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REVIEW ARTICLE

The peel essential oil composition of bergamot fruit (*Citrus bergamia*, Risso) of Reggio Calabria (Italy): a review

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ABSTRACT

Bergamot (*Citrus bergamia*, Risso) grows exclusively in the province of Reggio Calabria (Southern Italy). Most part of this territory lies on by the Ionian or the Tyrrhenian sea, with over 700 kilometers of coastline, it has one of the highest values of heliophany in Italy and it is protected, from the Northern winds, by the Aspromonte mountain massif. Most part of the bergamot cultivation area is located between Scilla (West) and Monasterace (East) in a coastal strip with a width up to 12 km from the coast. Bergamot fruit is produced between November and March in relation to the geographical area. Over the last decades, environmental climate changes occurred also in this area. This phenomenon, together with new applied industrial processes may have influenced the bergamot essential oil composition. In addition, the modern analytical apparatus gives more detailed information. The aim of this review is to describe the evolution of studies on the volatile fraction of the bergamot peel essential oil over the years.

Keywords: Flavedo; Flavour; Limonene; Linalyl acetate; Volatiles; Extraction methods

INTRODUCTION

Bergamot (*Citrus bergamia*, Risso) is a citrus fruit (Figures 1-7) of great interest whose production area is in continuous expansion.

Today it represents an important productive and economic component in the province of Reggio Calabria (Italy), despite the past period of productive crisis which has led to the drastic reduction of cultivated areas (Table 1) and the minimising of cultivation practices necessary to maintain the quality of the fruit (Nesci et al., 2010; Strano et al., 2017). Bergamot fruit goes through a moment of renewed interest and all the productive aspects show satisfactory growth rates, for example the price paid to growers. This positive moment is highlighted by the production trend which is very satisfactory although it can certainly be increased. Recently were recovered many agricultural areas dedicated to the cultivation of bergamot which in the past were partially abandoned (Sicari et al., 2016a).

In the past decades, bergamot fruit was produced almost exclusively for the extraction of the peel essential oil, as well as for ornamental purposes. Today, there is a new interest in bergamot juice and other derivatives fruit because of their nutraceutical properties (Di Donna et al., 2009; Di Donna et al., 2011; Di Donna et al., 2013; Di Donna et al., 2020; Sicari et al., 2016b; Giuffrè, 2019; Giuffrè et al. 2019), however the bergamot peel essential oil (BEO) remains, still today, the most requested bergamot derivative. The protected designation of origin "Bergamot of Reggio Calabria - essential oil" is reserved with essential oil of bergamot that meets the conditions and requirements established in a production specification (PDO, bergamot essential oil, 2015) Table 1.

The applications of the bergamot peel essential oil are mainly in perfumery, medicine, cosmetics manufacture and food flavouring. Bergamot peel essential oil has been recognised for its antimicrobial, anti-inflammatory, analgesic and anti-proliferative properties (Navarra et al., 2015b), neuroprotective effect as it reduces neuronal damage caused in vitro by excitotoxic stimuli (Corasaniti et al., 2007), anticancer activity on neuroblastoma cells (Celia et al., 2013; Navarra et al., 2015a), antimicrobial

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Fig 1. A bergamot tree (*Castagnaro* cv).

Fig 2. Leaves and fruits of a bergamot tree (*Castagnaro* cv).

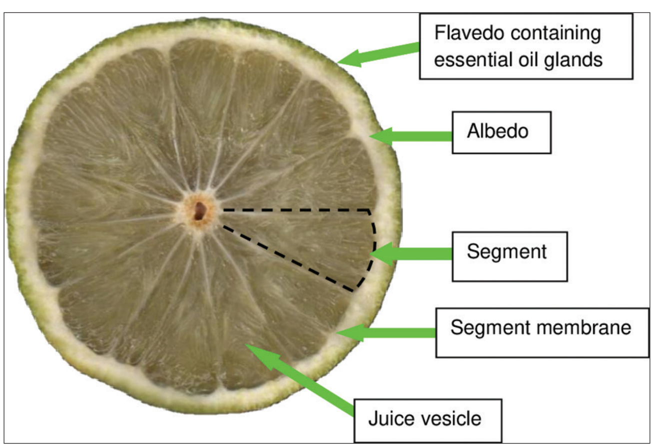

Fig 3. Cross section of a bergamot fruit.

activity against bacteria, fungi and larvae (Cirmi et al., 2016), neurobiological effects mainly for oxygenated compounds such as linalool and linalyl acetate (Bagetta et al., 2010), in aromatherapy is used to minimise the symptoms of cancer pain (Forlot and Pevet, 2012) moreover a mixture

Fig 4. The bergamot fruits are loaded by the conveyor belt for the washing process.

Fig 5. The peel of the bergamot fruits is scraped to extract the essential oil.

Fig 6. The bergamot essential oil is separated from water and impurities by means of a centrifuge.

of essential oils inhaled before sleeping was found to have a beneficial effect for patients in cardiac rehabilitation (McDonnell and Newcomb, 2019).

