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Review Article

Metadata review of variation in published chemical compositions of *Mentha piperita* essential oils

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Natural complex substance, chemical composition, natural variability, *Mentha piperita* , identification.

Abstract

In this review, we have sampled articles from the literature on *Mentha piperita* with the purpose of evaluating the magnitude of natural variation in the chemical composition of essential oils. Thus, 26 articles about *Mentha piperita* essential oil collected from those including complete gas chromatographic profiles and recently published, accounting for ca. 10% of total published literature on the topic, were analyzed. First of all, we found that none of the 26 reported profiles met all 12 chemical ranges set in the AFNOR standard for *Mentha piperita* essential oil. In terms of chemical diversity, we found 85 compounds reported only in one publication, and one single report had up to 13 single-handed. A total of 171 different compounds were described in these 26 publications, with some questionable practices in terms of identification. Beyond natural variability, the selection appeared too heterogeneous in quality and good analytical practices to account for the qualitative and quantitative diversity of results observed.

1. Introduction

In the world of essential oils and other natural products, regulatory guidelines and quality control struggle to delineate between the naturally occurring chemical variations of a raw material, degradation or transformation upon ageing, and the accidental or deliberate presence of unexpected substances or adulterants [1, 2]. Natural variation is the term used to describe the slight changes in chemical composition found in a particular natural product, including essential oils, fermented foodstuffs, meat, honey, or coffee. A natural product will be affected by the environment in which it was produced, the genetic variation of the producer (plant, yeast, animal or bee), and the processing conditions, typically distillation or cold-pressure extraction in the case of essential oils.

In order to maintain the value of these particular natural products, but also to support biological studies where these essential oils could be engaged (e.g. toxicity, ecotoxicity, biodegradability [3], or various beneficial effects for well-being), chemical markers which distinguish them from their more generic counterparts, must be identified and quantified. However, even the generic natural products must be defined both botanically and chemically to properly regulate them in the consumer market [4]. The regulatory bodies require that all raw materials must pass some minimum quality testing for identification and purity. In the case of essential oils, besides simple physico-chemical testing and olfactory evaluation, these requirements are generally met by GC analysis to assess chemical conformity to a recognized norm such as ISO, AFNOR or a national pharmacopeia [5].

Adulteration is the addition of anything to a raw material during or after the acquisition procedures. According to GMP definitions, this can include the contamination of batches during processing or the intentional addition of specific compounds or diluting the raw material in bulk to achieve an economic advantage, known as economically motivated adulteration (EMA). Essential oils can be diluted in vegetable oils or solvents to increase volume [6]. Essential oils can also be adulterated with synthetic compounds to increase volume, improve scent, or meet regulatory specifications [2]. Finally, essential oils can be mixed with lower cost oils, this practice can be very difficult to detect and is both the extension of expensive oil volume and up-selling of a lower value product [2, 6]. These adulterations become challenging to detect when the chemical definition of the oil must be broad enough to encompass the natural variation yet exclude what could be called "unnatural variation". In the case of Lamiaceae, there are many examples of plants producing essential oils with different chemotypes, like lavender, melissa, peppermint, basil, rosemary, sage and thyme. A chemotype describes the subspecies of a plant that has the same morphological characteristics but produces essential oils of different chemical compositions, generally as an expression of the diversity of geographical origins [7].

Peppermint, *Mentha piperita*, is one of the most commonly used essential oils with 3,300 tons produced in 2007 (Fig. 1) [8]. The mint essential oil can be found in chewing gum, dental care products, shampoo, and herbal remedies as well as baked goods and cleaning supplies. Many homes also keep mint essential oil in their medicine cabinets and its biological activities for potential development of a new medicinal and cosmetic products have been the subject of intense research [9]. Cornmint, produced at about 10 times the rate of peppermint, is used often in place of peppermint, resulting in a public perception that cornmint *is* peppermint, olfactively, reducing our ability to identify a cornmint sample being sold as peppermint. The International Trade Centre indicates that US grown mints sell for 95-120 USD/kg, while Indian cornmints are worth about half, at 55 USD/kg [10].

Figure 1. *Mentha piperita*. By Franz Eugen Köhler, Köhler's Medizinal-Pflanzen - List of Koehler Images, Public Domain.

Peppermint is a hybrid of *Mentha aquatica* and *Mentha spicata* with the botanical name *Mentha x piperita*. As it is a hybrid, the plant is usually sterile and is reproduced and cultivated by planting cuttings of the rhizome rootstock. There are numerous existing and proprietary newly bred varieties of peppermint, thus requiring adequate taxonomic and genetic profiling [11]. The herbaceous plant is harvested upon flowering and distilled directly or after a short drying time.

2. Materials and methods

2.1. Chemotypes

The 72-chromosome type peppermint can express two chemotypes, the menthone type with a characteristic peppermint odor, or a carvone dominant type [12]. There is another hybrid *M. piperita* var. *citrata,* a cross

Table 1. Comparison of *Mentha piperita* and *Mentha arvensis* of different geographical origins AFNOR/ISO ranges [12, 13].

Constituents	***Mentha piperita***		***Mentha arvensis***					
			China		**India**		**Other**	
	Min %	**Max %**	**Min %**	**Max %**	**Min %**	**Max %**	**Min %**	**Max %**
Menthol	32.0	49.0	33.0	45.0	33.0	45.0	30.0	46.0
Menthone	13.0	28.0	18.0	30.0	17.0	26.0	17.0	32.0
1,8-Cineole	3.0	8.0	0.3	1.5	0.2	1.0	0.1	2.0
Isomenthone	2.0	8.0	8.0	12.0	8.0	14.0	6.0	13.0
Menthyl acetate	2.0	8.0	1.5	4.0	1.5	5.0	2.0	7.0
Menthofuran	1.0	8.0	-	-	-	-	-	-
Neomenthol	2.0	6.0	4.0	8.0	4.0	10.0	3.0	11.0
β-Caryophyllene	1.0	3.5	0.5	2.0	0.5	2.0	0.5	2.0
Limonene	1.0	3.0	1.5	4.0	1.0	4.0	1.0	7.0
Pulegone	0.5	3.0	0.5	2.5	0.5	2.5	0.5	2.5
trans-Sabinene	0.5	2.3	-	-	-	-	-	-
3-Octanol	0.1	0.5	0.5	3.0	0.2	1.8	0.2	2.0

of *M. spicata* and *M. citrata*, that have a higher linalool and linalyl acetate content [13]. Additionally, Hefendehl and Murray characterized *M. citrata x M. spicata* L. var. *crispata*, a peppermint variety dominated by limonene and cineole, and less than 6.9% of the characteristic pulegone and menthone family compounds [12].

