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Antioxidant Activity of Rosemary Extract and its Synergistic Activity with Phospholipids in Oil and Fat

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ABSTRACT

The aim of this study was to evaluate the effectiveness of rosemary extract and the synergistic effect of phospholipids. In the first part of this study, rosemary extract was extracted with an alcoholic solvent. And its antioxidant activity was evaluated by rancimet method and compared with phospholipids. For this purpose, rosemary extract in three concentrations (0.02%, 0.05% and 0.1%) and phospholipids in two concentrations (0.1% and 0.3%) and a combination of these two substances to tallow to investigate the characteristics Synergistic was added and was tested for 15 days. The results of this study showed that the use of a combination of rosemary extract with phospholipids to improve the oxidative stability of Tallow will lead to the consumption of products with desirable properties and acceptable to consumers.

Keywords: Rosemary, Antioxidant, Tallow, Synergistic

Introduction

Fats and oils are among the most important nutrients that play a special role in human health. This group of foods spoils at high temperatures, during processing due to various reactions such as oxidation, which leads to loss of nutritional value as well as the production of products such as free radicals that endanger human health. Adding antioxidants to food is one of the most effective ways to reduce food spoilage this method is widely used to increase the shelf life of foods and improve the stability of fats and foods containing fat, thereby preventing the loss of sensory properties and nutritional value [1]. Consumers are increasingly aware of the use of synthetic antioxidants, as they find these synthetic compounds carcinogenic despite their high efficiency, low cost, and high stability [2]. Therefore, the strongest synthetic antioxidant (TBHQ) is not allowed for use in Japan, Canada and Europe. Similarly, BHA has been removed from the list of safe compounds (GRAS) in general [3]. Therefore, there is a way to use plant-derived natural antioxidants to replace these synthetic antioxidants.

Rosemary extract (Rosmarinus officinalis L) with its strong antioxidant activity was often the first choice for processed foods and is widely used in the lipid-containing food industry [4].

Materials and Methods

When we study the effect of different antioxidant products to change the stable oxidation of Tallow, we need to perform the melting process. The purpose of finding the effect of antioxidant products is to change the stable oxidation, which is treated by adding antioxidants to Tallow, by 7 possible antioxidant sample. In the first sample, control through the content is free of oxidants, in the second sample, the content composition of 0.02% rosemary extract and in the third sample, 0.05% rosemary extract, in the fourth sample, 0.1% rosemary extract, in the fifth sample, 0.1% Phospholipid is used in the sixth sample. 0.3% phospholipid with 100 g of Tallow is used. The antioxidant is soaked with Tallow in a 30 cm container and stirred twice a day at 30 °C. 6 will be measured and in the seventh sample, the concentration of the highest resistance time related to the combination of tallow with rosemary extract with the concentration of the highest resistance time of the combination of phospholipids with tallow is determined and finally the induction period is determined by rancimat method at 150 °C. All the above samples will be tested and evaluated 3 times and the result will be reported on average with standard deviation. Finally, it will be tested for peroxide number, thiobarbiotic acid number,

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oxidation resistance time for 15 days.

Table 1
Specifications of samples

Row	Sample
1	Control sample
2	Tallow and 0.02% rosemary extract
3	Tallow and 0.05% rosemary extract
4	Tallow and 0.1% rosemary extract
5	Tallow and 0.1% Phospholipids
6	Tallow and 0.3% Phospholipids
7	Phospholipids + rosemary extract+ Tallow

Production of rosemary extract

To extract rosemary extract by alcohol, 10 g of sample powder was extracted in three consecutive steps with a total of 200 ml (70, 70 and 60 ml, respectively) of ethanol. In the first two stages, which lasted a total of two hours, a shaker was used, and in the third stage, the remaining pulp from the previous two stages was exposed to ethanol for 22 hours at room temperature without the use of a shaker. The extract obtained from each step was added to the extract of the next steps after refining. For bleaching, activated carbon (ratio of extracted material to activated carbon: five to three) was added to the whole extract and then filtered using Buchner funnel and No. 1 filter paper. Most of the ethanol was removed using a Rotary Evaporator. The concentrated extract was spread on the surface of glass plates in a thin sheet and then transferred to a vacuum oven. The extract was calculated to dry constant weight and then the extraction efficiency was calculated. (Amount of rosemary plant / amount of extract obtained × 100 = extraction efficiency). The resulting powder was stored in the refrigerator until further testing [5].

