



## Scientific Research

## Using of Natural Antioxidant Resveratrol for Enhancement of Shelflife of Mayonnaise

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## ABSTRACT

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The research aimed to evaluate the antioxidant activity of pure resveratrol by testing DPPH radical scavenging activity and the solubility of resveratrol in various solvents, as well as to assess its application in extending the preservation of mayonnaise. Five mayonnaise treatments were prepared, including treatment A as a control sample without antioxidant and treatment B containing the synthetic antioxidant BHT added at a rate of 0.2%. Treatments C, D, and E contained the natural antioxidant resveratrol added at rates of 0.1, 0.2, and 0.3%, respectively. The mayonnaise samples were stored for 4 weeks at a temperature of  $28 \pm 2$  °C. The results showed that the resveratrol compound exhibited the highest free radical inhibition rate (94.25%) at a concentration of 500 mg/ml, compared to the same concentration of vitamin C, which exhibited an inhibition rate of 77.9%. The results indicated a low solubility of resveratrol in water, reaching 3.7 mg/ml. The solubility of resveratrol increased in vegetable oils and emulsions. It was noted that the mean values of peroxide, Thio barbituric acid (TBA), and pH of the oil samples separated from mayonnaise treated with BHT or resveratrol were lower compared to the mean values of the control sample during storage. The addition of resveratrol helped improve the sensory properties, and the treatment with 0.2% resveratrol (D) performed comparably to the sample containing the synthetic antioxidant BHT.

## 1- Introduction

Oxidation is one of the main causes of spoilage of oils, fats, or foods. It is considered one of the most important causes that lead to the appearance of rancidity. It also reduces the shelf life and nutritional value of oil or food products, rendering them potentially unsafe for consumption [1]. Therefore, various industrial antioxidants are added to prevent oxidative rancidity of edible oils and foods that are incorporated in their composition to increase their stability against oxidation [1].

Recently, the world has started to use natural sources as preservatives in the food, medical, and cosmetic fields for many reasons. These natural materials have many benefits, the most important of which is ensuring human health. In addition, it aligns with consumer preference for natural food ingredients [2]. The use of natural food additives that act as antioxidants is due to their ability to work inside the body safely without leaving side effects compared to chemicals. Experiments have shown that some synthetic chemicals have been implicated as carcinogens or cancer promoters. Some of them work to change the taste and affect the nutritional value or may leave toxic effects on the body in the long term [2].

Phenolic compounds are found in many plants [3]. They form an important part of the human diet. These compounds are important from a nutritional and health perspective due to their antioxidant properties. Polyphenols consist of an aromatic ring containing one or more hydroxy groups (-OH). They range from simple molecules to complex, high-molecular-weight polymers. The

antioxidant activity depends on the structure of phenolic compounds, especially the number of hydroxy groups and their location in the aromatic ring [4].

Resveratrol acts as a natural polyphenol in scavenging free radicals by scavenging hydrogen and oxygen radicals. It can eliminate lipid peroxidation caused by hydroxide radicals. It can also prevent DNA damage caused by hydrogen peroxide and hydroxide [5]. Resveratrol has many biological properties. However, the best-described property of resveratrol is its ability to act as a powerful antioxidant [6].

The antioxidant activity of resveratrol depends on the arrangement of functional groups in the structural composition. Therefore, the composition, substitution, and total number of hydroxyl groups greatly affect many mechanisms of antioxidant activity, such as free radical scavenging. Resveratrol is a powerful antioxidant. It is five times more effective than beta-carotene, 20 times more than vitamin C, and 50 times more than vitamin E. It is found in the peel of some fruits, such as grapes, blueberries, and raspberries [7]. Grapes are one of the most abundant sources of phenolic compounds. The total phenolic compounds of grape seeds range from 55 to 964 mg/100 g [8].

Resveratrol contributes to the regeneration and restoration of nerve cells. It also regenerates damaged areas of the brain. Thus, it helps restore mental abilities in neurological patients or the elderly [9]. In addition, resveratrol can also be used to reduce or prevent lipid oxidation in pharmaceutical products. It also delays the formation of toxic oxidation products,

maintains nutritional quality, and extends the shelf life of pharmaceutical preparations [10].

Mayonnaise is an appetizer that is widely spread on dining tables all over the world. The demand for mayonnaise has increased in recent years, coinciding with the increase in fast food. Mayonnaise contains a large amount of fats, proteins, and fat-soluble vitamins. It is an oil-water emulsion. The oil content of mayonnaise reaches 70–80% [11].

Mayonnaise is a consumer product that has a positive impact in many countries. It is one of the most important diet products [12]. The mayonnaise industry varies from one country to another according to the nature of the country and its food culture. In Switzerland, mayonnaise is an emulsion in water containing a high percentage of oil, reaching 75%. Egg yolk is used as an emulsifying agent. In Spain, the percentage of oil in the manufacture of mayonnaise should not exceed 65% as a minimum. The percentage of egg yolk should be more than 5%. The acidity index is 0.2, such as acetic acid, and the pH should be around 4.2 [13].

Recently, consumer concerns about harmful industrial preservatives have increased. This has led to an increasing demand for the use of natural preservatives in food systems. Therefore, the present study aimed to (1) evaluate the efficacy of pure resveratrol in scavenging free radicals, (2) identify its effective functional groups using infrared spectroscopy, and (3) assess its potential application as a natural antioxidant substitute for synthetic antioxidants in mayonnaise production.

## 2. Material and method

The method followed by [14] was adopted for estimating antioxidant activity using the DPPH test. The test was performed as follows:

A stock solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared by dissolving 4.0 mg of the reagent in 100 ml of methanol, resulting in a final concentration of 40 µg/ml. Then, the standard solution (vitamin C) and the resveratrol sample were prepared by dissolving 0.5 g of vitamin C in 100 ml of methanol and distilled water, resulting in a standard solution concentration of 5 µg/ml. Using the dilution law, serial concentrations of vitamin C and the resveratrol sample were prepared at 30, 60, 120, 250, and 500 µg/ml. Each mixture was shaken vigorously and left for 30 minutes at room temperature. Then, the absorbance was measured at 517 nm using a Shimadzu UV-VIS spectrophotometer. The IC<sub>50</sub> value of the sample was calculated as the concentration required to inhibit 50% of DPPH free radicals using the dose-inhibition logarithm curve. A lower absorbance of the reaction mixture indicates higher free radical scavenging activity [15].

