

The effect of Grape seeds and Olive Leaves on Shelf life of Sausage

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موبايل:

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Abstract:

This study was designed to evaluate the effect of grape seeds and olive leaves as natural antioxidants on shelf life of sausage during the storage period at refrigeration temperature (4°C). Minced meat was divided into 4 samples, the first sample (control): minced meat without any additives, the second sample: it was homogenized with grape seeds replacement with minced meat (1%), the third sample: it was homogenized with olive leaves replacement with minced meat (1%), and the fourth sample: it was homogenized with mixed of grape seeds and olive leaves replacement with minced meat (0.5%, 0.5%). The chemical composition and sensory evaluation of all samples was determined. The results showed that protein and ash contents decreased in grape seeds from olive leaves. The shelf life of these three test sausages were compared with a control sausage (1% minced meat) in chemical composition (moisture, ash, crude protein, fat, crude fiber, and total carbohydrates). There was a significant increase ($P \leq 0.05$) with increasing level of olive leaves in these parameters; fat and fiber contents. Olive leaves were more effective at all concentrations followed by grape seeds then mixed of grape seeds and olive leaves, indicating their stability and potential for longer shelf life. The sensory evaluation of sausage samples, is found to be sensorially acceptable, in taste – appearance – color – texture – odor – overall acceptability. There was no significant difference ($P \geq 0.05$) in all sensory evaluation when comparing with control.

Key words: Grape seeds, Olive leaves, Shelf life, Sensory evaluation, Chemical composition.

Introduction:

Grape a flowering plant is a member of the family *Vitaceae*. The species of *Vitis vinifera L.* is an endemic Mediterranean plant. A large amount of marc consisting of seeds, peels, remaining portion of pulp, and stalks are produced following pressing grape bunches for different applications. The phenolic constituents of grape pomace including peels and seeds are mostly concentrated in the seeds of both red and white grape marc Seyed et al., (۲۰۲۳). Grape seed extract is an excellent source of various polyphenols that exhibit highly potent antioxidant and disease prevention properties the presence of phenolic compounds in the seed aqueous extract make it a valuable natural antimicrobial component to avoid food spoilage and foodborne infections Hosam et al., (۲۰۲۲).

Olive leaves have long been known for their therapeutic and medicinal properties and they are used in both traditional and modern medicine; they are an important source of phenolic compounds, such as oleuropein, verbascoside, hydroxytyrosol, and tyrosol, which all exhibit important biological activity—for example, antioxidant and antimicrobial properties Graziana et al., (۲۰۲۲). The seed matrix by extraction with appropriate solvent it is also relevant to mention that some natural extracts rich in polyphenols such as those obtained from *Rosmarinus officinalis L.*, grape seed and skin, and olive pulp are generally recognized as safe GRAS can be applied in food as antioxidants. Due to the potential of natural extracts for the development of healthier muscle foods, this review aims to discuss the potential sources of polyphenols among seeds and their application in meat products Paulo et al., (۲۰۲۰).

Meat and related products are excellent sources of proteins, amino acids, fats and minerals such as zinc, iron and phosphorous, vitamins and other valuable nutrients; Therefore, it constitutes an integral part of human nutrition Georgios et al., (۲۰۲۰). Meat preservation technologies have been expanded through the global use of cold chain logistics, new heat treatments ultra-cooling, ultra-fast freezing, immersion vacuum cooling, freezing pressure, dielectric heating, ohmic heating Tim and Nathan, (۲۰۱۹). Oxidation processes are the major cause of deterioration of meat quality and shelf life of meat products, leading to negative changes in nutritive value and in sensory and physicochemical properties of meat Olaf et al., (۲۰۱۹). Traditional extracts from plants, spices and culinary herbs have been added to enhance flavor and increase

the food shelf life. Food additives are natural or industrial chemicals added to food and are widely used in the food industry to increase the shelf life of the product and/or attribute as well as to enhance certain properties in foods including preservation, coloring and sweetening. However, they have negative effects as some of them have been banned due to their toxicity Sanabel and Ali, (٢٠٢١).

