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Scientific Research

Identification of sterol content and quality evaluation of virgin olive oil of Arbequina, Mari, Shenge and Zard cultivars in Tarem region

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ABSTRACT

Olive oil is a unique edible oil that has attracted attention due to its high levels of monounsaturated fatty acids, pleasant taste, good stability, and special health benefits. In this study, the quality of virgin olive oils from the Arbequina, Mari, Shengeh, and Zard cultivars in the Tarem region was investigated, and the parameters of sterols, acidity, peroxide value, and iodine value were evaluated. Gas chromatography was used to determine and identify the sterols. The results showed that the amount of sterols in virgin olive oils from the Arbequina, Shengeh, Zard, and Mari cultivars differed significantly at the 5% level. The most abundant sterols found in all cultivars were beta-sitosterol, delta5-avenasterol, and campesterol. The free acidity level in the Mari cultivar had the highest amount, while the Arbequina cultivar had the lowest amount. The highest peroxide value was observed in the Zard cultivar, while the lowest was observed in the Mari cultivar. Finally, it can be concluded that the quality parameters evaluated for the Arbequina, Shengeh, Zard, and Mari cultivars were within the allowable standard range, and there was no significant difference in quality among these cultivars.

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1. Introduction

Iran is one of the most important olive growing areas in the world and the olive plant is considered as one of the genetic reserves of Iran. The olive tree is native to the Mediterranean region and constitutes one of the oldest tree species whose fruits and by-products, such as olive oil, have historically been the basis of nutrition for indigenous populations living in this region. The planting of olive trees, the exploitation of olives and the subsequent extraction of the liquid to obtain olive oil are intrinsically linked to the background and culture of Mediterranean populations.[1] Virgin olive oil is an oil that is produced by the fruit of the olive tree only by mechanical physical extraction processes under controlled temperature conditions, which leads to the preservation of its qualitative characteristics and bioactive compounds [2]. Olive oil consists of high amounts of natural antioxidants that are effective in preventing many diseases. Therefore, olive oil improves the fat profile by reducing the amount of low-density lipoprotein and increasing the amount of high-density lipoprotein, improving vascular oxidative damage, favorable changes in body homeostasis, reducing blood cholesterol, improving vascular function, and improving blood pressure control. becomes [3]. Iran's national standard divides olive oil into four categories: virgin, semi-refined, refined and sulfur. Virgin olive oil is an oil that is extracted from the fruit of the olive tree mechanically under certain conditions, especially at the right temperature. Also, operations such as washing, smoothing and separation centrifuge are performed on it. Due to its pleasant sensory and nutritional properties, olive oil is considered as one of the valuable sources of edible oil in many European, Asian and especially Mediterranean countries. For this reason, the area under olive cultivation in the world is expanding rapidly [4]. However, in many cases, consumers show an unfavorable level of knowledge and understanding in recognizing a special feature in a product, which may cause problems in identifying a high-quality food product from low-quality types, due to economic reasons and lack of consumer information. , the mixture of refined oil and virgin olive oil or mixed with other refined vegetable oils such as sunflower are marketed for frying purposes, therefore, according to the mentioned cases, the identification of compounds such as sterols in olive oil can improve the quality of the oil to an optimal extent. Consumer made sure [5]. Several ways have been proposed to identify and determine the authenticity of olive oil, including the identification of sterol compounds [6]. Virgin olive oil contains many of the important bioactive compounds of the olive fruit intact, this oil is widely welcomed by people of all ages due to its special flavor and high nutritional properties. . The quantitative and qualitative characteristics of olive oil are influenced by environmental factors and garden management. Climatic conditions, variety, cultivation method, harvest time and its processing method shape the final characteristics of olive oil and create the conditions to identify the product related to different geographical regions. Studies have shown that the effect of geographical region and plant origin has a significant difference in some variables such as fatty acids (linoleic acid percentage and oleic acid percentage) [7]. Considering the importance of this issue in this research, the sterol content and important quality factors of virgin olive oil obtained from four cultivars of Arabikina, Mari, Shenge and Zard were investigated in Tarem region.