The percentage of DPPH scavenging effect was calculated using the following equation:

$$\text{Inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where:

- Inhibition (%): Percentage inhibition rate of DPPH
- A<sub>0</sub>: Absorbance of the free radical in the absence of the sample after 30 minutes
- A<sub>1</sub>: Absorbance of the mixture (radical + sample) after 30 minutes

IC<sub>50</sub>:

The concentration of antioxidant required to inhibit 50% of the free radical [15].

## 2.2 Estimation of Resveratrol Solubility

The solubility of resveratrol was estimated according to [16].

### 2.2.1 Preparation of the Standard Solution

A 1 mg portion of pure resveratrol was weighed and placed in a 10 ml volumetric flask. The flask was filled with different solvents separately (water, sunflower oil, olive oil, Tween 80, and Tween 20) to the mark such that the final concentration was 100 ppm.

### 2.2.2 Measurement

The concentration of resveratrol dissolved in the **above-mentioned** samples was measured using a high-performance liquid chromatography (HPLC) system (SYKAM, Germany). A separation column of 250 mm × 4.6 mm was used. The mobile phase consisted of a mixture of methanol-water-acetic acid (10:90:1 v/v) at a flow rate of 1 ml/min and a wavelength of 306 nm. The final concentration of resveratrol was

calculated according to the following equation:

$$\text{Concentration of the substance} = \frac{\text{Concentration of the standard substance} \times (\text{Area of the sample} / \text{Area of the standard substance})}{1}$$

## 2.3 Diagnosis of the Active Groups Using Fourier Transform Infrared (FTIR) Spectrometry

The functional groups of the pure resveratrol compound were identified using a Shimadzu Fourier transform infrared (FTIR) spectrometer (Japan). The analysis was conducted in the laboratories of Science and Technology / Department of Environment and Water. The compound was prepared and placed on potassium bromide (KBr) slides in the IR device, and the light absorption was measured in the range of 400–4000 cm<sup>-1</sup> [17]. The compounds were identified according to the peaks obtained using the device.

## 2.4 Mayonnaise Manufacturing

### 2.4.1 Mayonnaise Ingredients

The following materials were used in the preparation of mayonnaise: sunflower oil, eggs, water, sugar, white vinegar, mustard, salt, and white pepper. All ingredients were obtained from local markets, in addition to pure resveratrol (commercial) and the synthetic antioxidant BHT, which were purchased from a **supplier** in Baghdad.

### 2.4.2 Mayonnaise Preparation

The method of [18] was followed for manufacturing mayonnaise. Table 1 shows the proportions of the ingredients used in manufacturing mayonnaise. Five mayonnaise treatments were prepared. Treatment A was the control sample, containing only the standard mayonnaise ingredients. Treatment B contained the synthetic antioxidant BHT added at a rate of 0.2%. Treatments C, D, and E contained the natural

antioxidant resveratrol added at rates of 0.1, 0.2, and 0.3%, respectively.

Salt, mustard, and white pepper were mixed with whole eggs and water at medium speed for 5 seconds to form the aqueous phase. Then, the oil representing the fatty phase was added in an uninterrupted flow to prevent the emulsion from breaking. The product was filled into sterile, tightly sealed glass bottles and stored at  $28 \pm 2^\circ\text{C}$  for 1 day, and 1, 2, 3, and 4 weeks to conduct physicochemical tests and sensory evaluation at each time point.

**Table 1.** Proportions of ingredients used in the manufacture of mayonnaise

Components	Percentage (%)
Oil (ml)	70
Eggs (g)	10
Water (ml)	4.5
Sugar (g)	2.5
White vinegar (ml)	5
Mustard (g)	0.3
Salt (g)	1.5
White pepper (g)	0.5

### 2.5 Chemical tests of mayonnaise

#### 2.5.1 Extraction of Oil from Mayonnaise

The method of [19] was adopted to extract oil from mayonnaise. A 20 g portion of mayonnaise was weighed, and 20 ml of chloroform, 40 ml of methanol, and 1 ml of distilled water were added and mixed for 2 minutes. Then, 20 ml of chloroform was added and mixed for 5 minutes. After that, the mixture was filtered twice using glass wool and then filter

paper. Subsequently, the filtrate was placed in a separating funnel to separate the upper layer (chloroform) from the lower layer (aqueous layer). The chloroform solvent was evaporated at  $40^\circ\text{C}$  under vacuum pressure using a rotary evaporator to obtain the oil.

#### 2.5.2 Estimation of Peroxide Value

The peroxide value of the different mayonnaise samples was estimated according to the A.O.A.C. method [20]. A 5 ml aliquot of the extracted oil was dissolved in 30 ml of a solvent (60% glacial acetic acid and 40% chloroform).

Then, 5.0 ml of saturated potassium iodide solution was added and left for 5 minutes with continuous stirring. Then, 30 ml of distilled water and 0.5 ml of 1% starch reagent were added. Then, the sample was titrated with 0.01 N sodium thiosulfate solution with vigorous shaking during the titration process. The peroxide value was calculated on the basis of the number of milliequivalents per 1 kg of oil according to the following equation:

Peroxide value (milliequivalents/kg oil) = (ml sodium thiosulfate × sodium thiosulfate normality × 1000) / (sample weight in grams)

### 2.5.3 Estimation of Thiobarbituric Acid

Thiobarbituric acid value was estimated according to the method of [21]. The absorbance of the resulting color was measured at a wavelength of 530 nm using a spectrophotometer. The TBA value was calculated by multiplying the absorbance value by the factor 5.2. The value was expressed as mg of malondialdehyde (MDA) per kg according to the following equation:

TBA value (mg MDA/kg) = Absorbance × 5.2

### 2.5.4 pH Value

The pH of the different mayonnaise samples was measured according to A.O.A.C. [20] using a pH meter. A 5 ml aliquot of the sample was taken, and 5 ml of hexane solvent was added prior to measurement.

