 doi:10.20944/preprints202206.0321.v1

Article

Coffee Leaf Tea from El Salvador: On-site Production Considering Influences of Processing on Chemical Composition

Marc C. Steger [1,2], Marina Rigling [1], Patrik Blumenthal [2], Valerie Segatz [3,4], Andrès Quintanilla-Belucci [5], Julia M. Beisel [3], Jörg Rieke-Zapp [6], Steffen Schwarz [2], Dirk W. Lachenmeier [3,*] and Yanyan Zhang [1,*]

[1] Department of Flavor Chemistry, University of Hohenheim, Fruwirthstr. 12, Verfügungsgebäude 221, 70599 Stuttgart, Germany; marcsteger2@googlemail.com (M.C.S.); marina.rigling@uni-hohenheim.de (M.R.); yanyan.zhang@uni-hohenheim.de (Y.Z.)
[2] Coffee Consulate, Hans-Thoma-Strasse 20, 68163 Mannheim, Germany; patrik.blumenthal@live.de (P.B.); schwarz@coffee-consulate.com (S.S.)
[3] Chemisches und Veterinäruntersuchungsamt (CVUA) Karlsruhe, Weissenburger Strasse 3, 76187 Karlsruhe, Germany; julia.beisel@cvuaka.bwl.de
[4] Hochschule für angewandte Wissenschaften Coburg, Friedrich-Streib-Strasse 2, 96450 Coburg, Germany; valerie.segatz@stud.hs-coburg.de
[5] Finca La Buena Esperanza, Pasaje Senda Florida Norte 124, San Salvador, El Salvador; coffeelbe@gmail.com
[6] Rubiacea Research and Development GmbH, Hans-Thoma-Strasse 20, 68163 Mannheim; Germany; joerg.rieke_zapp@yahoo.de
* Correspondence: lachenmeier@web.de; Tel.: +49-721-926-5434; yanyan.zhang@uni-hohenheim.de; Tel.: +49-711-459-24871

Abstract: The production of coffee leaf tea (*Coffea arabica*) in El Salvador and the influences of processing steps on non-volatile compounds and volatile aroma-active compounds were investigated. The tea was produced according to process steps of conventional tea (*Camellia sinensis*) with available possibilities on the farm. Influencing factors were the leaf type (old, young, yellow, shoots), processing (blending, cutting, rolling, freezing, steaming), drying (sun drying, oven drying, roasting) and fermentation (wild, yeast, *Lactobacillus*). Subsequently, the samples were analysed for the maximum levels of caffeine, chlorogenic acid, and epigallocatechin gallate permitted by the European Commission. The caffeine content varied between 0.37 g/100 g dry mass (DM) and 1.33 g/100 g DM, the chlorogenic acid between not detectable and 9.35 g /100 g DM and epigallocatechin gallate could not be detected at all. Furthermore, water content, essential oil, ash content, total polyphenols, total catechins, organic acids, and trigonelline were determined. Gas chromatography—mass spectrometry-olfactometry and calculating of the odour activity values (OAVs) were carried out to determine the main aroma-active compounds, which are *β*-ionone (honey-like, OAV 132-927), decanal (citrus-like, floral, OAV 14-301), *α*-ionone (floral, OAV 30-100), (*E*,*Z*)-2,6-nonadienal (cucumber-like, OAV 18-256), 2,4-nonadienal (melon-like, OAV 2-18), octanal (fruity, OAV 7-23), (*E*)-2 nonenal (citrus-like, OAV 1-11), hexanal (grassy, OAV 1-10), and 4-heptenal (green, OAV 1-9).

Keywords: coffee leaf tea; novel food; coffee by-products; *Coffea arabica*; caffeine; epigallocatechin gallate

1. Introduction

Coffee is one of the most consumed beverages in the world. Worldwide, over 10 million tons are produced each year with a turnover of more than 20 billion dollars [1]. However, the unstable price conditions of coffee put pressure on the farmers to keep their farms in good condition. The use of coffee by-products, i.e., products that are created during the production of coffee, could help to create a sustainable future [2]. One of these coffee by-products is the coffee leaf, which has been approved as a tea beverage in the EU

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

since July 2020 [3]. Coffee leaves are usually produced as a by-product during the pruning of the plants [4].

Coffee leaves from the coffee plant are typically light green (buds and young leaves) to dark green (matured leaves) with a size range of 15 cm (*Coffea arabica*) up to 50 cm (*Coffea liberica*). The lifetime of a leaf is about 7-10 months [5]. There is much evidence to suggest that the leaves of the coffee plant have long been used as a traditional food in the countries where it is grown. Von Pröpper observed in 1882 [6] that "The leaves of the coffee plant, roasted and poured over with hot water, make an excellent tea, which has long been one of the staple foods of the entire Indian archipelago, and is said to be not inferior in effect to the true Chinese tea, but apparently has not yet come into commerce" (authors' translation from German). Further mentioned in the literature are the countries Ethiopia, West Sumatra, Jamaica, Java, and South Sudan [7,8]. Novita et al. [9] described the traditional production of "Kahwa daun", a herbal tea from coffee leaves produced in West Sumatra. Herby branches with leaves were clasped on a bamboo stick and then smoked or dried over a cooking fire. In Indonesia the infusion of coffee leaves is called "copi daon" or "leaf coffee" and in Ethiopia it is called "Quti" whereby in both countries the leaves are sun dried [10]. Consumption of the tea may occur in the belief to prevent or treat diseases such as headache, stomach pains and diarrhoea but in most countries, it is just consumed for the taste or the effects of caffeine [11–13]. Positive properties of coffee leaves on the human health can be derived by the chemical compounds, which were determined in different studies [14–17]. These include carbohydrates, amino acids, organic acids, alkaloids, phenolic compounds, terpenes, carotenoids, phytosterols and flavour compounds such as aldehydes, alcohols, ketones, and esters. The chemical composition of a leaf is highly influenced by the light intensity, nitrogen concentration of the soil, age of the plant and the leaf, growing region and the coffee species [16].

Novel foods or traditional foods from third countries require an authorization to be placed on the market in the European Union (EU) [18]. The application to authorize the placing on the market of an infusion of coffee leaves of the species *Coffea arabica* and/or *Coffea canephora* as a traditional food was approved by the EU Commission on July 01, 2020. Since the applicant could not provide evidence of the use of coffee leaves as an ingredient in other beverages, only the infusion of coffee leaves was approved as a novel food. Critical values have been set for the substances caffeine, chlorogenic acid and epigallocatechine gallate [3].

In this study, the possibilities to produce coffee leaf tea in a country of origin were investigated. With the locally available resources, different coffee tea samples were produced. Furthermore, the effects of leaf types, coffee varieties, processing, and drying methods on the consumer acceptance, the aroma profile and the product characteristics should be understood and described. This understanding can later help to optimally adjust process and manufacturing parameters to the desired taste. For the analyses of non-volatiles, near-infrared spectroscopy (NIR), high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy were used. The water content, essential oil, ash content, caffeine, polyphenol content, catechins, organic acids, trigonelline and lactic acid were determined. Afterwards the samples were sensorically evaluated by a panel according to DIN 10809 [19], followed by aroma analysis using gas chromatography-olfactometry.

2. Materials and Methods

2.1 Coffee leaf tea production in El Salvador

The collected leaves are shown in Table 1. Used leaf types, their variety, and their collection place are given. Finca la Palma is located in Chinameca, San Miguel and Finca La Quintanilla is located on the north side of Cacahuatique mountain in Morazan, El Salvador. Furthermore, different leaf types are shown in Figure 1.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

Figure 1. From left to right: whole shoots, young leaves, old leaves (Bourbon Tekisic).

Table 1. Used leaf types, their variety, and their collection place.

Leaf type	*Coffea arabica* variety	Finca
Old leaf	Pacamara	La Palma
Yellow leaf	Pacamara	La Palma
Old leaf	Bourbon Tekisic	La Quintanilla
Young leaf	Bourbon Tekisic	La Quintanilla
Shooter	Bourbon Tekisic	La Quintanilla

The harvest of the leaves was conducted between February and March 2021. Old leaves were cut directly from the plant on the field. About five leaves were harvested per plant. All yellow leaves of a plant were picked directly from the branches. Shoots were completely cut off from the plants and then divided into old and young leaves. All leaves were cleaned with fresh water before further processing. For each sample 600 g of fresh leaves were picked. For determination of water content of fresh leaves, one sample of each leaf type was dried in the oven at approx. 80°C. The weight was determined before drying (approx. 2 g) and after drying with a precision balance. Each experiment was carried out in triplicates.

Different processing steps were applied to obtain a variety of different teas. These steps were related to the pre-treatment, drying and fermentation of the leaves. All leaves underwent a withering process where the leaves were stored on drying beds for 12 hours overnight at 20 °C. Subsequently, the leaves were either dried whole or processed by various mechanical methods. The methods are shown in Table 2. Furthermore, the processing steps are shown in Figure 2.

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

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

(c) (d)

Figure 2. (a) Leaves after blending **(b)** Leaves in the steaming pot **(c)** Leaves directly after crumbling **(d)** Crumbled, whole and cut leaves on the drying bed.

Table 2. Processing steps and their explanation.

Process	Explanation
Whole leaf	No further mechanical intervention
Blending	The leaves were blended in a kitchen blender. For 100 g leaves, around 400 mL tapped water were added.
Cutting	Leaves were cut with a kitchen knife to small strips (20 mm wide)
Rolling	The leaves were rolled by hand
Freezing	The leaves were frozen in a freezer at -20 °C for 2 days
Crumbling	Leaves were crumbled by hand
Steaming	Leaves were steamed in a 50 L pot. A sieve was placed in the centre of the pot and approximately 2 L of tapped water was boiled under the leaves. The temperature was measured at the lid of the pot. The process was stopped when the temperature reached around 100 degrees.

2.1.1 Drying methods

Three different drying methods were carried out for the samples, namely sun/air drying, oven drying and roasting. For sun drying the leaves were stored on a drying bed for at least 48 h until they were crispy. The oven drying was performed at 70°C in a gas oven for 4 h. The roasting was done on a gas stove until the leaves were crispy. The sun drying process is shown in Figure 3.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

Figure 3. Sun drying of the different samples on the drying bed in Chinameca.

2.1.2 Fermentation

The leaves were fermented by storing them in closed plastic buckets (anaerobe) for 12 h at a temperature of around 25 °C (overnight). 40 mL starter cultures were sprayed on the leaves. For pre-cultivation of the microorganisms (*Saccharomyces cerevisiae* var. bayanus, *Lactobacillus plantarum*), around 1 g of the dry culture was dissolved in 100 mL of water, one hour before mixing it with the samples. For the wild fermentation, the leaves were stored in the buckets without adding a starter culture.

2.1.3 Postprocessing and packaging

Samples that had not been mixed before were brought to approximately the same sheet size with the blender. Subsequently, all samples were packed into zip bags and vacuum sealed for transport.

2.2 Analysing of non-volatile compounds

2.2.1 Sample preparation for HPLC, NMR and Photometry

The preparation of the ground tea sample was carried out according to the international standard ISO 1572 [20]. In accordance with this standard, the samples were prepared using a comminution mill so that the ground material subsequently fell completely through a test sieve with a mesh size of 500 µm. For each sample, a small portion of the sample was first ground in the mill and discarded. Subsequently, the amount of sample required for further testing was ground and packed in a separate, airtight package.

2.2.2 Total Polyphenols and Catechins

5.0 mL of a warm methanol-water mixture was added to the extraction tubes containing 200 mg of sample, sealed with a plug, and mixed on a vortex mixer. The samples were heated in a water bath and mixed again after 5 min and 10 min. The extraction tubes are now brought to room temperature and centrifuged for 10 min at 3500 rpm. The supernatant liquid (the extract) was decanted into a 10 mL volumetric flask and the previous extraction steps were repeated once with the residue in the extraction tube. The two extracts were combined and tempered to 20°C in a water bath. Finally, the tempered extracts were filled up to the 10 mL mark of the volumetric flasks with the methanol-water extraction mixture.

For HPLC analysis, the solution was diluted (1:5) with a stabilizing solution (100 mg EDTA, 100 mg ascorbic acid in 10% acetonitrile), membrane filtered and transferred to special vials for HPLC. For this purpose, the plunger was removed from a 2 mL syringe and connected to a membrane filter. Then about 2 mL of the sample solution was transferred into the syringe and about 1 mL was filled into 2 vials by placing the plunger on

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

top. All vials were frozen until measurement. The procedure was previously described in more detail [21].

For the determination of total phenols, 1 ml of the diluted sample (1:100 with distilled water) was pipetted into each centrifuge tube. In addition, two centrifuge tubes were filled with 1 mL of distilled water each to determine the blank value. Then, 5 mL of the Folin-Ciocalteau reagent (1:10 dilution of Folin-Ciocalteau-Phenol and distilled water) was added to each of the centrifuge tubes. After 6 minutes, 4 ml of sodium carbonate solution was added to each centrifuge tube. The centrifuge tubes were sealed and slightly shaken. After one hour, the samples could be determined using a photometer. Each measurement was performed in duplicates.

2.2.3 Nuclear magnetic resonance (NMR)

200 mg of the ground coffee leaf tea was weighed into a 15 mL centrifuge tube and 8 mL of deionized water was added. The tubes were then placed on the combination shaker and shaken on level 8 for 20 min. 2 mL of the solution was membrane filtered into a 4 mL glass vial. Sodium dihydrogen phosphate buffer, pH 6,1, and Trimethylsilylpropanoic acid (TSP) were brought to room temperature and 70 µL each of TSP and 100 µL of buffer were pipetted into an empty vial. Then, 600µL of the membrane-filtered sample was pipetted into each of these prefilled vials. The solution was homogenized before 600 µL of each was pipetted into an NMR tube. The NMR tubes were finally sealed with a lid and a spinner for the NMR instrument. The measurements were done according to a previously described procedure developed for cold brew coffee [22].

2.3 Sensory analysis

The 24 tea samples from El Salvador of the Bourbon and Pacamara coffee varieties were tasted by a trained test panel composed of 7 experienced people. The tasting was done at room temperature with cupping spoons. The main questions of the tasting are: (i) Which teas exhibit the highest popularity among testers? (ii) What flavour profiles do each of the top eight tea samples exhibit?

The coffee leaf tea is prepared according to DIN 10809 [19] in infusion vessels. After 5 minutes, the tea is poured off into the bowl and can be tasted after a short cooling period.

The individual samples were rated according to the personal preference of the tasters with values from 0 to 5 (0 = likes very badly 5 = likes very well). The 8 highest scoring samples are brewed for a simple descriptive test, a profile test and a rank order test.

The tasted "best eight" were then to be evaluated in a ranking test by all participants. The samples are ranked from 1 to 8 according to personal preference (1= best / 8= worst). Multiple assignment of numbers is excluded in this test. A selection must be made even if there are only slight differences (forced-choice test).

The test material was tested for the perceptual attributes of colour, odour, and flavour. Participants are free to add other properties. The individual results are then shared. The terms are collected and either accepted or rejected by the testers. A minimum of 50% agreement is required to define a term.

Finally, the given characteristics (sweet, salty, sour, bitter, body and the dwell time of the taste (finish) are described with values ranging from 0 (absent) to 5 (strongly expressed).

2.4 Analysis of volatile, odour-active compounds

2.4.1 Tea preparation

To prepare the teas, 2 g of tea were filled into cellulose bags. These were infused with 200 mL of boiling distilled water in a beaker and infused for 5 minutes. The tea bag was then removed and the samples were frozen in aluminium bottles at -20 °C.

2.4.2 Direct-immersion stir bar sorptive extraction (DI-SBSE)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

To extract the compounds from the tea, DI-SBSE technique was applied. Therefore 10 mL of tea and 3.3 g of NaCl and 0.05 mL of thymol standard (4.2 mg/L) were transferred to a headspace vial (20 mL). The mixture was stirred by a Twister (PDMS) with 1000 rpm for 2 hours at room temperature. The Twister was then taken out and rinsed with distilled water and dried off with a lint-free tissue. Afterwards the Twister was placed in the autosampler of the gas chromatograph. Each Twister was conditioned for 1 hour at 250°C after use. Each measurement was done in triplicate.

2.4.3 Gas chromatography

Gas chromatography (GC) was performed according to Rigling et al. [23]. In short, an Agilent 7890 B gas chromatograph connected to a 5977 B mass spectrometry detector (Agilent Technologies, Waldbronn, Germany) was equipped with TDU, CIS as well as an olfactometry detection port (ODP 3, Gerstel, Mülheim an der Ruhr, Germany). An Agilent J&W DB-WAXms column (30 m × 0.25 mm ID × 0.25 µm film thickness) (Agilent Technologies) was installed. Helium (5.0) served as carrier gas with a constant flow rate of 1.62 mL/min. The gas flow was split 1:1 into the MS detector and the ODP by means of a µFlow-Manager Splitter (Gerstel) with a column outlet pressure of 20 kPa. The GC oven temperature was held at 40 °C (3 min), then ramped with 5 ◦C/min to 240 ◦C (10 min). The following parameters were applied: MS mode, scan; scan range, *m*/*z* 40–330; electron ionization energy, 70 eV; source temperature, 230 °C; quadrupole temperature, 150 °C; ODP 3 transfer line temperature, 250 °C; ODP mixing chamber temperature, 150 °C; ODP 3 makeup gas, N2 (5.0) (Westfalen). The data were collected using Gerstel ODP1 and Agilent Mass Hunter B07.06 combined with Gerstel Maestro.

