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Changes On Essential Oil Composition of Aniseed (*Pimpinella anisum* L.) During Ten Maturity Stages

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The essential oils extracted from aniseeds at ten different maturation stages were analyzed by GC-FID. The essential oil contents of the aniseed (2.38-4.60 %, v w^{-1}) showed remarkable variations during maturation. At the later maturation stages, the essential oil contents of aniseed decreased. A total 20 compounds were detected in the samples representing 93.34-97.31 % of the total essential oils. The (E)-anethole rates were found in between 85.14-92.28 % and the (E)-anethole was the main component of aniseed at all stages of harvesting. The obtained results indicate that the aniseed should be harvested at the 1st, 2nd or 3rd maturation stages, since the levels of both essential oils and (E)-anethole are the highest. If this is not the case, the 6th maturation stage should be considered as the appropriate harvesting period.

Key Words: Aniseed, *Pimpinella anisum* L., Harvesting stages, Plant maturity, Essential oil composition.

INTRODUCTION

Anise or aniseed (*Pimpinella anisum* L.) belongs to the *Apiaceae* Lindl. (*Umbeliferae*) family and is an annual plant. The fruits are used in production of essential oils, which have economical importance because of its wide application in food, drug, pharmaceutical, cosmetic and beverage industries. The fruits are likewise utilized as carminative, appetizer, sedative as well as stimulant in the milk production of breast-feeding mothers[1]. Additionally, the various extracts and essential oils of aniseeds have antimicrobial, antioxidative and antifungal activities[2,3]. The composition of essential oils determines its quality. The content and composition of essential oils of aniseed are affected by ecological conditions, growing techniques and the fruit maturity level. Several studies have been conducted to determine the effects of different growing techniques (*e.g.* sowing time, manure and irrigation) and origins of aniseed on the essential oils[1,4-8]. The content and composition of thyme, dill, coriander and *Stachys inflata* essential oils differed with harvesting time[9-12]. Similarly, it was demonstrated that the essential oil compositions of various fruits was affected by maturation stage[13,14]. On the other hand, with the exception of Omidbaigi *et al.*[15], no study has been caried out to determine the variation(s), if any, in the content and composition of the aniseed essential oils during maturation stages. Omidbaigi *et al.*[15] investigated the variations in the content and composition

of aniseed essential oils harvested at two different periods (waxy stage and ripe stage). The first developing flower of anise is the primary umbel located at the top of main stem followed by the second and third umbels developing from top to the bottom. Therefore, during maturation both mature and immature fruits are present in the same plant. In the late harvesting periods, the early mature fruits may drop off during the harvest.

The aim of the this study was to determine the essential oil content and composition of aniseeds harvested at different plant maturation stages.

EXPERIMENTAL

Plant material: The aniseeds, Denizli type, used in the present study were obtained from the bulk anise grown in the Harran Plain (Southeastern Anatolia, Turkey). Field experiments were conducted at the Agricultural Experiment Research Area of the Agricultural Faculty, Harran University in Sanliurfa, Turkey, during 2002 and 2003 vegetation periods. The experiment was designed as a Randomized Complete Block Design with three replications. The aniseeds were sown in February 2, 2002 and February 2, 2003, with 30 cm inter-row space and the seed level of 20 kg ha^{-1}. At the stage of sowing, N (50 kg ha^{-1}) and P (50 kg ha^{-1}) were swere applied to all plots. At the beginning of May, N (50 kg ha^{-1}) was given again. The experiment was set up as four rows each was 5 m in length, with 3 replications. The 2 middle row of the plot were harvested at 10 different maturation stage. The harvest periods and phenological properties are presented in Table-1.

TABLE-1
STAGES OF ANISE PLANT MATURITY AND DATES OF HARVEST

Stage	Phenological stages of plant	Harvest date	
		2002	2003
1	Primary umbel was at the beginning of waxy stage and all leaves were green	June 22	June 26
2	Colour changed to brownissh in the primary umbel	June 24	June 28
3	Seconder umbels colour changed to brownish and the colour of bottom leaves turned yellowish	June 26	June 30
4	The primary umbel full matured completely and middle leaves turned yellowish	June 28	July 2
5	Primary umbel became brown and main stem turned yellowish	June 30	July 4
6	Primary umbel was completely dry, 2/3 of the main stem turned yellow	July 1	July 6
7	Side umbels were brown, the main stem turned yellow and no leaf was visible in the plant	July 3	July 8
8	All the umbels were brown and the main stem was drying	July 5	July 10
9	Plant was completely dry, primary umbels dropped off easily and main stem was dried	July 7	July 12
10	Plant was completely dry and primary umbels dropped off	July 9	July 14

Essential oil isolation: Plants from each plots were dried at room temperature after harvesting. Samples were taken from the cleaned aniseeds were grinded and 50 g of each seed sample (3 replicates for each stage) was subjected to hydrodistillation for 2 h and essential oils were measured[16].

