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Development of bergamot flavors according to their enantiomeric state

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ABSTRACT: In our study, enantiomers of bergamot essence components were investigated. Afterwards, the flavoring effects of each of its optical isomers were investigated. Our aim is to find and develop the product with the strongest aroma effect. The effects of components that determine the quality of bergamot oil on values such as odor, taste and colour were investigated by gas chromatography-mass spectrometry (GC-MS) and sensory analysis evaluation. Moreover, application studies taken place in which shelf life studies be monitored depending on the use of antioxidants. Application and GC results were examined. In line with these results, it was determined that butylated hydroxy anisole (BHA), caffeic acid and gallic acid kept the product stable until the end of the 18th month.

KEYWORDS: bergamot oil, gas chromatography-mass spectrometry, sensory analysis, enantiomer, flavor

1. INTRODUCTION

Bergamot, or *Citrus aurantium bergamia*, is a citrus fruit and flowery olfactory characteristics, or 'notes', of the plant give to the bergamot oil a fresh and spicy tone, highly appreciated in perfumes and other cosmetic productions for its fragrant notes, fixative and antimicrobial properties. The main components in cold pressed bergamot oils are limonene, linalool and linalyl acetate. There are also β-pinene and α-terpinene. These substances and their enantiomers are divided into 2 classes depending on the market supply as those with enantiomer status and those without enantiomer status in Table 1 and 2 [1,2].

Table 1. The list and general information of the enantiomers used in the study are in the table below.

Compound Name	CAS	Percentage of The Enantiomeric Distribution (%)	% of Presence in Bergamot Oil
ALPHA PINENE L(-)	7785-26-4	65-76%	1.20%
ALPHA PINENE D(+)	7785-70-8	35-24%	1.20%
LINALOOL L(-)	126-91-0	99%	4-10%
LINALOOL D(+)	126-90-9	1%	4-10%
LYNALILE ACETATE R(-)	16509-46-9	99%	24-27%
LYNALILE ACETATE S(+)	51685-40-6	1%	24-27%
B-PINENE R(+)	19902-08-0	8%	-
B- PINENE S(-)	18172-67-3	92%	-
SABINENE R(+)	2009-00-9	15-20%	1,07%
SABINENE S(-)	10408-16-9	80-85%	1.07%
LIMONENE D(+)	138-86-3	97%	41-45%

Its organoleptic features are: [3,4]

• Colour: from green to yellowish-green to yellowish-brown. The colour fades with ageing, particularly when the oil is exposed to daylight.

• Odour: the top note is extremely rich, fresh, sweet-citrusy, lavandaceous, with a tea-like nuance. The dry-down is oily-herbaceous, balsamic, with a lemony-citrus character, and a slight tobacco/tea note, somewhat reminiscent of clary sage and neryl acetate.

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• The oil has a thinner odour, with p-cymene and linalyl acetate notes to the fore, and the dry-down has a pithy, citrusy lemony character, very clean and fresh.

Table 2. Substances that do not have enantiomer status are listed in the table below.

Compound Name
BENZYL BENZOATE
CITRAL 95
TERPINOLENE 90
GAMMA TERPINENE
P-CIMENE
CAMPHOR

According to the European Medicines Agency (EMA), the bergamot oils photosensitive and melanogenic properties, ascribed to its content in furocoumarins (psoralens) such as bergapten, which may be even photo-mutagenic [5]. The bergapten-free oil is often used in the fragrance industry. Bergapten-free oil is a reduced-bergapten content oil, with bergapten present at a content level below 30 ppm [6].
In recent years, Bergamot flavor prices have increased because of less production or downright adulteration. While the physicochemical standards set up by the International Organization for Standardization (ISO), The French Standardization Association (AFNOR), and the Experimental Station for the Citrus Essences and Derivatives Industry have been important to establish the genuineness of a product, they are not sufficient anymore [6].

