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Modifications of dried basil (*Ocinum basilicum*) leaf oil by gamma and microwave irradiation

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Dried basil leaves were irradiated with two different doses of gamma rays and with microwaves. Comparison with a blank sample was performed by capillary gas chromatography/mass spectrometry (GC/MS) which identified 47 peaks. Linalool and estragol showed the greatest increases with γ-radiation and dropped with microwaves. The composition of essential oils was different, except for a few compounds which increased or decreased regardless of the treatment. Gamma radiation caused the most evident changes in the composition profiles. A sensory test confirmed significant differences between the extracts. The panellists preferred the gamma treated sample, while the microwaved sample was the least appreciated. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Spices and herbs are often highly microbiologically contaminated and spoilage can occur during storage. Their use in food curing and seasoning can cause undesired high microbial contamination. Ethylene oxide was one of the most popular treatments for reducing the microbiological populations (Vajdi and Pereira, 1973). Nowadays this substance is banned by EC law (Uijl, 1992) because it is hazardous. Instead microwave, gamma radiation and ozone are now widely used to reduce microbiological contamination (Emam *et al.*, 1995; Farag *et al.*, 1995; Zhao and Cranston, 1995).

The aim of this study is to compare effects of different techniques of basil sterilisation on its essential oil composition.

MATERIALS AND METHODS

Sample origin

The dried basil samples came from Egypt and were provided by Cannamela s.r.l. (Castel Maggiore BO, Italy), who had subjected the samples to different treatments. Gamma radiation was done at Gamma Rad (Bologna, Italy) with doses of 5 and 10 kGy from a ^{60}Co source (samples 2 and 3, respectively). Microwave treatment was performed at Cannamela in a continuous plant consisting of a 2540 MHz wave oscillator which heated the herbs to a maximum temperature of 100°C for 15 min (sample 4). Untreated basil was used as control (sample 1).

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Oil extraction

The oil constituents were extracted from 10 g of a mixture of dried basil and anhydrous Na_2SO_4 (1:1 w/w) in a Soxtec apparatus (International PBI, Milan, Italy) for 2 hours with 50 ml of redistilled *n*-hexane. A portion of the extract (5 ml) was concentrated at room temperature with a stream of pure N_2 to about 0.5 ml and submitted to gas chromatographic analysis.

These operations were repeated twice.

Analysis of oil composition by GC and GC/MS

Gas chromatographic analyses were performed by injecting 1 ml of oil solutions in the split mode (split ratio 1:45) into a Carlo Erba Strumentazione gas chromatograph HRGC 5160 (Milan, Italy) equipped with a 25 m × 0.25 mm id, 0.25 μm film thickness SE 52 capillary column (Mega S.p.A., Legnano, Italy). The column was operated with hydrogen as the carrier gas (1 ml min^{-1}) with an initial temperature of 50°C which was then raised at 4°C per min to 70°C where it was held for 8 min. It was then raised at rate of 5°C per min to 200°C, holding this temperature for 6 min, and then ballistically to 300°C. The injector was kept at 250°C, and the flame ionisation detector was set at 300°C. Chromatograms were displayed and integrated by

Table 1. ANOVA results as peak area percentage of different basil treatments[a]