2- materials and methods

1-2- Materials

In this research, 4 olive samples from the four olive cultivars Arbikina, Shenge, Zard and Mari were sampled from the Tarem Olive Research Station in Mehr 1401. The sampling time is 150

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days after flowering and the base date is May 20. Extra virgin olive oil was produced from the olives of each variety by cold pressing method. Then the treatments were analyzed. The chemicals used are methanol, cyclohexane, diethyl ether, stane, ethanol alcohol, glacial acetic acid, sodium sulfate, sodium methoxide, toluene (Merck, Germany) and rhodamine G6 (Sigma, America), and the GC device is model 6500 of the company. YOUNGLIN of South Korea was used.

2-2-Measuring the acidity of virgin olive oil

Free acidity, based on milligrams of potassium hydroxide necessary (in milligrams) to neutralize free fatty acids in one gram of fat, which was measured according to the test method provided in the national standard and to determine the percentage of free fatty acids in the oil. This number is used according to the predominant type of fatty acid in different oils, which was reported based on oleic acid in this test. This index was also proposed as the percentage of free fatty acid. The amount of free fatty acid indicates the quality, purity, freshness and age of fats and oils. The principles of the test are to dissolve the sample in a suitable solvent mixture and titrate it with an ethanolic or methanolic solution of sodium or potassium hydroxide. The test was conducted according to the national standard number 4178.

2-3-Measuring the peroxide number of virgin olive oil

Peroxide number was measured in order to detect the amount of oxidation products and peroxides in oil. It should be considered that the amount of peroxide is a dynamic parameter and its value depends on the background of the sample. Also, measuring the amount of peroxide is an experimental method and the obtained amount depends on the weight of the sample. This index shows the amount of primary oxidation products in the oil. The test was conducted according to the national standard number 4179.

4-2- Identifying and determining the composition of sterols

In this method, alpha-cholestanol is first added to the sample as an internal standard, and it is saponified with potash ethanol, and unsaponifiable compounds are extracted with ethyl ether. Then sterols and triterpene alcohols are separated on thin layer chromatography plates 2 based on silica gel. The separated parts are silylized and analyzed by gas chromatography with capillary column. Sampling was done in accordance with Iranian National Standard No. 493, edible oils and fats.

The identification of individual peaks was done according to the inhibition times and by comparison with the mixture of sterols and TMSE triterpentylalkyls identified under the same conditions. The peak areas of alpha-cholestanol, sterol and triterpene di-alcohols are calculated using electronic integration.

The concentration of each sterol is calculated separately, in mg/kg of fat, using formula number 1:

$$\text{sterol} = \frac{A_x \times M_s \times 1000}{A_s \times m} \qquad \text{Formula 1}$$

5-2- Measurement of iodine index of virgin olive oil

Iodine index shows the degree of unsaturation of an oil and is determined by titration with active iodine solution. Under favorable conditions, halogens are absorbed by unsaturated fatty acids in oils. The amount of added halogen approximates the number of unsaturated bonds. Therefore, the number of fat double bonds is one of the ionic index.

2-6- Statistical analysis

The statistical analysis of the obtained results was done using the minimum significant difference test in the form of a completely randomized design. All the tests were performed in at least three repetitions and the results were evaluated using SPSS software at a confidence level of 95%.

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3- Results and discussion

1-3- The results of the evaluation of sterol compounds in olive oil

The amount of sterols in all the oil samples of the studied cultivars were within the standard range. And the amount of sterols in the virgin oils of Arbekina, Shenge, Zard and Mari cultivars had statistically significant differences with each other at the level of 5% ($P < 0.05$). Total sterols in all cultivars were higher than the minimum allowed for virgin olive oil i.e. 1000 ppm.

The highest amount of stigmasterol in the analyzed samples is related to Arabkina olive oil with 0.87% and the lowest value of this parameter is related to Mari olive oil with 0.66%. The amount of stigmasterol in Shenge variety olive oil was 0.8% and yellow olive oil was 0.72%.

The amount of β-sitosterol in the samples of Arabkina, Shenge, Zard and Mari olive oils was 85.9%, 82.1%, 80.9% and 71.1%, respectively.

The highest amount of β-sitosterol in the studied samples is related to Arabkina variety olive oil with 85.9% and the lowest value of this parameter is related to Mari variety olive oil with 71.1%.