### 2.6 Sensory Evaluation of Mayonnaise

The sensory evaluation of different mayonnaise treatments across storage periods (1 day, 2, and 4 weeks) was conducted by members of the Department of Home Economics, College of Education for Girls, University of Baghdad for the characteristics of color, flavor, smell, texture, and overall acceptability, according to the sensory evaluation form proposed by Reddy et al. [22].

### 2.7 Statistical Analysis

The Statistical Analysis System (SAS) [23] was used to analyze the data to study the effect of different treatments on the studied traits according to a completely randomized design (CRD). The significant differences between the means were compared using the least significant difference (LSD) test at a significance level of  $p \leq 0.05$ .

## 3. Results and Discussion

### 3.1 Estimation of the antioxidant activity of pure resveratrol

The results in Table 2 showed that a concentration of 500 mg/ml of resveratrol exhibited the highest inhibition rate of 94.25% compared to the same concentration of vitamin C, which exhibited an inhibition rate of 77.9%. The results of the statistical analysis in the same table indicated the presence of significant differences at the level ( $p \leq 0.05$ ). The results were consistent with those of [24], who found that orange peels and beta-carotene extracted from orange peels were more

effective in scavenging free radicals at a concentration of 250 mg/ml compared to vitamin C at the same concentration.

When comparing the antioxidant activity of resveratrol, it was observed to be more effective than vitamin C, confirming its status as a strong natural antioxidant. Previous studies have reported that resveratrol is five times more effective than beta-carotene, 20 times more effective than vitamin C, and 50 times more effective than vitamin E [7, 9]. [25] indicated that resveratrol extracted from grape waste provided higher antioxidant activity compared to synthetic resveratrol. The radical scavenging activity of the extracted resveratrol reached 71.14%, while it reached 62.21% for synthetic resveratrol. [26] reported that the lignan isolated from roasted sesame seed oil exhibited higher activity in inhibiting free radicals compared to the synthetic antioxidant BHT. This is due to the presence of

sesamol in sesame oil, which has high antioxidant activity. [27] indicated the ability of the extract of *Viola odorata* (Banafsha) to scavenge free radicals at rates ranging from 70.60% to 87.03% at concentrations of 0.125 and 0.500 mg/ml, respectively, compared to vitamin C, which exhibited rates of 41.33% and 53.00% at the same concentrations. According to [28], the methanolic extract of pumpkin peels exhibited significant free radical scavenging activity, reaching 92.68% at a concentration of 50 mg/ml. This was compared to natural antioxidants, such as vitamin C, which demonstrated a scavenging activity of 97.18%. [29] demonstrated that the phenolic compounds in ethanolic and aqueous extracts of sage leaves exhibited scavenging activities of 15.8% and 14.6%, respectively. The ethanolic extract was more effective in scavenging free radicals than the aqueous extract.

**Table 2.** Shows the estimate of the antioxidant activity of pure resveratrol compared to vitamin C using concentrations of (30, 60, 120, 250, and 500) mg/ml.

AA %	30 mg / ml	60 mg / ml	120 mg / ml	250 mg / ml	500 mg/ml	LSD value
Vit C	18.59	30.25	44.58	58.98	77.9	12.63 *
Natural resveratrol	23.65	51.25	74.89	86.59	94.25	14.08 *
LSD value	3.96 *	7.02 *	7.55 *	9.41 *	7.84 *	--

\*Values represent means of three replicates. **Different superscript letters within the same column indicate significant differences ( $p \leq 0.05$ ).**

The  $IC_{50}$  is defined as the concentration of antioxidant required to inhibit 50% of the free radical DPPH [15]. It is inversely proportional to antioxidant activity; i.e., a lower  $IC_{50}$  value indicates higher antioxidant activity. The

results in Table 3 indicate that the  $IC_{50}$  value for resveratrol was 58.5  $\mu$ g/ml, derived from the dose-response curve across concentrations of 30–500  $\mu$ g/ml. The  $IC_{50}$  value for vitamin C was 215.5  $\mu$ g/ml. This confirms that the

efficiency of the antioxidant activity is inversely proportional to the  $IC_{50}$  value. These findings indicate that resveratrol is a more effective

antioxidant than vitamin C. From the results of the statistical analysis in Table 3, it is clear that there are statistically significant differences at the probability level ( $p \leq 0.05$ ).

**Table 3.** Shows the  $IC_{50}$  value, which represents a 50% inhibition rate for both resveratrol and vitamin C.

IC 50	Con (mg/ml)
VIT C (250 mg/ml)	<b>211.93</b>
Resveratrol (60 mg/ml)	<b>58.53</b>
LSD value	<b>22.746 *</b>

$IC_{50}$  values were calculated from dose-response curves across concentrations of 30–500  $\mu\text{g/ml}$ .

### 3.2 Estimation of resveratrol solubility

Table 4 and Figures 1–5 show the solubility of resveratrol in water, sunflower oil, olive oil, Tween 20, and Tween 80. The results indicated a low solubility of resveratrol in water, reaching 3.7 mg/ml, while the solubility of resveratrol in vegetable oils and emulsions increased significantly. It reached 37.6 and 55.1 mg/ml in sunflower oil and olive oil, respectively. In the emulsions Tween 80 and Tween 20, it reached 92.9 and 84.6 mg/ml, respectively.

The results of the statistical analysis indicated that there were statistically significant differences at the probability level ( $p \leq 0.05$ ) between the different solvents. These results were somewhat consistent with those of [30], who found that the solubility of resveratrol in the oils tested increased to 23.58 mg/ml. Regarding surfactants (emulsifiers), Cremophor EL and Tween 20 showed high solubility, reaching 82.84 and 107.75 mg/ml, respectively, while Tween 80 reached 76.80 mg/ml.

