Chapter 1

Introduction

1.1 Background

Olive oil is one of the main basic factors of Mediterranean culture diet and one of the most nutritional and health oils that is highly accepted by consumers all over the world. The olive trees are highly cultivated in Mediterranean regions as it is the suitable environment for these plants growth.

Olive oil is composed from major component (fatty acids and triacylglycerol) and other minor component (include sterols, phenolics, vitamins …etc). Fatty acid is composed from long chain of carbon (in olive oil from 16 to 18 carbon atom); that could be saturated or unsaturated; with carboxyl group. Fatty acids in oil is attached in group of three fatty acids with a glycerol molecule forming triacylglycerol (TAG). The TAG may lose one or two of its fatty acids that will be found in oil as free fatty acid. Sterols are minor component of olive oil, they play nutritional role for consumer, and also they reduce the cholesterol absorption into the body. They include stigmasterol and campesterol. Sterols are very good test for olive oil adulteration as sterols are specific for each species. Olive oil classified according to its quality; table (1.3) illustrates this classification, (International olive oil council, 2008).

Olive oil forms the major product of many Palestinian villages and it is the source of income for many Palestinian families and a main component of these families

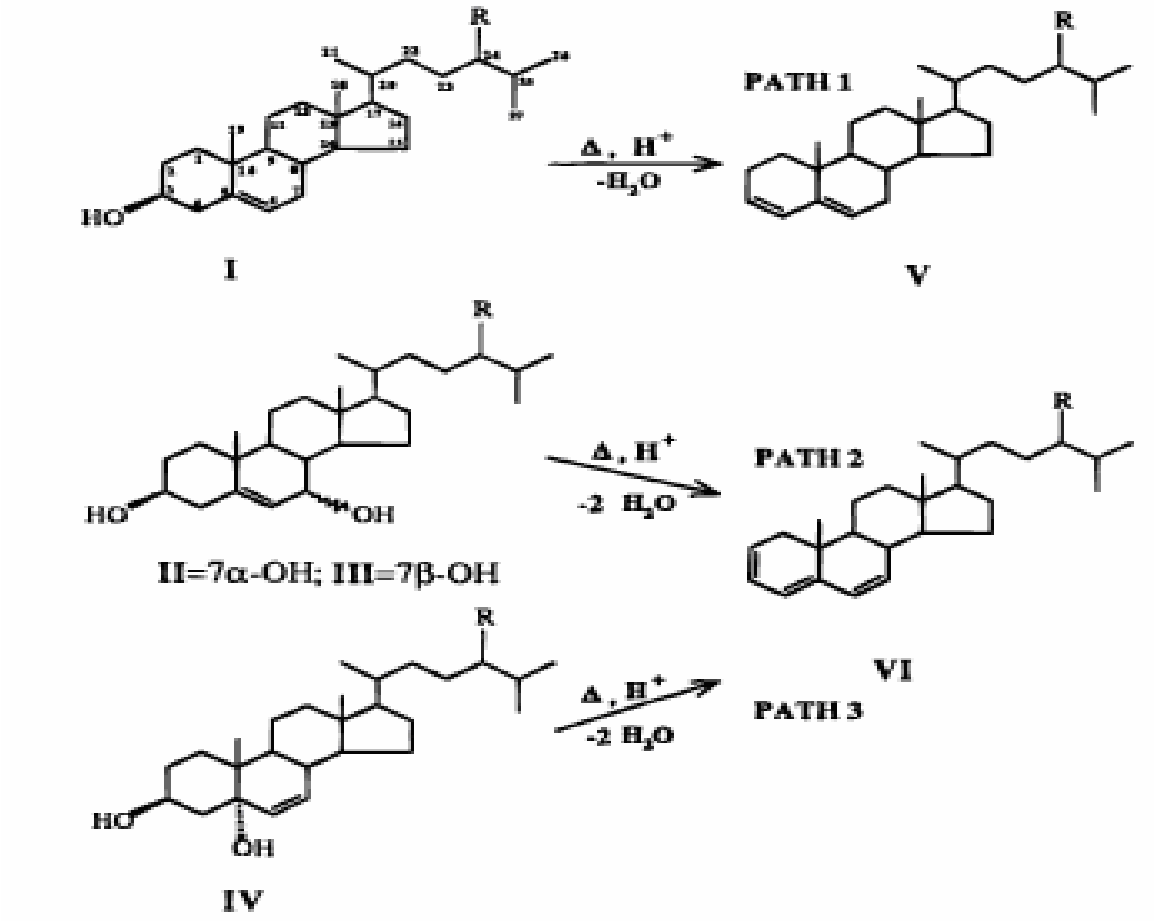
Mediterranean diet. As an example the amount of olive oil production in Palestine during the year of 2008 reached to 19429 ton, with an average consumption of 2.5 Kg per capita (for Palestine population), and with an excess production of 8758 ton, (Palestinian Ministry of Agriculture - G.D. of extension and rural development, 2008).

The total areas cultivated with olive trees in Palestine reached to 961268 dunum with an average production of 106369 ton of olive fruits and 19429 ton of olive oil. All of these olive tree farms depend on the farmers families labor workers and form a major source of these families income. The estimation for olive oil production cost is 2.31 $/ liter, which is considered to be high compared to the olive oil price in the Palestinian markets, (Palestinian Ministry of Agriculture - G.D. of extension and rural development, 2008).

In order to have better life style for families depending on olive oil production; we need to increase the competition power of Palestinian olive oil in order to get better chance and better prices in west markets which are suitable for the high production cost.

The European committee has introduced quality standards by the International Olive Oil Council (IOOC) for olive oil for being accepted to export to their markets. These standards include; among others; free acidity, peroxide value and iodine value. Steradienes content (especially $\Delta 7$ stigmastadienes) is another quality standard added to the olive oil international quality standards by IOOC as olive oil adulteration test. Steradienes formed by dehydration of beta sitosterols, Figure (1.1) shows the formation of sterenes compounds by dehydration process from sitosterols. Stigmastadienes considered as an olive oil adulteration and fraud indexes.

Additions to two parameters are important for evaluation of olive oil fraud; the content of Δ7 Stigmastenol where it should be ≤ 0.5%, and the stigmastadiene content (one of the sterenes compounds formed by the dehydration of Δ7 Stigmastenol) is determined to be ≤ 0.15 mg/kg for edible virgin olive oil, (Dimitrios Boskou, 2006). The European community has reduced this value of stigmastadiene content to ≤ 0.1mg/kg for edible virgin olive oil accepted for exportation to their markets, (COI/T.15/NC no.3/Rev.3, 2008).



R= H: Ia, IIa, IIIa, IVa, Va, Via

R= C_2H_5: Ib, IIb, IIIB, IVb, Vb, VIb

R= CH_3: Ic, IIc, IIIc, IVc, Vc, Vic

R= C_2H_5 and C_{22}-C_{23} double bound: IId, IIId, IVd, Vd, VId

R= C_2H_5 and C_{22}-C_{23} double bound: Ie, IIe, IIIe, IVe, Ve, VIe

Fig (1.1): Formation of steradienes by sterol dehydration, (R. Bortolomeazzi, *et al*, 2000).

The problems that facing the development of Palestinian olive oil sector are technical and environmental problems which decrease the quality of Palestinian olive oil and prevent its exportation. An example of environmental problems is the increase of olive fly infection that cause decrease in the quality of olive oil. Technical problems include fruits collection methods and the way of olive oil production and storage, (Palestinian Ministry of Agriculture - G.D. of extension and rural development, 2008).

Olive oil is classified into virgin olive oil, refined olive oil, refined olive - pomace oil and other refined blends. These olive oil classes have different quality characteristics and defined in table (1.1), (CODEX STA 33, 2001).

Table (1.1): Definitions of olive oil types, (CODEX STAN 33, 2001).

No.	Olive oil Type	Definition
1	**Olive oil**	oil obtained from the fruits of olive tree without have been subjected to manipulation or any treatment not authorized by this standard
2	**Virgin olive oil**	oil obtained from the fruits of olive tree by mechanical or other physical methods under conditions, particularly thermal, which do not lead to alteration of the oil. It is suitable for consumption in the natural state.
3	**Refined olive oil**	oil obtained from virgin olive oil, the acid content or organoleptic characteristics of which render it unsuitable for consumption in the natural state, by means of refining methods which do not lead to alteration in the initial glyceridic structure.
4	**Refined olive – pomace oil**	oil obtained from "olive pomace" by extraction by means of solvents and made edible by means of refining methods which do not lead to alteration in the initial glyceridic structure.

Olive oil quality parameters include chemical and physical parameters. These parameters depend on the olive oil classification. Table (1.2) shows physical and chemical quality parameters of different types of olive oil.

Table (1.2): Physical and chemical (quality characteristics) parameters of olive oil, (CODEX STA 33, 2001).

No.		Index	Value	Oil quality
1	Physical parameters	Refractive index (n_D^{20})	1.4677 – 1.4705	Virgin olive oil
				Refined olive oil
			1.4680 – 1.4707	Refined olive - pomace oil
		Iodine value	75 – 95	Virgin olive oil
			75 – 94	Refined olive oil
			75 – 92	Refined olive - pomace oil
2	Quality characteristics (chemical parameters)	Peroxide value (meq/kg)	= 20	Virgin olive oil
			= 10	Refined olive oil
			= 10	Refined olive-pomace oil
			= 20	Blends
		Free acidity (maximum % expressed as oleic acid)	≤ 3.3	Virgin olive oil
			≤ 0.3	Refined olive oil
			≤ 0.3	Refined olive-pomace oil
			≤ 1.5	Blends

The olive oil main quality parameters that used for olive oil classification are free acidity percentage and peroxide value. Table (1.3) shows this classification according to the values accepted for olive oil in European markets

Table (1.3): Olive oil Classification, (International olive oil council, 2008).

No.	Olive oil class	Free acidity (%)	Peroxide value (meq/kg)	Use
1	**Extra virgin olive oil**	**≤ 0.8**	**≤ 20**	**Edible**
2	**Virgin olive oil**	**≤ 2**	**≤ 20**	**Edible**
3	**olive oil**	**≤ 3.3**	**≤ 20**	**Edible**
4	**Lampante olive oil**	**> 3.3**	**No limit**	**Not Edible**
5	**Refined olive oil**	**≤ 0.3**	**< 5**	**Not Edible**

The study uses the following quality indexes to study olive oil quality in Palestine:

1) Acidity value percentage (AV) is the percentage of free fatty acids as oleic acid, (D.D. Ben Miled, *et al*, 2000). This parameter is affected by the time and storage method of Extra Virgin Olive Oil (EVOO). Table (1.2) & (1.3) shows the olive oil classification according to acid value.

2) Peroxide value (PV): Peroxide value is the number that expresses, in mill-equivalents of active oxygen, the quantity of peroxide contained in 1000 g of the olive oil, (M.F. Diaz, *et al* , 2006). Peroxide value is affected by the Varity of olive trees and by the storage time and conditions, (J. Guil-Guerrero and J. Urda-Romacho, 2009). Table (1.2) shows olive oil classification according to PV.
3) Iodine value (IV): is the weight of iodine absorbed by 100 parts by weight of the substance; it is known that iodine value is a measure of double bond content in oils, principally oleic acid, (M.F. Diaz, *et al*, 2006).Table (1.1) shows olive oil classification according to iodine value.
4) Refractive index of aqueous solutions and oil is of crucial importance in applications of adulteration of oils and purity, (W. Yunus, *et al*, 2009). Refractive index (n_D^{20}) values for olive oils are as follows: Virgin olive oil from 1.4677 to 1.4705 and for refined olive oil from 1.4677 to 1.4705, (CODEX STA 33, 2001).
5) Reaction of nitric acid with olive oil is a test to determine if the oil mixed with other oils or not. So that; by adding nitric acid to olive oil must give no change in oil color to give a positive test, but if the color of oil has been changed into yellowish or brown so its negative test and the oil is bad (mixed), (R. Benedikt, 2007).
6) Sterenes (especially stigmastadienes) content using the ISO 15788-2 method of analysis. Dehydration of sterols in the refining process of vegetable oils results in the formation of steroidal hydrocarbons (sterenes and steradienes). The determination of sterenes can detect the addition of refined oil to extra virgin olive oil as low as the 1% level, (R. Bortolomeazzi, *et al*, 2000).

The most important class of olive oil is the virgin olive oil because it is the edible type of olive oil. Virgin olive oil is classified according to its free acidity percentage into extra

virgin, virgin and ordinary virgin olive oil. Table (1.4) shows virgin olive oil classification according to free acidity.

Table (1.4): Classification of virgin olive oil according to free acid value, (International olive oil council, 2008).

No.	Class	Free Acidity (%)
1	**Extra virgin olive oil**	**≤ 0.8**
2	**Virgin olive oil**	**≤ 2.0**
3	**Ordinary virgin olive oil**	**≤ 3.3**

1.2 Theoretical Framework

Dehydration of sterols in the refining process of vegetable oils results in the formation of steroidal hydrocarbons (sterenes and steradienes). This process occurred under the effect of acidic earths (acid rain) and deodorization at high temperature. The determination of sterenes can detect the addition of refined oil to extra virgin olive oil as low as the 1% level, (R. Bortolomeazzi, *et al*, 2000).

Acidity value (%) is the percentage of free fatty acids as oleic acid, (D.D. Ben Miled, et al, 2000). According to another definition: acid value is the number of milligrams of potassium hydroxide required to neutralize the free acids in 1 g of the substance, (M.F.

Diaz, *et al*, 2006). This parameter is affected by the time and storage method of extra virgin olive oil (EVOO). Studies showed that the factors affecting olive oil acidity value are storage time and olive trees Variety, (J. Guil-Guerrero and J. Urda-Romacho, 2009).

Peroxide value is the number of expresses, in mill equivalents of active oxygen, the quantity of peroxide contained in 1000g of the substance, (M.F. Diaz, *et al*, 2006). Peroxide value is affected by the verity of olive trees and by the storage time and storage conditions, (J. Guil-Guerrero and J. Urda-Romacho, 2009).

Iodine value is the weight of iodine absorbed by 100 parts by weight of the substance; It is known that iodine value is a measure of double bond content in oils, principally oleic acid. The olive oil fatty acids are rich in oleic acid, which represents one unsaturated bond on the nine-carbon atom (C9). (M.F. Diaz, *et al*, 2006).

Knowledge of the refractive index of aqueous solutions and oil is of crucial importance in applications of adulteration of oil and purity of oils determination, (W. Yunus, *et al*, 2009). A refractive index (n_D^{20}) value for olive oils is as follows: Virgin olive oil from 1.4677 to 1.4705 and for refined olive oil from 1.4677 to 1.4705, (CODEX STA 33, 2001).

The reaction of nitric acid with olive oil presents a test to determine wither the oil mixed with other oils or not. If the addition of nitric acid to olive oil gives no change in oil color; the oil is not mixed with other oils. However if the color of oil changed into yellowish or brown; this indicates that the oil is mixed with other oils, (R. Benedikt, 2007).

