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Enzymatic mitigation of 5-*O*-chlorogenic acid for an improved digestibility of coffee

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ABSTRACT

A *p*-coumaroyl esterase from *Rhizoctonia solani* was used to decrease 5-*O*-chlorogenic acid (5-CQA) content in coffee powder. HPLC-UV showed a decline of up to 98% of 5-CQA after the enzyme treatment. Effects on aroma were determined by means of aroma extract dilution analysis. Flavour dilution factors of treated and control extract differed in four volatile compounds only. Effect on aroma and taste was evaluated by sensory tests. No significant differences were perceived, and no off-flavour nor off-taste was noted. As chlorogenic acids are suspected to cause stomach irritating effects in sensitive people, the enzyme treatment offers a technically feasible approach to improve the quality of coffee beverages by reducing 5-CQA concentration without significantly affecting the aroma and taste profile.

1. Introduction

Coffee is one of the most popular beverages and consumed in large amounts all over the world. However, sensitive people complain about heartburn and stomach irritation even after drinking a small cup of coffee (Boekema, Samsom, van Berge Henegouwen, & Smout, 1999; Cohen, 1980; Pehl, Pfeiffer, Wendl, & Kaess, 1997). The cause and mechanism of these symptoms are still unclear. Various substances are under suspicion. Among them are caffeine, pyrogallol, catechol, $^{\beta}$*N*-alkanoylhydroxytryptamide and *N*-methylpyridinium, but also chlorogenic acids. The latter refer to a related family of esters of hydroxycinnamic acids (caffeic acid, ferulic acid and *p*-coumaric acid) with quinic acid and plays an important role in gastric acid secretion. *Inter alia*, a high concentration of *N*-methylpyridinium and low concentrations of $^{\beta}$*N*-alkanoylhydroxytryptamide and chlorogenic acids led to lower gastric acid secretion after the ingestion of coffee (Rubach et al., 2012, 2014). Moreover, some people complained about headache and urinary tract infection after consumption of chlorogenic acids rich green coffee extracts (Blum, Lemaire, & Lafay, 2007).

Coffee is consumed due to its stimulating effect, pleasant flavour and attractive taste. More than 900 compounds contribute to the complex volatile composition (Buffo, & Cardelli-Freire, 2004). Among them are approximately 25 character impact components (Blank, Sen, & Grosch, 1992; Semmelroch, Laskawy, Blank, & Grosch, 1995). Besides the volatile aroma, bitterness and acidity are important factors of coffee quality (Drewnowski, 2001; Masi, Dinnella, Monteleone, & Prescott, 2015). Chlorogenic acids not only play a role as antioxidants of coffee, but also contribute to the aroma and taste profile (Moon, & Shibamoto, 2010). A low cup quality was related to a high 5-*O*-caffeoylquinic acid (5-CQA) concentration (Farah, Monteiro, Calado, Franca, & Trugo, 2006).

There is a continuing high interest in producing a “healthy and mild” coffee beverage (Clarke, 1987). Dewaxing, the Lendrich (low-temperature steaming) and the Darboven process (high-pressure steam and vacuum procedure) are supposed to produce a better digestible coffee. Since beans are treated before roasting, it cannot be claimed with certainty whether chlorogenic acid itself or its roasting products are responsible for the beneficial effects (Ehrlich, Lücker, & Schaefer, 1999). A reliable procedure to reduce chlorogenic acids concentrations is prolonged roasting at high temperatures (Moon, Yoo, & Shibamoto, 2009; Trugo, & Macrae, 1984). However, roasting neither selectively removes chlorogenic acids, nor can roasting conditions be decoupled from the formation of desired volatiles and undesired risk compounds, such as acrylamide and furans.

Enzymes are efficient tools to selectively convert a certain target compound. Since enzymes have always been a part of food processing, the benefits of their application in food industry are well-known (Dewdney, 1973). For that reason, enzymatic treatment of coffee presents a feasible approach to degrade chlorogenic acids without changing other coffee constituents. Ferulic acid esterases (FAE) are widespread enzymes which release phenolic acids *inter alia* by hydrolysis reactions in plant cell walls. This class of enzymes is already applied in the food, bioethanol and pharmaceutical industry. A great number of FAEs are of fungal origin, but also other sources are known (Ramos-de-

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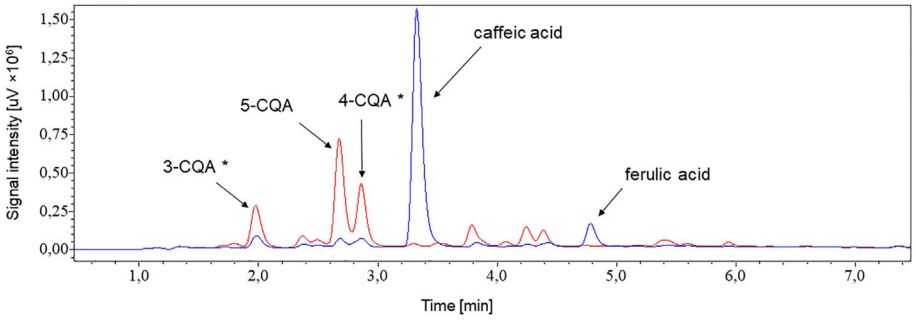

Fig. 1. HPLC-UV chromatogram (323 nm). The 5-CQA concentration decreased and simultaneous the caffeic acid concentration increased in enzymatically treated coffee powder (blue) compared to an untreated coffee powder (red). With an asterisk marked substances 3-caffeoylquinic acid (3-CGA) and 4-caffeoylquinic acid (4-CGA) were assigned according to Moon et al. (2009). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Aroma active compounds with FD-factors ≥ 100 of the enzymatically treated coffee ("treated") and the untreated coffee ("control").