**Table 4.** Shows the solubility of resveratrol in a number of solvents compared to water

Solvent	Solubility (mg/ml)
Water	3.7
Sunflower oil	37.6
olive oil	55.1
Tween 80	92.9
Tween 20	84.6
LSD value	11.68 *

\*Values represent means of three replicates. Different superscript letters indicate significant differences ( $p \leq 0.05$ ).

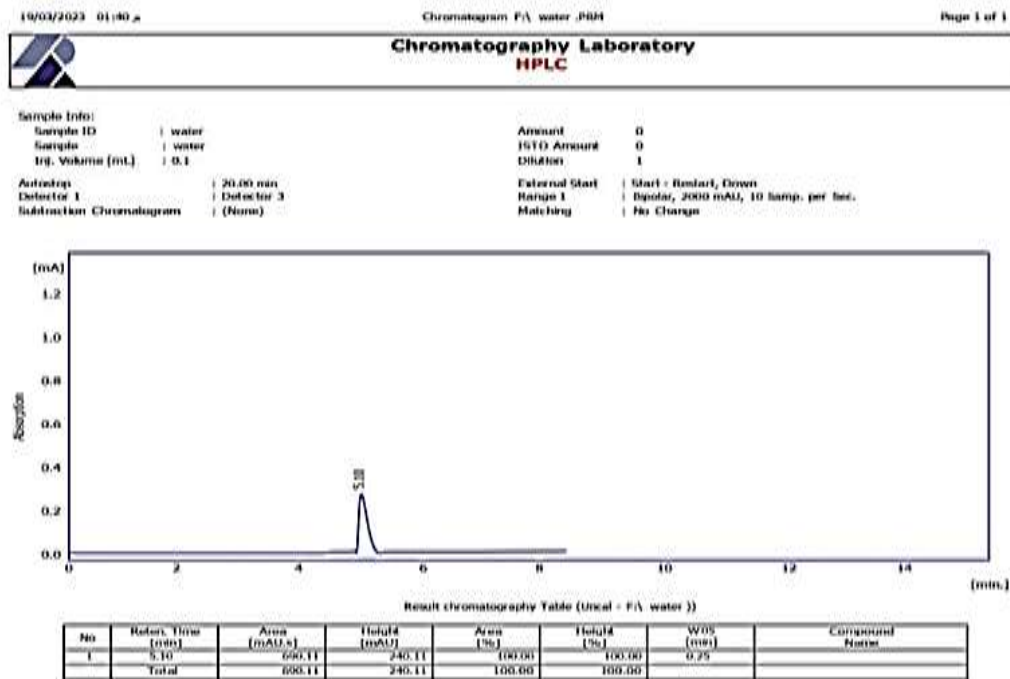


Figure 1. Estimation of resveratrol solubility in water by HPLC

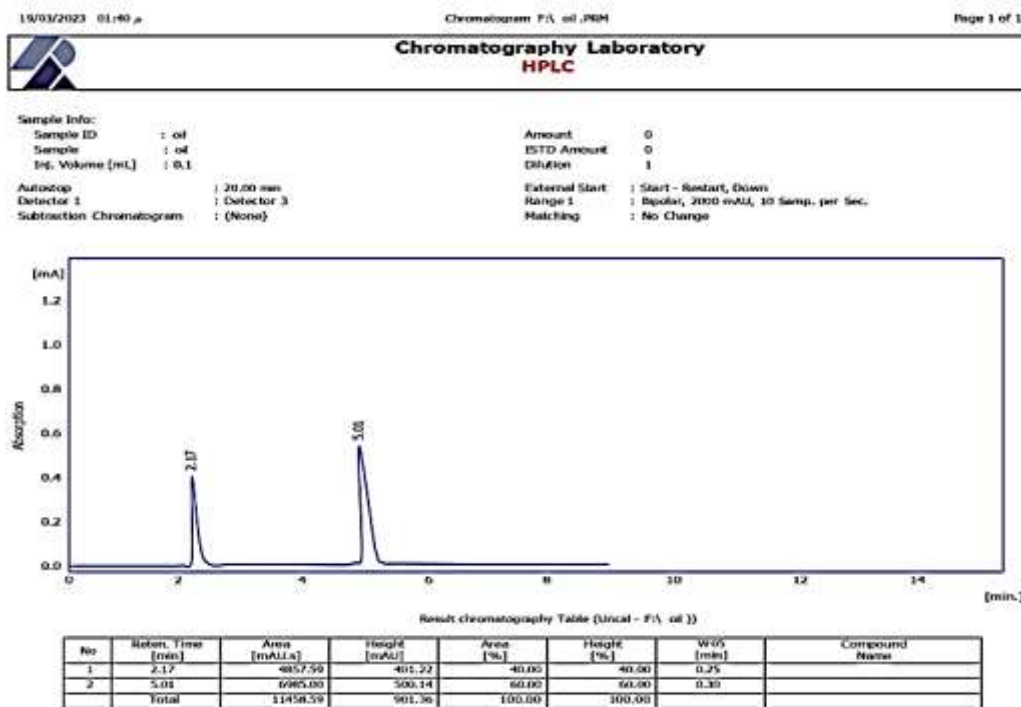


Figure 2. Estimation of resveratrol solubility in sunflower oil by HPLC

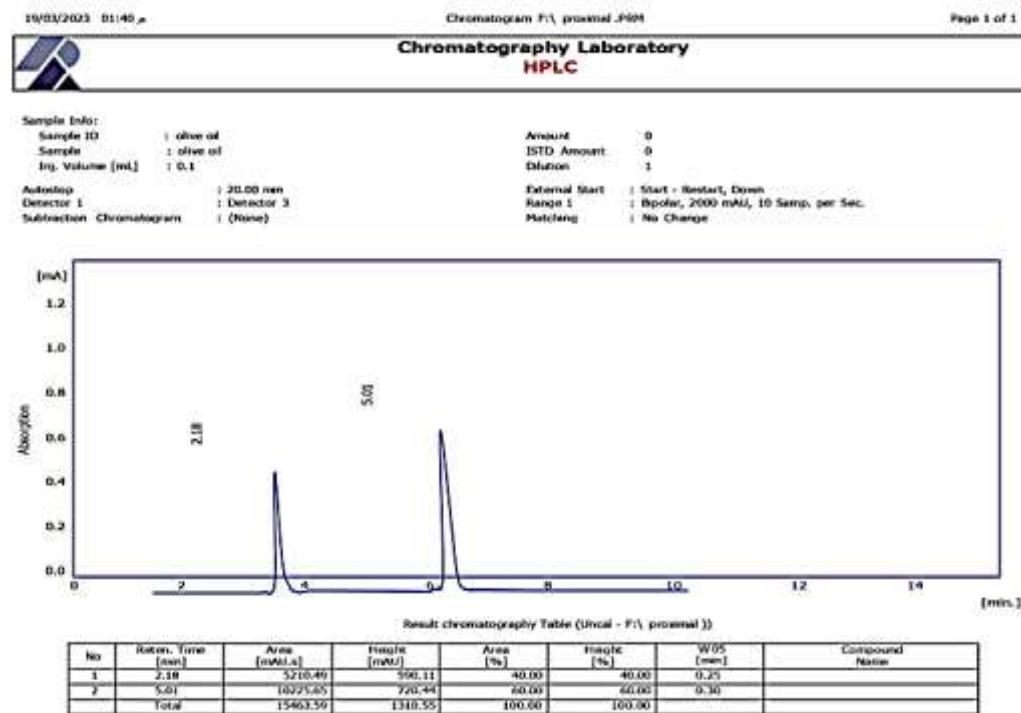


Figure 3. Estimation of resveratrol solubility in olive oil by HPLC

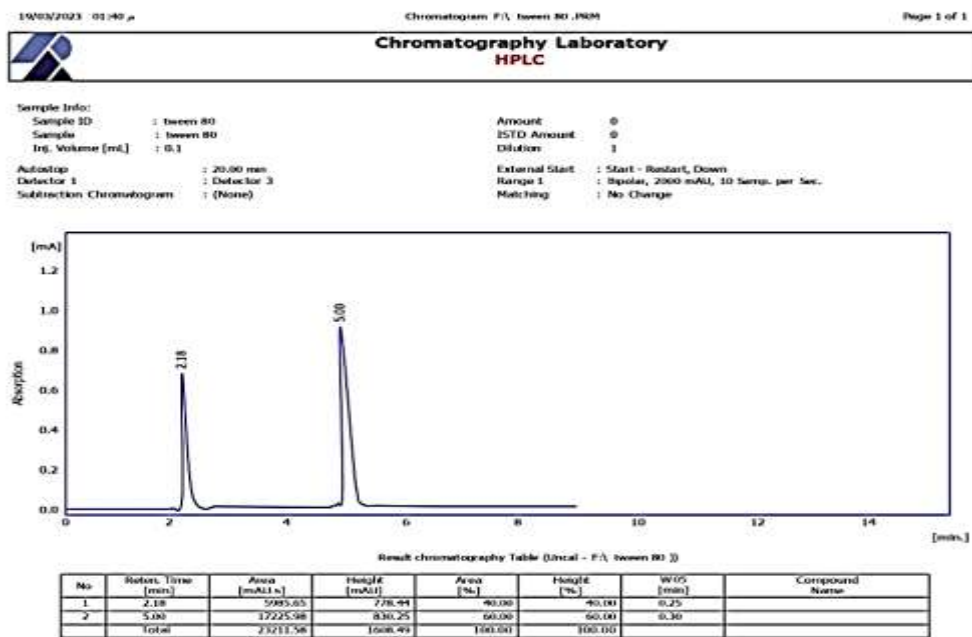


Figure 4. Estimation of the solubility of resveratrol in Tween 80 by HPLC

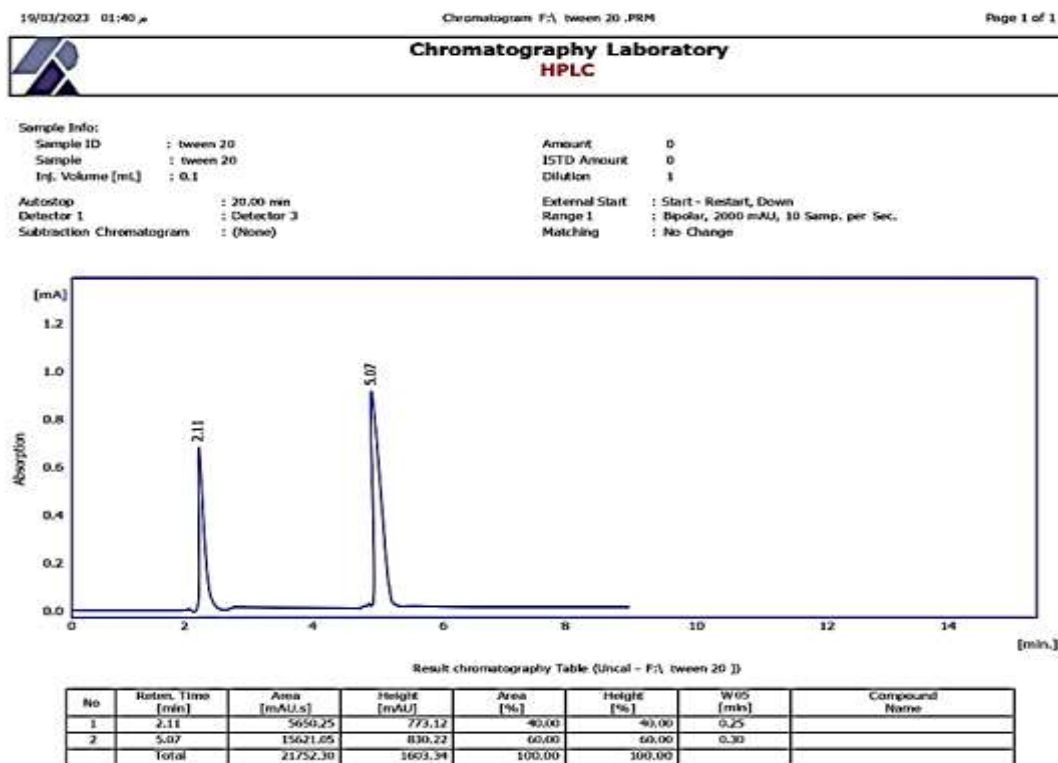


Figure 5. Estimation of the solubility of resveratrol in Tween 20 by HPLC

### 3.3 Infrared spectroscopy (FTIR)

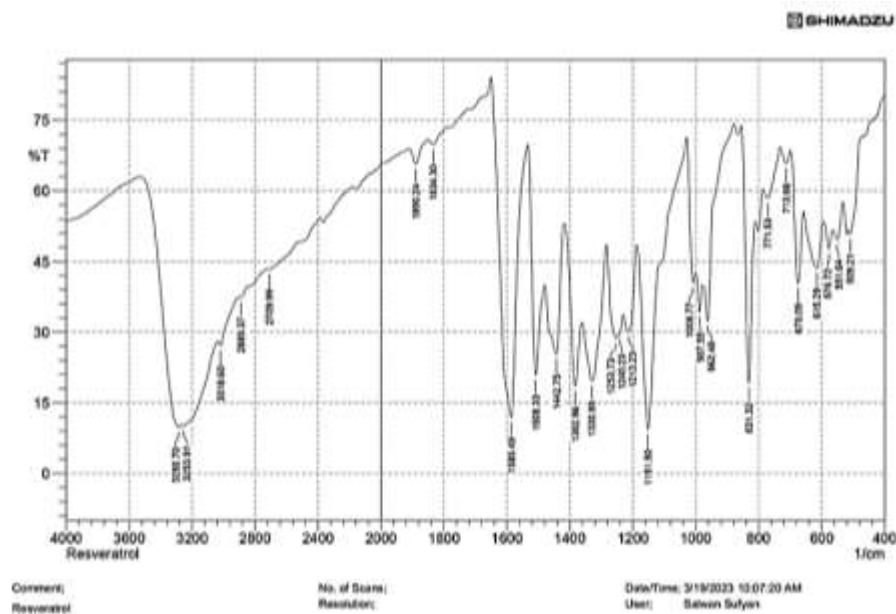
FTIR spectroscopy was used to identify the functional groups of resveratrol. The results in Figure 6 showed the presence of different functional groups. Resveratrol showed prominent absorption bands at the following wavenumbers: 3288.70, 3018.60, 2889.37, 2709.99, 1585.49, 1442.75, 1330.88, 1151.50, 962.48, 831.32, and 675.09  $\text{cm}^{-1}$ . The peaks falling within the range of 4000–3200  $\text{cm}^{-1}$  at 3288.70  $\text{cm}^{-1}$  indicate O–H stretching of phenolic hydroxyl groups [31]. The absorption band at 3018  $\text{cm}^{-1}$  corresponds to C–H stretching of aromatic hydrogen. The peak at 2889  $\text{cm}^{-1}$  is attributed to C–H stretching of aliphatic groups. The