In this study, different bergamot flavorings have been developed and their effectiveness has been investigated in order to reduce costs and produce flavorings that has given the same effect at low dosages, and to be sustainable. Evaluation of aromas kept in accelerated stability conditions have been done by looking at oxidation products with GC and sensory analysis. Shelf life has calculated by comparing the results.

2. RESULTS AND DISCUSSION

GC and MS are the most common tools used to analyze the constituents of oils and varying isomers and their chemical structures can be detected. Chromatograms obtained in oil analyzes are presented in Figures 1 and 2 and their evaluations are made in Table 3.

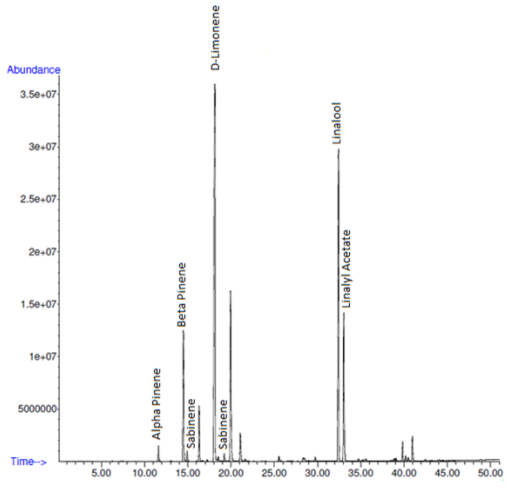

Figure 1. Chromatogram of the bergamot oil strain.

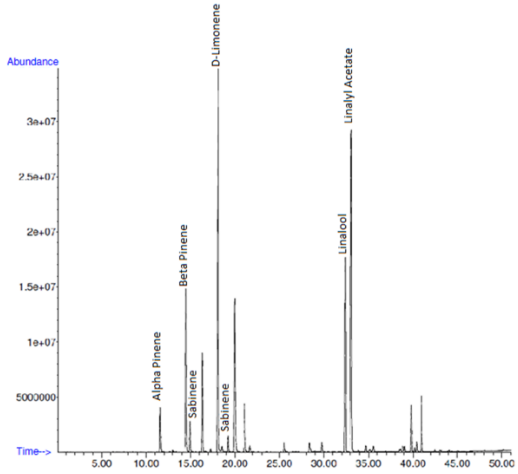

Figure 2. Chromatogram of the bergapthene free oil strain.

When we examine the % content results obtained from these chromatograms, we see that there are results in parallel with the values in the literature. The main reasons for the difference in the literature

reference intervals are the chemical reactions that occur depending on the bergamot harvest, the temperature applied during the obtaining methods, and the storage conditions [7, 8].

Table 3. Results of bergamot oil and bergapthene free oil obtained by GC-MS device.

Bergamot Oil	CAS	Composition%	Reference Range [4]		Reference Range [5]		Supplier Company Reference Interval	
The compounds			min.	max	min	max	min	max
Limonene	005989-27-5	35.90±0.7	31.36	43.23	30.00	50.00	25.00	50.00
Lynalyl acetate	000115-95-7	13.83±1.1	14.26	22.46	23.00	35.00	10.00	25.00
Linalool	000078-70-6	26.14±0.1	18.08	33.98	6.00	15.00	25.00	50.0
Beta pinene	000127-91-3	8.63±0.4	0.58	1.01	5.00	10.00	1.00	10.0
Sabinene	003387-41-5	1.25±1.0	0.56	0.85	0.50	2.00	-	-
Alfa pinene	000080-56-8	0.98±0.8	0.56	1.01	0.20	0.70	0.10	1.00
Bergamot Oil (bergapthene free)	CAS	Composition%	Reference Range [4]		Reference Range [5]		Supplier Company Reference Interval	
The compounds			min.	max	min	max	min	max
Limonene	005989-27-5	28.01±0.4	35.45	42.82	30.00	50.00	25.00	50.00
Lynalyl acetate	000115-95-7	25.98±1.1	27.61	29.55	23.00	35.00	25.00	50.00
Linalool	000078-70-6	13.09±0.3	10.02	13.73	6.00	15.00	10.00	25.00
Beta pinene	000127-91-3	8.69±0.1	4.72	5.70	5.00	10.00	1.00	10.00
Sabinene	003387-41-5	2.41±0.2	0.81	1.00	0.50	2.00	-	-
Alfa pinene	000080-56-8	2.43±0.3	0.90	1.11	0.20	0.70	0.10	1.00