The amount of campestrol in the samples of Arabkina, Shenge, Zard and Mari olive oils was 2.6%, 3.1%, 3.46% and 2.74%, respectively. The highest amount of campestrol in the analyzed samples is related to the oil of the yellow variety with 3.46% and the lowest value of this parameter is related to the olive oil of the Arbekina variety with 2.60%. The amount of delta-5-onesterol in the samples of Arabkina, Shenge, Zard and Mari olive oils is 6.47, 7.94, 6.43, and 6.82, respectively. The highest amount of delta-5-onesterol in the examined samples is related to Shenke variety olive oil with 7.94% and the lowest value of this parameter is related to Yellow variety olive oil with 6.43%.

The permitted amount of brassicastrol in virgin olive oil is less than or equal to 1% according to the Codex standard and the International Olive Oil Council. The amount of this sterol in all olive oil samples of Arabkina, Shenge, Zard and Mari cultivars was equal to zero.At Jimenez et al.'s research (2022) on Ampelter virgin olive oil, virgin olive oil has beta-sitosterol and Δ5 avenasterol content in the medium range, along with high amounts of campestrol and Δ7stigmasterol.[8].

Ganduz and Kanuskan (2023) found that the highest amount of beta-sitosterol in the green period of Saurani variety (91.66%) and the lowest amount of beta-sitosterol in the spotted period of Halhalı variety (86.16%) were observed in the study of sterols of local cultivars of Turkey. The maximum amountΔ5-Onesterol in the ripening period of Saurani variety 54.6% and the lowest amountΔ5-Avenosterol was observed in the green period of the Halhalı cultivar at 36.2%. The content of beta-sitosterol, stigmasterol and erythrodiol in olive oil changes with ripening[9]. Sterols play an important role in determining the authenticity of olive oil and their amount is influenced by the ripening and processing process[10]. The composition of sterols is used as a "fingerprint" to indicate the authenticity of olive oil.[8]. Ambient temperature affects the concentration of sterols and this affects the quality of olive oil[11].