absorption peak at 1585.49  $\text{cm}^{-1}$  is assigned to C=C stretching of the aromatic ring, confirming the presence of aromatic structures. The peak at 1442.75  $\text{cm}^{-1}$  further supports C=C stretching within the aromatic ring.

Absorption peaks of different intensities appear in the region between 1000–1300  $\text{cm}^{-1}$ , indicating C–O stretching, which may correspond to hydroxy or ether linkages. The appearance of absorption peaks between 1000–700  $\text{cm}^{-1}$  is due to C–H bending outside the aromatic ring, confirming the aromatic nature of resveratrol. These results are consistent with [32], who confirmed the presence of an absorption band at 3400–3200

$\text{cm}^{-1}$  attributed to the O–H bond and an absorption band at  $1640 \text{ cm}^{-1}$  indicating the presence of the C=C bond, as well

as a peak in the  $650 \text{ cm}^{-1}$  region attributed to aromatic C–H bending within the structural composition of resveratrol.



**Figure 6.** Far-field infrared spectroscopy (FTIR) for resveratrol

#### 3.4 Estimation of the peroxide value of manufactured mayonnaise treatments

The estimation of the peroxide value of foods and edible oils is one of the tests indicating the rate of the primary stages of oxidative rancidity (formation of peroxides and hydroperoxides). It was noted from Table 5 that the peroxide value of the oil separated from mayonnaise (control treatment A) was 2.55 milliequivalents/kg of oil. This value falls within the conditions set by the Iraqi standard specification [33]. The specification stipulates that the peroxide value should not exceed 10 milliequivalents/kg. The peroxide value of the oil separated from the mayonnaise used in this study was consistent

with those stated by many researchers, including [34, 35]. The mean estimated peroxide values of the oil separated from mayonnaise after manufacturing ranged between 2.1 and 3.62 mEq/kg oil.