Preparation of Tallow

Beef tallow was prepared from fresh carcasses in Sari city and after transferring to the laboratory, washing with water and removing meat residues, it was completely crushed by a blender and kept in the refrigerator until extraction [6]. After initial preparation of the sample (cleaning, drying and crushing), fat extraction was performed by a rotary evaporator at 80 °C at 60 rpm for 2.5 hours under vacuum. Fat extraction with the above conditions dilutes the extracted fat and optimizes the operating conditions of the extracted fat [7].

Physical and chemical tests

Determination of fatty acid composition

To determine the fatty acid composition, the sample is prepared as a methyl ester derivative by Christie method. In this way, 50 mg of fat is methylated after dissolving in toluene by 0.5 N sodium Methoxide. Excess sodium in the medium was neutralized by the addition of acetic acid and the fatty acid methyl ester was extracted with hexane. Then, to study the profile of fatty acids, a gas chromatograph model Shimadzu 14 A equipped with a flame detector and a diethylene glycol succinate column according to AOCS standard with the number Ce le-91 is used. The injection site sample temperature is 250 °C, the column temperature is 180 °C, the detector temperature is 220 °C, the carrier gas (nitrogen) flow rate is 50 ml / min, the device sensitivity is 6 and the sample injection rate is 5 µl. From the comparison of the peaks drawn by the device with standard peaks, based on the relative retention time of the peaks, the type of fatty acids will be identified and the amount of fatty acids will be determined by calculating the area below the resulting peaks curve [8].

Calculate Peroxide value

Weigh 5 g of the sample into an Erlenmeyer flask and then add 30 ml of acetic acid-chloroform solution (2: 3 ratios) and stir well to dissolve the sample. In the next step, 0.5 ml of saturated potassium iodide was added to it and left in the dark for 1 minute. Then 30 ml of distilled water was added to the above solution and finally 0.5 ml of starch adhesive reagent was added to it. Titration of the sample with 0.01 N sodium thiosulfate was continued until the blue color of the solution disappeared and it became colorless. Along with the titration of the samples, the titration of the control sample was also performed and the number of peroxide in terms of milliequivalents of peroxide per kg of sample was calculated with the following equation [9].

$$\text{Peroxide value} = \frac{V_2 - V_1 \times N \times 1000}{m}$$

In this equation," V1" and "V2" are the titration number of the sample and the control, respectively, "N" is the normality of sodium thiosulfate and m is the weight of the sample in terms of grams [10].

Resistance time

Fat oxidation resistance time is the time between the moment the sample reaches the desired temperature

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and the moment when the production of by-products resulting from fat oxidation increases rapidly and is reported in hours. Although the determination of oxidation resistance time is usually done at 110 °C, it is also possible to test at higher and lower temperatures of 150-100 °C. It should be noted that with each increase of 10 degrees Celsius in temperature, this time is halved. Oxidation resistance time was measured according to the Iranian standard method No. 3734 using a Metrohm 743 Rancimat at a temperature of 120 °C with an air flow of 20 liters per hour [6].

Measurement of thiobarbituric acid (TBA) value

Thiobarbituric acid number was measured by colorimetry to measure oxidation by-products by AOCS (2007) method. 200 mg of the sample was transferred to a 25 ml balloon and then volumized with 1-butanol. Then 5 ml of this mixture was mixed with 10 ml of TBA reagent (0.2%) in a 15 ml Falcon and placed in a water bath at 95 °C for 2 hours, then under cold water at temperature 25 °C was delivered. The absorbance of the samples was read at 532 nm against distilled water. The number of thiobarbituric acid in milligrams of malondialdehyde per kilogram of oil was calculated according to the following equation.

$TBA\ Value = 50 \times (A - B)/ m$

In this equation, "A" is the sample absorption, "B" is the control absorption and "m" is the fat weight (mg).

Results

Fatty acid composition

The results of the profile analysis of tallow fatty acids are presented in Table 2. Findings of this study showed that the highest levels of saturated fatty acids (SFA) related to Palmitic acid (C16: 0) 17.24%, the highest levels of monounsaturated fatty acids (MUFA) related to Oleic acid (C18: 1) 46.55% and the highest levels of polyunsaturated fatty acids (PUFA) were related to linoleic acid (C18: 2) 4.99%.