2.2. Pharmacopeas and standards

ISO and AFNOR as well as the British Pharmacopeia offer guidelines for the identification and qualification of essential oils. For mint, there are standards for *M. piperita, M. arvensis, M. pulegium,* and *M. spicata*, which are all commonly referred to as mint, although they are unique genetically. *Mentha pulegium* can contain as high as 83% of pulegone [14-16].

In following the AFNOR/ISO guidelines, outlined in Table 1, the determining factors between *M. piperita* and *M. arvensis* are isomenthone, 1,8-cineole, and 3-octanol, where all other controlled compounds have overlapping ranges [17, 18].

The addition of *M. arvensis* in *M. piperita* essential oil is considered a possible adulteration, identified by enantioselective-GC analysis of isopulegol and menthyl acetate [2]. The AFNOR/ISO documents do not include specifications for these enantiomers, and the optical rotation ranges on the whole essential oil overlap among all groups of peppermint and cornmint.

In this paper, we present the results of an analysis of 26 articles about *M. piperita* essential oil collected from those including complete gas chromatographic profiles and published in the years 2003-2018. This selection accounted for ca. 10% of total published literature and our intention was to try to evaluate the magnitude of natural variation in the chemical composition of the essential oil of this plant. Besides technical variation, i.e., what and how parts of the plant were collected and processed, and how the essential oil was analyzed and data processed, variation remains significant.

3. Results and discussion

To create a picture of the chemical composition of *Mentha piperita* essential oil, an extensive literature review was conducted till 2021. Search terms included peppermint, *Mentha piperita*, mint, GC and composition to reach a maximum of relevant articles. From 274 articles, 26 were collected from those including complete gas chromatographic profiles, in a tentative to cover various geographical origins and the profile data were transferred into Table 2. The ISO norm is also presented in the table for the sake of comparison, as well as results obtained when asking ChatGTP "what is the chemical composition of the essential oil of *Mentha piperita*" then "give more details in the form of a table" then "I want at least 25 compounds". This might be considered trivial, but it is a reflection of the unfiltered digital knowledge available that people not trained as scientists might be tempted to use.

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Table 2. Chemical compositions of various *M. piperita* essential oils found in the literature, compared with ISO norm and ChatGTP.[a]