2.4.4 Semi quantification

Semi quantification was performed using the internal standard thymol (c = 4.2 mg/L) and the weighed-in standard solutions. The response factor of the respective substances could then be calculated using the peak areas of the standard.

2.4.5 Odour activity value (OAV)

To determine the odour activity value (OAV), the odour threshold of each substance was retrieved from the literature. Values above 1 indicate the possibility of sensory perception of the respective substance.

2.5 Statistical analysis

Microsoft Excel was used to calculate means and standard deviation and for graphical illustrations. The statistical evaluation of the sensory test was performed using the Friedmann test. The calculations (One way ANOVA (confidence level $p < 0.05$) were applied using SPSS (IBM Corporation, Armonk, USA). For the analytical data, the statistical evaluation was carried out using the program Design Expert 12 (Stat-Ease, Inc, MN, USA). Hereby an ANOVA for selected factorial model (confidence level $p < 0.05$) was applied. The results are presented as mean value ± standard deviation of the respective parameters.

3. Results

3.1 Moisture content of fresh leaves

The moisture content of the different types of fresh leaves is shown in Table 3.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

Table 3. Moisture content of the different coffee leaves directly after picking shown as mean ± standard deviation.

Leaf type	Moisture content [%]
Pacamara yellow	56.28 ± 1.02
Shoots Bourbon whole	72.74 ± 1.73
Bourbon old	62.83 ± 2.22
Bourbon young	72.89 ± 0.99

Young leaves and shoots showed the highest water content with 72.89 ± 0.99% and 72.74 ± 1.73%, respectively. Weatherley [24] described a correlation between leaf age and its water content. The water content decreased in all plants during the aging process. Yellow leaves show the lowest water content with 56.28 ± 1.02%. This effect was to be expected as the plant tries to extract all nutrients and water from the dead leaf before dropping it [25]. To obtain enough quantity for the analyses, 600 g of fresh leaves were collected for each sample. Since the pruning of the plants was already done, it was important not to cut off too many leaves and especially not the fresh buds. This could lead to deterioration of the plant [26].

3.2 Preparation of coffee leaf tea samples

In total, 24 different samples were produced during this field study. All samples are shown in Table 4.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

Table 4. Produced coffee leaf tea samples in this study including the test number, the variety, the leaf type, the processing, the drying and the fermentation process.

	Test No.	*Coffea arabica* variety	Leaf type	Processing	Drying	Fermentation
1	936	Pacamara	old	whole	air	none
2	324	Pacamara	yellow	whole	air	none
3	742	Pacamara	old	crumbled	air	none
4	183	Pacamara	yellow	crumbled	air	none
5	502	Pacamara	old	cutted	air	none
6	643	Pacamara	old	blended	air	none
7	842	Pacamara	old	crumbled	air	Yeast
8	234	Pacamara	old	whole	oven	none
9	238	Pacamara	old	blended	air	*Lactobacillus*
10	182	Pacamara	old	blended	air	Yeast
11	789	Pacamara	old	blended	air	Wild
12	156	Pacamara	yellow	blended	air	Wild
13	687	Pacamara	yellow	blended	air	Yeast
14	463	Pacamara	yellow	blended	air	*Lactobacillus*
15	289	Bourbon	Shooter	blended	air	Wild
16	138	Bourbon	old	blended	air	Yeast
17	147	Bourbon	young	blended	air	Wild
18	392	Bourbon	young	steamed/rolled	air	none
19	305	Bourbon	young	rolled/fermented	air	Wild
20	930	Bourbon	Shooter	blended/steamed	air	none
21	743	Bourbon	old	whole	air	Wild
22	901	Bourbon	old	whole/frozen	air	none
23	369	Bourbon	old	whole	roasted	none
24	220	Bourbon	Shooter	whole	air	none

3.3 Water content, essential oil content and ash content

The results of the NIR analysis for water, essential oil and ash are shown in Table A1 in the appendix. Furthermore, the influences of the manufacturing methods are shown in Figure 4.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

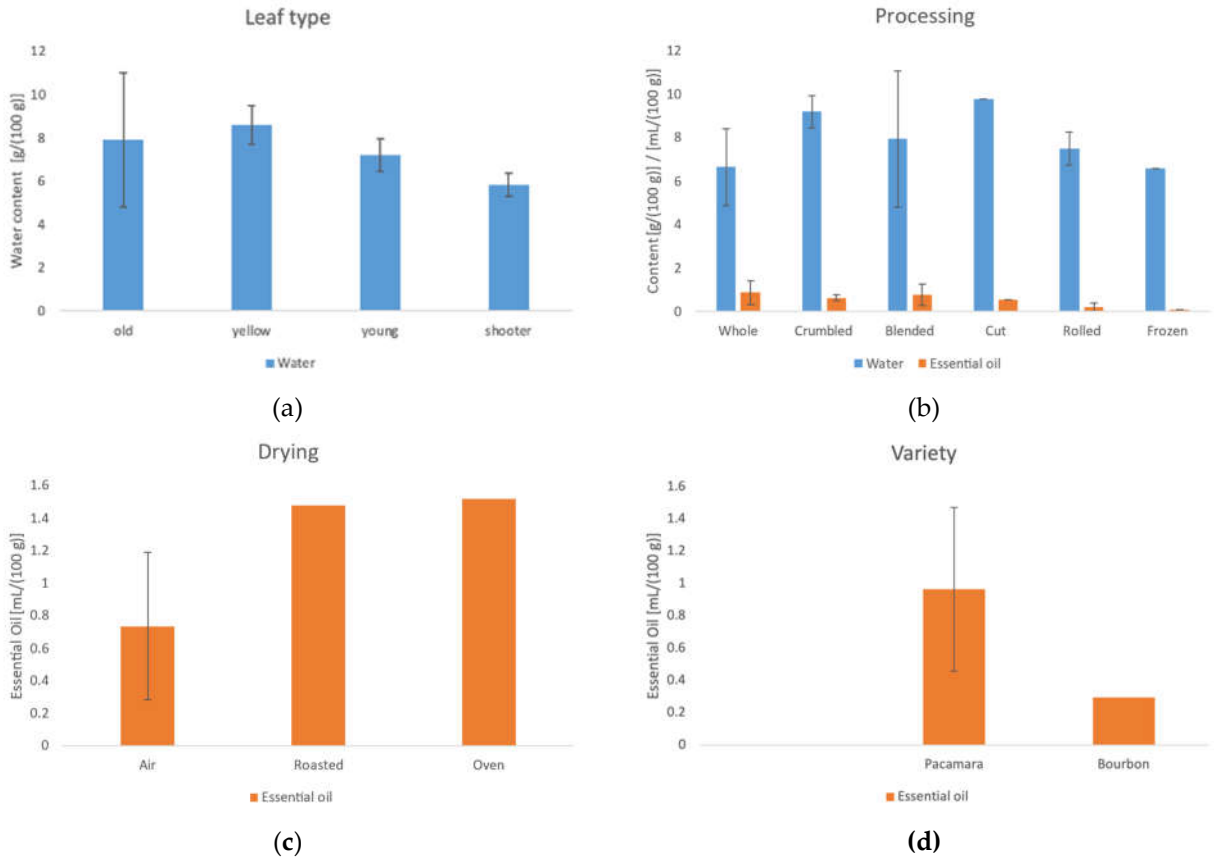


Figure 4. (a) Influence of the leaf type on the water content **(b)** Influence of the processing methods on the water and the essential oil content **(c)** Influence of the drying method on the essential oil content **(d)** Influence of the variety on the essential oil content.

The water content of the different samples varied from 3.92 g/100 g to 17.42 g/100 g. Statistical analysis of the individual samples showed significant indication of an influence of the leaf type, the processing and the drying method. On average, yellow leaves on average show the highest water content (8.59 g/100 g), while shoots show the lowest water content (5.83 g/100 g). In processing, freezing (6.58 g/100 g), the use of the whole leafs (6.65 g/100 g) and blending (7.93 g/100 g) lead to lower water content. These values are up to 33% lower compared to the other processing steps. The different surface area plays a role here. Blended samples will dry much faster than less processed ones [27]. Furthermore, up to 46% lower water contents could be achieved during drying by roasting and by the oven. According to Arslan et al. [28] oven drying has a more than double drying rate compared to sun drying. However, the water content of sample 16 with the highest water content was significantly higher compared to the other samples, indicating an error during sun drying. Here, the sample was probably removed from the drying bed too early. Furthermore according to German guidelines [29], the water content of a tea or tea-like product must not exceed 8%. In future production, special care must be taken to ensure that the sun-drying process is not terminated too early.

The essential oil content of the leaves in this experiment varies from not detectable to 1.52 mL/100 g. The statistical analysis showed a significant influence of the variety and the drying method. Leaves from Pacamara have a significant higher oil content compared to Bourbon. The effects of drying methods on the essential oil content have already been investigated in many previous studies for different plants. Here, gentle drying in the oven resulted in a higher oil content than drying under direct sunlight [30–32].

The ash content of the leaves showed no significant influencing factors. The values of all samples ranged between 7.81 g/100 g and 10.20 g/100 g regardless of the influences.

 doi:10.20944/preprints202206.0321.v1

3.4 Content of caffeine and catechins

The results of the HPLC analysis for caffeine and catechins are shown in Table A2 in the appendix. Furthermore the influence of the manufacturing methods on caffeine and the total catechins are shown in Figure 5 and Figure 6.

3.4.1 Caffeine

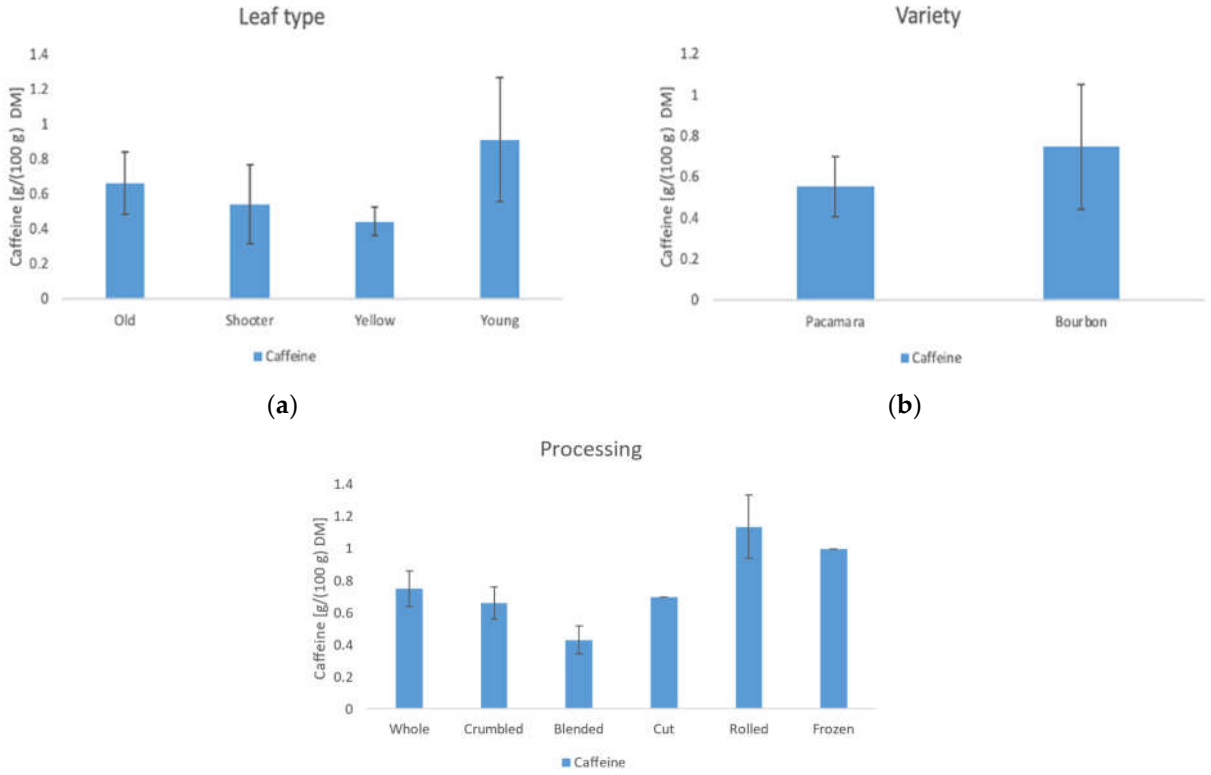
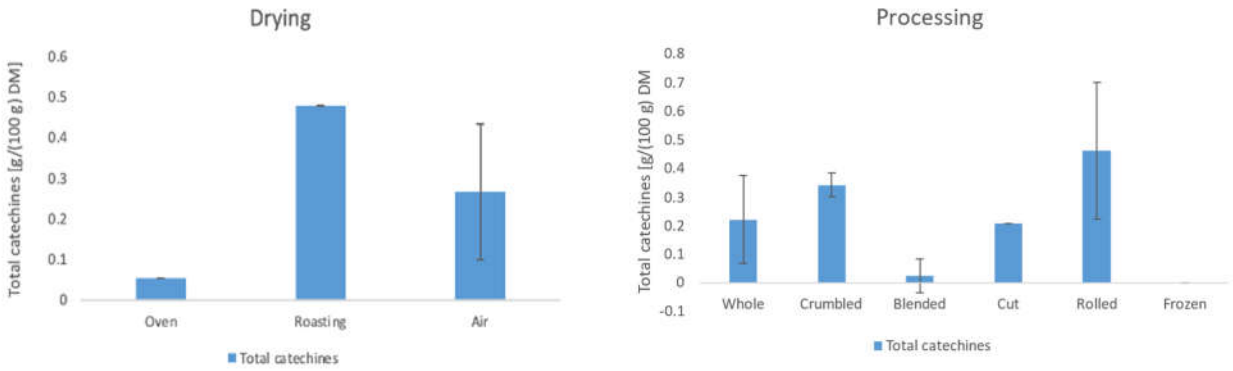


Figure 5. Influence of the leaf type **(a)**, the variety **(b)**, and the processing method **(c)** on the caffeine content.

The caffeine content of the leaves varies between 0.37 g/100 g DM and 1.33 g/100 g DM. In this case, the leaf type and the variety show significant influences on the caffeine content of the tea. Young leaves show the highest caffeine value (0.91 g/100 g DM) while yellow leaves show the lowest (0.44 g/100 g DM). The caffeine levels are approximately the same as those detected by Ratanamarno et al. [33]. The effect of caffeine reduction with leaf age has already been observed in some studies with different plants [34,35]. Song et al. [36] explained this effect mainly by the function of caffeine as a pesticide. Younger leaves of the plant must be more protected compared to old ones; therefore, the plant builds up higher concentrations in those leaves. Furthermore, the processing is a significant variable towards the caffeine content. Here, rolling (1.2 g/100 g DM) and freezing (1.00 g/100 g DM) of the leaf show the highest contents of caffeine. According to Astill et al. [37], the caffeine content decreases during the fermentation and drying stage. In case of freezing, it is possible that metabolic pathways are stopped, which result in less degradation during drying. Since the rolling process was only carried out on young leaves, further tests would be needed to determine whether this has an influence on the caffeine content. Furthermore, the low caffeine content of the blended samples may be due to the addition of water. Some of the caffeine may have been dissolved in the water during processing and then dripped off through the drying bed during drying. Theobromin, which is described in the metabolic pathway as a precursor of caffeine [38], could only be detected in small amounts in three samples.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

3.4.2 Catechins



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

Figure 6. Influence of the drying method **(a)** and the processing method **(b)** on the total catechin content.

The results of the total catechin content show a clear influence of the blending process. All blended samples, with exclusion of sample 6, have no catechins at all. According to scientific findings, this could be due to the oxidation of catechins by polyphenol oxidase to theaflavin [39]. This effect occurs during the fermentation of black tea [40]. The large surface area and added water of the mixed samples could be responsible for an enhanced enzymatic reaction. Furthermore, the drying parameters show an influence on the total catechin content. Air drying has a significantly higher average value (0.266 g/100 g DM) than oven drying (0.054 g/100 g DM) and a significantly lower value than roasting (0.479 g/100 g DM). Li et al. [41] investigated the correlation of temperature and duration of thermal treatment on catechin content. Accordingly, the low content of the oven-dried sample can be attributed to the 4-hour drying time. The roasted sample had a much shorter drying time (20 min), which resulted in the highest content.

The epigallocatechine gallate mentioned in the European Commission's novel food approval could not be detected in any sample. Since Ratanamarno et al. [33] have already detected epigallocatechine gallate in fresh coffee leaves, it can be concluded that it was degraded during processing, transport or storage. Turkmen et al. [42] stated an absence of epigallocatechine gallate in black tea because of oxidation and fermentation processes [42]. This effect could also have occurred in the coffee leaf tea samples during the withering process.

