

**The relationship between the structure and functionality of the essential PUFA delivery systems based on sodium caseinate with phosphatidylcholine liposomes without and with a plant antioxidant: an in vitro and in vivo study**

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**Methods**

**Estimation of the oxidation level of the liposomes PC–FO and PC–FO–EOC both in a pure form and in the complexes with SC**

We have controlled the secondary (malonic dialdehyde (MDA)) oxidation products of the studied combinations of the lipids PC–FO and PC–FO–EOC both in a pure form and in the complexes with SC during their storage at room temperature and light exposure. The MDA was determined using the TBA (2-thiobarbituric acid) test in the presence of trichloroacetic acid (TCA).<sup>1,2</sup>

Briefly, the TBA-TCA reagent was prepared by dissolving 15 g of TBA and 0.67 g of TCA in 100 ml of a double distilled water. This reagent (3 ml) was vigorously mixed with the test sample (0.5 ml) and thereafter the resulting mixed solution was heated in a boiling water bath for 30 min. The absorbance ( $A$ ) of the colored thiobarbituric acid reactive substances (TBARS) was measured by spectrophotometry (SF-2000, UKB Spectrum, Russia) at two different wavelengths:  $\lambda = 532$  nm (the maximum absorbance of the TBARS) and  $\lambda = 580$  nm (the minimum absorbance of the TBARS). The MDA concentrations were calculated in the test samples by the following equation:

$$C_{\text{MDA}} = (A_{532} - A_{580}) \times 7 \times 1000/155 \quad (1)$$

where  $A_{532}$  and  $A_{580}$  are the absorbance values at 532 nm and 580 nm, respectively;  $C_{\text{MDA}}$  is the concentration of MDA (nmol/ml); 155 is the molar extinction coefficient (1000 cm<sup>2</sup>/mol) of MDA

at  $\lambda = 532$  nm in a cuvette with a path length of 1 cm; 7 is the dilution factor of the sample with the TBA-TCA reagent; 1000 is the conversion factor to the concentration (mol/ml).

The estimated experimental error in measurements of the secondary lipid oxidation product was not higher than  $\pm 10\%$ .

### References

- 1 J. M. Gutteridge and T. R. Tickner, The thiobarbituric acid-reactivity of bile pigments. *Med. Biochem.*, 1978, 19, 127–132.
- 2 J. Fernandez, J. A. Perez-Alvarez, J. A. Fernandez-Lopez, Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chem.*, 1997, 59(3), 345–353.