Table 2
Fatty acid composition

Fatty acid formula	Tallow
C14:0	3.65
C15:0	1.56
C16:0	17.24
C17:0	1.97
C18:0	14.79
C16:1	3.65
C17:1	2.79
C18:1	46.55
C20:1	0.88
C18:2	4.99
C18:3	0.78

Peroxide value

The results for peroxide number changes are presented in Table 3. According to the results, with increasing time, the values of peroxide number increased in all samples and these changes were more in the control sample ($P < 0.05$). According to the results of statistical analysis in most days, the highest amounts of peroxide in the control sample, and with increasing concentrations of extracts and phospholipids, better results were observed. Also, at the end of the storage period, the lowest values of peroxide number were observed in Tallow + phospholipid treatment of 0.3% ($P < 0.05$).

Table 3
Peroxide values in different sample during storage in terms of milliequivalents / kg of fat

Sample	Storage time (days)					
	0	3	5	7	10	15
Control sample	1.25±0.06 ^{Af}	3.45±0.10 ^{Ad}	3.06±0.07 ^{Ae}	3.83±0.07 ^{Ac}	5.02±0.06 ^{Ab}	5. 81±0.07 ^{Aa}
Tallow and 0.02% rosemary extract	1.23±0.08 ^{Af}	3.05±0.07 ^{Bd}	2.84±0.06 ^{Be}	3.22±0.03 ^{Bc}	4.03±0.02 ^{Bb}	4.90±0.08 ^{Ba}
Tallow and 0.05% rosemary extract	1.27±0.07 ^{Af}	2.83±0.05 ^{Cd}	2.52±0.03 ^{Ce}	3.43±0.03 ^{Cc}	3.77±0.09 ^{Cb}	4.55±0.04 ^{Ca}
Tallow and 0.1% rosemary extract	1.23±0.07 ^{Ad}	2.50±0.05 ^{Dc}	2.45±0.08 ^{Cc}	3.18±0.02 ^{CDb}	3.57±0.02 ^{Db}	4.08±0.03 ^{Da}
Tallow and 0.1% Phospholipids	1.20±0.05 ^{Af}	2.38±0.03 ^{Ee}	2.47±0.04 ^{Cd}	3.12±0.03 ^{Dc}	3.36±0.03 ^{Eb}	3.84±0.06 ^{Ea}
Tallow and 0.3% Phospholipids	1.25±0.04 ^{Ae}	2.07±0.03 ^{Fd}	2.10±0.01 ^{Dd}	2.58±0.01 ^{Ec}	3.04±0.08 ^{Fb}	3.60±0.05 ^{Fa}
0.3%Phospholipids +0.05% rosemary extract+ Tallow	1.25±0.05 ^{Af}	1.8±0.03 ^{Ge}	1.90±0.01 ^{Gd}	2.31±0.03 ^{Gc}	2.42±0.06 ^{Gb}	2.70±0.05 ^{Ga}

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Resistance time

The results related to oxidation resistance time and induction time in different Tallow treatments are presented in Table 4. According to the results, by adding the extract and phospholipids, it significantly increased the oxidation resistance time and induction time ($P < 0.05$). And with increasing concentration, better results were observed ($P < 0.05$). In general, phospholipids were more effective than rosemary extract ($P < 0.05$). Also, the percentage of synergism was equal to -1.00.

Table 4
Oxidation resistance time and induction period

Sample	Oxidation resistance time	Induction time
Control sample	5.53±0.08 ^{f}	2.29±0.08 ^{f}
Tallow and 0.02% rosemary extract	6.27±0.21 ^{e}	3.05±0.06 ^{e}
Tallow and 0.05% rosemary extract	6.51±0.09 ^{d}	4.40±0.25 ^{d}
Tallow and 0.1% rosemary extract	6.64±0.08 ^{c}	5.38±0.09 ^{c}
Tallow and 0.1% Phospholipids	5.83±0.09 ^{c}	5.56±0.03 ^{c}
Tallow and 0.3% Phospholipids	5.94±0.05 ^{b}	6.21±0.10 ^{b}
0.3%Phospholipids +0.05% rosemary extract+ Tallow	7.34±0.09 ^{a}	7.12±0.03 ^{a}

Thiobarbituric acid (TBA) value

The results of thiobarbiotic acid number changes are presented in Table 5. According to the results, with increasing time, the values of thiobarbiotic acid increased in all treatments and these changes were more in the control treatment ($P < 0.05$). According to the results of statistical analysis in most days, the highest levels of thiobarbiotic acid in the control treatment, and with increasing concentrations of extracts and phospholipids, better results were observed. Also, at the end of the storage period, the lowest values of thiobarbiotic acid number were observed in tallow + phospholipid sample of 0.3% ($P < 0.05$).