1.3 Problem Statement

Olive oil forms a major agricultural product in many Palestinian villages, also it is the main source of income for many Palestinian families. The international olive oil council (IOOC) appointed quality standards for olive oil export to west markets. These standards have prevented Palestinian olive oil exportation to Europe during the year of 2004/ 2005 as it did not satisfy the requirements of trade standard applying to olive oils and olive-pomace oils (COI/T.15/NC no.3/Rev.3). This problem is a major barrier that prevents the increase of Palestinian olive oil production and exportation to have better income for Palestinian families that depends on olive farming. In addition, the steradienes content of olive oil (especially stigmastadiene) as adulteration index and quality parameter for olive oil not studied before for Palestinian olive oil.

1.4 Objectives

The general objective of this study is to measure the quality parameters and environmental characteristics of olive oil in northern West Bank and to compare them with international standards. The specific objectives of this study are to:

1. Measure the steradienes (especially stigmastadiene) concentration of olive oil in Northern West Bank and to compare them with international standards.
2. Measure free acidity, iodine value, refractive index, peroxide value and nitric acid test (to test oil samples purity) for study area - olive oil.
3. Determine the olive oil quality according to international standards.

4. Test international standard complains to the Palestinian olive oil parameters, especially for stigmastadiene.
5. Study the environmental factors that effect on olive oil quality especially on the sterenes concentration of the study area olive oil.

1.5 Hypotheses of the study

The hypotheses of this study are:

- Palestinian virgin olive oil in northern part of the West Bank area is satisfying the international quality standards.
- Environmental factors of cultivation, extraction and storage of olive oil affect olive oil quality parameters.
- Stigmastadiene content of olive oil increases with the increase of storage time.
- Stigmastadiene content of olive oil is affect by storage temperature.

1.6 Study Overview

Literature review used by the study is in chapter two. Each one includes an idea about the subject of this study. The third chapter gives a theoretical framework of the study and discusses practical parts of the study. Fieldwork, sampling procedure, instrumentation, analytical techniques and questionnaire used for collecting environmental and cultivation information are explain in third chapter. The results and discussion obtained by this study are in the fourth chapter. The study recommendations and conclusions illustrated in chapter five.

Chapter Two

Literature Review

The following studies explain different issues, like the limits of stigmastadiene content of olive oil, the formation of stigmastadiene in olive oil, the effect of extraction, cultivation and storage factors on olive oil quality, as well as some environmental factors that effect on olive oil quality.

J. Guil-Guerrero and J. Urda-Romacho, (2009), the study was applied in Tabernas Desert in Spain by using Picual, Arbequina and Hojiblanca extra virgin olive oil. According to the study; Mediterranean diet characterizes a lifestyle and culture that has been contributes better health and quality of life. The Mediterranean food tradition has three basic essentials that include olives and its oils. The extra virgin olive oil (EVOO) quality considered from diverse points of view: normative, commercial, nutritional, therapeutic and sensorial. The last three parameters related to chemical composition; therefore, some chemical parameters must appear on the label of the product like: acidity and peroxide value (PV). These parameters are affected by the time and storage method of extra virgin olive oil (EVOO). The PV value increases over the allowed European Union law if the olive oil is stored in bottles partly field and the air is the conditioner gas. Therefore, the factors that affect extra virgin olive oil (EVOO) in storage stage are temperature, contact with oxygen and exposure to light. The free acid value was expressed as the amount of oleic acid as percentage (%), PV was expressed as mill-equivalents of active oxygen per kilogram of oil. These tests were used as quality index during storage

stage for 12 months in different storage bottles. The quality of the oil depends on the variety of the oil and it was found that oil is more stable when stored in dark bottles.

K. H. Wilm, (2009), Phytosterols are the sterols, which are present in plants such as beta-sitosterol, stigmasterol and campesterol. Examples of plant sterols are shown in figure (2.2). Raffination of oils and fats removes components with bad taste and smell, such as free fatty acids, products of oxidation, ketones, and aldehydes, waxes, and phosphatides. It also removes environmental contaminants, herbicides, pesticides, fungicides, polycyclic hydrocarbon, heavy metals and products of the metabolism of microorganism leaving traces which are tolerable. Raffination of oils remove part of the steroles. Refined oils have 0.1 to 0.45% of steroles left.

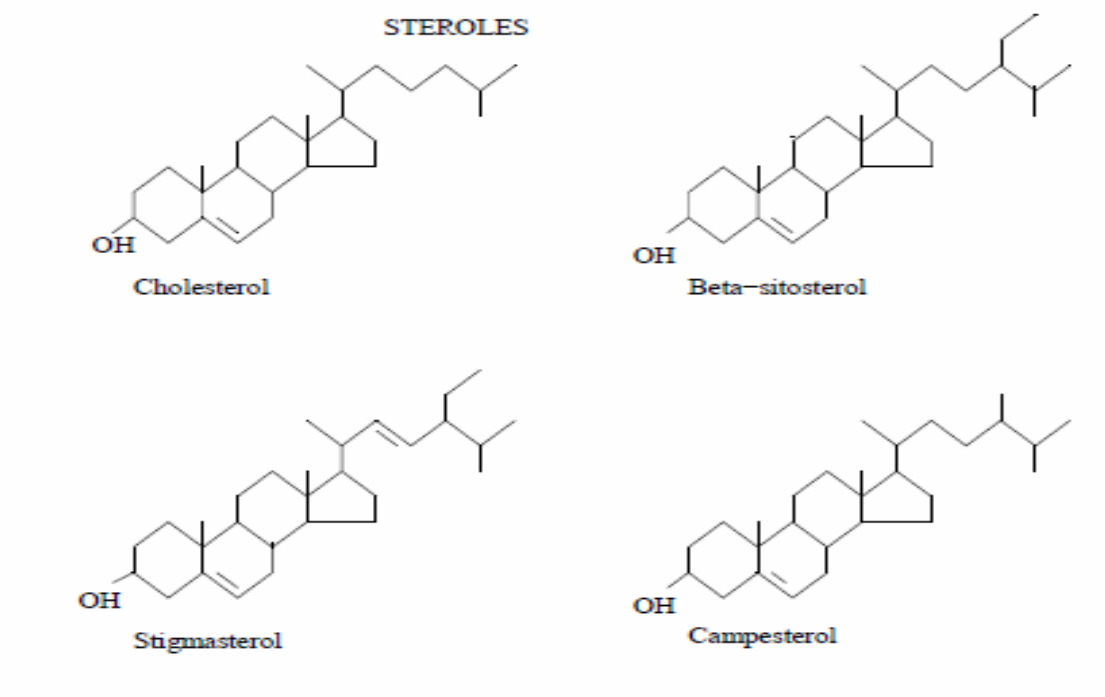


Fig (2.2): Examples of plant sterols, (K. H. Wilm, 2009).

Por M. Soledad Gracia Gómez, et. al. , (2009), in this work the variability of the parameters of chemical composition of virgin olive oil, "Empeltre" variety, in 472 samples coming from the (Bajo Aragón) region, in three seasons (1996/97, 1997/98 and 1998/99) was studied. The free acidity, peroxide values, ultraviolet absorbance's, fatty acid composition, sterolic composition and the level of waxes and polyphenols were analyzed. Considering their chemical composition, the majority of the analyzed oil samples are of great quality. The oil of the 1997/98 season differ more from those of other seasons, having been affected by the attack of the fly of (Dacus oleae), which gave a higher acidity level, raising the peroxides and the waxes as well. The sterolic composition was also affected. The "Empeltre" variety from the Bajo Aragón region shows high values of Delta7 Stigmastenol, which in many cases exceed the maximum value of the European Union Commission Regulation.

Xiao Qianewen, et. al. , (2009), this study was conducted in China and deals with the suitable areas for olive trees cultivation and distribution of olive trees in china environment. Olive trees adapted to Mediterranean climate; rainfall precipitation , high humidity and low temperature satisfy its flower differentiation, while in summer rich sunlight and low humidity is favorable for production and oil accumulation. In addition, the soil in Mediterranean is rich in calcium. In determination, the suitable regions for olive tree cultivation, attention paid to temperature, sunlight hours and relative humidity. Temperature affects flower differentiation, if temperature is not low enough; olive tree will not flower, but if the temperature is too low; cold injury will happen. Sunlight hours affect yield and oil content. Relative humidity affects the growth of flower and fruit, high relative humidity cause disease and insect easily.

Physiological differentiation of olive bud happened in December to following year February, which needs certain temperature. 12.5^{o} C is critical condition to end bud dormancy. Morphological differentiation happened after physiological differentiation, which need a stable temperature and 10^{o} C to 15^{o} C is the most suitable. Bud starts morphological differentiation at the end of February to middle third of March. High humidity and rainfall occurrence in March cause flower falling and fruit dropping. In April wind is common and temperature over 15◦ C, olive florescence occurs as theses conditions are good for pollen spreading. June to December is the period for oil accumulation and August to December is the critical; 50% of oil will be accumulated during this period. So long sunlight hours and low humidity are favorable for oil formation.

Trade standard applying to olive oils and olive-pomace oils, (2008), this standard applies to olive oil and olive-pomace oil that is object of international trade or food aid transactions. Virgin olive oil is the oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration. Virgin olive oil classified into three classes fit for consumption according to its free acidity value as shown in table (1.4) of page (10).

The other part is virgin olive oil not fit for consumption as it is, designated lampante virgin olive oil, is virgin olive oil which has a free acidity, expressed as oleic acid, of more than 3.3 grams per 100 grams and/or the organoleptic characteristics and other characteristics of which correspond to those fixed for this category in this standard. It is intended for refining or for technical use. Furthermore, according to this standard the stigmastadiene content in

(mg/kg) is ≤0.10 for edible virgin olive oils and ≤0.50 for lampante virgin olive oil. In addition, peroxide value for edible virgin olive oil is determined to be ≤20 meq peroxide oxygen per kg of oil. Table (2.1) classifies olive oil according to free acidity and peroxide values.

Table (2.1): Classification of olive oil according to free acid value and peroxide value, (International olive oil council, 2008).

No.	Quality Parameter	Extra virgin olive oil	Virgin olive oil	Ordinary virgin olive oil	Lampante virgin olive oil	Refined olive oil	Olive oil	Crude olive-pomace oil	Refined olive-pomace oil	Olive-pomace oil
1	**1. Free Acidity (% m/m expressed in oleic acid)**	**≤ 0.8**	**≤ 2.0**	**≤ 3.3**	**≤ 3.3**	**≤ 0.3**	**≤ 1.0**	**No limit**	**≤ 0.3**	**≤ 1.0**
2	**2. Peroxide value (in milleq. Peroxide per kg/oil)**	**≤ 20**	**≤ 20**	**≤ 20**	**No limit**	**≤ 5**	**≤ 15**	**No limit**	**≤ 5**	**≤ 15**

M. Hmidat, (2007), this study was conducted in West Bank/ Palestine. The obtained results from this study showed a great influence of soil type, region, maturity index and olive fly infection, which are the main factors that affect Δ 7 Stigmastenol, where the pressing temperature, the olive storage before pressing, olive variety and oil storage showed a mild affect. Olive seed, the land topographic and olive leaves found to have a negligible affect on Δ 7 stigmastenol.

The acid value has been increased with ripening progress (increase of ripening period and harvested in late of November). This increase is due to the decrease in sterolic compounds includes Delta 7 Stigmastenol and increase in the end results of lipase enzyme like free fatty acids that mean increase the acid value. The same effect found with Peroxide value; increase of PV with increase of ripening progress. In addition, the infection with olive fly was found to cause increase in acidity and peroxide value because of the damage of fruit tissue, which increase the amount of lipase enzyme and increase its activity and holes formed since infection cause the entry of microbes (bacteria and fungi). While the olive fly infection cause decrease in iodine value due to chemical and enzymatic reactions that took place, and due to the holes and chemical materials added by olive fly. In addition, increase of ripening period cause decrease in iodine value due to oxidization of fatty acids double bonds.

S.A. Vekiari, et. al., (2007), the study was done in the fields of the institute of Subtropical plants and olives in Messara in the island of Crete using olive trees Varity Koroneiki. According to this study, the olive oil quality indexes are affected by extraction methods and storage conditions. During the storage of olive oil the peroxide value was significantly affected by type of extraction machinery, packing material and light intensity. The exposed

oil to diffused or artificial light reaches to maximum PV in the second or third month of storage and decreased thereafter, while the oil stored in dark bottles attained maximum PV after six months of storage. The samples that extracted using centrifugal machines and stored in glass bottles in dark have higher PV values than samples extracted by classical method. The extraction method, storage and packing materials affect the olive oil quality. The pressing method of oil extraction is the oldest method, which is still a widespread method in use. The new method of extraction, which is centrifugation system, requires the addition of warm water to the oil paste to separate oil from other phases. This new system cause production of more vegetation water and decrease content of olive oil phenols that effect on sensory and nutritional quality and affect on the resistance of virgin olive oil autoxidation. The PV, acidity and phenols content tests show higher values in the classical system than in the centrifugal system.

Dimitrios Boskou, (2006), this book reported that several unsaturated hydrocarbons with a steroideal structure, known as sterenes, are formed by dehydration of sterols, during olive oil refining. Among them, stigmasta-3,5-diene which originates from the dehydration of β-sitosterol, and it is considered as an effective marker of oils subjected to a bleaching process or to a thermal treatment. Limits set by International bodies for stigmastadienes are 0.15 ppm for virgin olive oil, and 0.5 ppm for lampante. Stigmastadiene determination is useful especially to check the addition of desterolized oils since the high temperatures needed for the removal of sterols during refining process promotes the formation of sterenes. The official method for stigmastadiene determination involves extraction of unsaponifiable matter, fractionation of steroidal hydrocarbons with silica gel column chromatography and then GC analysis. Sterenes can also be determined by RP-HPLC

coupled with a UV detector since they have characteristic absorptions due to the presence of a conjugated double bond system.

M.F. Diaz, et. al., (2006), the study where conducted in Spain to compare the Ozonized olive oil and sunflower oil chemically and microbiologically. It contains a fatty acid composition of oleic acid of (65-85%). The pure olive oil is widely used for its nutritional and therapeutic effects. The quality indexes for olive oil include peroxide value, acid value and iodine value. Peroxide value is the number that expresses, in mill-equivalents of active oxygen, the quantity of peroxide contained in 1000 g of the olive oil. Acid value is the number of mg of potassium hydroxide required to neutralize the free acids in 1.0 g of the substance. Iodine value of a substance is the weight of iodine absorbed by 100 parts by weight of substance. It is known that iodine value is a measure of double bond content in oils, principally oleic acid. The olive oil fatty acids are rich in oleic acid which represents one unsaturated bond on the nine carbon atom (C9).

CODEX STAN 33, (2001), this standard applies to virgin olive oil, refined olive oil, refined olive - pomace oil (and other refined blends). The definition of these olive oil types are in table (1.1) in page (4). The physical parameters and quality characteristics of olive oil according to this standard are summarized in table (1.2) of page (6).

R. Amirante, et. al., (2001), the study was applied in Tuscany olive varieties (Moraiolo, Leccino and Frantoio); they have reported that the quality of olive oil originates from the choice of cultivars and related cultural practices and from the harvest time. Oil is dispersed in very minute drops in the cells of olive pulp. After the crushing, the subsequent mixing of the obtained paste breaks the entire oil cells and causes the oil drops to disperse in the

vegetable water to aggregate. The parameters, which are liable to change in the extraction process, are the mixing time, temperature, and degree of dilution of the olive paste. Oil samples were taken in 250ml dark glass bottles and stored at 4 ºC till quality tests (include acidity and peroxide value) were performed. The best results in terms of extraction yield obtained with 32% water added to olive paste with average yield of 15.8% (w/w oil to olive). The change in percentage of added water has harmful effects on yield and quality.