The shelf life of fresh meat is integrally related to the product condition, packaging system, and storage temperature. Loss of product's shelf life results from human error, poor management, and technical malfunctions leading to rejection, as the food product no longer meets the standards of the consumer with food safety and quality being the prime importance to present-day consumers, modern meat supply chain management systems focus on having a holistic approach towards providing high-quality products by taking preventive measures to ensure hygiene and safety at every stage of the supply chain. A minor mishap in any stage of the supply chain can significantly impact the product's shelf life Perichitala et al.,(٢٠٢٣).

Materials and Methods

Materials

Source of samples

Grape seeds and olive leaves were obtained from Agricultural Research Center, Giza, Egypt. Casings (small intestine) fat was purchased from butcher's shop in Assiut city, Egypt. Citric acid were purchased from the local market in Assiut city, Egypt. Spices, onions and garlic, salt and black pepper (Alsalam company, Assiut , Egypt.

Methods

Preparation of materials

Grape seeds

Grape seeds were cleaned to remove all impurities, and washed, then dried in a drying oven at temperature ٥٠°C for ٣ days. The seeds were milled into a fine powder using a laboratory mill (LM dp`١٢٠ Perten Instruments, USA) Nahed et al., (٢٠٢٠). The ground powder sieves on a ٣٥٥ mesh sieve. The powder was

packed in polyethylene bags and stored at ambient temperature until use Anju et al., (2018).

Olive Leaves

The fresh olive leaves samples were collected. After collection, fresh olive leaves are washed with tap water to eliminate any traces of dust. Then removing of the bitter taste from olive leaves by was soaking in 3% citric acid solution for 24 h. Then washing three times by tap water and dried in a hot air oven at 60 °C (Vebmlw Medizinische, Gerete, Berlin, Germany). All previous drying operations were carried out until constant weight, then grinded, sifted and stored at 20°C in tight packs Maha et al., (2021).

Table (1): Sausage formula and ingredients

Ingredients	Quantity (%)
Meat	60,0
Ice water	10
Fat	0
Salt	3
Black pepper	0,3
Coriander	0,3
Nutmeg	0,3
Garlic	0,3

Preparation of sausage

Casings (small intestine) were cleaned with water, salt and sodium bicarbonate. Then prepared the meat and spices for stuffing, they stuffed into casing sausage by the filling machine manually, The sausage samples were executed as follow:

Sample (1): control sample 100% minced meat

Sample (2): sausage supplemented with 1% grape seeds powder

Sample (3): sausage supplemented with 1% olive leaves powder

Sample (4): sausage supplemented with 5% grape seeds powder and 5% olive leaves powder.

then packaged them in foam dishes. Also, they wrapping by polyethylene bags. Immediately, part of the samples was carried out to sensory evaluation, chemical and microbial evaluation remaining samples were kept at 4 °C up to two months Maha et al., (2021). Natural casings preserved in dry salt must first be soaked in lukewarm water before use. Flush each casing by putting the end of the casing over the cold water tap and running cold water through the casing. Unused casings can be drained, covered again with fine salt, and frozen. Soak natural casings which come in a brine, in cold water before use. Some artificial casings should be soaked in warm tap water (100 °F) at least 30 minutes, but not over 4 hours before use. Puncture with a knife point before sausage is stuffed unless the casings are pre-stuck. These holes eliminate air and fat pockets in the finished product Jan et al.,(2003).

Determination of moisture content

Moisture content was determined by using the air oven drying method by AOAC Official Method 990.19 AOAC ,(2016). The measurement was done by drying the samples in a Model 600 air oven (Mettler, Germany) at 100 °C for overnight. The dish was then cooled in a desiccator and weighed and weighed and the process was repeated until constant weight reached.

Determination of ash content

Ash content of the samples was determined using the dry ashing method AOAC Official Method 999.11 AOAC ,(2016). Ashing of sausage sample (10 g) was done in a Thermo Scientific

Thermolyne 62700 muffle furnace at 550 °C. Before ashing, the crucible was dried in an oven at 100 °C for 3 hrs.

Determination of protein content

Kjeldahl Method was used to determine protein content based on the standard procedure in AOAC Official Method 973.48 AOAC ,(2016). This method is performed based on an automated Kjeltac instrument (Foss, Germany) to determine protein content in sausage samples.

Determination of fat content

Fat content was determined based on the Soxhlet extraction method by using AOAC Official Method 960.39 AOAC.,(2016). Instead, Fat content was measured by weight loss of the sample or by weight of the fat removed.

