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Residues of herbal hemp leaf teas – How much of the cannabinoids remain?

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ABSTRACT

Herbal teas of fiber-type hemp varieties (*Cannabis sativa* L.) rich in cannabidiolic acid (CBDA) and cannabidiol (CBD) and poor in Δ9-tetrahydrocannabinol acid (THCA) and Δ9-tetrahydrocannabinol (THC) are very popular today. The conditions for preparing herbal infusions are not well standardized and analysis of the lipophilic cannabinoids in infusions is difficult. Therefore, we analyzed the hemp leaf residues after tea preparation by using a response surface modelling approach to estimate the effects of variations in temperature, water volume and extraction time on the residual content of five cannabinoids (CBDA, CBD, THCA, THC, cannabinol (CBN)) in the hemp leaves after extraction. The quantity of remaining cannabinoids was mainly influenced by temperature in the first order. Volume and extraction time were only exerting minor influences under usual tea preparation processes. At elevated water temperatures CBD and THC values were even higher than in the original drug material presumably due to decarboxylation of CBDA and THCA. Rising temperatures increased extraction of CBDA and CBD, as opposed to THCA and THC. The degradation of THC to CBN was not significant at the conditions of infusion preparation.

Analyzing herbal residues after tea brewing is just an approximation to the true values of valuable or unfavorable compounds in tea, overestimating the true values. However, that approach offers a good control for further improvements of herbal tea analysis and gives reliable indications for risk assessment.

1. Introduction

Hemp (*Cannabis sativa* L., Cannabaceae) is a plant well known for its content of bioactive cannabinoids, a group of secondary metabolites unique for the genus *Cannabis*. These compounds are produced by specialized glands in the leaf epidermis, occurring more densely in the inflorescence and less in the leaves (Petri, Oroszlán, & Fridvalszky, 1988). Four cannabinoids are present at high quantities, cannabidiolic acid (CBDA) and Δ-9-tetrahydrocannabinolic acid (THCA), which decarboxylate at elevated temperatures to form cannabidiol (CBD) and Δ-9-tetrahydrocannabinol (THC) respectively. Cannabinol (CBN) is a degradation product of THC. Due to their interactions with the human endocannabinoid system, these compounds are of special interest for their therapeutic potential in neurological disorders (Maroon & Bost, 2018).

THC is a psychoactive compound with effective doses between 10 and 20 mg THC, while the other three cannabinoids are non-psychoactive compounds or exert only a low psychoactivity (Lachenmeier & Rehm, 2015). Therefore, it is the presence of THC in hemp food products that is of special interest for food control purposes. However, it is not only the original THC content of the starting material that is being considered, but also the formation of additional THC due to conversions in the course of industrial processing or preparation by the consumer, such as that of THCA into THC. It is known that hot temperatures increase THCA decarboxylation to form THC, whereas this conversion does not occur in the human body (Jung et al., 2009; Raikos et al., 2014). Formation of THC could also possibly occur by conversion of CBD under acidic conditions. However CBD-to-THC conversion was never actually observed, neither under specific light or temperature regimes nor in a simulated gastric juice. Consequently, THC formation by this route is likely not relevant for food products (Lachenmeier, Habel, et al., 2019).

In Austria, cultivars of *Cannabis sativa* with a THC-content below 0.3% are not regulated by the Narcotic Substances Act

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(Suchtmittelgesetz – SMG, in the version of May 14, 2020). However, cultivation in Europe is only subsidized when using cultivars from the Common EU Catalogue of Varieties of Agricultural Plant Species (with a THC content below 0.2%, 'fiber hemp').

In a fiber hemp, CBDA is the major cannabinoid. However, even these varieties are never completely free of THC (Broséus, Anglada, & Esseiva, 2010). That is one of the reasons why EFSA assessed the human exposure to THC and established an acute reference dose (ARfD) of 1 μg Δ9-THC/kg body weight for the use of fiber type hemp in food (as e.g. herbal teas) (EFSA, 2015). Hemp leaves have become very popular as ingredients for herbal teas (infusions), either as hemp-only tea or as part of tea mixtures. These herbal teas are usually prepared as infusions by pouring boiling water on the plant material and allowing to steep for a defined period of time (usually 5–15 min) (European Medicines Agency).