Reference	[19]	[20]	[21]	[22]	[23]	[24]	[25]	[26]	[27]	[28]	[29]	[30]	[31]	[32]	[33]	[34]	[35]	[36]	[37]	[38]	[39]	[40]	[41]	[42]	[43]	[44]	[12]		N.A.	
Year (20xx)	14	15	14	11	11	12	09	13	03	06	18	17	17	17	10	18	17	18	14	14	13	03	12	07	10	13	16		24	
Region[b]	CN	BR	IR	IN	IN	IR	RS	TW	RS	IR	SA	IR	CM	IR	KR	DZ	BJ	IN	IR	N.D.	RU	IT	IN	BG	EG	TR	ISO		ChatGTP	
Number of compounds in ISO range (out of 12)	3	1	8	3	9	3	5	3	6	1	3	8	3	8	8	2	7	4	7	6	5	3	4	2	5	6	Min	Max	Min	Max
α-Pinene	0.2	1.08	0.55	4.80	0.71	0.32			0.12		0.98	0.79	8.21	0.69	0.42	2.09	0.80	1.50	0.80	0.80	0.36	0.20	1.64	1.20	0.20	0.37			tr	1.5
2-Bromocyclohexanol																							0.08							
(*E*)-Hex-3-enol																		0.10												
2,5-Diethyltetrahydrofurane																		0.10												
α-Thujene											0.03		0.23						0.04											
Tricyclene										1.20																				
Camphene				0.30						1.20	0.14	0.02	0.32						0.02										tr	0.1
Camphor													0.23	0.16				0.10								1.50				
Sabinene	0.2	0.81	0.38	0.10	1.47[e]	0.26	2.50		0.20	1.80	0.90	0.42		0.46				0.70	0.50		0.29				0.01					
β-Pinene	0.46	1.50	0.80	5.70		0.58			0.33	2.50	1.22	0.97	0.69	0.93		1.59	1.20	1.70	1.10	0.90	0.59	0.40	1.61	2.40	0.36	0.12			tr	2.0
β-Phellandrene			0.06	2.80											0.36								0.82							
β-Terpinene															0.66															
δ-3-Carene				0.40								0.18	0.90																	
β-Myrcene	0.52	1.21	0.32	1.30	0.26		0.50		0.11		0.77	0.18	1.24[d]	0.21				0.80[c]	0.20[c]		0.24	0.10[d]	0.37			0.14[d]			tr	0.2
3-Octanone				0.20									0.18																	
3-Octanol			0.29	3.50	0.21		0.10		0.05		0.27	0.14		0.24				0.80	0.10		0.27		0.44				0.1	0.5		
α- Phellandrene											0.08	0.06[d]																		
α-Terpinene			0.32		0.23		0.10			19.70		0.29	0.21	0.33	0.25		0.30[d]		0.20				1.02							
p-Mentha-1,3,8-triene											0.11																			
Paracymene	0.22		0.25	0.60	0.19		0.10			0.15		0.09	0.28	0.15	0.63			0.20	0.10	0.90	0.45		0.70						tr	0.1
Limonene	1.76	37.18	3.29	10.60	2.23		6.90		0.10	0.70	9.92	2.22	13.36	2.46		2.97[d]	2.80	7.10	2.30	0.90	6.03		3.49				1	3	tr	6.0
1,8-Cineole	2.91	2.41	3.75	3.60	5.44	6.69	5.60	5.33	0.80	0.17	5.03	4.46	1.28	7.07	0.12	6.73	6.50	0.50	4.50	4.60	5.24	3.50	5.53	2.60	3.45	5.59	3	8	3.0	6.75
Eucalyptol															5.80[f]															
n-Octanol																		0.10												
3-Octanol																							0.44							
Isovaleric acid																							0.16							
2-Methylbutyl ester[c]																							0.10							
Linalool oxide																									0.05					
α-Terpinolene			0.11	0.30	0.19		0.10			0.08	0.08	0.14	0.44[d]	0.15	0.16			0.10[d]	0.10[d]				0.23		0.38				tr	0.1
Santolina triene															0.45															
(Z)-β-Ocimene					0.19		0.10				0.28		0.09	0.12							0.24	0.2								
(E)-β-Ocimene	0.07		0.20		0.06		0.20				0.11								0.10		0.06									
γ-Terpinene					0.46		0.30			0.60	0.09	0.47	0.34	0.59	0.42											0.11			tr	0.1
γ-Terpinene																			0.30											
cis-β-Terpineol	0.71																						0.57							
Cis-*sabinene hydrate*						0.50					0.54			0.33					1.40	1.40[d]									tr	0.1
Trans-*sabinene hydrate*			0.51						1.60			0.61			1.12												0.5	2.3	tr	0.1
Linalool	0.42		0.39	0.10			0.20		0.31		0.06		0.40	0.39				0.20	0.10	0.50	0.43		0.40		0.14					
2-Methylbutyl 2-methylbutanoate																					0.06									
(*Z*)-Epoxy-ocimene													0.44																	
(*E*)-Pinocarveol													0.22																	
trans-*p*-Menth-2-enol	0.07														0.55							0.10								
Cis-*p*-menthen-1-ol																					0.05									
Isoamyl valerianate[c]											0.02																			
Amyl isovalerate											0.02	0.15																		
Limonene oxide											0.08																			
Isopulegol	0.15			3.00	0.04											0.41					0.09		0.26		0.04					
Neoisopulegol																		1.80												
Menthone[f]	14.51		28.8		29.78	2.45	12.70	21.12	8.93	0.80	0.01	16.04	18.47	8.28	21.41	20.84	7.40	20.70	15.80	21.70	32.75	40.70	25.83	25.90	15.61	20.15	13	28	0.4	45.6
Isomenthone				14.80	4.77				3.66	10.30	0.08			1.95	5.34	7.25	4.80	11.60		7.40	6.49				0.90		2	8	1.3	15.5
Menthofuran			1.90		1.12	11.18	6.80			2.30		8.91		4.07	6.37		1.60		4.10				5.04			6.13	1	8	1.0	17.0
Isomenthol				6.40[d]	0.17			6.26		0.20			0.60	0.94			0.80	0.70	0.90	0.70	0.40		1.00							
Neomenthol	9.26		3.80		3.46	2.79			2.11			4.24		4.27			8.28	5.30	6.0						1.70		2	6	3.2	9.2
Menthol	30.69	0.84	36.90	19.10	36.51	53.28	37.40	30.35	39.63	3.60		45.34	33.59	43.95	33.37	49.89	46.70	26.0	46.40	47.50	33.37	24.90	26.53	35.00	34.29	35.12	32	49	28.5	69.1
β-Fenchyl alcohol			0.63																											
Mint furanone								2.49															0.3							
L-Borneol											0.64															0.06				
Isoborneol													3.50																	
Isoneomenthol	0.45			1.50					0.63					0.32				0.10	0.40	0.60					0.20					
4-Terpineol					0.50						0.28[d]							0.30		1.50	0.98		0.1						tr	3.01
α-Terpineol	0.41[d]	0.30		0.80	0.14				3.00		0.48		0.55	0.44	0.38			0.60		0.40	0.29		1.42						tr	0.5
β-Terpineol																							0.10							
γ-Terpineol										2.70																				
δ-Terpineol																					0.10									
(+)-Sabinol																							0.16							
cis-Dihydrocarvone		0.80									0.68																			
trans-Dihydrocarvone											0.08																			
Dihydrocarveol			0.17																											
cis-Carveol				0.05							0.48																			
trans-Carveol				0.05						14.50	0.30																		tr	58.98
Verbenone				0.1																										
Estragole	0.30																													
cis-Carane												8.70				4.99														
trans-Carane	0.07							10.99				0.18																		
Octyl acetate																		0.10			0.09				0.04[d]					
Pulegone	4.36			2.30			1.20	2.12	1.59		0.94	0.80		2.63	1.06	0.42	3.60	1.70	0.04	1.20	1.08	0.70	4.11		0.62	2.12	0.5	3	tr	6.9
Isopulegone											0.16[d]												0.26							
Pinocarveol				0.20																										
Thujol				0.40																										
Geranyl formate				0.60																										
Neryl acetate				0.40																										
Geranyl acetate										0.12																				
Linalyl acetate																						0.20								
Linalyl isobutyrate																									0.11					
Carvone		49.27	3.82	0.30				5.60		0.46	70.26										0.05[d]		0.55			10.6			tr	2.07
cis-3-Hexenyl isovalerate			0.57									0.15						1.0												
Sabinyl acetate				0.20																										
Hexyl isovalerate																		0.10												
Piperitone	2.31[d]	1.19	0.76	2.10	0.28		0.80		0.88			0.61	4.66	0.37	0.57		1.70	1.40	0.30	0.30	1.06	1.30	1.01							