A. Pasqualone and M. Catalano, (2000), the study where done on natural virgin olive oils collected directly from miles in Italy, the study found that when the ratio of free sterols/total sterols exceeds 70%; it can exclude the presence of neutralized oils in extra virgin ones. They have reported that virgin olive oil represents the best food within the category of all vegetable and animal alimentary fats. Alimentary fats contain both free and esterified sterols. Studies found that the ratio of free / esterified sterols in virgin olive oil is 2.2; which could characterized by higher quantity of stigmasterol within free sterols. The study found that in virgin and lampante oil, when focusing on campesterol and stigmasterol, there is a very slight difference in the composition of free compared to total sterols. Campesterol is comprised between 2.8 and 4% (average 3.34%) in the total sterol fraction; while in free sterols ranged from 2.1 to 3.8% (average 3.19%). Stigmasterol ranged from 1.2 to 3.1% (average 2.2%) in total fraction and from (1.2 to 3.4%) average (2.56%) in free fraction.

D.D. Ben Miled, et. al., (2000), the study was done in Tunis; they have studied different extraction procedures of olive oil to determine the effect of extraction method on the olive oil quality and stability. This study found that changes in olive oil quality depend on olive oil varieties (like chemlali and chetoui) rather than extraction system. The ideal extraction

method is one that gives the highest yield of oil without altering the oils original composition and quality. The two common extraction methods are pressing system (solid-liquid mass of olive paste is subjected to pressure and expressed to separate oily from solid phase), and centrifugation system. Olive oil is the only vegetable oil, which contain appreciable amount of polyphenols acting as antioxidant and conferring to a greater stability against oxidation during storage.

R. Bortolomeazzi, et. al., (2000), a laboratory study applied in Italy found that the dehydration of hydroxyl sterols dissolved in extra virgin olive oil and in the presence of 1% bleaching earths at 80◦C for 1 hour results in the formation of the same steroidal hydrocarbons (sterenes and steradienes) found in the refined oils. The dehydration of sterols in refining process of vegetable oils results in the formation of steroidal hydrocarbons (sterenes and steradienes) with two double bonds in the ring system. The bleaching with acidic earths (acid rains) and deodorization at high temperature cause sterols to degrade by dehydration forming steroidal hydrocarbons (sterenes or steradienes). The determination of sterenes can detect the addition of refined oil to extra virgin olive oil as low as the 1% level. Figure (1.1) in page (3) shows the sterenes formation from sterols dehydration process.

M.C. Dobarganes, et. al., (1999), this study is a technical report for International Union of Pure and Applied Chemistry, chemistry and the environment division – commission on oils and fats derivatives. The study were done for the development of standardized method for the determination of stigmastadienes content in vegetable oils. Moreover, the study reported that significant amounts of hydrocarbons are formed in vegetable oils as a consequence of thermal treatments during the refining processes. Among these hydrocarbons, the stigmasta-3,5-diene, a steroidal compound, is the most abundant in all

refined vegetable oils since it derives from the ß-sitosterol by dehydration. The 3,5-stigmastadiene is formed with minor amounts of the 2,4-isomer and it was found that both substances have a single and well defined gas chromatographic peak when hydrocarbon fraction is analyzed on a low polar column. Therefore the sum of both isomers can be easily quantified by gas-chromatographic analysis of the steroidal hydrocarbon fraction. For virgin olive oil, the usual production processes (pressure or centrifuging) do not produce measurable amounts of stigmastadienes (less than 0.01 mg/kg). In crude olive residue oil, small concentrations of stigmastadienes are found (ranging between 0.2 and 3 mg/kg) due to the high temperatures applied during the drying of the raw olive residue. In the refining processes, stigmastadienes are formed in all steps involving high temperatures, such as bleaching and deodorizing. Depending on the conditions applied during the refining process, commercial refined vegetable oils show stigmastadienes concentrations ranging between 1 and 29 mg/kg, and therefore the assessment of stigmastadienes allows not only the identification of thermally treated oils but also the detection of minor amounts of refined vegetable oils in virgin olive oils. A method for the determination of stigmastadienes and the results of a collaborative study carried out under the auspices of the International Olive Oil Council were presented to IUPAC Commission on Oils, Fats and Derivatives by the Spanish representative. The Commission considered the question of desirability of introducing the stigmastadiene content as a criterion for virgin olive oil and put forward the proposal of study the analytical method which could be adopted as an international standard method.

M. Amelio, et. al., (1998), the study apply an HPLC determination procedure for stigma-3,5-diene and wax esters, and suggested a routine and quick screening method for these compounds. Stigmastadienes are dehydration compounds of sitosterol and belong to

sterenes that are of wider family of dehydration compounds from sterols. They are produced during refining process mainly during bleaching and deodorization steps. This is why they must not present in virgin olive oil. The presence of steradienes in declared virgin oil means there is a fraudulent of olive oil with refined oils.

Chapter Three

Methodology

3.1 Overview

Techniques of analysis and sampling procedures are explained as follows and according to the official methods of analysis of the Association of Official Analytical Chemists (AOAC) official method of analysis and literature review chapter.

The olive oil samples were collected directly from the mills using 250ml new clean glass dark bottles. Samples were stored at 4°C until be analyzed, (R. Amirante, *et al*, 2001). Samples were analyzed in the lab quickly as possible to avoid further reactions that could affect on olive oil characteristics.

Chemical and physical analysis of olive oil samples was performed according to AOAC official method of analysis including: free acidity, iodine value, refractive index, peroxide value (K.Helrich, 1990) and nitric acid test (R. Benedikt, 2007). Sterenes and especially stigmastadiene content were analyzed on HPLC using the ISO 15788-2 method. These methods are explained In the following paragraphs.

Refractive Index of aqueous solutions and oil is of crucial importance in applications of adulteration of oils and purity. It measured at 20ºC to 25ºC for oils. In this procedure 2 to 3 drops filtered oil sample is placed on the surface of lower prism, then the prism is closed and the mirror is adjust until it gives sharpest reading, then the instrument reading is convert to **(n)** from the tables, (K.Helrich, 1990).

Iodine Absorption number is the weight of iodine absorbed by 100 parts by weight of the substance; It is known that iodine value is a measure of double bond content in oils, principally oleic acid. The olive oil fatty acids are rich in oleic acid, which represents one unsaturated bond on the nine-carbon atom (C9). (M.F. Diaz, Et al, 2006). Iodine Absorption number was determined using Hanus Method. In this method; 0.25 g oil is placed into 500ml glass flask and dissolved in 10ml chloroform, then 10ml Hanus I solution (BrI solution) is added. The resulting solution is mixed by pipette draining, left stand for 30 min in dark, and shacked occasionally. 10 ml of 15% KI solution is then added, and shacked roughly, and then 100ml of freshly boiled and cooled distilled water was added and the iodine was titrated with 0.1 N $Na_2S_2O_3$ until the yellow solution turns to almost colorless. Afterwards, few drops of starch indicator were added and the titration continued until blue entirely disappeared. and Two blanks were also conducted in the same manner. Percentage Of iodine absorbed (by weight) was calculated as follows:

I number = [(blank titr. Vol. – Sample titr. Vol.)x Nx 12.69]/sample weight (g) , N: $Na_2S_2O_3$ normality, (K.Helrich, 1990).

Acid value was determined by titration method as follows: 7.05 g of well-mixed oil was placed into 250 ml flask, then 50 ml alcohol was added, then this solution was titrated with 0.25 N NaOH and shacked till a permanent faint pink color appeared and persisted for at least one minute, (K.Helrich, 1990).

Peroxide value was determined by titration method, where 5 g of olive oil was taken in 250 ml Erlenmeyer flask, then 30 ml of acetic aced – chloroform (3:2 ratio v/v) was added , then swirled to dissolve, 0.5 ml KI solution was added and lift to stand with occasionally

shaking for 1 minute, then 30ml of distilled water was added. Afterward, the solution was titrated with 0.1 N $Na_2S_2O_3$ with vigorously shaking until yellow color almost gone. 0.5 ml of 1% starch solution was then added and the titration was continued with shaking until blue color disappeared. The PV value calculated as follows:

PV (milli-equevalent peroxide/ kg sample) = V x N x 1000/ sample weight (g)
Where V= ml $Na_2S_2O_3$ used for sample titration minus the bank volume; and N = normality of $Na_2S_2O_3$, (K.Helrich, 1990).

Nitric acid test was done to determine if the olive oil is mixed with other oils or not. An equal measure of nitric acid volume is mixed with the oil sample and shacked in a test tube and left to stand for some time. If the color changed, the oil is mixed; color intensity increased by the increase of the fraud oil percentage. (R. Benedikt, 2007).

Olive oil samples were analyzed for sterenes content using the ISO 15788-2 method of analysis. In this method 0.5 g sample was dissolved with petroleum ether and extracted on glass chromatographic column of silica gel 60 and eluted with 20 ml petroleum ether. The collected eluent is evaporated and the residue is dissolved in 0.5ml of acetonitrile/ tert-butyl methyl ether (50:50). Samples are stored in freezer at -20◦C till be analyzed as soon as possible on RP- HPLC using acetonitrile/tert-butyl methyl ether (70:30) as mobile phase and 235 nm UV detector, (ISO 15788-2).

3.2 Study area: Location & Climate

Bet-dajan and Assera –Al-shmaleh villages are Palestinian villages that locate in Nablus area in the northern of west bank. These villages' populations; as all of the northern west bank villages populations; are farmers with the main cultivation crop is olive trees. Peoples in these villages depend on olive oil as a source of income for their families and as a main component of their traditional food. Bet-dajan village locates on the Eastern foothills with Palestinian grid coordination of 178185 N and 185165 E. While Assera – Al-shmaleh villages locates on the Western foothills with Palestinian grid coordination of 175677 N and 184349 E. Map in figure (3.1) explains study area location.

Northern west Bank has a Mediterranean climate characterized by: rainy winter season and dry summer season. Rain season extend from October to May, the main months of precipitation are December, January, and February. Winter climate is affect by passing Cyprus Cyclones. Summer season is a dry hot season that affected by the Persian Gulf trough. Spring and autumn seasons are moderate seasons due to the winds of Sharv Cyclone which moves quickly a long the North African Shores. Figures (4.5) to (4.8) shows different metrological factors measurements in study area during the year of 2007/2008, ((http://www1.cbs.gov.il/reader/ & Palestine national authority ministry of transport metrological authority 2007 & 2008 climatic bulletin, and Http://www1.cbs.gov.il/reader/, 28.09.2009).

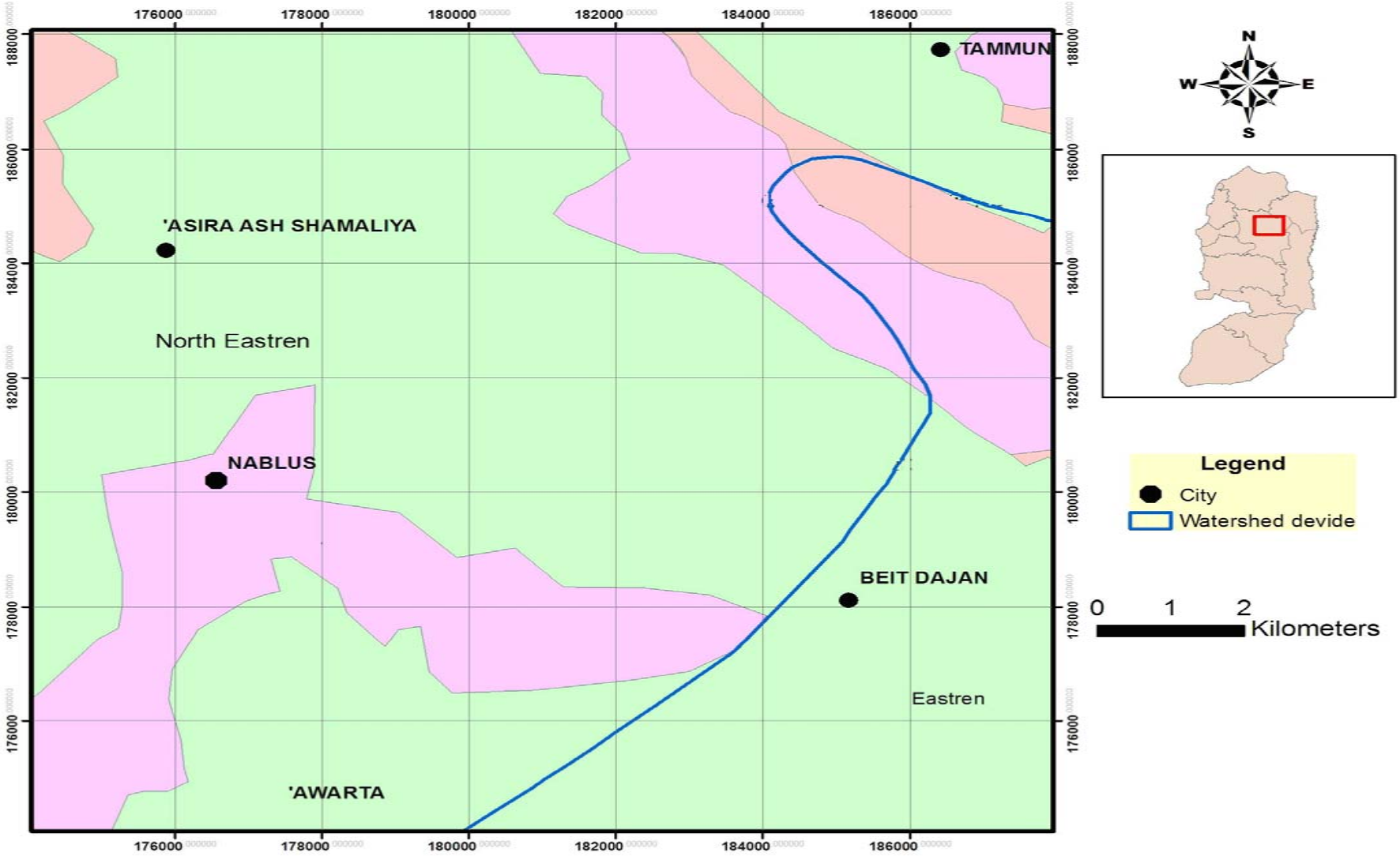
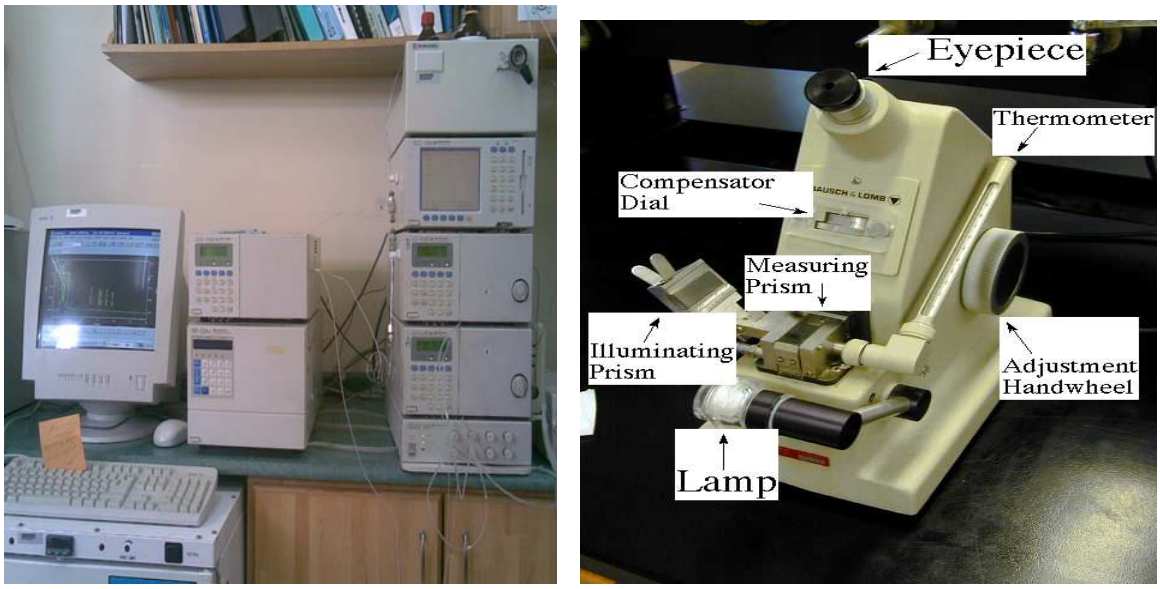


Figure (3.1): Location of study area (Bet Dagan & Assera Al Shamalia) in West Bank**.**

3.3 Instruments

The main instruments that used in this study are HPLC with UV detection and Refractometer. Figure (3.2) show pictures of these instruments.