The preparation of herbal infusions is not well standardized, specifically, water temperature will shift while cooling down during the extraction process. It can be expected, that because of their lipophilic nature, even under optimum extraction conditions, only a fraction of the cannabinoids are transferred into a herbal infusion. However, there is no validated, generally recognized method for analyzing cannabinoids in infusions, partially due to issues in recovering lipophilic compounds from water (Federal Institute for Risk Assessment BfR, 2018). In order to address the former challenge a response-surface modelling approach was used to cover a range of water temperatures, water volumes and steeping times for the preparation of an infusion. To address the latter, rather than attempting to determine the cannabinoid contents in the tea liquid, the cannabinoid contents of the starting plant material before infusion and the residue after infusion were compared and loss or gain of the major cannabinoids for this aqueous extraction was calculated.

2. Material and methods

2.1. Plant material

The upper leaves of *Cannabis sativa* L. var. 'Fiona' were harvested before flowering and dried at room temperature. Each extraction experiment was performed with an analysis sample of approximately 1.5 g that represented a portion of a laboratory sample of approximately 800 g. These portions were arrived at by dividing the total laboratory sample with a riffle divider in nine serial steps, at each step into halves. In order to establish that the resulting portions were actually true aliquots of the original laboratory sample, 14 randomly chosen presumptive aliquots were tested for their cannabinoid contents. For CBD and CBDA the relative standard deviations were 3.8% and 2.7%, respectively (Table 1). For THC and THCA the relative standard deviations were 3.6% and 3.0% respectively. From this we concluded that our laboratory analysis samples were in fact true aliquots, that the measured cannabinoid values were in fact reliable base values for the following recovery experiments and that the observed RSD% values for repeatability likely represented but measurement uncertainty.

2.2. Preparation of the herbal infusions

Infusions of *C. sativa* samples were prepared under different conditions of temperature (43 °C, 55 °C, 70 °C, 85 °C and 97 °C), using different volumes of water (59 ml, 100 ml, 150 ml, 200 ml and 241 ml) and steeping times (5.9 min, 10 min, 15 min, 20 min and 24 min). Aliquots of *C. sativa* (1.5 g) were added to the different volumes of water at different temperatures and allowed to steep for selected times. Subsequently, the residue of the plant material was separated from the water by filtration (Rotilabo®-folded filter, type 113p with ø185mm) and dried in a drying chamber (Memmert, Schwabach, Germany) at 45 °C.

2.3. Analysis of the plant residues

The dried plant material from each of the preparations was suspended in 25 ml methanol, treated in an ultra-sonic bath for 10 min and then left for further extraction in a tumbler overnight for approximately 14 h. The following day, each extract was filtered using a syringe-filter (ø 0.2 μm; VWR). The samples were then analyzed by high performance liquid chromatography within the same day.

High performance liquid chromatography was carried out on a Nexera XR LC-20AD liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degassing unit, a SIL-20A autosampler, a CTO-20AC column oven at a constant temperature of 30 °C and a SPD-M20A diode array detector equipped with a D2/W lamp. The mobile phases consisted of pure acetonitrile (eluent A) and 0.85% phosphoric acid in water (eluent B).

To separate the various cannabinoids, a gradient program was

Table 1
Mean cannabinoids content in Cannabis sativa leaves before and after their infusion as well as ANOVA results with regression coefficients, significance levels and multiple R^2 values of five cannabinoids. Blocks and interactions were not significant and are therefore not presented (FO … first-order, PQ … pure quadratic, significance levels: *** … <0.001, ** … <0.01, * … <0.05, n.s. … not significant).