3.5 Content of total polyphenols

The results of the photometric analysis of the total polyphenol content are shown in Table A3 in the appendix. Furthermore, the influence of the manufacturing methods is shown in Figure 7.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

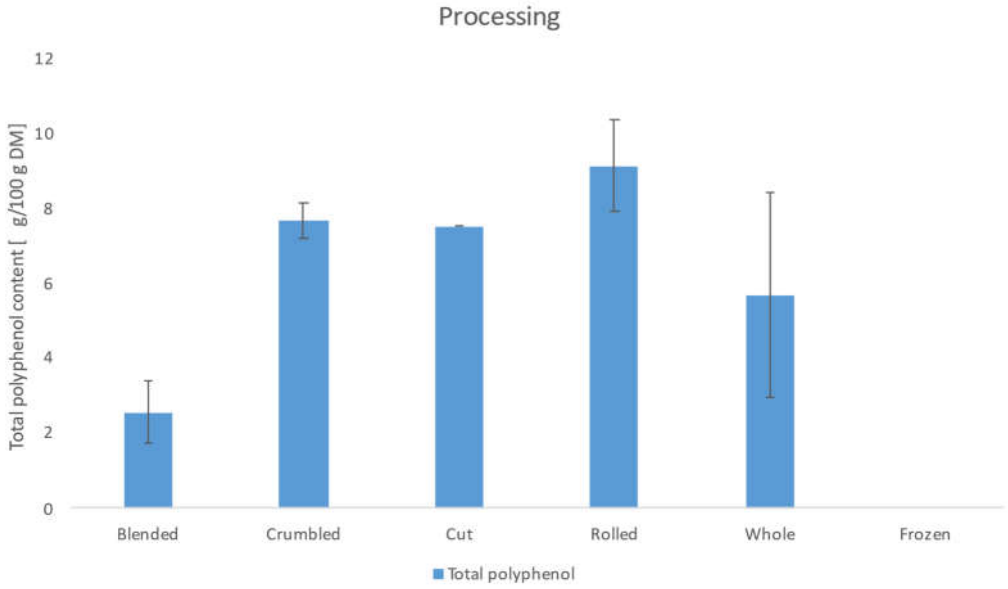


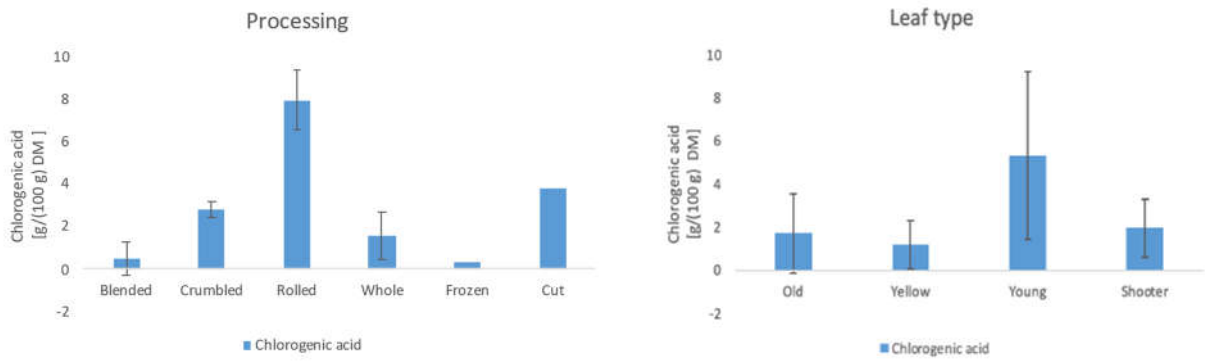
Figure 7. Total polyphenol content depending on process parameters.

The total polyphenol content varies between not detectable and 10.36 g/100 g DM and depends mainly on the processing. The blended samples show a significantly lower phenol content with 2.55 g/100 g DM compared to crumbling, cutting, rolling and whole leaves. Furthermore, no phenol could be detected in the frozen sample. Existing literature shows the influence of freezing on the polyphenol content before. Oszmiański et al. [43] stated a loss of up to 33.6% and Loncaric et al. [44] up to 48% after a freezing pretreatment. The low content in the blended samples can be explained by an increased oxidation process. Due to the big surface of the blended leaves, the polyphenol oxidase can degrade the polyphenols faster compared to the other samples. Turkmen et al. [42] described a decrease in polyphenol content in fermented black tea compared to green tea. In this case, the polyphenol oxidase has not been deactivated by a heat treatment, resulting in a loss that depends on the duration of the fermentation.

3.6 Content of organic acids and Trigonellin

The results of the NMR analysis for organic acids and trigonellin are shown in Table A4 in the appendix. Furthermore the influence of the manufacturing methods are shown in Figures 8-11.

3.6.1 Chlorogenic acid



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

Figure 8. (a) Influence of the processing method on the chlorogenic acid content **(b)** Influence of the leaf type on the chlorogenic acid content.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

The content of chlorogenic acid varies between not detectable and 9.35 g/100 g DM. The significant influences here are the leaf type and the processing. The young leaves show the highest amount of chlorogenic acid with 5.33 g/100 g DM followed by shooter with 1.96 g/100 g DM, the old leaves with 1.71 g/100 g DM and the yellow leaves with 1.21 g/100 g DM. As with caffeine, chlorogenic acid exerts a protective effect on the leaf through its antioxidant property. Therefore, here it is also present in an increased amount in the young leaves. This data also coincide with the analyses already carried out by Monteiro et al. (2020) [45]. Furthermore it is shown, that freezing (0.31 g/100 g DM) and blending (0.47 g/100 g DM) have a negative effect on the amount. The negative effect of freezing contradicts a study by Ścibisz et al. [46] where freezing had no effect.

3.6.2 Lactic acid

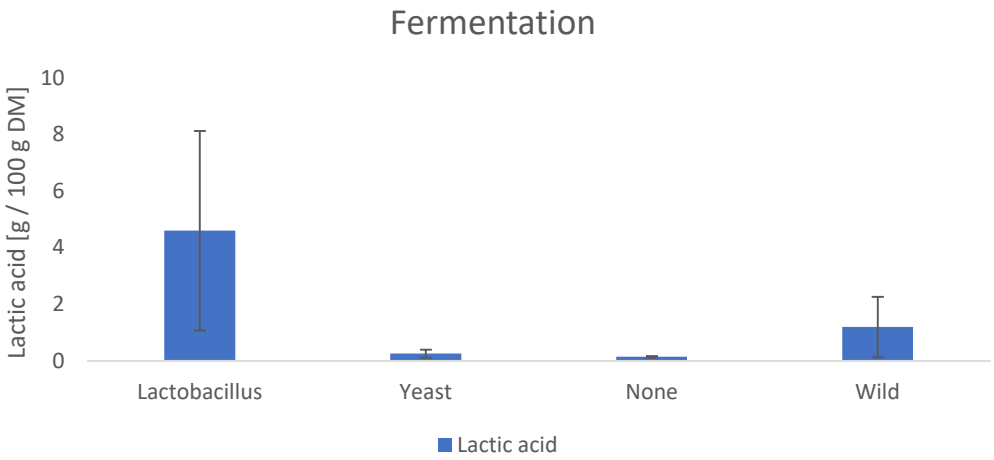
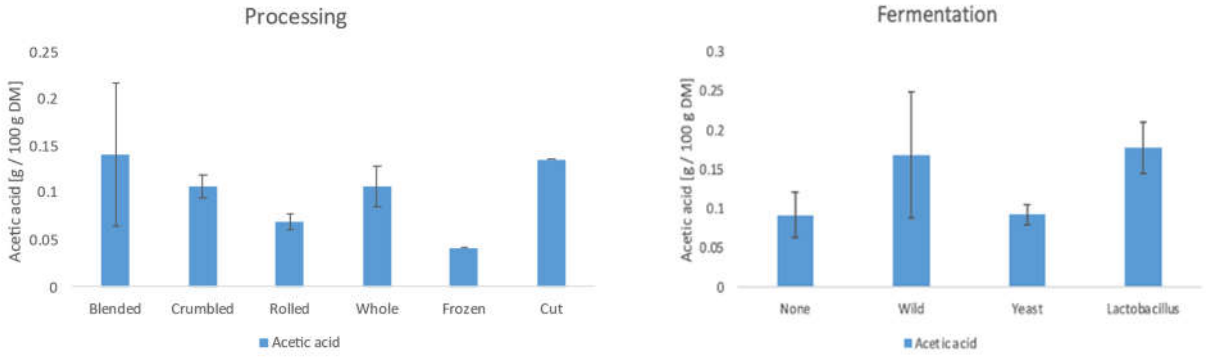


Figure 9. Influence of the fermentation on the lactic acid content.

Lactic acid varied from 0.11 g/100 g DM to 8.12 g/100 g DM in the samples. As expected, the samples fermented with the lactobacillus showed the highest value. Furthermore, an increased amount is found in the wild fermented samples. This suggests that a certain percentage of lactic acid bacteria is present in the microbiota of the coffee leaf.

3.6.3 Acetic acid



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

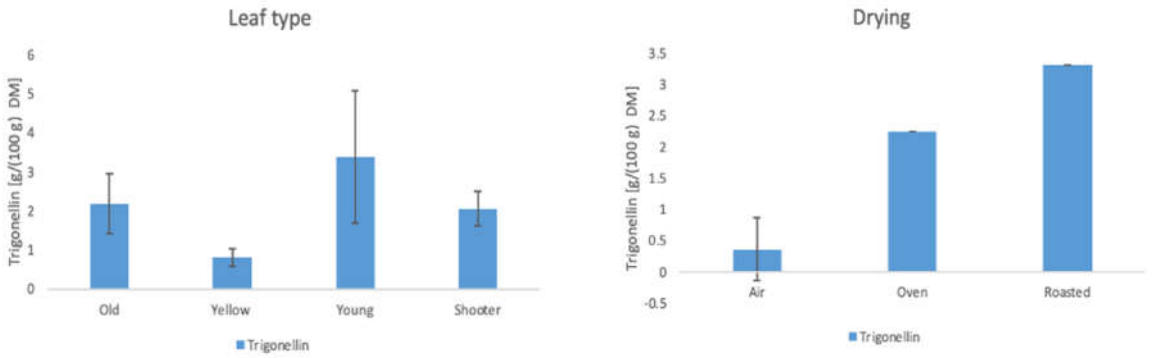
Figure 10. (a) Influence of the processing method on the acetic acid content **(b)** Influence of the fermentation on the acetic acid content.

Acetic acid varied from 0.04 g/100 g DM to 0.29 g/100 g DM. Above all, the processing and the type of leaf influence the amount significantly. As with the other acids, freezing gives a significantly lower value (0.042 g/100 g DM) than the rest. Samples with larger

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

surface area such as blended and cut samples show the highest values (0.14 g/100 g DM and 0.13 g/100 g DM. Samples fermented wild and with Lactobacillus have significantly higher values (0.17 g/100 g DM / 0.18 g/100 g DM) than those fermented with yeast or not fermented (0.09 g/100 g DM). The yeast does not differ from the non-fermented samples, indicating that this yeast strain produces few organic acids.

3.6.4 Trigonelline



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

Figure 11. (a) Influence of the leaf type on the trigonelline content (b) Influence of the drying method on the trigonelline content.

Trigonelline is a substance, which is mainly found in the seeds of many plants. Quantities between 0.62 g/100 g DM and 4.87 g/100 g DM could be detected in the tea samples. The trigonelline content results in this study are up to four times higher than those found in a recent study by Monteiro et al. [45]. In the coffee beans, however, only 1-1.2 g/100 g DM are contained in the untreated state. The trigonelline content is influenced by the leaf type and the drying method. The effect of a higher content in young leaves coincides with the result of Monteiro et al. [45]. Furthermore the roasting lead to a high trigonelline content. Zhu et al. [47] showed a similar relationship with hemp seeds where roasting had the highest influence on trigonelline levels.

3.7 Sensory evaluation

3.7.1 Personal acceptance

The results of the ranking test with the eight best rated teas are shown in Table 5. An evaluation of the results using the Friedmann test showed no statistical differences between the teas. Nevertheless, an improvement in flavour by yeast fermentation can be inferred by these results. The top 2 were both treated with the Anaferm yeast. For sample 369, however, a strong polarization could be detected within the panel. It was described as "the best" by two people and as "the most disliked" by two other people from the panel.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

Table 5. Results of ranking test and descriptive terms for colour, odour (via orthonasal detection) and flavour (via retronasal detection) of the panel for the best 8 tea samples.

Test number	Ranking	Colour	odour	flavour
687	1	sediment turbid red-brown	peach-like	peach-like
182	2	clear red	floral woody	sweet peach-like
369	3	clear yellow-brown	popcorn-like smoky roasty	popcorn-like roasty
147	4	very clear yellow-brown	chestnut flower-like floral	honey-like grassy
156	5	red clear	honey floral	honey-like acacia flower-like
234	6	clear amber	grassy rooibos-like	green bean-like vegetal
930	7	clear yellow-green	floral sweet	floral basil-like
743	8	turbid light orange	green bean-like	grassy green bean-like broccoli-like

3.7.2 Simple descriptive test

The results of the simple descriptive test are shown in Table 5. The results of the simple descriptive test show a wide range of flavours perceived through the panel. It can be recognized how the fermented samples 182, 687, 156 and 147 differ from the unfermented samples in the type of aromas. While fermentation tends to produce sweetish fruity notes, the unfermented samples tend to have green and vegetal aromas. Wang et al. [48] also found a correlation between fermentation and a loss of green flavour compounds and an increase of fruity flavours. There was also a wide difference in the various colours. Here, as shown in Figure 12, especially the fermented teas show a considerably darker colour compared to the rest. The exception is sample 147, which despite fermentation has the lightest colour of all teas. The change of the colour was also reported by Borah et al. [49]. In the study the tea changed the colour from green to a darker copper red during the fermentation process.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

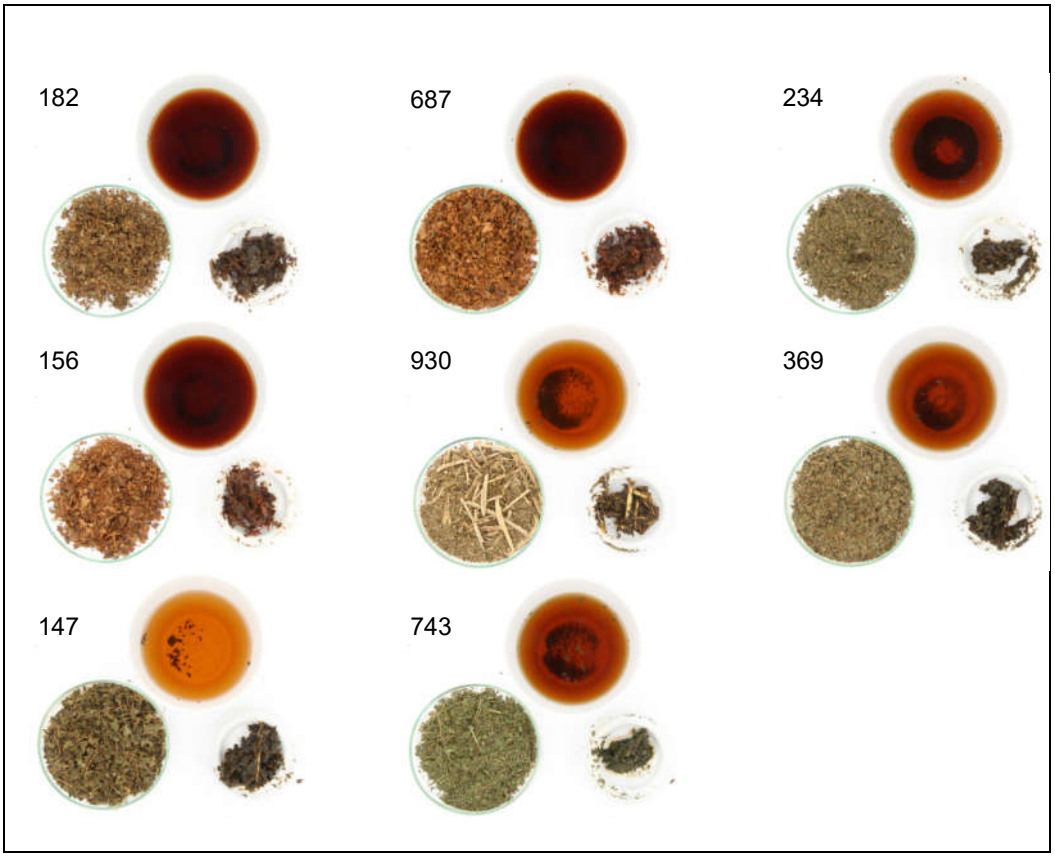


Figure 12. Brewed tea samples along with brewed and unbrewed tea leaves of the best eight teas.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

