tx_ttnews%5Btt_news%5D=30&cHash=a5dd067b6f5812047845d93f073f661f; cited 2013 Sep 17.

Yamanishi T. **1967**. *Flavor of black tea*. Jap. Agr. Res. Quart. 2(4):25–32.

Yamanishi T, Kobayashi A, Nakamura H, Uchida A, Mori S, Ohsawa K, Sasakura S. **1968**. *Flavor of black tea. V. A comparison of the aroma of various types of black tea*. Agr. Biol. Chem. 32(3):379–86.

Yamanishi T, Kita Y, Watanabe K, Nakatani Y. **1972**. *Flavor of black tea. VII. Constituents and composition of steam volatile aroma from ceylon tea*. Agr. Biol. Chem. 36(7):1153–8.

Yamanishi T. **1978**. *The aroma of various teas. Flavor of foods and beverages. Chemistry and technology: Proceedings of a conference sponsored by the Agricultural and Food Chemistry Divisioin of the American Chemical Society, held in Athens, Greece, June 27-29, 1978*. New York: Academic Press. p. 305–28.

Young AR, Walker SL, Kinley JS, Plastow SR, Averbeck D, Morlière P, Dubertret L. **1990**. *Phototumorigenesis studies of 5-methoxypsoralen in bergamot oil: Evaluation and modification of risk of human use in an albino mouse skin model*. J. Photochem. Photobiol. B. 7(2-4):231–250.

Young KWH, Danielewska-Nikiel B, Munro IC. **2006**. *An evaluation of the maximized survey-derived daily intake (MSDI) as a practical method to estimate intake of flavouring substances*. Food Chem. Toxicol. 44(11):1849–1867.

Zaynoun ST, Johnson BE, Frain-Bell WW. **1977**. *A study of oil of bergamot and its importance as a phototoxic agent*. Br J Dermatol 96(5):475–482.

Zeller A, Rychlik M. **2006**. *Character Impact Odorants of Fennel Fruits and Fennel Tea*. J. Agric. Food Chem. 54(10):3686–3692.

8 APPENDIX

8.1 Tea samples

Table 37. Tea samples.

no.	country[a]	type[b]		G[c]	brand name	labelling	ingredients (original wording)	price/ 100 g[d]	date of purchase	shop / city	best before	weight per tea bag
Earl Grey tea samples												
1	D	B1	tb	+	Teekanne	Earl Grey Schwarzer Tee	Schwarzer Tee, Bergamotte-Aroma	4.83 €	13.8.2009	Real Freising	06.2011	1.75 g
2	D	B1	tb	-	Teekanne	Earl Grey Schwarzer Tee	Schwarzer Tee, Bergamotte-Aroma, Bergamotte-Öl	4.83 €	2.12.2009	Real Freising	09.2011	1.75 g
3	D	B1	ll	-	Teekanne	Earl Grey Finest Selection F.B.O.P.	Schwarzer Tee, Bergamotte-Öl	2.68 €	5.8.2009	Real Freising	11.2010	-
4	D	B2	tb	+	Meßmer	Feinster Earl Grey frisch-pikant	Schwarzer Tee, natürliches Bergamotte- und Zitronenaroma mit anderen natürlichen Aromen	3.42 €	29.3.2012	Real Freising	09.01.2014	1.75 g
5	D	B2	ll	-	Meßmer	Feinster Earl Grey	Schwarzer Tee, Aroma	2.66 €	25.7.2009	Kaufland Freising	4.8.2010	-
6	D	B3	tb	-	Ronnefeldt	Earl Grey	Schwarzer Darjeeling Tee, Aroma	10.53 €	17.12.2009	Karstadt Munich	17.9.2011	1.50
7	D	B3	ll	-	Ronnefeldt	Special Earl Grey FOP Herbst	Tee, Aroma	3.95 €	17.12.2009	Karstadt Munich	7.11.2011	-
8	D	B4	tb	-	GEPA	Ceylon Bio Earl Grey	Schwarzer Tee, natürliches Bergamotte-Öl	4.57 €	21.11.2009	Karieu Kronach	15.7.2011	1.75 g
9	D	B4	ll	-	GEPA	Bio Earl Grey	Schwarzer Tee, natürliches Bergamotte-Öl	4.00 €	21.11.2009	Karieu Kronach	31.10.2011	-
10	D	B5	ll	-	Sir Winston Tea	Earl Grey	Tee, natürliches Bergamotte-Öl	2.40 €	5.8.2009	Real Freising	05.2011	-
11	D	B6	tb	+	Goldmännchen	Earl Grey Schwarzer Tee Bergamottaroma Feine Qualität	Schwarzer Tee, Bergamottaroma	3.18 €	25.7.2009	Kaufland Freising	04.2012	1.75 g
12	D	PLB1	tb	+	K-Klassik	Earl Grey Schwarzer Tee aromatisiert-Bergamotte	Schwarzer Tee, Bergamotte-Aroma	0.99 €	25.7.2009	Kaufland Freising	02.2011	1.75 g
13	D	PLB2	tb	+	Westcliff	Earl Grey	Schwarzer Tee, natürliches Aroma (Bergamotte)	0.99 €	20.7.2009	Aldi Freising	1.7.2011	1.75 g
14	D	PLB3	tb	+	ja!	Earl Grey	Schwarzer Tee, Aroma	0.99 €	15.7.2009	Rewe Freising	2.6.2011	1.75 g
15	D	PLB4	tb	+	real Quality	Earl Grey	Schwarzer Tee, natürliches Aroma	4.26 €	2.12.2009	Real Freising	30.10.2011	1.75 g
16	D	PLB5	tb	+	Lord Nelson	Earl Grey	Schwarzer Tee, natürliches Bergamotte-Aroma	1.81 €	29.3.2010	Lidl Freising	23.6.2011	1.75 g
17	D	Ts1	ll	-	-	Earl Grey	China/Ceylon-Blend, natürliches Aroma Bergamotte	3.20 €	11.7.2009	Teeparadies Freising	-	-

Table 37. continued

no.	country[a]	type[b]		G[c]	brand name	labelling	ingredients (original wording)	price/ 100 g[d]	date of purchase	shop / city	best before	weight per tea bag
18	D	Ts2	II	-	-	Earl Grey	Darjeeling und natürliches Aroma	4.00 €	11.7.2009	Teeparadies Freising	-	-
19	D	Ts3	II	-	-	Earl Grey Spezial	Schwarze China/Indien Tees, Bergamotteöl	5.44 €	10.10.2009	Tee Blatt Munich	-	-
20	D	Ts4	II	-	-	Earl Grey Typ II	Java/Indien-Tees, Bergamotte-Öl	5.44 €	10.10.2009	Tee Blatt Munich	-	-
21	D	Ts5	II	-	-	Earl Grey	Schwarztee, Bergamotte-Öl	4.00 €	5.12.2009	Hasselbach Natur- und Trendpro-dukte München	12.2011	-
22	D	Ts6	II	-	Sinensis	Earl Grey	Schwarzer Tee, natürliches Bergamotteöl	4.30 €	7.9.2010	Backhaus Friedrich, Limburg a. d. Lahn	21.4.2012	-
23	D	Ts7	II	-	-	Earl Grey mit Tips	Schwarzer Tee, Aroma	3.60 €	27.9.2010	Abraham´s Tea House Hannover	-	-
24	D	Ts8	II	-	-	Earl Grey mit Tips	Schwarzer Tee, Bergamotte Aroma	3.70 €	27.9.2010	Abraham´s Tea House Hannover	-	-
25	D	Ts9	II	-	-	Earl Grey	Bergamotteöl (oral communication)	n.k.[e]	09. 2010	Jasmin Tee und Keramik Kiel	-	-
26	D	Ts10	II	-	-	Schwarztee Earl Grey Spezial	schwarzer Tee, natürliches Bergamotteöl (oral communication)	n.k.	n.k.	Rendsburger Teehaus Kiel	-	-
27	D	Ts11	II	-	-	Earl Grey, mit Bergamotteöl der Klassiker	n.k.	2.80 €	4.10.2010	Teehaus Berlin	-	-
28	D	Ts12	II	-	-	Earl Grey Green, frisch, mit Malvenblüten	n.k.	2.90 €	4.10.2010	Teehaus Berlin	-	-
29	H	B7	tb	+	Lipton	Earl Grey	black tea, bergamot flavour: 7%		n.k.	n.k.	1.9.2011	1.5 g
30	H	B7	tb	+	Lipton	Earl Grey Tea	black tea (89,3%), natural flavouring (10,2%), blue petals (0,3%), jasmine petals (0,2%)		n.k.	n.k.	1.10.2011	1.8 g
31	H	B8	tb	+	Dilmah	Earl Grey	Ceylon black tea with Bergamot flavour		n.k.	n.k.	1.9.2012	1.5 g
32	H	B9	tb	+	Tetley	Earl Grey	fekete tea, bergamot aroma (természetazonos (6,5%))		n.k.	n.k.	1.6.2012	2 g
33	H	B10	tb	+	Pickwick	Earl Grey	tea, bergamot flavouring		n.k.	n.k.	1.9.2001	2 g
34	H	B11	tb	+	Sir Morton	Earl Grey	fekete tea, bergamot aroma; tea, bergamot flavouring		n.k.	n.k.	1.10.2011	1.5 g
36	H	PLB5	tb	+	Lord Nelson	Earl Grey	czarna herbate, aromat		n.k.	n.k.	4.11.2011	1.75 g
37	H	PLB6	tb	+	S Budget	Earl Grey Tee	Schwarzer Tee, Bergamotte-Aroma		n.k.	n.k.	1.4.2011	4.5 g
38	H	Ts13	II	-	-	Earl Grey Special	n.k.	n.k.	08. 2010	Big Ben Teaház	30.8.2011	-