	CBDA	CBD	*CBDA + CBD*	THCA	THC	CBN	*THCA + THC + CBN*
Cannabis leaves before infusion (mean ± std.dev. in μg/g)	4,073 ± 109	802 ± 31	*4,875 ± 124*	111 ± 4.0	76 ± 2.2	52 ± 6.4	*238 ± 13*
Cannabis leaves after infusion with 150 ml water at 70 °C for 15 min mean ± std.dev. in μg/g)	1,004 ± 21	1,087 ± 24	*2,091 ± 39*	46 ± 5.9	78 ± 1.7	66 ± 1.7	*190 ± 7.9*
(Intercept)	−977.16 ***	1,073.16 ***	*2,050.32 ****	44.387 ***	77.82 **	65.12 ***	*187.32 ****
first order							
temperature	−69.92 ***	77.37 ***	*7.44*	2.634 ***	6.17 ***	0.51	*9.31 ****
volume	−8.31 *	−1.90	*−10.21*	0.084	−0.22	−0.14	*−0.27*
time	−31.40 *	54.04 ***	*22.63 **	0.647	2.57 ***	1.41	*4.63*
second order							
temperature^2	5.98 ***	4.17 **	*10.15 ****	0.038	0.22 *	−0.53 **	*−0.28*
volume^2	0.27	−0.09	*0.18*	−0.056	−0.02	−0.12 *	*−0.18*
time^2	−4.05	−30.86 **	*−34.91 **	−1.990	−2.39 *	−3.10 *	*−7.47 **
multiple R^2	0.95	0.98	*0.86*	0.79	0.97	0.71	*0.90*
FO	***	***	*	***	***	n.s.	***
PQ	*	**	***	n.s.	n.s.	n.s.	*
lack of fit	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

performed on a 4.6 × 150 mm C-18 column (Waters: Atlantis T3 3 μm) starting with a mixture of 53% eluent A and 47% eluent B ramping up evenly to 20% A and 80% B within 15 min to hold there for another 11 min. Subsequently the gradient was further ramped up to 100% B within 2 min to hold there for 5 min. Finally, the program returned to the starting conditions within 2 min and held there for 5 min.

The cannabinoids were identified using purified, certified reference compounds (Lipomed). Although Δ8-THC was only a minor compound and not evaluated later on, the reference compound of Δ8-THC was used to demonstrate the separation from Δ9-THC. Initially, standard solutions of CBD, CBDA, Δ9-THC, Δ9-THCA, Δ8-THC and CBN were used to determine the relative response factors of the cannabinoids in relation to Δ9-THC, in order to simply rely on just Δ9-THC standard solutions for quantification of later runs. All the recorded quantitative data were adjusted using these factors. The response factors were 1.12 (CBD), 1.27 (CBDA), 1.13 (Δ9-THCA), 0.99 (Δ8-THC) and 1.82 (CBN). The limit of detection (LOD) of THC was 0.81 μg/g and the limit of quantitation (LOQ) 2.68 μg/g. The coefficients of variation of intra- and inter-day validation (n = 8) were between 3.9% and 13%.

2.4. Statistical design and evaluation

The central composite design with two blocks as well as model fit (level of significance = 0.05) were performed in R with the rsm package (Lenth, 2009). The results were visualized with contour plots using the same package.

3. Results

To quantify CBDA, CBD, THCA, THC and CBN in hemp leaves residues after their infusion, aliquots of homogeneous plant material were submitted to infusions with varying water temperatures, water volumes and steeping times. The cannabinoids content in the starting homogeneous hemp leaves was 4,073 ± 109 μg/g (CBDA), 802 ± 31 μg/g (CBD), 111 ± 4.0 μg/g (THCA), 76 ± 2.2 μg/g (THC) and 52 ± 6.4 μg/g (CBN) (Table 1).

