# Characterization of Fennel Fruits: Types and Quality (I)

Mokhtar M. Bishr[1], Eman G. Haggag[2]*, Mohamed M. Moawed[3] and Osama M. Salama[4]

[1]Research and Development Dept., Arab Co. for Pharm. and Med. Plants (MEPACO)
[2]Pharmacognosy Dept., Faculty of Pharmacy, Helwan University, Cairo, Egypt
[3]Botany Dept., Faculty of Science, Ain Shams University, Cairo, Egypt
[4]Pharmacognosy Dept., Faculty of Pharm. Sci. and Pharm. Ind., Future University in Cairo, Egypt
wemisr@hotmail.com

**Abstract:** Four samples of different fennel fruit cultivars (F **1**-F **4**), obtained from El-Fayoum, Egypt (F **1**), El-Menia, Egypt (F **2**), Sudan, El-Khartoum (F **3**), and Germany (F **4**) were cultivated in MEPACO`s Farm (Arab Co. for Pharm. and Med. Plants, Cairo, Egypt) and the obtained fruits were subjected to macro- and micro-morphological stereomicroscopic examination as well as GC-MS analysis of their volatile oils. **The aim** of the study is to determine the differences in the macro- and micro- characters of different fruit cultivars as well as their oil constitutes. **The results** showing different exomorphic parameters *viz*. shape, color, dimensions and surface sculpture. Also the stereomicroscopic examination showed differences in the epicarp, mesocarp; vitti and endosperm. GC-MS analysis of volatile oils of (F **1**-F **4**) showed on comparing three parameters; fenchone, estragole and *trans*-anethole that F **4** has the highest percentage of *trans*-anethole (78.98%), while F **1** and F **2** have close values (1.05 and 1.02%, respectively) followed by F **3** (3.02%). F **4** has the lowest percentage of estragole (3.97%); while (F **1**-F **3**) have higher values (78.58, 64.81 and 25.79%, respectively). Also F **4** has doubled the percentage of fenchone (6.73%) of F **1** and F **2** (2.54 and 2.57%, respectively), while F **3** has 0.69%. Thus results show that the two cultivars growing in Egypt (F **1** and F **2)** have almost the same ratios of the compared parameters while, the Sudan cultivar F **3** is closer to F **1** and F **2** than it is to F **4**. Also the three cultivars (F **1**-F **3**) are far from specification of sweet fennel oil but close to bitter fennel oil. The German cultivar (F **4)** has the best oil quality as a sweet fennel. Investigation of the powdered samples (F **1**-F **4**) showed that only F **4** is different in having higher abundant fragments of reticulate parenchyma cells with ratio of 1:3 {F **4**:(F **1**-F **3**)}. **In conclusion:** These findings are of pharmaceutical-industrial value helping in the production of herbal pharmaceutical products of fennel fruit and/or oil of known higher quality.
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**Key Words:** Foeniculum vulgare, Fenchone, Estragole, Anethole, Stereomicroscopic examination.

## 1. Introduction

Fruit of Foeniculum vulgare Mill. (Fam. Umbellifereae) is cultivated in Europe and much is imported from India, China and Egypt (**Trease and Evan, 1976**). Fennel oil has long been used as an aromatic stomachic (**Gunn, 1920**). Fennel oil is a natural antispasmodic agent (**Plant and Miller, 1926**), it also has anti-foaming and carminative effect (**Harries *et al.,* 1978**). It is used in treatment of irritable bowel syndrome as it exerts gastric relief due to its local anesthetic effect on the gastrointestinal tract (**Holt, 1990**). Also it is used in management of bronchial asthma due to the strong diaphoretic effect of anethole (**Haggag *et al.,* 2003**). Fennel oil emulsion showed to reduce intestinal spasms and increase motility of small intestine in infantile colic (**Alexandrovich *et al.,* 2010**). Fennel oil have been investigated for its constituents as sweet and bitter oil (**Wichtl and Bissett, 1994; Leung and Foster, 1996**). The therapeutic value of fennel fruit as well as its presence in several commercial cultivars, varying considerably in size and appearance (**Barthlott, 1981 & 1990; Barthlott *et al.,* 1998 & 2003; Javadi and Yamaguchi, 2004; Salimpour *et al.,* 2007**), encouraged the authors to make close investigation of macro- and micro- characters of different fruit cultivars as well as their oil constitutes.