Table 1: Production of bergamot fruit in the Region of Calabria (ISTAT, 2020)

Year	Growing area (hectares)	Fruits produced (q)	Fruits harvested (q)
2010	1,461	254,736	254,736
2012	1,800	423,000	401,850
2013	1,800	423,000	410,000
2014	1,800	430,200	430,200
2015	1,500	300,000	298,000
2016	1,500	375,000	372,000
2017	1,500	187,500	187,500
2018	1,500	300,000	300,000

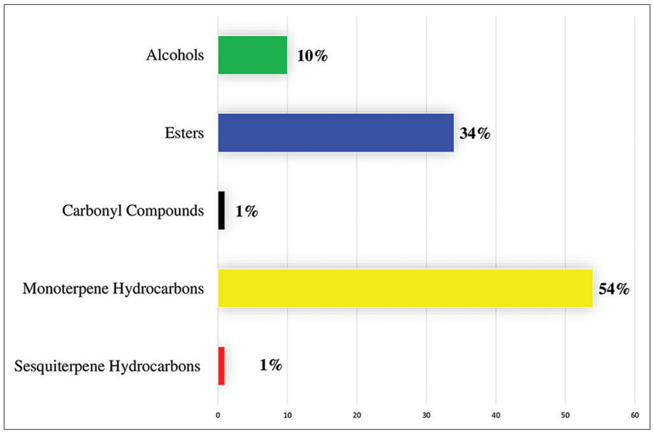

Fig 7. Volatile composition (%) of the bergamot essential oil. Data are the means of values from scientific works listed in the table 2.

Over the decades, bergamot essential oil extraction techniques have changed, as well as the analytical techniques, this allowed to improve the detection of compounds in bergamot essential oil. In addition, the climatic conditions varied over the years, with an increasing average temperature and a decreasing rainfall and it is well known that the microclimatic and environmental conditions influence the chemical properties of a vegetable (Abewoy, 2018). All this being said and considered, the purpose of this work is to describe the evolution of the analytical results that have been published over time with respect to the bergamot fruit peel essential oil composition from Reggio Calabria (South Italy), since 1984 to 2019.

MATERIALS AND METHODS

The essential oil extraction

The cross section is that typical of a citrus fruit (Figure 3). The peel essential oil extraction process was widely modified and improved over years. In the past, the extraction process of the bergamot essential oil was carried out for a long time by hand with the ‘sponge process’ which consisted in the following phases: cutting the fruit in two halves, extracting the pulp and then compressing the peel with a rotary movement of the hand against a natural sponge in order to make the essence sprout from oil glands contained in the peel. From the sponges, the essential oil, together with the whole liquid phase coming from the peel, was squeezed into a ‘concolina’ (i.e. a specific tool) and separated by decantation. Today almost all the industrial plants processing bergamot fruits for the essential oil extraction apply a cold extraction system consisting of peeling machines coupled with a water shower. The peel essential oil extraction process consists of an automatic system for the loading and unloading of fruits (Figure 4). Once inside, the fruits are sprayed with water, which allows a better extraction, and then they are conveyed in adherence to rough walls similar to large graters. The peel is scraped off, the oil glands break and the essential oil comes out and it is removed along with the water and any other debris (parts of peels) that have formed from this continuous rubbing of the flavedo (Figure 5). The essential oil is immediately separated from water and solids, by centrifugation (Figure 6). This process is carried out in a room that is poorly lit by sunlight to reduce the risk of oxidation but it would be advisable to evaluate the possibility of operating in a controlled atmosphere to reduce the presence of oxygen also in this case to counteract oxidation. At this point, the obtained essential oil, is subjected to chemical analysis and it is stored in specific sealed and labelled steel containers before to be sold. In the past years it was studied a different extraction technique involving the supercritical carbon dioxide extraction which was found to obtain an aroma fraction very similar to that of the fresh bergamot juice (Sicari and Poiana, 2017). The application of this technique moved by the need to reduce the dimension of the extraction plant and by the possibility to remove bergapten during essential oil extraction but this process was very expensive and not yet applied in an industrial scale so far.

RESULTS

The essential oil composition

Citrus essential oils are of considerable importance not only for their use in the agro-food, pharmacology and perfume industries, but also for scientific uses. Essential oils are contained in different tissues of citrus plants, first of all in the fruit flavedo (the peel) but also in the leaves in the buds and in the flowers. The qualitative and quantitative composition of essential oil is influenced by many factors, such as the climatic condition, the graft holder, the cultural practices, the age of the plant, the degree of fruit ripening, the extraction technique, but above all it depends by the genotype (Branca, 2012). The bergamot essential oil differs from the other citrus essential oils for the high content of oxygenated compounds about 38% (mainly esters and alcohols), and

Table 2: Volatile components of the bergamot peel essential oil expressed as % of the peak areas: Belsito et al., 2007[1]; Costa et al., 2010[2]; Di Giacomo, and Mincione, 1994[3]; Dugo et al., 1987[4]; Dugo et al., 2012[5]; Gionffrido et al., 2000[6]; Gioffrido et al., 2003[7]; Siano and Cautela, 2012[8]; Verzera et al., 1996[9] (* in the table is included the sum of sabinene + β-pinene); Russo et al. 2012[10] (* in the table octanal is given as the sum with α-Phellandrene); Giuffrè and Nobile, 2020[11]; Sawamura et al., 2006. (In bold is reported the harvest year)

