

# Comparison Physicochemical Properties of Hexane Extracted Aniseed Oil from Cold Press Extraction Residue and Cold Press Aniseed Oil

Derya Ören Duran, Şeyma Akalin Benderli

**Abstract**—Cold press technique is a traditional method to obtain oil. The cold-pressing procedure involves neither heat nor chemical treatments; therefore, cold press technique has low oil yield. The cold pressed herbal material residue still contains some oil after cold press. In this study, the oil that is remained in the cold pressed aniseed was extracted with hexane; and it was analyzed to determine physicochemical properties and quality parameters. It was found that the aniseed after cold press process still contained 10% oil. The values of other analysis parameters were 2.1 mgKOH/g for free fatty acid (FFA) and 7.6 meqO<sub>2</sub>/kg for peroxide. Cold pressed aniseed oil values were determined as 2.1 mgKOH/g for FFA and 4.5 meqO<sub>2</sub>/kg for peroxide, respectively. In addition, fatty acid composition was analyzed, and it was found that both types of oil had same fatty acid compositions. The main fatty acids were oleic, linoleic and palmitic acids.

**Keywords**—Aniseed oil, cold press, extraction, residue.

## I. INTRODUCTION

**A**NISEED (*Pimpinella anisum* Linn.) is an annual flowering plant, which belongs to the Apiaceae family native to the Eastern Mediterranean and South Asia. This family is known for its distinctive flavor, which is a mixture of volatile fragrant compounds that come from essential oils and f make the essence of the plant. Aniseed contains about 1.5–5.0% essential oil; and, the main component of the aniseed essential oil is the trans-anethole, which gives this plant a sweet herbaceous odor and taste [1]. Anethole component in aniseed is affected by the genotype, ecological conditions, and agricultural practices such as irrigation, plant population, fertilizer and planting date. Apart from anethole, aniseed is well known for its essential oil, which is the source of its fragrance and flavor [2]-[5].

Aniseed plant is an annual plant. The leaves are at the plant and simple. The flowers are white and about 3 mm diameter [5]. The aniseed fruits and seeds contain essential oils [6].

Although the main component of aniseed oil is trans-anethole (75-90%), coumarins (umbelliferone, umbelliprenin, bergapten and scopoletin), lipids (fatty acids, beta-amyrin, stigmasterol and salts), flavonoids (flavonols, flavon, glycosides, rutin, isoorientin and izovitexsin), protein and carbohydrates are also listed among the other components [7], [8].

Today, aniseed is an important raw material used in the

pharmaceutics, perfume, food and cosmetic industries [9]. It has been reported that Apiaceae seed is used as an ingredient in the food industry such as bread, biscuits and cookies, and that Apiaceae are suitable spices in the meat industry [2]. Recently, this spice plant has begun to attract even more attention from consumers due to its antimicrobial, antifungal, insecticidal and antioxidant effect on human health [10].

It is known from the literature that aniseed has an effect on the digestive system by increasing the secretion of the salivary glands and reducing the acidity of gastric juice. Anise is known to have an estrogenic effect, thereby helping to reduce bleeding duration and menstrual pains as well as reducing hot flashes in postmenopausal women. Aniseed has an effect on microorganisms that cause many diseases. Anise has a wide range of effects on various bacteria, fungi, viruses and amoebae [11].

Aniseed is traditionally used in the form of herbal drug, in the solid form as herbal preparation or in liquid form dissolved in water for oral use (defined according to the standard terminology published in the European Pharmacopoeia) [12].

There is a growing interest in cold press within the last few years since it preserves the nutritional properties in oils. Cold press process preserves the natural taste and nutritional properties desired by the consumer [13]. Cold press is method that does not require heat and chemical treatment in oil extraction and thereby it contains many healthy components such as the natural antioxidants. Cold press oils are the best oils, which contain many phenolic compounds such as antioxidants and useful phytochemicals [14]-[16]. Although the oil productivity is low, it is can be safely used by the consumers and they could achieve the desired taste since there is no solvent extraction and it does not contain any components such as chemical residues etc. [13].