Table 37. continued

no.	country[a]	type[b]		G[c]	brand name	labelling	ingredients (original wording)	price/ 100 g[d]	date of purchase	shop / city	best before	weight per tea bag
39	P	B7	tb	+	Lipton	Earl Grey	black tea (89,3%), natural flavouring (10,2%), blue petals (0,3%), jasmine petals (0,2%)	19.30 zl	19.1.2010	Tesco Opole	1.11.2011	1.8 g
40	P	B7	ll	-	Lipton	Earl Grey	Herbata czarna, aromat bergamotowy 1,5%, platki nagietka 0,5%;	6.35 zl	19.1.2010	Tesco Opole	1.10.2011	-
41	P	B8	tb	+	Dilmah	Earl Grey	czarna herbate cejlonska, aromat bergamoty (7%); Schwarzer Ceylon Tee, Bergamottearoma	18.00 zl	19.1.2010	Tesco Opole	1.11.2012	1.5g
42	P	B9	tb	+	Tetley	Earl Grey	herbata czarna, aromat bergamotki identyczny naturalnym (6,5%)	10.90 zl	19.1.2010	Tesco Opole	1.6.2012	2 g
43	P	B13	tb	+	Twinings	Earl Grey Tea	black tea, bergamot flavouring (4,3%)	13.00 zl	19.1.2010	Handlowa Opole	28.02.2011	2 g
44	P	B14	tb	+	Chelton	Earl Grey	herbata czarna z naturalnym dodatkiem aromatu bergamotki z plantacji w. Cejlon; 100% Ceylon schwarzer Tee mit Bergamottearoma	12.00 zl	19.1.2010	Handlowa Opole	25.8.2011	2 g
45	P	B15	tb	+	Irving	Traditional Earl Grey tea	black tea, bergamot flavour - 12% (contains gluten), lemon flavour	13.30 zl	19.1.2010	Tesco Opole	1.3.2011	1.5 g
46	P	B16	ll	-	Sir Roger	Earl Grey	mieszanka herbat czarnych, aromat	2.99 zl	19.1.2010	Tesco Opole	1.7.2011	-
47	P	PLB7	tb	-	Tesco Value	Earl Grey Tea	herbata czarna, aromat	2.50 zl	19.1.2010	Tesco Opole	1.12.2011	1.5 g
48	P	Ts14	ll	-	Herbata Swiata Akro	-	n.k.	n.k.	n.k.	Tesco Opole	-	-
49	GB	B13	tb	+	Twinings	Earl Grey	tea, bergamot flavouring	2.39 £	n.k.	Scotland	-	2.5 g
50	GB	B13	tb	+	Twinings	Earl Grey	tea, bergamot flavouring	3.9 £	n.k.	Scotland	1.8.2011	2 g
51	GB	B17	tb	-	Stash	Earl Grey black tea	blended black teas with oil of bergamot	9.87 £	16.7.2009	Teaworld Ltd Hertfordshire	21.04.2012	1.90
52	GB	B17	tb	-	Stash	Green Tea Earl Grey	Premium black teas with oil of bergamot	9.87 £	16.7.2009	Teaworld Ltd Hertfordshire	04.02.2012	1.9 g
53	GB	B18	tb	+	Taylors of Harrogate	Naturally Decaffeinated Earl Grey Tea	Black decaffeinated tea, natural Bergamot flavour (10%)	3.70 £	16.7.2009	Teaworld Ltd Hertfordshire	31.3.2010	2.5 g
54	GB	B19	tb	+	Ahmad	Earl Grey Tea	Tea, bergamot flavouring; natur-identisches Bergamotte-aroma	4.88 £	16.7.2009	Teaworld Ltd Hertfordshire	02.2012	2 g
55	GB	B20	tb	-	Tazo	Earl Grey scented Black Tea	High-grown black teas (97%) blended with the essence of bergamot	6.81 £	16.7.2009	Teaworld Ltd Hertfordshire	15.01.2010	2.42 g
56	GB	B21	tb	-	teapigs	Darjeeling Earl Grey	Darjeeling tea, cornflowers, natural bergamot flavour	9.31 £	16.7.2009	Teaworld Ltd Hertfordshire	21.08.2010	2.5 g
57	GB	B22	tb	-	Numi	Aged Earl Grey Organic - Bergamot Assam robust with fruity notes	Fair Trade Certified org. Assam Black Tea TGFOP Grade 1, org. Italian Bergamot fruit	10.69 £	16.7.2009	Teaworld Ltd Hertfordshire	12.1.1900	2 g
58	GB	B23	tb	+	Fairtrade	Earl Grey Tea	tea, bergamot flavouring	n.k.	n.k.	Scotland	1.8.2011	2.5 g

Table 37. continued

no.	country[a]	type[b]		G[c]	brand name	labelling	ingredients (original wording)	price/ 100 g[d]	date of purchase	shop / city	best before	weight per tea bag
59	I	B5	tb	+	Sir Winston Tea	Earl Grey Tea	Schwarzer Tee, Bergamotte-Aroma	8.29 €	14.3.2010	Rome	1.1.2012	1.75 g
60	I	B7	tb	+	Lipton	Earl Grey Tea	tè nero, aroma naturale	5.55 €	14.3.2010	Rome	1.8.2001	1.8 g
61	I	B13	tb	+	Twinings	Lady Grey Tea	Tea, Orange peel (3%), Lemon peel (3%), citrus flavouring	7.80 €	14.3.2010	Rome	28.7.2012	2 g
62	I	B24	tb	-	altromercato	Tè nero Earl Grey	Tè - Aromi naturali		n.k.	Rome	10.7.2012	1.75 g
63	I	PLB8	tb	+	Carrefour	Tè Earl Grey	Tè, aromi		n.k.	Rome	1.11.2012	1. 75 g
64	I	Ts15	ll	-	-	Earl Grey Tee	n.k.	5.00 €	19.3.2010	Rome	-	-
65	E	B13	tb	+	Twinings	Earl Grey Tea	black tea, bergamot flavouring (3%)	n.k.	n.k.	Madrid	28.01.2013	2 g
66	E	PLB5	tb	+	Lord Nelson	Earl Grey	Black tea, flavouring	2.72 €	25.9.2010	Lidl Valencia	11.3.2012	1.75 g
67	E	PLB9	tb	-	Hacendado	Té Earl Grey	Té negro (98%), aroma de bergamota (2%), Trazas de gluten.	2.83 €	25.9.2010	Mercadona Valencia	06.2012	1.75g
68	E	Ts16	ll	-	-	Earl Grey Superior	From the ancient Chinese recipe of black tea with bergamot lime oil: this is the most traditional aromatic tea.	5.50 €	10. 2010	Teashop Mantocada Barcelona	04.2011	-
69	E	Ts17	ll	+	Sans & Sans	Earl Grey Luxus	Té negro de china y darjeeling con aroma de bergamota	5.00 €	10. 2010	Sans & Sans Fine Tea Merchants Barcelona	n.k.	-
70	E	Ts18	ll	-	-	Earl Grey negro	n.k.	4.53 €	10. 2010	Mr. Coffee	n.k.	-
71	DK	B13	tb	+	Twinings	Earl Grey Tea	organic tea, Bergamot Flavouring (2.2%)	57.90 kr	n.k.	Super Best Copenhagen	28.3.2013	2.0g
72	DK	B25	tb	+	Fredsted	Økologisk earl grey TE	Økologisk sort te (97%), bergamotolie	30.00 kr	n.k.	Super Best Copenhagen	31.1.2012	2.0g
73	DK	PLB10	ll	-	coop Änglamark	Earl grey the	Økologisk og Fairtrade sort the fra Indien 98%, aroma 2%	22.36 kr	n.k.	SuperBrugsen Copenhagen	12.4.2012	-
74	DK	Ts19	ll	-	-	Earl Grey Øko	Organic "black tea" from China, "natural bergamot oil"	32.00 kr	n.k.	Tante T Copenhagen	-	-
75	DK	Ts20	ll	-	-	-	organic "black tea" from Ceylon, Bergamot oil	35.00 kr	n.k.	Vinbørsen Copenhagen	-	-
76	FI	B7	tb	+	Lipton	Earl Grey Tea	black tea, natural flavour (10,2%), petals (0,5%) (Bluet and Jasmine)	n.k.	09. 2010	K-supermarket	03.2012	1.8 g
77	FI	B7	ll	-	Lipton	tea Earl Grey	Black Tea (98%), Flavouring (1,5%), Marigold Petals (0.5%) (Ringelblumenblätter)	n.k.	09. 2010	Stockmann	06.2012	-