	Compound	1987[4] 1984-1985	1994[3] 1984-1992	1998[9] 1996-1997	2000[6] 1998-1999	2003[7] 2000-2001	2006[12] 2000	2007[1] 2005-2006	2012[5] 2008-2009	2012[5] 2009-2010	2010[2] 2009	2012 2010-2011	2012[8] 2010-2011	2012[10] 2011	2020[11] 2018-2019 Castagnaro	2020[11] 2018-2019 Fantastico	2020[11] 2018-2019 Femminello
1	Tricyclene	-	0.004	<0.01	-	0.001	-	0.00	<0.01	<0.01	0.01	<0.01	0.000	-	0.01	0.01	0.01
2	α-Thujene	0.361	0.357	0.325	0.332	0.175	-	0.27	0.33	0.29	0.24	0.29	0.180	0.31	0.22	0.32	0.11
3	α-Pinene	1.375	1.354	1.240	1.343	0.678	1.3	1.01	1.23	1.11	0.95	1.11	0.690	1.16	0.53	0.21	0.33
4	Camphene	0.039	0.038	0.035	0.034	0.018	tr	0.02	0.03	0.03	0.03	0.03	0.020	0.03	0.04	0.07	0.02
5	Sabinene	1.324	1.278	8.750*	0.978	0.720	1.1	0.91	1.04	1.01	0.81	1.04	0.750	1.01	0.66	0.48	0.47
6	β-Pinene	7.836	7.517	8.750*	7.083	4.380	6.2	5.20	6.01	5.84	5.08	5.94	6.450	5.75	4.12	5.22	5.78
7	6-Methyl-5-hepten-2-one	-	0.006	0.010	0.006	<0.001	tr	-	0.02	0.02	0.02	0.02	-	0.01	0.01	0.01	0.01
8	Myrcene	0.959	0.998	0.930	1.152	0.879	0.8	1.10	1.09	0.99	0.83	0.90	0.910	1.04	0.73	0.57	0.54
9	Octanal	0.053	0.050	0.055	0.048	0.020	tr	0.05	0.04	0.05	0.05	0.05	0.020	0.06	0.04	0.04	0.03
10	α-Phellandrene	0.030	0.030	0.030	0.054	0.022	tr	0.10	0.03	0.02	0.02	0.03	0.020		0.07	0.09	0.01
11	Esile Acetate	-	-	<0.01	-	-	-	-	-	-	0.02	-	-	-			
12	δ-3-Carene	-	0.003	-	0.007	0.003	-	<0.01	<0.01	<0.01	-	<0.01	-	-	0.01	0.01	tr
13	α-Terpinene	0.018	0.171	0.170	0.149	0.120	-	-	0.10	0.13	0.10	0.14	0.120	0.16	0.23	0.35	0.28
14	p-Cymene	0.130	0.236	0.365	-	0.113	1.4	0.18	0.37	0.140	0.33	0.09	0.120	0.36	0.17	0.30	0.28
15	Limonene	38.387	39.722	35.395	41.398	38.176	37.2	32.52	44.48	41.92	38.89	36.83	38.340	42.44	39.40	35.03	31.10
16	β-Phellandrene	-	-	-	-	-	0.2	-	-	-	-	-	-	-			
17	Eucalyptol	-	-	-	-	-	tr	-	-	-	-	-	-	-			
18	(Z)-β-Ocimene	0.023	0.025	0.030	0.221	0.024	tr	0.05	0.06	0.05	0.04	0.05	0.020	0.03	0.06	0.11	0.09
19	(E)-β-Ocimene	0.254	0.253	0.245	0.221	0.199	-	0.21	0.24	0.19	0.16	0.24	0.220	0.23	0.25	0.24	0.16
20	γ-Terpinene	8.458	8.350	8.210	8.638	6.155	6.8	5.49	7.53	6.91	5.62	6.99	7.220	7.20	7.42	5.00	8.13
21	(E)-Linalool oxide	-	-	-	-	-	tr	-	-	-	-	-	-	-			
22	cis-Sabinene hydrate	0.038	0.039	0.030	0.038	0.032	0.1	-	0.04	0.04	0.03	0.04	0.031	-			
23	Octanol	0.002	0.002	0.010	-	-	-	0.10	-	-	-	-	<0.001	tr	0.09	0.07	0.05
24	Terpinolene	0.359	0.350	0.335	0.300	0.297	0.3	-	0.28	0.28	0.21	0.30	0.300	-	0.42	0.41	0.31
25	trans-Sabinene hydrate	-	0.022	-	0.038	0.032	-	0.00	0.00	0.00	0.00	0.00	0.001	0.03			
26	(E)-Solanone	-	-	-	-	-	0.1	-	-	-	-	-	-	-			
27	Linalool	8.571	7.756	13.155	6.079	7.417	8.8	14.41	7.04	9.65	6.55	12.01	7.540	9.98	6.00	8.67	9.00
28	Nonanal	0.038	0.035	0.040	0.000	0.021	tr	-	0.03	0.04	0.03	0.05	0.030	0.03	0.03	0.03	0.01
29	4,8-dimethyl-1,3(E),7-nonatriene	-	-	-	-	-	-	-	0.01	<0.01	-	0.01	-	-			
30	Hepthyl acetate	-	-	0.020	-	-	-	-	0.01	<0.01	0.01	0.01	-	-	0.01	0.01	0.01
31	Citronellal	0.016	0.015	0.020	0.020	0.025	-	-	0.01	0.01	0.01	0.01	0.020	-			
32	cis-Limonene oxide	-	0.005	<0.01	0.008	0.009	-	-	0.01	<0.01	-	<0.01	0.010	tr	0.01	0.02	0.04
33	trans-Limonene oxide	-	0.005	<0.01	0.003	0.008	-	-	0.01	<0.01	0.01	<0.01	0.010	tr	0.02	0.01	0.02
34	Camphor	-	0.003	0.010	-	0.004	-	-	<0.0	<0.01	-	<0.01	0.010	-	0.01	0.01	0.01
35	Isopulegol	-	0.003	<0.01	-	0.003	-	-	<0.01	<0.01	-	<0.01	-	-			
36	cis-isocitral	-	-	-	-	-	-	-	<0.01	<0.01	-	<0.01	-	-			
37	Terpinen-4-ol	0.019	0.022	0.020	0.025	0.021	tr	0.05	0.03	0.02	0.03	0.02	0.020	0.03	0.04	0.04	0.09
38	α-Terpineol	0.055	0.059	0.085	0.066	0.042	0.1	0.13	0.09	0.10	0.07	0.09	0.040	0.09	0.08	0.07	0.07
39	Decanal	0.066	0.006	0.075	0.063	0.054	tr	0.07	0.05	0.05	0.06	0.06	0.050	0.05	0.06	0.01	0.11
40	Dodecane	-	-	0.010	-	-	-	-	-	-	-	-	0.100	-			
41	Octyl acetate	0.011	0.114	0.135	0.113	0.099	-	0.14	0.10	0.09	0.13	0.10	-	0.10	0.11	0.11	0.10