Table 2. *(Continued).*

Reference	[19]	[20]	[21]	[22]	[23]	[24]	[25]	[26]	[27]	[28]	[29]	[30]	[31]	[32]	[33]	[34]	[35]	[36]	[37]	[38]	[39]	[40]	[41]	[42]	[43]	[44]	[12]	**N.A.**		
Year (20xx)	14	15	14	11	11	12	09	13	03	06	18	17	17	17	10	18	17	18	14	14	13	03	12	07	10	13	16	24		
Region[b]	CN	BR	IR	IN	IN	IR	RS	TW	RS	IR	SA	IR	CM	IR	KR	DZ	BJ	IN	IR	N.D.	RU	IT	IN	BG	EG	TR	ISO	ChatGTP		
Number of compounds in ISO range (out of 12)	3	1	8	3	9	3	5	3	6	1	3	8	3	8	8	2	7	4	7	6	5	3	4	2	5	6	Min	Max	Min	Max
Piperitone oxide										1.90															0.18					
p-Menthon-3,8-diol[c]										0.20																				
Pipertitinone oxide[c]										19.30																				
p-Menth-1-en-9-ol										0.16																				
Cubenol				0.20																										
trans-Anethole			1.26																											
Bornyl acetate									0.66																0.02					
Neomenthyl acetate						0.65								0.69				0.10	0.40	0.90	0.16				0.22					
Dihydroedulane																					0.06									
n-Decanol																		0.20												
Menthyl acetate	12.86		4.54	6.60	3.38	15.10	17.40	0.81	10.44				3.73	8.35	6.71		6.70	9.50	9.70	3.50	3.05	13.90	8.07[h]	8.80	2.49		2	8	0.3	17.4
Thymol														0.13																
Isomenthyl acetate						0.61			0.64					0.43				0.20	0.40		0.07				30.47					
Neoisopulegyl acetate																					0.05[d]									
Neoisomenthyl acetate												0.51																		
Δ4(8)-Menthene																0.42														
p-Menth-3-ene			0.25									0.42			0.29															
Mono-(2-ethyl hexyl) ester[c]								0.85																						
Dihydrocarveol acetate			0.14																											
Levomenthol	0.17																													
Neoiso-isopulegyl acetate																		0.10												
Isopulegyl acetate	0.08[d]																													
Nonyl acetate																		0.10												
Bicyclogermacrene	0.06[d]		0.27			0.22	1.30					0.15		0.28				0.30	0.10		0.08									
Eugenol	0.11																													
Isoeugenol													0.80																	
α-Bourbonene	0.23			0.50							0.54																			
β-Bourbonene		1.00	0.33		0.15	0.37	0.40			1.20		0.21		0.52	0.56			0.30	0.20	0.60	0.15[d]	0.40								
α-Gurjunene										0.15	0.06																			
β-Elemene	0.29								2.48			0.06		0.19				0.10				0.30								
n-Decyl acetate			0.11																											
Jasmone	0.09																													
Nerolidol																							4.26							
β-Caryophyllene	2.52	1.16	1.61	0.80	1.60	2.06	0.30		3.08	7.60	0.73	0.79	0.30	2.69	3.02		2.10	1.50	0.80	0.70	0.49[d]	3.50		3.60	0.08	1.71[d]	1	3.5	tr	2.5
β-Cedrene													0.31																	
β-Copaene				0.10										0.11						0.10										
β-Farnesene	0.54		0.24		0.22	0.30	0.70		0.54			0.18		0.32					0.20		0.10		0.90							
α-Humelene									0.46					0.12							0.08	0.40				0.86				
γ-Muurolene				0.10																		0.20								
Myrtenol				0.20																	0.08[d]									
cis-Muurola-4(14)-5-diene											0.61																			
α-Amorphene	0.06																													
9-*epi*-β-caryophyllene													0.76																	
Decyl acetate																		0.20												
Octyl isovalerate																		0.10												
Germacrene D	1.13	0.59		0.30	1.71	2.01	0.50		0.25		0.69	0.82	0.24	2.05	1.96			1.10	0.90		0.34[d]		1.38						tr	1.0
Germacrene A							0.50																							
α-Cubebene											0.28		0.21																	
β-Cubebene			1.30						1.80																					
(-)-Mint lactone	1.69																													
γ-Gurjunene											0.52											0.10								
α-Eudesmene											0.29																			
α-Selinene													0.26																	
γ-Cadinene	0.18		0.06								0.08											3.70								
(-)-Calamenene											0.18																			
α-Cadinene											0.07																			
δ-Cadinene												0.03	0.35		0.19			0.10			0.10		0.23							
β-(3-Thienyl)acrylic acid	2.09																													
Ledene oxide (II)															0.15															
Aromadendrene															0.16															
10-epi-γ-Eudesmol											0.52																			
τ-Cadinol											0.11																			
Spathulenol	0.41[d]			0.20					1.02											0.50	0.10[d]	0.10								
Caryophyllene oxide	1.37		0.12	0.20								0.05		0.20						0.80		0.10	0.32							
Isocaryophyllene ⊚-oxide[c]																					0.14									
Viridiflorol			0.39				0.20		1.11			0.23		0.64					0.30		0.24				0.05				tr	2.9
τ-Muurolol									0.21													0.10								
Ledol	1.05																													
3-Hexadecyne	0.11																													
T-Cadinol																					0.17	0.50								
α-Cadinol	0.16										0.15									0.10	0.23									
Aristolene oxide	0.10																													
Globulol																				0.30										
Epiglobulol	0.35[d]			0.80																			0.25							
Mint furanone																							0.30		0.06					
p-Menthone-1,2,3-triol[c]																									0.05					
cis-α-Bisabolene epoxide	0.2																													
Hexahydrofarnesyl acetone	0.15[d]																													
Phytol	0.14																													
Eicosane	0.06[d]											0.36																		
(Z)-Hex-3-enyl phenyl acetate																		0.10												
Epizonarene									0.50																					

[a]Compounds belonging to the AFNOR norm are italicized. [b]ISO 3166 country codes. [c] Name is incomplete or erroneous, but this is how it appears in the publication. [d]Mispelled or partial name in the publication, corrected by the authors. [e]Co-elution. [f]Eucalyptol and 1,8-cineole are synonyms, but they were presented separately in the publication. [g]Sum of *cis*- and *trans*-. [h]Sum of two values given in the publication for this single compound.