## 2. Materials and Methods

**Plant materials:**

Four samples of different fennel fruit cultivars (F **1**-F **4**), obtained from El-Fayoum, Egypt (F **1**), El-Menia, Egypt (F **2**), Sudan, El-Khartoum (F **3**), and Germany (F **4**) were cultivated in MEPACO`s Farm (Arab Company for Pharmaceutical and Medicinal Plants, Cairo, Egypt) and the obtained fruits were subjected to this study.

**Macromorphological Investigation:**

The investigated fruits were dried, cleaned and examined by Stereomicroscope and photographed by Digital Camera 7.2 mega pixels to show the different exomorphic parameters viz. shape, dimensions and

color. For stereo-electro-microscopic (SEM) examination, the fruits were dried, fixed in 70% alcohol and were mounted on brass stubs and coated with a thin layer of gold using Edwards Sputter coater and examined using different magnifications by JEOL- T100 scanning electron microscope at the SEM unit, Faculty of Science, Ain Shams University. The terminology used for the description of leaves as examined by SEM is that of **Stearn (1966); Barthlott (1981 & 1990); Barthlott *et al.* (1998 & 2003); Javadi and Yamaguchi (2004) and Salimpour *et al.* (2007).**

**Micromorphological Investigation:**

Mature fruits were softened in warm water for 12 – 72 hrs and then dehydrated using a tertiary butyl alcohol series and sectioned at a thickness of 15 - 20 µm; sections were double stained with safranin and light green according to the traditional methods of **Johanson (1940).** The fruit sections were described as LM, and photographed using Digital Camera (Sony cyber-shot DSC.W55, 7.2 mega pixels). The anatomical descriptive terms of fruit coat in the present study based on the terms of **Corner (1976).**

**Preparation of the essential oils:**

The fresh fruits (500g) were separately hydro-distilled for 6 hours in a Clevenger type apparatus. The resulting oils were collected, dried over anhydrous sodium sulphate and stored in refrigerator until analyzed. Percentage yields were determined according to the Egyptian Pharmacopoeia, 1984.

**GC/MS Analysis:**

GC/CMS analysis was performed on a GC/MS system (Shimadzu GC/MS- QP2010) with software (Class 5000). Gas chromatograph equipped with a TR-5MS (5% Phenyl Polysil Phenylene Siloxane), column (DB 30m × 0.25 mm i.d × 0.25 um film thickness). The analyses were carried out under the following conditions: Carrier gas: He with flow rate 1 ml/min; 235°C; Detector temp. FID: 250°C; Injector temp.: 235°C; split ratio; 1:10; Oven temp. Program: initial temp.; 40°C (0.5 min) increasing to 150°C (at 7.5°C/min), 150°C (1min) then increasing to 250°C (at 5°C/min)- 250°C (2min). The capillary column was directly coupled with mass spectrometer HP 5973 (Agilent). EI-MS were recorded at 70 ev. The analysis has been done at the Quality Control Department, Chemistry Section of Arab Co. for Pharm. and Med. Plants (MEPACO), Cairo, Egypt. Identification of the components were performed by aid of the computer library search (Class 5000 lab software package) comparison of mass spectra with literature data and by comparison of their retention times and mass fragmentation patterns with those of the library data base (**Massada, 1967; Egyptian Pharmacopoeia, 1984; Adams, 1995; Guido *et al.*, 2005**).

**3. Results**

Macromorphological characters of the different cultivars (F **1**-F **4**) whole mount are shown in Figure 1, microphotographs of the fruit surface sculpture of the different cultivars (F **1**-F **4**) are shown in Figure 2 and microphotographs of fruit anatomy of the different cultivars (F **1**-F **4**). The results of macromorphological and micromorphological characters of the four cultivars (F **1**-F **4**) are summarized in Table **1** and GC-MS analysis of volatile oils of the four cultivars (F **1**-F **4**) is summarized in Table **2**.