(Contd...)

Table 2: *(Continued)*

	Compound	1987[4] 1984-1985	1994[3] 1984-1992	1998[9] 1996-1997	2000[6] 1998-1999	2003[7] 2000-2001	2006[12] 2000	2007[1] 2005-2006	2012[5] 2008-2009	2012[5] 2009-2010	2010[2] 2009	2012 2010-2011	2012[8] 2010-2011	2012[10] 2011	2020[11] 2018-2019 Castagnaro	2020[11] 2018-2019 Fantastico	2020[11] 2018-2019 Femminello
42	Nerol+Citronellol	0.041	0.038	0.050	0.028	0.028	-	0.17	0.02	0.04	0.02	0.05	0.030	0.08	0.02	0.06	0.09
43	Neral	0.229	0.228	0.190	0.216	0.223	0.2	0.14	0.19	0.19	0.18	0.20	0.230	0.19	0.12	0.31	0.44
44	cis – Sabinene hydrate acetate	-	0.089	10.970	-	0.087	0.1	0.06	-	-	-	-	0.090	0.08			
45	Carvone	-	-	-	-	-	-	-	0.01	<0.01	0.01	0.01	-	-	0.01	0.01	0.01
46	Linalyl acetate	28.118	27.946	41.360	27.929	36.479	30.1	31.01	26.13	28.01	37.00	30.62	32.470	26.30	33.40	37.73	38.61
47	Geranial	0.361	0.363	-	0.338	0.339	-	0.69	0.29	0.29	0.28	0.29	0.340	-	0.68	0.26	0.39
48	Perillaldehyde	-	-	-	-	-	-	-	0.01	<0.01	<0.01	<0.01	<0.001	-			
49	Isobornyl acetate	0.018	0.019	0.020	0.023	0.019	-	-	0.02	0.01	0.02	0.01	0.020	tr	0.02	0.02	0.03
50	Ascaridole	-	-	-	-	-	-	-	<0.01	<0.01	-	<0.01	-	-			
51	Undecanal	0.007	0.008	0.020	0.011	0.016	-	-	0.02	0.01	0.01	0.01	0.010	-			
52	Nonyl acetate	0.017	0.018	0.020	0.020	0.034	-	-	0.02	0.02	0.02	0.02	0.030	-	0.11	0.05	0.07
53	Methyl geranoate	-	0.005	0.010	0.005	0.008	-	-	0.01	<0.01	0.01	<0.01	<0.001	tr	0.02	0.01	0.01
54	Linalyl propionate	0.032	0.032	0.040	0.024	0.003	-	-	0.03	0.03	0.03	0.04	0.060	0.04	0.05	0.04	0.03
55	2-Decen-1-al	-	0.000	0.105	-	-	-	-	-	-	-	-	-	-			
56	δ-Elemene	-	0.029	0.010	0.023	0.067	-	-	0.01	0.01	0.01	0.02		-	0.02	0.03	0.02
57	α-Terpenyl acetate	0.169	0.172	0.145	0.206	0.210	0.2	-	0.17	0.14	0.19	0.14	0.220	0.15	0.31	0.34	0.15
58	Geranial + Perialdehyde	-	-	0.225	0.000	-	-	-	-	-	-	-	-	-			
59	Indole	-	-	0.010	0.000	-	-	-	-	-	-	-	-	-			
60	Citronellyl acetate	0.022	0.027	0.020	0.397	0.018	0.1	-	0.05	0.01	-	0.01	0.020	0.03	0.11	0.03	0.09
61	Neryl acetate	0.303	0.345	0.385	0.494	0.392	tr	0.28	0.42	0.36	0.41	0.35	0.410	0.42	0.67	0.26	0.50
62	Geranyl acetate	0.366	0.381	0.035	-	0.393	-	0.57	0.42	0.30	0.42	0.25	0.390	0.40	0.74	0.52	0.32
63	β-Elemene	-	-	-	-	-	-	-	0.01	0.01	<0.01	<0.01	-	-			
64	cis-α-Bergamotene	0.306	-	0.030	-	0.004	tr	-	0.02	0.02	0.02	0.02	-	-			
65	α-Humulene	0.028	0.027	0.020	-	0.037	tr	-	-	-	-	-	-	0.03			
66	Dodecanal	-	0.000	0.290	-	0.028	-	0.21	<0.01	<0.01	-	<0.01	0.310	0.05	0.09	0.09	0.03
67	Decyl acetate	-	0.031	0.260	-	0.039	tr	0.03	0.04	0.03	0.05	0.03	0.400	-	0.06	0.07	0.08
68	β-Caryophyllene	0.369	0.352	0.230	0.385	0.406	0.3	0.55	0.35	0.35	0.23	0.32	0.420	0.39	0.25	0.32	0.30
69	trans-α-Bergamotene	-	0.301	0.260	0.355	-	0.3	-	0.31	0.29	0.22	0.26	<0.001	0.32	0.46	0.54	0.55
70	cis -β-Farnesene	-		0.310	-	0.107	0.1	0.45	0.01	0.01	0.01	0.01	0.10	0.07	0.02	0.01	0.01
71	Germacrene D	-	0.061	0.110	0.062	0.118	tr	-	0.04	0.04	0.03	0.05	0.130	tr	0.11	0.21	0.08
72	α-Farnesene	-	0.039	<0.01	-	0.053	-	-	0.05	0.05	<0.01	0.06	0.050	0.03			
73	β-Santalene	-	-	-	-	-	-	-	0.01	0.01	0.01	0.01	-	-			
74	γ-Curcumene	-	-	-	-	-	-	-	<0.01	0.01	-	0.01	-	-			
75	trans-β-Bergamotene	-	-	-	-	-	-	-	0.02	0.01	0.01	0.01	-	-	0.04	0.02	0.01
76	Bicyclogermacrene	-	-	-	-	-	-	-	0.02	0.02	0.01	0.02	-	-	0.01	0.01	0.01
77	(Z)-α-Bisabolene	-	-	-	-	-	-	-	0.04	0.03	0.03	0.03	-	-	0.06	0.08	0.07
78	β-Bisabolene	0.437	0.426	0.360	0.535	0.608	-	-	0.047	0.44	0.33	0.39	0.650	0.46	0.83	0.73	0.31
79	(Z)-γ-Bisabolene	-	-	-	-		-	-	<0.01	<0.01	<0.01	0.01	<0.001	-			
80	Tridecanal	-	-	-	-	0.014	-	-	-	-	-	-	-	-			
81	δ-Cadinene	-	-	-	-	-	-	-	<0.01	<0.01	<0.01	<0.01	<0.01	-			
82	Germacrene B	-	0.012	0.010	-	-	-	-	-	-	-	-	-	tr			
83	β – Sesquiphellandrene	-	0.007	-	-	0.047	-	-	0.02	<0.01	<0.01	<0.01	-	0.02			