Table 5 shows the effect of adding BHT as a synthetic antioxidant as well as pure resveratrol on the development of peroxide values during storage for 4 weeks at a temperature of  $28 \pm 2 \text{ }^\circ\text{C}$  for samples of oil separated from mayonnaise at the concentrations used. The results of the statistical analysis indicated that there were no significant differences in the peroxide values between the different treatments after manufacturing. However, during storage, a gradual increase in the peroxide values of the oil samples

separated from mayonnaise was observed. The peroxide value increased in the control treatment (A) to 8.98 mEq/kg oil after 4 weeks of storage compared to the **mean** peroxide values of the samples treated with BHT (treatment B) or pure resveratrol (treatments C, D, and E). The mean peroxide values reached 7.00, 6.20, 7.11, and 6.14 mEq/kg oil, respectively. It was noted that the mean peroxide values of the oil samples separated from mayonnaise treated with BHT or resveratrol were lower compared to the mean values of the control sample during storage for 4 weeks at  $28 \pm 2$  °C. This is attributed to the fact that the synthetic antioxidant BHT and resveratrol are phenolic compounds that can donate an electron or a hydrogen atom to the free radicals formed during the initiation stage, converting them into inactive groups and reducing the rate of

oxidative rancidity and prolonging the initiation duration. This reduced the rate of formation of peroxides and hydroperoxides [36], resulting in the samples of oil separated from mayonnaise treated with BHT and resveratrol exhibiting lower peroxide values compared to the control treatment.