The practical experiment was performed over two days. Individual results of the two days did not differ from each other. Furthermore, no interactions were observed between temperature, volume and time. Since volume always had the lowest effect (Table 1), the values described in the text below relate to a fixed volume of 150 ml and are

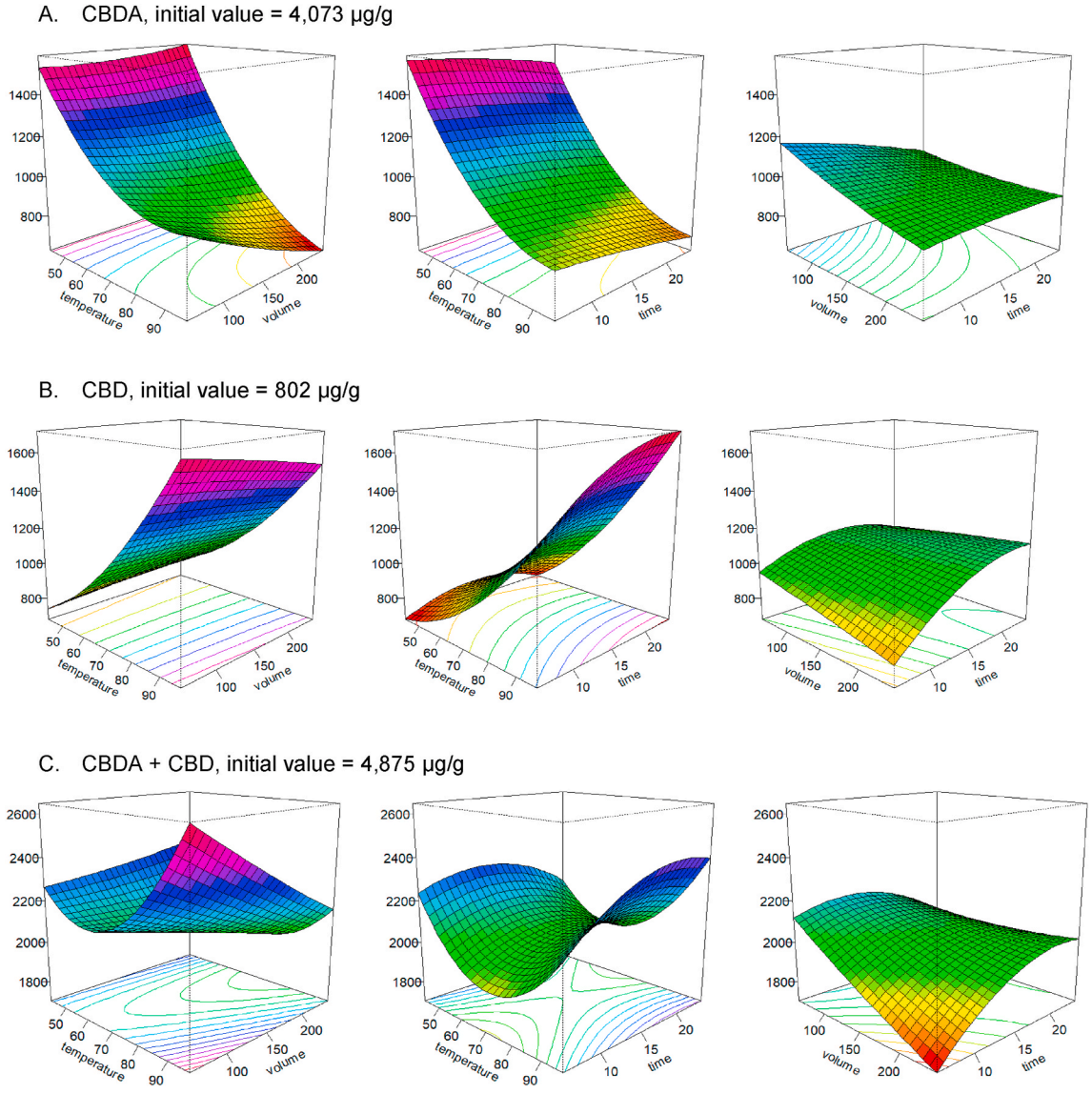

Fig. 1. Contour plots for CBDA **(A)**, CBD **(B)** and the sum of both **(C)** (values in μg/g) of *C. sativa* leaf residuals after infusion (temperature [°C], volume [ml], time [min.]).

accompanied by abbreviated experimental conditions in brackets (temperature [°C]/time [min.]).