GC-FID Analysis: Analytical gas chromatography was carried out on a Thermo Quest-Finnigan Trace GC gas chromatography equipped with a flame ionization detector (FID) and a AS 2000 autosampler. A polyethylene glycol ZB-wax capilar column (30 m × 0.25 mm, 0.25 μm film thickness) were used. The flow of the carrier gas (He) was 1.5 mL min^{-1}. The split ratio was 50:1. The analysis was performed using the following temperature program; oven temperatures isotherm at 60 °C, from 60 to 220 °C at the rate of 4 °C min^{-1} and from 220 to 240 °C at the rate of 1 °C min^{-1}. Both temperatures of injector and dedector were held at 250 °C. The injection volume was 0.5 μL.

Quantitative data were obtained electronically from FID area data without using correction factors. All the test were performed in triplicate. The identity of some of the essential oil components was confirmed by GC analaysis by co injection with authentic substances. The compounds were as follows; α-pinene (Fluka, 80605), camphene (Supelco, 442505), β-pinene (Fluka, 80607), (+)-3-carene (Fluka, 21986), phellandrene (Fluka, 47729), ocimene (Fluka, 74730), γ-terpinene (Fulka, 86476), *p*-cymene (Fulka, 30039), terpinolene (Fluka, 86484), linalool (Fulka, 62140), estragole (Fluka, 05818), valencene (Fluka, 75056), (Z)-anethole, (E)-anethole (Fulka, 10368), anisaldehyde (Fulka, 10440), *p*-anisic acid (Fluka, 84688), acetanisole (Aldrich, W200506), carvacrol (Aldrich, 282197), anisyl alcohol (Aldrich, W209910) and isoeugenol (Fluka, 58850).

RESULTS AND DISCUSSION

As seen in Fig. 1, the essential oil content of aniseed varied between 2.38 and 4.68 %. The essential oil content of aniseed decreased with delaying harvest time up to the 5th harvets period and then remained almost constant. This was attributed to the fact that the number of fruits per unit weight was higher in the early harvest periods. These findings were in harmony with Callan *et al.*[10] and Omidbaigi *et al.*[15] who demonstrated that the essential oil content of fruits decreased with delaying harvest time. On the contrary, Msaada *et al.*[11] found higher ratio of essential oils in the mature coriander furits. This may be due to that these authors determined the essential oil content of fresh coriander in which the total solids content was low.

The composition of essential oils at different stages of aniseed maturity is listed in Table-2. According to the harvesting stages, a total 20 compounds (96.68-98.61 %) detected at the all maturity stages. At the all stages, the major constituens of aniseed essential oils were (E)-anethole 85.14-92.28 %, anisaldehyde 1.31-4.45 %, estragole 1.47-1.72 % and isoeugenol 0.02-2.21 %. Other constituents were present in amount less than 1 % (Table-2).

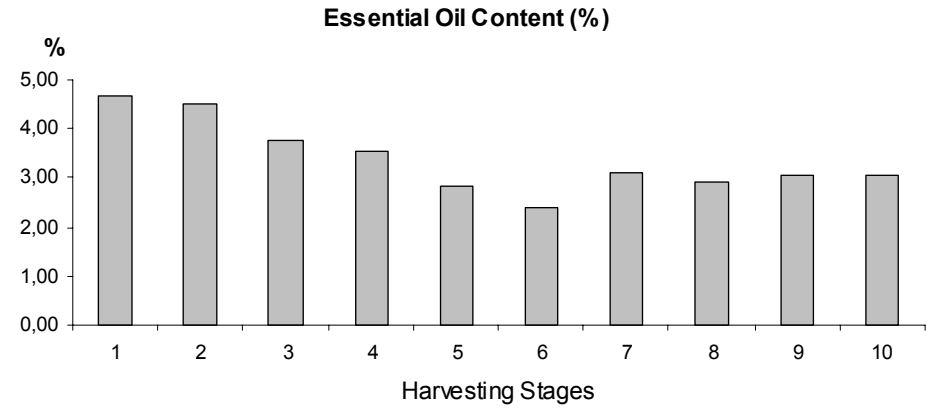

Fig. 1. Changes on the essential oil content of aniseed during the different harvesting stages (Average of 2 years)

TABLE-2
CHANGES ON THE COMPOSITION (%) OF ANISEED HARVESTED AT DIFFERENT MATURITY STAGES (AVERAGE OF TWO YEARS)