Compound		1	2	3	4
1	α-pinene	0.29 A	0.34 A	0.34 A	0.52 B
2	camphene	0.02 A	0.03 A	0.03 A	0.13 B
3	β-phellandrene	0.18 B	0.27 C	0.27 C	0.01 A
4	β-pinene	0.46 B	0.61 C	0.62 C	0.13 A
5	β-myrcene	0.10 a	0.24 b	0.22 b	0.10 a
6	p-cymene	0.03 A	0.16 C	0.16 C	0.12 B
7	1,8-cineole	5.14 a	6.06 b	6.29 b	5.98 b
8	γ-terpinene	0.01 A	0.09 B	0.11 B	0.01 A
9	*cis*-p-2-menthen-1-ol[b]	0.21 A	0.36 B	0.39 B	0.19 A
10	*cis*-linalool oxide	0.08 A	0.16 B	0.19 B	0.13 AB
11	*trans*-linalool oxide	0.08 A	0.13 AB	0.18 B	0.09 A
12	*trans*-p-2-menthen-1-ol[b]	0.07 A	0.15 B	0.08 AB	0.06 A
13	linalool	14.92 B	28.23 C	27.16 C	8.84 A
14	camphor	1.41 B	0.34 A	0.33 A	4.39 C
15	borneol	0.72 B	0.33 A	0.36 A	2.77 C
16	myrcenol[b]	0.19 A	0.18 A	0.19 A	0.36 B
17	menthol	0.07 B	0.16 C	0.07 B	0.01 A
18	terpin-1-en-4-ol	0.88 B	0.73 A	0.71 A	1.02 C
19	α-terpineol	1.25 A	1.02 A	0.99 A	2.25 B
20	estragole	4.54 B	13.06 C	13.69 C	1.58 A
21	allylphenol	0.37 A	0.67 AB	0.63 AB	0.83 B
22	neral	0.18 B	0.21 B	0.36 C	0.04 A
23	bornyl acetate	1.05 B	0.77 A	0.95 B	1.24 C
24	safrole[b]	0.05 A	0.05 A	0.09 A	1.44 B
25	*cis*-methyl cinnamate	0.66 A	1.10 B	1.25 B	2.27 C
26	thymol	0.68 A	1.10 AB	1.25 B	2.40 C
27	eugenol	12.13 B	9.63 A	10.52 A	10.31 A
28	α-cubebene	0.01 ns	0.01 ns	0.01 ns	0.01 ns
29	*trans*-methyl cinnamate	9.08 C	5.14 B	5.74 B	1.83 A
30	elemene[b]	2.19 b	1.83 ab	1.56 a	1.43 a
31	methyl eugenol	2.08 B	1.79 AB	1.87 B	1.44 A
32	β-caryophyllene	1.24 B	0.92 A	0.91 A	3.16 C
33	α-bergamotene[b]	6.88 D	5.89 C	5.41 B	3.97 A
34	α-caryophyllene	0.73 B	0.62 A	0.56 A	0.80 B
35	a cadinene isomer[b]	0.35 ns	0.30 ns	0.26 ns	0.22 ns
36	a cubebene isomer[b]	2.42 C	2.04 B	1.53 A	1.56 A
37	β-farnesene[b]	0.77 B	0.56 A	0.52 A	0.55 A
38	sesquiterpene[b]	0.33 ns	0.33 ns	0.16 ns	0.16 ns
39	eremophyllene[b]	0.39 ns	0.37 ns	0.32 ns	0.29 ns
40	β-bisabolene[b]	0.22 A	0.18 A	0.22 A	0.32 B
41	γ-cadinene[b]	3.77 C	2.42 A	2.68 AB	3.00 B
42	calamenene[b]	2.31 C	0.64 B	0.03 A	0.01 A
43	myristicine[b]	1.34 ns	0.83 ns	0.94 ns	1.60 ns
44	trimethyltetrahydrobenzofuranone[b]	0.65 B	0.61 B	0.42 A	0.43 A
45	spathulenol[b]	1.04 B	0.64 A	0.68 A	1.98 C
46	δ-cadinol[b]	1.28 B	0.68 A	0.65 A	1.63 C
47	α-cadinol[b]	9.89 B	5.06 A	5.44 A	17.31 C
48	other unidentified	7.39 ab	3.09 a	2.77 a	11.19 b

[a]: 1: no treatment; 2: γ-rays (5 kGy); 3: γ-rays (10 kGy); 4: microwave. Numbers with different following letters differ at $p \leq 0.01$ (capital letters) and at $p \leq 0.05$ (lower-case letters). ns: not significant.
[b] Tentatively identified by the MS library (NIST/NBS, 1990 version).

Chrom-Card data handling (Fisons Instruments, Milan, Italy).

The same GC conditions were maintained for GC/MS, which was carried out on a QMD 1000 (Fisons Instruments, Milan, Italy) instrument. Mass spectra were recorded at 70 eV with a mass range from 33 to 350 m/z.

Chromatographic peaks were identified by retention time, mass spectrometry library (NIST/NBS, 1990 version) and mass spectra of authentic compounds when available.

To compare the results, analysis of variance and Tukey's test (Miller and Miller, 1993) were used.

Sensory evaluation

The four different essential oils were evaluated by 17 inexperienced people. The test asked for the name of the herb submitted to sensory evaluation and a ranking of the samples in ascending order of preference. The ranks were summed and then compared as suggested by Kramer (1960) and reported by Amerine *et al*. (1965).

RESULTS AND DISCUSSION

The analyses (Table 1) documented 28 substances identified by GC, and GC/MS, 19 compounds tentatively identified by the GC/MS library, and other unidentified substances.