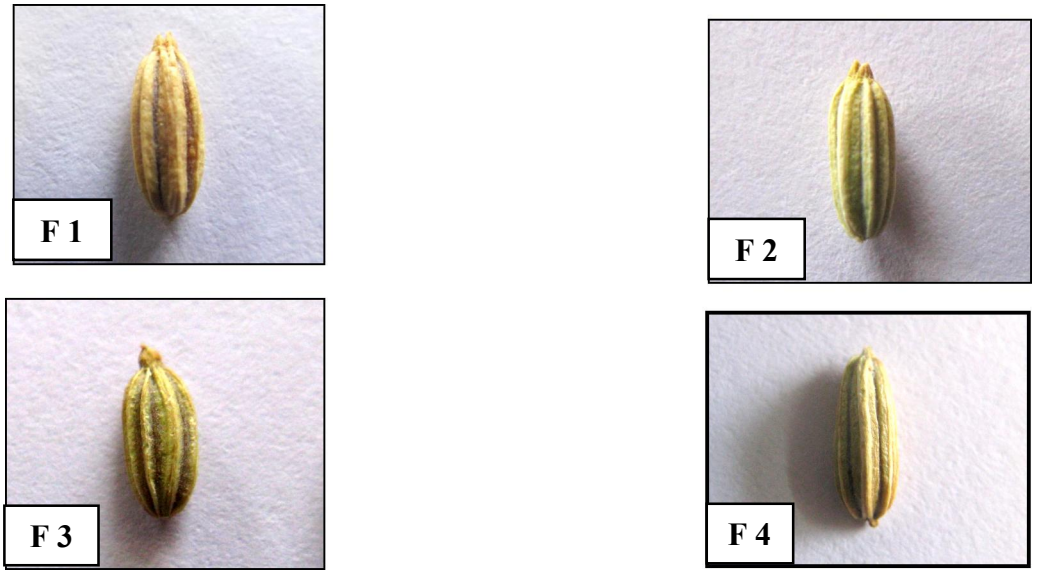
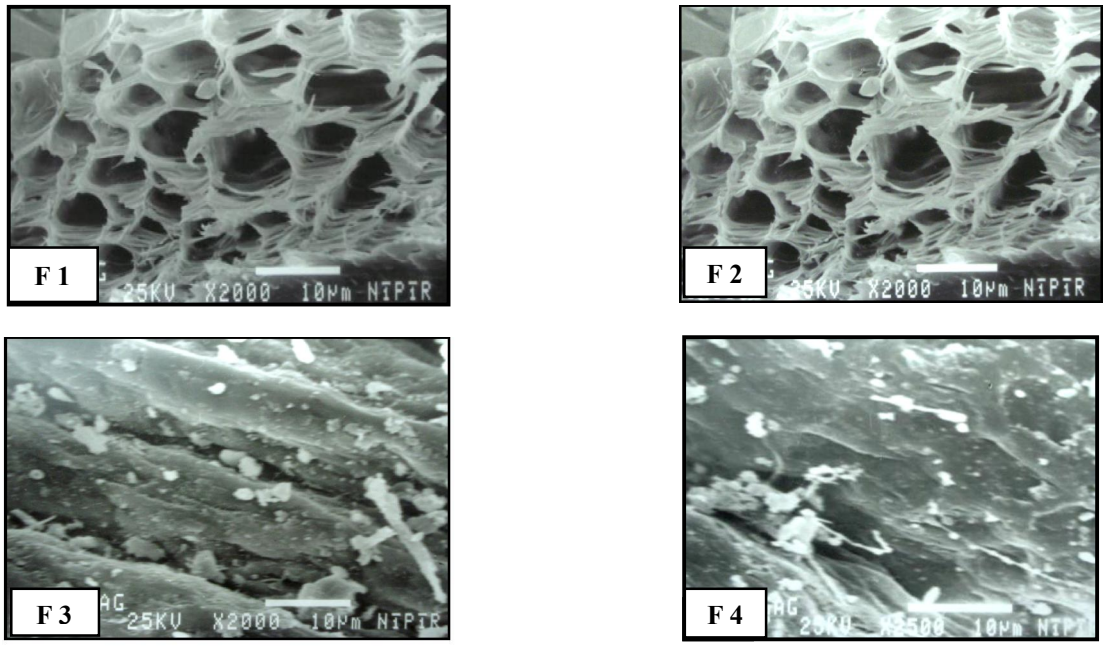



