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Determination of antioxidant activity of cold-pressed edible oils from Republic of North Macedonia by Hemoglobin assay

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Introduction

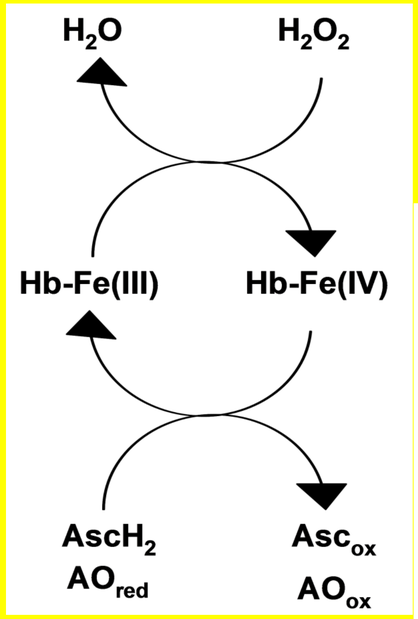
In the present study a new approach, based on cell-free hemoglobin (Hb) analysis, is proposed to evaluate antioxidant activity of cold-pressed edible oils from sunflower, flax and sesame seeds. The new modified method is based on the spectrophotometric measurement of Hb concentration at specific wavelength (412 nm) [1-3].

Materials and Methods

For the determination of the antioxidant potential of extracts from cold pressed oils a modified procedure of the HAPX assay was used (Benedec et al., 2013). In brief, 2.5 µL of 11 264 sodium ascorbate (concentration 50 mM) was mixed with 2.3 µL of H_2O_2 and 3.7 µL purified hemoglobin (concentration 1.7 mM). Furthermore, sodium acetate buffer (956 L, 50 mM, pH 5.5) was mixed with ascorbic acid (7 L, 50 mM), H_2O_2 (20 L, 50 mM) and 2.5 L of the extracts obtained from cold pressed oils. After 12–15 s, met-hemoglobin (met-Hb) from a 1.4 mM stock solution (7 L) was added to the reaction mixture and the absorbance at 290 nm was monitored. A measurable significant inhibition of the ascorbic acid consumption was observed compared to the reference (run in four different experiments) in which the extract was replaced by an equal amount of extraction solvent. The slope of each sample was calculated at the tested concentration and also without extracts from cold pressed oils (blank). The inhibition of ascorbic acid consumption was determined as follows: HAPX = 100 − [(slope of the sample/slope of the blank) × 100]. All the spectroscopic measurements were performed using a Jasco V-530 UV-Vis spectrophotometer (Jasco International Co., Ltd., Tokyo, Japan).

Results and Discussion

Hemoglobin ascorbate peroxidase activity inhibition (HAPX) assay was applied for the first time in order to compare the antioxidant activity of cold pressed edible oils. HAPX assay shows the capability of an extract obtained from an oil to quench the HbFeIV resulted by hydrogen peroxideinduced damage upon HbFeIII. Transportation of oxygen via hemoglobin is possible by bonding of an oxygen molecule to iron in the ferrous state (Fe2+). Oxyhemoglobin produced by side reaction undergoes autoxidation forming methemoglobin (Fe3+) and in this oxidation state hemoglobin cannot bind any oxygen. In this case Fe3+ from methemoglobin activates hemoglobin and form ferryl Fe4+. Ferryl hemoglobin is produced in the body in case of stress, illness and induces peroxidation of lipids (Benedec, Vlase, Oniga, Moţ, Damian, Hanganu, Duma & Silaghi-Dumitrescu, 2013). Hemoglobin ascorbate peroxidase activity inhibition (HAPX) assay measure the capability of the extracts from cold-pressed oils to quench the HbFeIV resulted by hydrogen peroxide-induced damage upon HbFeIII. (Fig.1 and 2) The results showed the highest value of the antioxidant potential for extract from flaxseed oil (590.1±3.9 mg Trolox/L oil). The extract from sunflower oil showed a lower value and the lowest antioxidant potential measured by this assay was obtained for sesame seed oil (280.2±1.9 mg Trolox/L oil).