In following with the current industrial procedures, none of the 26 reported profiles met all 12 chemical ranges set in the AFNOR standard. Surprisingly, 85 compounds are reported only in one report, and one single report had up to 13 single-handed. This makes for a challenge to researchers and quality control labs alike. One must ask if it is the norm or the literature which more accurately represents mint essential oil. Perhaps the mint itself, widely grown and popular for selective breeding, varies so broadly that norms based on the chemical composition of a distilled derivative of the plant will struggle to span the breadth of the variation. A total of 171 different compounds were described in these 26 publications, with 6 compounds erroneously named, and at least 26 misspelled.

Compounds such as α- and β-pinene are both present in 23 out of 26 publications, known by ChatGTP but not listed in the norm. *trans*- and *cis*-sabine hydrates are present in 4 and 5 publications out of 26, respectively. While only *trans* isomer is in the norm, ChatGTP expects both isomers and there is a risk depending on how the composition was determined (see below) that authors have switched *cis* and *trans* isomers in their identification. On the contrary, *trans*-carveol appears in 3 out of 26 publications, which is not in the norm, but is predicted by ChatGTP to be likely to appear in up to 58.98%. A probable digital confusion with *Mentha spicata* essential oil for the bot. There appears to be a bad habit within the essential oil research field, that any outlying result can be explained away with "natural variation" without any deeper investigation. One example reports a *M. piperita* L. sample from Brazil containing 51.8% linalool and no menthol or menthone and contributes the differences to geographic region [17]. The methods for identification used included MS spectra comparison with database, retention indices, and literature review, but there was no further investigation into the plant material or chemical analysis. Another paper looking at the antimicrobial activity of Moroccan essential oils reports *M. piperita* with 41.4% linalool and 39.5% linalyl acetate and 0% menthone nor isomenthone [13]. These extreme deviations from the accepted norms, which are often mentioned in the texts, without verification of the identification of the plant material, or often even any express discussion of the variation is concerning (the reason why these two examples were not used in Table 2). A secondary evaluation of the plant material or a commentary of a potential chemotyping error would be the minimum. Or, if the blame is to be placed at the normalization level, a peppermint norm should include multiple chemical profiles for specific chemotypes.

In terms of natural variation, the commonly cited sources of variation are genetics, geography, and growing conditions. Some studies have looked specifically at the extent to which these changes affect the essential oils [20, 29, 37]. Intuitively, drought conditions reduced the essential oil yield of the plants under stressed conditions, as water is a starting material in the metabolism and less water would result in fewer secondary metabolites. Most compound changes in a study on peppermint essential oil for Iran were considered significant ($p<0.05$) but few consistent trends exist in the data [18]. The application of salicylic acid to the leaves had no significant effect on the oil composition [37]. The geographic origin of *M. piperita* from Spain has a wide variation in the terpene composition, but very consistent enantiomeric ratios [29].

As reported in Table 2, the mint literature has notable consistencies and glaring outliers. Samples 2 [20] and 11 [29] are carvone chemotypes from Brazil and Saudi Arabia, respectively. Another Iranian *M. piperita* sample seems to mark another menthol chemotype [28], or misidentification, characterized by high α-terpinene, isomenthone, *trans*-carveol and piperitone/piperitenone oxides (unlisted), rather than the typical menthone dominated chemical profile [19]. Sample 4, from India, is high in iso-menthone, closer to the high end of the *M. arvensis* ISO/AFNOR range, and limonene, exceeding all mint ranges, suggesting possible adulteration in this commercially purchased oil [21].

3.1. PCA score plot of reference data

In the hope of mining the data for some insight into more useful characteristics to monitor, preliminary PCA analysis was applied to the dataset using BioVinci software. Missing peak areas were replaced with '0' and compounds reported in less than 2 samples were excluded from the dataset. In this preliminary attempt, the first principal component

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contained 53.14% of the total variance; PC2, 13.66% and PC3, 11.34%. Coloring is representative of the number of components which fall into the ISO/AFNOR ranges, the darkest red having 9 of 12 compounds in the range. However, there was very little grouping, aside from excluding the 2 carvone chemotypes from the other samples.

The fairly uniform PCA clustering may indicate that the samples are not statistically different from each other, and yet, none of the samples pass the 12 compound ISO/AFNOR requirements of *M. piperita*. This may be a sign that the norms should be revisited to match the scientific data presently available. What is made visible in the PCA analysis, Fig. 2, is that the data in Table 3 does not have particularly strong patterns.

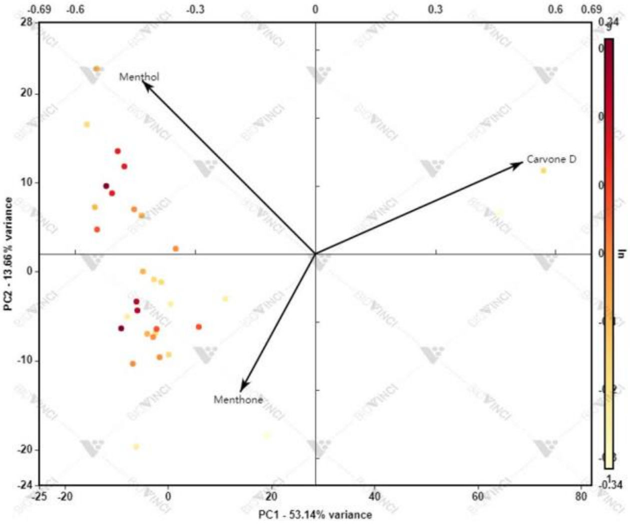

Figure 2. PCA plot of *M. piperita* references colored by accordance with ISO/AFNOR norms. The plot was created with BioVinci software (BioTuring Inc., San Diego, CA, USA).