Compound	1	2	3	4	5	6	7	8	9	10
α-Pinene	0.066	0.045	0.044	0.049	0.051	0.036	0.051	0.043	0.047	-
Camphene	0.022	-	0.025	0.030	0.012	-	0.022	-	0.030	-
β-Pinene	0.029	-	0.046	0.019	0.111	0.014	0.017	0.016	0.018	0.016
(+)-3-Carene	0.148	0.010	0.160	0.132	0.011	0.014	0.013	0.013	0.111	0.011
Phellandrene	0.264	0.096	0.053	0.052	0.063	0.090	0.057	0.053	0.074	0.067
γ-Terpinene	-	0.015	0.007	0.011	0.012	0.011	0.008	0.010	0.010	0.033
Ocimene	0.040	0.048	0.046	0.046	0.053	0.038	0.034	0.039	0.049	0.059
p-Cymene	0.020	0.017	0.020	-	0.027	-	-	0.024	0.025	0.020
Terpinolene	0.081	0.081	0.083	0.097	0.082	0.094	0.086	0.082	0.087	0.081
Linalol	0.143	0.134	0.148	0.191	0.135	0.136	0.134	0.134	0.135	0.149
Estragole	1.540	1.594	1.628	1.637	1.720	1.468	1.536	1.491	1.583	1.647
Valencene	0.408	0.351	0.323	0.286	0.254	0.381	0.354	0.276	0.262	0.264
(Z)-Anethole	0.130	0.160	0.179	0.188	0.181	0.187	0.207	0.205	0.215	0.210
(E)-Anethole	92.276	90.958	89.564	87.421	86.868	91.255	89.173	87.577	87.862	85.136
Anisaldehyde	1.308	2.073	2.958	3.667	4.187	1.784	2.727	3.260	3.130	4.452
p-Anisic acid	0.121	0.120	0.141	0.153	0.302	0.153	0.151	0.166	0.294	0.170
Acetanisole	0.099	0.159	0.238	0.391	0.222	0.073	0.265	0.137	0.146	0.423
Carvacrol	0.037	0.048	0.054	0.087	0.136	0.042	0.074	0.089	0.083	0.119
Anisyl Alcohol	0.558	0.581	0.577	0.568	0.353	0.559	0.644	0.490	0.512	0.504
Isoeugenol	0.021	0.052	0.041	0.781	1.642	0.028	0.389	1.586	0.518	2.214
Total	97.311	96.542	93.335	95.806	96.422	96.352	95.934	95.681	95.191	95.565

Variation in the levels of constituents depending on the harvesting stages was remarkable. The highest (E)-anethole level was recorded in the firts harvesting stage, follwed by the 2nd, 3rd and 6th harvesting stages, in decreasing order. Overall, the (E)-anethole content decreased continuously until the 5th harvesting period.

Afterwards, an increase was observed in the levels of this compound and, finally, during the late harvesting periods the (E)-anethole content decreased again. In contrast, the level of anisaldeyhde (Fig. 2), *p*-anisic acid, acetoanisole, carvacrol, isoeugenol, ocimene and *p*-cymene gradually increased in the same periods and reached the highest levels in the 5th and 10th harvesting stages. In general, the effect of the furit maturity on the level of the valencene was found to have similar to that of (E)-anethole. The level of the (+)-3-carene, γ-terpinene, β-pinene, camphene and anisyl alcohol were varied depending on the harvesting stages. While (Z)-anethole contents was increased from 1st to10th harvesting satages, phellandrene and α-pinene contents decreased. The level of the terpineol, linalol, estragole and γ-himachalene did not change during the plant maturity stages.

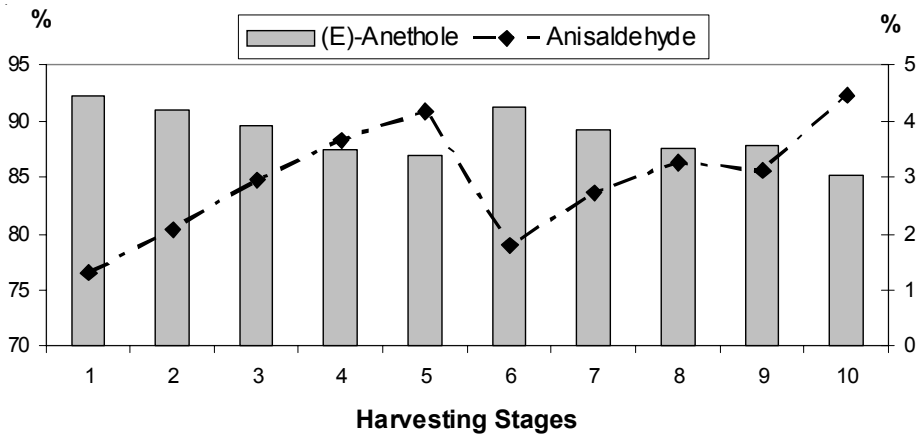

Fig. 2. Changes on the content of (E)-anethole and anisaldehyde during the harvesting stages (Average of 2 years)

These findings were in harmony with the findings of other researchers who demonstrated that maturaty process affected the essential oil composition of various fruits[9-15]. On the other hand, fluctuation in the (E)-anethole level during the maturation period may have resulted from the growing properties of anise plant. The umbels of anise become mature at different periods from top to bottom. Therefore, aniseeds harvested at different maturation stages eventually include fruits having different maturation levels.

Generally, the essential oils of the aniseed during their maturity stages revealed occuring great differences during the maturity of the plant. The obtained results indicate that aniseeds should be harvested at the 1st, 2nd or 3rd mauration stages, since the levels of both essential oils and (E)-anethole are the highest. If this is not the case, the 6th maturation stage should be considered as the appropriate harvesting period.

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