Table 37. continued

no.	country[a]	type[b]		G[c]	brand name	labelling	ingredients (original wording)	price/ 100 g[d]	date of purchase	shop / city	best before	weight per tea bag
78	FI	B13	ll	-	Twinings	Classics Earl Grey Tea	Schwarztee, Bergamotte-Aroma (4.3%)	n.k.	n.k.	K-supermarket	28.6.2012	-
79	FI	PLB5	tb	+	Lord Nelson	Earl Grey	Black tea, flavouring	n.k.	09. 2010	Lidl Finnland	4.4.2012	1.75 g
80	FI	PLB11	ll	-	Rainbow	Earl Grey Tee	tea, bergamot flavour	n.k.	09. 2010	S-market	25.3.2012	-
81	F	B7	tb	+	Lipton	Thé Russian Earl Grey	thé, arôme (2,6%)	6.25 €	25.1.2010	Shopi Briançon	1.10.2011	2 g
82	F	B7	ll	+	Lipton	thé Finest Earl Grey	thé, arômes (6,1%), pétales de fleur (0,5%)	30.40 €	25.1.2010	Shopi Briançon	1.8.2010	-
83	F	B7	ll	+	Lipton	thé Russian Earl Grey	thé, écorces d´agrumes (citron et orange):6.4%, arôme (5,2%), pétales de fleur (0,5%)	32.45 €	25.1.2010	Shopi Briançon	1.9.2011	-
84	F	B13	tb	+	Twinings	Original Earl Grey	thé, arôme bergamote (3%)	2.92 €	25.1.2010	Shopi Briançon	28.12.2011	2 g
85	F	B26	tb	+	Escale Equitable	Thé Earl Grey	Thé noir : 93%, arôme bergamote 7%	4.76 €	25.1.2010	Shopi Briançon	28.4.2011	2 g
86	IRL	B13	tb	+	Twinings	Earl Grey	tea, bergamot flavouring	n.k.	23.7.2010	n.k.	09.2011	2.5 g
87	IRL	PLB5	tb	+	Lord Nelson	Earl Grey	Black tea, flavouring	0.43 €	18.11.2009	Lidl Dublin	8.9.2011	1.75 g
88	IRL	PLB12	tb	+	Diplomat	Green Tea Earl Grey	Green Tea, Flavourings, Bergamot Oil (0.5%)	n.k.	23.7.2010	n.k.	05.2011	2.5 g
89	IRL	Ts21	ll	-	-	Earl Grey	n.k.	5.50 €	18.11.2009	M&D Dublin	-	-
90	IRL	Ts22	ll	-	-	Earl Grey & Blue Flowers	n.k.	5.50 €	18.11.2009	M&D Dublin	-	-
Black tea samples												
101	D	B2	tb	-	Meßmer	Klassik	schwarzer Tee	4.00 €	25.06.2011	Real Freising	29.1.2014	1.75 g
102	D	B2	tb	-	Meßmer	Klassik	schwarzer Tee	4.00 €	25.6.2011	Real Freising	10.1.2014	1.75 g
103	D	B1	tb	-	Teekanne	Darjeeling	schwarzer Tee	3.70 €	25.6.2011	Real Freising	03.2015	1.8 g
104	D	B13	tb	-	Hillcrest	Ostfriesen Teefix	n.k.	3.12 €	28.6.2011	Teeparadies Freising	n.k.	2.5 g
105	D	B1	ll	-	Teekanne	Pure Ceylon Tea Bags	Schwarzer Tee, Blattsortierung Flowery Broken Orange Pekoe	2.64 €	25.6.2011	Teeparadies Freising	11.2014	-

Table 37. continued

no.	country[a]	type[b]		G[c]	brand name	labelling	ingredients (original wording)	price/ 100 g[d]	date of purchase	shop / city	best before	weight per tea bag
106	D	Ts23	ll	-	-	Assam Finest Selection F.B.O.P.	n.k.	~4€	28.6.2011	Teeparadies Freising	n.k.	-
107	D	Ts24	ll	-	-	Koslanda Bio-Ceylon	n.k.	~4€	28.6.2011	Teeparadies Freising	n.k.	-
Fruit tea sample												
201	D	B2	tb	+	Meßmer	Waldbeere	Hibiskus, Hagebutten, Äpfel, Aroma (Erdbeere, Himbeere, Brombeere), Holunderbeeren, Heidelbeeren	3.50 €	21.6.2011	Real Freising	24.2.2013	2.5 g

[a] country of purchase: Germany (D), Hungary (H), Poland (PL), United Kingdom (UK), Italy (I), Spain (E), Denmark (DK), Finland (FIN), France (F), Ireland (IRL); [b] type: B: product assignable to specific brands (B1-26); PLB: products assignable to private label brands exclusively used by specific supermarket chains (PLB1-12); Ts: products not assignable to a specific brand / private label brand, but sold in speciality tea-shops (Ts1-22). tb: products offered in tea bags; ll: products offered as loose leaves; [c] tea samples containing granulate (G) were signed with (+), those without with (-); [d] prices are given in Euro (€),Złoty (zl), Danish krones (kr), Pound sterling (£); [e] not known (no description was given on the package).

8.2 Recovery rates

Table 38. Recovery rates used for quantification corrections of substances determined in tea infusions and residual tea materials (squeezed tea bag from tea infusion). Preparations see 3.2.3.6.

substance	recovery rate [%]	
	tea infusion	residual tea material
aliphatic linalyl esters		
linalyl formate	93.8 ± 0.6	112.9 ± 1.3
linalyl acetate	108.2 ± 0.5	121.1 ± 1.0
linalyl propanoate	98.2 ± 0.1	107.9 ± 7.0
linalyl butanoate	103.4 ± 2.2	104.5 ± 2.7
linalyl isobutanoate	99.1 ± 0.6	102.3 ± 1.4
linalyl isovalerate	105.2 ± 1.3	95.3 ± 7.6
linalyl hexanoate	89.7 ± 0.5	70.4 ± 9.6
linalyl octanoate	41.8 ± 4.3	48.8 ± 12.3
aromatic linalyl esters		
linalyl cinnamate	92.4 ± 0.9	115.1 ± 7.4
linalyl benzoate	107.6 ± 0.3	92.4 ± 12.1
linalyl phenylacetate	87.4 ± 0.3	82.8 ± 3.3
monoterpene alcohols		
linalool [a]	103.3 ± 2.9	115.8 ± 5.2
α-terpineol	83.3 ± 0.6	86.5 ± 3.1
monoterpene esters		
geranyl acetate	89.2 ± 0.2	95.4 ± 1.2
neryl acetate	91.3 ± 1.4	97.9 ± 2.0
terpinyl acetate	87.8 ± 0.3	95.2 ± 1.0
menthyl acetate	89.6 ± 0.1	103.6 ± 19.2
monoterpene hydrocarbons		
limonene [b]	106.9 ± 0.5	104.1 ± 6.1
β-pinene	108.8 ± 0.4	110.1 ± 5.9
γ-terpinene	106.0 ± 1.0	111.7 ± 1.1

[a] values adopted for geraniol and nerol; [b] values adopted for myrcene. Data represent means ± standard deviations from triplicate experiments.

8.3 Response factors and retention indices

Table 39. GC-response factors (Rf´s) and linear retention indices (RI´s) of flavouring substances on DB-5.

substance	Rf[a]	linear retention index (RI)	
		experimental[b]	literature
pinene, β-	0.80	982	978[c] / 978[d] / 980[e] / 990[f] / 934[g] / 990[h] / 981[i]
myrcene	-	989	989[c] / 990[d] / 991[e] / 994[h] / 988[i]
limonene	0.80	1021	1030[c] / 1030[d] / 1031[e] / 1031[g]/ 1036[h] / 1027[i]
(*Z*)-ocimene	-	1030	1040[e]
(*E*)-ocimene	-	1039	1050[e]
γ-terpinene	0.81	1048	1072[d] / 1062[e] / 1074[h] / 1055[i]
terpinolene	-	1081	1088[e]
linalool	0.94	1098	1103[c] / 1098[e] / 1104[f] / 1101[g] / 1107[h] / 1095[i]
α-terpineol	0.89	1181	1195[c] / 1192[d] / 1199[f] / 1198[g] / 1187[i]
linalyl formate	1.07	1218	
nerol	-	1235	1233[c]/ 1228[e]
geraniol	-	1264	1256[c] / 1255[e] / 1276[h]/ 1252[i]
linalyl acetate	1.10	1266	1259[d] /1257[e]
neryl formiate	-	1284	
menthyl acetate	0.89	1294	
geranyl formiate	-	1301	1300[e]
linalyl propanoate	0.98	1341	
terpinyl acetate	0.88	1349	
neryl acetate	0.98	1368	1365[e]
linalyl isobutanoate	0.97	1377	1374[e]
geranyl acetate	0.91	1386	1383[e] / 1377[f]
linalyl butanoate	1.01	1423	1422[e]
neryl propanoate	-	1456	1454[e]
linalyl isovalerate	1.00	1471	
geranyl propanoate	-	1476	1475[e]
neryl isobutanoate	-	1490	1491[e]
geranyl isobutanoate	-	1515	1514[e]
neryl butanoate	-	1540	
geranyl butanoate	-	1562	
neryl isovalerate	-	1585	
linalyl hexanoate	0.96	1607	
geranyl isovalerate	-	1611	
neryl hexanoate	-	1731	
geranyl hexanoate	-	1755	

substance	Rf[a]	linear retention index (RI)	
		experimental[b]	literature
linalyl octanoate	0.98	1802	
linalyl benzoate	1.03	1803	
linalyl phenylacetate	0.79	1848	
neryl octanoate	-	1927	
neryl benzoate	-	1943	
geranyl octanoate	-	1950	
geranyl benzoate	-	1964	
neryl phenylacetate	-	1992	
geranyl phenylacetate	-	2018	
linalyl cinnamate	0.91	2117	
neryl cinnamate	-	2265	
geranyl cinnamate	-	2289	

[a] Response factor determined in relation to on the internal standard 2-decanol on a DB-5 column and GC-System I (see Materials and Methods), for some substances not analysed due to the lack of pure reference compounds, a Rf=1 was supposed; [b] linear retention index determined with authentic reference compounds on GC-system I and on a DB-5 column; [c] Rychlik et al. 1998; [d] Goodner. 2008; [e] Adams 1995; [f] Gomez et al. 1993; [g] Jordán et al. 2002; [h] Högnadóttir and Rouseff. 2003; [i] Jirovetz et al. 2003.

Table 40. GC-response factors (Rf´s) and linear retention indices (RI´s) of flavouring substances on DB-WAX.

substance	Rf[a]	linear retention index (RI)	
		experimental[b]	in-house table
2-methylpropyl acetate	- [c]	1008	1004
3-methylbutyl acetate	-	1119	1115
(*Z*)-3-hexenyl acetate	-	1311	1318
heptanol, 2- *(ESD)*	1.12	1324	1319
(*Z*)-3-hexenol	-	1384	1387
benzaldehyde	-	1501	1496
2-nonanol *(CSD)*	1.01	1524	1526
decanol, 2- *(ISD)*	-	1624	1619
benzyl acetate	-	1723	1703
β-damascenone	-	1816	1813
α-ionone	-	1847	1832
β-ionone	-	1936	1921
eugenol	-	2161	2166
methyl isoeugenol	-	2163	2172
γ-undecalactone	-	2247	2237
vanillin	-	2588	2570
raspberry ketone	-	2975	2942

[a] Response factor determined dependent on the injection standard (ISD) 2-decanol on a DB-WAX column and GC-System I (see Materials and Methods); [b] linear retention index determined with authentic reference compounds on GC-system I and on a DB-WAX column; [c] no Rfs required because of the application of a standard addition method.