## II. MATERIAL AND METHOD

### A. Seed and Oil Samples

Seed samples were provided from Central Anatolian suppliers and oil was extracted from the seeds by using the cold pressed method at Zade Vital Pharmaceuticals Inc. The temperature was kept below 40 °C and no chemical and heating process were used. The cold-press machine was used by 3-4 kg seed/h capacity depending on the differences of the seeds. Settling out the sediments was performed by storing the cold-pressed oil in stainless steel intermediate tanks for one day.

Step1. Extraction of Oil by Cold Press: Cold press oil was

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obtained by using mechanic extraction without heat treatment. Aniseed oil was obtained by the method of cold press extraction process in Zade Vital Pharmaceuticals Inc. extracted oils were hermetically closed under the nitrogen.

Step2. Milling of Herbal Material after Cold Press: Aniseed herbal material was milled by blender about ten minutes.



Fig. 1 Residue of aniseed after cold press extraction

Step3. Evaporation: Milled aniseed material was evaporated in incubator at 105 °C about 2 hours.

Step4. Weighting Milled Aniseed: The milled seeds are weighted and put in to the filter cartridge.

Step5. Extraction of Oil by Hexane Extraction: Aniseed oil was obtained by soxhlet extraction. Hexane was used in the soxhlet system

#### B. Chemicals

Chloroform, acetic acid, potassium hydroxide, ethanol, nitric acid, methanol were used in analyses. These chemicals were supplied from companies such as T. Baker, Sigma Aldrich. In addition, pure water and ultra-pure water were used by pharmacopeial criteria.

#### C. Analyses of Peroxide Value, FFA, Specific Gravity, Refractive Index

In this study, the oil that remained in the cold pressed aniseed was extracted with hexane and analyzed to determine physicochemical properties and quality parameters. In the analyses, EP 2.5.5 method was used for the peroxide values, EP 2.5.1 was used for the FFA, and 2.2.5 method was used for [17]. Refractive index was measured by an automatic refractometer of Rudolph J257 brand.

#### D. Fatty Acid Composition

Fatty acid composition is performed with a validated method in the company [18]. First of all, 60 g oil was weighed and then 2 mL KOH was added. They were mixed for a few minutes. After that potassium hydroxide was added and centrifuged. Organic phase was filtered and put into GC vial. The analyses lasted about 50 minutes.

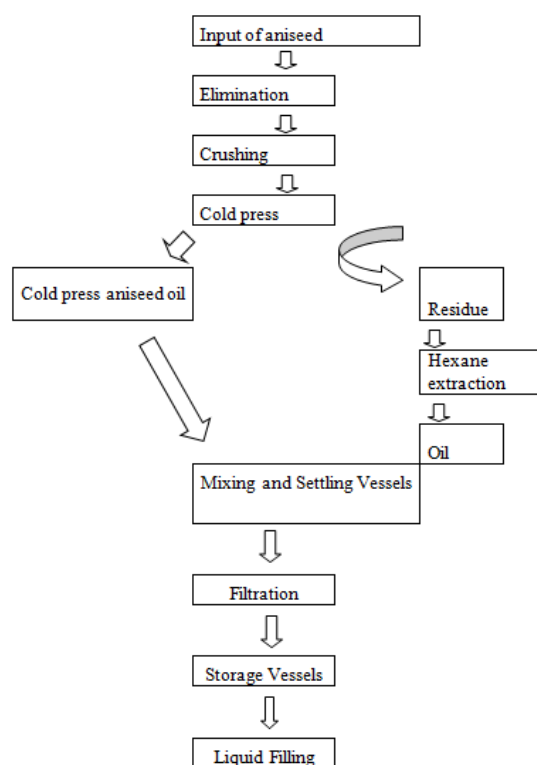


Fig. 2 Flow chart of oils obtained through cold press and extracted with hexane

Schimadzu GC conditions:

- Column: SUPELCO SP 2560
- Max. Temperature: 325 °C
- Initial Flow: 1,1 mL/min, N<sub>2</sub> (Constant Flow)
- Nominal Initial. Pressure: 163,5 kPa
- Average Velocity: 26.9 cm/sec n
- Inlet Mode: Split
- Temperature: 250 °C
- Injection Volume: 1,0 µL
- Pressure: 163,5 kPa
- Split Average: 1:100
- Total Flow: 114,1 mL/min
- Gas Type: N<sub>2</sub>
- Front Detector: Flame Ionization
- Temperature: 260 °C
- Hydrogen Flow: 40,0 mL/min
- Air Flow: 400 mL/min
- Mode: Constant Makeup Flow
- Makeup Flow: 30,0 mL/min
- Makeup Gas Type: Nitrogen

### III. RESULT AND DISCUSSION

In this study, the aniseed oil obtained by the cold press technique and the vegetable oil in the residue that was released after the cold press were extracted with hexane; and they were compared by considering certain physicochemical properties of the two oils. These physicochemical properties were FFAs,

peroxide value, refractive index and specific gravity. In addition, the fatty acid composition that had an important role in determining the characteristics of the oil was examined; and, major saturated and unsaturated fatty acids were determined.

In a study conducted by [19], the density of aniseed oil was found to be 0.9677 g/cm<sup>3</sup>, its acid value was 0.94 KOH/g, and refractive index was 1.4961. In another study conducted by Sun et al., the specific gravity was 0.998, the refractive index was 1.55 and the acid value was 2.55 [22].

In the study conducted by Satibeş et al., aniseeds were obtained from various locations in Turkey, and the analyses performed on these seeds were concluded as follows: The specific gravity of the seeds obtained from Çeşme region was initially 0.9765 at 20 °C, which became 0.9759 after 9 months; their refraction index was initially 1.555 at 20 °C, which became 1.550 after 9 months. The acid value was found to be 1.29. In the same study, the specific weight, refractive index and acid number of aniseeds collected from Burdur and Tavsanli region were determined as 0.988 and 0.9856, 1.553 and 1.550, and 1.16 and 1.24, respectively [20].

In a study by Naher et al., the refractive index was found to be 1.559, the specific gravity was 0.824, and the acid value was 8.09 [10].

In a study by Albulushi et al., the refractive index was found to be 1.4765, and the acid value was 0.3927. In addition, the odor, color and appearance of the aniseed oil at room temperature were defined; it was found that it had a spicy odor, homogenous light yellow color and a transparent appearance with a density close to water [21].

TABLE I  
SOME PHYSICO-CHEMICAL PROPERTIES OF COLD PRESS ANISEED OIL AND RESIDUE BY HEXANE EXTRACTED ANISEED OIL

Analysis	Cold press aniseed oil	Residue by hexan extracted aniseed oil
FFA (mgKOH/g oil)	2,4	2,1
Peroxide Value (meqO <sub>2</sub> /Kg Oil)	4,5	7,6
Specific Gravity (g/cm <sup>3</sup> )	0,976	0,982
Refractive Index (20°C)	1,4881	1,4877

TABLE II  
COMPARISON OF FATTY ACID COMPOSITIONS OF COLD PRESS ANISEED OIL AND RESIDUE BY HEXANE EXTRACTED ANISEED OIL

Fatty acids	Cold press aniseed oil	Residue by hexan extracted aniseed oil
C 6:0 (Caproic acid)	0,00	0,00
C 8:0 (Caprilic acid)	0,04	0,04
C 10:0 (Capric acid)	0,62	0,00
C 12:0 (Laurik acid)	0,70	0,00
C 14:0 (Myristic acid)	22,92	24,12
C 16:0 (Palmitic acid)	3,16	4,26
C 16:1 (Palmitoleic acid)	0,20	0,15
C 18:0 (Stearic acid)	0,70	0,99
C 18:1 (Oleic acid)	48,61	53,28
C 18:2 (Linoleic acid)	16,08	25,06
C 18:3 (Linolenic acid)	0,37	0,10
C 20:0 (Arachidic acid)	0,14	0,09
C 20:1 (Eicosanoic acid)	0,22	0,22
C 22:0 (Behenic acid)	0,11	0,11