3.7.3 Profile test

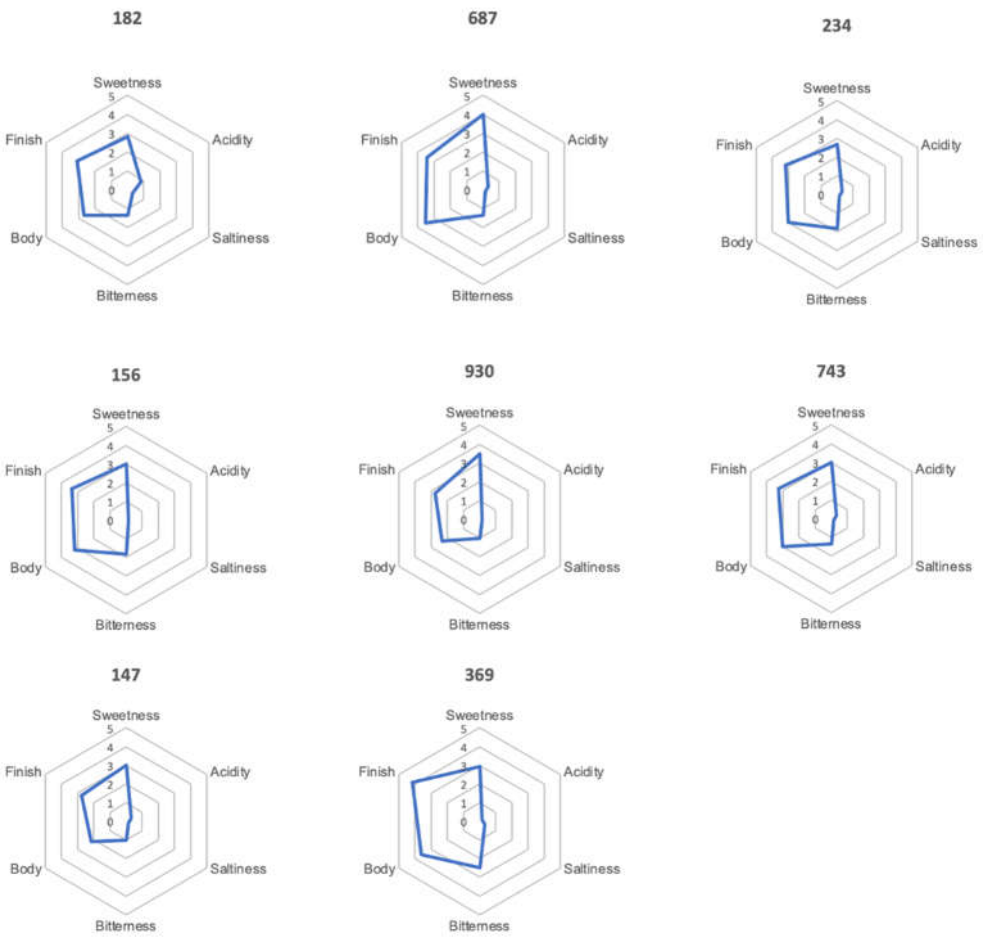


Figure 13. Graphical illustration of the results of the profile test.

The results of the profile test (Figure 13) show that the taste is mainly dominated by sweet and partly bitter impressions. Sweetness ranged from 2.8 to 4 in all samples, bitterness ranged from 1.5 to 2. Acidity was only detected with a value of 0.8 in sample 182 and saltiness could not be detected. Values of less than 0.5 were not considered in this study. This coincides with the description of Yuwono et al. [50] in which he describes the tea as sweetish, green and woody. The evaluation with the Program Design Expert showed no significant influence of the different process parameters on sweetness, saltiness, bitterness, acidity, body and finish.

3.8 Aroma analysis via DI-SBSE-GC-MS-O

3.8.1 Identification of odour-active compounds

A total of 68 different olfactory impressions could be detected from the 8 samples (Table A5). Of these, 44 could be identified by mass spectrum, RI, and odour. Exemplary total ion chromatograms and mass spectra are shown in Figures A1-A2. Of the 44 substances identified, 16 are aldehydes, 10 are ketones, 8 are alcohols, 3 are organic acids, 2 are pyrazines, 2 are ionenes, 1 is a terpene, 1 is an aromatic heterocyclic amine, and 1 is a fatty acid ester. A total of four substances were detected in all samples. These are (*E*,*E*)-3,5-octadien-2-one, 2,6-nonadienal ,α-ionone and β-ionone. Pyrazines, which are typical roast aromas, could only be identified in the roasted sample 369. 4-Heptanal could only be detected in fermented samples and γ-dodecalactone just in yeast fermented samples.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

The perceived odours could be described to a large extent as green and grassy (22 substances). Furthermore, some sweetish notes (11 substances) and notes in the area of melon or cucumber (11 substances) could be identified. In the area of fruity and citrus, 7 and 5 impressions, respectively, were perceived. Other attributes were roasty, herbal, honey, vanilla, aquarium, unique, nutty, forest and stable.

3.8.2 Semi-quantification and odour activity values

The results of the semi-quantification and the calculated OAVs of each sample are shown in the Table A6 to Table A13 in the appendix. The associated OAVs can be found in Table A14 in the appendix. In addition, the percentage of the OAVs in the total aroma for each sample is shown in Figures 14-21.

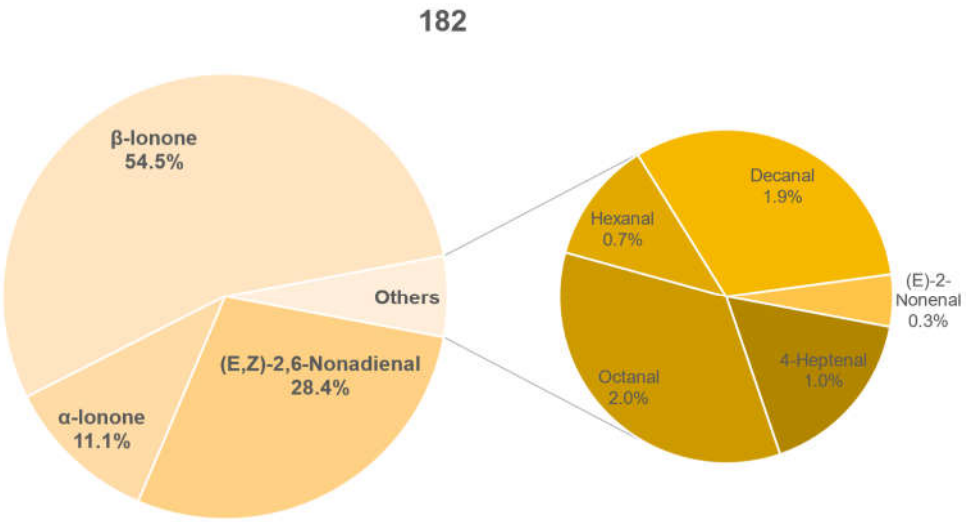


Figure 14. Percentage of OAV of each compound in the total aroma of sample 182.

Sample 182 showed a total of nine substances with an OAV greater than 1. Three of these substances, *β*-ionone (54.4%), (*E*,*Z*)-2,6-nonadienal (28.4%), and *α*-ionone (11.1%) accounting for 93.9% of the total aroma. Furthermore, the substances decanal, 4-heptenal, hexanal and (*E*)-2-nonenal are partly responsible for the aroma.

The smell of the tea was described in the previous tasting as floral and woody while the flavour was sweetish and peach via retronasal detection. The sweetness here can most likely be attributed to the *β*-ionone while the green and floral tones come from (*E*,*Z*)-2,6-nonadienal and *α*-ionone. Another role is probably played by hexanal (0.7%) which is according to Zhu and Xiao [51] one of the key aroma compounds of peach. These, together with decanal (fruity) and octanal (citrus like) could have caused the perceived peach-like flavour.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

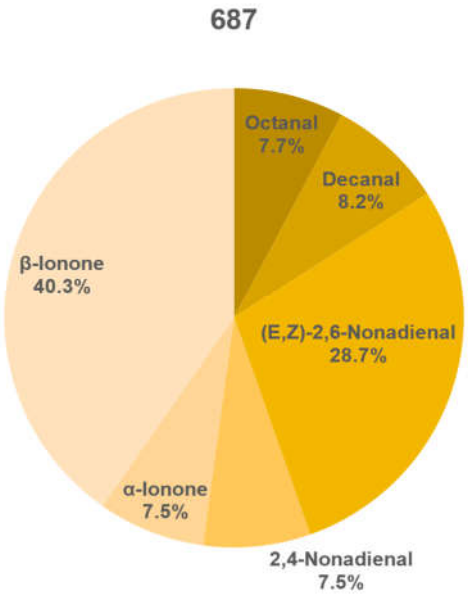


Figure 15. Percentage of OAV of each compound in the total aroma of sample 687.

Sample 687 shows six compounds with a OAV over 1. Here the main compounds are *β*-ionone (40.3%) and (*E,Z*)-2,6-nonadienal (28.7%). Furthermore, decanal (8.2%), octanal (7.7%) (*E,Z*)-2,4-nonadienal (7.5) and *α*-ionone (7.5%) have a relatively large share. However, overall the sample showed significantly lower total concentrations than all other samples. This could be due to the fact that the plant has extracted the substances from the yellow leaves before dropping them[52]. The resulting high percentage of fruity substances, such as (*E,Z*)-2,6-nonadienal and (*E,Z*)-2,4-nonadienal most likely leads to the detected fruity peach-like flavour of the tea.

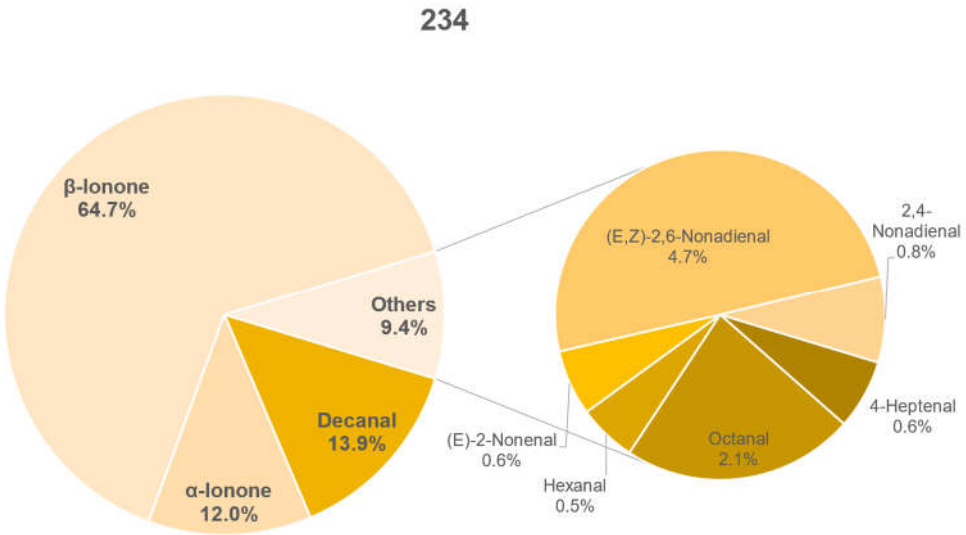


Figure 16. Percentage of OAV of each compound in the total aroma of sample 234.

A total of 9 aroma compounds with a OAV more than 1 were detected in sample 234. The main aroma substances are *β*-ionone (64.7%), decanal (13.9%) and *α*-ionone (12%). Furthermore, the substances (*E,Z*)-2,6-nonadienal, octanal, 2,4-nonadienal, 4–heptenal, (*E*)-2-nonenal and hexanal are partly responsible for the aroma. The strongly pronounced floral tones can therefore probably be attributed to Decanal and *α*-ionone, together with (*E,Z*)-2,6-nonadienal (4.7%), hexanal (0.5%) and 4-heptanal (0.6%). The sensory tones of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

the green bean and the rooibos do not coincide here, however, with the aroma components of these two products described in the literature [53,54].

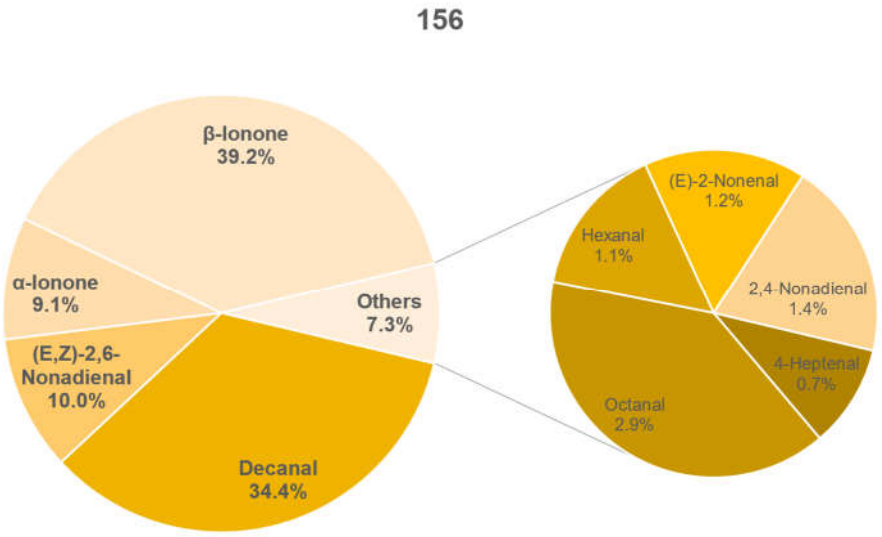


Figure 17. Percentage of OAV of each compound in the total aroma of sample 156.

In sample 156, 9 aroma compounds could be identified with an OAV of more than 1. *β*-ionone (39.2%) and decanal (34.4%) form the main part of the aroma compounds. Furthermore, (*E,Z*)-2,6-nonadienal with 10% and *α*-ionone with 9.1% are main aroma compounds, while the substances octanal, 2,4-nonadienal, (*E*)-2-nonenal, hexanal and 4-heptenal account for the remaining 7.3%. Overall, the total concentrations of the various compounds are significantly higher compared to the comparable sample 687 which also consists of yellow leaves and was fermented. The difference here is in the type of fermentation, which in this sample was wild and uncontrolled. Microorganisms could have caused the increase in concentrations in this sample; however, this would have to be verified by further studies.

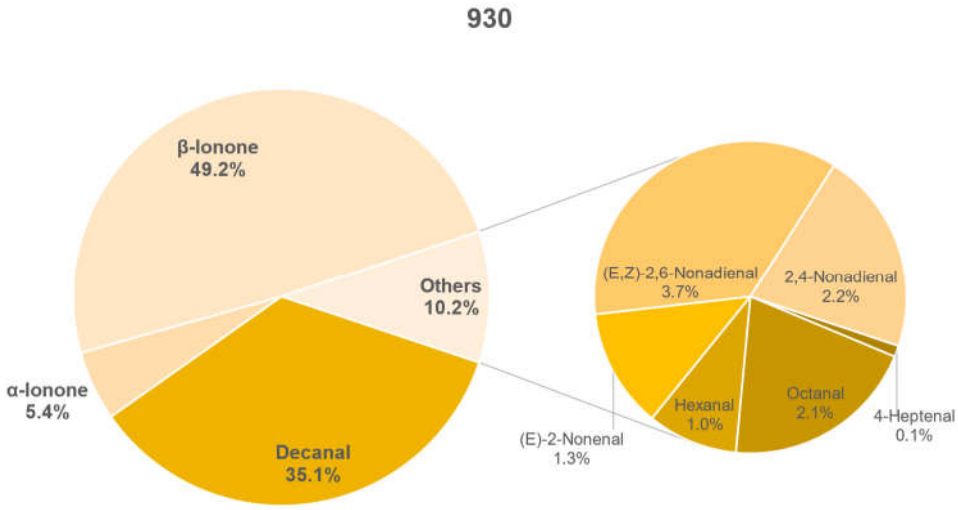


Figure 18. Percentage of OAV of each compound in the total aroma of sample 930.

Similar to the previous sample, 9 aroma forming substances were detected in sample 930. Here, *β*-ionone (49.2%) and decanal (35.1%) make up the main component. Including

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

α-ionone (5.4%), the substances (*E,Z*)-2,6-nonadienal, 2,4-nonadienal, octanal, (*E*)-2-nonenal, hexanal and 4-heptenal account for a total of 15.6% of the aroma. The aroma described as sweetish and floral is assumed to be composed mainly of the substances β-ionone, decanal, α-ionone, hexanal and 4-heptenal. Furthermore, no influence of the heat treatment (steaming) on the volatile substances can be identified for this sample.

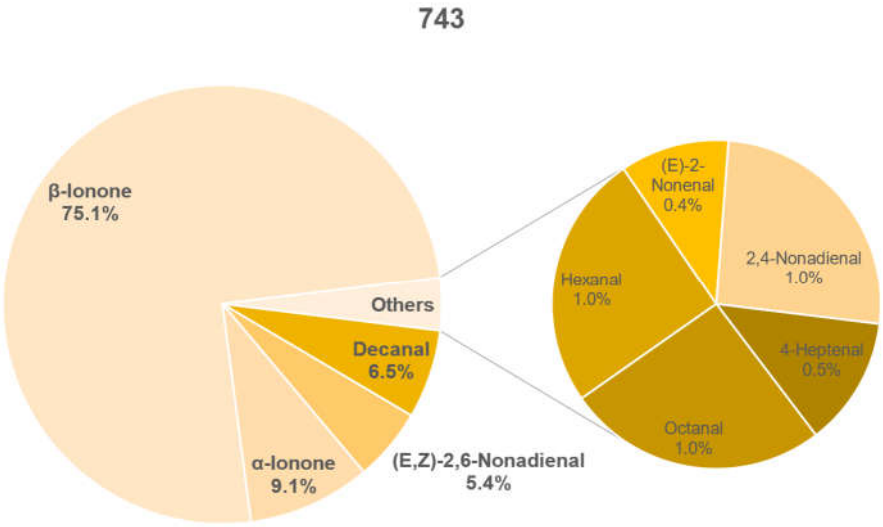


Figure 19. Percentage of OAV of each compound in the total aroma of sample 743.