It was expected that there would be a correlation between samples which more closely conform to the norm guidelines, but this is not the case. Further, it might be expected that samples that were extracted by steam distillation might be notably different in composition from those that were hydrodistilled, but this is not the case either. Granted, this is possibly due to at least one of the steam distilled samples also being a different chemotype. In terms of algorithm needs, more samples are needed within this distillation method to establish a statistical difference between these groups, if there is one.

Table 3 shows the statistical review of the literature values displays a range of relevant statistics extracted from the literature data.

Plus, or minus one standard deviation of the mean of the sample set results in about a 95% confidence interval for other samples of the same sample type. One would expect the regulatory ranges and the literature mean +/- 1 standard deviation, should be similar. The table includes this calculation for all compounds appearing in 3 or more references, while the ISO/AFNOR defines 12 compound ranges. In several cases, like 1,8-cineole, isomenthone, menthofuran, neomenthol, pulegone and menthone, the calculated ranges and the norms are in general agreement, though not exactly equal. While others, like D-limonene and 3-octanol are less in agreement. Standard deviation, and therefore the accepted ranges, appeared generally quite broad.

It is surprising how close this data set agrees with the ISO/AFNOR norm ranges, while not having a single reference meet all 12 requirements. In most, though not all, cases, the reference generated ranges are broader than those of the norm. Arguably, the ranges are tightened to control for better quality oils and reduce the risk of false identification. But in this review, the norms are now exclusive of much of the published analysis of mint, and rather a consensus on a selection of qualities found on the market, reflected by the vision the technical committee has on it.

3.2. Methodology review

Technical variation can introduce changes in chemical profiles. Table 4 outlines the important extraction and analysis particularities of the references included in this discussion. Eight references did not use hydrodistillation, and of those 8, 6 references used purchased essential oils or otherwise did not describe the extraction process.

While the extraction and GC-MS methods are fairly standardized, plant identification is much less overt. The primary approach is identification by a taxonomist on intact plant material. One paper grew plants from cuttings, which may be the most certain method of acquiring mint. Contrarily, one paper planted seeds [41], which for mint is a challenge, as the plant is a cross between water mint (*Mentha aquatica*) and spearmint (*Mentha spicata*), and is a triploid chromosome, resulting in sterile seeds.

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Table 3. Statistical figures from literature data in Table 2.[a]

Constituents	Min	Ave	Max	SD	Rel SD	Ave – 1 SD	Ave + 1 SD	ISO low	ISO high	Count
α-Pinene	0.12	1.25	8.21	1.81	1.44	-0.55	3.06			23
Camphene	0.02	0.33	1.2	0.44	1.33	-0.11	0.78			6
Camphor	0.1	0.50	1.5	0.67	1.35	-0.17	1.17			4
Sabinene	0.01	0.64	2.5	0.68	1.07	-0.04	1.31			15
β-Pinene	0.12	1.26	5.7	1.18	0.94	0.08	2.44			22
β-Phellandrene	0.06	1.01	2.8	1.23	1.22	-0.22	2.24			4
δ-3-Carene	0.18	0.49	0.9	0.37	0.75	0.12	0.86			3
β-Myrcene	0.11	0.50	1.3	0.40	0.79	0.10	0.90			12
3-Octanol	0.05	0.53	3.5	0.96	1.79	-0.42	1.49	0.1	0.5	12
α-Terpinene	0.1	2.27	19.7	6.13	2.71	-3.87	8.40			10
Paracymene	0.09	0.33	0.9	0.26	0.77	0.08	0.59			15
Limonene	0.1	6.30	37.18	8.57	1.36	-2.27	14.87	1.0	3.0	18
1,8-Cineole	0.12	3.98	7.07	2.11	0.53	1.87	6.09	3.0	8.0	26
α-Terpinolene	0.08	0.17	0.38	0.10	0.55	0.08	0.27			11
β-*trans*-Ocimene	0.06	0.11	0.2	0.06	0.54	0.05	0.18			7
γ-Terpinene	0.09	0.38	0.6	0.18	0.49	0.19	0.56			9
cis-Sabinene hydrate	0.33	0.69	1.4	0.48	0.69	0.21	1.17	0.5	2.3	4
Linalool	0.06	0.29	0.5	0.15	0.52	0.14	0.44			14
Isopulegol	0.04	0.57	3	1.08	1.89	-0.51	1.65			7
Menthone	0.01	17.95	40.7	10.16	0.57	7.79	28.10	13.0	28.0	24
Isomenthone	0.08	6.10	14.8	4.25	0.70	1.85	10.36	2.0	8.0	13
Menthofuran	1.12	4.96	11.18	3.09	0.62	1.87	8.05	1.0	8.0	12
Isomenthol	0.17	1.15	6.26	1.72	1.49	-0.57	2.87			11
Neomenthol	1.7	4.66	9.26	2.40	0.52	2.25	7.06	2.0	6.0	11
Menthol	0.84	34.01	53.28	12.72	0.37	21.29	46.73	32.0	49.0	25
Isoneomenthol	0.1	0.53	1.5	0.43	0.83	0.09	0.96			8
4-Terpineol	0.1	0.68	1.5	0.56	0.84	0.11	1.24			5
α-Terpineol	0.14	0.73	3	0.79	1.07	-0.05	1.52			12
trans-Carane	0.07	3.75	10.99	6.27	1.67	-2.53	10.02			3
Pulegone	0.04	1.72	4.36	1.23	0.72	0.48	2.95	0.5	3.0	19
Carvone-D	0.3	17.61	70.26	26.84	1.52	-9.23	44.45			8
cis-3-Hexenyl isovalerate	0.15	0.57	1	0.43	0.74	0.15	1.00			3
Piperitone	0.28	1.13	4.66	1.04	0.92	0.09	2.18			17
Neomenthyl acetate	0.1	0.45	0.9	0.31	0.69	0.14	0.75			7
Menthyl acetate	0.81	7.77	17.4	4.66	0.60	3.10	12.43	2.0	8.0	19
Isomenthyl acetate	0.07	4.69	30.47	11.37	2.43	-6.68	16.06			7
Bicyclogermacrene	0.08	0.34	1.3	0.40	1.18	-0.06	0.74			8
β-Bourbonene	0.15	0.48	1.2	0.31	0.65	0.17	0.79			13
β-Elemene	0.06	0.57	2.48	0.94	1.65	-0.37	1.51			6
β-Caryophyllene	0.08	1.93	7.6	1.69	0.88	0.24	3.62	1.0	3.5	21
β-Copaene	0.1	0.10	0.11	0.01	0.06	0.10	0.11			3
β-Farnesene	0.1	0.39	0.9	0.25	0.65	0.13	0.64			11
α-Humelene	0.08	0.38	0.86	0.31	0.82	0.07	0.70			5
Germacrene D	0.24	1.04	2.05	0.65	0.62	0.39	1.69			15
γ-Cadinene	0.06	1.01	3.7	1.80	1.79	-0.79	2.80			4
d-Cadinene	0.03	0.17	0.35	0.11	0.69	0.05	0.28			6
Spathulenol	0.1	0.46	1.02	0.41	0.91	0.04	0.87			4
Caryophyllene oxide	0.05	0.40	1.37	0.46	1.16	-0.06	0.85			8
Viridifloral	0.05	0.40	1.11	0.34	0.85	0.06	0.73			8
α-Cadinol	0.1	0.16	0.23	0.05	0.33	0.11	0.21			4
Epiglobulol	0.25	0.53	0.8	0.39	0.74	0.14	0.91			2