A B

Figure (3.2): Instruments used in this study for analysis of olive oil samples:

A- HPLC with UV detection, (Shimadzu, Cat No. 228-34736-91).
B- Refractometer, (Zeiss Butyrefractometer).

3.3.1 HPLC

Typical HPLC system consists of the following main components: Solvent Reservoirs, Pump, Injector, Column, Detector and Data Acquisition and Control System, (Y. Kazakevich, *et al*, 2007).

The four main types of HPLC techniques are normal phase (NP), reverse phase (RP), ion exchange (IEX), and Size-exclusion chromatography (SEC). There are three basic types of

molecular forces inside the chromatographic column: ionic forces, polar forces, and dispersive forces. Each specific technique capitalizes on each of these specific forces:

1. Polar forces are the dominant type of molecular interactions employed in normal-phase HPLC.
2. Dispersive forces are employed in reversed-phase HPLC.
3. Ionic forces are employed in ion-exchange HPLC.
4. Size-exclusion HPLC is based on the absence of any specific analyte interactions with the stationary Phase, (Y. Kazakevich, et al, 2007).

3.3.1.1 Reversed-Phase HPLC (RP HPLC or RPLC)

As opposed to normal-phase HPLC, reversed-phase chromatography employs mainly dispersive forces (hydrophobic or van der Waals interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed. Reversed-phase HPLC is by the most popular mode of chromatography. Almost 90% of all analyses of low-molecular-weight samples are carried out using RP HPLC. One of the main drivers for its enormous popularity is the ability to discriminate every closely related compounds and the ease of variation of retention and selectivity, (Y. Kazakevich, *et al*, 2007).

3.3.2 Refractometer

A refractometer measures the extent to which light is bent (i.e. refracted) when it moves from air into a sample and is typically used to determine the index of refraction of a liquid sample.

The refractive index is a unit-less number, between 1.3000 and 1.7000 for most compounds, and is normally determined to five-digit precision. Since the index of refraction depends on both the temperature of the sample and the wavelength of light used these are both indicated when reporting the refractive index as follows:

$$n(_{D}^{20})\ 1.3742$$

The italicized n denotes refractive index, the superscript indicates the temperature in degrees Celsius, and the subscript denotes the wavelength of light (in this case the D indicates the sodium D line at 589 nm).

The refractive index is commonly determined as part of the characterization of liquid samples, in much the same way that melting points are routinely obtained to characterize solid compounds. It is also commonly used to:

- Help identify or confirm the identity of a sample by comparing its refractive index to known values.
- Assess the purity of a sample by comparing its refractive index to the value for the pure substance.
- Determine the concentration of a solute in a solution by comparing the solution's refractive index to a standard curve.

3.4 Sampling

Olive oil samples for this research were collected directly from olive extraction mills in 250ml new clean dark glass bottles. Samples were stored at 4ºC till be analyzed, (R. Amirante, *et al*, 2001), from two villages extraction mills in northern west bank; from \Nablus district; which are First one is Beit-dajan village mill, the second one is Assera – al-shmaleh village mill. Samples were divided in the lab into two small glass bottles one for stigmastadiene analysis and another for chemical and physical parameters analysis. All samples kept in dark conditions at 4ºC.

Samples were taken from the mill of Assera –al-shmaleh in five successive days; in which at least three samples were taken every day. This system of sampling were done in order to study the correlations between extraction process activities of cleaning and changing extraction water and other parameters of extraction with olive oil quality parameters and stigmastadiene content. Information about extraction process were taken from the the engineer who manages the millwork.

Part of the collected samples were divided in two glass bottles and stored in dark conditions for six months where one part is stored at room temperature and the other part at 4ºC, then samples were analyzed for stigmastadiene content to study the effect of storage temperature on the stigmastadiene content of olive oil.

In order to determine the environmental factors that affect olive oil quality parameters; information about culturing environment, type of soil, fruit collection steps till extraction of oil , cultivation environment, varieties of olive tree cultured and extraction method were

collected through a questionnaire filled by farmers whom oil samples were taken from their crop. Also further information about the hydrological year, olive oil production and olive sector during the season of 2008 has been taken through the Palestinian Authority Departments that corresponding to this sector.

3.5 Chemical analysis

In this study, olive oil samples were analyzed for: peroxide value, Fatty acids (free), Nitric acid test, Sterenes (especially stigmastadiene) content, Refractive index and Iodine absorption number according to reference methods of analysis as shown in table (3.1).

Table (3.1): Tests and official analysis methods used in this study.

No.	Test	Official Method	Reference
1	**Refractive index**	AOAC official method of analysis No. 921.08 Zeiss Butyrorefractometer	**K.Helrich, 1990**
2	**Iodine absorption number**	AOAC official method of analysis No. 920.158 of Hanus method	**K.Helrich, 1990**
3	**Peroxide value**	AOAC official method of analysis No. 965.33 titration method	**K.Helrich, 1990**
4	**Fatty acids (free)**	AOAC official method of analysis No. 940.28 titration method	**K.Helrich, 1990**
5	**Nitric acid test**	To test oil samples purity; add equal volumes of nitric acid and olive oil sample; mix and let stand 24 hours, if the color of oil changed into yellow or brown so the oil is fraud by other oils.	**R. Benedikt, 2007**
6	**Sterenes (especially stigmastadiene) content**	International standard ISO 15788-2 procedure, animal and vegetable fats and oils determination of stigmastadienes in vegetable oils part 2: method using high – performance liquid chromatography (HPLC)	**ISO 15788-2**

3.7 Statistical analysis

3.7.1 Quality assurance:

Samples were analyzed three times to check the reliability and reproducibility of the results and the average, and standard detection was calculated. Standard of Delta – 3, 5 - Cholestadiene was analyzed in each run on HPLC with different concentration in order to check data quality and instrument reliability. Physical and chemical parameters were analyzed three times and the average value was taken.

Chapter Four

Results and Discussion

4.1 General characteristics of data

Forty (40) olive oil samples have been analyzed for 2008 olive oil production season in Bet-dajan (18 samples) & Assera – al-shmaleh (22 samples) villages in the northern area of west bank. Physical and chemical parameters (iodine value, refractive index, free acidity, peroxide value and nitric acid test), and sterenes, (especially stigmastadiene content) were analyzed directly after the samples reached to the lab. Table (4.1) presents the analysis methods. Arithmetic mean, standard deviation, minimum and maximum values were calculated for the samples and presented in Table (4.1); and table (a.4) in the appendix; illustrated detailed results of samples analysis.

Table (4.1): Summary data for olive oil quality parameters of samples collected from the mills of Bet-dajan & Assera – Al-shmaleh villages for the production season of 2008.

No.	Variable	Average	Standard deviation	Minimum	Maximum
1	Iodine value (cg/g)	91.78	34.6	50.7	165.5
2	Refractive index (n_D^{20})	1.4696	0.0005	1.4687	1.4707
3	Acid Value (%)	1.22	0.87	0.38	3.78
4	Peroxide value (meq/kg)	19.10	5.52	8.54	35.5
5	Nitric acid test	All samples have negative test			
6	Stigmastadiene (mg/kg)	0.13	0.165	0.00	0.63
7	Cholestadien (mg/kg)	0.28	0.341	0.00	1.33
8	Stigmastatriene (mg/kg)	0.01	0.022	0.00	0.08
9	Campestadiene (mg/kg)	0.03	0.075	0.00	0.45
10	Total sterenes (mg/kg)	0.45	0.474	0.00	1.44

4.2 Chemical analysis

Table (4.1) presents the arithmetic average value of the measured parameters. The average of free acidity percentage is 1.22, which is in the range of virgin olive oil. The minimum value of acidity is 0.38 that is in the range of extra virgin olive oil, while the maximum value was 3.78, which is in the range of Lampante virgin olive oil. The average of peroxide value is 19.1 (meq/kg)which is in the range of extra virgin olive oil, where the minimum value is 8.54 (meq/kg) that in the rage of olive oil, while the maximum value is 35.5 (meq/kg), which in the range of Lampante virgin olive oil (International olive oil council, 2008).

Olive oil analyzed samples was classified according to free acidity percentage and peroxide value parameters to determine the oil quality depending on the Trade standard applying to olive oils and olive-pomace oils, 2008 as shown in table (4.2).

In general, Samples of Bet Dagan and Assera were extracted using centrifugation systems. The extraction in Assera was done in the range of thermal conditions from 25 to 30 º C and in Bet Dagan ranged from 30 to 35 º C. The person whom manages the work of Assera mill was more qualified and interest in olive oil quality.

Table (4.2): Classification of analyzed olive oil samples according to free acidity percentage and peroxide value tests, (International olive oil council, 2008).

Olive Oil Class	Sample Source	No. of Samples	Total No. of samples	Percentage from total analyzed samples
Extra virgin olive oil	**Bet Dagan**	**5**	**10**	**25%**
	Assera	**5**		
Virgin olive oil	**Bet Dagan**	**6**	**17**	**42.5%**
	Assera	**11**		
Ordinary virgin olive oil	**Bet Dagan**	**0**	**3**	**7.5%**
	Assera	**3**		
Lampante olive oil	**Bet Dagan**	**0**	**2**	**5%**
	Assera	**2**		
Olive oil	**Bet Dagan**	**7**	**8**	**20%**
	Assera	**1**		
Total	**Bet Dagan**	**18**	**40**	**100%**
	Assera	22		

4.2.1 Iodine value (cg/g):

The Iodine value of the analyzed samples ranged from 50.7 to 165.5 cg/g. Ten samples have an Iodine value higher than 95 cg/g and another ten samples have and iodine value less than 75 cg/g; most of these samples were taken from Bet Dagane village, which fields is located on the Eastern foothills. The last group includes twenty samples have an Iodine value ranged from 75 to 95 cg/g; all of theses samples was takes from Assera village olive trees fields which locates on western foothills. The variation of Iodine value could be related to the location on Eastern or Western foothills. Also that in Bet Dagane village no pesticide or fisheries is used against olive fly (Dacus oleae), while in Assera village

farmers use Fisheries. It is concluded that the infection with olive fly cause increase in Iodine value in Bet Dagan samples, (Por M. Soledad Gracia Gómez, est. al, 2009).

Ripening progress of olive fruits affects also the iodine value. For example, the low ripening progress of olive fruits of Bet Dgan causes higher iodine vale of olive oil. Similarly, the high ripening progress of olive fruits in Assera causes decrease in iodine value due to oxidization of fatty acids double bonds. Few samples in Bet Dagan have very low iodine values even the ripening time was low. This can be attributed to the olive fly infection which chemical and enzymatic reactions resulted from the holes and chemical materials added by olive fly, (M. Hmidat, 2007).

4.2.2 Refractive index (n_D^{20}):-

The average of refractive index measurements was 1.4696, which measured at room temperature and 589 nm wavelength. All values were in the range of virgin olive oil according to the Physical parameters and quality characteristics of Olive oil, (CODEX STA 33, 2001) except two samples from Bet Dagane village that have a higher value of refractive index and with high peroxide and Iodine values and low acid value due to infection with olive fly Dacus oleae, (Por M. Soledad Gracia Gómez, est. al, 2009).

4.2.3 Acid Value (%):

Acid values for all samples are from 0.38% to 3.78% with an average of 1.22%. The olive oil acidity values for Assera samples are from 0.38% to 3.78% with an average of 1.56%;

while the acidity values of Bet Dagane olive oil samples range from 0.42% to 1.93% with an average of 0.8%. this difference in the acid values of the olive oil of the two villages can be attributed to the difference in the harvesting time (October for Beit Dagan and November for Assera). It is known that the acid values increases with increasing the ripening period due to the decrease in the sterolic compounds as example Delta 7 Stigmastenol, and due to the increase in the end results of lipase enzyme like free fatty acids which means increase in the acid value of olive oil and; also it is known that the increase of ripening time lead to better maturity index, (M. Hmidat, 2007).

The high acid values of some of Bet Dagan samples is related to the infection possibility of fruits with olive fly. The olive fruit could be infected with olive fly, however; although not been a studied factor in this investigation; the existence of olive fly tapes was good indication for the possibility of such infection, which causes damage of fruit tissue, and increase the amount of lipase enzyme and the holes in the olive fruits which causes the entry of microbes (bacteria and fungi), (M. Hmidat, 2007).

4.2.4 Peroxide value (meq/kg):

Peroxide values for all samples found to be in the range of 8.54 to 35.5 meq/kg with an average of 19.1 meq/kg. The peroxide values for Assera samples are from 13.17 to 35.5 meq/kg with an average of 21.9 meq/kg; while the peroxide values of Bet Dagane samples are from 8.54 to 24.42 meq/kg with an average of 16.67 meq/kg This variation between the peroxide values of the olive oil of the two villages is related to the location, storage time; that wasn't controlled and ranged from 8 hours in Assera to 3 to 4 days in Bet Dgane; of olive fruits before extraction and amount of rainfall precipitation.

Peroxide value increase with increase of ripening time. Olive fruits in Bet Dagan were collected in October while in Assera they were collected in November. Therefore, the ripening time in Assera was higher, which causes higher values of peroxide due to lipase enzyme activity, (M. Hmidat, 2007).

4.2.5 Nitric acid test:

The nitric acid test gives no reaction (no color change) for all olive oil samples. This result shows that the olive oil samples are not mixed with other oils (pure oil samples).