Looking at the results, 2 aromas in Table 4 worked by extracting the dominant limonene, the bergamot aroma can be felt more clearly.

Table 4. The percentage components of the ingredients in the flavor formulation was listed.

BERGAMOT FLAVORS	L-LINALOOL LEAVO	R-LINALYL ACETATE	S-SABINENE	BENZYL BENZOATE	CITRAL 95	TERPINOLENE 90	L- ALPHA PINENE	L-BETA PINENE
201509	18	49	2	1	17	0.5	2.5	10
201510	15	53	4	3	14	3	2	6

When our panelists compared bergamot flavorings with bergamot oils, they found Bergamot 201509 flavoring to be suitable. Pilot production of the Bergamot 201509 formula was made. Panelists evaluated the flavors of Bergamot 201509, our targeted Natural Bergamot Oil Italy, and the market products X and Y bergamot flavours by application. According to the results from the panelists, the taste-smell-color diagrams of the products are given in Figure 3. In the study, some scores were used for the spider web graph: 1-9 for taste (1 bad, 6-9 acceptable, 9 excellent), 1-5 for smell (1 very weak 3-5 acceptable, 5 very strong) and color1-3 (1 cloudy, 3 clear) and it is determined with the help of the spider web graphic that the product very close to both the targeted product and the competitor product is obtained.

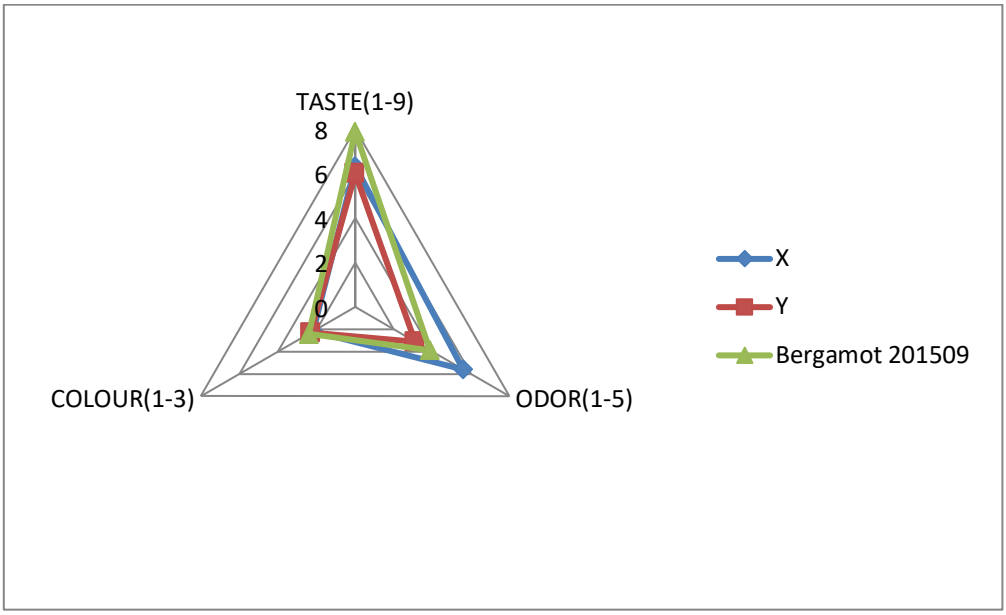

Figure 3. Bergamot tea application spidernet graph was shown.