Semi-quantification also identified 9 compounds of importance for the aroma in sample 743. β-Ionone and α-ionone are the main substances here with 75.1% and 9.1%. Furthermore, decanal (6.5%), (*E,Z*)-2,6-nonadienal (5.4%) and with 1% or less 2,4-nonadienal, octanal, hexanal, 4-heptenal,(*E*)-2-nonenal are odour-active. The aroma described as grassy and floral is assumed to be composed mainly of the substances, decanal, β-ionone, α-ionone, hexanal and 4-heptenal.

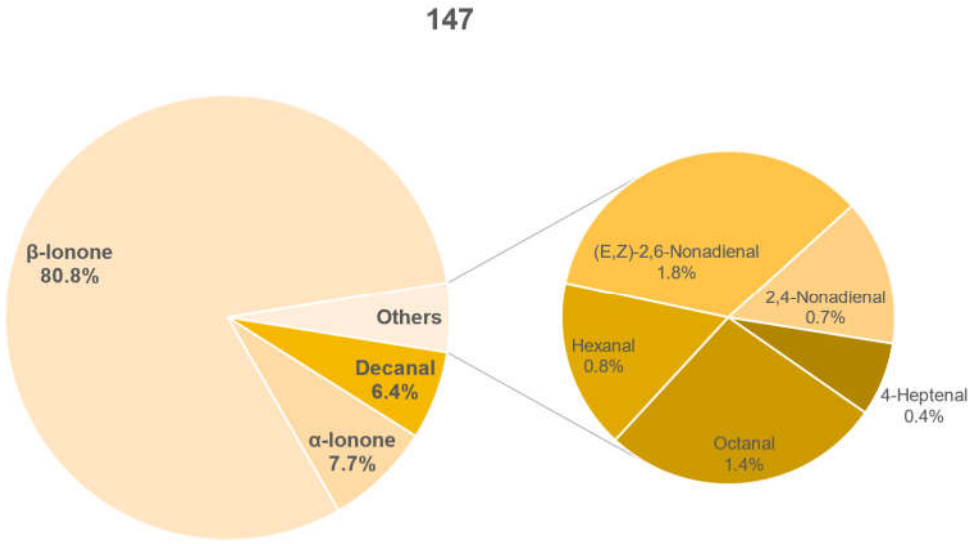


Figure 20. Percentage of OAV of each compound in the total aroma of sample 147.

A total of 8 different substances with an OAV higher than 1 were detected in sample 147. The main flavouring agent is β-ionone with 80.8%. This is followed by α-ionone

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

(7.7%), decanal (6.4%) and the others, (E,Z)-2,6-nonadienal, octanal, hexanal, 2,4-nonadienal, and 4-heptenal with (5.1%). In general, the sample had the highest concentration of *β*-ionone (6.49 µg/L, Table X). In comparison to the comparable, wild fermented sample 156 even the triple. This high level of *β*-ionone also seems to be confirmed in the odour perception which would be described as chestnut blossom and the flavour which was determined by the panel to be honey.

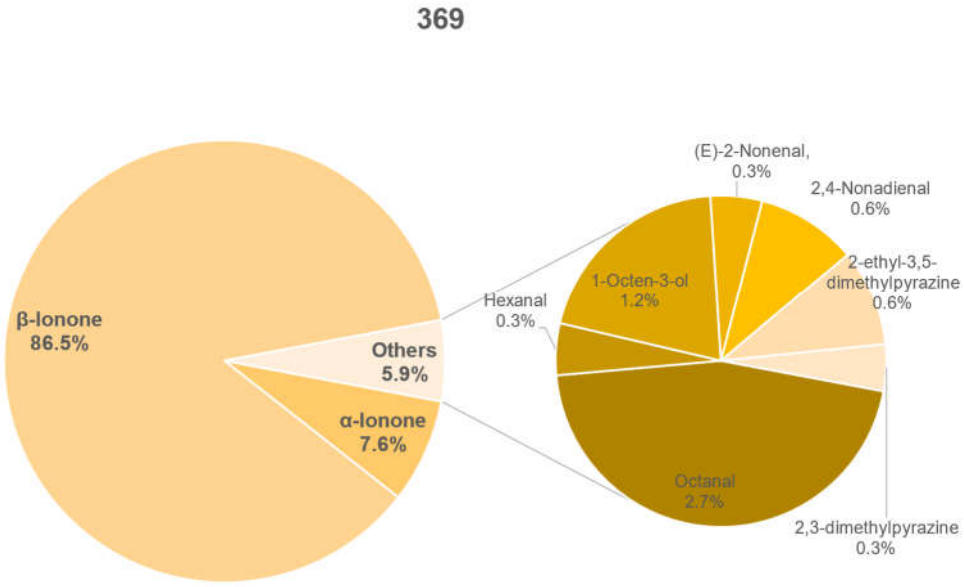


Figure 21. Percentage of OAV of each compound in the total aroma of sample 369.

In roasted sample 369, a total of 9 aroma-active compounds with an OAV above 1 could be detected. Here, β-ionone is the main aroma substance with 86.5%. Furthermore, 2,3-dimethylpyrazines (0.3%) and 2-ethyl-3,5-dimethylpyrazines (0.6%) with an OAV of 1 and 2 are found in the sample. However, the smoky and roasted flavour of this tea is strongly influenced by these components despite their very small contribution to the overall aroma. One reason for the strong perception of smoke is a natural protective instinct of humans against fire. The sensory cells perceive it very strongly in order to detect the danger of fire at an early stage [55].

In total, 9 main aroma compounds (Figure 22) were identified in coffee leaf tea via semi quantification and calculation of OAV.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

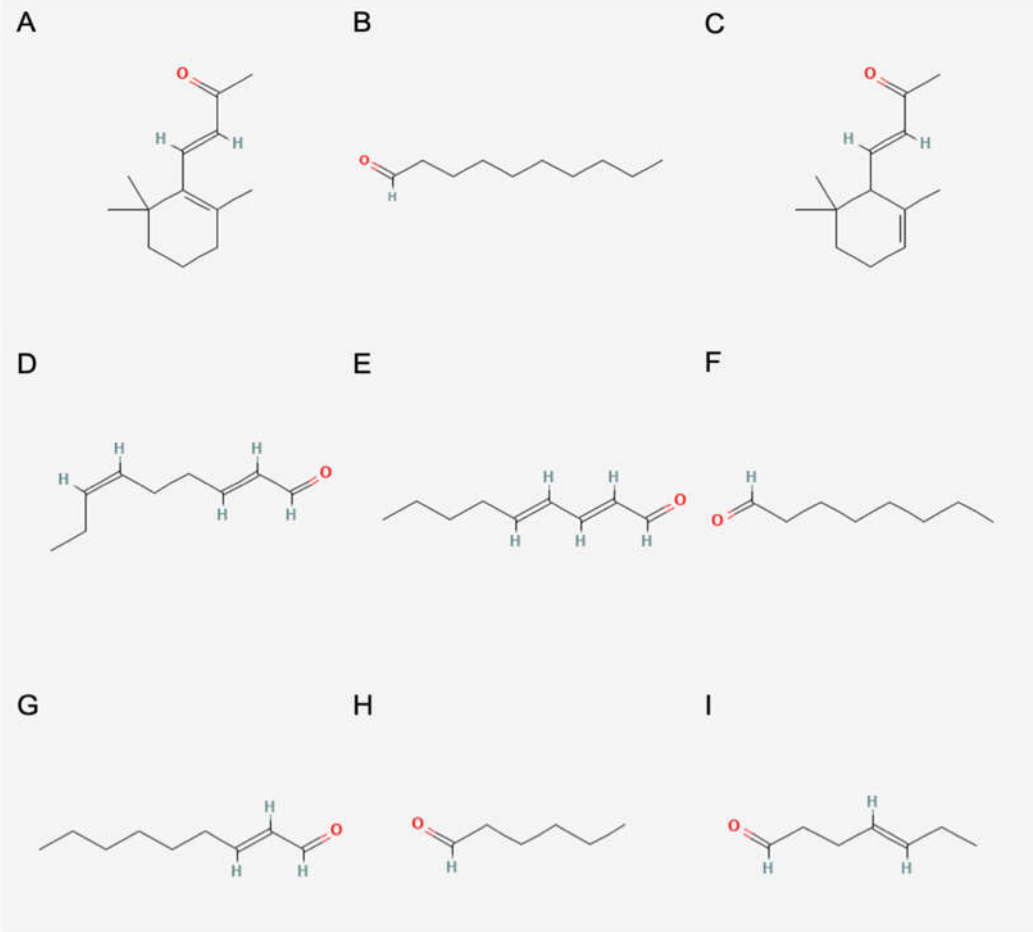


Figure 22. Structure of the main aroma compounds. A: *β*-ionone, B: decanal, C: *α*-ionone, D: (*E*,*Z*)-2,6-nonadienal, E: 2,4-nonadienal, F: octanal, G: (*E*)-2 nonenal, H: hexanal, I: 4-heptenal [56].

Three of them, *β*-ionone, *α*-ionone and 2-ethyl-3,5-dimethylpyrazines are according to Ho et al. [57] also main aroma substances of the tea plant. *β*-Ionone has the largest overall share in all samples and varies between 39.2% (156) and 86.5% (369). Furthermore, the percentage of decanal in two samples with a *β*-ionone value below 50% is very high (34.4% (156) and 35.1% (930). *α*-Ionone which is also present in all samples varies from 5.4% (930) to 12% (234). Moreover, (*E*,*Z*)-2,6-nonadienal was found as a main aroma component in some samples. As in the case of decanal, the value here varies greatly between 1.8% (147) and 28.7% (687) depending on the sample. The citrusy note of (*E*)-2-nonenal could not be noted in any of the samples during tasting by the panel. Despite the large variation between the samples, it was not possible to correlate the differences with the parameter in this study. Further studies are needed to provide comparable conditions between samples. Only the roasting process showed a large difference in these tests compared to the other samples, as the pyrazines also contribute a large proportion to the aroma.

The main disadvantage of the calculated OAV is that the interaction of individual aroma components is not taken into account. It is possible that individual aroma components, which were excluded because of an OAV below 1, may well contribute to the aroma together with other substances [58]. In addition, the perceived intensity of an aroma is not proportional to the value of the OAV. A doubling of the OAV around the threshold can have a greater impact on the aroma than a doubling of an OAV very far above the threshold [59].

4. Conclusions

In this study, different ways of producing teas from coffee leaves directly on the farm were investigated. The samples differed both in popularity among consumers and in the

 doi:10.20944/preprints202206.0321.v1

chemical composition of the active ingredients and flavourings. In future, the data obtained in this study may help to adjust process parameters directly to consumer preferences and allow farmers to earn an extra income from this by-product. For this purpose, on-site experiments should be carried out to upscale the processes.

Young leaves showed a positive correlation on various plant protective ingredients such as caffeine content, chlorogenic acid and trigonelline. The variety played a role in essential oils and caffeine content in these experiments. Pacamara had an increased level of essential oil and a slightly lower level of caffeine compared to Bourbon. In the processing parameters, blending of the samples resulted in a strong decrease in caffeine, catechins, the polyphenols and chlorogenic acid. In contrast, cell disruption processes such as rolling or crumbling led to increased values. Among the drying methods, roasting and oven drying had a positive effect on the essential oil and trigonelline content in the samples. Fermentation mainly had an effect on the acidity of the samples. Increased levels of lactic and acetic acid were found here, particularly in wild-fermented and Lactobacillus-fermented samples.

The sensory analysis revealed that fermented teas in particular are ahead in terms of popularity with consumers. Green tones in particular are masked by more fruity notes (e.g. peach). In addition, the roasting of the tea seems to lead to a polarizing product.

In the aroma analysis by gas chromatography, 68 aroma active compounds were detected by the ODP. By calculation of the OAV, 6-9 aroma compounds could be determined for each tea, which are the main components of the aroma profile. These are β-ionone (honey-like), decanal (citrus, floral), α-ionone (floral), (*E,Z*)-2,6-nonadienal (cucumber-like), 2,4-nonadienal (melon-like), octanal (fruity), (*E*)-2 nonenal (citrus), hexanal (grassy) and 4-heptenal (green). In addition, the two substances 2,3-dimethylpyrazines and 2-ethyl-3,5-dimethylpyrazines were found in the roasted sample.

Author Contributions: Conceptualization, Steffen Schwarz and Dirk W. Lachenmeier; Data curation, Marc C. Steger; Formal analysis, Marc C. Steger, Patrik Blumenthal and Valerie Segatz; Investigation, Marc C. Steger, Patrik Blumenthal and Valerie Segatz; Methodology, Marina Rigling, Andrès Quintanilla-Belucci, Jörg Rieke-Zapp, Steffen Schwarz and Dirk W. Lachenmeier; Resources, Steffen Schwarz, Dirk W. Lachenmeier and Yanyan Zhang; Supervision, Steffen Schwarz, Dirk W. Lachenmeier and Yanyan Zhang; Validation, Marina Rigling and Dirk W. Lachenmeier; Visualization, Marc C. Steger and Dirk W. Lachenmeier; Writing – original draft, Marc C. Steger; Writing – review & editing, Marc C. Steger, Marina Rigling, Patrik Blumenthal, Valerie Segatz, Andrès Quintanilla-Belucci, Julia M. Beisel, Jörg Rieke-Zapp, Steffen Schwarz, Dirk W. Lachenmeier and Yanyan Zhang. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Data Availability Statement: All relevant data are within the paper and its appendices.

Acknowledgments: The teams of the plant-based food and NMR laboratories at CVUA Karlsruhe are warmly thanked for excellent technical assistance.

Conflicts of Interest: S.S. is owner of and P.B. and M.C.S. are consultants for Coffee Consulate, Mannheim, Germany. Coffee Consulate is an independent training and research centre. A.Q.B. is owner of Finca La Buena Esperanza, a coffee plantation in El Salvador. J.R.Z. is managing director of Rubiacea Research and Development GmbH, Mannheim, Germany, which is providing consulting, research and development in the field of food and food technology with focus on coffee and coffee by-products. Coffee Consulate, Finca La Buena Esperanza and Rubiacea Research and Development GmbH are currently researching the potential of coffee by-products for production of tea and other foods. However, P.B., M.C.S., S.S., J.R.Z., and A.Q.B. report that there is no conflict of interest related to the work under consideration. The other authors declare no conflict of interest.

References

1. International Coffee Organisation (ICO) *Coffee Market Report*; London, United Kingdom, 2021.
2. Murthy, P.S.; Madhava Naidu, M. Sustainable Management of Coffee Industry By-Products and Value Addition—A Review. *Resour. Conserv. Recycl.* **2012**, *66*, 45–58, doi:10.1016/J.RESCONREC.2012.06.005.