**Fig. (1):** Macrophotographs of fennel different cultivars fruits (whole mount) X=10




uit surface sculpture of fennel different cultivars X=400

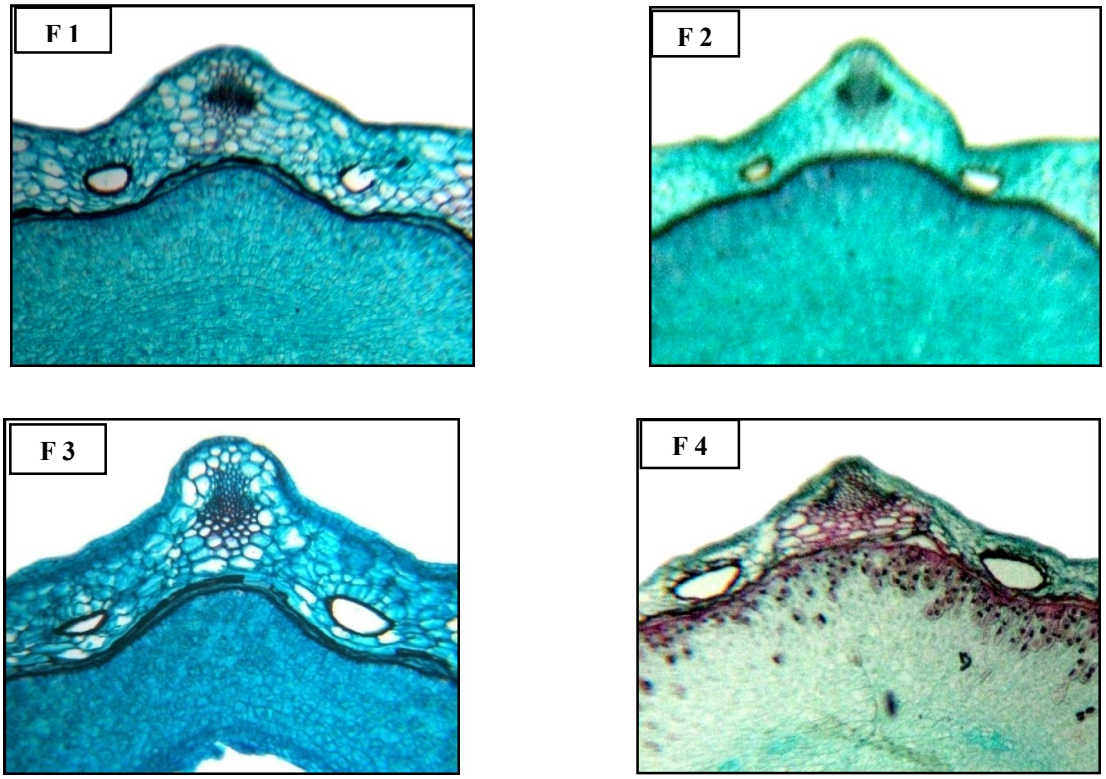


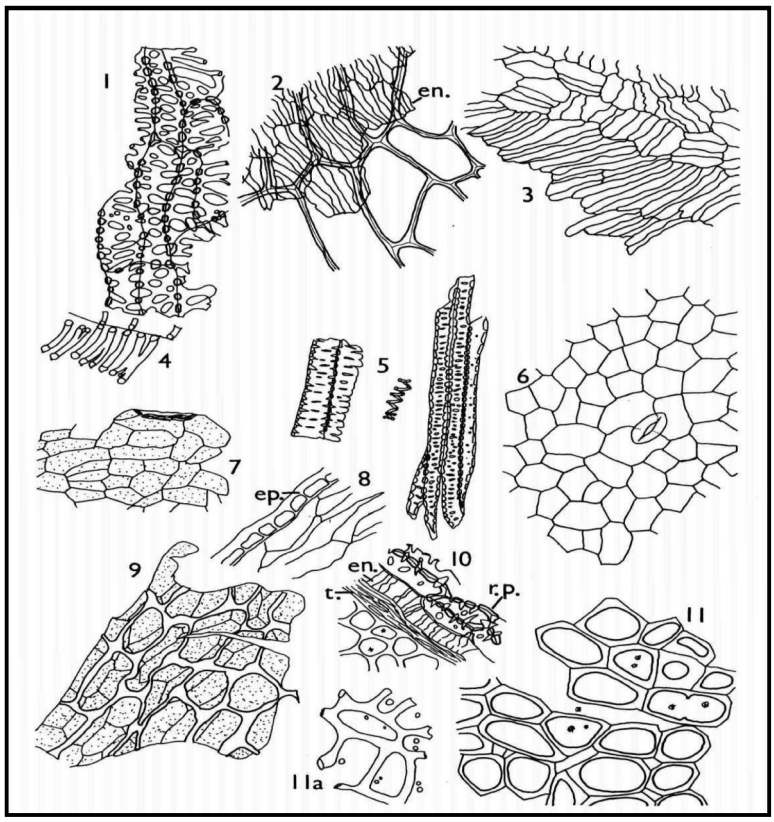

**Fig. (3):** Microphotographs of fruit anatomy of fennel different cultivars X=400

**Table (1):** Macro- and micro-morphological characters of the four fennel cultivars

| Description | | | F 1 | F 2 | F 3 | F 4 |
|---|---|---|---|---|---|---|
| Macromorphological Characters | Shape | | Elliptical | Elliptical | Oval | Oval |
| | Color | | Brownish green | Greenish yellow | Brownish green | Light brown |
| | Dimensions | | 9 (±1)x 2 (±1) mm | 9 (±2)x 3 (±1) mm | 5 (±2)x 2 (±1) mm | 6 (±1)x 2 (±1) mm |
| | Fruit surface sculpture | | Rugose | Verrucate | Striated | Reticulate-Foveate |
| | Anticlinal wall | Level | Elevated | Elevated | Elevated | Elevated |
| | | Surface sculpture | Smooth | Wrinkled | Wrinkled | Smooth |
| | | Margin | Straight | Straight | Straight | Straight |
| | Periclinal wall | Level | Slightly Depressed | Slightly Depressed | Slightly Depressed | Slightly Depressed |
| | | Surface sculpture | Smooth | Wrinkled | Wrinkled | Wrinkled |