When the application and GC results were examined, BHA, Gallic Acid and caffeic acid kept the product stable until the end of the 18th month (Table 5-7). When BHA was examined (Table 5), the amount of linalyl acetate and citral 95 to rise rapidly at 18 months. Sabinen shown to decline rapidly at 18 months. When gallic acid was analyzed (Table 6), the amount of acetate linalyl to rise rapidly at 18 months. Sabinen shown to decline rapidly at 15 months. Citral 95 reached the lowest level in the 21st month. As for caffeic acid (Table 7), the situation is as follows, the amount of acetate linalyl to rise rapidly in the 21st month. Sabinen remained stable from 12th to 18th months, sabinen shown to decline rapidly after 18th month and citral after 21th month. Consistent with other application results. Shelf life was determined as 18 months using BHA, gallic acid and caffeic acid. The shelf life of market products is about 12 months (5).

Table 5. Change of main components of bergamot flavoring with BHA according to months. (The number of experiment repeats (n=3))

Month	Linalool Leavo	Linalyl acetate	Sabinene	Benzyl benzoate	Citral 95	Terpinolene 90	L-Alpha pinene	L-Beta pinene
0	17.97±0.1	49.24±1.3	1.68±0.8	1.36±3.1	17.50±1.1	0.36±2.0	2.06±3.1	6.98±0.9
3.	18.66±1.7	49.88±2.1	1.67±3.5	1.22±2.7	17.10±2.1	0.36±0.8	2.60±0.5	9.40±2.6
6.	18.62±0.6	50.02±1.2	1.68±1.2	1.10±1.8	16.93±0.4	0.32±1.4	2.57±3.2	9.37±1.7
9.	18.92±3.1	50.96±0.9	1.67±3.2	1.03±2.1	16.95±3.1	0.31±0.3	2.57±1.1	9.54±3.4
12.	18.67±1.9	50.88±1.3	1.66±1.9	1.07±3.0	16.78±0.7	0.30±0.2	2.54±3.7	9.51±3.1
15.	18.93±2.4	51.69±3.1	1.66±2.1	0.95±2.4	16.83±3.6	0.26±2.4	2.53±2.1	9.61±3.3
18.	18.30±1.7	54.81±3.4	1.62±1.6	1.14±1.8	16.25±3.2	0.32±0.9	2.46±2.6	9.29±2.2
21.	18.19±1.5	55.22±0.1	1.62±3.1	0.91±2.1	16.10±0.1	0.24±1.1	2.41±0.9	9.41±2.4

Table 6. Change of main components of bergamot flavoring with gallic acid according to months. (The number of experiment repeats (n=3))

Month	Linalool Leavo	Linalyl acetate	Sabinene	Benzyl benzoate	Citral 95	Terpinolene 90	L-Alpha pinene	L-Beta pinene
0	18.39±0.1	49.17±2.1	1.72±3.1	1.15±0.5	17.42±1.4	0.35±0.7	2.67±3.1	9.23±0.1
3.	18.77±0.1	50.47±3.1	1.70±3.5	1.01±0.1	17.25±0.1	0.33±2.4	2.63±0.2	9.51±3.2
6.	18.84±1.4	51.47±1.9	1.68±2.1	1.00±1.7	16.95±3.1	0.31±3.1	2.56±1.6	9.59±1.1
9.	18.74±1.1	51.02±3.0	1.67±2.4	1.00±2.9	16.91±0.8	0.30±1.1	2.57±2.4	9.52±0.1
12.	18.82±0.9	51.59±3.0	1.66±0.1	0.95±0.8	16.80±3.0	0.30±2.4	2.53±3.3	9.57±0.8
15.	19.18±0.1	51.59±1.4	1.64±1.8	0.93±0.1	16.67±1.9	0.25±3.1	2.50±1.0	9.59±1.1
18.	18.91±1.2	53.14±1.1	1.60±2.7	1.04±2.1	16.59±2.6	0.25±3.0	2.50±2.0	9.70±1.2
21.	19.15±2.1	53.42±0.8	1.58±0.9	0.87±2.1	16.47±0.8	0.24±0.5	2.47±0.4	9.79±2.8

3. CONCLUSION

In the study, it was determined with the help of spider web graphics that a product very close to both the targeted product and the competitor product was obtained. Moreover, a good effect was tried to be obtained at almost half the dose, extended shelf life and the aim of sustainability was tried to be achieved. Thus, an effective and low-cost product was obtained that can be used in the food and pharmaceutical-cosmetics industry.

