Validity of marker compounds for authenticity control case study: Methyl cinnamate

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Abstract

Methyl 3-methoxy-3-phenyl-propanoate (3-MMPP) is seen as a potential marker, which is used by analytical laboratories and NGO´s to conclude on the correct production of natural methyl cinnamate (MC) qualities according to the EU flavour directive 1334/2008. However, there have been questions about its validity, as a marker for adulteration is only valid, if it can unmistakably be associated with non- permitted starting materials and/or process conditions. There are two obvious routes to get access to natural methyl cinnamate, which would be in full compliance with the requirements for natural flavouring substances according EU flavour regulation 1334/2008. As potential starting materials natural cinnamic acid or *Alpinia malaccensis* roots can be used. Within this study we shed light on the potential formation of 3-MMPP during processing and proved that this trace compound is not appropriate as a marker for adulteration.

Introduction

There is a global trend towards natural food solutions. Hence the industry is looking for ways to increase their portfolio of natural aroma compounds. Good examples are the esters of cinnamic acid, which are widely used for fragrance compositions as well as for the creation of flavours. Especially the methyl ester of cinnamic acid is widely used due to its sweet, aromatic and balsamic notes combined with a fruity odour. Methyl cinnamate occurs naturally in a variety of plants, including fruits like strawberry and some culinary spices, such as thyme and basil. However, the isolation from these species is not economically feasible. Alternative sources are essential oils of rhizomes of various *Alpinia* species, e.g. *Alpinia malaccensis* [1] containing approximately 78% methyl cinnamate (Figure1). [2,3]

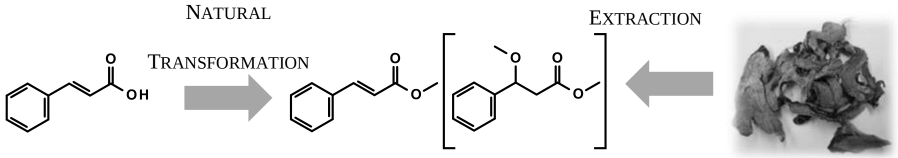

Figure 1: Potential ways to generate methyl cinnamate from different sources.

Another alternative would be the esterification of widely available natural cinnamic acid with methanol form natural sources under conditions permitted by corresponding national or international laws. Natural aroma compounds are mostly more expensive than their synthetic equivalents and are therefore prone to adulteration. Several sophisticated methods, e.g. isotopic ratio mass spectrometry [4] or SNIF-NMR, have been developed to identify adulterated material. As these methods are not always conclusive, the presence of trace components is often considered as an additional marker. The presence of 3-

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MMPP is often referenced as an indicator for potential adulteration, when found in natural methyl cinnamate samples. At the same time there is no published evidence supporting the quality of this potential indicator. Within this study we attempted to check the suitability of 3-MMPP by answering the question whether or not 3-MMPP can be formed during processing of a suitable raw material. In this study it was also investigated, whether the formation of 3-MMPP could serve as a potential indicator for the use of a mineral acid during the esterification of cinnamic acid.

Experimental

Materials and methods

All chemicals were purchased from commercial suppliers. Dried roots of *A. malaccensis* were purchased from Indonesia *via* a German distributor.

GC/MS-Analysis was conducted using a HP 6890N (Agilent, Santa Clara, CA, USA), fitted with a DB-WAXms capillary column (Phenomenex, Torrance, CA, USA) (30m*0.25mm i.d., df 0.25µm), coupled with an MSD 5975C (Agilent, Santa Clara, CA, USA). The GC conditions for the GC/MS-analysis were: split injection (split ratio 20:1), injector temperature 230°C; initial oven temperature at 60°C for 1min, ramp at 4°C/min to 230°C for 20min. Helium was used as the carrier gas and the flow rate was 2.0mL/min. Quantities of 3-MMPP were determined via semi-quantification against the internal standard 2-Nonanol using the GC-FID signal.

All stable isotope measurements were performed using a gas chromatography-combustion/ high temperature conversion-isotope ratio mass spectrometry (GC-C/HTC-IRMS) system consisting of an HP 7890B gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with a robotic autosampler (CTC Analytics, Zwingen, Switzerland), coupled to a BiovisION isotope ratio mass spectrometer (Elementar, Langenselbold, Germany) via an oxidation reactor ($\delta^{13}C$) or via a pyrolysis reactor ($\delta^{2}H$) in a GC5 interface (Elementar, Langenselbold, Germany). All $\delta^{13}C$ and $\delta^{2}H$ values were normalized relative to V-PDB or V-SMOW by a two-point calibration using two vanillin working standard with distinct isotope signatures.