The content of CBDA in the plant material before infusion was 4,073 μg/g (Table 1). In tea preparation, water temperature (significant effects of the first-order and second-order polynomials) and to a lesser extent water volume and steeping time (first-order effects only) influenced CBDA content in the residues after tea brewing. The remaining CBDA decreased with temperature from 1,524 μg/g at 43 °C/6 min to 959 μg/g in the center of experimental conditions (@70 °C/15 min) to 617 μg/g at 97 °C/24 min (Fig. 1).

CBD content in the leaves before tea preparation was 802 μg/g. CBD in the leaf residues after tea preparation was significantly influenced by

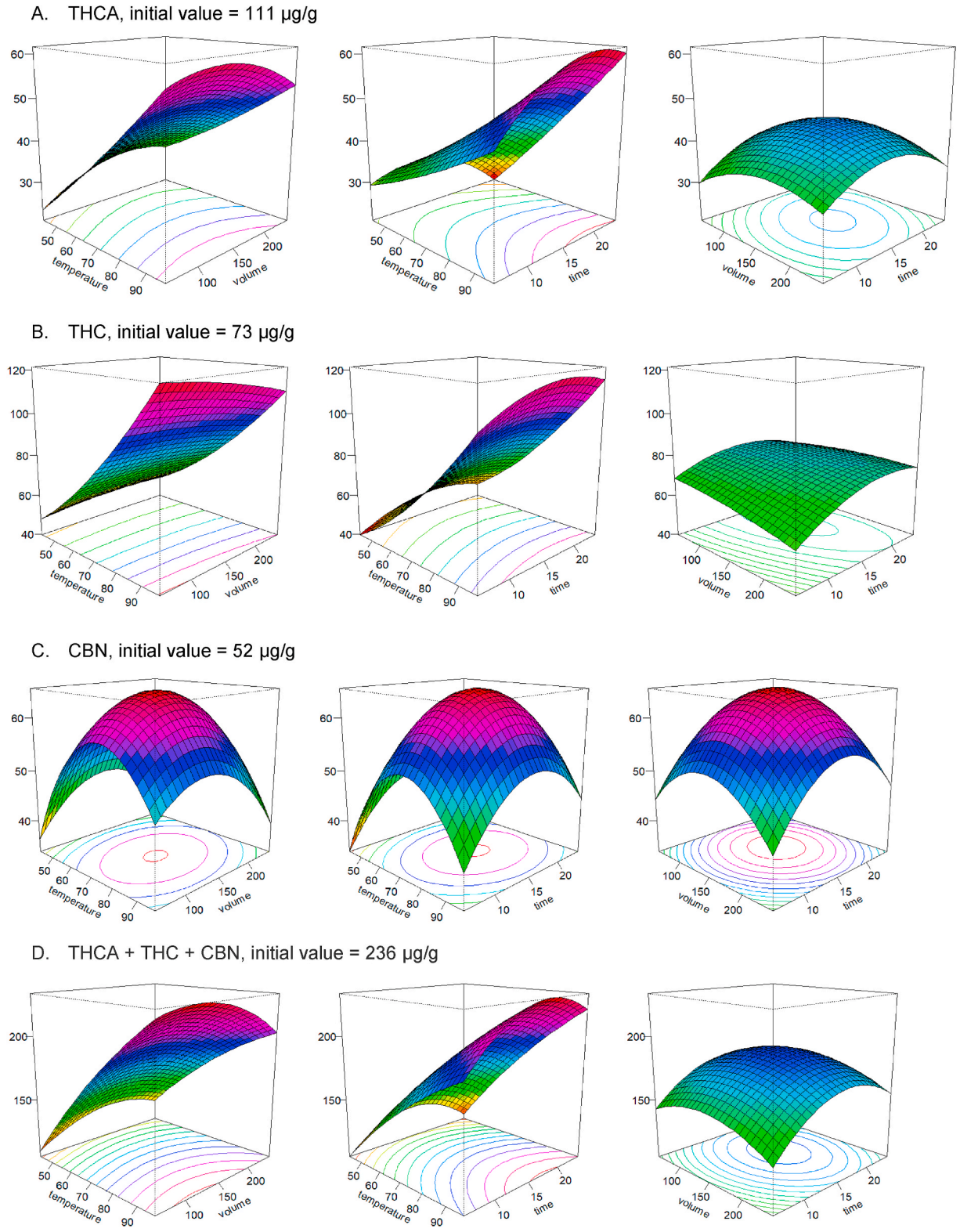

Fig. 2. Contour plots for THCA **(A)**, THC **(B)**, CBN **(C)** and the sum of all three **(D)** (values in μg/g) of *C. sativa* leaf residuals after infusion (temperature [°C], volume [ml], time [min.]).

water temperature and steeping time with higher effects in the first-order than in the second-order polynomial. CBD in the remaining leaves increased with temperature from 678 μg/g (@ 43 °C/6 min) to 1,093 μg/(@ 70 °C/15 min) to 1,710 μg/g (@ 97 °C/24 min) (Fig. 1).

While the observed contours for CBDA and CBD are almost planar (corresponding to much higher first order than second order effects), the sum of the two compounds (CBDA + CBD) shows pronounced second order effects, presumably due to the heat induced conversion of CBDA to CBD (Fig. 1, Table 1). From a value of 4,875 μg/g before tea preparation, the content of CBDA + CBD after tea preparation varied with 2,236 μg/g at 43 °C/6min. to 2,054 μg/g at 70 °C/15 min and 2,399 μg at 97 °C/24 min. At high temperatures and low volumes the amount in the residues increased to 2,645 μg/g (@ 97 °C/60 ml/15 min) (Fig. 1).

The amount of THCA in the initial plant material was 111 μg/g. The residue levels after infusion were significantly influenced only by water temperature (first-order polynomial only) (Table 1). After tea preparation, the content in the residues fell to 29 μg/g (@ 43 °C/6 min), 45 μg/g (@ 70 °C/15 min) and 60 μg/g (@ 97 °C/24 min) (Fig. 2). THC was at 76 μg/g in the starting material and was highly