8.4 Limits of detection (LOD) and quantification (LOQ)

Table 41. Exemplary calculations of LOD´s and LOQ´s for the individual isolation methods.

	linalyl acetate	linalool	limonene	β-pinene	γ-terpinene	dihydro-linalool
	calculations for the extraction of tea material					
limit of detection						
[µg/mL][a]	0.039	0.034	0.031	0.036	0.029	0.037
[µg/2 mL *n*-hexane][b]	0.078	0.069	0.062	0.072	0.057	0.074
[µg/g tea][c]	0.078	0.069	0.062	0.072	0.057	0.074
[µg/g tea] in 1:5 dilution[d]	0.391	0.345	0.309	0.358	0.287	0.371
limit of quantification						
[µg/mL][a]	0.119	0.104	0.094	0.109	0.087	0.113
[µg/2 mL *n*-hexane][b]	0.238	0.208	0.188	0.218	0.175	0.226
[µg/g tea][c]	0.238	0.208	0.188	0.218	0.175	0.226
[µg/g tea] in 1:5 dilution[d]	1.190	1.038	0.941	1.090	0.873	1.128
	calculations for the extraction of tea material					
limit of detection						
[µg/mL][a]	0.039	0.034	0.031	0.036	0.029	0.037
[µg/2 mL *n*-hexane][b]	0.078	0.069	0.062	0.072	0.057	0.074
[µg/6 mL tea infusion][e]	0.078	0.069	0.062	0.072	0.057	0.074
[µg/200 mL tea infusion][f]	2.61	2.30	2.06	2.38	1.91	2.47
[µg/tea bag] without transfer rate	2.61	2.30	2.06	2.38	1.91	2.47
transfer rate [%][g]	1.93	66.39	26.45	25.72	25.72	66.39
[µg/tea bag] + transfer rate	135.44	3.46	7.78	9.27	7.43	3.72
[µg/g tea], tea bag = 1.5 g	90.29	2.31	5.19	6.18	4.95	2.48
[µg/g tea], tea bag = 1.75 g	77.39	1.98	4.45	5.30	4.24	2.13
[µg/g tea], tea bag = 2.8 g	48.37	1.24	2.78	3.31	2.65	1.33
limit of quantification						
[µg/mL][a]	0.119	0.104	0.094	0.109	0.087	0.113
[µg/2 mL *n*-hexane][b]	0.238	0.208	0.188	0.218	0.175	0.226
[µg/6 mL tea infusion][e]	0.238	0.208	0.188	0.218	0.175	0.226
[µg/200 mL tea infusion][f]	7.93	6.92	6.27	7.26	5.82	7.52
[µg/tea bag] without transfer rate	7.93	6.92	6.27	7.26	5.82	7.52
transfer rate [%][g]	1.93	66.39	26.45	25.72	25.72	66.39
[µg/tea bag] + transfer rate	412.06	10.42	23.71	28.24	22.64	11.33
[µg/g tea], tea bag = 1.5 g	274.71	6.95	15.81	18.83	15.09	7.55
[µg/g tea], tea bag = 1.75 g	235.46	5.96	13.55	16.14	12.94	6.47
[µg/g tea], tea bag = 2.8 g	147.16	3.72	8.47	10.09	8.09	4.05

*[a] limit concentrations in the final extract; [b] limit concentrations in 2 mL extraction solvent (n-*hexane*); [c] limit concentrations in the tea material when 1 g initial weight was used; [d] limit concentrations in the tea material when the extract was diluted 1:5 (1:50 dilutions are not listed because the 1:5 dilution was injected in case of too low peak areas in the 1:50 dilution); [e] an aliquot of 6 mL out of 200 mL was extracted; [f] the tea bag subjected to the hot water infusion refer to a total of 200 mL tea beverage; [g] transfer rates see 4.2.3 and footnote of Table 53 (transfer rate of linalool also used for dihydrolinalool).*

8.5 Stability of linalyl acetate in cold water and tea infusion

Table 42. Degradation of linalyl acetate (starting concentration: 0.04 mg/mL) into hydrolysis and isomerisation products in water at RT (20°C) in the course of 144 h, respectively. The contents [%] were calculated with the sum of all measured substances (as linalyl acetate equivalents) set to 100%.

	content [%]					
time	linalyl acetate	linalool	α-terpineol	geraniol	nerol	geranyl acetate
0 min	99.9 ± 0.01	0.1 ± 0.01	n.d.[a]	n.d.	n.d.	n.d.
8 min	99.7 ± 0.3	0.3 ± 0.04	n.d.	n.d.	n.d.	n.d.
15 min	99.0 ± 0.05	0.6 ± 0.04	0.3 ± 0.01	n.d.	n.d.	0.1 ± 0.01
30 min	98.1 ± 0.1	1.1 ± 0.06	0.6 ± 0.03	n.d.	n.d.	0.1 ± 0.01
45 min	97.0 ± 0.06	1.6 ± 0.06	0.9 ± 0.02	n.d.	n.d.	0.2 ± 0.02
60 min	95.9 ± 0.09	2.2 ± 0.08	1.1 ± 0.03	n.d.	n.d.	0.3 ± 0.01
120 min	91.9 ± 0.1	4.4 ± 0.07	2.3 ± 0.05	n.q.[b]	n.q.	0.6 ± .02
180 min	87.4 ± 0.2	6.5 ± 0.02	3.5 ± 0.02	0.2 ± 0.08	0.2 ± 0.02	0.8 ± 0.03
240 min	83.5 ± 0.2	8.5 ± 0.1	4.6 ± 0.04	0.3 ± 0.04	0.2 ± 0.01	1.1 ± 0.01
300 min	79.1 ± 0.2	10.8 ± 0.06	5.9 ± 0.01	0.5 ± 0.08	0.3 ± 0.02	1.4 ± 0.05
420 min	71.9 ± 0.09	14.3 ± 0.08	7.8 ± 0.02	0.7 ± 0.05	0.4 ± 0.03	1.9 ± 0.02
24 h	27.2 ± 0.2	34.0 ± 0.04	23.0 ± 0.1	3.5 ± 0.2	1.0 ± 0.2	5.2 ± 0.04
48 h	7.8 ± 0.2	46.5 ± 0.2	25.6 ± 0.06	5.8 ± 0.5	1.8 ± 0.3	5.9 ± 0.1
144 h	1.3 ± 0.2	51.3 ± 0.2	28.4 ± 0.1	9.0 ± 0.1	2.4 ± 0.1	2.8 ± 0.2

time	neryl acetate	myrcene	(*E*)-ocimene	(*Z*)-ocimene	terpinene	limonene
0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15 min	n.q.	n.q.	n.q.	n.d.	n.d.	n.d.
30 min	0.1 ± 0.01	n.q.	n.q.	n.q.	n.d.	n.q.
45 min	0.1 ± 0.01	0.2 ± 0.01	n.q.	n.q.	n.d.	n.d.
60 min	0.2 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	n.q.	n.d.	n.q.
120 min	0.4 ± 0.02	0.3 ± 0.01	0.2 ± 0.00	n.q.	n.q.	n.q.
180 min	0.5 ± 0.01	0.4 ± 0.01	0.3 ± 0.03	0.2 ± 0.01	n.q.	n.q.
240 min	0.6 ± 0.04	0.5 ± 0.01	0.5 ± 0.02	0.2 ± 0.01	n.q.	n.q.
300 min	0.8 ± 0.01	0.5 ± 0.03	0.4 ± 0.01	0.2 ± 0.00	n.q.	n.q.
420 min	1.1 ± 0.02	0.7 ± 0.02	0.7 ± 0.05	0.3 ± 0.02	n.q.	0.1 ± 0.004
24 h	3.0 ± 0.1	1.1 ± 0.04	1.3 ± 0.07	0.6 ± 0.03	n.q.	0.2 ± 0.02
48 h	3.4 ± 0.04	1.0 ± 0.03	1.3 ± 0.07	0.6 ± 0.05	n.q.	0.2 ± 0.01
144 h	2.6 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	0.5 ± 0.08	n.q.	0.2 ± 0.03

[a] below limit of determination (LOD); [b] below limit of quantification (LOQ), see 3.2.3.7 and 8.4.