(Contd...)

Table 2: *(Continued)*

	Compound	1987[4] 1984-1985	1994[3] 1984-1992	1998[9] 1996-1997	2000[6] 1998-1999	2003[7] 2000-2001	2006[12] 2000	2007[1] 2005-2006	2012[5] 2008-2009	2012[5] 2009-2010	2010[2] 2009	2012 2010-2011	2012[8] 2010-2011	2012[10] 2011	2020[11] 2018-2019 Castagnaro	2020[11] 2018-2019 Fantastico	2020[11] 2018-2019 Femminello
84	(E)-γ-Bisabolene	-	-	-	-	-	-	0.47	0.02	<0.01	<0.01	0.01	-	-			
85	(E)-α-Bisabolene	-	-	-	-	-	-	-	0.01	0.01	0.01	0.01	-	-			
86	cis-Sesquisabinenehydrate	-	-	-	-	-	-	-	<0.01	<0.01	<0.01	<0.01	0.050	-			
87	(E) -Nerolidol	-	0.019	0.020	-	0.026	-	0.02	0.03	0.03	0.030	0.02	0.030	tr	0.04	0.03	0.03
88	Spathulenol	-	-	-	-	-	-	-	0.01	0.01	0.010	0.00	-	-			
89	trans-Sesquisabinenehydrate	-	-	-	-	-	-	-	0.01	<0.01	<0.01	<0.01	-	-			
90	2,3-Dimethyl-3-(4-methyl-3-pentenyl)-2-norbonanol	-	0.011-	0.015	-	-	-	-	0.01	0.01	-	0.01	-	-			
91	Tetradecanal	-	0.007	-	-	0.044	-	-	-	-	-	-	0.040	0.02			
92	Campherenol	-	0.015	0.015	-	0.011	-	-	0.01	0.01	0.01	0.01	0.010	0.02	0.02	0.01	0.01
93	α-Bisabolol	-	0.016	0.015	-	0.010	-	0.01	0.02	0.02	0.02	0.02	0.010	0.02	0.02	0.01	0.01
94	Nootkatone	-	0.031	0.050	0.059	0.063	-	-	0.05	0.05	0.07	0.05	0.060	-	0.09	0.02	0.06

for the hydrocarbon content, about 62%, clearly lower than that of the other oils (Ahmed et al., 2019; Ainane et al., 2018; Al Jabri and Hossain, 2018). It has a pleasant aroma similar to a fresh fruit. It is composed of a volatile fraction 93-96% and a non-volatile residue 4-7%.