influenced by temperature and time in the first-order polynomial and weakly so in the second orders of temperature and time (Table 1). After tea preparation the content in the residues increased from 39 μg/g (43 °C/6 min) to 79 μg/g (70 °C/5min.) and to 116 μg/g (97 °C/24 min) (Fig. 2). CBN, rather an "artefact" as a non-enzymatic degradant of THC, was present in the starting material at 52 μg/g. Tea brewing resulted in significant effects in all second order parameters (Table 1). CBN was characterised by a clear maximum at 65 μg/g around 70 °C/150 ml/15 min in the plant material after tea preparation (Fig. 2).

With increasing temperature THCA is gradually and continuously converted to THC. The accumulated total content of THCA, THC and CBN, their values being interdependent, was 238 μg/g in the starting material, the remaining content in the residues was highly influenced by first-order temperature and only moderately by time in the second-order polynomial (Table 1). The shape of the contour plot (Fig. 2) for the aggregate totals of the three compound starts from 102 μg/g (@ 43 °C/6 min) and goes over 190 μg/g (@ 70 °C/15 min) to 220 μg/g (@ 97 °C/24 min). However, at 97 °C and only 6 min steeping time the residues' contents are only 190 μg/g increasing with time to a maximum of 232 μg/g (@ 80 °C/18 min).

4. Discussion

Food products prepared from fiber-type hemp cultivars, poor in THC, but rich in CBD, have become very popular. Besides hemp oil, beverages like hemp beer and CBD rich food supplements, infusions from inflorescences and/or leaves that are widely used either as single ingredient or in herbal tea mixtures. Lipophilic cannabinoids are only marginally soluble in water, and to some extent, the cannabinoids in a herbal infusion may become adsorbed onto small particles suspended in the tea (Zoller, Rhyn, & Zimmerli, 2000). Thus, due to their lipophilicity and potential adsorption issues, analysis of cannabinoids in an infusion is difficult and not reproducible (Lachenmeier, Bock, et al., 2019). Therefore, this paper focuses on the quantitation of cannabinoids in the residue of the hemp leaves after preparing the infusion, using HPLC/UV.