Determination of Chemical composition

Gross chemicals composition for beef sausage samples had been done according to William and George,(2005). while carbohydrate was determined by difference as follows: Carbohydrate was calculated by difference. Total carbohydrates % = 100 -(moisture% ash % + fat + % protein + % fiber content) Total calories were calculated using the equation: $E = (4.18 \times \% \text{ protein}) + (9.37 \times \% \text{ fat}) + (4.18 \times \% \text{ carbohydrate})$ Where: E = Energy as calories per 100 grams sample Crisan and Sands, (1978).

Sensory evaluation of sausage.

The samples to be used for sensory evaluation in zero time were randomly selected. 24 Experienced judges from the were asked to evaluate the treatment effects on, cooked. taste, appearance, color, texture , odor ,and overall acceptability of the studded sausage samples. Cross et al., (1978). Sausage samples were separately cooked 15-20 minutes by deep fat frying in vegetable oil then sausages were turned every two minutes to prevent excessive browning. Samples were kept warm in aluminum foil for evaluation test. They were put in coded plates and served warm in special pan to the panelists. From each treatment a sample was randomly placed in a dish divided to under natural light. A six point hedonic scale was used Siham, (2019). The data collected were subjected to statistical analysis by using complete randomized design used to analyze the results obtained from this study and subjected to ANOVA followed by Least significant difference test (LSD) using the SPSS, (2008).

Statistical analysis

Data were presented as the mean of duplicate standard deviation (mean \pm SD). T test was used to establish the significance of differences among mean values at ($p < 0.05$). Analysis of variance (ANOVA) was carried out using Proc Mixed of SAS package

version 9.2 SAS(2008) and means were compared by Duncan test of significant Steel and Trrie,(1981).

Results and Discussion

The gross chemical composition of grape seeds and olive leaves is tabulated in Table (1). The results indicate that a highly significant at ($P < 0.01$) in moisture, crude protein, fat, ash, crude fiber, and total carbohydrates between grape seeds and olive leaves. These results are in agreement with Hanaa, (2010) The average values for the chemical composition of grape seeds in moisture ,fat, . and Ibrahim et al, (2016). for olive leaves in moisture, fiber and ash between grape seeds and olive leaves. The moisture content is low in grape seeds compared to olive leaves. Food products with high moisture content are susceptible to microbial attack and therefore have limited shelf life Muyanja et al., (2012).

Table (2): chemical composition of grape seeds and olive leaves on dry weight basis (g/100g)

Sample s	Moisture %	Protein %	Fat %	Ash %	Fiber %	Carbohydrate %
Grape seeds	9.82± 0.29	11.01 ± 1.37	16.81± 1.49	2.83± 0.19	33.48± 0.50	20.00± 0.89
Olive Leaves	30.82± 1.03	10.9± 0.07	7.9± 0.01	6.9± 0.02	18.93± 0.09	24.00± 0.2

-Carbohydrates were calculated by difference.

Caloric value

Total calories were calculated using the equation: $E = (2.62 \times \% 11.01) + (8.37 \times 16.81) + (4.2 \times \% 20.00) = 278.16$ Where: E = Energy as calories per 100 grams sample in grape seeds . $E = (2.62 \times \% 10.9) + (8.37 \times 7.9) + (4.2 \times \% 24.00) = 197.79$ in olive leaves .

Table (2) reveals that moisture content was found of 9.82, and 30.82% in grape seeds and olive leaves. Hence the low moisture content of seeds indicates their stability against microbial attack and potential longer shelf life Nishant and Neeraj, (2018). The protein content was low in olive leaves, followed by grape seeds. The fat content in grape seeds and olive leaves were 16.81, and 7.9%; respectively. The high-fat content of seeds makes them valuable for their use as a vibrant commercial source of edible oil

Arunima and Vivek, (2021). The ash content of grape seeds and olive leaves varied from 2.83 to 6.9%. Fiber plays several roles including increasing the shelf life of food products Kurek and Wyrwicz, (2015). Ash content of a food product is an index to the nutritive value mineral content, safety, and quality Agoreyo et al., (2011). According to Schneeman, (2002). crude fiber contributes to a healthy digestive and metabolic system in human. Carbohydrate values were 25.55% and 24.55% in grape seeds and olive leaves; respectively. The high carbohydrate content is a good source of energy for the body Sultana et al., (2015).