The main constituents of the non-volatile fraction of the bergamot essential oil are coumarinic and psoralenic compounds: about 2-4% of this essential oil. They are heterocyclic nucleus molecules containing oxygen. The most representative is citropten (5,7-dimethoxycoumarin), bergaptene, bergamottin and 5-geranyloxy-7-methoxycumarin. The non-volatile fraction is very important because it influences the olfactory characteristics of the essence, acting as a natural fixative of the perfumes, providing indications and useful information on the quality of the essential oil (Statti et al, 2004). The essential oil for cosmetic industry is deprived of constituents such as bergaptene, a furocumarin with a high photo-reactivity and its mutagenic activity increases if exposed to light. This exposure, if prolonged, could cause even deep skin burns and the onset of melanoma (Patel et al., 2009). Bergamot essential oil was proved to have analgesic, antimicrobial, anti-proliferative, and anti-inflammatory effects, and also positive effects on the central nervous and cardiovascular systems (Navarra et al., 2015).

The volatile fraction of the bergamot peel essential oil (Figure 7) contains monoterpene and sesquiterpene hydrocarbons and oxygenated derivatives (alcohols, esters, aldehydes and oxides), small quantities of aliphatic alcohols, aldehydes and also esters, which can vary within ranges more or less wide.

The first compound to be identified in the bergamot peel essential oil was δ-limonene by Wallach in 1885, followed by linalyl acetate by Bertram and Walbaum and Semmler and Tieman in 1892, and linalool by Elze in 1910.

These three compounds are also the main ones from a quantitative point of view (Dugo et al., 1991). The volatile fraction of bergamot essential oil differs significantly from that of other citrus peel oils due to the large amount of oxygenated compounds, mainly linalyl acetate and linalool, which can be more than 50% of the whole volatile fraction. In all citrus peel oils, the oxygenated volatile compounds vary from 1% in sweet orange, grapefruit and clementine, to 6% in lime oils (Di Giacomo and Mincione 1994). A relevant aspect of the composition of the volatile fraction of bergamot peel essential oil is its variability in quantitative data, in fact it is characterised by a limonene content which is about 34% lower than that of all the other citrus peel essential oils and a high content of linalool (around 6-10%) and linalyl acetate (around 33%).

Volatile composition variation over the years

In the table 2 are shown the bergamot peel essential oil components of the volatile fraction of oils described in the scientific studies carried out from the 1984-1985 until the 2018-19 harvest year. In the first published studies, only about 50 compounds were identified. Most of the studies on the bergamot peel essential oil composition are dedicated to the quantitative determination of the identified components. Gas chromatography techniques have evolved over time together with the development of knowledge on the composition of the volatile fraction of bergamot oil and that of other citrus oils. In the past, gas-chromatographic instruments were coupled with packed columns, then with steel capillary columns, then with glass capillary columns, and finally with fused silica capillary columns that are actually used. As regards the instrumentation, the gas chromatographs coupled with flame ionisation detector (FID) were replaced by gas chromatographs coupled with mass spectrometry (GC / MS) and multidimensional techniques which recently have allowed the highlighting of a greater number of compounds in the bergamot essential oil, and are identified by the use of comparative libraries but also (in the most doubtful cases) by comparison with pure standard substances injected individually or in groups, or by the 'addition method', that is conducted by adding pure single substances to an oil to be analysed to verify which peak increases in size. Bergamot peel essential oil has also been used as a matrix to develop and optimize advanced methods and analytical techniques (Cartoni et al., 1987; Micali et al., 1990). Since 1987, Mazza (1987), through the coupling of mass spectrometry and gas chromatography, identified many components of the bergamot peel essential oil.

As can be seen in table 2, from the 1996/97, much more compounds were revealed than in previous years, this was due to the evolution of instrumental analysis techniques. Later, Mondello et al. (1994, 1995, 1998) using HPLC-HRGC and HRGC-MS interactively, by capillary column SE-52 and Carbowax 20M, further improved the separation between the compounds in the chromatographic profile and therefore, consequently the knowledge on this essential oil.

In this review, the analytical results of the volatile components in bergamot essential oil from the 80s to 2018-2019 were studied in order to compare the percentage of each compound identified and to know its variation over 35 years of studies. The quantitative differences can be related to: i) climatic variations that occurred in the last decades; ii) difference in the composition existing in the three cultivars (*Castagnaro*, *Fantastico* and *Femminello*) and as a consequence of the genetic characters; iii) harvest time of fruits; iv) cultivation area; v) analytical techniques. In all the published studies, the most represented compound was limonene, a hydrocarbon monoterpene ranging between 31.10% and 44.48% (Table 2). If the ripening stage is considered, the lowest limonene content (27.38%) was found in the bergamot fruits picked in the second half of December and the highest content (52.02%) was found in the fruits picked in the second half of February 16-28d. More in detail, limonene showed a slight increase during ripening of bergamot fruit and accounted for 37-38.5% from 1st of December to 15th of February and accounted for 39.1-39.3% from February, 16 to March, 15 (Dugo et al., 1987), in similar amount to other citrus fruits as *Citrus aurantifolia* peel oil (Venkanteshwarlu and Selvaraj, 2000).