3. European Comission Commission Implementing Regulation (EU) 2020/917 of 1 July 2020 Authorising the Placing on the Market of Infusion from Coffee Leaves of Coffea Arabica L. and/or Coffea Canephora Pierre Ex A. Froehner as a Traditional Food from a Third Country under Regula. *Off. J. Eur. Union* **2020**, *L209*, 10–13.
4. Lachenmeier, D.W.; Rajcic de Rezende, T.; Schwarz, S. An Update on Sustainable Valorization of Coffee By-Products as Novel Foods within the European Union. *Biol. Life Sci. Forum* **2021**, *6*, 37, doi:10.3390/Foods2021-10969.
5. Wintgens, J.N. *Coffee: Growing, Processing, Sustainable Production. A Guidebook for Growers, Processors, Traders, and Researchers*; WILEY-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2004; ISBN 3527307311.
6. von Pröpper, L. *Der Kaffee- Und Theetisch Nebst Rezepten Und Servierkarten*; Verlag von J. Engelhorn: Stuttgart, Germany, 1882.
7. Klingel, T.; Kremer, J.I.; Gottstein, V.; Rezende, T.R. De; Schwarz, S.; Lachenmeier, D.W. A Review of Coffee By-Products Including Leaf, Flower, Cherry, Husk, Silver Skin, and Spent Grounds as Novel Foods within the European Union. *Foods* **2020**, *9*, 665, doi:10.3390/foods9050665.
8. Tritsch, N.; Steger, M.C.; Segatz, V.; Blumenthal, P.; Rigling, M.; Schwarz, S.; Zhang, Y.; Franke, H.; Lachenmeier, D.W. Risk Assessment of Caffeine and Epigallocatechin Gallate in Coffee Leaf Tea. *Foods* **2022**, *11*, 263, doi:10.3390/foods11030263.
9. Novita, R.; Kasim, A.; Anggraini, T.; Putra, D.P. Kahwa Daun: Traditional Knowledge of a Coffee Leaf Herbal Tea from West Sumatera, Indonesia. *J. Ethn. Foods* **2018**, *5*, 286–291, doi:10.1016/j.jef.2018.11.005.
10. Cramer, P.J.S. *A Review of Literature of Coffee Research in Indonesia.*; SIC Editorial, Inter-American Institute of Agricultural Sciences: Turrialba, Costa Rica, 1957.
11. Lamorde, M.; Tabuti, J.R.S.; Obua, C.; Kukunda-Byobona, C.; Lanyero, H.; Byakika-Kibwika, P.; Bbosa, G.S.; Lubega, A.; Ogwal-Okeng, J.; Ryan, M.; et al. Medicinal Plants Used by Traditional Medicine Practitioners for the Treatment of HIV/AIDS and Related Conditions in Uganda. *J. Ethnopharmacol.* **2010**, *130*, 43–53, doi:10.1016/j.jep.2010.04.004.
12. Ross, I.A. Coffea Arabica. *Med. Plants World* **2005**, *3*, 155–195, doi:https://doi.org/10.1007/978-1-59259-887-8_4.
13. Giday, M.; Asfaw, Z.; Woldu, Z. Ethnomedicinal Study of Plants Used by Sheko Ethnic Group of Ethiopia. *J. Ethnopharmacol.* **2010**, *132*, 75–85, doi:10.1016/j.jep.2010.07.046.
14. Martins, S.C. V; Araújo, W.L.; Tohge, T.; Fernie, A.R.; DaMatta, F.M. In High-Light-Acclimated Coffee Plants the Metabolic Machinery Is Adjusted to Avoid Oxidative Stress Rather than to Benefit from Extra Light Enhancement in Photosynthetic Yield. *PLoS One* **2014**, *9*, e94862, doi:10.1371/journal.pone.0094862.
15. Pompelli, M.F.; Martins, S.C. V; Antunes, W.C.; Chaves, A.R.M.; DaMatta, F.M. Photosynthesis and Photoprotection in Coffee Leaves Is Affected by Nitrogen and Light Availabilities in Winter Conditions. *J. Plant Physiol.* **2010**, *167*, 1052–1060, doi:10.1016/j.jplph.2010.03.001.
16. Chen, X. A Review on Coffee Leaves: Phytochemicals, Bioactivities and Applications. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 1008–1025, doi:10.1080/10408398.2018.1546667.
17. Chaves, A.R.M.; Ten-Caten, A.; Pinheiro, H.A.; Ribeiro, A.; Damatta, F.M. Seasonal Changes in Photoprotective Mechanisms of Leaves from Shaded and Unshaded Field-Grown Coffee (Coffea Arabica L.) Trees. *Trees - Struct. Funct.* **2008**, *22*, 351–361, doi:10.1007/s00468-007-0190-7.
18. The European Parliament and the Council of the European Union Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on Novel Foods, Amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and Repealing Regulation (EC) No 258/97. *Off. J. Eur. Union* **2015**, *L327*, 1–22.
19. DIN 10809:1988-08 *Analysis of Tea; Preparation of Liquor for Use in Sensory Tests*; Beuth Verlag, Berlin, Germany; doi:10.31030/2251247.
20. ISO 1572:1980-08 *Tea; Preparation of Ground Sample of Known Dry Matter Content*; International Organization for Standardization, Geneva, Switzerland; doi:10.31030/2251247.
21. Segatz, V.; Steger, M.C.; Blumenthal, P.; Gottstein, V.; Rigling, M.; Schwarz, S.; Zhang, Y.; Lachenmeier, D.W. Evaluation of

Analytical Methods to Determine Regulatory Compliance of Coffee Leaf Tea. *Biol. Life Sci. Forum* **2021**, *6*, 45, doi:10.3390/Foods2021-10937.

22. Claassen, L.; Rinderknecht, M.; Porth, T.; Röhnisch, J.; Seren, H.Y.; Scharinger, A.; Gottstein, V.; Noack, D.; Schwarz, S.; Winkler, G.; et al. Cold Brew Coffee-Pilot Studies on Definition, Extraction, Consumer Preference, Chemical Characterization and Microbiological Hazards. *Foods* **2021**, *10*, 865, doi:10.3390/foods10040865.
23. Rigling, M.; Yadav, M.; Yagishita, M.; Nedele, A.K.; Sun, J.; Zhang, Y. Biosynthesis of Pleasant Aroma by Enokitake (Flammulina Velutipes) with a Potential Use in a Novel Tea Drink. *LWT* **2021**, *140*, 110646, doi:10.1016/J.LWT.2020.110646.
24. WEATHERLEY, P.E. Studies in the Water Relations of the Cotton Plant: I. the Field Measurement of Water Deficits in Leaves. *New Phytol.* **1950**, *49*, 81–97, doi:10.1111/j.1469-8137.1950.tb05146.x.
25. Chabot, B.F.; Hicks, D.J. The Ecology of Leaf Life Spans. *Annu. Rev. Ecol. Syst. Vol. 13* **1982**, *13*, 229–259, doi:10.1146/annurev.es.13.110182.001305.
26. Zerbini, S. The Pruning of Ornamental Plants: Why Topping Is Such a Common Practice. *Alberi e Territ.* **2006**, *2*, 26–28.
27. Scherer, G.W. Theory of Drying. *J. Am. Ceram. Soc.* **1990**, *73*, 3–14, doi:10.1111/j.1151-2916.1990.tb05082.x.
28. Arslan, D.; Özcan, M.M. Evaluation of Drying Methods with Respect to Drying Kinetics, Mineral Content and Colour Characteristics of Rosemary Leaves. *Energy Convers. Manag.* **2008**, *49*, 1258–1264, doi:10.1016/j.enconman.2007.08.005.
29. BMEL *Leitsätze Für Tee, Teeähnliche Erzeugnisse, Deren Extrakte Und Zubereitungen Vom 2.12.1998 (BAnz. Nr. 66a Vom 9.4.1999, GMBl. Nr. 11 S.228 Vom 26.4.1999), Zuletzt Geändert Am 5.3.2013 (BAnz. AT Vom 12.12.2013 B6, GMBl. Nr. 63 S.1265 Vom 17.12.2013)*; Berlin, Germany, 2013.
30. Hanaa, A.R.M.; Sallam, Y.I.; El-Leithy, A.S.; Aly, S.E. Lemongrass (Cymbopogon Citratus) Essential Oil as Affected by Drying Methods. *Ann. Agric. Sci.* **2012**, *57*, 113–116, doi:10.1016/j.aoas.2012.08.004.
31. Morsy, N.F.S. Quality Evaluation of the Essential Oil of Basil Plant Dried by Different Methods. **2004**.
32. Omidbaigi, R.; Sefidkon, F.; Kazemi, F. Influence of Drying Methods on the Essential Oil Content and Composition of Roman Chamomile. *Flavour Fragr. J.* **2004**, *19*, 196–198, doi:10.1002/ffj.1340.
33. Ratanamarno, S.; Surbkar, S. Caffeine and Catechins in Fresh Coffee Leaf (Coffea Arabica) and Coffee Leaf Tea. *Maejo Int. J. Sci. Technol.* **2017**, *11*, 211–218.
34. Bowers, M.D.; Stamp, N.E. Effects of Plant Age, Genotype, and Herbivory on Plantago Performance and Chemistry. *Ecology* **1993**, *74*, 1778–1791, doi:10.2307/1939936.
35. Blum-Silva, C.H.; Chaves, V.C.; Schenkel, E.P.; Coelho, G.C.; Reginatto, F.H. The Influence of Leaf Age on Methylxanthines, Total Phenolic Content, and Free Radical Scavenging Capacity of Ilex Paraguariensis Aqueous Extracts. *Rev. Bras. Farmacogn.* **2015**, *25*, 1–6, doi:10.1016/j.bjp.2015.01.002.
36. Song, R.; Kelman, D.; Johns, K.L.; Wright, A.D. Correlation between Leaf Age, Shade Levels, and Characteristic Beneficial Natural Constituents of Tea (Camellia Sinensis) Grown in Hawaii. *Food Chem.* **2012**, *133*, 707–714, doi:10.1016/j.foodchem.2012.01.078.
37. Astill, C.; Birch, M.R.; Dacombe, C.; Humphrey, P.G.; Martin, P.T. Factors Affecting the Caffeine and Polyphenol Contents of Black and Green Tea Infusions. *J. Agric. Food Chem.* **2001**, *49*, 5340–5347, doi:10.1021/jf010759+.
38. Ashihara, H.; Monteiro, A.M.; Gillies, F.M.; Crozier, A. Biosynthesis of Caffeine in Leaves of Coffee. *Plant Physiol.* **1996**, *111*, 747–753, doi:10.1104/pp.111.3.747.
39. Friedman, M.; Levin, C.E.; Choi, S.H.; Lee, S.U.; Kozukue, N. Changes in the Composition of Raw Tea Leaves from the Korean Yabukida Plant during High-Temperature Processing to Pan-Fried Kamairi-Cha Green Tea. *J. Food Sci.* **2009**, *74*, C406–C412, doi:10.1111/j.1750-3841.2009.01185.x.
40. Toschi, T.G.; Bordoni, A.; Hrelia, S.; Bendini, A.; Lercker, G.; Biagi, P.L. The Protective Role of Different Green Tea Extracts after Oxidative Damage Is Related to Their Catechin Composition. *J. Agric. Food Chem.* **2000**, *48*, 3973–3978, doi:10.1021/jf000499g.

41. Li, N.; Taylor, L.S.; Ferruzzi, M.G.; Mauer, L.J. Kinetic Study of Catechin Stability: Effects of Ph, Concentration, and Temperature. *J. Agric. Food Chem.* **2012**, *60*, 12531–12539, doi:10.1021/jf304116s.
42. Turkmen, N.; Sarı, F.; Velioglu, Y.S. Factors Affecting Polyphenol Content and Composition of Fresh and Processed Tea Leaves. *Akad. Gıda* **2009**, *7*, 29–40.
43. Oszmiański, J.; Wojdyło, A.; Kolniak, J. Effect of L-Ascorbic Acid, Sugar, Pectin and Freeze-Thaw Treatment on Polyphenol Content of Frozen Strawberries. *LWT - Food Sci. Technol.* **2009**, *42*, 581–586, doi:10.1016/j.lwt.2008.07.009.
44. Loncaric, A.; Dugalic, K.; Mihaljevic, I.; Jakobek, L.; Pilizota, V. Effects of Sugar Addition on Total Polyphenol Content and Antioxidant Activity of Frozen and Freeze-Dried Apple Purée. *J. Agric. Food Chem.* **2014**, *62*, 1674–1682, doi:10.1021/jf405003u.
45. Monteiro, Â.; Colomban, S.; Azinheira, H.G.; Guerra-Guimarães, L.; Silva, M.D.C.; Navarini, L.; Resmini, M. Dietary Antioxidants in Coffee Leaves: Impact of Botanical Origin and Maturity on Chlorogenic Acids and Xanthones. *Antioxidants* **2020**, *9*, 6, doi:10.3390/antiox9010006.
46. Ścibisz, I.; Mitek, M. The Changes of Antioxidant Properties in Highbush Blueberries (Vaccinium Corymbosum L.) During Freezing and Long-Term Frozen Storage. *ACTA Acta Sci. Pol., Technol. Aliment* **2007**, *6*, 75–82.
47. Zhu, X.; Wang, C.; Wang, X.; Cai, M.; Deng, S. Effects of Different Processing Methods on the Determination of Trigonelline in Fructus Cannabis Decoction Pieces. *J. Pharm. Res.* **2016**, *35*, 19–21.
48. Wang, L.F.; Lee, J.Y.; Chung, J.O.; Baik, J.H.; So, S.; Park, S.K. Discrimination of Teas with Different Degrees of Fermentation by SPME-GC Analysis of the Characteristic Volatile Flavour Compounds. *Food Chem.* **2008**, *109*, 196–206, doi:10.1016/j.foodchem.2007.12.054.
49. Borah, S.; Bhuyan, M. Non-Destructive Testing of Tea Fermentation Using Image Processing. *Insight Non-Destructive Test. Cond. Monit.* **2003**, *45*, 55–58, doi:10.1784/insi.45.1.55.52593.
50. Yuwono, S.S.; Fibrianto, K.; Wahibah, L.Y.; Wardhana, A.R. Sensory Attributes Profiling of Dampit Robusta Coffee Leaf Tea (Coffea Canephora). *Carpathian J. Food Sci. Technol.* **2019**, *11*, 165–176, doi:10.34302/crpjfst/2019.11.2.13.
51. Zhu, J.C.; Xiao, Z.B. Characterization of the Key Aroma Compounds in Peach by Gas Chromatography–Olfactometry, Quantitative Measurements and Sensory Analysis. *Eur. Food Res. Technol. 2018 2451* **2019**, *245*, 129–141, doi:10.1007/S00217-018-3145-X.
52. Galmés, J.; Flexas, J.; Savé, R.; Medrano, H. Water Relations and Stomatal Characteristics of Mediterranean Plants with Different Growth Forms and Leaf Habits: Responses to Water Stress and Recovery. *Plant Soil 2007 290* **2007**, *290*, 139–155, doi:10.1007/S11104-006-9148-6.
53. Kawakami, M.; Kobayashi, A.; Kator, K. Volatile Constituents of Rooibos Tea (Aspalathus Linearis) As Affected by Extraction Process. *J. Agric. Food Chem.* **1993**, *41*, 633–636, doi:10.1021/JF00028A023.
54. Oomah, B.D.; Liang, L.S.Y.; Balasubramanian, P. Volatile Compounds of Dry Beans (Phaseolus Vulgaris L.). *Plant Foods Hum. Nutr. 2007 624* **2007**, *62*, 177–183, doi:10.1007/S11130-007-0059-3.
55. Bryan, J.L.; DiNenno, P.J.; Drysdale, D.; Beyler, C.L. Behavioral Response to Fire and Smoke. In *Handbook of Fire Protection Engineering*; DiNenno, P.J., Drysdale, D., Beyler, C.L., Eds.; The Society of Fire Protection Engineers (SFPE), 2002; pp. 315–341.
56. PubChem National Library for Medicine Available online: https://pubchem.ncbi.nlm.nih.gov/ (accessed on 10 March 2022).
57. Ho, C.T.; Zheng, X.; Li, S. Tea Aroma Formation. *Food Sci. Hum. Wellness* **2015**, *4*, 9–27, doi:10.1016/j.fshw.2015.04.001.
58. Paravisini, L.; Guichard, E. Interactions between Aroma Compounds and Food Matrix. In *Flavour: From Food to Perception*; Guichard, E., Salles, C., Morzel, M., Le Bon, A.-M., Eds.; John Wiley & Sons, Ltd: Chichester, UK, 2016; pp. 208–234 ISBN 9781118929384, doi:10.1002/9781118929384.
59. Doty, R.L. Psychophysical Measurement of Odor Perception in Humans. In *The Human Sense of Smell*; Springer Berlin Heidelberg: Berlin, Heidelberg, 1991; pp. 95–134, doi:10.1007/978-3-642-76223-9_5.

60. Takeoka, G.R.; Flath, R.A.; Mon, T.R.; Teranishi, R.; Guentert, M. Volatile Constituents of Apricot (Prunus Armeniaca). *J. Agric. Food Chem.* **1990**, *38*, 471–477, doi:10.1021/jf00092a031.
61. Pal, P.; Mitra, I.; Roy, K. QSPR Modeling of Odor Threshold of Aliphatic Alcohols Using Extended Topochemical Atom (ETA) Indices. *Croat. Chem. Acta* **2014**, *87*, 29–37, doi:10.5562/cca2284.
62. Buttery, R.G.; Turnbaugh, J.G.; Ling, L.C. Contribution of Volatiles to Rice Aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006–1009, doi:10.1021/jf00083a025.
63. Buttery, R.G.; Ling, L.C. 2-Ethyl-3,5-Dimethylpyrazine and 2-Ethyl-3,6-Dimethylpyrazine: Odor Thresholds in Water Solution. *LWT - Food Sci. Technol.* **1997**, *30*, 109–110, doi:10.1006/fstl.1996.0139.
64. Guadagni, D.G.; Buttery, R.G.; Turnbaugh, J.G. Odour Thresholds and Similarity Ratings of Some Potato Chip Components. *J. Sci. Food Agric.* **1972**, *23*, 1435–1444, doi:10.1002/jsfa.2740231207.
65. Teranishi, R.; Buttery, R.G.; Gaudagni, D.G. Annals New York Acad. *Sci* **1974**, *237*, 209–216.
66. Guadagni, D.G.; Buttery, R.G.; Okano, S. Odour Thresholds of Some Organic Compounds Associated with Food Flavours. *J. Sci. Food Agric.* **1963**, *14*, 761–765, doi:10.1002/jsfa.2740141014.
67. Engel, K.H.; Flath, R.A.; Buttery, R.G.; Mon, T.R.; Teranishi, R.; Ramming, D.W. Investigation of Volatile Constituents in Nectarines. 1. Analytical and Sensory Characterization of Aroma Components in Some Nectarine Cultivars. *J. Agric. Food Chem.* **1988**, *36*, 549–553, doi:10.1021/jf00081a036.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

Appendix A

Table A1. Results of the NIR analysis for water, essential oils and ash.