Table (3): Gross chemical composition of sausage sample (control) during frozen storage at -18°C at zero time and after three months on wet wet basis (g/100g)

Samples %	Control	M ± SD	P.value
Moisture	• time	62,16 ± 0,01	P ≤ 0,05
	After 3 months	61,95 ± 0,01	
Ash	• time	4,98 ± 0,01	P ≤ 0,05
	After 3 months	4,99 ± 0,01	
Protein	• time	14,95 ± 0,01	P ≤ 0,05
	After 3 months	13,99 ± 0,01	
Crude Fat	• time	13,89 ± 0,01	P ≤ 0,05
	After 3 months	12,98 ± 0,01	
Crude Fiber	• time	2,95 ± 0,01	P ≤ 0,05
	After 3 months	4,95 ± 0,01	
Carbohydrates	• time	1,07 ± 0,01	P ≤ 0,05
	After 3 months	1,14 ± 0,01	

Means ± SD. in the same column indicate significant differences at (P ≤ 0,05).

The obtained data in Table (3) indicates that a significant differences at d(P ≤ 0,05) between control in zero time and after 3 months in (moisture,

ash, crude protein, fat, crude fiber, and total carbohydrates). These results are in agreement with Nady et al., (2021). The moisture content is low in after 3 months compared to zero time. High moisture content is susceptible to microbial attack and therefore has limited shelf life Muyanja et al., (2012). Additionally, the ash content had a mean value of $4.98 \pm 0.01\%$ in zero time, while after three months $4.99 \pm 0.01\%$, the protein content with a mean value of $14.90 \pm 0.01\%$ in zero time, while after three months $13.99 \pm 0.01\%$. Also the fat content with a mean value of $13.89 \pm 0.01\%$ in zero time, while after three months $12.98 \pm 0.01\%$, the fiber content had a mean value of $2.90 \pm 0.01\%$, while after three months $4.90 \pm 0.01\%$. Finally, the carbohydrates content with a mean value of $1.07 \pm 0.02\%$ in zero time and $1.14 \pm 0.01\%$ after three months. These results are in agreement with Nady et al., (2021) in moisture, protein, ash.

Table (4): Gross chemical composition of sausage supplemented 10% grape seeds during frozen storage at -18°C at zero time and after three months on wet wet basis (g/100g)

Samples%	10% grape seeds	M ± SD	P.value
Moisture	0 time	62.26 ± 0.01	P ≤ 0.05
	After 3 months	60.01 ± 0.01	
Ash	0 time	4.98 ± 0.01	P ≤ 0.05
	After 3 months	4.99 ± 0.01	
Protein	0 time	14.90 ± 0.01	P ≤ 0.05
	After 3 months	13.99 ± 0.01	
Crude Fat	0 time	13.89 ± 0.01	P ≤ 0.05
	After 3 months	12.98 ± 0.01	
Crude Fiber	0 time	2.90 ± 0.01	P ≤ 0.05
	After 3 months	4.90 ± 0.01	
Carbohydrates	0 time	1.07 ± 0.02	P ≤ 0.05
	After 3 months	1.14 ± 0.01	

Means ± SD. in the same column indicate significant differences at a(P ≤ 0.05).

The chemical composition of beef sausage supplemented 10% grape seeds is showed in Table (4). Moisture, ash, protein, crude fat, crude fiber and carbohydrate were in zero time (62.26%, 4.21%, 12.02%, 16.23%, 4.34%, and .94 %). After three months were (60.01, 3.05, 15.26, 15.06, 6.04 and 0.58 %; respectively). The results agreement with Carmen et al., (2023). Grape seed extract, oleoresin rosemary, and water-soluble oregano extract were used for the oxidative and color stability of raw beef. Grape seed also contains extractable phenolic antioxidants, such as phenolic acid, flavonoids, procyanidins and resveratrol.

shows that the impacts of grape seeds on the moisture levels were significant on the 4th day but not on the 1st or 14th day. obtained similar results by applying grape seed to Turkish sucuk. Furthermore, the impacts of storage period on the moisture levels were determined to be significant. This is mainly due to the delay in the basturma production season and the gradual rise in temperatures. These results are in agreement with those suggested by Al-Mara-zany., (2008) as they reported a loss in the moisture value of basturma during storage.Yaman.,(2023).