4.2.6 Stigmastadiene content (mg/kg):

Stigmastadiene content of all analyzed samples was found to be from 0.00 to 0.63 mg/kg with an average of 0.13 mg/kg. The range of stigmastadiene content in Bet Dagane samples was from 0.00 to 0.63 mg/kg with an average of 0.27 mg/kg and standard deviation of 0.15 mg/kg; while stigmastadiene content for Asera samples are from 0.00 to 0.2 mg/kg with an average of 0.01 mg/kg and standard deviation of 0.04 mg/kg. In all of Bet Dagane samples, stigmastadiene was detected; while in Assera most of the samples are free of stigmastadiene. This variation related to the extraction conditions and the use of fisheries in farms of Assera and weather temperatures in ripening period. In Assera mill extraction process done at 25 to 30 º C, while in Bet Dagan extraction process don at temperature from 30 to 35 º C which cause the formation of sterenes by dehydration of sterols, (M.C. Dobarganes, est. al, 1999). Maximum and minimum temperatures in the ripening period in Assera is lower than maximum and minimum temperatures of ripening period of Bet Dagan; the difference ranged from 0 to 6 º C; which cause higher rate of

dehydration reaction of sterols that cause increase of sterenes content of olive oil in Bet Dagan. Also the use of Fishers in Assera decrease the infection by the olive fly, this infection in Bet Dagan olive trees cause increase in sterenes content, (Por M. Soledad Gracia Gómez, et. al, 2009).

It was found that 3 samples from 21 samples of Assera contain stigmastadiene and they satisfy the limits set by International bodies for stigmastadienes content of olive oil (0.15 ppm for virgin olive oil and 0.5 ppm for lampante olive oil), (Dimitrios Boskou, 2006), and the international olive oil council standards for virgin olive oil stigmastadiene content (0.1mg/kg for edible virgin olive oil and 0.5mg/kg for lampante virgin olive oil). While for Bet Dagan, was found that 15 samples from the 18 samples contain stigmastadiene higher than 0.15 mg/kg. This difference in stigmastadiene content of the olive oil of the two villages is related to the difference in weather conditions between the two villages due to their locations in different foothills (Bet Dagan on Eastern foothills and rain shadow area while Assera in Western foothills) this difference in location cause differences in weather conditions that includes temperature, humidity and precipitation.

Furthermore, the use of fishers in Assera reduces the infection with olive fly (Dacus oleae), and it is known that this infection leads to higher Delta 7 stigmastenol content, (Por M. Soledad Gracia Gómez, est. al, 2009), which dehydrate to form higher amounts of stigmastadiene, (R. Bortolomeazzi, *et al*, 2000).

For virgin olive oil, the usual production processes (pressure or centrifuging) do not produce measurable amounts of stigmastadienes (less than 0.01 mg/kg), but if higher temperature is used during extraction process higher concentrations of stigmastadiene is

formed, (M.C. Dobarganes, *et al*, 1999), therefore higher amounts of stigmastadiene was found in Bet Dagan samples compared to Assera samples as the extraction process is done on 30 to 35 degree Celsius range of thermal conditions, while in Assera extraction wasv done in the range of 25 to 30 degree Celsius.

4.2.7 Total sterenes content (mg/kg):

Total sterenes content of all olive oil samples were found to be from 0.00 to 0.63 mg/kg with an average of 1.44 mg/kg. The range of total sterenes content in Bet Dagane samples is from 0.47 to 1.44 mg/kg with an average of 0.91 mg/kg; while stigmastadiene content for Asera samples are from 0.00 to 0.55 mg/kg with an average of 0.06 mg/kg. It was found that Bet Dagane samples contain higher concentration of total sterenes compared to Assera sample. This difference is related to the extraction conditions and the use of fisheries in farms of Assera village reduce the infection with olive fly (Dacus oleae). The olive fruit could be infected with olive fly, however; although not been a studied factor in this investigation; the existence of olive fly tapes was good indication for the possibility of such infection. Infection affects the sterolic compounds composition and cause higher Delta 7 Stigmastenol and other sterols content which leads to increase in the amount formed sterenes from sterol dehydration, (Por M. Soledad Gracia Gómez, est. al, 2009). In addition, higher amounts of sterenes is found in Bet Dagan samples as the extraction process is done in the range of 30 to 35 º C while in Assera extraction done in range of 25 to 30 º C, (M.C. Dobarganes, *et al*, 1999).

4.4 Determination of relationships between olive oil quality parameters & environmental factors

4.4.1 Quality parameters correlations:

It was interesting to see if there is a relationship between the: acid value and stigmastadiene/ sterenes content, the peroxide value and the stigmastadiene/ sterenes content, stigmastadiene content and sterenes content. The relationship between different metrological factors; as example humidity, precipitation and temperature; and olive oil quality parameters was also studied.

4.4.1.1 Relationship between total sterenes and stigmastadiene:

Ageneral trend was found between the content of total sterenes (Stigma stadiene, cholestadiene, stigmastatriene and campestadiene) and stigmastadiene content. This relation is due to the formation of all sterenes compounds by dehydration of sterols in the same process and under same conditions, (R. Bortolomeazzi, *et al* , 2000).

4.4.1.2 Relationship between sterenes content & acidity of olive oil in different Harvesting times:

It found that Stigmastadiene and total sterenes content decrease with the increase of acidity under the effect of ripening time increase as shown in Figure (4.1). This increase in

stterenes content in Bet dagan olive oil; due to the decrease in sterolic compounds and increase in the end results of lipase enzyme like free fatty acids that mean increase in the acid value and degradation of sterenes compounds like other olive oil components, in addition to rain fall occurrence in November and October (ripening period) before collection of olive fruits; while no rainfall occurred in bet dagane before olive fruits collection; which increase the water content of olive fruits in Assera and dilute its constituents, this hydrated conditions increases the activity of lipase enzyme that increase its end products content in olive oil., (M. Hmidat, 2007).

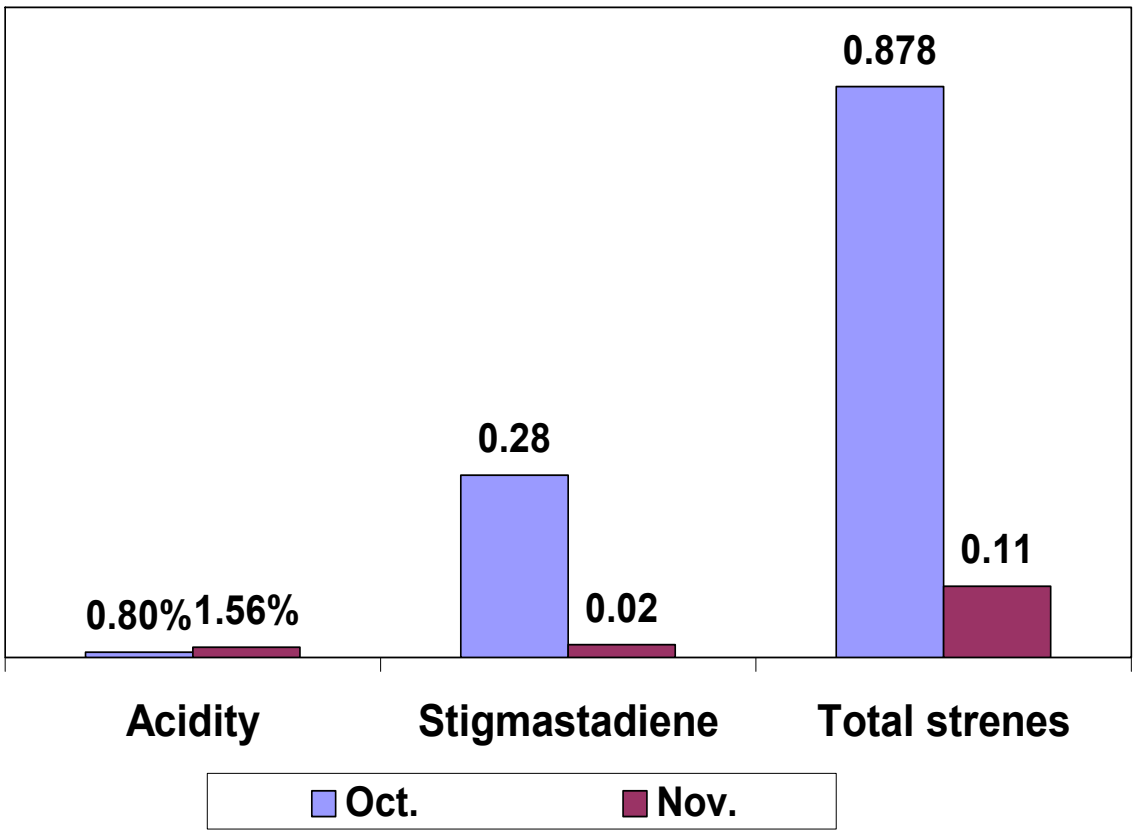


Fig (4.1): Relationship of sterenes & acidity in different Harvesting times.

4.4.1.3 Relationship of sterenes & Iodine value in different Harvesting times:

Stigmastadiene and total sterenes content found to be in a positive coloration with iodine value. Both sterenes content and iodine value found to be decreased with the increase of ripening time as shown in figure (4.2). Rainfall occurrence in November & October in Assera; while no rainfall occurred in Bet Dagan; increases the water content of Assera olive fruits and dilutes its constituents; this hydrated conditions increases the activity of lipase enzyme that increase its end products content in olive oil. Furthermore, oxidization of fatty acid double bonds causes decrease of Iodine value with the increase of ripening time, (M. Hmidat, 2007).

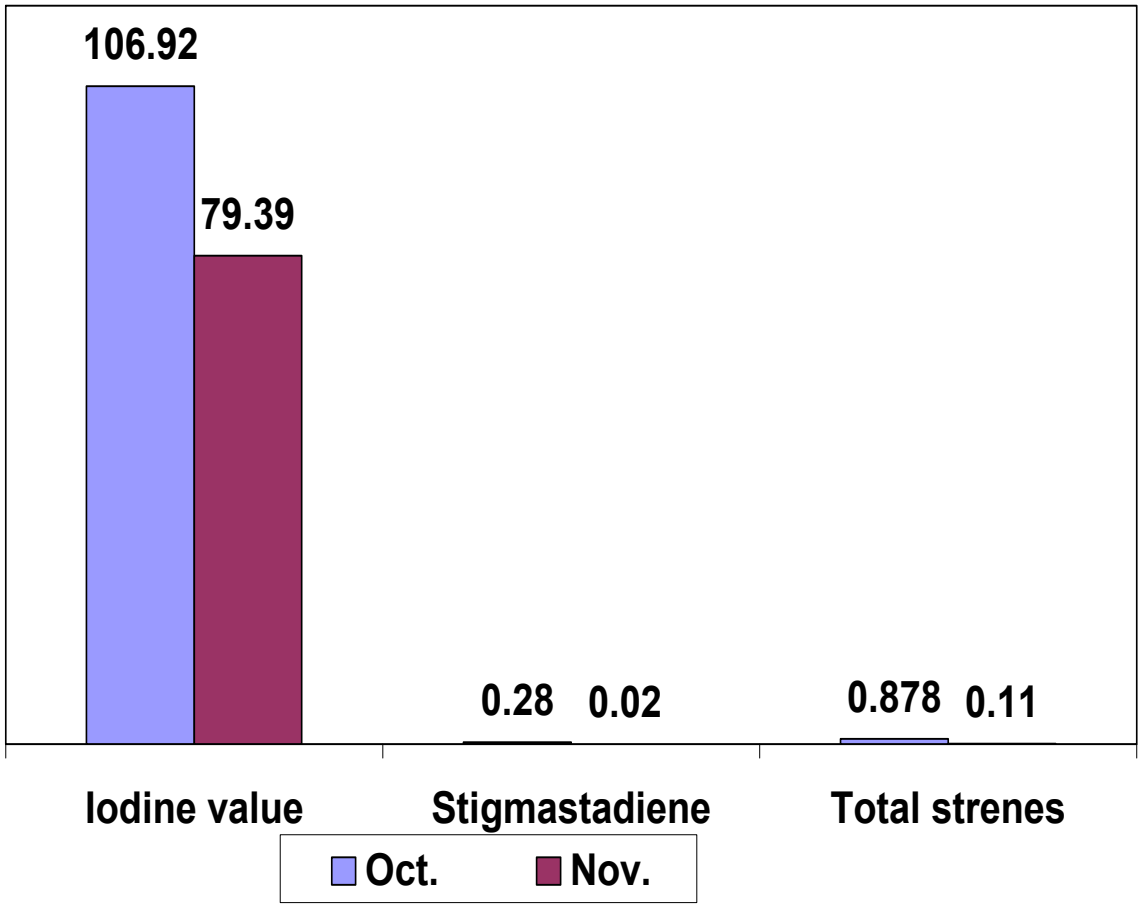


Fig (4.2): Relationship of sterenes & Iodine value in different Harvesting times.

4.4.1.4 Relationship between sterenes content & Peroxide value in different Harvesting times:

Stigmastadiene and total sterenes content was found to be decreased with the increase of peroxide under the effect of ripening time increase as shown in Figure (4.3). This is due to the decrease in sterolic compounds with the activity of olive ripening enzymes. In addition, peroxide value increases with the increase of ripening time due to the activity of lipase enzyme, (M. Hmidat, 2007).

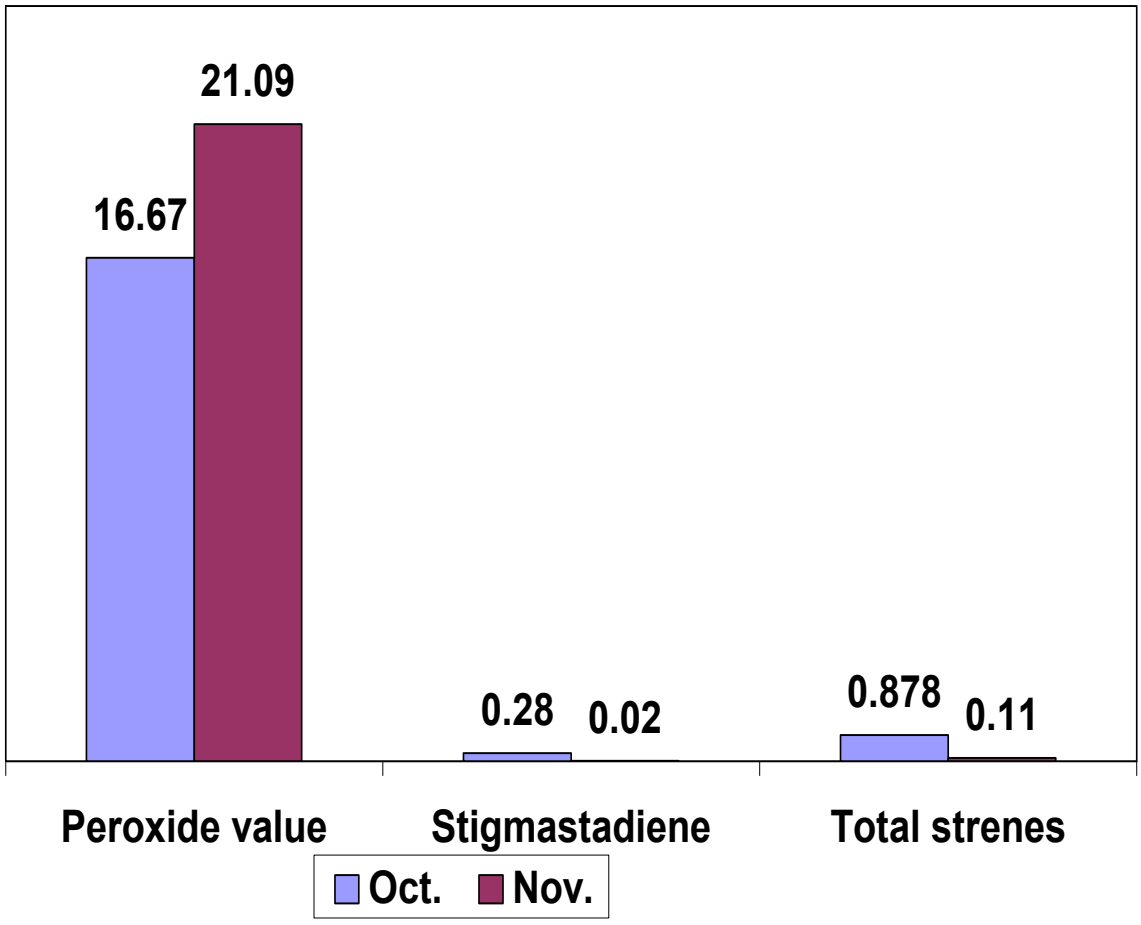


Fig (4.3): Relationship of sterenes & Peroxide value in different Harvesting times.