The second compound by quantity was linalyl acetate, grouped with esters and detected for a minimum quantity of 17.07% in the sampling of February 16-28d and for a maximum of 40.37% in the sampling of March 1-15d (Dugo et al., 1987). Other studies on bergamot peel essential oil report average linalyl acetate values ranging between 26.13% and 41.36% (Table 2). Linalyl acetate showed a more evident increasing trend with respect to linalool during bergamot fruit ripening, in fact it increased constantly with the following trend: 24.50% > 25.24% > 27.49% > 28.05% > 29.47% > 29.30% > 30.50% from 1-15d of December to 1-15d of March (Dugo et al., 1987), similarly to findings of other studies on *Citrus aurantifolia* peel oil (Venkanteshwarlu and Selvaraj, 2000). It is well known that bergamot is cultivated almost exclusively in the Reggio Calabria province (Southern Italy), however a study was published on bergamot plants growing in Turkey, in which it was not specified the bergamot cultivar and plants were not identified by a botanist. In this Turkish peel essential oil, it was found a high linalyl acetate content (38.7%) (Kirbaslar et al., 2000), i.e. in a higher quantity if compared with the peel essential oil of Reggio Calabria (Table 2).

The third compound by quantity was linalool which is also the main alcohol and results showed a content of 1.91% of linalool in the first fifteen days of February and a content of 20.26% in the last 15 days of December (Dugo et al., 1987) with average values between 6.00% and 14.41% (Table 2). It can be observed that if the minimum value of linalool is considered, it shows a different content during the harvest year, varying between 1.91% (1-15 February) and 6.07% (1-15 December) (Dugo et al., 1987). Oxygenates compounds are important flavouring molecules which are found in high quantity when the peel of citrus fruits is green whereas they decrease in quantity when the peel turns yellow; this was found in essential oil of grape fruit peel (Moshonas, 1971) and in the *Citrus aurantifolia* Swingle peel oil (Venkanteshwarlu and Selvaraj, 2000). In addition, the bergamot peel is green in October and gradually it changes its colour and turns to yellow (more or less strong) in March. According to these findings, the linalool content decreased with bergamot ripening and the mean quantity varied with this sequence: 12.12% > 11.31% > 10.59% > 8.49% >7.17% > 6.44% > 6.07% from the first two weeks of December to the first two weeks of March (Dugo et al., 1987).

γ-Terpinene (a monoterpene hydrocarbon) is another representative component and showed a low variability between the minimum quantities detected during fruit maturation from 5.84% (1-15 February) to 7.54% (1-15 December). Also the maximum quantities of γ-terpinene had a low variability ranging between 9.51% (1-15 December) and 11.38% (1-15 February) (Dugo et al., 1987). The γ-terpinene was quantified between 5.00% and 8.64% in the 35-year study (Table 2).

The β-pinene (monoterpene hydrocarbon) content was stable during fruit ripening and it was closed between 7.99% (1-15 December) and 7.67% (1-15 March), (Dugo et al., 1987). In the studies conducted over the years, β-pinene was found to be from a low 4.12% to a high 7.84% (Table 2) in higher quantity than the 3% found in the Turkish bergamot peel oil (Kirbaslar et al., 2000), in higher quantity than in other Citrus peel essential oil of grapefruit (0.1%) (Pino and Sánchez, 2000), and in lower quantity than in *Citrus aurantifolia* peel oil (13.5-23.8%), (Venkanteshwarlu and Selvaraj, 2000).

The fourth predominant monoterpene hydrocarbon was α-pinene whose mean content showed a low variability during bergamot fruit ripening and varied from 1.39% (1-15 December) to 1.35% (1-15 March). The minimum values were closed between 0.97% on the 1-15d of January sampling period and 1.24% on the 1-15d of December sampling period (Dugo et al., 1987). In the studies conducted until 2019 harvest year, it was found an α-pinene content ranging between 0.21% and 1.38% (Table 2).

The fifth predominant monoterpene hydrocarbon was sabinene which was found in lowest quantity in the most recent published paper 0.47-0.66% (Table 2) and whose mean content studied during bergamot ripening was found to range between 1.37% on 1-15d of December and 1.26% on 1-15d of March (Dugo et al., 1987). Sabinene quantity in bergamot peel essential oil is higher than in grapefruit peel essential oil of fruits cultivated in India 0.44-0.83% (Ahmed et al., 2019), or than in lemon peel essential oil 0.25% of fruits cultivated in Sicily (Southern Italy) (Bonaccorsi et al., 2009), or than in mandarin peel essential oil of fruits cultivated in India 0.08% (Goyal and Kaushal, 2018).

The sixth predominant monoterpene hydrocarbon was myrcene with a minimum of 0.54% and a maximum of 1.152% (Table 2), in very lower amount with respect to 4.0-6.24% in peel oil of grapefruit cultivated in India (Ahmed et al., 2019), and in similar amount with respect to peel oil of *Citrus aurantium* (0.6%) and to peel oil of *Citrus reticulata* (1.5%) grown in Morocco (Ainane, 2018).

All other volatile components were found in quantity lower than 1% (Table e 2).