Table 43. Degradation of linalyl acetate (starting concentration: 0.04 mg/mL) into hydrolysis and isomerisation products in black tea infusion at RT (20°C) in the course of 48 h, respectively (no values could be collected for 144 h due to a fungi infestation of the solution). The percentages were calculated with the sum of all measured substances (as linalyl acetate equivalents) set to 100%.

	content [%]					
time	linalyl acetate	linalool	α-terpineol	geraniol	nerol	geranyl acetate
0 min	99.9 ± 0.005	0.1 ± 0.005	n.d.[a]	n.d.	n.d.	n.d.
8 min	99.8 ± 0.02	0.2 ± 0.02	n.q.[b]	n.d.	n.d.	n.d.
15 min	99.2 ± 0.02	0.4 ± 0.02	0.3 ± 0.04	n.d.	n.d.	0.1 ± 0.004
30 min	98.5 ± 0.03	0.8 ± 0.06	0.5 ± 0.05	n.d.	n.d.	0.1 ± 0.007
45 min	97.7 ± 0.07	1.2 ± 0.02	0.8 ± 0.05	n.d.	n.d.	0.2 ± 0.002
60 min	96.8 ± 0.1	1.6 ± 0.007	1.0 ± 0.07	n.d.	n.d.	0.2 ± 0.01
120 min	93.0 ± 0.2	3.5 ± 0.07	2.3 ± 0.07	n.d.	n.d.	0.5 ± 0.004
180 min	88.6 ± 0.2	5.4 ± 0.07	3.6 ± 0.09	0.2 ± 0.07	0.2 ± 0.03	0.8 ± 0.02
240 min	84.3 ± 0.2	7.3 ± 0.07	4.9 ± 0.1	0.5 ± 0.03	0.3 ± 0.02	1.1 ± 0.02
300 min	79.8 ± 0.2	9.4 ± 0.1	6.4 ± 0.04	0.7 ± 0.02	0.4 ± 0.03	1.4 ± 0.02
420 min	72.3 ± 0.2	12.7 ± 0.05	8.7 ± 0.02	1.0 ± 0.07	0.5 ± 0.05	2.0 ± 0.02
24 h	30.0 ± 0.4	32.1 ± 0.07	22.0 ± 0.2	3.9 ± 0.2	1.2 ± 0.06	5.0 ± 0.01
48 h	8.3 ± 0.1	42.3 ± 0.1	29.3 ± 0.1	5.9 ± 0.3	1.8 ± 0.1	6.0 ± 0.3

time	neryl acetate	myrcene	(*E*)-ocimene	(*Z*)-ocimene	terpinene	limonene
0 min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8 min	n.d.	n.q.	n.d.	n.d.	n.d.	n.d.
15 min	n.q.	n.q.	n.q.	n.d.	n.d.	n.d.
30 min	n.q.	n.q.	n.q.	n.d.	n.d.	n.d.
45 min	0.1 ± 0.01	n.q.	n.d.	n.d.	n.d.	n.d.
60 min	0.1 ± 0.02	0.1 ± 0.007	n.q.	n.d.	n.d.	n.d.
120 min	0.3 ± 0.02	0.2 ± 0.007	0.2 ± 0.02	n.q.	n.d.	n.q.
180 min	0.5 ± 0.01	0.3 ± 0.009	0.3 ± 0.02	0.1 ± 0.02	n.d.	n.q.
240 min	0.6 ± 0.01	0.4 ± 0.01	0.4 ± 0.03	0.2 ± 0.02	n.q.	n.q.
300 min	0.8 ± 0.02	0.5 ± 0.02	0.5 ± 0.02	0.2 ± 0.007	n.q.	n.q.
420 min	1.1 ± 0.03	0.6 ± 0.03	0.6 ± 0.02	0.3 ± 0.01	n.q.	0.1 ± 0.004
24 h	2.8 ± 0.09	1.1 ± 0.03	1.1 ± 0.03	0.6 ± 0.02	n.q.	0.2 ± 0.02
48 h	3.4 ± 0.06	1.0 ± 0.05	1.0 ± 0.05	0.6 ± 0.01	n.q.	0.2 ± 0.010

[a] below limit of determination (LOD); [b] below limit of quantification (LOQ), see 3.2.3.7 and 8.4. Data represent means ± standard deviations from triplicate experiments.

8.6 Impact of the infusion time

Table 44. Distribution of reaction products obtained by hot water infusions (200 mL) of tea bags (2 g) containing 4045 µg linalyl acetate (median value obtained for 90 Earl Grey teas) with 0.5, 1, 2, 3, 5 and 10 minutes brewing time.

time [min]	transfer rate [%]					
	linalyl acetate	linalool	α-terpineol	geraniol	nerol	geranyl acetate
0.5	6.5 ± 1.5	13.8 ± 2.5	6.4 ± 1.2	2.2 ± 0.5	0.73 ± 0.08	2.3 ± 0.4
1	5.1 ± 0.3	14.3 ± 1.5	6.7 ± 0.7	2.1 ± 0.4	0.66 ± 0.09	2.3 ± 0.3
2	3.2 ± 0.3	15.9 ± 1.5	7.6 ± 0.7	2.5 ± 0.2	0.78 ± 0.06	2.5 ± 0.2
3	1.9 ± 0.4	17.0 ± 1.7	8.2 ± 0.8	2.8 ± 0.2	0.97 ± 0.12	2.7 ± 0.3
5	2.5 ± 0.1	18.9 ± 1.2	9.0 ± 0.6	3.1 ± 0.5	0.85 ± 0.13	2.6 ± 0.2
10	2.9 ± 0.2	20.3 ± 0.2	10.3 ± 0.07	3.9 ± 0.09	1.04 ± 0.11	2.3 ± 0.07

[min]	neryl acetate	myrcene	(*E*)-ocimene	(*Z*)-ocimene	terpinene	limonene	sum
0.5	1.2±0.2	1.3±0.2	1.0±0.2	0.58±0.09	0.30±0.07	0.23±0.03	36.6±6.1
1	1.2±0.2	1.4±0.1	1.1±0.2	0.59±0.06	0.31±0.05	0.22±0.02	36.1±3.8
2	1.3±0.1	1.4±0.1	1.1±0.1	0.61±0.06	0.32±0.05	0.22±0.01	37.5±3.4
3	1.4±0.2	1.5±0.2	0.9±0.4	0.81±0.11	0.38±0.04	0.26±0.05	38.8±3.5
5	1.4±0.1	1.4±0.11	1.1±0.07	0.62±0.04	0.36±0.02	0.23±0.02	42.1±2.1
10	1.2±0.02	1.1±0.01	0.8±0.02	0.49±0.02	0.28±0.04	0.18±0.002	44.9±0.6

Data represent means ± standard deviations from triplicate experiments.

Table 45. Distribution of reaction products determined in squeezed tea bags after hot water infusions (200 mL) of tea bags (2 g) containing 4045 µg linalyl acetate (median value obtained for 90 Earl Grey teas) with 0.5, 1, 2, 3, 5 and 10 minutes brewing time.

time	transfer rate [%]					
	linalyl acetate	linalool	α-terpineol	geraniol	nerol	geranyl acetate
0.5 min	34.5±3.6	0.7 ± 0.2	0.33 ± 0.06	n.d.	n.d.	0.18±0.03
1 min	32.4±1.7	1.1 ± 0.1	0.53 ± 0.07	n.d.	0.04 ± 0.01	0.27±0.03
2 min	30.6±3.6	1.7 ± 0.08	0.82 ± 0.06	n.d.	0.06 ± 0.002	0.49±0.03
3 min	23.0±2.3	1.8 ± 0.08	1.06 ± 0.05	0.18 ± 0.01	0.12 ± 0.004	0.61±0.10
5 min	23.2±0.4	2.6 ± 0.04	1.15 ± 0.02	0.22 ± 0.01	0.10 ± 0.01	0.86±0.08
10 min	17.6±1.4	2.9 ± 0.1	1.31 ± 0.04	0.28 ± 0.12	0.14 ± 0.001	1.36±0.13

time	neryl acetate	myrcene	(*E*)-ocimene	(*Z*)-ocimene	terpinene	limonene	sum
0.5 min	0.10±0.02	0.11±0.02	0.08±0.02	n.q.	n.d.	n.q.	36.1±4.0
1 min	0.14±0.02	0.18±0.02	0.12±0.02	0.06±0.01	0.05±0.005	0.04±0.003	34.9±1.9
2 min	0.26±0.02	0.32±0.03	0.24±0.03	0.13±0.02	0.07±0.01	0.07±0.009	34.7±3.8
3 min	0.32±0.05	0.33±0.04	0.25±0.03	0.14±0.02	0.02±0.01	0.07±0.009	28.0±2.5
5 min	0.44±0.04	0.58±0.06	0.44±0.05	0.23±0.03	0.11±0.03	0.11±0.01	30.0±0.2
10 min	0.71±0.07	0.96±0.10	0.75±0.07	0.37±0.04	0.19±0.03	0.17±0.017	26.7±2.0

Data represent means ± standard deviations from triplicate experiments.

8.7 Impact of the amount of added flavouring substance

Table 46. Distribution of a) reaction products in the final tea beverage and b) in the residual tea bag obtained by hot water infusion (200 mL) of a tea bag (2 g) containing 100 µg linalyl acetate.

			content		transfer rate
	no.[a]		[µg]	linalyl acetate equivalent [µg]	linalyl acetate equivalent [%]
a)	10	linalyl acetate	n.q.	n.q.[b]	n.q.
		hydrolysis product			
	6	linalool	12 ± 1	16 ± 1	15.6 ± 0.8
		reaction products			
	7	α-terpineol	6 ± 1	8 ± 1	7.6 ± 1.4
	9	geraniol	n.q.	n.q.	n.q.
	8	nerol	n.d.	n.d.[c]	n.d.
	12	geranyl acetate	n.d.	n.d.	n.d.
	11	neryl acetate	n.d.	n.d.	n.d.
	1	myrcene	n.d.	n.d.	n.d.
	3	(*E*)-β-ocimene	n.d.	n.d.	n.d.
	4	(*Z*)-β-ocimene	n.d.	n.d.	n.d.
	5	terpinolene	n.d.	n.d.	n.d.
	2	limonene	n.d.	n.d.	n.d.
		sum	**18 ± 2**	**23 ± 2**	**23.2 ± 2.1**
b)	10	linalyl acetate	21 ± 2	21 ± 2	20.8 ± 1.8
		hydrolysis product			
	6	linalool	2 ± 0.03	2.5 ± 003	2.5 ± 0.03
		reaction products			
	7	α-terpineol	n.q.	n.q.	n.q.
	9	geraniol	n.q.	n.q.	n.q.
	8	nerol	n.q.	n.q.	n.q.
	12	geranyl acetate	n.q.	0.4 ± 0.03	n.q.
	11	neryl acetate	0.4 ± 0.03	n.q.	0.4 ± 0.03
	1	myrcene	0.2 ± 0.001	0.3 ± 0.001	0.3 ± 0.001
	3	(*E*)-β-ocimene	0.2 ± 0.05	0.3 ± 0.1	0.3 ± 0.1
	4	(*Z*)-β-ocimene	n.q.	n.q.	n.q.
	5	terpinolene	n.q.	n.q.	n.q.
	2	limonene	n.q.	n.q.	n.q.
		sum	**24 ± 2**	**24 ± 2**	**24.3 ± 1.9**

[a] Peak numbers according to Figure 25; [b] below limit of quantification (LOQ); [c] below limit of determination (LOD), see 3.2.3.7 and 8.4. Data represent means ± standard deviations from triplicate experiments.