In the process of preparing an infusion of plant material, the solvent will extract the soluble constituents thus reducing the remaining content in the plant residue, but no extraction occurs for insoluble compounds. In our case, the contents of CBD and THC in hemp leaves residues after infusion were even increased, when compared to the starting plant material, presumably due to decarboxylation of their corresponding acid-forms at higher temperatures. Increase or decrease of a particular cannabinoid's content in the hemp leaf residues appear primarily related to the lipophilicity of the compound (THC > THCA > CBD > CBDA, based on partition coefficients (log P) of 5.95 (THC), 6.25 (THCA), 6.33 (CBD) and 6.63 (CBDA) (Anderson, Low, Banister, McGregor, & Arnold, 2019)) and the extent of decarboxylation.

Some peculiar differences were observed for different extraction temperatures regarding the cannabinoids content before and after tea preparation. The following observations are for 15 min steeping time: CBDA decreased in the plant material when extracted at 43 °C from 4,073 μ/g to 1,527 μg/g (−62%) and at 97 °C to 617 μg/g (−85%), while CBD in plant material decreased at 43 °C only from 802 μg/g to 678 μg/g (−15%), but increased at 97 °C by more than twofold to 1,710 μg/g. The absolute difference of CBD between 43 °C and 97 °C with +908 μg/g corresponds almost to the loss of CBDA of −910 μg/g (≅ −800 μg/g CBD) indicating that the differences found in the plant material between the two temperature extremes are probably only due to decarboxylation.

For the THCA/THC-pair, the situation was not so straight-forward. THCA decreased from a starting point of 111 μg/g to 29 μg/g (−75%) at 43 °C and to 60 μg/g (−54%) at 97 °C, so a difference of 31 μg/g between low and high temperature. THC decreased from 76 μg/g to 39 μg/g (−49%) at 43 °C and increased at 97 °C to 116 μg/g (+53%) with an absolute gain of +40 μg/g. The absolute increase of THC can only be explained by decarboxylation of THCA at higher temperatures if THC is also formed from THCA in water together with THC precipitation from water. Analysis of the water after adding pure THCA to water and 15 min in boiling water showed a decarboxylation of 6.6% (Hazekamp, Bastola, Rashidi, Bender, &). Taschwer and Schmid (2015) found in plant material dried at 50 °C no conversion of THCA to THC, whereas complete decarboxylation was recorded at 100 °C and 150 °C, but only after 2 h and 1 h, respectively. Therefore decarboxylation cannot be expected to play an important role when preparing a herbal infusion by pouring boiling water over the plant material with a steeping time of 10 min (mean water temperature 70 °C), but has to be considered for decoctions (preparation in constantly boiling water for longer than 10 min).

THC may also degrade to CBN under certain conditions. However, degradation of THC to CBN is insignificant at boiling temperature of water and 15 min extraction time (Hazekamp et al., 2007), as also confirmed by our study.