Table 5
Thiobarbiotic acid levels in different treatments during storage in terms of Malondialdehyde / kg fat

Sample	Storage time (days)					
	0	3	5	7	10	15
Control sample	1.5±0.05 ^{Af}	2.04±0.05 ^{Ae}	2.98±0.07 ^{Ad}	3.99±0.05 ^{Ac}	4.46±0.10 ^{Ab}	5. 24±0.09 ^{Aa}
Tallow and 0.02% rosemary extract	1.53±0.08 ^{Af}	1.86±0.02 ^{Be}	2.81±0.04 ^{Bd}	3.63±0.04 ^{Bc}	4.05±0.04 ^{Bb}	4.50±0.05 ^{Ba}
Tallow and 0.05% rosemary extract	1.52±0.04 ^{Af}	1.76±0.03 ^{Ce}	2.34±0.06 ^{Cd}	3.05±0.06 ^{Cc}	3.57±0.04 ^{Cb}	4.04±0.05 ^{Ca}
Tallow and 0.1% rosemary extract	1.51±0.03 ^{Af}	1.68±0.03 ^{De}	2.05±0.06 ^{Cd}	2.85±0.06 ^{Dc}	3.08±0.03 ^{Db}	3.78±0.03 ^{Da}
Tallow and 0.1% Phospholipids	1.52±0.03 ^{Af}	1.73±0.06 ^{CDe}	1.89±0.05 ^{Dd}	2.60±0.05 ^{Ec}	2.76±0.03 ^{Eb}	3.20±0.05 ^{Ea}
Tallow and 0.3% Phospholipids	1.49±0.03 ^{Af}	1.67±0.03 ^{De}	1.83±0.04 ^{Dd}	2.47±0.04 ^{Fc}	2.66±0.02 ^{Fb}	3.07±0.02 ^{Fa}
0.3%Phospholipids +0.05% rosemary extract+ Tallow	1.51±0.05 ^{Af}	1.61±0.02 ^{Ge}	1.82±0.07 ^{Gd}	2.33±0.05 ^{Gc}	2.42±0.02 ^{Gb}	2.70±0.03 ^{Ga}

Discussion

As Table 3 shows, the peroxide value decreases with increasing concentrations of the extract and phospholipids. These numerical values of peroxide in phospholipids with a concentration of 0.3% are lower compared to the concentration of 0.02%, and as the storage time increases, the values of the peroxide number increase. To study lipid reactions, peroxides are considered as the primary measurement indicator [11]. This study was in line with the research of Yaqub and Chi Man in 2000 and showed that the process of oil oxidation can be reduced. The reason for this is the low oxidation number of rosemary essential oil compared to other titles of antioxidant structures such as palm oil [12].

By adding rosemary and phospholipid extracts on the tallow; Oxidation resistance time and induction time increase and a significant correlation has been observed and it has been shown that the higher the concentration of rosemary extract, the better the results will be. But compared to phospholipids, it has experienced less oxidation resistance time and more induction time. As a result, it can be concluded that phospholipids increase the duration of oxidation. And the combination of rosemary extract and phospholipid produces better effects. This argument is consistent with Herandaz's 2020 study, which states that antioxidants such as rosemary combined with phospholipids are more effective than tocopherol antioxidants. Hernandez has shown that tocopherols are effective at concentrations of 1000 ppm, while

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rosemary-phospholipid mixed antioxidants are more effective at concentrations above 5000 ppm [13].

Conclusion

Fats and oils are among the most widely consumed food products among human societies and different countries, and this issue is also reflected in Iran, and given that this type of food products are prone to oxidation and spoilage to prevent their spoilage in industry, Many synthetic antioxidants are used. But these types of synthetic antioxidants can be dangerous to your health. And endanger a person's health, so researchers have considered natural antioxidants as a suitable alternative to prevent fat oxidation. In this study, the antioxidant and synergistic effects of rosemary extract with phospholipids were studied. This study has shown that rosemary has significant effects on ireducing oxidation, and the combination of this extract with phospholipids can reduce the oxidation of tallow, and this reduction has been very significant. Therefore, from the results of this study, it can be inferred that the use of high concentration rosemary extract with phospholipids can be used to prevent talc spoilage. The results of this study showed that the use of a combination of rosemary extract with phospholipids to improve the oxidative stability of tallow will lead to the consumption of products with desirable properties and acceptable to consumers. Therefore, this study considers the use of a combination of rosemary extract to improve the properties of tallow.

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