Sample Number	Water [g/100 g]	Essential Oil [mL/100 g]	Ash [g/100 g]
1	7.71 ± 0.15	1.08 ± 0.09	8.41 ± 0.19
2	9.68 ± 0.36	0.79 ± 0.07	10.20 ± 0.20
3	8.26 ± 0.13	0.46 ± 0.21	8.88 ± 0.99
4	10.07 ± 0.11	0.66 ± 0.11	9.85 ± 0.11
5	9.77 ± 0.24	0.52 ± 0.5	9.46 ± 1.01
6	7.86 ± 0.03	0.47 ± 0.06	7.94 ± 0.23
7	9.24 ± 0.23	0.76 ± 0.06	8.14 ± 0.08
8	5.99 ± 0.13	1.52 ± 0.08	9.98 ± 0.08
9	6.27 ± 0.11	1.28 ± 0.20	9.64 ± 0.10
10	7.11 ± 0.26	1.16 ± 0.07	9.00 ± 0.09
11	6.84 ± 0.14	1.20 ± 0.03	8.95 ± 0.10
12	8.12 ± 0.24	1.04 ± 0.29	9.29 ± 0.88
13	7.85 ± 0.22	1.31 ± 0.06	8.53 ± 0.16
14	8.35 ± 0.13	1.25 ± 0.16	8.52 ± 0.37
15	5.52 ± 0.26	0.00 ± 0.00	9.89 ± 0.14
16	17.42 ± 0.04	0.30 ± 0.01	3.22 ± 0.00
17	6.56 ± 0.12	0.02 ± 0.03	8.54 ± 0.09
18	6.75 ± 0.13	0.39 ± 0.02	7.82 ± 0.14
19	8.25 ± 0.30	0.00 ± 0.00	7.81 ± 0.05
20	5.39 ± 0.04	0.37 ± 0.31	9.00 ± 0.86
21	5.99 ± 0.1	0.01 ± 0.02	9.85 ± 0.92
22	6.58 ± 0.06	0.09 ± 0.04	8.62 ± 0.21
23	3.92 ± 0.06	1.48 ± 0.05	8.10 ± 0.16
24	6.58 ± 0.02	0.30 ± 0.26	9.55 ± 0.81

Table A2. Results of the HPLC analysis for caffeine and catechins.

Sample Number	Caffeine [g/100 g DM]	Epigallocatechine gallate [g/100 g DM]	Total catechins [g/100 g DM]	Theobromin [g/100 g DM]
1	0.740 ± 0.003	-	0.306 ± 0.006	-
2	0.565 ± 0.004	-	0.299 ± 0.002	0.021 ± 0.001
3	0.747 ± 0.010	-	0.390 ± 0.023	-
4	0.520 ± 0.001	-	0.290 ± 0.002	0.015 ± 0.001
5	0.699 ± 0.027	-	0.208 ± 0.090	-
6	0.693 ± 0.026	-	0.206 ± 0.089	-
7	0.713 ± 0.006	-	0.345 ± 0.004	-
8	0.669 ± 0.001	-	0.054 ± 0.003	-
9	0.396 ± 0.003	-	-	-
10	0.406 ± 0.003	-	-	-
11	0.444 ± 0.001	-	-	-
12	0.392 ± 0.003	-	-	-
13	0.370 ± 0.001	-	-	-
14	0.368 ± 0.004	-	-	-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

15	0.380 ± 0.004	-	-	-
16	0.449 ± 0.001	-	-	-
17	0.462 ± 0.003	-	-	-
18	0.939 ± 0.596	-	0.221 ± 0.157	-
19	1.333 ± 0.019	-	0.699 ± 0.009	0.009 ± 0.003
20	0.381 ± 0.003	-	0.045 ± 0.007	-
21	0.761 ± 0.012	-	0.092 ± 0.008	-
22	1.000 ± 0.004	-	-	-
23	0.894 ± 0.008	-	0.479 ± 0.001	-
24	0.858 ± 0.003	-	0.091 ± 0.128	-

Table A3. Results of the photometric analysis of the total polyphenol content.

Sample Number	Total Polyphenol [g/100 g DM]
1	8.28 ± 0.33
2	9.34 ± 0.36
3	7.62 ± 0.26
4	8.31 ± 0.22
5	7.54 ± 0.67
6	3.73 ± 0.28
7	7.15 ± 0.18
8	4.76 ± 0.13
9	0.91 ± 0.12
10	1.68 ± 0.75
11	1.50 ± 0.81
12	2.35 ± 0.66
13	2.04 ± 0.82
14	2.18 ± 0.82
15	0.91 ± 0.08
16	1.08 ± 0.00
17	1.15 ± 0.00
18	7.91 ± 0.77
19	10.36 ± 0.25
20	2.73 ± 0.45
21	0.79 ± 0.11
22	-
23	5.93 ± 0.27
24	5.08 ± 0.19

Table 1A4. Results of the NMR analysis of the organic acid contents.

Sample	Chlorogenic acid [g/100 g DM]	Acetic acid [g/100 g DM]	Lactic acid [g/100 g DM]	Trigonellin [g/100 g DM]
1	2.92	0.107	0.18	2.79
2	2.58	0.088	0.16	0.98
3	2.38	0.101	0.13	2.66
4	2.81	0.097	0.15	1.15
5	3.78	0.136	0.14	2.76

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

6	1.17	0.068	0.11	1.80
7	3.32	0.124	0.13	2.66
8	1.44	0.114	0.11	2.25
9	0.20	0.145	8.12	1.26
10	0.16	0.102	0.21	1.21
11	0.08	0.266	0.92	1.28
12	0.23	0.115	0.21	0.62
13	0.18	0.093	0.20	0.68
14	0.24	0.21	1.08	0.62
15	0.08	0.137	2.76	1.44
16	0.00	0.051	0.49	1.11
17	0.06	0.29	2.59	1.01
18	9.35	0.061	0.16	4.87
19	6.58	0.078	0.22	4.25
20	2.82	0.078	0.14	2.34
21	0.31	0.122	0.49	2.01
22	0.31	0.042	0.12	3.30
23	6.20	0.14	0.14	3.31
24	3.00	0.074	0.23	2.41

Table A5. Odour-active compounds of the eight tea samples sorted by their retention indices.

Number	Compound	RI	RI (lit/std)	Odour	Identification	182	687	234	156	930	743	147	369
1	Hexanal	1079	1079	green, grassy	RI,MS,O	X	X	X	X	X	X	X	X
2	4-Heptenal	1242	1239	green	RI,MS,O	X	X		X			X	
3	Octanal	1285	1286	fruity	RI,MS,O			X					
4	n.i.	1298	-	green	/					X			
5	1-Hydroxy-2-propanone[a]	1307	1305	sweetish	RI,O								X
6	2,3 Dimethylpyrazin	1347	1347	-	RI,MS								X
7	1-Hexanol[a]	1357	1357	sweetish green	RI,O						X		
8	n.i.	1370	-	green/cucumber	/								X
9	(*E*)-2-Octenal	1430	1430	cucumber	RI,MS,O		X	X	X				
10	(*E*)-4-Nonenal[a]	1435	1435	fruity	RI,O						X		
11	n.i.	1449	-	melon	/							X	
12	1-Octen-3-ol	1453	1452	forest	RI,MS,O			X					
13	n.i.	1465	-	fruity	/			X					
14	3-Ethyl-3,5-dimethyl-pyrazine[a]	1468	1466	roasty, coffee	RI,O								X
15	Decanal	1502	1497	citrus, floral	RI,MS,O	X	X	X	X	X	X	X	X
16	2-Ethyl-3,5-dimethylpyrazine	1512	1512	-	RI,MS								X
17	(*E*,*E*)-3,5-Octadien-2-one[a]	1519	1521	green, grassy	RI,O							X	
18	Benzaldehyd	1523	1522	sweetish fruity	RI,MS,O			X					
19	n.i.	1530	-	green, grassy	/								X
20	(*E*)-2-Nonenal	1536	1537	citrus like	RI,MS,O					X			
21	n.i.	1560	-	green, grassy	/							X	
22	n.i.	1562	-	green, grassy	/	X							
23	2,6-Nonadienal	1587	1586	cucumber	RI,MS,O	X	X	X	X	X	X	X	X
24	5H-5-Methyl-6,7-dihydrocyclopentap-yrazine[a]	1629	1630	peanut	RI,O								X

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

25	(*E*,*E*)-2,4-Nonadienal[a]	1699	1698	melon,cucumber	RI,O						X			
26	α-Terpineol	1699	1699	citrus like	RI,MS,O					X			X	
27	n.i.	1700	-	citrus like	/			X	X					
28	2,4-Nonadienal	1702	1700	melon,cucumber	RI,MS,O	X								
29	2,3-Nonadienal[a]	1703	1703	melon,cucumber	RI,O	X								
30	4-Ethyl-benzaldehyde[a]	1709	1711	fruity	RI,O									X
31	n.i.	1712	-	pineapple	/						X			
32	(*E*,*Z*)-2,6-Nonadien-1-ol[a]	1769	1770	green, cucumber	RI,O				X				X	X
33	3,7,11-Trimethyl-1-dodecanol[a]	1770		green	RI,O			X						
36	3,4-Dimethylbenzaldehyde[a]	1811	1790	sweetish, green	RI,O	X	X	X			X	X		
37	2,5-Dimethylbenzaldehyde[a]	1812	1812	sweetish, green	RI,O					X				
38	Hexanoic acid[a]	1844	1844	fatty	RI,O						X			
39	Dodecanoic acid, ethyl ester[a]	1846	1846	sweetish, floral	RI,O									X
40	α-Ionone	1857	1855	sweetish, floral	RI,MS,O	X	X	X	X	X	X	X	X	
41	n.i.	1874	-	green	/					X				
42	Benzyl alcohol	1878	1877	fruity	RI,MS,O				X					
43	β-Ionone	1942	1943	honey, flower	RI,MS,O	X	X	X	X	X	X	X	X	
44	1-Dodecanol	1969	1971	cocutnut	RI,MS,O				X					
45	n.i.	2005	-	green	/		X		X	X				
46	n.i.	2007	-	green	/	X								X
47	4-Methoxybenzaldehyde	2027	2025	vanilla	RI,MS,O		X		X			X		
48	n.i.	2034	-	cocutnut	RI,MS,O									X
49	n.i.	2078	-	green, cucumber	/				X	X				
50	n.i.	2081	-	green, cucumber	/		X							
51	1-Ethylundecylbenzene[a]	2092	2094	herbal	RI,O					X				
52	n.i.	2094	-	herbal	/						X	X		
53	n.i.	2096	-	herbal	/	X	X	X						
55	n.i.	2155	-	green, cucumber	/		X	X	X					
56	1-Methyldodecylbenzene[a]	2158		mint	RI,O					X				
57	Nonanoic acid	2168	2164	sweetish green	RI,MS,O		X	X						X
58	Tetrahydro-6-pentyl-2H-pyran-2-one[a]	2201	2201	cocotnut	RI,O									X
59	Isopropyl palmitate[a]	2242	2237	sweetish	RI,O	X								
60	n.i.	2251	-	citrus like	/						X			
61	Dihydro-5-pentyl-2(3H)-furanone[a]	2264	2266	cocutnut	RI,O					X				X
62	5-Heptyldihydro-2(3H)-furanone[a]	2264		roasty, coffee	RI,O				X					
63	γ-Dodecalactone	2381	2379	fruity	RI,MS,O	X	X							
64	n.i.	2397	-	aquarium	/							X		
65	(*Z*)-Dihydro-5(2-octenyl)-2(2H)-furanone[a]	2402	2390	green, sweetish	RI,O		X							
66	n.i.	2402	-	green, sweetish	/	X			X	X	X			
67	Indole	2449	2443	Nutty	RI,MS,O									X
68	n.i.	2471	-	Stable	/				X					

 doi:10.20944/preprints202206.0321.v1

Abbreviations: RI, retention index; X, found in the sample; [a], provisionally identified; n.i.: not identified, MS: mass spectrometry, O: olfactometry.

Table A6. Results of semi-quantification and calculated OAVs for sample 182.

Compound	Concentration in µg/L	OAV
β-Ionone	3.44 ± 0.33	491
(*E*,*Z*)-2,6-Nonadienal	2.56 ± 0.36	256
α-Ionone	3.01 ± 0.59	100
Octanal	12.87 ± 0.93	18
Decanal	1.69 ± 0.15	17
4-Heptenal	7.15 ± 1.22	9
Hexanal	28.4 ± 4.19	6
(*E*)-2-Nonenal	0.22 ± 0.02	3
1-Dodecanol	0.33 ± 0.09	<1
3-Hexen-1-ol	28.91 ± 3.76	<1
α-Terpineol	0.76 ± 0.08	<1
1-Dodecanol	0.3 ± 0.03	<1
(*E*)-2-Octenal	1.78 ± 0.19	<1
Nonanoic acid	638.8 ± 36.55	<1
Benzyl alcohol	147.73 ± 26.28	<1
(*E*,*E*)-2,4-Heptadienal	101.17 ± 6.95	<1
γ-Dodecalactone	2.54 ± 0.43	<1

Table A7. Results of semi-quantification and calculated OAVs for sample 687.

Compound	Concentration in g/L	OAV
β-Ionone	0.93 ± 0.05	132
(*E*,*Z*)-2,6-Nonadienal	0.49 ± 0.06	49
α-Ionone	0.9 ± 0.04	30
Decanal	1.41 ± 0.16	14
Octanal	6.72 ± 1.05	10
2,4-Nonadienal	0.76 ± 0.13	8
Hexanal	23.46 ± 3.89	5
(*E*)-2-Nonenal	0.26 ± 0.01	3
4-Heptenal	2.21 ± 0.37	3
1-Dodecanol	0.38 ± 0.07	<1
3-Hexen-1-ol	41 ± 8.15	<1
α-Terpineol	0.82 ± 0.11	<1
1-Dodecanol	0.13 ± 0.01	<1
(*E*)-2-Octenal	0.39 ± 0.06	<1
Nonanoic acid	730.46 ± 13.3	<1
Benzyl alcohol	53.87 ± 7.15	<1
(*E*,*E*)-2,4-Heptadienal	25.07 ± 1.45	<1
γ-Dodecalactone	2 ± 0.36	<1
Benzaldehyd	2.71 ± 0.28	<1

Table A8. Results of semi-quantification and calculated OAVs for sample 234.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

Compound	Concentration in µg/L	OAV
β-Ionone	1.75 ± 0.07	249
Decanal	5.37 ± 0.91	54
α-Ionone	1.39 ± 0.04	46
(*E,Z*)-2,6-Nonadienal	0.18 ± 0.03	18
Octanal	5.75 ± 0.71	8
2,4-Nonadienal	0.28 ± 0.03	3
4-Heptenal	1.97 ± 0.34	2
(*E*)-2-Nonenal	0.19 ± 0.02	2
Hexanal	9.28 ± 1.6	2
1-Dodecanol	0.24 ± 0.03	<1
1-Octen-3-ol	0.63 ± 0.1	<1
3-Hexen-1-ol	14.35 ± 2.77	<1
α-Terpineol	1.13 ± 0.06	<1
(*E*)-2-Octenal	0.1 ± 0.02	<1
Nonanoic acid	1199.03 ± 347.21	<1
Benzyl alcohol	33.6 ± 3.78	<1
γ-Dodecalactone	0.05 ± 0	<1
Benzaldehyd	5.66 ± 0.37	<1
Indole	3.32 ± 0.13	<1

Table A9. Results of semi-quantification and calculated OAVs for sample 156.

Compound	Concentration in µg/L	OAV
β-Ionone	2.14 ± 0.15	306
Decanal	26.83 ± 3.29	268
(*E,Z*)-2,6-Nonadienal	0.78 ± 0.21	78
α-Ionone	2.13 ± 0.06	71
Octanal	15.84 ± 2.13	23
2,4-Nonadienal	1.01 ± 0.01	11
(*E*)-2-Nonenal	0.74 ± 0.04	9
Hexanal	38.98 ± 1.25	9
4-Heptenal	4.65 ± 0.72	6
1-Dodecanol	0.73 ± 0.08	<1
3-Hexen-1-ol	40.6 ± 1.07	<1
α-Terpineol	1.46 ± 0.15	<1
(*E*)-2-Octenal	0.67 ± 0.08	<1
Nonanoic acid	1508.67 ± 310.48	<1
Benzyl alcohol	130.01 ± 14.89	<1
(*E,E*)-2,4-Heptadienal	57 ± 4.83	<1
γ-Dodecalactone	0.16 ± 0.03	<1
Benzaldehyd	6.5 ± 0.76	<1
Benzaldehyde, 4-methoxy-	1.83 ± 0.07	<1

Table A10. Results of semi-quantification and calculated OAVs for sample 930.