The solubility of THC in water at 23 °C is 2.8 mg/l (Garrett & Hunt, 1974). The solubility of the other cannabinoids in water should theoretically increase in the order THC < THCA < CBD < CBDA (see log P values above) but are not yet experimentally proven. Solubility of cannabinoids at higher water temperatures is also unknown, but likely increasing as e.g. observed for solubility of saturated fatty acid in water (Khuwijitjaru, Adachi, & Matsuno, 2002). However, a spiking experiment with pure THCA and THC to boiling water (Hazekamp et al., 2007) showed only 63% recovery or THCA (plus an additional 6.6% THC originated by decarboxylation) and only 17% for THC (while observing THC precipitation on the glass surface). Calculating for better comparability the differences between hemp leaves before and after tea preparation at 43 °C for 15 min in a volume of 150 ml (= 'losses' to water/suspended particles/glass surface) resulted in 26.46 mg/l CBDA, 1.24 mg/l CBD, 0.82 mg/l THCA and 0.37 mg/l THC. All cannabinoids are structurally very similar and thus the individual solubility of a particular cannabinoid (in water) is possibly also dependent on the concentration of other cannabinoids in the water. Zoller et al. (2000) detected 1 mg/l THC analyzing the infusions from herbal hemp samples containing 1,250 μg/g THC (a content 16-fold higher than in our sample, prepared with 3g hemp in 200 ml water at 90 °C for 30 min). Theoretically adjusting their THC content in leaves to ours would correspond to 0.063 mg/l THC in the tea. The tea prepared from a drug-type *C. sativa* with a THC content of 6 mg/g contained 10 mg THC per liter tea (Hazekamp et al., 2007). Again adjusting their THC leaf content to our THC leaf content would correspond to 0.132 mg/l THC in the tea. In another study of a fiber-type hemp, 0.040 mg/l THC were detected in an infusion from hemp leaves containing 80 μg/g THC, so the same amount of THC as in our leaves (CVUA, 2008). The same level of THC in the tea (0.040 mg/l) were found from a fiber-type hemp tea with 15 μg/g THC in raw material (0.203 mg/l after adjustment to our THC content) (Lachenmeier, Kroener, Musshoff, & Madea, 2004). That comparisons demonstrate the difficulty of analysing lipophilic compounds in herbal

teas and the overestimation by concluding from herbal residuals to compounds indeed present in the tea.

In view of these results and relatively little conversion of THCA to THC even at higher water temperatures, the assumption of complete transfer of THC from the starting material into the tea - as practiced by the German BfR to overcome analytical uncertainties (BfR, 2018b) – must lead to a substantial overestimation of the true THC content of infusions from hemp leaves. Even our approach, - analyzing the residual THC content in the hemp leaves after extraction and assuming the difference to have migrated into the tea, is likely still an overestimation of the true THC concentration in the infusion but at least allows a more realistic estimation of the concentration that may at worst be present in the tea. Certainly the approach is preferable to the proposed and entirely unsubstantiated assumption of total transfer.

The overall difference of cannabinoids that can be observed between tea leaves before and after tea preparation may be the combined effect of cannabinoids dissolved in water, cannabinoids adsorbed onto particles suspended in solution, cannabinoids undissolved or precipitated, degradation of cannabinoids and cannabinoids bound to the pot/cup surfaces. Garrett and Hunt (1974) determined the binding of THC on a glass surface of a 50 ml flask to 20% and 40% at 0.1 and 0.05 μg/ml, respectively, figures indicating a binding saturation at rather low cannabinoid levels. Compared to the high levels of cannabinoids in our experiment, surface binding was not a significant factor. However, this part should not be neglected in further studies of transfer rates from plant material into a specific matrix. Thus, the concentration of a cannabinoid that can be determined in solution will be the result of a dynamic equilibrium between dissolution and phase joining (e.g. precipitation of solids). Cannabinoids may be adsorbed back out of solution onto plant material or the inner surfaces of cups or pots. Considering all of these factors that potentially influence analyses, estimation of lipophilic amounts in tea based on differences in plant residues before and after tea brewing are at best approximations. Nevertheless, the values received may serve as useful reference for further improvements in herbal tea analyses and a realistic basis for risk assessment in food control.

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CRediT authorship contribution statement

Filip Knezevic: Investigation, Writing – original draft. **Andreas Nikolai:** Methodology, Investigation, Writing – review & editing, Project administration. **Rudolf Marchart:** Conceptualization, Resources, Writing – review & editing, Supervision. **Silvio Sosa:** Methodology, Writing – review & editing. **Aurelia Tubaro:** Writing – review & editing, Supervision, Funding acquisition. **Johannes Novak:** Conceptualization, Resources, Writing – original draft.

Declaration of competing interest

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

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