As noted from the same table and after 4 weeks of storage, the sample treated with BHT (treatment B) had a lower peroxide value compared to the samples treated with resveratrol. Treatment D (0.2% resveratrol) was comparable to the sample treated with the synthetic antioxidant BHT. The results of the statistical analysis indicated the presence of significant differences at the level ( $p \leq 0.05$ ) between the mean peroxide values of the different treatments.

**Table 5.** The effect of adding pure resveratrol on the peroxide number values (mEq/100 g fat) for different mayonnaise treatments during the storage period of 4 weeks at a temperature of  $28 \pm 2$  °C.

Treatments	POV values (meq/100 g fat)				
	Storage period (one week)				
	First Day	1	2	3	4
A control treatment	2.55	3.55	4.87	6.99	8.98
0.2 BHT (B %)	2.54	2.62	3.14	4.11	6.14
C (RESVERATROL 0.1%)	2.56	2.88	3.77	4.90	7.11
D (RESVERATROL 0.2%)	2.55	2.69	3.20	4.18	6.20
E (RESVERATROL 0.3%)	2.54	2.77	3.65	4.77	7.00
LSD	0.307 NS	0.718 *	0.833 *	0.896 *	0.937 *

\*Values represent means of two replicates. Different superscript letters within the same column indicate significant differences ( $p \leq 0.05$ ).

NS: Non-significant.

### 3.5 Estimation of the value of thiobarbutyric acid (TBA) for manufactured mayonnaise treatments

The results of Table 6 indicate that there were no significant differences in the TBA values of the oil samples separated from mayonnaise immediately after manufacturing. However, the TBA values

in all treatments were significantly lower than the control sample after 4 weeks of storage at  $28 \pm 2$  °C. A significant increase in the TBA values of mayonnaise samples was observed, with values reaching 0.085, 0.079, and 0.088 mg malondialdehyde/kg oil for mayonnaise with resveratrol added at 0.3%, 0.2%, and 0.1%, respectively. At the same time, the TBA value reached 0.075 mg malondialdehyde/kg oil for the mayonnaise sample with BHT added at 0.2%. These values were significantly lower than the control sample, which reached 0.118 mg malondialdehyde/kg oil. This represents a significant improvement in the TBA values. All treatments maintained TBA values within the acceptable limits compared to the control treatment, according to the ISO (2006) specification, which states that the TBA value should not exceed 0.09 mg malondialdehyde/kg oil. The results of the statistical analysis also indicated the presence of significant differences between all mayonnaise treatments and the control

treatment after 4 weeks of storage at the probability level ( $p \leq 0.05$ ).

It was also noted that treatment D (0.2% resveratrol) performed comparably to treatment E (0.3% resveratrol) and treatment C (0.1% resveratrol) and was close to treatment B (0.2% BHT). These results were consistent with those of [37] in a study on the manufacture of mayonnaise from bran oil and coconut oil. They found that there was an increase in the TBA value in the control sample compared to the treatments in which the oil was replaced with coconut oil and rice bran oil. The findings also agreed with the results of [38] that the increase in TBA value for mayonnaise samples made by replacing sunflower oil with flaxseed oil fortified with black seed oil was less than that of the control treatment. Additionally, [39] indicated that adding proanthocyanidin at a concentration of 1% to mayonnaise inhibited malondialdehyde formation with an effect similar to that of the synthetic antioxidant BHT.

**Table 6.** Effect of adding pure resveratrol on TBA values (milliequivalent/100 g fat) for different mayonnaise treatments during a storage period of 4 weeks at a temperature of  $28 \pm 2$  °C.

Treatments	POV values (meq/100 g fat)				
	Storage period (one week)				
	First Day	1	2	3	4
A control treatment	0.035	0.048	0.064	0.098	0.118
0.2 BHT (B %)	0.032	0.035	0.038	0.060	0.075
C (RESVERATROL 0.1%)	0.034	0.045	0.048	0.070	0.088
D (RESVERATROL 0.2%)	0.034	0.038	0.041	0.066	0.079
E (RESVERATROL 0.3%)	0.033	0.041	0.045	0.068	0.085
LSD	0.014 NS	0.018 NS	0.019 *	0.0198 *	0.022 *

Values represent means of two replicates. Different superscript letters within the same column indicate significant differences ( $p \leq 0.05$ ).

NS: Non-significant.

### 3.6 Measurement of pH

Table 7 shows the pH of different mayonnaise samples. The pH value of mayonnaise for the control treatment (A) after manufacturing was 3.83. For the other treatments containing the synthetic antioxidant BHT at 0.2% and pure resveratrol at 0.3%, 0.2%, and 0.1%, the pH values were 3.80, 3.84, 3.81, and 3.82, respectively. The results of the statistical analysis indicated that there were no significant differences at  $p \leq 0.05$  between the treatments in the pH values immediately after manufacturing.

pH is an important factor in mayonnaise preparation. By observing the pH values of the treatments, they are close to the control treatment. These results are consistent with [39], who found no significant difference in the pH

values of samples prepared by adding different percentages of proanthocyanidin immediately after manufacturing. The pH reached 4.35. The findings were also similar to those of [40], who indicated that the pH of commercial mayonnaise samples ranged between 3.64 and 3.14. During storage, a decrease in the pH values was observed for all mayonnaise treatments. After 4 weeks, the control treatment was 3.13, while the treatments containing the synthetic antioxidant BHT at 0.2% and pure resveratrol at 0.3%, 0.2%, and 0.1% were 3.33, 3.36, 3.31, and 3.37, respectively. It is clear from the results that the pH values of the mayonnaise treatments to which resveratrol was added were higher than the control treatment despite the absence of significant differences.

**Table 7.** The effect of adding pure resveratrol on the pH values of different mayonnaise treatments during the storage period of 4 weeks at a temperature of  $28 \pm 2^\circ\text{C}$ .