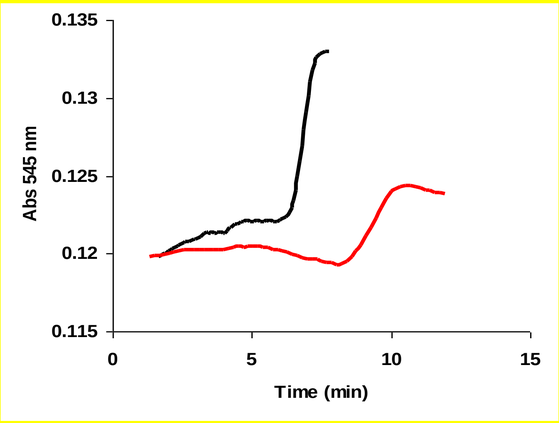


Fig. 1. Hemoglobin assay and calibration curves

Conclusion

HAPX assay showed the highest antioxidant activity for flaxseed oil and the lowest antioxidant potential for sesame oil. However the value for the antioxidant activity of sunflower oil was similar to that of flaxseed oil. Those results indicate that HAPX assay is not the most suitable assay for extracts of cold-pressed oils obtained by a mixture of methanol-water (80:20 v/v) or the results reflect a quite different picture of antioxidant activity not yet covered by the other assays. A relationship with cyclolinopeptides might be further investigation for freshly cold pressed linseed oils in order to proof this possibility.

Table 1. Calibration curve for hemoglobin assay

Calibration curves for antioxidant assays	
Calibration curve of Trolox for HAPX assay	y = 0.0067-0.4097x R^2=0.9804

Table 2. Hemoglobin assay

Sample	HAPX assay (mg trolox/L oil)
Sunflower oil	566.8 ±13.5[b]
Flaxseed oil	590.1 ± 3.9[a]
Sesame seed oil	280.2 ± 1.9[c]

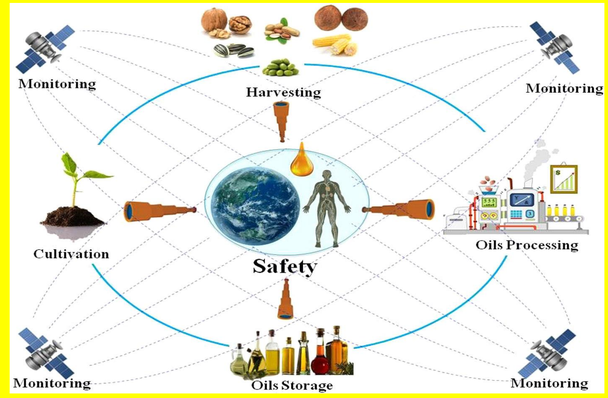


Fig. 3. Health benefits and production of cold-pressed edible oils [3]

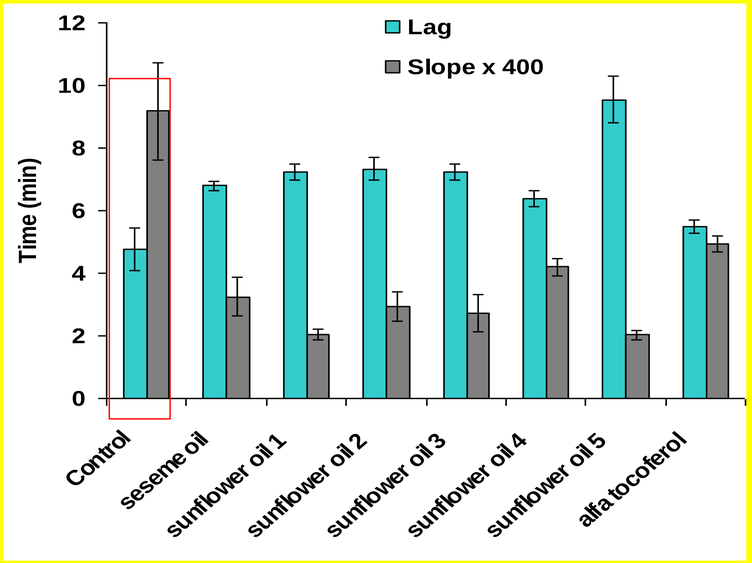


Fig. 2. Antioxidant activity of cold-pressed edible oils determined by Hemoglobin assay

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