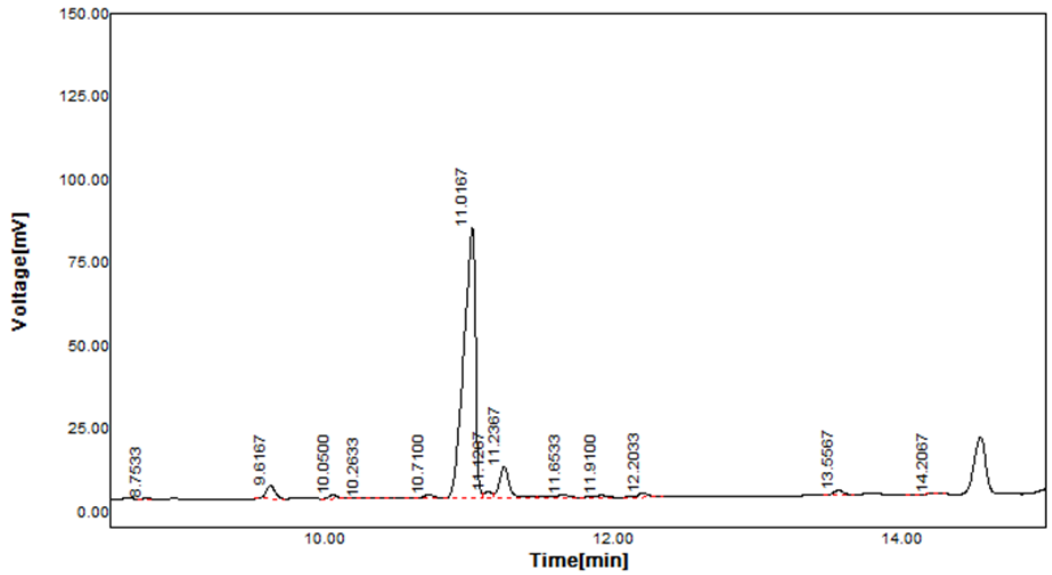

Fig1 Chromatograph of virgin olive oil sample of Shenge variety

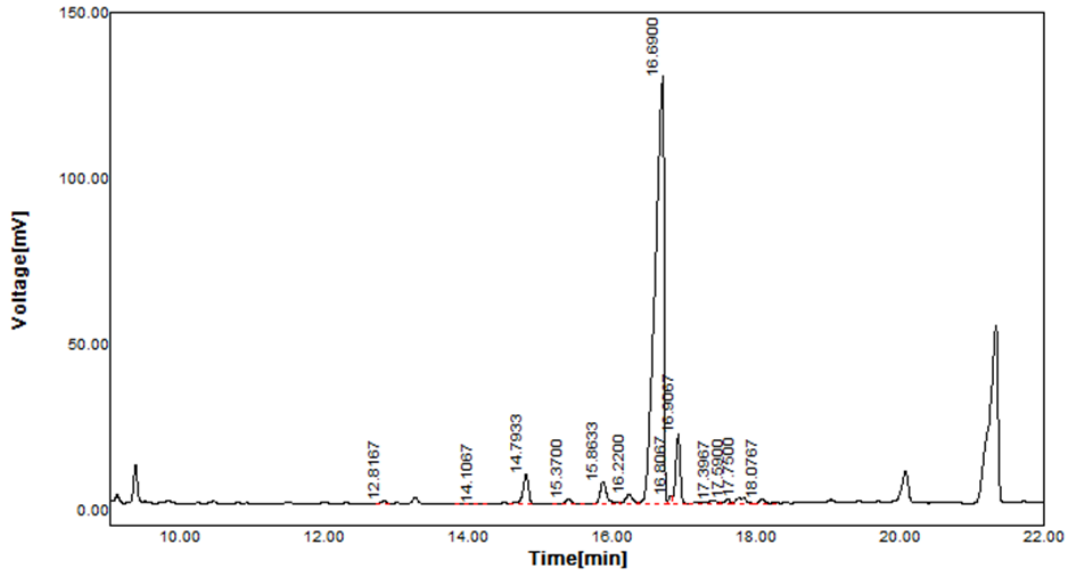

Fig 2 Chromatograph of virgin olive oil sample of Zard variety

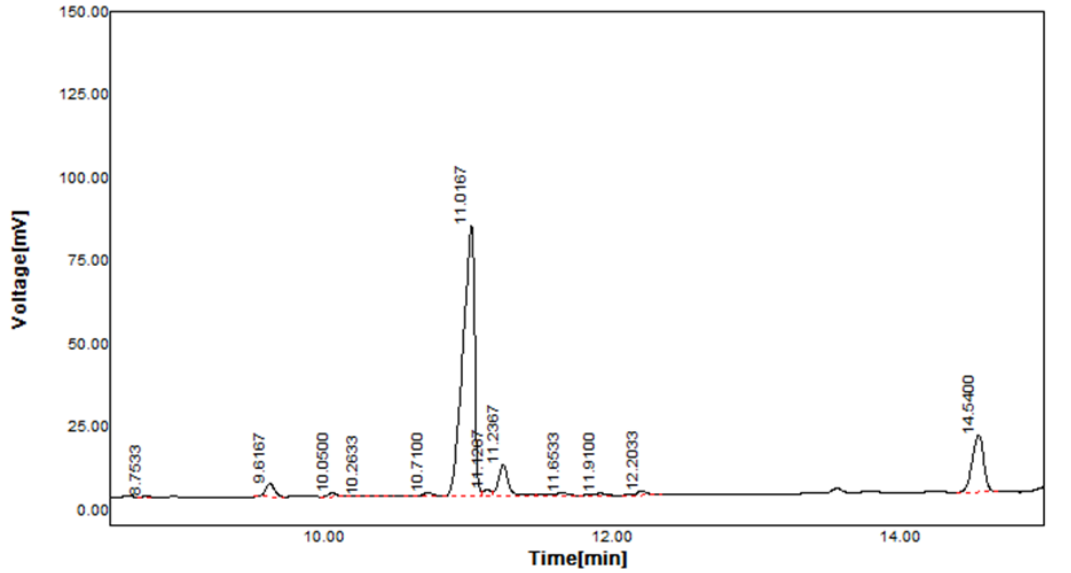

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Fig 3 Chromatograph of virgin olive oil sample of Mari variety