Treatments	PH Values				
	First Day	Storage period (one week)			
		1	2	3	4
A control treatment	3.83	3.67	3.46	3.34	3.13
0.2 BHT (B %)	3.80	3.75	3.52	3.45	3.37
C (RESVERATROL 0.1%)	3.84	3.69	3.46	3.37	3.31
D (RESVERATROL 0.2%)	3.81	3.73	3.53	3.48	3.36
E (RESVERATROL 0.3%)	3.82	3.70	3.57	3.51	3.33
LSD	0.307 NS	0.298 NS	0.329 NS	0.271 NS	0.288 NS

\*Values represent means of two replicates. Different superscript letters within the same column indicate significant differences ( $p \leq 0.05$ ).

NS: Non-significant.

### 3.7 Sensory evaluation

The sensory evaluation process was conducted for the mayonnaise treatments manufactured with the addition of the natural antioxidant resveratrol to

determine the extent of the evaluators' acceptance of these treatments. The sensory evaluation form included five basic characteristics: color, smell, flavor, texture, and overall acceptability. The

results in Table 8 indicated that all evaluated treatments obtained close total scores regarding the different sensory characteristics immediately after manufacturing. No significant differences appeared between them at the probability level ( $p \leq 0.05$ ). However, after 4 weeks of storage, the results indicated the emergence of significant differences at the probability level ( $p \leq 0.05$ ) between the control treatment A and the other treatments containing the synthetic antioxidant BHT and the natural antioxidant resveratrol, while there were no significant differences between the treatments containing resveratrol. Based on the results of the sensory evaluation, treatment D (0.2% resveratrol) performed

better than treatments C and E (0.1% and 0.3% resveratrol) in all the studied sensory properties and was comparable to treatment B (0.2% BHT).

The results of the current study agreed with those of [41] regarding the composition of fatty acids and oxidative stability of peanut oil and sesame oil in the manufacture of mayonnaise. The sensory properties of mayonnaise include overall appearance, smell, color, and taste. The sensory evaluation results showed that all samples and all sensory properties were accepted positively over a storage period of 6 weeks, consistent with the study of [39].

**Table 8.** The effect of adding pure resveratrol on the sensory properties of different mayonnaise treatments during a storage period of 4 weeks at a temperature of  $28 \pm 2$  °C.

Treatments	Storage period (one week)	Sensory traits				
		Colour	Smell	Flavor	Texture	General Acceptance
A control treatment	First day	8.5	8.3	8.2	8.6	8.5
	2 weeks	7.8	6.3	6.2	6.6	5.7
	4 weeks	7.6	5.3	5.6	5.1	4.5
B BHT (%0.2)	First day	8.4	8.3	8.4	8.4	8.4
	2 weeks	8.2	8.0	8.3	8.2	8.1
	4 weeks	7.7	7.6	7.9	7.9	7.4
C (Resveratrol 0.1%)	First day	8.4	8.2	8.2	8.5	8.5
	2 weeks	7.9	7.1	6.8	7.6	7.2
	4 weeks	7.2	6.7	5.8	6.4	5.7
D (Resveratrol 0.2%)	First day	8.5	8.4	8.1	8.5	8.4
	2 weeks	8.2	8.3	8.1	8.2	8.2
	4 weeks	7.6	7.4	7.6	7.6	7.2
E (Resveratrol 0.3%)	First day	8.5	8.2	8.3	8.4	8.3
	2 weeks	8.0	7.8	7.8	7.7	7.2

	4 weeks	7.3	7.2	7.1	6.8	6.7
LSD	---	1.08 *	1.74 *	1.597 *	1.063 *	2.058 *

\*Values represent means of evaluator scores. Different superscript letters within the same column and storage period indicate significant differences ( $p \leq 0.05$ ).

#### 4-Conclusions

This study provides a robust, multi-faceted evaluation of pure resveratrol as a potent natural antioxidant and its practical efficacy in preserving a complex food matrix, specifically mayonnaise. The key findings conclusively demonstrate that resveratrol exhibits significantly superior *in vitro* antioxidant activity compared to the benchmark ascorbic acid (vitamin C), evidenced by a markedly higher DPPH inhibition rate (94.25% vs. 77.9% at 500 mg/ml) and a substantially lower  $IC_{50}$  value (58.5  $\mu$ g/ml vs. 215.5  $\mu$ g/ml). This intrinsic potency is further corroborated by FTIR spectroscopic analysis, which confirmed the characteristic functional groups, including phenolic hydroxyls, responsible for its radical-scavenging capacity. A critical practical hurdle for resveratrol application—its limited aqueous solubility—was investigated. The data confirm its poor solubility in water (3.7 mg/ml) but significantly enhanced solubility in lipid-based and emulsified systems, particularly in Tween 80 (92.9 mg/ml) and olive oil (55.1 mg/ml). This solubility profile directly informs its successful deployment in an oil-in-water emulsion like mayonnaise. The core application research reveals that incorporating resveratrol into mayonnaise effectively retards oxidative rancidity during storage at  $28 \pm 2^{\circ}\text{C}$ . All tested concentrations (0.1%, 0.2%, and 0.3%) significantly suppressed the formation of primary (peroxide value) and secondary

(thiobarbituric acid reactive substances) oxidation products compared to the control, performing comparably to the synthetic antioxidant BHT. Notably, the 0.2% resveratrol treatment emerged as the most efficacious, showing no significant difference from the 0.2% BHT treatment in final POV (6.20 vs. 6.14 mEq/kg) and TBA (0.079 vs. 0.075 mg MDA/kg) values after four weeks, while also maintaining superior sensory attributes (color, smell, flavor, texture) closest to the BHT standard. In conclusion, this work substantiates that natural resveratrol is not only a powerful antioxidant *in vitro* but also a highly effective preservative in a real food system. The 0.2% concentration is identified as the optimal level, offering oxidative stability and sensory protection equivalent to a common synthetic antioxidant. These findings strongly advocate for the potential of resveratrol as a natural, functional alternative to synthetic antioxidants in lipid-rich foods, contributing to cleaner labels and enhanced product quality without compromising shelf life or consumer acceptance.

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