4.4.2 Metrological Data and correlations with olive oil quality parameters:-

4.4.2.1 Metrological data & quality parameters

Table (4.4) compares metrological factors in Bet Dagan and Assera villages from December 2007 to November 2008. Results of this study have shown that olive oil of Bet Dagan, has higher stigmastadiene, total sterenes content and iodine values compared to olive oil of Assera.. However, acidity (%) and peroxide values of olive oil in Assera was found to be higher compared to Bet Dagan olive oil. Figure (4.4) illustrates these results.

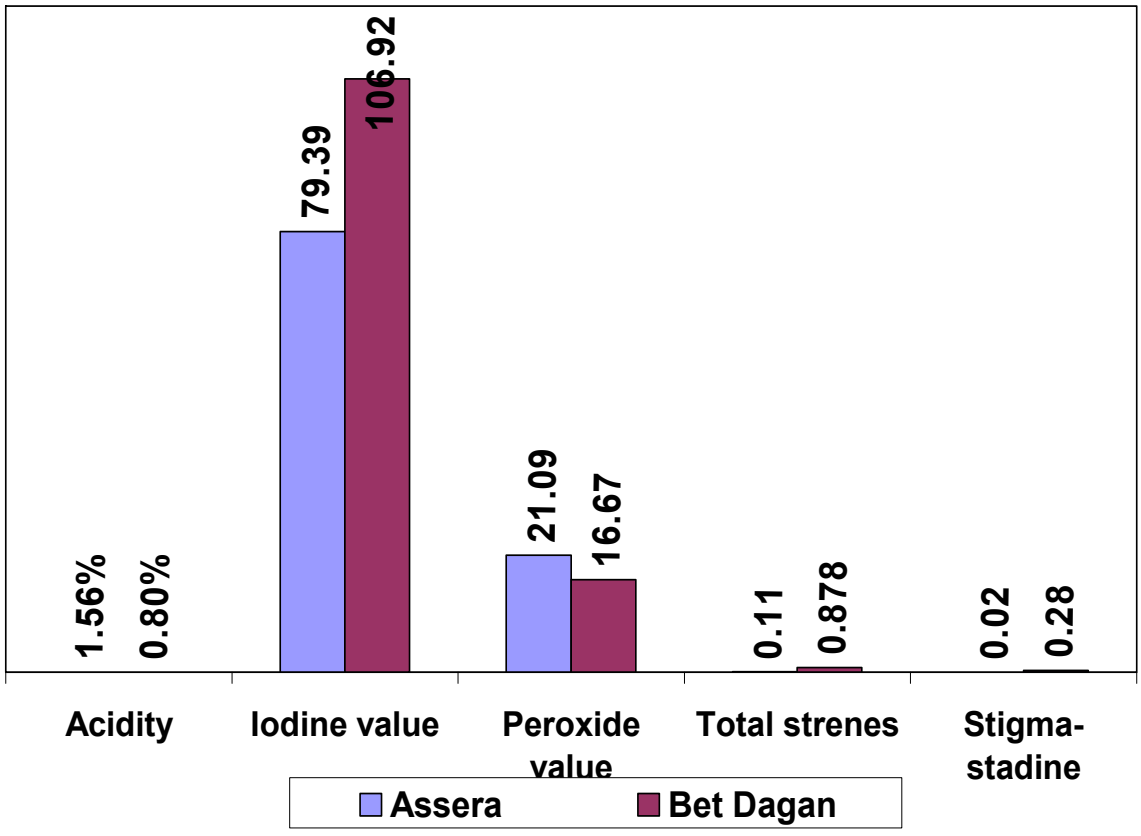


Fig (4.4): Comparison of olive oil quality parameters average values in Bet Dagan & Assera villages.

Table (4.3) illustrate the average values of different olive oil quality parameters in different harvesting times. It is now interesting to study the correlation between different metrological factors like temperature, rainfall precipitation, humidity and quality parameters of olive oil of the villages.

Table (4.3): comparison of olive oil quality parameters (Acid values, Iodine values, Peroxide values and content of stigmastadiene and total sterenes) in Bet Dagan & Assera villages.

No.	Village	Harvest time	Acid value (%)	Iodine value (cg/g)	Peroxide value (meq/kg)	Stigmastadiene (mg/kg)	Total sterenes (mg/kg)
1	Assera – Al-Shamallia	November	1.56	79.4	21.1	0.02	0.11
2	Bet Dagan	October	0.8	106.9	16.7	0.28	0.88

Table (4.4): Measures of metrological factors from Dec. 2007 to Nov. 2008 in Bet Dagan, (Http://www1.cbs.gov.il/reader/, 28.09.2009) & Nabluse city as a representative metrological data for Aserra (both on northern eastern foothills), (http://www1.cbs.gov.il/reader/ & Palestine national authority ministry of transport metrological authority 2007 & 2008 climatic bulletin).

	Bet Dagan		Assera		Bet Dagan	Assera	Bet Dagan	Assera
Date	Max. Temp. (°c)	Min. Temp. (°c)	Max. Temp. (°c)	Min. Temp. (°c)	Precipita-tion (mm)	Precipita-tion (mm)	Relative humidity (%) 8 a.m.	Relative humidity (%)
Nov-07	*	*	21.6	12.6	96.1	85.3	*	61
Dec-07	*	*	15.8	8.3	87.4	78.4	*	69
Jan-08	16.3	5.8	10.9	3.6	137.7	157.7	77	64
Feb-08	18.6	7.3	14.4	6.4	84.2	104.7	78	65
Mar-08	24.9	11.9	22.2	12.6	0.9	6	62	52
Apr-08	26.2	13.8	25.2	14.3	0	0	57	49
May-08	26.2	15.5	25.8	14.6	3.3	0	61	58
Jun-08	30.5	20.2	30.3	19.1	0	0	60	53
Jul-08	31.5	22.4	31.1	20.4	0	0	64	62
Aug-08	31.9	23.5	31.2	21.1	0	0	68	56
Sep-08	30.8	21.9	29.3	20	0	11	61	67
Oct-08	27.4	17.7	25.5	16.6	0	23.3	67	68
Nov-08	25.4	13.2	21.5	14.2	0	4.2	67	58

4.4.2.2 Correlation of olive oil sterenes content & temperature:-

From Figure (4.5) & (4.6) we found that the maximum and minimum temperatures from January to November is higher in Bet Dagan than in Assera village; especially from August to November period during which 50% of olive oil is accumulated (Xiao Qianewen, *et al*, 2009). The higher exposer of olive fruit to higher tempreture in Bet Dagan compared to Assera enhances the dehydration of sterols and formation of stigmastadiene and sterenes in olive oil, (R. Bortolomeazzi, *et al*, 2000).

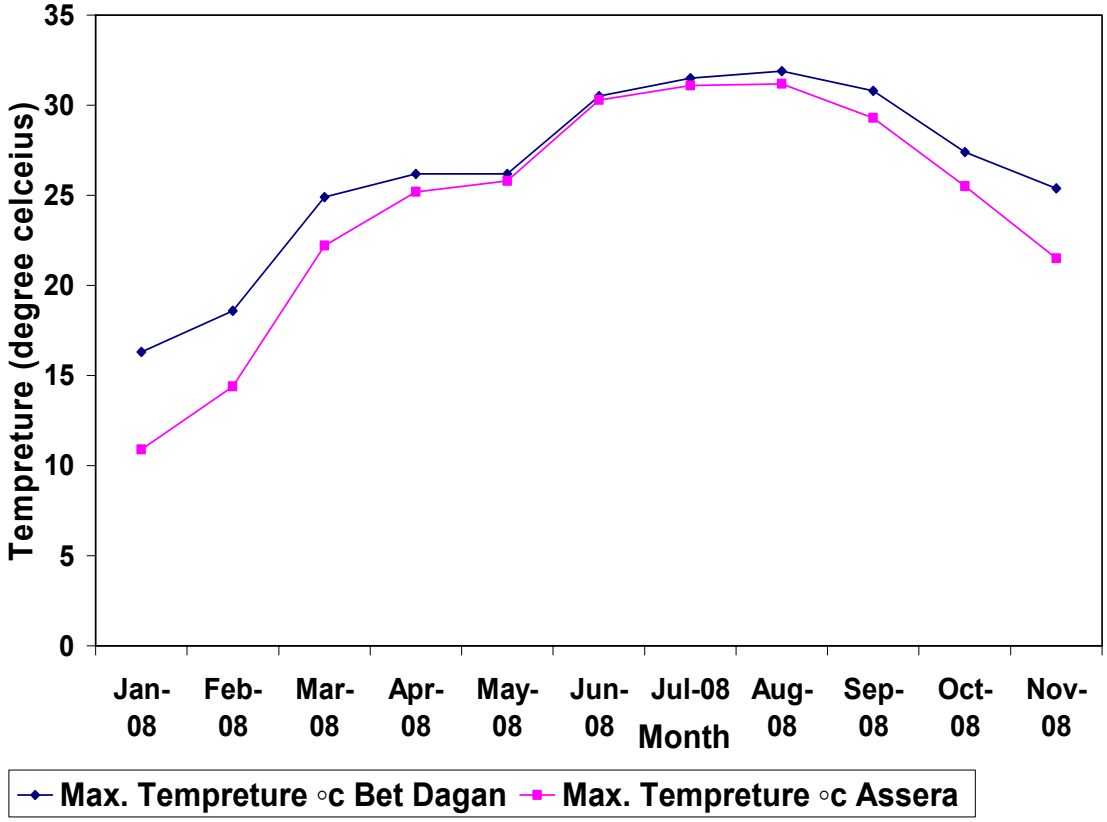


Fig (4.5): Maximum temperature in Bet Dagan & Assera during the year of 2008.

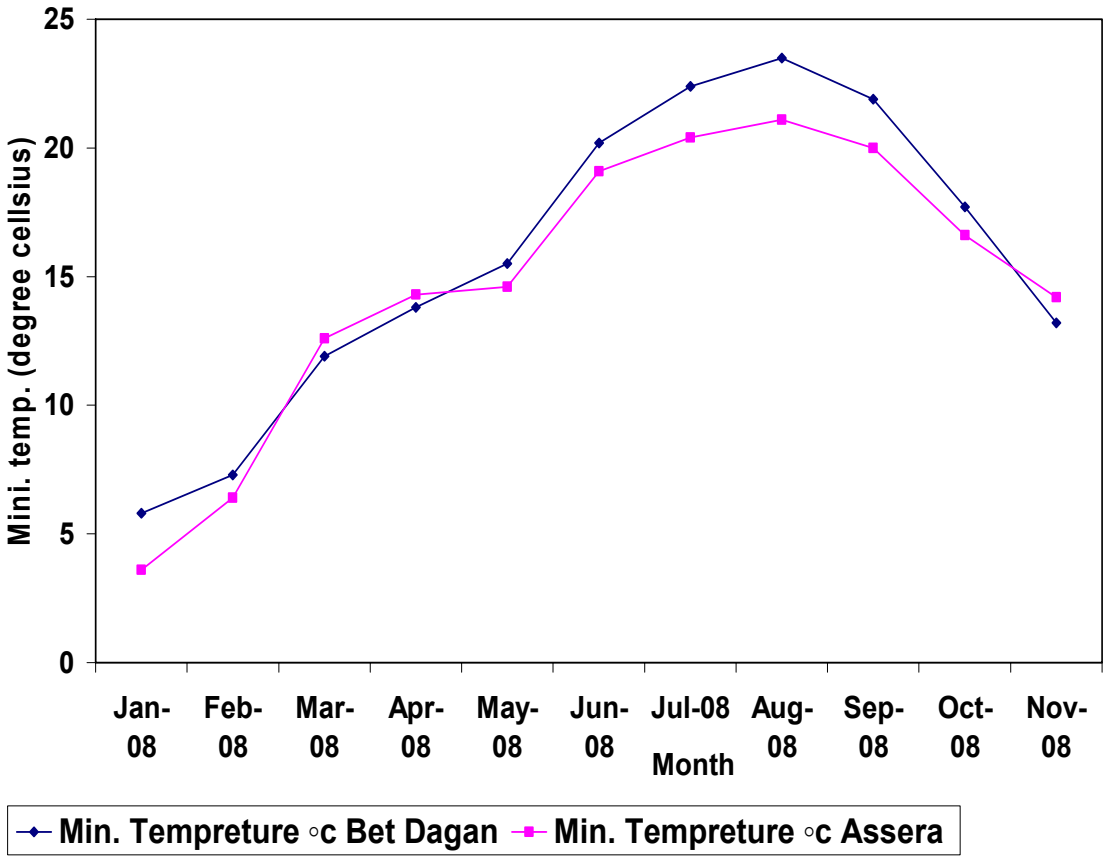


Fig (4.6): Minimum temperature in Assera & Bet Dagan during the year of 2008.

4.4.2.3 Correlation of rainfall Precipitation & olive oil sterenes content:-

Figure (4.7) explains the distribution of monthly rainfall for Assera and Bet Dagan for the hydrological year of 2007/2008. From this figure, we found that Assera has better hydrological year and higher precipitation values. The higher amount of precipitation; which is correlated with longer day hours- due to its location in western foothills- cause higher quality and quantity of produced olive oil in Assera compared to Bet Dagan, (Xiao

Qianewen, *et al*, 2009). It is also noted that rainfall which occurs before olive fruits collection is corroborated with lower content of sterenes and stigmastadiene.