Results and Discussion

Isolation of methyl cinnamate from Alpinia malaccensis

The processing of the dried rhizomes of *Alpinia malaccensis* can either be done by extraction with a suitable solvent (e.g. methanol) or hydrodistillation with subsequent rectification to obtain sensorially acceptable products. Dried *Alpinia malaccensis* rhizomes from Indonesia were extracted by simultaneous distillation extraction (SDE) employing water and a mixture of diethylether/pentane to mimic hydrodistillation conditions. In addition, the rhizomes were extracted with methanol and the solvent removed with and without the addition of Na_2CO_3 to prevent the decomposition by acidic byproducts during rectification. As expected, it was not possible to distinguish the different processing conditions *via* IRMS data (Table 1).

Table 1: IRMS data and formation of 3-MMPP during different extraction conditions.

work-up procedure	*Methyl Cinnamate*		*3-MMPP*
	delta ^{13}C	delta ^{2}H	
SDE	− 26.5 ± 1.0 ‰	− 150 ± 15 ‰	./.
methanolic extract w/o addition of Na_2CO_3	− 26.6 ± 1.0 ‰	− 151 ± 15 ‰	./.
methanolic extract with addition of Na_2CO_3	− 27.2 ± 1.0 ‰	− 146 ± 15 ‰	720 ppm

However, the addition of Na_2CO_3 led to the formation of significant amounts of 3-MMPP which is in line with related reactions described in chemical literature. [5] So even during processing of a widely accepted raw material, traces of 3-MMPP can be formed and good communication with the corresponding supplier is mandatory.

Generation of methyl cinnamate via esterification

Our studies showed that the esterification of cinnamic acid and methanol is possible under various conditions. Surprisingly, simple heating of a mixture of both starting materials led to significant formation of methyl cinnamate without the need of any additional additives (Table 2). Moreover, there is no significant difference regarding the formation of 3-MMPP neither between the use of a mineral or organic acid nor without the use of any additive.

Table 2: Generation of methyl cinnmate starting from cinnamic acid and methanol.

Cinnamic acid [mmol]	*Methanol [mmol]*	*Additive [mmol]*	*Conditions*	*Conversion [%]*	*MC : 3-MMPP*
34	244	Sulfuric acid 16.9	6h reflux	> 90	50:1
27.0	244	Tartaric acid 2.7	9h, 120 °C/ 4.2 bar	15	67:1
67	280	none	26h, 120 °C/ 4.2 bar	19	63:1

In many regulations heating of methanol and cinnamic acid under slightly elevated temperature and pressure is a process which is allowed for the generation of natural aroma compounds. Hence the use of 3-MMPP as a potential marker for authenticity is highly questionable, as there is no reliable evidence for unequivocal differentiation between the use of permitted and non-permitted process conditions according to the EU flavour regulation 1334/2008 during the generation of natural methyl cinnamate.

Summary

Available natural methyl cinnamate (European legislation) can at least be generated *via* two different processes. Firstly, an extraction/distillation of *Alpinia malaccensis* roots should be feasible. Here the generation of 3-MMPP could be traced back to the potential use of processing aids (e.g. Na_2CO_3) which are widely used and comply with current legislation for the generation of natural aroma chemicals. Secondly esterification of methanol and cinnamic acid delivers a suitable product, providing the used raw materials are sourced in a natural quality. Here we could show that methyl cinnamate could be formed easily as assumed under the addition of a mineral acid. However, it could also be formed just by addition of an organic acid or even without any additives. In all cases 3-MMPP could be detected. Taking this into account, the use of 3-MMP as potential marker for adulteration should be challenged.

References

1. Lim, T. K. (2016) Alpinia malaccensis. Edible Medicinal and Non-Medicinal Plants: Volume 12 Modified Stems, Roots, Bulbs. Cham, Springer International Publishing: 172-177.
2. Nor Azah, M. A., Sam, Y. Y., Mailina, J., and Chua, L. S. L. (2005) Journal of Tropical Forest Science 17(4): 631-633
3. Sirat, H. M., Basar, N., andJani, N. A. (2011) Natural Product Research 25(10): 982-986
4. Fink, K., Richling, E., Heckel, F., and Schreier, P. J. (2004) Agric. Food Chem. 52(10): 3065-3068
5. Guo, S.-H., Xing, S.-Z., Shuai Mao, S., Ya-Ru Gao, Y.-R., Wen-Liang Chen, W.-L., and Yong-Qiang Wang, Y.-Q. (2014) Tetrahedron Letters 55(49): 6718-6720