Table (°): Gross chemical composition of sausage supplemented 1.0% olive leaves during frozen storage at -18°C at zero time and after three months On wet wet basis (g/100g).

Samples%	1.0% olive leaves	M ± SD	P.value
Moisture	• time	64,33 ± 0,01	P ≤ 0,05
	After 3 months	63,12 ± 0,01	
Ash	• time	0,31 ± 0,01	P ≤ 0,05
	After 3 months	0,46 ± 0,01	
Protein	• time	14,20 ± 0,01	P ≤ 0,05
	After 3 months	10,24 ± 0,01	
Crude Fat	• time	13,31 ± 0,01	P ≤ 0,05
	After 3 months	12,21 ± 0,01	
Crude Fiber	• time	2,32 ± 0,01	P ≤ 0,05
	After 3 months	3,06 ± 0,01	
Carbohydrates	• time	0,03 ± 0,01	P ≤ 0,05
	After 3 months	0,91 ± 0,01	

Means ± SD. in the same column indicate significant differences at a (P ≤ 0,05).

The results mentioned in the Table (°) indicated that beef sausage supplemented 1.0% olive leaves was in zero time (64,33, 0,31, 14,20, 13,31, 2,32, and 0,03) for moisture, ash, protein, crude fat, crude fiber and carbohydrate ; respectively and after three months was (63,12, 0,46, 10,24, 12,21, 3,06, and 0,91%) ; respectively. The results agreement with Kazem et al., (2016) Who reported the addition of natural ingredients such as green tea, stinging nettle and olive leaves extracts to Frankfurter type sausage could provide functional components to improve the healthier properties, sensory attributes and shelf life extension of novel meat products .

Table (٦):Gross chemical composition of sausage supplemented ٥% grape seeds +٥% olive leaves during frozen storage at -١٨°C at zero time and after three months on wet wet basis (g/١٠٠g)

Samples%	٥% grape seeds + ٥% olive leaves	M ± SD	P.value
Moisture	٠ time	٦٤,١٦ ± ٠,٠٠١	P ≤ ٠,٠٥
	After ٣ months	٦٠,٠٦ ± ٠,٠٠١	
Ash	٠ time	٣,٥٧ ± ٠,٠٠١	P ≤ ٠,٠٥
	After ٣ months	٥,٦١ ± ٠,٠٠١	
Protein	٠ time	١٢,٥١ ± ٠,٠٠١	P ≤ ٠,٠٥
	After ٣ months	١٤,٠٦ ± ٠,٠٠١	
Crude Fat	٠ time	١٥,٦٧ ± ٠,٠٠١	P ≤ ٠,٠٥
	After ٣ months	١٦,٢٣ ± ٠,٠٠١	
Crude Fiber	٠ time	٣,٣٥ ± ٠,٠٠١	P ≤ ٠,٠٥
	After ٣ months	٤,١٠ ± ٠,٠٠١	
Carbohyd rates	٠ time	٠,٧٤ ± ٠,٠٠١	P ≤ ٠,٠٥
	After ٣ months	٠,٣٩ ± ٠,٠٠١	

Means ± SD. in the same column indicate significant differences at (P≤٠,٠٥).

The obtained data in Table (٦) showed that moisture, ash, protein, crude fat, crude fiber and carbohydrate for supplemented ٥%grape seeds +٥% olive leaves was in zero time (٦٤,١٦, ٣,٥٧, ١٢,٥١, ١٥,٦٧, ٣,٣٥ and ٠,٧٤) %; respectively and after three months were (٦٠,٠٦, ٥,٦١, ١٤,٠٦, ١٦,٢٣, ٤,١٠ and ٠,٣٩) %; respectively. The addition of plant material in meat technology can inhibit the development of probiotics in the product and change the direction of bio physicochemical changes. In the case of raw-fermented ripening meats-the technology of which uses a hurdle method-some uncontrolled changes in the direction of ripening may threaten product safety Justyna et al., (٢٠٢٠).

Table (V): Sensory evaluation of meat sausage groups in control , 1.0% grape seeds, 1.0% olive leaves and 0.5%+0.5%grape seeds , olive leaves at zero time.