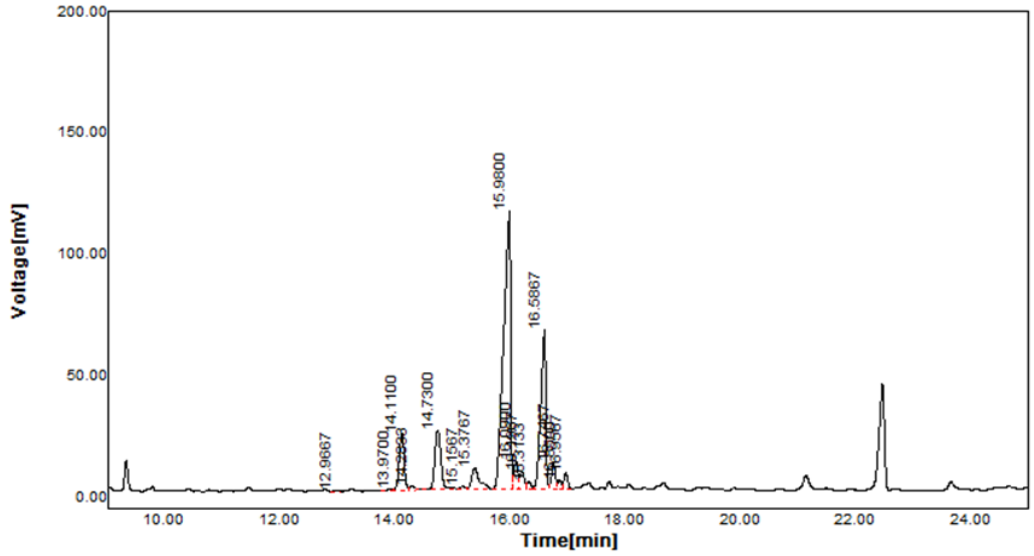

Fig 4 Chromatograph of virgin olive oil sample of Arbequina variety

2-3- The results of peroxide index evaluation in olive oil

The peroxide index of oil samples of Arbekina, Shenge, Zard and Mari cultivars has a statistically significant difference at the level of 5%. The amount of peroxide in olive oil samples of Arbekina, Shenge, Zard and Mari cultivars was 9.54, 9.25, 9.61 and 9.12 milliequivalents of oxygen per kg, respectively. The highest amount of peroxide was observed in olive oil of the yellow variety with 9.61 milliequivalents of oxygen per kg, and the lowest amount was observed in olive oil of the mary variety with 9.12 milliequivalents of oxygen per kilogram. The permissible amount of peroxide index in virgin and extra virgin olive oil is 20 milliequivalents of oxygen per kilogram of oil. All the results obtained were within the range set by the National Standard Organization for extra virgin olive oil. At first, the amount of peroxide in the oil increases until it reaches the maximum level, then this amount decreases because these compounds turn into second products of autoxidation such as ketones, aldehydes and conjugated dienes. These products, which are the second products of auto-oxidation, are responsible for the spicy flavor in the oil. In the research of Gutierrez et al. in 2022, which was conducted on virgin olive oil, the peroxide content of virgin olive oil was within the standard range. Peroxide content is checked for oxidative stability[12-16].

3-3- The results of manual index evaluation in olive oil

manual index The oil samples of Arabkina, Shenge, Zard and Mari cultivars have statistically significant differences at the 5% level. Hand index in all samples were within the allowed range of the governor. The obtained values of iodine index in the oil samples of Arabkina, Shenge, Zard and Mari cultivars are 78/58, 75/54, 75/49 and 78/74, respectively. The highest amount of iodine index in the studied samples is related to Mari olive oil with an iodine index of 79.34 and the lowest value of this parameter is related to yellow olive oil with an iodine index of 75.49. The amount of this parameter was at a similar level between Arbekina, Shenge, Zard and Mari cultivars, and no significant difference was observed between these cultivars in terms of quality. Iodine number indicates the degree of unsaturation of an oil. Unsaturated fats, like other unsaturated compounds, bind with iodine at the place of double bonds and produce an additional compound, so the iodine number can determine the degree of saturation or unsaturation of fats.