Oil composition was characterised by linalool (15%), eugenol (12%), α-cadinol (10%), *trans*-methyl cinnamate (9%), α-bergamotene (7%), 1,8-cineole (5%), and estragole (5%). This composition pattern is not in good agreement with the literature which, in turn, indicates estragole (Baritaux *et al.*, 1992; Pino *et al.*, 1993), linalool (Sheen *et al.*, 1991; Perez-Alonso *et al.*, 1995) or methyl cinnamate (Morales *et al.*, 1993) as the main compound. On the other hand, agronomical and environmental factors could affect the basil composition (Adler *et al.*, 1989; Bonnardeaux, 1992; Simon *et al.*, 1992) as well as different cultivar types.

The sterilisation requirements were achieved by each treatment (Modelli *et al.*, 1996) but they caused strong changes in the spice. In fact, only five substances [28], [35], [38], [39] and [43] seemed not to be influenced by the treatments.

The largest variations are reported in Fig. 1, while Fig. 2 shows the smallest variations. Linalool [13] and estragol [20] showed the greatest increases with γ-radiation, and an opposite behaviour with microwave. These variations could be explained, in turn, by glycoside

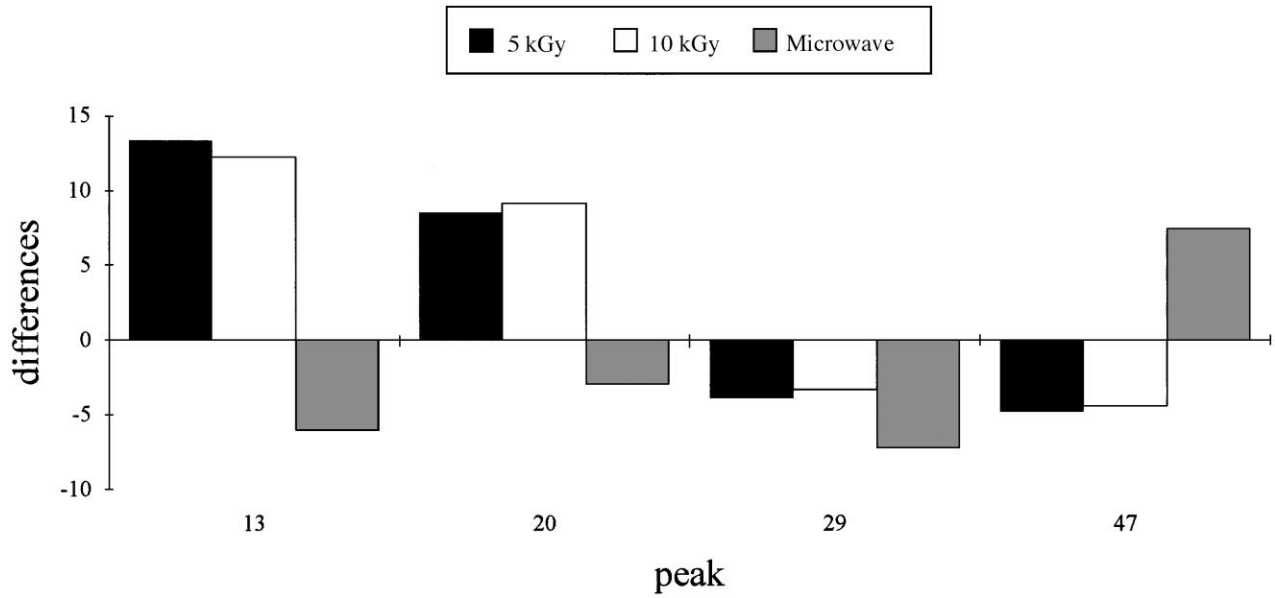

Fig. 1. Substances showing the largest variations, obtained by the % subtraction of the blank from each treatment.