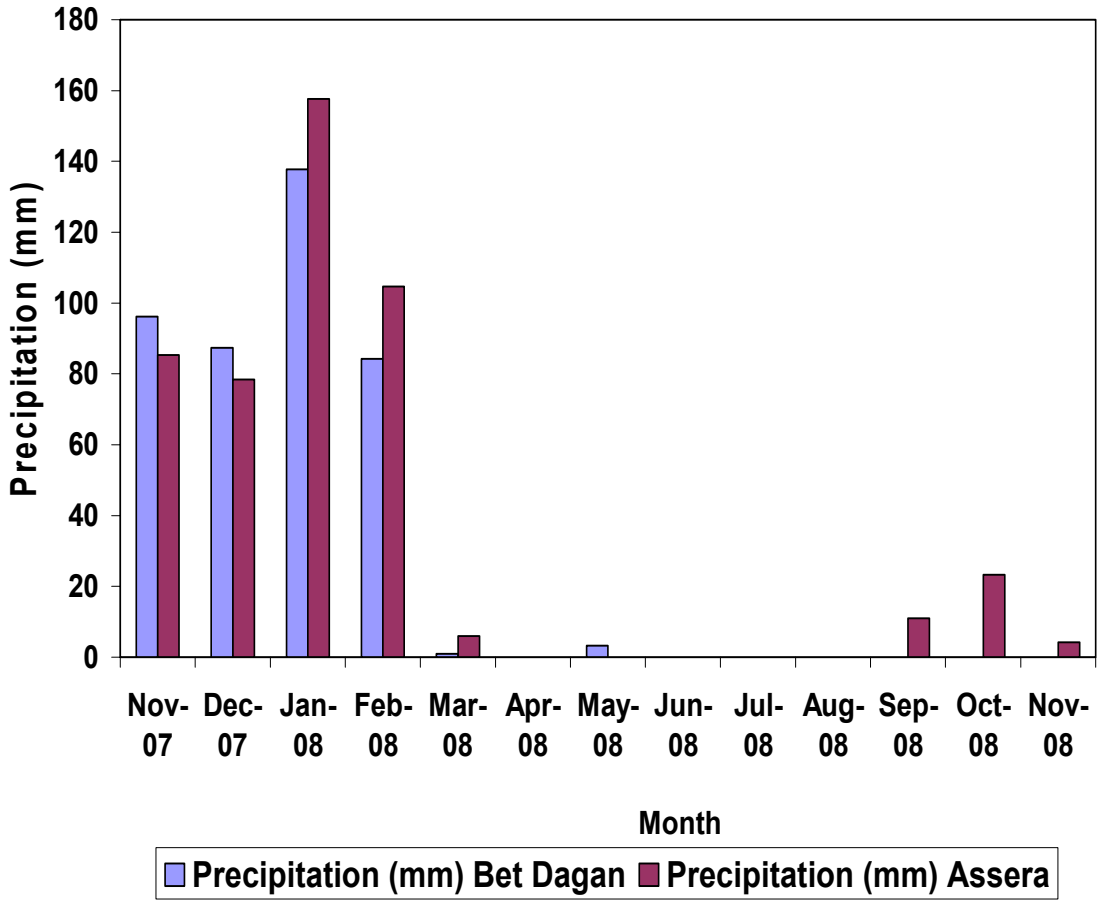


Fig (4.7): Distribution of rainfall precipitation in Assera & Bet Dagan.

4.4.2.4 Correlation of Humidity & olive oil sterenes content:-

From figure (4.8), we found that from June to November 2008 relative humidity is higher in Bet Dagan compared to in Assera. The lower relative humidity and longer day hours;

due to location in western foothills; in Assera village enhances higher amounts of olive oil accumulation and better quality as this is the favorable conditions for olive oil formation, (Xiao Qianewen, *et al*, 2009). Higher values of humidity in Bet Dagan was correlated with higher sterenes content.

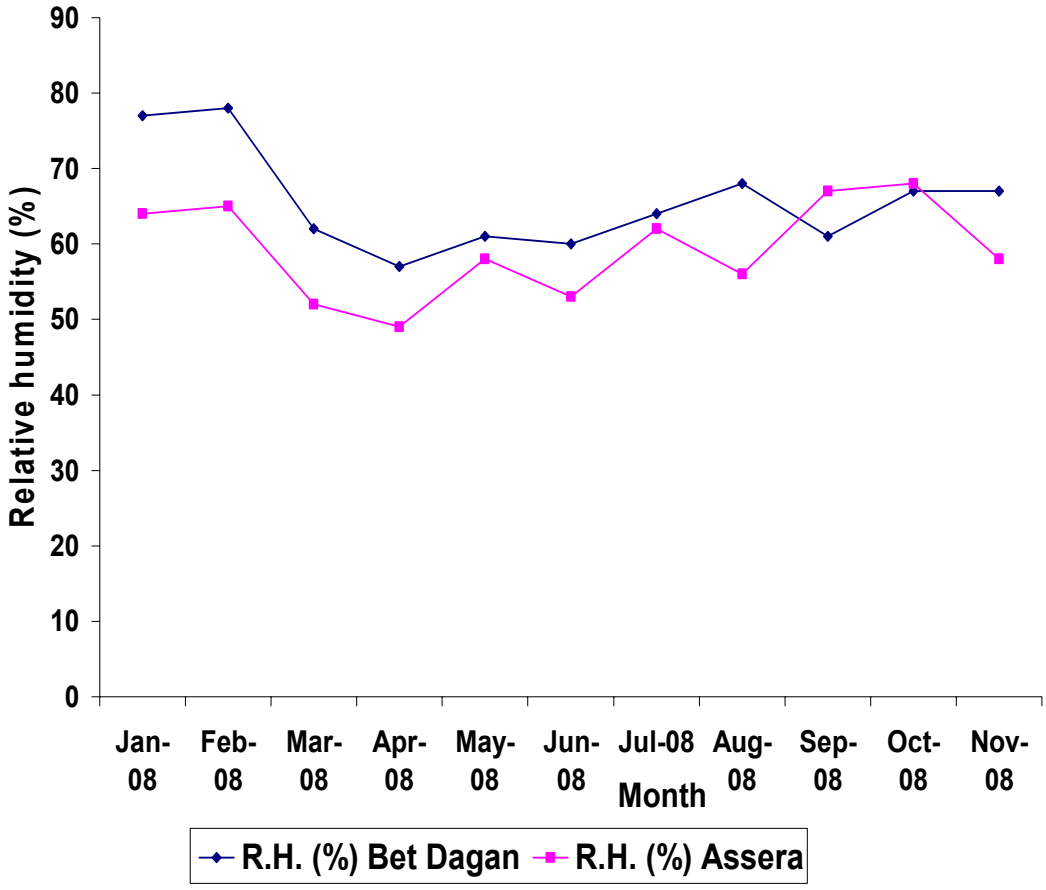


Fig (4.8): Relative humidity of Assera & Bet Dagan during 2008 year.

4.4.3 Storage test:-

Five olive oil samples from Assera were stored in dark condition and glass bottles for 9 months in two different temperatures, (at room temperature and at 4 degree Celsius). After storage period, samples were analyzed for sterenes content. Table (4.5) shows sterenes content of these samples for direct analysis after collection and after storage period in both temperatures.

This test performed in order to determine the effect of olive oil storage temperature on the sterenes content in order to in improving methods of olive oil storage during transfer and in market shops.

From these results, it is seen that there is no direct relationship between the sterenes content and the storage condition.

Table (4.5): Sterenes content of storage test samples.

Storage Temperature	Sample ID	Stigmastadiene (mg/kg)	Cholestadiene (mg/kg)	stigmastatriene (mg/kg)	Campestadiene (mg/kg)	TOTAL sterenes (mg/kg)
Room Temperature Storage	1	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00
	4	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.02	0.00	0.00	0.02
4 degree Celsius Storage	1	0.01	0.09	0.00	0.04	0.14
	2	0.02	0.13	0.00	0.04	0.19
	3	0.00	0.12	0.00	0.00	0.12
	4	0.73	0.30	0.00	0.08	1.12
	5	0.00	0.04	0.00	0.01	0.06
Direct Analysis after collection	1	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.03	0.00	0.00	0.03
	3	0.00	0.00	0.00	0.00	0.00
	4	0.00	0.01	0.00	0.00	0.01
	5	0.02	0.3	0	0.01	0.33

4.4.4 Comparison of olive oil quality indexes between Palestinian olive oil and other countries olive oil:-

Different olive tree verities cultured in Palestine includes (manzalino, nabali, modifeid nabali and k18). These varieties differ from the verities cultured in other countries. From the compression between Cuba, Crete, Spain and Italy olive oil quality (according to acidity and peroxide); which is produced from different olive trees varieties; with Palestinian olive oil quality in table (4.6), we found that Palestinian olive oil is classified into extra virgin, ordinary virgin and olive pomace oil, (International olive oil council, 2008). Palestinian olive oil shows good competition quality parameters for Acidity and Peroxide values according to the International olive oil council limits compared with these countries olive oil acidity and peroxide values.

Table (4.6): Compression of Palestinian olive oil quality with other countries olive oil (this table illustrate average values of quality indexes). Oil classification deepened on Acidity & Peroxide values, (International olive oil council, 2008).

No.	Country	Reference	Olive tree verity	Iodine value (cg/g)	Free Acidity	Peroxide value meq/kg	Olive oil Classification
1	Cuba	Maritaza F. Diaz, *et al*, 2006	*	81.8	0.14%	10	Olive pomace
2	Crete Island	S. A. Vekiari, *et al*, 2007	Koroneiki	*	0.37%	2.75	Extra virgin
3	Spain/ season 2004/2005	J. Luis & J.Urda, 2009	Picula	*	0.12%	1.8	Refined
			Arbequina	*	0.14%	5.9	Refined
			Hojiblanca	*	0.12%	3.7	Refined
4	Italy	A. Pasqualone & M. Catalono, 2000	**Surely,** Extra virgin	*	0.75%	*	Extra virgin
			Surely, Current virgin and lampante	*	5.98%	*	Crude olive pomace
5	Palestine	Himadate 2007	k18	84.3	0.44%	9.6	Extra virgin
			modifeid nabali	84.7	0.36%	7.8	Extra virgin
			nabali	84.2	0.32%	4.4	Olive pomace
			manzalino	87.4	0.44%	8.2	Extra virgin
6	Palestine (North west bank)	Current study 2008/2009	Nabalei	91.78	1.22%	19.1	Ordinary virgin
	Palestine (Assera)	Current study 2008/2009	Nabalei	79.39	1.56%	21.09	Ordinary virgin
	Palestine (Bet Daga)	Current study 2008/2009	Nabalei	106.9	0.80%	16.67	Extra virgin

Chapter Five

Conclusions and Recommendations

5.1 Main Conclusions

Environmental characteristics of olive oil in northern west bank analyzed for production season of 2008 in Bet Dagan and Assera villages as a case study for Palestinian olive oil. Main conclusions summarized as following:

- Results of this study have shown that olive oil of Bet Dagan, has higher stigmastadiene, total sterenes content and iodine values compared to olive oil of Assera.. However, acidity percentage and peroxide values of olive oil in Assera was found to be higher compared to Bet Dagan olive oil. These differences is due to differences in metrological factors, extraction conditions and use of fishers against olive fly in Assera village.
- Classification of Palestinian olive oil (represented by Assera and Bet Dagan olive oil) includes the following classes: Extra virgin olive oil (25%), Virgin olive oil (42.5%), Ordinary virgin olive oil (7.5%), Lampante olive oil (5%) and Olive oil (20%).
- It was found that 3 samples from 21 samples of Assera contain stigmastadiene and they satisfy the limits set by International bodies for stigmastadienes content of olive oil (0.15 ppm for virgin olive oil and 0.5 ppm for lampante olive oil) and limits of the international olive oil council standards for virgin olive oil stigmastadiene content (0.1mg/kg for edible virgin olive oil and 0.5mg/kg for

lampante virgin olive oil). While for Bet Dagan, 15 out of 18 samples was found containing stigmastadiene higher than 0.15 mg/kg. This difference in stigmastadiene content of the olive oil of the two villages is related to the difference in weather conditions between the two villages due to their locations in different foothills; this difference in location cause differences in weather conditions that includes temperature, humidity and precipitation, and also due to use of fisheries against olive fly in Assera.

- Stigmastadiene and total sterenes content was found to be decreased with the increase of peroxide under the effect of increase ripening time.
- Stigmastadiene and total sterenes content was found to be in a positive coloration with iodine value; both sterenes and stigmastadienes content and iodine value were found to be decreased with the increase of ripening time.
- It was found that Stigmastadiene and total sterenes content decrease with the increase of acidity under the increase of ripening time.
- The higher monthly averages of minimum and maximum temperatures of Bet Dagan cause the increase of Stigmastadiene content of olive oil.
- The higher amount of precipitation In Assera; which is correlated with longer day hours- due to its location in western foothills- cause higher quality and quantity of produced olive oil in Assera compared to Bet Dagan. It is also noted that rainfall which occurs before olive fruits collection is corroborated with lower content of sterenes and stigmastadiene.
- The lower relative humidity and longer day hours; due to location in western foothills; in Assera village enhances higher amounts of olive oil accumulation and better quality as this is the favorable conditions for olive oil formation.

5.2 Recommendations

From the results of this Study, we recommend the following:

1. Supply farmers with olive fly Fisheries.
2. Increase the knowledge of farmers about the farming activities (that related to olive culturing and fruit collection) which effect on olive oil quality.
3. Determine olive fruits harvesting time.
4. To use new technologies in order to control the temperature of olive oil extraction.
5. Increase the knowledge of mills engineers about the extraction factors effect on olive oil quality.
6. To apply further studies on olive oil quality in Palestine, by designing field experiments to study certain factors (include irrigation factor, olive fly infection, harvesting time, metrological factors … etc) in order to determine its effect on olive oil quality.
7. The extraction of olive oil shall done in a range of temperature of 25 - 30º C, and the ripening time shall be increased especially in the areas that may have rainfall during November.

References

A. Pasqualone and M. Catalano, (2000), Free and total sterols in olive oils effects of nutralization. Grasas y Aceites 51, pages 177-182.

Codex standard for olive oil, (2001), Virgin and refined, and for refined olive-pomace oil (CODES STAN 33-1981[Rev.1-1989]). Codex Alimentarius, vol 8, pages 25 – 39.

D.D. Ben Miled, A. Smaoui, M. Zarrouk and A. Cherif, (2000), Do extraction procedures affect olive oil quality and stability?. Biochemical society transactions 28, pages 929-933.

Dimitrios Boskou, (2006), Olive Oil Chemistry and Technology, Second Edition, AOCS Press, page 140.

International standard, (2003), Animal and vegetable fats and oils determination of stigmastadienes in vegetable oils part 2: method using high – performance liquid chromatography (HPLC), ISO 2003, first edition, reference number ISO 15788-2:2003(E).

International olive oil council, (2008), Trade standard applying to olive oils and olive-pomace oils, COI/T.15/NC no.3/Rev.3, pages 1-16.

J. Guil-Guerrero and J. Urda-Romacho, (2009), Quality of extra virgin olive oil affected by several packaging variables, Grasas y Aceites 60, pages 125-133.

K.Helrich, (1990), Official methods of analysis of the association of official analytical chemists, Fifteenth edition, Association of official analytical chemists, Inc., Virginia 22201 USA.

K. H. Wilm, (2009), Our food database of food and related sciences, Text book, Five edition, page 400.

M.A.A. Hmidat, (2007), Factors affecting in Palestinian olive oil quality, master theses, Alquds university.

M. Amelio, R. Rizzo and F. Varazini, (1998), Separation of stigmasta-3,5-diene, squalene isomers and wax easter from olive oils by single high-performance liquid chrpmatography, JAOCS, 75, pages 527-530.

M. C. Dobarganes, A. Cert and A. Dieffenbacher, (1999), The determination of staigmastadienes in vegetable oils, Pure & Appl. Chem., 71, pages 349.