[a] Min=Mimimum; Ave=Average; Max=Maximum; SD=Standard deviation; Rel SD=Relative standard deviation. 3 samples were excluded from statistical analysis due to clear chemotypic differences.

Table 4. Method comparison.

FID	MS	Column 1	Column 2	Compound Identification	Botanical Indentification	Distillation	Ref.
	X	HP-1	-	MS spectra matching with NIST library.	Leaves of *M. piperita* were collected from the Experimental Halophytes Growing Base of Shandong Academic of Sciences (Jinan, China).	Hydrodistillation Clevenger 6 h	[19]
	X	DB-5 MS	-	Retention times. Co-injection of standards. MS spectra matching with NIST library.	Plant material was cultivated and collected, botanic and genetic certification were obtained from the databank of germoplasm of Embrapa genetic products (Brasilia, Brazil)	Hydrodistillation	[20]
X	X	HP-5MS	-	Retention times. MS spectra matching with NIST and WILEY librairies, and literature data.	Collected at Medicinal Plant Research Center of Barij in June 2011 and authenticated by H. Hosseini; herbarium sample kept under the number 174-1.	Hydrodistillation Clevenger 3 h	[21]
X	X	AB-Innowax 7031428	-	MS spectra matching with NIST05 & WILEY8 libraries and standard compounds.	None – essential oils purchased from a commerical source.	N.D.	[22]
X	X	CP-Wax 52 CB	PE-5	Retention times. MS spectra matching with NIST and WILEY libraries, and literature data.	Fresh samples (*cv. Kukrail*) obtained from an experimental field of Central Institute of Medicinal and Aromatic Plants, Research Centre Pantnagar (India).	Hydrodistillation Clevenger 3 h	[23]
X	X	DB-5 fused silica column	-	Retention times. MS spectra matching with Wiley library.	N.D.	Hydrodistillation Clevenger 2.5 h	[24]
X	X	Fused silica capillary coated with cross-linked methyl silicone gum	-	Retention times. Comparison with authentic samples, MS spectra matching with Wiley/NBS libraries.	N.D.	Hydrodistillation Clevenger 2 h	[25]
	X	HP-5MS	-	MS spectra matching with Wiley 7n and NIST02 libraries.	Identified and deposited in the herbarium of the Department of Cosmetic Science, Chin Nan University of Pharmacy and Science, Tainan, Taiwan.	Steam distillation Clevenger	[26]

Table 4. *(continued).*

FID	MS	Column 1	Column 2	Compound Identification	Botanical Indentification	Distillation	Ref
	X	HP 5MS	-	Retention times. MS spectra matching with authentic samples, and with NIST, NBS, and Wiley libraries.	Confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS), Faculty of Natural Sciences and Mathematics, University of Novi Sad, Serbia.	Hydrodistillation	[27]
X	X	DB-5	-	Retention times. Comparison with authentic samples, MS spectra matching *computer* library.	Collected from Damavand district of Tehran-Iran during June/July 2005, not certified.	Steam distillation Clevenger 1.5 h	[28]
	X	VF-5 fused silica capillary	-	Retention times. MS spectra matching with Wiley & NIST libraries and with literature data.	Taxonomically identified at Department of Biology, Faculty of science, Taif University, KSA.	Hydrodistillation Clevenger 2.5 h	[29]
	X	HP5-MS	-	MS spectra matching *computer* library or standard compounds (similarity index).	N.D.	Hydrodistillation	[30]
X	X	DB5	HP1	Retention times. MS spectra matching with literature (Adams, 2007).	Plants were harvested in September 2013 and were identified in the National Herbarium in Yaounde – Cameroon where voucher specimens were kept.	Hydrodistillation Clevenger	[31]
X	X	BP5	BP-5MS	Retention times. MS spectra matching Wiley library.	Collected the research farm of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, in July 2013. Voucher specimens deposited in the Medicinal Plants Institute Herbarium (MPIH) of Iran.	hydrodistillation Clevenger 3 h	[32]
	X	HP5MS	-	Retention times compared with authentic standards. MS spectra matching with NIST and Wiley libraries.	None – essential oils purchased from a commerical source.	Steam distillation	[33]
	X			Retention times matching with literature and authentic samples. MS spectra matching with NIST08 and W8N08 libraries.	Plants were harvested at their flowering stage and sampled from the National Park of El-Kala, North-East Algeria (El Tarf region, 36°49′N 8°25′), not certififed.	Hydrodistillation Clevenger 3 h	[34]

