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Original Article

Determination of Antioxidative Effect of *Achillea Millefolium* Essential Oil on Mayonnaise Stability by Rancimat Method

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Abstract

Background and Aim: Lipid oxidation is the main chemical process affecting mayonnaise deterioration.
Materials and Methods: In this research, the effect of different concentrations of *Achillea millefolium* essential oil in concentrations of 7.20, 5.85, and 3.83 mg/ml on the oxidative stability of mayonnaise was studied by Rancimat technique.
Results: The induction time of the mayonnaise containing essential oil with concentration of 7.20 mg/ml was longer than that of mayonnaise with chemical antioxidant TBHQ in concentration of 0.12 mg/ml as positive control, while the control sample had a lower protective factor (P<0.05).
Conclusion: This study suggests that the essential oil of *Achillea millefolium* could be used as natural antioxidant in mayonnaise, as a substitute to chemical antioxidant.
Keywords: *Achillea millefolium*, Essential oil, Mayonnaise, Rancimat

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Introduction

Nowadays, mayonnaise is the most widely used sauce in the world. Mayonnaise is an oil in water (O/W) emulsion containing 65–70% oil and egg yolk. It is susceptible to deterioration due to autoxidation. (15). The lipid oxidation of mayonnaise leads to the reduction of shelf life and deterioration of flavour, aroma, colour, and nutritive value (10-11, 15, 17, 21). In order to control and decrease lipid oxidation, antioxidants are added to foods. Synthetic antioxidants such as Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA) and tert-Butylhydroquinone (TBHQ) have been used in food industry to prevent oxidation. These compounds are more economical than natural antioxidants. Nevertheless, these products make a negative impression for being chemical products (8, 15, 18). Plant materials have gained much attention because they exhibit a wide range of activities such as antioxidant, antimicrobial, anti mutagenic and antiinflammatory activities (Li et al., 2014). The antioxidant effects of purple corn extract (15), ginger powder (14), zenyan essential oils (9), black glutinous rice (20), hot paprika, ground black pepper fruits, dried basil herb (17), *Zataria multiflora* Boiss, and *Satureja hortensis* L. essential oils (22) in mayonnaise have been considered previously. There are still many plants which have various values but not explored and used in foods yet. Among them, *Achillea millefolium* is more known for food applications. The genus *Achillea*, which is represented by approximately 100 species, is a perennial, rhizomatous medicinal plant with aromatic leaves and flowers. These plants are mostly found in Europe, Asia and North America. Species of *Achillea* are widely distributed in Iran (16). Alsohaili, and Al-fawwaz (3) investigated the composition of Achillea Fragrantissima essential oil and its antimicrobial activity in cheese, meat milk and tomato, but not many references have been found concerning the effect of the essential oil of *Achillea millefolium* on the oxidative stability of mayonnaises.

The Rancimat method or oxidation stability index (OSI) is a temperature-accelerated method, that detects the formation of oxidation products using the conductivity probe. Air passes through the sample in the reaction vessel at constant elevated temperature. In this process, fatty acids are oxidized. At the end of the test volatile, secondary reaction products are formed, which are transported into the measuring vessel by the air stream and absorbed in the measuring solution (19). The continuously recorded electrical conductivity of the measuring solution is increasing due to the absorption of the reaction products. Thus their appearance can be detected. The time that elapses until secondary reaction products are detected is called induction time (IP). It characterizes the oxidation stability of oils. Furthermore, in various food products, high temperature will also change the structure and properties of the food product, which adds another level of complexity to the assessment of shelf life. The good predictability of shelf life in emulsions can be attributed to the similarity between the conditions of the accelerated oxidation and the natural oxidation process (19).

This study investigated the antioxidative effects of the essential oil of *Achillea millefolium* in stored mayonnaise by Rancimat method and then compared it with synthetic TBHQ antioxidant.

Materials and Methods

Materials

A traditional sort of refined soy bean oil with the composition of unsaturated fatty acids: 18:1: 23.34%, 18:2: 52.59%, 18:3: 7.23% and without the synthetic antioxidants, was obtained from Naz Company, Isfahan., Iran. TBHQ was purchased from Sigma-Aldrich (USA). Other ingredients required for mayonnaise formulation such as egg, sugar and salt were all purchased from a local supermarket (1).

Preparation and Storage of Mayonnaise

In this study, extracted essential oil of *Achillea millefolium* as a natural condiment was selected to investigate its antioxidant properties (1). First, mayonnaise was produced in 5 kg batches and was formulated with soy bean oil (65% w/w), water, vinegar, egg, NaCl, sugar, citric acid, mustard powder, potassium sorbate and sodium benzoate. Then mayonnaise samples were divided into five experimental treatments and stored at 4°C (1). An attempt was made to develop a useful formula of mayonnaise free from chemical preservatives. According to the calculated amounts of antioxidant activity of essential oil in 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) test (1), the different concentrations of essential oil (3.83, 5.85 and 7.20 mg/ml) were added to mayonnaise samples (EO). Synthetic antioxidant TBHQ in concentrations 0.12 mg/ml (T) was used as a reference substance for comparative purposes (1).

Lipid Extraction from Mayonnaise

Oil was extracted from mayonnaise samples according to the method of Bligh and Dyer (5).

1. For each 1 ml of sample, 3.75 ml 1:2 (v/v) $CHCl_3$:MeOH was added, then it was vortexed well.
2. Then 1.25 ml $CHCl_3$ was added and it was vortex well.
3. Finally 1.25 ml dH_2O was added and it was vortex well.
4. It was centrifuged at 1000 RPM in IEC table-top, and then centrifuged for 5 minutes at room temperature to give a two-phase system (aqueous top, organic bottom).