Table 7. Change of main components of bergamot flavoring with caffeic acid according to months. (The number of experiment repeats (n=3))

Month	Linalool Leavo	Linalyl acetate	Sabinene	Benzyl benzoate	Citral 95	Terpinolene 90	L-Alpha pinene	L-Beta pinene
0	18.23±1.0	48.62±0.1	1.82±2.6	1.19±0.1	17.53±0.1	0.36±0.7	2.69±0.7	9.12±2.1
3.	18.67±1.1	50.25±2.1	1.72±2.7	1.12±0.5	17.21±2.0	0.33±2.1	2.62±2.6	9.43±1.1
6.	18.30±0.2	49.81±1.1	1.68±0.1	1.28±2.1	17.00±3.6	0.28±3.1	2.55±3.6	9.21±0.8
9.	19.03±0.6	51.41±3.6	1.63±1.1	1.05±1.5	17.10±0.5	0.29±3.1	2.50±0.7	9.42±1.1
12.	18.98±0.5	51.88±2.0	1.59±0.5	0.99±2.2	16.84±0.1	0.27±0.1	2.45±2.1	9.40±0.9
15.	18.99±0.6	52.32±3.1	1.58±2.1	0.96±0.1	16.82±3.1	0.24±0.7	2.43±3.1	9.49±0.2
18.	18.97±0.1	52.53±2.1	1.60±0.7	0.97±0.1	16.76±2.1	0.3±0.6	2.45±0.1	9.46±0.7
21.	19.08±1.7	54.97±0.6	1.53±3.1	0.94±3.1	16.39±2.1	0.21±3.1	2.36±3.1	9.64±2.1

4. MATERIALS AND METHODS

4.1. Materials

In the study, 2 cold-pressed oils (Bergamot oil Italy and Bergamot oil bergapthene free) were used for the development of bergamot flavorings for content analysis. All other materials used are of technical quality. Çaykur brand unscented tea named Rize was used in sensory analyzes. X and Y brand bergamot flavors were used for challenge studies. All chemicals were used as received without further purification.

4.2. Analysis of oils

Qualitative analyzes by gas chromatography (GC) and % content/composition values were tested by mass spectrometry (MS by GC-MS Device (AgilenT, GC /INTUVO 9000, MS/5977B (G7077B) Autosampler / 7693A (G4513A)). Thus, reference contents were created for new flavorings to be designed. The method values [9] used are as follows in Table 8. Method validations were performed according to the ICH Q2 (R1) guideline. The standards were prepared at different concentrations (1–10 g/mL) to obtain the standard curve. The r^2 value for method was above 0.987.

Table 8. The method values were listed.

Temperature rise step	3°C
Column temperature change points	50-250°C
Residence time at determined change points	0-10 min.
Column equilibration time	1 min
Syringe volume	10 µL
Injection volume	0.1 µL
Pressure	4.4382 psi
Split ratio	50 :1
Split flow	31,688 mL/min
Column equilibration time	1 min
Syringe volume	10 µL

4.3. Formulation studies

Looking at the analysis of oils results, 2 aromes in Table 4 worked by extracting the dominant limonene, where the bergamot aroma can feel more clearly. 1% w/w solutions of antioxidants in ethyl alcohol were used as 2% in the formula.