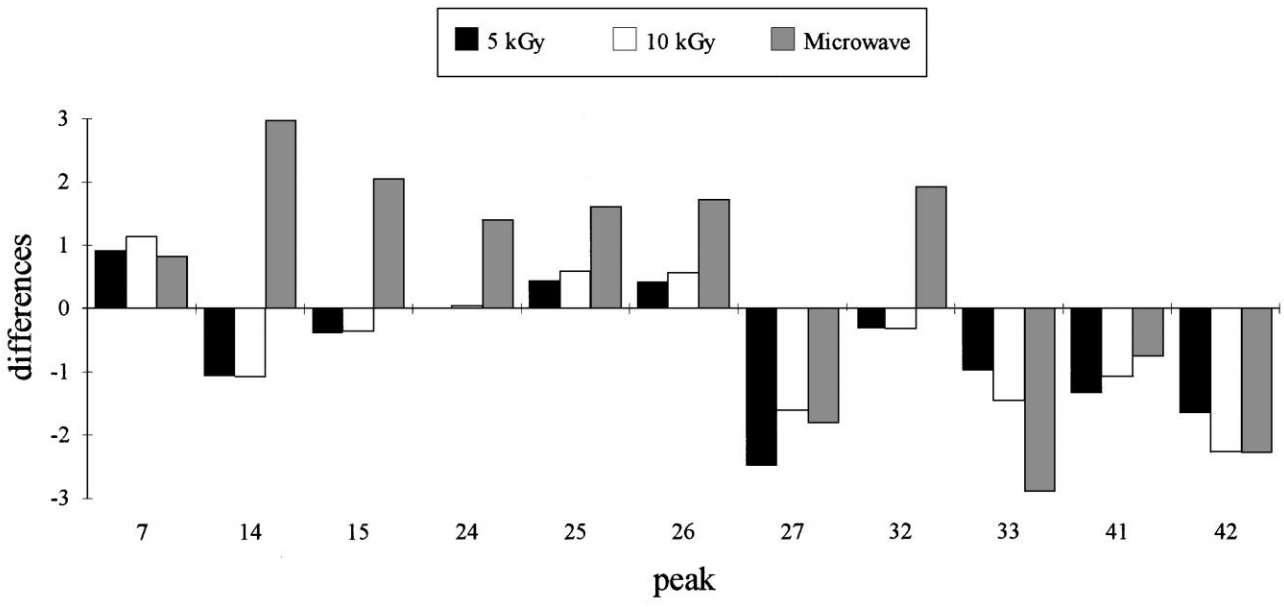

Fig. 2. Substances showing the smallest variations, obtained by the % subtraction of the blank from each treatment.

cleavages (γ-rays) or by heat-related losses (microwaves). Furthermore, it is worth mentioning that *trans*-methyl cinnamate [29] may be partially converted into the *cis*-isomer [25].

The composition profiles were different for each treatment, except for six compounds [6], [7], [10], [21], [25] and [26] which increased, and 7 compounds [27], [29], [33], [36], [37], [41] and [42] which decreased. A great number of sesquiterpene hydrocarbons belong to this latter group.

Independent of the radiation doses, there was significant increase of the monoterpene hydrocarbons [3], [4], [5], [6] and [8]. On the other hand, in addition to the above-mentioned sequiterpene hydrocarbons, [32] and [34], also decreased. Microwave treatment causes severe losses of [3] and [4]. These data do not completely agree with other authors (Emam *et al.*, 1995), even though they reported different behaviour for the same substances submitted to the same treatment in different herbs (Farag *et al.*, 1995). Other findings demonstrated that β- and γ-irradiation had almost no effect on basil composition (Venskutonis *et al.*, 1996).

The influence of the oil extraction technique on oil composition must be carefully considered. For example, it is well-known that hydrodistillation enhances the hydrocarbon content in essential oils, while solvents extract also waxes and pigments.

Monoterpenols had different behaviours when submitted to γ-radiation or microwave. In the first case, [9] and [12] increased, but the same compounds were not influenced by microwaves. An opposite trend was evident for [15], [16], [18], [19] and for all sesquiterpenols [45], [46], [47], which considerably increased with microwave and were depressed by γ-radiation.

Hydrocarbon and alcohol variations were not independent, even if it is not clearly evident. In fact, the sum of the first group increased with γ-radiation, but was not influenced by mcrowave, even if there were large modifications of some individual components. The alcohol sum, excluding [13], showed a slight decrease with γ-radiation and a large increase with microwave. The same trend was even more evident for [14], but [22], the only other carbonyl compound present, displayed great sensitivity to heat and increased its concentration at 5 kGy.

Compounds with an aromatic ring did not have the same behaviour. Estragole [20] underwent a remarkable increase with γ-radiation and a decrease of the same order of magnitude with microwave. On the other hand, [43] was not influenced by any treatment.

Safrole [24] had a particular rise with mcrowave, while it was unaffected by γ-rays. It is interesting to note that mcrowave caused a comparable decrease in [27]. It might be supposed that heat promoted the formation of a dioxolane ring from the hydroxyl and the methoxyl group of [27]. However, a sample of [27], heated under the same conditions of microwave treatment in the presence of 5% of water, did not generate [24]. If our hypothesis is true, the reaction was catalysed by a transition metal, or, more probably, by an enzymatic system, before heat denaturation. The invariability of [43] indirectly confirmed the transformation of [27] into [24]. In fact, 4-allyl-2,6-dimethoxyphenol, an hypothetical precursor of [43], was not detected.