Compound	Concentration in µg/L	OAV
β-Ionone	2.95 ± 0.23	421

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

Decanal	30.06 ± 3.49	301
α-Ionone	1.39 ± 0.07	46
(*E,Z*)-2,6-Nonadienal	0.31 ± 0.03	31
2,4-Nonadienal	1.66 ± 0.06	18
Octanal	12.37 ± 1.9	18
(*E*)-2-Nonenal	0.86 ± 0.12	11
Hexanal	36.85 ± 3.52	8
4-Heptenal	0.81 ± 0.06	1
1-Dodecanol	0.96 ± 0.09	<1
3-Hexen-1-ol	57.73 ± 10.8	<1
α-Terpineol	6.01 ± 0.84	<1
(*E*)-2-Octenal	0.37 ± 0.06	<1
Nonanoic acid	1462.18 ± 228.82	<1
Benzyl alcohol	20.44 ± 0.05	<1
(*E,E*)-2,4-Heptadienal	21.27 ± 1.36	<1
γ-Dodecalactone	0.06 ± 0.01	<1
Benzaldehyd	3.56 ± 0.31	<1
Indole	1.9 ± 0.07	<1

Table A11. Results of semi-quantification and calculated OAVs for sample 743.

Compound	Concentration in µg/L	OAV
β-Ionone	3.79 ± 0.31	541
α-Ionone	1.96 ± 0.06	65
Decanal	4.7 ± 0.54	47
(E,Z)-2,6-Nonadienal	0.39 ± 0.04	39
2,4-Nonadienal	0.65 ± 0.06	7
Octanal	5.02 ± 0.19	7
Hexanal	31.71 ± 2.48	7
4-Heptenal	2.84 ± 0.29	4
(E)-2-Nonenal	0.24 ± 0.03	3
1-Dodecanol	0.66 ± 0.05	<1
1-Octen-3-ol	0.58 ± 0.11	<1
3-Hexen-1-ol	50.56 ± 6.39	<1
α-Terpineol	0.89 ± 0.08	<1
(E)-2-Octenal	0.19 ± 0.01	<1
Nonanoic acid	949.75 ± 121.15	<1
Benzyl alcohol	35.32 ± 3.23	<1
(E,E)-2,4-Heptadienal	24.74 ± 1.93	<1
γ-Dodecalactone	0.05 ± 0.01	<1
Benzaldehyd	6.07 ± 0.93	<1
Indole	3.14 ± 0.34	<1

Table A12. Results of semi-quantification and calculated OAVs for sample 147.

Compound	Concentration in µg/L	OAV
β-Ionone	6.49 ± 0.67	927
α-Ionone	2.66 ± 0.25	89

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

Decanal	7.38 ± 0.86	74
(*E,Z*)-2,6-Nonadienal	0.2 ± 0.03	20
Octanal	11 ± 0.89	16
Hexanal	42.82 ± 2.57	10
2,4-Nonadienal	0.74 ± 0.09	8
4-Heptenal	3.31 ± 0.07	4
1-Dodecanol	0.38 ± 0.04	<1
1-Octen-3-ol	0.3 ± 0.05	<1
3-Hexen-1-ol	44.86 ± 5	<1
α-Terpineol	1.7 ± 0.24	<1
(*E*)-2-Octenal	0.19 ± 0.02	<1
Nonanoic acid	1235.09 ± 11.07	<1
Benzyl alcohol	35.18 ± 2.8	<1
(*E,E*)-2,4-Heptadienal	11.5 ± 1.07	<1
γ-Dodecalactone	0.05 ± 0	<1
Benzaldehyd	5.84 ± 0.68	<1
Indole	1.61 ± 0.13	<1
Benzaldehyde, 4-methoxy-	0.9 ± 0.15	<1

Table A13. Results of semi-quantification and calculated OAVs for sample 369.

Compound	Concentration in µg/L	OAV
β-Ionone	2.57 ± 0.12	367
α-Ionone	0.97 ± 0.03	32
Octanal	7.97 ± 0.41	11
1-Octen-3-ol	5.07 ± 0.19	5
2,4-Nonadienal	0.22 ± 0.01	2
2-ethyl-3,5-dimethylpyrazine	0.1 ± 0.01	2
(*E*)-2-Nonenal	0.1 ± 0.01	1
Hexanal	5.66 ± 0.24	1
2,3-dimethylpyrazine	2.85 ± 0.11	1
1-Dodecanol	0.43 ± 0.02	<1
3-Hexen-1-ol	29.8 ± 1.1	<1
α-Terpineol	0.2 ± 0.02	<1
1-Dodecanol	0.29 ± 0.01	<1
(*E*)-2-Octenal	0.1 ± 0.01	<1
Nonanoic acid	951.9 ± 121.64	<1
Benzyl alcohol	47.66 ± 5.34	<1
(*E,E*)-2,4-Heptadienal	0.93 ± 0.05	<1
γ-Dodecalactone	0.06 ± 0.01	<1
Benzaldehyd	2.92 ± 0.2	<1
Indole	6.58 ± 0.43	<1
2,5-Dimethyl-3-(2-methylpropyl)-pyrazine	2.6 ± 0.03	<1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

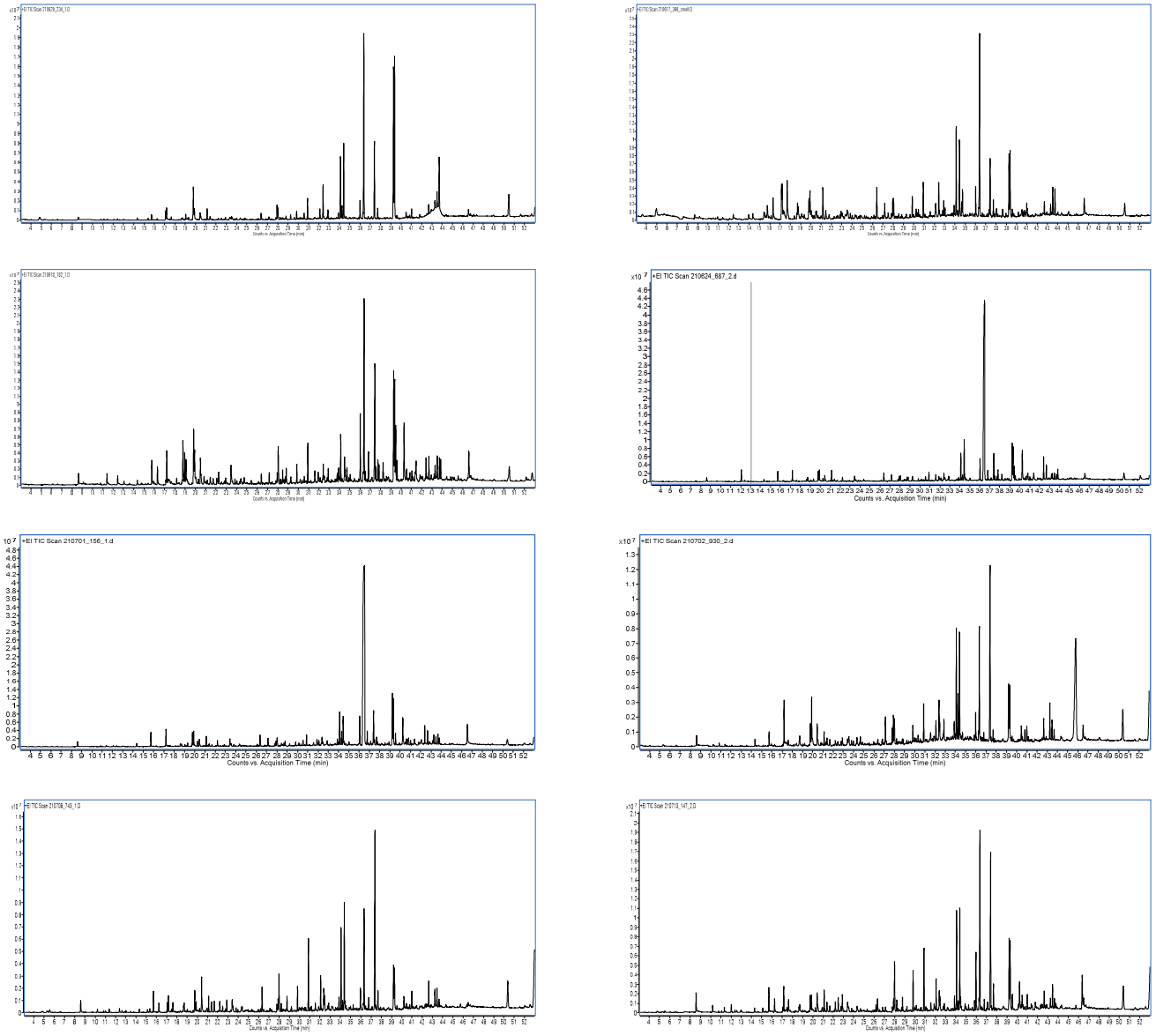
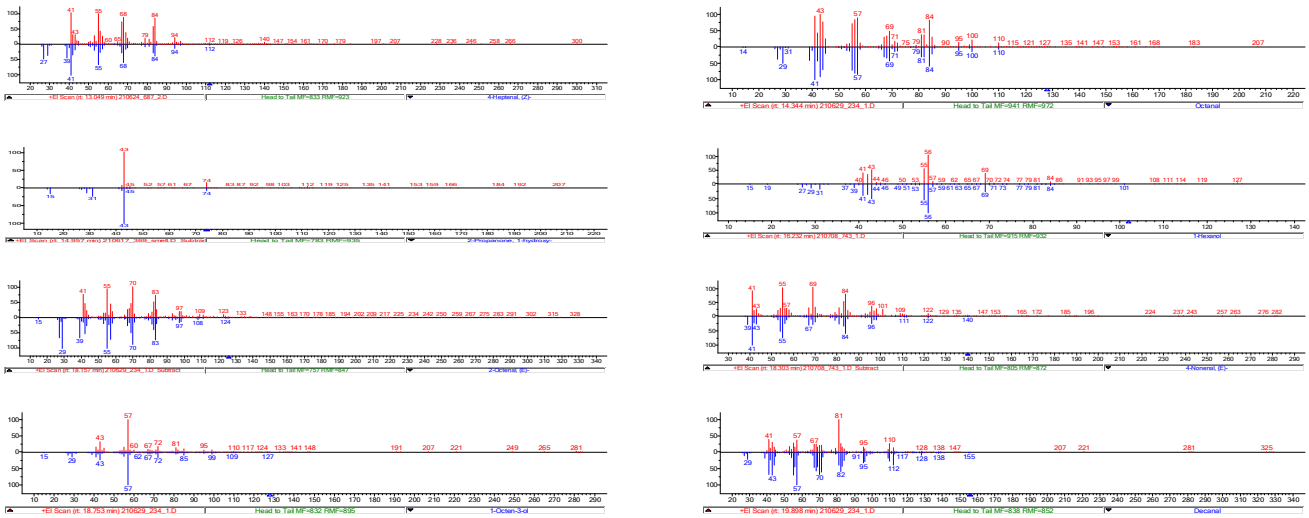
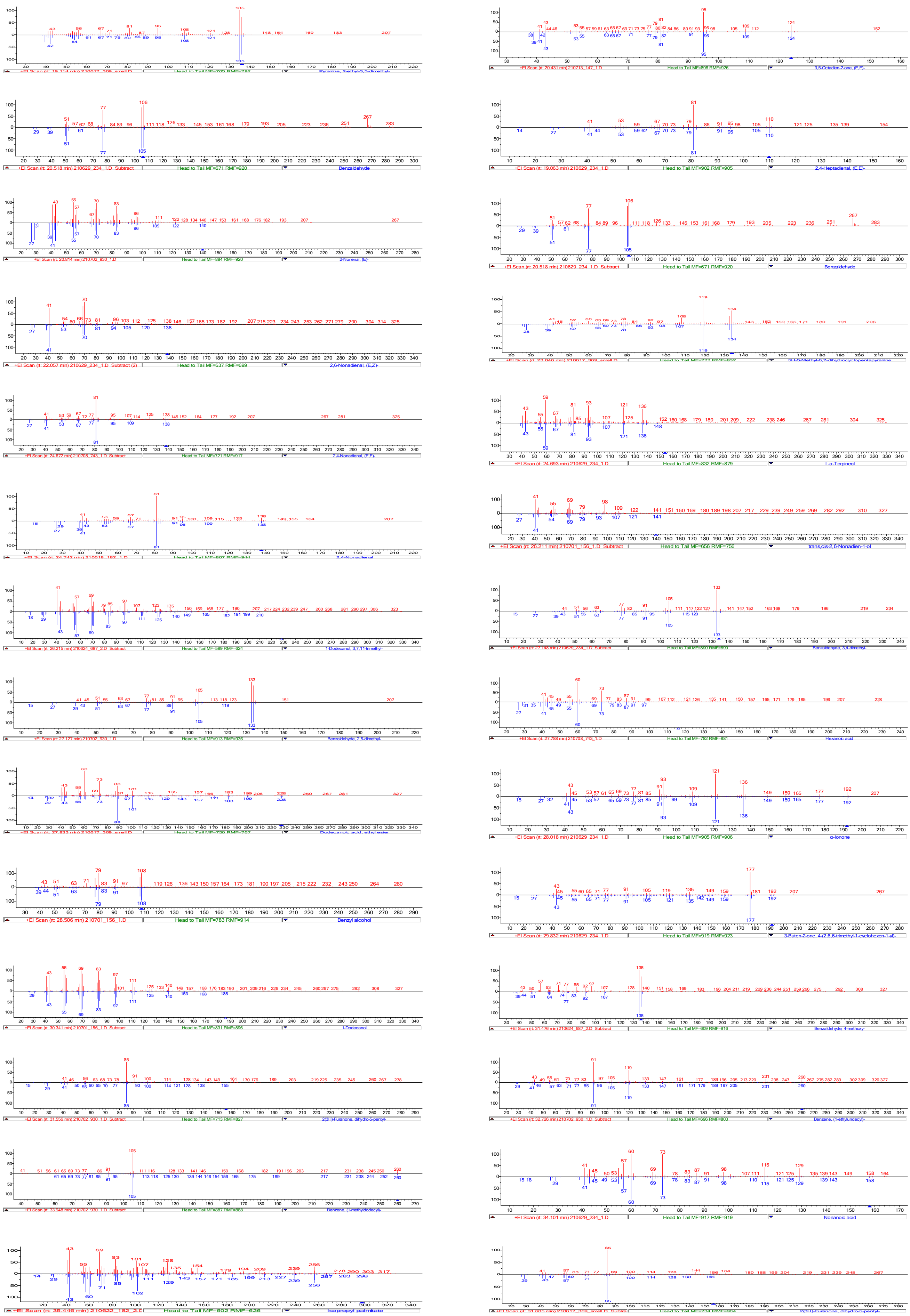


Figure A1. Total ion chromatograms of the tea samples.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 June 2022 doi:10.20944/preprints202206.0321.v1

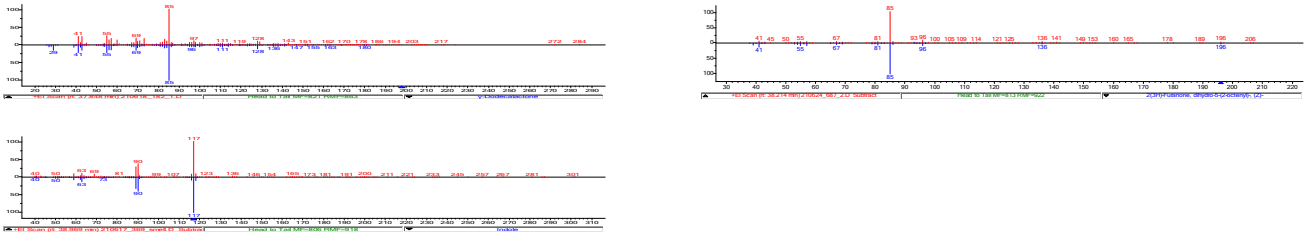


Figure A2. MS spectra of the identified substances (red beams) and the corresponding substance of the NIST Mass Spectral Library (blue beams).

Table A14. Chemical compounds and their associated odour thresholds.

Compound	Odour Threshold [ppb]	
α-Terpineol	330	Takeoka et al., 1990 [60]
1-Dodecanol	7.1	Pal et al., 2014 [61]
1-Octen-3-ol	1	Buttery et al., 1988 [62]
2-ethyl-3,5-dimethylpyrazine	0.04	Buttery and Ling, 1997 [63]
2-Nonenal, (E)-	0.08	Buttery et al., 1988 [62]
2-Octenal, (E)-	3	Guadagni et al., 1972 [64]
2,3-dimethylpyrazine	2.5	
2,4-Heptadienal, (E,E)-	778	
2,4-Nonadienal	0.09	Teranishi et al., 1974 [65]
2,5-Dimethyl-3-(2-methylpropyl)-py-	800	
2,6-Nonadienal, (E,Z)-	0.01	Teranishi et al., 1974 [65]
3-Hexen-1-ol	70	Takeoka et al., 1990 [60]
4-Heptenal	0.8	
Benzaldehyde	350	Buttery et al., 1988 [62]
Benzaldehyde, 4-methoxy-	47	
Benzyl alcohol	10000	Buttery et al., 1988 [62]
Decanal	0.1	Guadagni et al., 1963 [66]
Hexanal	04.05.05	Buttery et al., 1988 [62]
Indole	140	Buttery et al., 1988 [62]
Nonanoic acid	3000	
Octanal	0.7	Buttery et al., 1988 [62]
α-Ionone	0.03	
β-Ionone	0.007	
γ-Dodecalactone	7	Engel et al., 1988 [67]