4.4 Stability studies

Evaluation of aromas with or without antioxidants kept in accelerated stability conditions in the cabinet (Nuve, TK120, Turkey) will be done by looking at oxidation products with GC along 21 months. A freshly made aroma sample was kept as a witness at room conditions without any aging.

4.5. Sensory analysis

A panel team was formed from employees of Elso Chemistry Co. (Turkey) for sensory analysis. The people in the panel team were informed about the project by the application specialist before the tasting. Two g of tea leaves were used for tasting and 4 g of tea leaves for odor analysis. For 100 ml of water, 2 g of tea leaves were sprayed with essence from 8 cm away and mixed with the help of a glass baguette and kept closed for 1 day. Flavor is generally used in the market at a rate of 1- 0.70%. However, it was used at a rate of 0.40% in this study.

For tasting, the tea was infused for 15 minutes and filtered. At this stage, the temperature of the tea is 60 °C [10, 11]. The ten panelists rated the color, smell and taste of the brewed tea.

Tea drinking temperature should be 57-58°C [8]. Odor detection was made by smelling the tea and the pulp after brewing. Evaluation of aromas kept in accelerated stability conditions will be done by looking at oxidation products with sensory analysis.

4.6. Statistical analysis

The results of all the experiments were calculated as mean ± standard error of mean. P-values < 0.05 were measured statistically significant.

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REFERENCES

[1] Costa R, Dugo P, Navarra M, Raymo V, Dugo G, MondelloL. Study on the chemical composition variability of some processed bergamot (*Citrus bergamia*) essential oils. Flav Frag J. 2010, 25(1): 4-12. [CrossRef]

[2] Casabianca H, Graff JB. Separation of linalyl acetate enantiomers: Application to the authentication of bergamot food products. J High Res Chrom. 1994. [CrossRef]

[3] Burfield, T. Natural aromatic materials–Odours & origins. Int J Aromather. 2001; 11: 47–48.

[4] Arctander S. Perfume and Flavor Materials of Natural Origin: A Perfumer's and Flavorist's Practical Description of Available Materials, Their Origin, Production and Processing, Appearance, Odor and Flavor Type, Evaluation, Application and Availability with Brief Notes on Their Main Constituents, Replacements and Most Common Adulterants; Allured Publishing: Carol Stream, IL, USA, 1994.

[5] European Medicines Agency Assessment Report on Citrus Bergamia Risso et Poiteau, Aetheroleum. Available online: https://www.ema.europa.eu/en/documents/herbal-report/draft-assessment-report-citrus-bergamia-risso-et-poiteau-aetheroleum_en.pdf (accessed on 24 November 2020).

[6] Valussi M, Donelli D, Firenzuoliand F, Antonelli M. Bergamot Oil: Botany, Production, Pharmacology, Encyclopedia 2021; 1: 152–176. [CrossRef]

[7] Dugo G, Bonaccorsi I, Sciarrone D, Schipilliti L, Russo M, Cotroneo A, Dugo P, Mondello L, Raymo V. Characterization of cold-pressed and processed bergamot oils by using GC-FID, GC-MS, GC-C-IRMS, enantio-GC, MDGC, HPLC and HPLC-MS-IT-TOF. J Ess Oil Res. 2012; 24(2): 93-117. [CrossRef]

[8] Brown F, Diller KR. Calculating the optimum temperature for serving hot beverages. Burns : J Int Soc Burn Inj. 2008 ; 34(5): 648-654. [CrossRef]

[9] Güneştürk S. Çay kültürü ve ürünleri. Millî Folklor 2012; 24 (93).

[10] Peterson J, Dwyer J, Jacques P, Rand W, Prior R, Chui K. Tea variety and brewing techniques influence flavonoid content of black tea. J Food Compos Anal. 2004; 17(3–4): 397-405. [CrossRef]

[11] Turkish Food Codex Regulation on Food Additives.

https://leap.unep.org/countries/tr/national-legislation/turkish-food-codex-regulation-food-additives, (accessed on 18 July 2021).