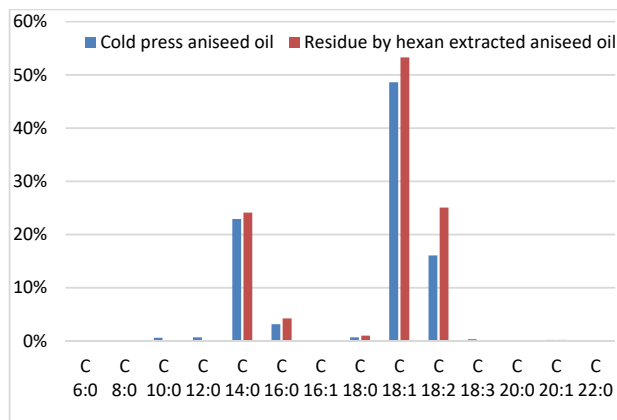


Fig. 3 Comparison of fatty acid composition

In our study, certain physicochemical properties of cold pressed aniseed oil and the aniseed that was extracted from the residue with hexane are presented in Table I. When all the values in Table I were compared to other studies, the results were found to be very close to each other.

In the study by Sun et al., among the fatty acid composition values, mean capric acid was found to be 0.14%, mean lauric acid was 0.50%, mean palmitic acid was 8.00%, mean stearic acid was 0.70%, mean arachidic acid was 0.07%; in the unsaturated fatty acids, mean oleic acid was found to be 20%, mean linoleic acid was 23%, and mean linolenic acid was 1% on average [22].

In our study, the fatty acid values obtained as a result of the two extractions were close to each other. When compared to other studies in the literature, the values obtained were close to the values obtained in our study. Aniseed oil is rich in unsaturated fatty acids.

#### IV. CONCLUSION

Aniseed oil is aromatic oil with many health benefits. In this study, firstly, oil was extracted through cold press. Then, it was identified that there was still 10% oil present in the residue; therefore, oil was extracted from the residue with hexane extraction. Later, major physicochemical parameters were analyzed in these vegetable oils obtained, and it was concluded that the parameters were at the optimum level in cold pressed aniseed oil.

Aniseed oil is rich in polyunsaturated fatty acids, and it has benefits in many areas due to its content.

Among the extraction techniques, cold press has been determined as the most useful method. In this way, the components in the aniseed could be transferred into the oil without being destroyed and thereby brought into use in the healthiest way for human life.