| | Wax Shape | | Granulate / Rodlets | Granulate / Rodlets | Granulate | Granulate |
| Micromorphological Characters | Epicarp | Cuticle | Thick | Thick | Thin | Thin |
| | | No. of Layer | Two | Two | One | One |
| | | Shape | Tangential | Tangential | Tangential/ Radial | Tangential |
| | Mesocarp | Reticulate Parenchyma | Above the V.B | Above the V.B | Above the V.B | Above and below the V.B |
| | | No. of Layer | 6 -1 0 | 8 - 12 | 6 - 10 | 4 - 6 |
| | | Type of cells | Polyhedral parenchyma / Collenchyma | Polyhedral parenchyma / Collenchyma | Polyhedral parenchyma / Collenchyma | Polyhedral parenchyma / Collenchyma |
| | | No. of Vascular Bundle | 5 | 5 | 5 | 5 |
| | | Size of V.B. | 5 small bundles | 5 small bundles | 3 small + 2 medium | 3 small + 2 large |
| | | No. of Vitti | 6 | 6 | 6 | 6 |
| | | Shape | Terete | Terete | Compressed oval | Oval |
| | Endocarp | No. of Layer | One | One | One | One |
| | | Shape | Polyhedral | Compressed | Polyhedral | Compressed |
| | Seed Coat | No. of Layer | One | One | One | One |
| | | Shape | Compressed | Compressed | Tangential | Compressed |
| | Endosperm | No. of Layer | 18 - 20 | 13 - 18 | 15 - 18 | 18 - 22 |
| | | Shape | Elliptic - Square | Elliptic - Square | Globoid | Globoid |