No.	Retention index[a]				Odour[d]	FD-factor[e]		Substance	Identification criteria[f]
	DB-WAX		HP-5			treated	control		
	Exp[b]	Lit.[c]	Exp[b]	Lit.[c]					
1	1357	1357	965	936	Musty	100	10	Ethyl pyrazine	A, C
2	1413	1415	–	–	Vinegar	1000	1000	Acetic acid	A, C
3	1445	1446	1072	1079	Grassy, earthy	1000	1000	2-Ethyl-3,5-dimethylpyrazine	A*
4	1478	1494	1177	1170	Earthy	1000	1000	2,3-Diethyl-5-methylpyrazine	A*
5	1507	1512	1182	1181	Pea-like	100	100	2-/3-Isobutyl-3-methoxypyrazine	A*
6	1514	1514	1155	1150	Grassy, flower stem	100	100	(*Z*)-2-Nonenal	A*
7	1602	1602	814	818	Cheesy	100	100	Butanoic acid	A, C
8	164	1641	852	850	Cheesy	1000	1000	2-/3-Methylbutanoic acid	A, C
9	1781	1820	1198	1199	Roasty	10	100	1-Furfurylpyrrol	A, C
10	1803	1819	1388	1380	Fruity	1000	1000	*β*-Damascenone	A, B
11	1840	1840	1091	1091	Spicy	1000	1000	Guaiacol	A, B, C
12	1943	1943	1116	1133	Caramel	100	10	Maltol	A, B, C
13	2016	2015	1062	1062	Caramel	10,000	10,000	Furaneol	A, B
14	2081	2085	1177	1151	Leather-like	10	100	2,5-Dimethylphenol	A*
15	2160	2160	1339	1334	Burnt	100	100	4-Vinylguaiacol	A, B, C
16	2195	2196	1139	1127	Spicy	1000	1000	Sotolon	A, B

[a] Retention indices determined on DB-WAX and HP-5 column according to van den Dool and Kratz (1963).
[b] Experimentally determined retention indices.
[c] Retention indices from reference library (NIST chemistry WebBook).
[d] Odour description perceived on DB-WAX column.
[e] Flavour dilution factor determined on DB-WAX column.
[f] All of the compounds have previously been identified in coffee. Besides odour quality following criteria were used for identification: A = comparision of retention indices on two different columns with reference library, B = reference substance, C = mass spectrum compared with commerical mass spectra database NIST 14.
* Tentatively identified.

la-Peña, & Contreras-Esquivel, 2016). Bel-Rhlid, Thapa, Kraehenbuehl, Hansen and Fischer (2013) demonstrated the enzymatic hydrolysis of chlorogenic acids with a FAE from *Lactobacillus johnsonii*. Other esterases were used to hydrolyse chlorogenic acid lactones to reduce the bitterness in roasted coffee extracts (Kraehenbuehl et al., 2017). Nieter, Kelle, Linke and Berger (2017) identified, produced and characterized a *p*-coumaroyl esterase from *Rhizoctonia solani* (R*sp*CAE) with a chlorogenic acid esterase side activity possessing the ability to hydrolyse the ester linkage between caffeic and quinic acid. In the present work, R*sp*CAE was used to reduce 5-CQA content in coffee. The effect of an enzymatic degradation of chlorogenic acids on the aroma and taste profile of coffee was determined by combining analytical and sensory methods. It should be investigated if it was possible to decouple the mitigation of chlorogenic acids from the well-established and optimized conditions of the various roasting processes.

2. Material and methods

2.1. Chemicals

All chemicals were obtained from Carl Roth (Karlsruhe, Germany) except caffeic acid, which was from Fluka (Buchs, Switzerland). Ultrapure water was used for chemical analysis (TKA-GenPure, Labor- und Analysen-Technik GmbH, Garbsen, Germany). Solvents (GC grade) were rectified before usage.

2.2. Coffee

Green coffee beans (100% Arabica from Columbia) were purchased from a local coffee shop. Coffee beans were roasted at 240 °C for 14 min with a Gene Café Coffee bean roaster (CBR-101, Ansan, Korea) and ground to grinding degree three (according to operating manual for the use of filter coffee) with a WMF KÜCHENminis® handheld coffee grinder (WMF consumer electric GmbH, Jettingen-Scheppach, Germany).

2.3. Enzyme

The *p*-coumaroyl esterase from *Rhizoctonia solani* (R*sp*CAE) was produced heterologously in *Komagataella phaffii* and purified as described elsewhere (Nieter et al., 2017). The activity of enzyme needed to release 1 μmol min^{-1} of free ferulic acid at 37 °C was defined as one Unit (U). Enzyme activity was determined using methyl ferulate as substrate. The applied enzyme solution had an activity of 2480 U L^{-1}.

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