Table 47. Distribution of a) reaction products in the final tea beverage and b) in the residual tea bag obtained by hot water infusion (200 mL) of a tea bag (2 g) containing 400 µg linalyl acetate.

			content		transfer rate
	no.[a]		[µg]	linalyl acetate equivalent [µg]	linalyl acetate equivalent [%]
a)	10	linalyl acetate	6 ± 0.2	6 ± 0.2	1.5 ± 0.05
		hydrolysis product			
	6	linalool	52 ± 4	66 ± 5	16.6 ± 1.3
		reaction products			
	7	α-terpineol	26 ± 2	33 ± 3	8.3 ± 0.8
	9	geraniol	12 ± 1	15 ± 2	3.7 ± 0.4
	8	nerol	n.q.[b]	n.q.	n.q.
	12	geranyl acetate	10 ± 1	10 ± 1	2.5 ± 0.2
	11	neryl acetate	n.q.	n.q.	n.q.
	1	myrcene	n.d.[c]	n.d.	n.d.
	3	(*E*)-β-ocimene	n.d.	n.d.	n.d.
	4	(*Z*)-β-ocimene	n.d.	n.d.	n.d.
	5	terpinolene	n.d.	n.d.	n.d.
	2	limonene	n.d.	n.d.	n.d.
		sum	**106 ± 8**	**131 ± 10**	**32.7 ± 2.6**
b)	10	linalyl acetate[b]	109 ± 5	109 ± 5	27.3 ± 1.3
		hydrolysis product			
	6	linalool[c]	8 ± 0.6	10 ± 1	2.4 ± 0.2
		reaction products			
	7	α-terpineol[c]	4 ± 0.4	5 ± 0.6	1.2 ± 0.1
	9	geraniol[c]	2 ± 0.06	3 ± 0.1	0.7 ± 0.02
	8	nerol[c]	1.1 ± 0.04	1.4 ± 0.04	0.3 ± 0.01
	12	geranyl acetate[c]	3 ± 0.1	2 ± 0.06	0.7 ± 0.03
	11	neryl acetate[c]	2 ± 0.06	3 ± 0.1	0.4 ± 0.01
	1	myrcene[d]	1.1 ± 0.04	2 ± 0.05	0.4 ± 0.01
	3	(*E*)-β-ocimene[d]	0.9 ± 0.09	1.3 ± 0.1	0.3 ± 0.03
	4	(*Z*)-β-ocimene[d]	0.5 ± 0.04	0.7 ± 0.06	0.2 ± 0.01
	5	terpinolene[d]	0.2 ± 0.02	0.3 ± 0.03	0.1 ± 0.008
	2	limonene[d]	0.2 ± 0.01	0.3 ± 0.02	0.1 ± 0.004
		sum	**131 ± 5**	**136 ± 5**	**34.1 ± 1.1**

[a] Peak numbers according to Figure 25; [b] below limit of quantification (LOQ); [c] below limit of determination (LOD), see 3.2.3.7 and 8.4. Data represent means ± standard deviations from triplicate experiments. Data represent means ± standard deviations from triplicate experiments.

Table 48. Distribution of a) reaction products in the final tea beverage and b) in the residual tea bag obtained by hot water infusion (200 mL) of a tea bag (2 g) containing 20000 µg linalyl acetate.

			content		transfer rate
	no.[a]		[µg]	linalyl acetate equivalent [µg]	linalyl acetate equivalent [%]
a)	10	linalyl acetate	554±83	554 ± 377	2.6 ± 0.4
		hydrolysis product			
	6	linalool	1619±202	2060 ± 1281	9.6 ± 1.2
		reaction products			
	7	α-terpineol	789±95	1004 ± 121	4.7 ± 0.6
	9	geraniol	320±38	407 ± 48	1.9 ± 0.2
	8	nerol	99±12	126 ± 15	0.6 ± 0.1
	12	geranyl acetate	319±42	319 ± 42	1.5 ± 0.2
	11	neryl acetate	172±23	172 ± 23	0.8 ± 0.1
	1	myrcene	124±17	178 ± 25	0.8 ± 0.1
	3	(*E*)-β-ocimene	99±14	142 ± 20	0.7 ± 0.1
	4	(*Z*)-β-ocimene	53±7	76 ± 10	0.4 ± 0.04
	5	terpinolene	27±3	39 ± 4	0.2 ± 0.02
	2	limonene	19±3	28 ± 4	0.1 ± 0.02
		sum	**7839±870**	**5106 ± 577**	**23.9 ± 2.7**
b)	10	linalyl acetate	11110±1187	11110 ± 1187	51.9 ± 5.5
		hydrolysis product			
	6	linalool	248±14	315 ± 17	1.5 ± 0.1
		reaction products			
	7	α-terpineol	111±8	141 ± 11	0.7 ± 0.05
	9	geraniol[b]			
	8	nerol	12±0.9	15 ± 1	0.1 ± 0.006
	12	geranyl acetate	92±7	49 ± 4	0.4 ± 0.03
	11	neryl acetate	49±4	92 ± 7	0.2 ± 0.02
	1	myrcene	40±3	57 ± 5	0.3 ± 0.02
	3	(*E*)-β-ocimene	30±3	43 ± 4	0.2 ± 0.02
	4	(*Z*)-β-ocimene	8±0.7	11 ± 1	0.1 ± 0.005
	5	terpinolene[c]	n.q.[d]	n.q.	n.q.
	2	limonene[c]	n.q.	n.q.	n.q.
		sum	**11698 ±1189**	**11833 ± 1191**	**55.3 ± 5.6**

[a] Peak numbers according to Figure 25; [b] not quantifiable due to overlapping peaks (linalyl acetate); [c] not quantifiable reasoned by a higher dilution of the extract in favour of the too high peak area of linalyl acetate; [d] below limit of quantification (LOQ), see 3.2.3.7 and 8.4. Data represent means ± standard deviations from triplicate experiments.

8.8 Transfer of aliphatic and aromatic linalyl esters

Table 49. Distribution of reaction products obtained by hot water infusions (200 mL) of tea bags (2 g) containing 4045 µg linalyl ester (median value of linalyl acetate obtained for 90 Earl Grey teas).

	transfer rate [%]					
substance	linalyl ester	linalool	α-terpineol	geraniol	nerol	geranyl ester
aliphatic linalyl ester						
l. formiate	n.d.	30.6±0.3	12.6±0.4	4.7±0.1	1.4±0.09	1.5±0.07
l. acetate	1.9±0.4	18.9±1.8	8.2±0.8	2.8±0.2	1.0±0.1	2.7±0.3
l. propanoate	7.0±0.6	11.0±2.0	5.1±0.9	1.9±0.5	0.6±0.1	2.2±0.2
l. butanoate	9.9±1.6	8.7±1.7	4.1±0.9	1.5±0.4	0.4±0.1	1.6±0.3
l. isobutanoate	11.0±0.7	5.1±0.6	2.6±0.3	1.0±0.2	0.3±0.03	1.1±0.1
l. isovalerate	17.4±2.5	5.8±0.2	2.7±0.1	0.9±0.03	0.3±0.02	1.1±0.02
l. hexanoate	25.6±3.4	2.5±0.2	1.1 ±0.1	0.4±0.005	n.q.	0.8±0.04
l. octanoate	48.7±9.7	0.4±0.2	0.2 ±0.07	n.d.[a]	n.d.	0.4±0.3
aromatic linalyl ester						
l. benzoate	3.3±0.5	14.5±2.9	6.3±1.2	1.7±0.2	0.5±0.04	2.6±0.5
l. phenylacetate	4.1±0.4	11.1±1.8	5.2±1.0	2.3±0.4	0.7±0.1	2.5±0.4
l. cinnamate	13.3±0.7	11.1±0.5	3.3±2.7	2.0±0.3	0.6±0.07	2.3±0.07

substance	neryl ester	myrcene	(*E*)-ocimene	(*Z*)-ocimene	terpinene	limonene
aliphatic linalyl ester						
l. formiate	0.6 ± 0.04	0.7±0.03	0.7±0.06	0.4±0.01	0.4±0.03	0.2±0.02
l. acetate	1.4 ± 0.2	1.5±0.2	0.9±0.4	0.8±0.1	0.4±0.04	0.3±0.05
l. propanoate	0.9 ± 0.1	1.1±0.2	0.9±0.1	0.5±0.09	0.2±0.04	0.1±0.02
l. butanoate	0.9 ± 0.2	1.1±0.2	0.8±0.2	0.4±0.09	0.2±0.05	0.1±0.02
l. isobutanoate	0.6 ± 0.05	0.8±0.07	0.5±0.06	0.3±0.04	0.1±0.03	n.q.
l. isovalerate	0.6 ± 0.003	0.8±0.05	0.6±0.03	0.4±0.03	0.2±0.02	n.q.
l. hexanoate	0.3 ± 0.02	0.4±0.04	0.3±0.02	0.2±0.02	n.q.	n.q.
l. octanoate	0.3 ± 0.07	n.q.[b]	n.d.	n.d.	n.d.	n.d.
aromatic linalyl ester						
l. benzoate	1.2 ± 0.2	0.9±0.1	0.8±0.1	0.4±0.07	0.3±0.04	0.2±0.03
l. phenylacetate	1.3 ± 0.2	1.1±0.2	0.9±0.2	0.5±0.06	0.3±0.03	0.2±0.03
l. cinnamate	1.3 ± 0.04	0.8±0.01	0.7±0.03	0.4±0.03	0.3±0.02	0.2±0.01

substance	sum	sum alcohols	sum esters
aliphatic linalyl ester			
l. formiate	24.6 ± 0.3	2.4 ± 0.1	2.1 ± 0.1
l. acetate	13.3 ± 1.0	3.8 ± 0.1	4.1 ± 0.5
l. propanoate	8.4 ± 1.6	2.8 ± 0.5	3.1 ± 0.1
l. butanoate	6.6 ± 1.4	2.6 ± 0.6	2.5 ± 0.6
l. isobutanoate	4.1 ± 0.5	1.7 ± 0.2	1.6 ± 0.2
l. isovalerate	4.1 ± 0.1	2.0 ± 0.1	1.7 ± 0.02
l. hexanoate	1.7 ± 0.1	0.9 ± 0.07	1.0 ± 0.06
l. octanoate	0.4 ± 0.06	n.q.	0.6 ± 0.4
aromatic linalyl ester			
l. benzoate	32.7 ± 5.5	8.5 ± 1.0	2.6 ± 0.4
l. phenylacetate	30.1 ± 4.6	8.2 ± 1.5	2.9 ± 0.5
l. cinnamate	36.1 ± 2.9	5.3 ± 1.8	2.2 ± 0.1

[a] below limit of determination (LOD); [b] below limit of quantification (LOQ), see 3.2.3.7 and 8.4. Data represent means ± standard deviations from triplicate experiments.