**Table (2):** GC-MS analysis of volatile oils of the four fennel cultivars

| Active principle | $R_t$ (min) | Relative percentage | | | |
|---|---|---|---|---|---|
| | | F 1 (El-Fayoum) | F 2 (El-Menia) | F 3 (El-Khartoom) | F 4 (Germany) |
| Fenchone | 11.66 | 2.54 | 2.57 | 0.69 | 6.73 |
| Estragole | 15.02 | 78.58 | 64.81 | 25.79 | 3.97 |
| Trans-Anethole | 17.54 | 1.05 | 1.02 | 3.02 | 78.98 |




**1** Reticulate parenchyma of the mesocarp.
**2** Endocarp (en.) with overlying cells of the innermost layer of the mesocarp, in surface view.
**3** Endocarp in surface view.
**4** Fragment of a reticulately thickened vessel.
**5** Elements from the fibro-vascular tissue.
**6** Epicarp in surface view showing a stoma.
**7** Fragment of a vitta.
**8** Epicarp (ep.) and parenchyma of the mesocarp in sectional view.
**9** Fragment of a vitta with overlying thick-walled cells of the inner- cells of the most layer of the mesocarp, in surface view.
**10** Part of the pericarp and seed in sectional view showing the reticulate parenchyma (r.p.), endocarp (en.), testa (t.) and endosperm.
**11** Endosperm containing microrosette crystals of calcium oxalate.
**11a** Thick-walled cells of the endosperm

**Fig. (4):** Items of powdered fennel sample X=80

## 4. Discussion

The four samples of the different fennel fruit cultivars (F 1-F 4), showed macro-morphological differences in parameters *viz*. shape, color, dimensions and surface sculpture. Also the micro-morphological stereomicroscopic examination showed differences in the epicarp, mesocarp; vitti and endosperm. GC-MS analysis of volatile oils of (F 1-F 4) showed on comparing three parameters; fenchone, estragole and *trans*-anethole that F 4 has the highest percentage of *trans*-anethole (78.98%), while F 1 and F 2 have close values (1.05 and 1.02%, respectively) followed by F 3 (3.02%). F 4 has the lowest percentage of estragole (3.97%); while (F 1-F 3) have higher values (78.58, 64.81 and 25.79%, respectively). Also F 4 has doubled the percentage of fenchone (6.73%) of F 1 and F 2 (2.54 and 2.57%, respectively), while F 3 has 0.69%. Thus results showed that the two cultivars growing in Egypt (F 1 and F 2) have almost the same ratios of the compared parameters while, the Sudan cultivar F 3 is closer to F 1 and F 2 than it is to F 4. Also the three cultivars (F 1-F 3) are far from specification of sweet fennel oil but close to bitter fennel oil and the German cultivar (F 4) has the best oil quality as a sweet fennel when all compared with reported data *(*Wichtl and Bissett, 1994; Leung and Foster, 1996*).* Investigation of the powdered samples (F 1-F 4) showed that only F 4 is different in having higher abundant fragments of reticulate parenchyma cells with ratio of 1:3 {F 4:(F 1-F 3)} (Figure 4).

**Conclusion**

GC-MS analysis of volatile oils of (F 1 - F 4) showed on comparing the three parameter; fenchone, estragole and *trans*-anethole that the two cultivars growing in Egypt (F 1 and F 2) have almost the same ratios of the compared parameters while, the Sudan cultivar F 3 is closer to F 1 and F 2 than it is to F 4. Also the three cultivars (F 1 - F 3) are far from specification of sweet fennel oil but close to bitter fennel oil. The German cultivar (F 4) has the best oil quality as a sweet fennel. Thus these findings are of pharmaceutical-industrial value helping in the production of herbal pharmaceutical products of fennel fruit and/or oil of known higher quality.

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**Corresponding author**

Eman G. Haggag

Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, 11795, Cairo, Egypt.

wemisr@hotmail.com

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