





In silico prediction of metabolism as a tool to identify new metabolites of dietary monoterpenes

Jarlei Fiamoncini

Food Metabolome and the Metabolism of Food Compounds

Food metabolome is the part of the metabolome derived from the digestion and metabolism of food.

The more we know about **food compounds metabolism**, the better we can study the effects of **diet** in **health**.

Dietary monoterpenes are a part of the food metabolome that remains poorly studied.



Dietary Monoterpenes



- Formed by the condensation of 2 isoprene units
- Low molecular weight and relatively high lipophilicity
- Found in the essential oil of herbs and citrus fruits
- Daily intake up to 200 mg

Demonstrated effects

Antinociceptive Antimicrobial Hypotensive Anti-inflammatory Hypoglycemic (STZ diabetic mice) Antioxidant Antineoplasic Modulators of the activity of ion channels Toxic effects



Pharmacokinetics of Monoterpenes

- ✓ Both in humans and rats, dietary terpenes reach effective concentrations in plasma within 1 hour
- ✓ Their metabolites are detected in circulation up to 24 hours after intake
- ✓ Topic administration of terpenes is also effective to increase their concentration in plasm



300 mg. Data are mean + s.d., n = 4.

Fig. 4. Plasma levels of M-G (*expressed as menthol after enzymatic hydrolysis) in a human volunteer after ingestion of 100 mg menthol.

Problems



✓ Despite recognized health effects, the metabolism of dietary terpenoids is poorly known

✓ Different isomers for each compound make terpenoids analysis very complex.

Aims of the study

- ✓ Identify enzymatic reactions involved in the metabolism of terpenoids
- ✓ Validate metabolism predictions
- ✓ Identify new metabolites of dietary terpenoids





Investigation of Metabolism of Monoterpenes





defining the reactions involved in the the metabolism of dietary monoterpenes





http://phytohub.eu/



https://www.lhasalimited.org





defining the reactions involved in the the metabolism of dietary monoterpenes

Biotransformation Name	Phase	Enzyme								
			Compounds that undergo the specific reactions							
Allylic Hydroxylation	Phase I	CYP450	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol	linalool		
Conjugation of Alkyl Carboxylic Acids with Glycine	Phase II	ACS, AANAT	geraniol	terpinen-4-ol	perillyl alcohol					
Conjugation of Carboxylic Acids with Glutamine	Phase II	ACS, AANAT	geraniol							
Epoxidation of 1,1,2-Trisubstituted Alkenes	Phase I	CYP450	limonene	geraniol	terpinen-4-ol	perillyl alcohol	linalool			
Epoxidation of 1,1-Disubstituted Alkenes	Phase I	CYP450	limonene	nootkatone	perillyl alcohol					
Epoxidation of Monosubstituted Alkenes	Phase I	CYP450	linalool							
Glucuronidation of Aromatic Alcohols	Phase II	UGT	thymol							
Glucuronidation of Carboxylic Acids	Phase II	UGT	thymol	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol cuminaldehyde	linalool	menthol
Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	Phase II	UGT	thymol	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol cuminaldehyde	linalool	menthol
Hydroxylation of Alkyl Methine	Phase I	CYP450	nootkatone	terpinen-4-ol	menthol					
Hydroxylation of Aromatic Methine	Phase I	CYP450	thymol	cuminaldehyde	e					
Hydroxylation of Methyl Carbon Adjacent to an Aliphatic Ring	Phase I	CYP450	nootkatone	menthol						
Hydroxylation of Methyl Carbon Next to an Aromatic Ring	Phase I	CYP450	thymol							
Hydroxylation of Terminal Methyl	Phase I	CYP450	thymol	terpinen-4-ol	cuminaldehyde	linalool	menthol			
Hydroxylation of Unfunctionalised Alicyclic Methylene	Phase I	CYP450	limonene	nootkatone	perillyl alcohol	menthol				
Oxidation of Aldehydes	Phase I	ALDH	cuminaldehyde	•						
Oxidation of Primary Alcohols	Phase I	ADH	thymol	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol cuminaldehyde	linalool	menthol
Oxidation of Secondary (Alicyclic) Alcohols	Phase I	ADH	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol	menthol		
Reduction of Aldehydes	Phase I	ALDR	cuminaldehyde	•						
Reduction of Alicyclic Ketones	Phase I	ADH	menthol							
Reduction of alpha, beta-Unsaturated Compounds	Phase I	abKDBR	nootkatone							
Vicinal Diols from Epoxides	Phase I	EH	limonene	nootkatone	geraniol	perillyl alcohol	linalool			



LIMONENE



in vivo experiment

feeding rats isolated monoterpenes and collecting metabolites-rich urine



5 cycles – same rats were exposed to different food compounds



non-targeted LC-MS analysis in search of predicted metabolites



Investigation of Metabolism of Dietary Monoterpenes







citral





Conclusions

- Considering the selected 22 biotransformations, more than 1500 metabolites were predicted from the 23 tested terpenoids.
- ✓ The predicted metabolites were helpful for the annotation of the peaks detected after the rats were exposed to the terpenoids.
- Next step is to validate the hypothetical structures of known and *new* metabolites using qToF MS/MS.
- ✓ The knowledge generated is being used to improve *in silico* prediction tools (BioTransformer)
- ✓ The generated data will be made available in food compounds databases (PhytoHub, HMDB)

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Workflow for experimental data analysis





carvacrol





1,8-cineole