Table 50.Distribution of products determined in squeezed tea bags after hot water infusions (200 mL) of tea bags (2 g) containing 4045 µg linalyl ester (median value of linalyl acetate obtained for 90 Earl Grey teas). Data represent means ± sd´s from triplicate experiments.

	transfer rate [%]					
substance	linalyl ester	linalool	α-terpineol	geraniol	nerol	geranyl ester
aliphatic linalyl ester						
l. formiate	5.3±0.4	7.6±0.9	4.1±0.5	1.3±0.1	0.4 ± 0.04	2.1±0.2
l. acetate	23.1±2.3	1.8±0.08	1.1±0.05	0.2±0.01	0.1 ± 0.004	0.6±0.1
l. propanoate	26.0±3.6	0.8±0.1	0.5±0.05	0.1±0.03	0.04 ± 0.008	0.2±0.03
l. butanoate	20.6±3.4	0.6±0.1	0.5±0.06	0.07±0.02	n.d.[a]	0.2±0.02
l. isobutanoate	26.5±4.5	0.3±0.04	0.3±0.03	n.q.[b]	n.d.	0.1±0.02
l. isovalerate	20.9±6.9	0.3±0.02	0.2±0.03	n.q.	n.d.	0.1±0.04
l. hexanoate	32.9±6.9	0.2±0.01	0.2±0.01	n.d.	n.d.	0.1±0.02
l. octanoate	44.3±6.4	0.1±0.03	0.1±0.01	n.d.	n.d.	0.2±0.04
aromatic linalyl ester						
l. benzoate	28.3±9.5	1.3±0.3	0.5±0.08	0.1±0.01	0.03 ± 0.008	0.6±0.1
l. phenylacetate	28.4±3.3	0.9±0.09	0.4±0.05	0.1±0.01	0.04 ± 0.008	0.4±0.04
l. cinnamate	17.8±4.4	0.8±0.009	0.3±0.02	0.09±0.01	n.q.	0.2±0.04

substance	neryl ester	myrcene	(*E*)-ocimene	(*Z*)-ocimene	terpinene	limonene
aliphatic linalyl ester						
l. formiate	1.1±0.08	0.8±0.06	0.8±0.05	0.4±0.03	0.4±0.03	0.3±0.02
l. acetate	0.3±0.05	0.3±0.04	0.2±0.03	0.1±0.02	0.02±0.01	0.07±0.009
l. propanoate	0.13±0.02	0.2±0.03	0.1±0.01	0.07±0.004	0.03±0.003	0.03±0.002
l. butanoate	0.08±0.01	0.2±0.03	0.1±0.03	n.q.	n.d.	n.q.
l. isobutanoate	0.1±0.02	0.09±0.03	0.05±0.02	0.04±0.01	n.d.	n.q.
l. isovalerate	0.07±0.02	0.06±0.02	n.q.	n.q.	n.d.	n.q.
l. hexanoate	0.04±0.01	n.q.	0.11±0.01	n.d.	n.d.	n.d.
l. octanoate	0.1±0.002	n.q.	n.d.	n.d.	n.d.	n.d.
aromatic linalyl ester						
l. benzoate	0.2±0.04	0.2±0.02	0.1±0.02	0.07±0.02	0.05±0.003	0.04±0.003
l. phenylacetate	0.2±0.02	0.2±0.04	0.1±0.05	0.07±0.02	0.04±0.005	0.03±0.009
l. cinnamate	0.1±0.03	0.1±0.02	n.q.	n.q.	n.q.	n.q.

substance	sum	sum alcohols	sum esters
aliphatic linalyl ester			
l. formiate	24.6 ± 2.1	5.9 ± 0.6	2.6 ± 0.2
l. acetate	28.0 ± 2.5	1.4 ± 0.04	0.8 ± 0.09
l. propanoate	28.3 ± 3.3	0.7 ± 0.08	0.4 ± 0.04
l. butanoate	22.3 ± 3.3	0.5 ± 0.08	0.3 ± 0.05
l. isobutanoate	27.4 ± 4.7	0.3 ± 0.03	0.2 ± 0.05
l. isovalerate	21.7 ± 7.0	0.2 ± 0.03	0.06 ± 0.02
l. hexanoate	33.5 ± 6.9	0.2 ± 0.01	0.1 ± 0.01
l. octanoate	44.9 ± 6.5	0.1 ± 0.01	n.q.
aromatic linalyl ester			
l. benzoate	31.4 ± 9.4	0.6 ± 0.07	0.4 ± 0.05
l. phenylacetate	31.0 ± 3.1	0.5 ± 0.06	0.4 ± 0.1
l. cinnamate	19.4 ± 4.5	0.4 ± 0.02	0.1 ± 0.02

[a] *below limit of determination (LOD);* [b] *below limit of quantification (LOQ), see 3.2.3.7 and 8.4.*

8.9 Transfer of flavouring substances occurring in commercial Earl Grey teas (actual vs. predicted values in the residual tea bag)

Table 51. Contents of flavouring substances of Earl Grey teas in tea bags flavoured with granules (a: tea no. 101; b: tea no. 102), without granules (c: tea no. 103) and of an Earl Grey tea purchased as loose leaves tea (d: tea no. 104). Data predicted on the basis of the transfer rates from model experiments vs. data actually determined for Earl Grey tea infusions.

		tea bag	tea beverage	
	substances	[µg / 2 g]	predicted[a,b] [µg / 200 mL]	actual [µg / 200 mL]
a)	*Tea no. 101: tea bags, flavoured with granulates*			
	linalyl acetate	4652 ± 129	1073 ± 109 [b]	919 ± 31
	linalool	1941 ± 45	331 ± 16	500 ± 67
	other reaction products			
	limonene	3095 ± 79	880 ± 73	670 ± 11
	terpinolene	119 ± 3	34 ± 3	22 ± 2
	γ-terpinene	86 ± 4	24 ± 2	18 ± 0.7
	myrcene	70 ± 1	31 ± 2	21 ± 1
	β-pinene	38 ± 2	11 ± 0.9	7 ± 0.2
	(*E*)-β-ocimene	n.d.	8 ± 1	8 ± 0.9
	(*Z*)-β-ocimene	n.d.	4 ± 0.5	4 ± 0.6
	α-terpineol	111 ± 10	55 ± 3	71 ± 7
	geraniol	n.d.	7 ± 0.3	3 ± 1
	nerol	n.d.	4 ± 0.2	5 ± 1
	geranyl acetate	n.d.	28 ± 5	26 ± 2
	neryl acetate	n.d.	15 ± 2	15 ± 1
	sum others [c]	3520 ± 80	1102 ± 74	872 ± 7
	sum	10112 ± 253	2506 ± 132	2291 ± 91

Table 51 continued.

substances	tea bag [µg / 2 g]	tea beverage predicted[a,b] [µg / 200 mL]	tea beverage actual [µg / 200 mL]
b) *Tea no. 102: tea bags, flavoured with granulates*			
linalyl acetate	2402 ± 112	554 ± 56	578 ± 48
linalool	2214 ± 88	336 ± 18	476 ± 46
other reaction products			
limonene	2398 ± 109	681 ± 57	737 ± 54
terpinolene	14 ± 1	4 ± 0.4	3 ± 0.2
γ-terpinene	436 ± 20	124 ± 10	126 ± 9
myrcene	64 ± 3	24 ± 2	24 ± 2
β-pinene	483 ± 23	137 ± 11	131 ± 9
(*E*)-β-ocimene	n.d.	4 ± 0.6	6 ± 0.7
(*Z*)-β-ocimene	n.q.	2 ± 0.3	3 ± 0.3
α-terpineol	169 ± 6	45 ± 4	67 ± 7
geraniol	52 ± 2	11 ± 0.5	20 ± 1
nerol	n.d.	2 ± 0.1	3 ± 0.2
geranyl acetate	109 ± 6	51 ± 3	62 ± 5
neryl acetate	35 ± 4	19 ± 2	24 ± 2
sum others [c]	3758 ± 171	1104 ± 59	1205 ± 91
sum	8374 ± 371	1994 ± 84	2259 ± 183
c) *Tea no. 103: tea bags, flavoured without granulates*			
linalyl acetate	1742 ± 21	402 ± 41	655 ± 19
linalool	1394 ± 14	215 ± 11	400 ± 18
other reaction products			
limonene	506 ± 7	144 ± 12	212 ± 6
terpinolene	23 ± 1	7 ± 0.6	3 ± 0.3
γ-terpinene	n.d.		
myrcene	6 ± 0.2	6 ± 0.5	6 ± 0.5
β-pinene	2 ± 0.2	0.5 ± 0.004	n.d.
(*E*)-β-ocimene	n.q.	3 ± 0.4	n.d.
(*Z*)-β-ocimene	n.d.	2 ± 0.2	n.d.
α-terpineol	1394 ± 14	42 ± 4	77 ± 2
geraniol	28 ± 2	6 ± 0.3	15 ± 0.3
nerol	5 ± 0.5	2 ± 0.1	2 ± 0.3
geranyl acetate	109 ± 6	15 ± 2	20 ± 0.7
neryl acetate	35 ± 4	7 ± 0.9	8 ± 0.3
sum others [c]	774 ± 9	235 ± 13	344 ± 9
sum	3910 ± 43	851 ± 44	1399 ± 42