In the group of monoterpene hydrocarbons were detected: α-thujene (0.11-0.36%), camphene (0.018-0.039%), α-phellandrene (0.01-0.054%), δ-3-carene (tr-0.01%), α-terpinene (0-0.35%), terpinolene (0-0.42%), (Z)-β-ocimene (0.02-0.11%), (E)-β-ocimene (0.16-0.25%), (Table 2).

In the group of aliphatic hydrocarbons, the following elements were found: dodecane (0-0.1%), and in the group of aromatic hydrocarbons was found: *p*-cymene (0-0.365%), (Table 2).

In the group oxygenated ketones, the following elements were found: carvone (0-0.01%) and nootkatone (0-0.07%), (Table 2).

In the group of sesquiterpenes, the following elements are ascribed: germacrene D (0-0.21%), β-elemene (0-0.01%), α-humulene (0-0.037%), β-caryophyllene (0-0.42%), α-farnesene (0-0.45%), (Table 2).

In the group of aldehydes, the following elements were found: octanal (0.02-0.055), nonanal (0-0.05), decanal (tr-0.075%), undecanal (0-0.02%), dodecanal (0-0.29%), neral (0.12-0.44%), citronellal (0-0.025%), geranial (0-0.69%), (Table 2). The bright green citrus note of the bergamot essential oil is due to geranial and neral.

In the group of alcohols, the following elements are ascribed: terpinen-4-ol (tr-0.09%), α-terpineol (0.04-0.13%), octanol (0-0.10%), nerol + citronellol (0-0.17%), (E)-nerolidol (0-0.03%), (Table 2).

In the group of esters, the following elements were detected: octyl acetate (0-0.14%), neryl acetate (tr-0.49%), geranyl acetate (0-0.74%), (Table 2).

DISCUSSION

The physico-chemical properties of fruits, vegetables and their derivatives are to be ascribed to many pre- and post-harvest practices (Mele et al., 2018). In particular, pre-harvest factors such as climate (Nawaz et al., 2019; Otero et al., 2011), genus (Sicari et al, 2016a), cultivar (Giuffrè and Capocasale, 2015), rootstock (de Castro Machado et al., 2015), organic and conventional farming (Maggio et al., 2013), fertilisation (Quaggio et al., 2002), irrigation (Anastasi et al., 2010; Aguado et. al., 2012; Li et al., 2018), harvest date (Giuffrè, 2014), harvest year (Giuffrè, 2013), harvest maturity stage (Sun et al., 2019) were found to have a significant influence.

The improvement of the analytical methods has allowed obtaining a BEO chromatographic profile more detailed and including more information. Gas chromatographic columns more and more performing and the development of GC-MS and HPLC-MS techniques allowed to improve the detail and the precision of findings (Mondello et al., 1994; Dugo and Bonaccorsi, 2013; Marzocchi et al., 2019).

In addition, the changes of climate (Archivio Meteo, 2020) have influenced the analytical data on BEO. The average temperature is increased over years and the BEO has changed, this was evident in the evolution of data over years in almost all the detected volatiles. Sensory characteristics of BEO are also related to environment and climate.

Several studies have focused the sensory characteristics and the peculiar olfactory/aromatic notes which supply the various components to an essential oil (Sawamura et al., 2006; González-Mas et al., 2019; Russo et al, 2012; Palazzolo et al., 2013). More particularly, limonene gives citrus, fresh, herbaceous, acid and mint fragrances; (Z) -β-ocimene gives spicy, citrus and herbaceous notes; β-myrcene gives metallic, pungent, fruity, herbaceous, fresh and mushroom-like notes; β-pinene gives woody, herbaceous, sweet and smoky fragrances; γ-terpinene gives bitter, resinous, herbaceous, fruity, oily and smoke notes; geranyl acetate gives citrus and herbaceous fragrances; linalool gives floral, sweet, fruity and herbaceous notes; linalyl acetate gives floral, sweet, fruity, resinous fragrances.

The last two volatile components are some of those with higher percentages, moreover, their ratio is an important indicator for the evaluation of the aromatic characteristics of the essential oil and for the evaluation of the genuineness of the extract. According to the literature the linalool/linalyl acetate ratio is a quality parameter and in the bergamot essential oil of Reggio Calabria is around 0.3 (Sawamura et al., 2006; Bouzouita et al., 2010). During fruit maturation, linalool has a tendency to transform into the corresponding acetate and therefore to decrease. This behaviour gives the oil fresh aromatic and lavender notes while linalyl acetate fruity notes.

CONCLUSIONS

In this review it was analysed the variation in the volatile fraction representing 99% of the aroma of bergamot essential oil over thirty-five years of researches. In the observed studies, 94 compounds were identified. It was observed that the volatile fraction of bergamot peel essential oil was very variable. This means that the bergamot peel essential oil is different year by year, and this depends on many factors such as: the geographical area of cultivation (microclimate), the year of production, the cultivar, the age of the plant, the agronomic techniques (fertilisation, irrigation), the harvest date of fruits, the conditions and the storage duration. For this reason, in order to have a product with constant values of its composition elements, over the years, as required by many buyers, it is necessary to carry out a preliminary analysis on each batch produced. Then, if necessary, a mix of the quantities of essential oil of different batches in the right proportions, can be made in order to prepare a final product with the characteristics desired by customers.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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