1. Radical Scavenging Capacity of Walnut Oil: Effect of some Antioxidants on Storage Period

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Introduction. Among the nuts, walnut is characterized by its important amount of oil (60-65% of flesh weight). The oil extracted from walnut exhibits high contents of polyunsaturated fatty acids ω-6 (linoleic acid) and ω-3 (linolenic acid), as well as relevant amounts of vitamins (vitamin E). However, the storage period of this type of oil is limited due to its low stability and as the result moderate radical scavenging capacity. To avoid this stability problem, in walnut oil could be added some food antioxidants, thus oil will be protected while storage and characterized by significant radical scavenging capacity. This research work aims to apply response surface methodology to model and optimize *type*, *ratio* and *quantity* of the added food antioxidants to the cold pressed walnut oil with maximum oil stability and radical scavenging capacity, after 5 and 50 days of storage.

Materials and Methods. Walnut fruits of varieties *Calarash* and *Kogylnichanu* from the State Enterprise "*Forestry Iargara*" were purchased. The walnuts were cracked, shelled, and then milled into a fine powder in an electric mill. Oil was extracted using cold pressing with an electrical lab press. Extracted oil samples had a light yellow color and very characteristic nutty flavor. Oil samples with different amount of antioxidants were stored in dark-glass bottles at 25±3ºC until analysis. A Full three-Factor, two-level Experiment, FFE 2^3, was applied to estimate the effects of independent factors: n-Octyl Gallate, OG, X_1 (25-75 mg/g), racemic DL-α-Tocopherol, DLTP, X_2 (65-195 mg/g) and L-Ascorbic Acid 6-palmitat, AAP, X_3 (40-100 mg/g). Radical Scavenging Capacity, RSC, was measured by assay involving the 1,1-diphenyl-2-picrylhydrazyl, $DPPH^{\bullet}$, stable free radical.

Results. Radical scavenging capacity value of all walnut oil samples after 5 days of storage were in the range of 88.13-92.85% in comparison with reference sample, 50.45%. The contributions of different "pure" and "mixed" factors show following equations:

$$RSC_{5d} = 83.4X_0 - 1.8X_1 - 0.7X_2 - 2.1X_3 - 0.4X_{12} - 2.8X_{13} - 0.4X_{23} - 1.2X_{123},$$

$$RSC_{50d} = 48.0X_0 - 3.0X_1 + 0.7X_2 - 2.0X_3 - 3.9X_{12} + 0.0X_{13} - 3.9X_{23} + 0.1X_{123},$$

where RSC_{5d} is antiradical activity of walnut oil after 5 days of storage, RSC_{50d} – after 50 days. These and other linear regressions, pay attention to three important circumstances:

1. Apparently, activity of pure antioxidants satisfies the inequality: OG ≈ AAP ≠ DLTP.
2. Couples of antioxidants act differently at the initial stage and after the storage time Therefore, pairs OG-DLTP and DLTP-AAP do not show its influence at RSC on the fifth day, but its interaction clearly observes on the fiftieth day. Vice versa, initial influence of pair OG-AAP is significant, but after 45 days diminishes to zero.
3. The mixtures of antioxidants manifest the synergetic action to the RSC values, i.e., moderate super-addition effects of all antioxidant pairs is observed. The triple interaction between all antioxidants used, X_{123}, is less, then its interaction in pairs.

Conclusions. Walnut oil samples, added with antioxidants as OG, DLTP and AAP, maintain an acceptable quality at least up to 50 days of storage, its residual RSC values being at the range of 43.55-69.03%. The addition of the investigated antioxidants significantly increased RSC values and improves the storage period of walnut oil.