Table 51 continued.

	substances	tea bag [µg / 2 g]	tea beverage predicted[a,b] [µg / 200 mL]	tea beverage actual [µg / 200 mL]
d)	*Tea no. 104: loose tea leaves, flavoured without granulates*[d]			
	linalyl acetate	8087 ± 106	1865 ± 189	1494 ± 159
	linalool	3982 ± 51	1625 ± 116	1259 ± 138
	other reaction products			
	limonene	1548 ± 22	454 ± 37	253 ± 34
	terpinolene	17 ± 3	26 ± 2	4 ± 0.1
	γ-terpinene	159 ± 3	45 ± 4	25 ± 3
	myrcene	36 ± 2	94 ± 12	11 ± 1
	β-pinene	312 ± 9	89 ± 7	42 ± 6
	(*E*)-β-ocimene	n.d.	49 ± 22	5 ± 0.5
	(*Z*)-β-ocimene	n.q.	45 ± 6	3 ± 0.6
	α-terpineol	14 ± 3	523 ± 48	55 ± 4
	geraniol	n.d.	179 ± 11	n.d.
	nerol	65 ± 4	71 ± 7	20 ± 8
	geranyl acetate	8 ± 0.8	218 ± 27	18 ± 3
	neryl acetate	103 ± 2	151 ± 17	38 ± 5
	sum others[c]	2263 ± 37	1943 ± 74	474 ± 65
	sum	14332 ± 191	5433 ± 234	3228 ± 361

[a] calculated on the basis of reaction rates out of linalyl acetate (see 4.2.3.2) in combination with transfer rates determined in spiking experiments with linalyl acetate (23.1%); linalool (13.6%), also applied to nerol, geraniol; α-terpineol (14.9%); geranyl acetate (33.1%); neryl acetate (33.7%); γ-terpinene (28.4%), also applied to the other hydrocarbons; [b] standard d Limits of detection (LOD) and eviations of predicted values were calculated using the Gaussian propagation of uncertainty ($\sigma_{total} = ((\sigma_{1})^2 + (\sigma_{2})^2 + \ldots. (\sigma_{n})^2))^{1/2}$. [c] 'sum others' encompasses the compounds listed as 'other reaction products'. [d] tea leaves were weighted into a one-way tea bag for the tea infusion process. Data represent means ± standard deviations from triplicate experiments.

8.10 Degradation of linalyl acetate in water and tea infusions (both at 20°C)

Table 52. Degradation of linalyl acetate in water (starting concentration: 0.04 mg/mL) stored at RT (20°C) (values from 8 min to 24 h).

time [min]	time [h]	ln $_{time[h]}$	$c_{linalyl\ acetate}$ [%]	ln $c_{actual\ [\%]}$ [a]	ln $c_{calculated\ [\%]}$ [b]	residuals[c]
8	0.1	-2.01	99.7	4.60	4.62	0.02
15	0.3	-1.39	99.0	4.60	4.57	-0.03
30	0.5	-0.69	98.1	4.59	4.57	-0.02
45	0.8	-0.29	97.0	4.57	4.57	0.00
60	1.0	0.00	95.9	4.56	4.58	0.01
120	2.0	0.69	91.9	4.52	4.55	0.03
180	3.0	1.10	87.4	4.47	4.49	0.02
240	4.0	1.39	83.5	4.42	4.43	0.00
300	5.0	1.61	79.1	4.37	4.36	-0.01
420	7.0	1.95	71.9	4.28	4.23	-0.05
1440	24.0	3.18	27.2	3.30	3.31	0.01

[a] logarithmic values calculated for actual linalyl acetate concentrations at the appropriate time points; [b] values calculated with the polynomial equitation shown in Figure 50A for the appropriate time points; [c] deviations of the measurements values from their values predicted by the regression line = ln $c_{calculated\ [\%]}$ – ln $c_{actual\ [\%]}$.

Table 53. Degradation of linalyl acetate in tea infusion (starting concentration: 0.04 mg/mL) stored at RT (20°C) (values from 8 min to 24 h).

time [min]	time [h]	ln $_{time[h]}$	$c_{linalyl\ acetate}$ [%]	ln $c_{actual\ [\%]}$ [a]	ln $c_{calculated\ [\%]}$ [b]	residuals[c]
8	0.1	-2.01	99.8	4.60	4.60	0.005
15	0.3	-1.39	99.2	4.60	4.56	-0.04
30	0.5	-0.69	98.5	4.59	4.57	-0.02
45	0.8	-0.29	97.7	4.58	4.58	0.002
60	1.0	0.00	96.8	4.57	4.58	0.01
120	2.0	0.69	93.0	4.53	4.56	0.03
180	3.0	1.10	88.6	4.48	4.51	0.03
240	4.0	1.39	84.3	4.43	4.45	0.02
300	5.0	1.61	79.8	4.38	4.39	0.01
420	7.0	1.95	72.3	4.28	4.27	-0.01
1440	24.0	3.18	30.0	3.40	3.44	0.04

[a] logarithmic values calculated for actual linalyl acetate concentrations at the appropriate time points; [b] values calculated with the polynomial equitation shown in Figure 50B for the appropriate time points; [c] margin of [a] and [b].

9 CURRICULUM VITAE

PERSONAL INFORMATION

Name:	Anne-Marie Beate Orth
Date of birth:	28.12.1984
Place of birth:	Limburg an der Lahn
Current address:	Artilleriestraße 13 80636 München Germany

PROFESSIONAL EXPERIENCE

06/2014 – today	RDA Scientific Consultants GmbH Consultant Regulatory Affairs & CIO
11/2012 – 04/2013	Assistance at the marketing department of GQSystems (SAP®-industry solutions for medium-sized food companies)
11/2012 – 09/2013	Written Composition of the PhD Thesis
02/2009 – 10/2012	Research assistant (PhD Student) Technische Universität München, Chair of General Food Technology, Univ.-Prof. Dr. Karl-Heinz Engel PhD Thesis: “Assessment of Dietary Exposure to Flavouring Substances via Consumption of Flavoured Teas”

EDUCATION

10/2013 – 05/2014	Degree in Food Chemistry (2. Staatsexamen) Post-graduation, Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit
10/2004-10/2008	Degree in Food Chemistry (1. Staatsexamen) Academic studies of Food chemistry, Julius-Maximilians-Universität Würzburg, Univ.-Prof. Dr. Peter Schreier
08/2004 – 10/2004	Voluntary Social Year (Freiwilliges Soziales Jahr), Caritas-Altenzentrum St. Josefshaus Elz
08/1991 – 06/2004	Allgemeine Hochschulreife (Abitur) Gymnasium der Fürst-Johann-Ludwig-Schule Hadamar

Publications

Orth AM, Yu L, Poplacean I, Engel KH. **2014***. Chapter 103 - Assessment of the Intake of Flavouring Substances via Consumption of Flavoured Teas – Analysis of Earl Grey Teas Marketed in the European Union.* In: Ferreira V, Lopez R, editors. Flavour Science: Proceedings from XIII Weurman Flavour Research Symposium. San Diego: Academic Press. p. 563–6.

Orth AM, Poplacean I, Fastowski O, Engel KH. **2014**. *Assessment of dietary exposure to flavouring substances via consumption of flavoured teas. Part II: transfer rates of linalool and linalyl esters into Earl Grey tea infusions*. Food Addit. Contam. Part A 31(2):207–17.

Orth AM, Yu L, Engel KH. **2013**. *Assessment of dietary exposure to flavouring substances via consumption of flavoured teas. Part I: occurrence and contents of monoterpenes in Earl Grey teas marketed in the European Union*. Food Addit. Contam. Part A 30(10):1701–14.

Orth AM, Yu L, Poplacean I, Engel KH. **2011**. *Assessment of the intake of flavouring substances via consumption of flavoured teas – analysis of Earl Grey teas marketed in the European Union*. In: XIII Weurman Flavour Research Symposium, Sep 27-30 2011, Zaragoza, Spain (ed.). 2011. Book of Abstracts. p. 27.

CONFERENCES

10/26/2012 (Brussels, B)	FACET: Closing Conference, Poster presentation: Dietary exposure to flavouring substances via flavoured teas.
04/19 - 20/2012 (Dublin, IRL)	Creme Global: Crème Exposure: Food Safety and Nutrition Conference: Poster presentation: Intake assessment of flavouring substances via consumption of Earl Grey Tea – transfer rates upon hot water infusion.
03/06/2012 (Erlangen, D)	63. Arbeitstagung des Regional-Verbandes Bayern der Lebensmittelchemischen Gesellschaft, Fachgruppe in der GDCh, Talk: Transfer von Aromastoffen beim Aufguss von Earl Grey Tee.
09/27 - 30/2011 (Zaragoza, E)	XIII. Weurman Flavour Research Symposium. Talk participation: Assessment of the dietary exposure to flavouring substances via consumption of flavoured teas - Analysis of Earl Grey teas marketed in the European Union.
12/12/2011 (Brussels, B)	General assembly of the European project FACET (Food Additives and Contact materials Exposure Task): Presentation of work-results (talk): Assessment of the dietary exposure to flavouring substances via consumption of flavoured teas - Analysis of Earl Grey teas marketed in the European Union.