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Table 4. *(continued).*

FID	MS	Column 1	Column 2	Compound Identification	Botanical Indentification	Distillation	Ref
				Retention times matching with literature and authentic samples. MS spectra matching with NIST08 and W8N08 libraries.	Plants were harvested at their flowering stage and sampled from the National Park of El-Kala, North-East Algeria (El Tarf region, 36°49′N 8°25′), not certifed.	Hydrodistillation Clevenger 3 h	[34]
	X	DB-1	CWX 20M	Retention times.	Plants were harvested at Abomey-calavi (Bénin), not certified.	Hydrodistillation Clevenger	[35]
	X	HP-5 MS		Retention times. Co-injection with standards when available in the laboratory and MS spectra matching with ADAMS, NIST 08 and FFNSC2 libraries.	None – essential oils obtained from a commerical source.	N.D.	[36]
X	X	HP-5	HP-5MS	Retention times. MS spectra matching library or authentic compounds.	Peppermint rhizomes, identified and authenticated by A.R. Khosravi, a plant taxonomist at the Shiraz University, Herbarium, planted, obtained from the Botanical Garden, Shiraz University, Shiraz, Iran).	Hydrodistillation Clevenger	[37]
X	X	HP-5	HP-5MS	Retention times. MS spectra matching with Wiley 275 and NIST/NBS libraries.	None – essential oils obtained from a commerical source.	N.D.	[38]
	X	HP-5 column		Retention times MS spectra matching Tkachev, A.V., Issledovanie letuchikh veshchestv rastenii (The Study of Plant Volatiles), Novosibirsk, 2008.	Grown from the seeds provided by the Russian Research Institute of Essential Oil Plants (Simferopol, RU).	Hydrodistillation 3-4 h	[39]
X	X	DB-5	HP-1	Retention times. MS spectra matching with NIST 98 and Wiley 5 libraries, co-injections with authentic compounds when available.	Authenticated by Prof. V. De Feo (Faculty of Pharmacy, University of Salerno, IT). Voucher specimens deposited at the Herbarium of the Botany chair, Faculty of Pharmacy, Naples University, IT.	Hydrodistillation 3 h	[40]

Table 4. *(continued)*.

FID	MS	Column 1	Column 2	Compound Identification	Botanical Indentification	Distillation	Ref
X	X	AB-Innowax 7031428		MS spectra matching NIST12, NIST62 & Wiley 229 libraries.	None – essential oils obtained from a commerical source.	N.D.	
X	X	CP WAX 52	N.D.	Retention times. MS spectra matching NIST 98 & Wiley 5 libraries, co-injections with authentic compounds when available.	Institute for Roses and Aromatic Plants (IRAP, Kazanlak, Bulgaria)	Hydrodistillation 2 h	[42]
X	X	CPWA X 52 CB		N.D.	Medicinal Plant Program in the Department of Plant, Soil, and Insect Sciences at the University of Massachusetts, Amherst, MA.	Hydrodistillation, Clevenger, 3 h.	
X		DB-Wax		Relative retention times (RT) compared with those of authentic compounds found in the laboratory.	None – essential oils obtained from a commerical source.	N.D.	[44]

Only one reference included genetic testing of the plant material [28].

Lab distillation practices use a Clevenger apparatus as a standard method, this allows for better interlaboratory comparison. However, the lab scale and industrial scale distillation processes differ in temperature and distillation time, as well as distiller materials; glass versus steel, and could result in variations of the final product. If the 6 purchased samples are assumed to be industrially distilled, there is no observable pattern among their variation.

The papers were chosen for the express purpose of agglomerating the chemical profile of *Mentha piperita*. Papers without a table of compounds, and their relative % area in the oil were not included. Likewise, headspace GC analysis was excluded, as the results of the direct injection and headspace methods can vary. The methods were in general agreement as far as extraction and analysis methods, leaving the oil as the major variable. Unfortunately, species identification and chemotyping appear to be the least standardized practice in the evaluation of mint. As this is not a botany article, the only recommendation herein would be to increase the genetic testing of plant matter in conjunction with botanical authentication, including microscope analysis. As for the essential oil publications, identification of chemotype and analysis of multiple samples should be standard methodological practice [45-47]. Analytical chemists know that triplicate analysis is imperative, however, in the analysis of natural products, it seems the instrumental triplicate has overshadowed the sampling triplicate. To be concrete, the sample plant material should be collected in triplicate and distilled into 3 samples, in addition to single distillates analyzed by GC in 3 injections.

4. Conclusions

A set of 26 publications selected between 2003 and 2018 dealing with *Mentha piperita* essential oil chemical composition were examined and compared with the corresponding ISO norm. A relatively large dispersion was observed both qualitatively (171 different compounds listed) and quantitatively (relative proportion in the essential oil). The ISO norm should not be taken as a summary of existing literature data, and this is indeed how they are built, relying on a technical committee capturing the most relevant descriptor of products on the market, norms being intended primarily for business use. It would be relevant to have more frequent updates of the norms and to include data from the latest scientific literature. Although the publications were relatively recent, not all adopted generally accepted standards of good practices in the field. None neither considered predicted or corrected response factors for quantification in GC-FID.

Besides analytical considerations to explain the dispersion of results, when a plant can contain several chemotypes, and is closely related to similar plants, botanical characterization is imperative. Genetic profiling, although not widespread and presenting some risks of false positives, has been used to detect adulteration issues. Conversely, it may be worth questioning why the market value for *M. piperita* is higher than that of *M. arvensis* if the chemical composition and oil quality are thus closely related.

Authors' contributions

Conceptualization, M.P. S.A.; Methodology, M.P., S.A.; Investigation, M.P., S.A.; Resources, S.A.; Data curation, M.P., S.A.; Writing – original draft preparation, M.P., S.A.; Writing – review and editing, S.A.; Supervision, S.A.; Project administration, S.A.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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