Attribute s	Samples	M ± SD	P.value
Taste (1.0)	Control	9.28 ± 0.28	
	1.0% grape seeds	8.10 ± 0.38	n.s
	1.0% olive leaves	9.33 ± 0.27	n.s
	0.5% grape seeds + 0.5% olive leaves	8.10 ± 0.20	n.s
Appearance(1.0)	Control	9.32 ± 0.37	
	1.0% grape seeds	8.40 ± 0.27	n.s
	1.0% olive leaves	9.42 ± 0.37	n.s
	0.5% grape seeds + 0.5% olive leaves	8.12 ± 0.23	n.s
Color (1.0)	Control	9.48 ± 0.20	
	1.0% grape seeds	8.30 ± 0.22	n.s
	1.0% olive leaves	9.71 ± 0.30	n.s
	0.5% grape seeds + 0.5% olive leaves	8.34 ± 0.23	n.s
Textural (1.0)	Control	9.16 ± 0.20	
	1.0% grape seeds	8.06 ± 0.10	n.s
	1.0% olive leaves	9.22 ± 0.30	n.s
	0.5% grape seeds + 0.5% olive leaves	8.02 ± 0.20	n.s
Odor (1.0)	Control	9.38 ± 0.20	
	1.0% grape seeds	9.36 ± 0.22	n.s
	1.0% olive leaves	9.48 ± 0.36	n.s
	0.5% grape seeds + 0.5% olive leaves	9.06 ± 0.26	n.s
Overall acceptability (0.0)	Control	9.40 ± 0.23	
	1.0% grape seeds	8.30 ± 0.21	n.s
	1.0% olive leaves	9.68 ± 0.31	n.s
	0.5% grape seeds + 0.5% olive leaves	8.30 ± 0.26	n.s

Values followed in the same column are not significantly different at $P \geq 0.05$.

The present study showed Table (V) that the treatments differ significantly ($P < 0.05$) in the sensory parameters measured (taste – appearance – color – texture – odor) except color and all scores obtained were above moderately desirable. There were no significant ($P > 0.05$), in any of the parameters measured. The appearance of the food product in or out of the packaging is the main attribute used to make a rapid decision on the quality of the product or its conformity with consumer expectations. Based on that, appearance must be played detailed attention to when assessing the samples in laboratory environment Meilgaard et al., (2007). The texture can be defined as a manifestation of the mechanical, structural, and surface properties of food products Szczesniak, (2002).

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تأثير بذور العنب وأوراق الزيتون علي مدة صلاحية السجق

مستخلص البحث

تهدف هذه الدراسة إلي تقييم أثر استخدام مسحوق بذور العنب وأوراق الزيتون كمضادات أكسدة طبيعية وتأثيرها علي فترة صلاحية السجق خلال فترة التخزين عند الاعداد وبعد ثلاثة أشهر درجة حرارة التبريد (١٨ درجة مئوية). وتم تقسيم العينات اللحم المفروم الي (٤ عينات، العينة الأولى الكنترول : بدون اي إضافات (الكنترول) العينة الثانية: تم تجانسها مع مسحوق بذور العنب باللحم المفروم (١٠%) العينة الثالثة : ثم تجانسها مع مسحوق أوراق الزيتون باللحم المفروم (١٠%) والمجموعة الرابعة: تم تجانسها مع مسحوق خليط من بذور العنب وأوراق الزيتون باللحم المفروم (٥% - ٥%). تم تقدير التركيب الكيميائي والتقييم الحسي لجميع العينات. أظهرت النتائج انخفاض محتوى البروتين والرماد في بذور العنب عن أوراق الزيتون. وكانت هناك زيادة معنوية عند مستوي $0.05 \geq$ بزيادة نسبة أوراق الزيتون في هذه العينات في الدهون والالياف. كانت اوراق الزيتون اكثر فاعلية في جميع النسب تليها بذور العنب ثم خليط من بذور العنب واوراق الزيتون وهذا يشير الي احتمال زيادة فترة صلاحيتها. ووجد أن التقييم الحسي لعينات السجق مقبول حسيأ في (الطعم - المظهر - اللون - القوام - الرائحة - القبول العام) لا يختلف بشكل كبير عند مستوي $0.05 \leq$ في جميع العينات للتقييم الحسي عند مقارنتها بالاكنترول علي الرغم من أختلاف النسب المضافة ومدة الحفظ .

الكلمات المفتاحية : بذور العنب ، أوراق الزيتون ، مدة الصلاحية ،التقييم الحسي ،التركيب الكيميائي .