4-3- The results of free acidity evaluation in olive oil

There were statistically significant differences in the acidity parameter of the samples. The legal limit of free acidity according to the national standard of Iran for extra virgin olive oil is 0.8%. The free acidity of all the samples of all the oil samples of the studied cultivars were within the standard range. The obtained values of free acidity in olive oil samples of Arabkina, Shenge, Zard and Mari cultivars were 0.27, 0.34, 0.32 and 0.38, respectively. The highest amount of acidity was related to the variety Mari with 0.38% and the lowest value was related to the variety Arbekina with 0.27%. which indicates the higher lipolytic activity in Mari variety. In Arbequina, the lipolytic activity is less. Increasing the percentage of free fatty acids by increasing the heating time causes

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various chemical reactions, especially the hydrolysis of triglycerides. In addition to hydrolysis reactions, free fatty acids are also formed as a final product during the oxidation process and reduce the smoke point of frying oil. On the other hand, the accumulation of food particles in the fryer accelerates the production of free fatty acids and the presence of free fatty acids in the frying oil catalyzes the hydrolysis of triglycerides.[16-19]. Hydrolysis of triglycerides by lipolytic enzymes (lipases) leads to the production of mono and diacylglycerol and free fatty acids. The products obtained from the lipolytic reaction are odorless and tasteless and therefore do not cause defects in sensory characteristics. When the olive is damaged by mechanical action, the lipases that are present in some vacuoles of the mantle cells or the olive core come into contact with the oil. The reaction is intensified with increasing temperature and is a function of the contact time between lipase and oil. Lipase is hydrophilic and is active only in the presence of aqueous phase. When water is separated from the oil by decantation or centrifugation, the lipolysis reaction is slowed down or stopped. The type of olive variety, the conditions of planting and the conditions of olive storage after harvesting are parameters that affect the acidity of olive oil. In the research of Arjamand Far et al., which took place in the Darab area of Shiraz, the studied virgin olive oils had high lipolytic activity and the free acidity of the treatments was higher than the standard limit.[13]. In Gutierrez et al.'s research on virgin olive oil, the amount of free acidity was within the standard limit[12].

4 - Conclusion

According to the parameters tested in the virgin olive oil of Arbekina, Shenge, Zard and Mari cultivars, all the analyzed samples are of suitable quality and all the analyzed items in the 4 investigated treatments are within the standard range and according to the analysis and Observing a slight difference among the investigated parameters, it can be said that the Mari variety has better oxidative properties due to its lower peroxide index. In terms of the iodine index, the treatments were at the optimal level in terms of homogeneity, and in free acidity, the acidity of the four treatments was under ideal conditions. In terms of sterol content, which plays an important role in determining the quality and nutritional properties of olive oil, Arabkina and Shenge olive oil had better quality.

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چکیده

روغن زیتون به عنوان یک روغن خوراکی منحصر به فرد به دلیل داشتن مقادیر زیادی اسیدچرب غیر اشباع تک زنجیره‌ای، طعم دلپذیر، پایداری خوب و اثرات ویژه سلامت بخش مورد توجه قرار گرفته است. در این پژوهش، روغن های زیتون بکر ارقام آربیکینا، ماری، شنگه و زرد در منطقه طارم به لحاظ کیفی مورد بررسی قرار گرفت و پارامترهای استرول ها، اسیدیته، اندیس پراکسید و اندیس یدی ارزیابی گردید. کروماتوگرافی گازی برای تعیین و شناسایی استرول ها استفاده شد. نتایج نشان داد، میزان استرول در روغن های بکر ارقام آربکینا،شنگه، زرد و ماری با یکدیگر در سطح ۵درصد دارای اختلاف معنادار آماری بودند. فراوانترین استرول های موجود در تمام تیمارهای مورد بررسی شامل بتا سیتوسترول، دلتا۵اوناسترول و کمپسترول می باشد. میزان اسیدیته آزاد در نمونه روغن رقم ماری دارای بیشترین مقدار بود و در رقم آربکینا کمترین میزان را نشان داد. بیشترین میزان اندیس پراکسید در روغن رقم زرد و کمترین میزان، در رقم ماری مشاهده گردید. در نهایت میتوان گفت، پارامترهای مورد ارزیابی ارقام آربکینا، شنگه، زرد و ماری در محدوده مجاز استاندارد قرار داشتند و به لحاظ کیفی تفاوت قابل ملاحظه‌ای در بین این ارقام مشاهده نگردید.