M.F. Diaz, R. Hernandez, G. Martinez, G. Vidal, M. Gomez, H. Fernandez and R. Garcez, (2006), Comparative study of ozonized olive oil and ozonized sunflower oil, J. Braz. Chem. Soc. 17, pages 403 – 707.

Palestine national authority ministry of transport metrological authority, (2007), Climatic bulletin year 2007.

Palestine national authority ministry of transport metrological authority, (2008), Climatic bulletin year 2008.

Palestinian national authority - ministry of agriculture - G.D. of extension and rural development, 2008.

Por Mª Soledad Gracia1, Antonio Royo y Mónica Guillén, (2009), Chemical composition of virgin olive oil "Empeltre" variety from the Bajo Aragón region. Grasas Y Aceites, 60 (4), pages 321 – 329.

R. Amirante, E. Cini, G.L. Montel and A. Pasqualone, (2001), Influence of mixing and extraction parameters on virgin olive oil quality, Grasas Y Aceites 52, pages 198 – 201.

R. Benedikt, (2007), Chemical analysis of oils fats waxes and of commercial products derived therefrom, Read Book, pages 310.

R. Boggia, F. Evangelisti, N. Rossi, P.Salvadeo and P. Zunin, (2005), Chemical composition of olive oils of the cultivar Colombaia, Grasas y Aceites, 56, pages 276.

R. Bortolomeazzi, M. Zan, L. Pizzale and L. S. Conte, (2000), Identification of new steroidal hydrocarbons in refined oils and the role of hydroxyl sterols as possible precursors, Agric. Food chem., 48, pages 1101-1105.

S.A. Vekiari, P. Papadopoulou and A. Kiritsakis, (2007), Effect of processing method and commercial storage conditions on the extra virgin olive oil quality indexes, Grasas y Aceites 58, pages 237 – 242.

W. Mahmood Mat Yunus, Yap Wing Fen and Lim Mei Yee, (2009), Refractive Index and Fourier Transform Infrared Spectra of Virgin Coconut Oil and Virgin Olive Oil, American Journal of Applied Sciences, 2, pages 328 – 331.

X. Qianewen, Z. Li, Z. Lanying & W. Kaizhi , (2009), Study on olive development in China, Agricultuare & Environmental sciences, 5(3), pages 414-419.

Y. Kazakevich and R. Lobrutto, (2007), HPLC for pharmaceutical scientists, John Wiley & Sons, Inc.

(Http://www.pmd.ps/ar/climateaverage.htm, 28.09.2009).

(Http://www1.cbs.gov.il/reader/, 28.09.2009).

Appendix

Table (a.1): Acid value and Sterenes content in different Harvest times.

Harvest time	Acid value	Total sterenes (mg/kg)	Stigmastadiene (mg/kg)
October	0.80%	0.878	0.28
November	1.56%	0.11	0.02

Table (a.2): Iodine value and Sterenes content in different Harvest times

Harvest time	Iodine value (cg/g)	Total sterenes (mg/kg)	Stigmastadiene (mg/kg)
October	106.9	0.878	0.28
November	79.39	0.11	0.02

Table (a.3): Iodine value and Sterenes content in different Harvest times

Harvest time	Peroxide value (meq/kg)	Total sterenes (mg/kg)	Stigmastadiene (mg/kg)
October	16.67	0.878	0.28
November	21.09	0.11	0.02

Table (a.4): Summary data for olive oil quality parameters of samples collected from the mills of Bet-dajan & Assera – Al-shmaleh villages for the production season of 2008.

Source of Sample	Sample ID	Farmer Name	Iodine value (cg/g)	Nitric acid test	Refractive index	Acid Value (%)	Peroxide value (meq/kg)	Stigma stadiene (mg/kg)	Cholesta-dien (mg/kg)	Stigmast - atriene (mg/kg)	Campest -adiene (mg/kg)	TOTAL sterenes content (mg/kg)
Bet Dagan	081129 OI2	Abu-Hassan	109.10	- ve	1.46971	1.12	14.76	0.11	1.33	0.00	0.00	1.44
	081129 OI3	Abu-Ahmad	165.50	- ve	1.47072	0.43	21.75	0.28	0.44	0.00	0.00	0.72
	081130 OI4	Tawfeq Abu-Jeash	163.70	- ve	1.46971	0.46	16.79	0.31	0.40	0.05	0.07	0.83
	081130 OI5	Azam labasee	131.90	- ve	1.46971	1.12	20.94	0.26	0.39	0.00	0.04	0.69
	081130 OI6	Sameh Abu-Alabed	161.60	- ve	1.46871	0.58	14.76	0.16	0.23	0.02	0.06	0.47
	081213 OI7	Seleman Saed Amer	137.80	- ve	1.46871	0.81	13.69	0.25	0.38	0.06	0.10	0.79
	081213 OI8	Abu-Zahe	64.50	- ve	1.46971	0.66	13.80	0.44	0.64	0.01	0.03	1.12
	081213 OI9	Mezed Abed Alqader (1)	54.90	- ve	1.46971	0.42	11.74	0.34	0.44	0.03	0.06	0.87
	081213 OI10	Majed Abu-Holwan	138.20	- ve	1.46971	1.93	8.54	0.25	0.38	0.01	0.02	0.66
	081214 OI11	Rabeh Hussne Abed Aljalel	55.80	- ve	1.46971	1.04	8.54	0.24	0.45	0.05	0.07	0.81
	081214 OI12	Tawfeq Wajeh Abu-Jeash	53.60	- ve	1.46871	0.97	14.31	0.48	0.59	0.00	0.00	1.07
	081215 OI13	Abed Alkarem Mustafa Abed Aljalel	163.10	- ve	1.46971	0.42	19.84	0.26	0.40	0.04	0.12	0.82

Source of Sample	Sample ID	Farmer Name	Iodine value	Nitric acid test	Refractive index	Acid Value %	Peroxi-- de value	Stigma stadiene (mg/kg)	Cholesta- dien (mg/kg)	Stigmast - atriene (mg/kg)	Campest a- diene (mg/kg)	TOTAL sterenes content (mg/kg)
Bet Dagan	081215 OI14	Mezed Abed Alqader (2)	50.70	- ve	1.46871	1.16	18.26	0.34	0.47	0.02	0.10	0.93
	081215 OI15	Ahmad Amer	58.60	- ve	1.46971	0.93	24.42	0.63	0.36	0.00	0.00	0.99
	081216 OI16	Abu-Adel	128.10	- ve	1.46871	0.58	18.49	0.31	0.58	0.08	0.09	1.06
	081216 OI17	Sabah Abu-Jesh	163.50	- ve	1.47071	0.46	23.67	0.00	1.02	0.00	0.00	1.02
	081217 OI18	Imad Rebhe	69.50	- ve	1.46971	0.46	17.34	0.21	0.31	0.08	0.45	1.05
	081217 OI19	Abu-Samer	54.40	- ve	1.46871	0.81	18.42	0.11	0.35	0.01	0.00	0.47
Assera Al Shamalia	081113 OI20	Nasser abed alah Yassen	79.69	- ve	1.4697	0.73	15.95	0	0	0	0	0.00
	081113 OI21	Saed Abu-sara	80.65	- ve	1.4687	2.30	34.60	0	0	0	0	0.00
	081113 OI22	Rashed Talwza	80.46	- ve	1.4697	1.60	18.92	0	0.01	0	0	0.01
	081114 OI23	Yaser Al-tawfeq	80.01	- ve	1.4697	1.70	18.97	0	0.02	0	0	0.02
	081114 OI24	Amer Fawaz Jararah	78.70	- ve	1.4697	1.00	15.60	0	0	0	0	0.00
	081114 OI25	Zahe Sawalha	70.89	- ve	1.4697	1.20	17.38	0	0.1	0	0	0.10
	081114 OI26	Jalal Al sowfe	78.47	- ve	1.4697	1.40	20.90	0	0	0	0	0.00
	081114 OI27	Meflh abu-albasha	85.38	- ve	1.4697	3.50	16.98	0	0.03	0	0	0.03

of Sample	Sample ID	Farmer Name	Iodine value	Nitric acid test	Refractive index	Acid Value %	Peroxi-- de value	Stigma stadiene (mg/kg)	Cholesta- dien (mg/kg)	Stigmast - atriene (mg/kg)	Campest a- diene (mg/kg)	TOTAL sterenes content (mg/kg)
Assera Al Shamalia	081114 OI28	Yaser izat saleh yassen	75.62	- ve	1.4697	1.70	23.11	0	0.01	0	0	0.01
	081115 OI29	Mohamad rashed jaharia	67.96	- ve	1.4697	1.60	22.60	0	0.1	0	0	0.10
	081115 OI30	Nasser Abedalah	80.90	- ve	1.4697	1.00	19.91	0	0.01	0	0	0.01
	081115 OI31	Hussam Saleh Yusef	80.19	- ve	1.4697	0.58	19.30	0	0	0	0	0.00
	081115 OI32	Mohamad Faiq musa sawalha	81.93	- ve	1.4697	1.00	13.17	0	0.01	0	0	0.01
	081115 OI33	Anes mohamad yasen	81.18	- ve	1.4697	2.20	24.20	0	0.02	0	0	0.02
	081116 OI34	Issmat hamed showli	81.51	- ve	1.4697	0.54	19.71	0.2	0.33	0	0.02	0.55
	081116 OI35	Ahmad Abu-zahe	75.05	- ve	1.4697	0.38	17.00	0	0	0	0	0.00
	081115 OI36	Ramiz taher sawallha	79.92	- ve	1.4697	0.89	20.15	0	0.01	0	0	0.01
	081116 OI37	jafer abedalah atalwze	81.10	- ve	1.4697	3.78	20.92	0.02	0.03	0	0	0.05
	081116 OI38	Issam alsswas	77.42	- ve	1.4697	3.47	35.50	0	0	0	0	0.00
	081117 OI39	Husam Saleh	76.37	- ve	1.4697	0.58	20.96	0	0.02	0	0	0.02
	081117 OI40	Moner abed alatef hamarshe	89.98	- ve	1.4687	0.81	20.20	0	0.01	0	0	0.01
	081117 OI41	mohamad alhaj abed	83.21	- ve	1.4697	2.30	27.90	0.02	0.3	0	0.01	0.33

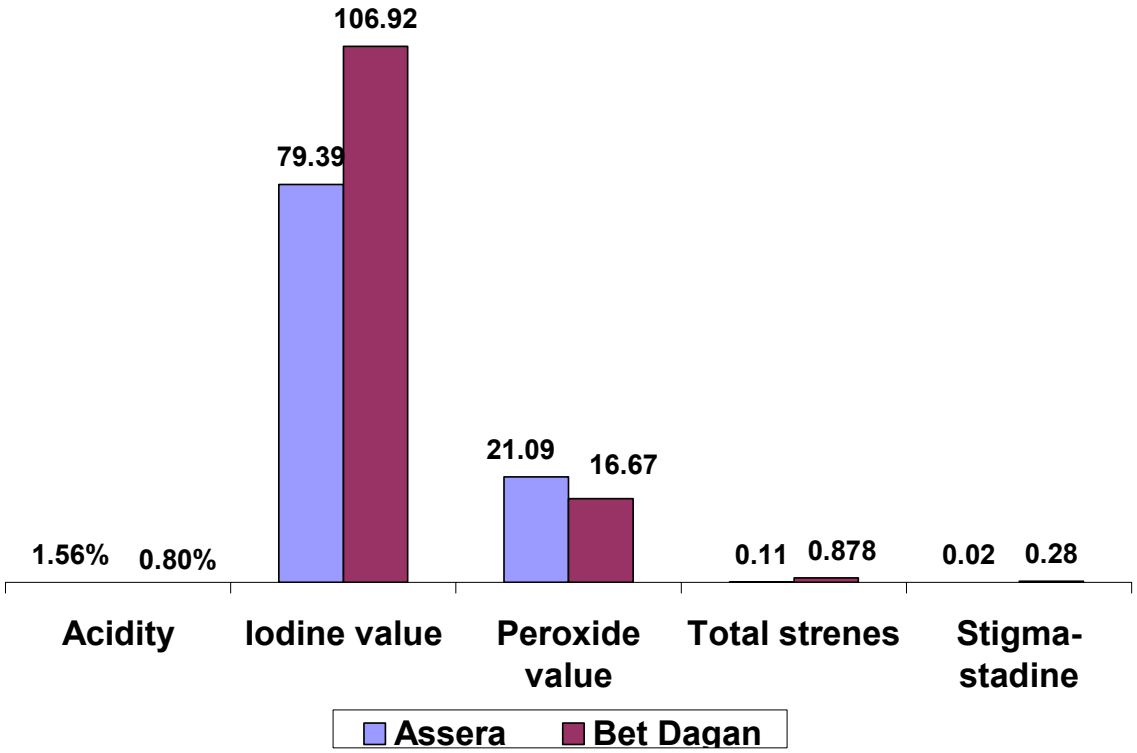


Fig (a.1): olive oil quality parameters Comparison in Assera and Bet Dagan.

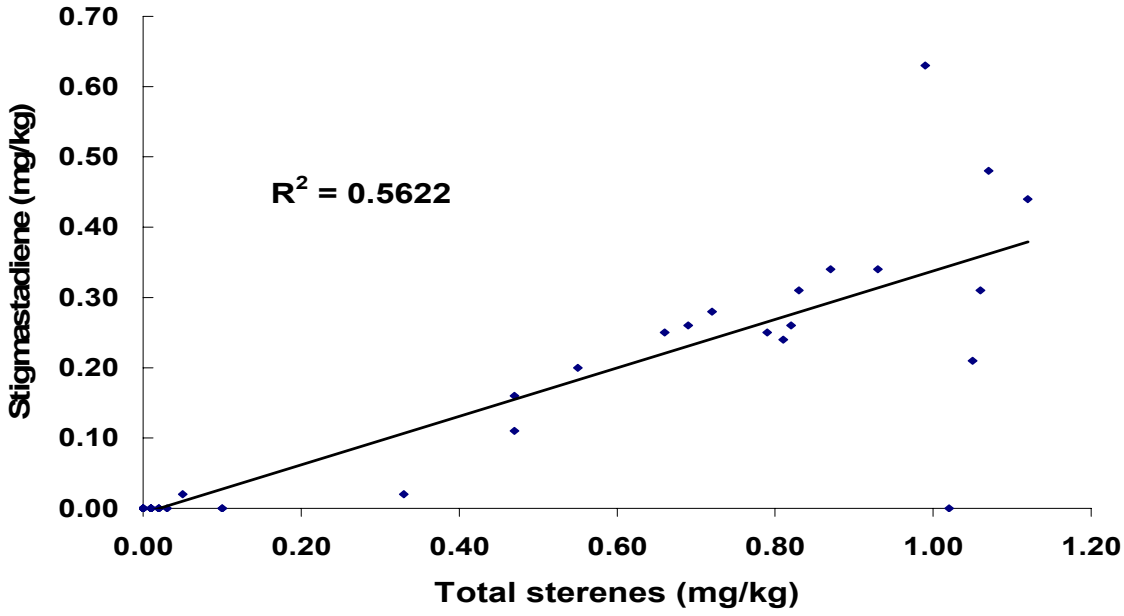


Fig (a.2): Relationship of Stigmastadiene & total sterenes.