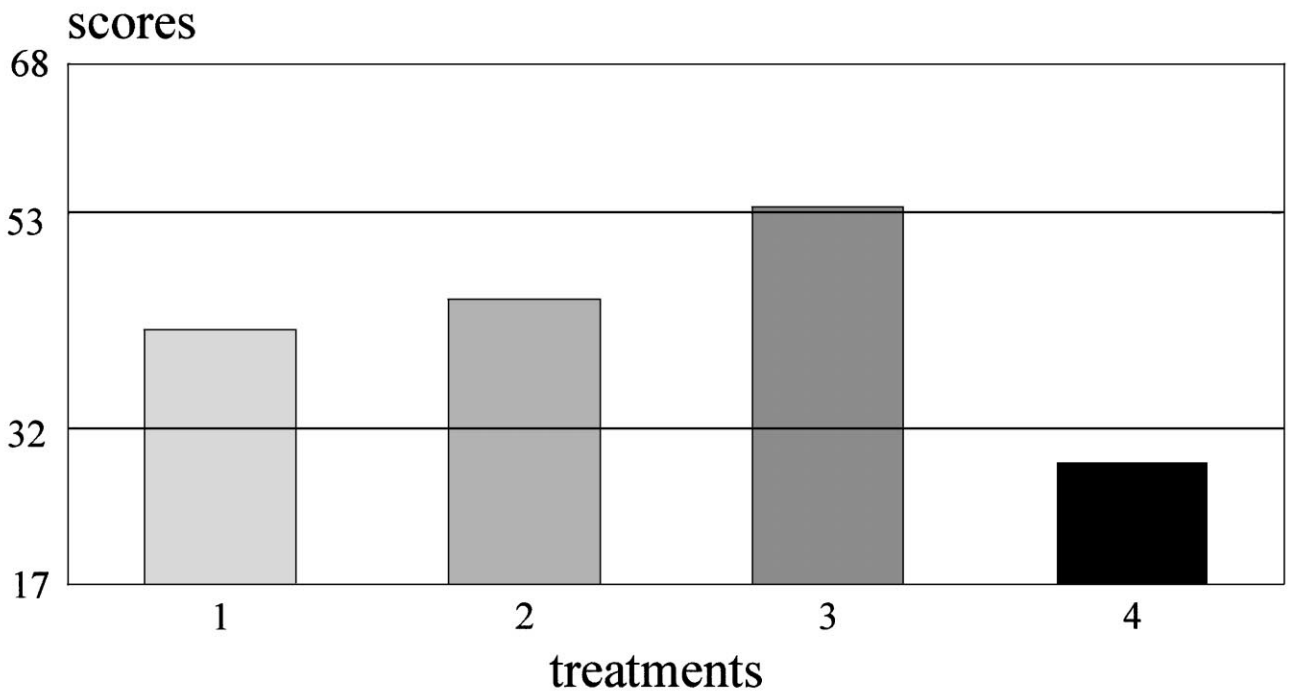

Fig. 3. Scores for each treatment; 32 and 53 represent the lowest and the highest insignificant rank sum, respectively (1: no treatment; 2: γ-rays 5 kGy; 3: γ-rays 10 kGy; 4: microwave).

Generally speaking, almost all analytes gave the same response, both at 5 and 10 kGy, except for [17], [33], [42] and [44], which showed greater instability at higher dose. The modifications induced by mcrowave were not simply related to heat losses. In fact, apart from 16 compounds which decreased, 22 other substances increased.

As a consequence of these strong modifications in the composition of the samples, the panellists indicated differences between the samples (Fig. 3). Sample 3 obtained the best score, while sample 4 the worst one. Samples 1 and 2 had no differences, obtaining a middle score. Eleven panellists (64.7%) recognised the extracts as basil, while the rest (35.3%) gave wrong assignations. Two people identified pepper, 1 thyme, 1 cinnamon, 1 rosemary and another one marjoram. Some panellists found sample 3 a bit harsh: as a matter of fact it had the most intense smell. All these considerations suggest that γ-radiation enhanced the organoleptic characteristics of the herb.

CONCLUSIONS

The sterilising treatments induced strong changes in the essential oil profile, which were not always in agreement with those in the literature. This discordance with the literature data indicates that it is necessary to standardise the analytical procedures, in order to have more comparable results.

Moreover, although it is not certain that the different treatments of the spices affect human health, the original sensory characteristics are strongly modified. Nowadays, γ-radiation and microwave treatment seem to be acceptable methods for preventing microbiological spoilage.

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