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## REMARKS

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## REFERENCES

- [1] Ullah H & Honermeier B., "Fruit yield, essential oil concentration and composition of three aniseed cultivars (*Pimpinella anisum* L.) in relation to sowing date, sowing rate and locations." *Industrial Crops and Products*, vol.42, pp. 489- 499, 2013.
- [2] M. Acimovic et al. "Essential oils of *Pimpinella anisum* and *Foeniculum vulgare*" *Botanica Serbica*, vol.39 (1), pp. 09-14, 2015.
- [3] Asadi-kavan, Z., Ghorbanli, M., Pessarakı, M., & Sateci, A., "Effect of polyethylene glycol and its interaction with ascorbate on seed germination index in *Pimpinella anisum* L." *Journal of Food, Agriculture and Environment*, vol. 7(3&4), pp.662–666, 2009.
- [4] Acimovic, M. G., Korac, J., Jacimovic, G., & Oljaca, S., "Influence of ecological conditions on seeds traits and essential oil contents in aniseed (*Pimpinella anisum* L.)" *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 42(1), pp.232–238, 2014.
- [5] Ozel, A., "Anise (*Pimpinella anisum*): Changes in yields and component composition on harvesting at different stages of plant maturity". *Expl Agric*, vol.45, pp.117–126, 2009.
- [6] Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC. Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour Fragr J*;23:213-226, 2008.
- [7] Picon, P. D., Picon, R. V., Costa, A. F., Sander, G. B., Amaral, K. M., Aboy, A. L., & Henriques, A. T. "Randomized clinical trial of a phytotherapeutic compound containing *Pimpinella anisum*, *Foeniculum vulgare*, *Sambucus nigra*, and *Cassia augustifolia* for chronic constipation." *BMC Complementary and Alternative Medicine*, vol:17 pp.10-17, 2010.
- [8] Yamini, Y., Bahramifar, N., Sefidkon, F., Saharkhiz, M. J., & Salamifar, E. "Extraction of essential oil from *Pimpinella anisum* using supercritical carbon dioxide and comparison with hydrodistillation". *Natural Product Research*, vol.22(3), pp.212–218, 2008.
- [9] Ross I.A., "Medicinal plants of the world: chemical constituents, traditional and modern medicinal uses". Humana press, Volume 2, pp.363-37, 2001.
- [10] Naher S., Ghosh A., Aziz S., "Comparative Studies on Physicochemical Properties and GC-MS Analysis of Essential Oil of the Two Varieties of the Aniseed (*Pimpinella anisum* Linn.) in Bangladesh". *Int.J. Pharm. Phytopharmacol. Res.* vol.2(2): pp.92-95, 2012.
- [11] Shojaii A and Fard M.A., "Review of Pharmacological Properties and Chemical Constituents of *Pimpinella anisum*" *ISRN Pharmaceutics* Volume, Article ID 510795, pp.1-8, 2012.
- [12] EMEA Committee on Herbal Medicinal Products (HMPC) (2012) Assessment report on *Pimpinella anisum* L., *fructus* and *Pimpinella anisum* L., *aetheroleum*, EMEA/HMPC/321181/2012.
- [13] Fawzy R. M., and Mohsen M. S., "Antiradical and antimicrobial properties of cold-pressed black cumın and cumın oils" *Eur. Food res. Technol.*, vol: 234 pp.833-844, 2012.
- [14] Crews C., Hough P., Godward J., Brereton P., Lees, M. and Guiet, S., "Quantitation of the main constituents of some authentic grape-seed oils of different origin". *Journal of Agricultural and Food Chemistry*, vol: 54, pp.6261–6265, 2006.
- [15] Yu, L., Zhou, K. And Parry, J. "Antioxidant properties of coldpressed black caraway, carrot, cranberry, and hemp seed oils". *Food Chem.* Vol: 91, pp.723–729 2005.
- [16] Bail S, Stuebiger G, Krist S, Unterweger H, Buchbauer G.. "Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity". *Food Chem.* Vol: 108, pp.1122-1132, 2008.
- [17] British Pharmacopoeia. Herbal Drugs and Herbal Drug Preparations Milk-thistle Fruit. Volume III. pp: 7173p. 2009.
- [18] Duran D., Ötleş S., Karasulu E., "Determination Amount of Silymarin and Pharmaceutical Products from Milk Thistle Waste Obtained from Cold Press" *Acta Pharm. Sci.* Vol 57(1), pp 85-101, 2019.
- [19] Rajab A. "Comparative Study of Physical and Chemical Properties of the

Oil Extracted from Fresh and Storage Aniseed and Determination of the Optimal Extraction Method" *IJPPR*, Volume 8, Issue 9: September 2016.

- [20] Satibeşe E, Doğan A., Yavaş İ., "The effects of storage period on aniseed essential oil composition" *Gıda*. vol: 19 (5), pp.295-299. 1994.
- [21] Albulushi S.,M.,Saidi H., Amaresh N., Mullaicharam A., Study of Physicochemical Properties, Antibacterial and GC-MS Analysis of Essential Oil of the Aniseed (*Pimpinella anisum* Linn.) in Oman, *RRJPP vol:2*, 24-33, 2014
- [22] Sun et al., "Anise (*Pimpinella anisum* L.), a dominant spice and traditional medicinal herb for both food and medicinal purposes". *Cogent Biology*, Vol: 5 pp. 5-25. 2019.

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