Oil Stability Index (OSI)

Oxidation stability index (OSI) was performed by AOCS official Method CD 12b- 92 using an oxidative stability instrument (473, Rancimat, model Metrohm, Switzerland). The temperature was adjusted at 110^{o}C and the air stream was 20 Lit/h.

Statistical Analysis

The statistical analysis of experimental results was performed by the software Design Expert 8.0 (Stat-Ease, Inc., Minneapolis, MN, USA). The ANOVA technique was performed to determine the statistical significance of samples.

Results and Discussion

Oil stability index (OSI) is used to compare the efficiency of antioxidants that are added to the edible oils and fats. The Oil Stability Index is defined as the point of maximum change of the rate of oxidation. Table 1shows distinct ranges of induction time estimated for samples at the tested concentrations. All of antioxidants had comparable efficacies. The highest stabilization factor was observed in the sample with EO. 3 and TBHQ and the lowest in the control sample without added antioxidant. It is related to the used soy bean oil in prepared mayonnaise that contained around 52.59 % linoleic acid and was hereby susceptible to oxidation (1). This means that essential oil can increase the resistance to oxidation, due to the function of its phenolic compounds, e.g., camphor, pinene, borneol, terpinen-4-ol, thymol, α-terpineol and 1,8 cineole (1). Antioxidant activity of these components was previously reported (6-7, 12-13). As it is revealed, there is a direct correlation between the essential oil concentration and induction time of different mayonnaises containing essential oil. Moreover, an increase in the amount of essential oil concentration results in the expansion of induction time and the consequent increase in the resistance to oxidation.

(Table 1). The fact that high temperatures reduce the antioxidant activity of antioxidant compounds is probably related to the predicative properties. That is to say, at high temperatures, their volatility increased and at the same time their activity and efficiency decreased (19). The results of measurements for OSI value are very similar to the results of DPPH measurements and confirm the findings of DPPH assay and other oxidation parameters (1).

The ANOVA technique was performed to determine

Table 1: The effect of caffeic acid on the serum level of fasting blood glucose, triglyceride, total cholesterol, LDL, VLDL, HDL and atherogenic index in alloxan-induced diabetic rats.

Antioxidant	Antioxidant addition (mg/ml)	IP (h)
EO.1	3.83	4.65
EO.2	5.85	4.76
EO.3	7.2	5.67
T	0.12	4.83
C	0.00	4.2

EO: Essential oil; T: TBHQ; C: Control (no antioxidant)

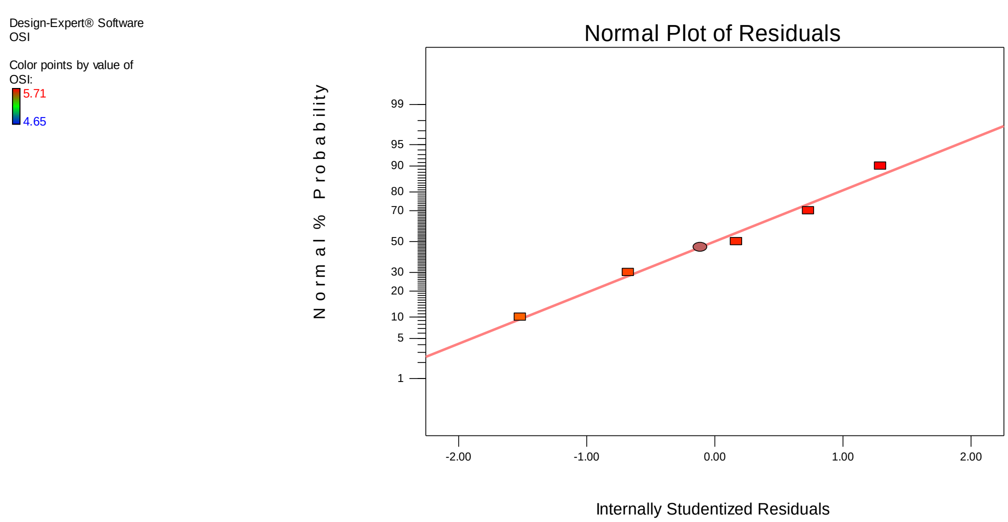

Figure 1. Diagnostic or normal probability plot of the residuals for all samples.

Table 2: ANOVA of the response surface optimal model for the response variables (actual values).

Sum of squares	DF	Mean of squares	F value	Pr > F	Standard deviation	R-Squared	Adj R-Squared	C.V. %
1.32	2	0.66	417.68	< 0.0001	0.040	0.9952	0.9929	0.74

The "Pred R-Squared" is in reasonable agreement with the "Adj R-Squared"; Values of "Prob > F" less than 0.0500 indicate model terms are significant

the statistical significance of OSI for essential oils, synthetic antioxidant and control sample. The model F-values implies that the model is significant. When p-value is less than 0.0500, model terms are significant (Table 2). Figure 1 shows the diagnostic of OSI for all samples. The diagnostic details provided by Design-Expert, can best be analyzed by inspection of various plots. The most important diagnostics will be the normal probability plot of the studentized residuals. The data points are approximately linear. There is no sign of any problem in our data.

Conclusion

This work clarified that the oxidative stability of prepared mayonnaise can be improved by the incorporation of essential oil of *Achillea millefolium* at concentrations 3.83, 5.85 and 7.20 mg/ml. Synthetic antioxidants like TBHQ can be substituted with this essential oil. This investigation was performed to study a greater application of essential oil as an additive to improve the oxidative stability of emulsified foods such as mayonnaise.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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