**Antioxidant Efficiency and Oxidizability of Mayonnaise by Oximetry and Isothermal Calorimetry**

Rajat Suhag (rsuhag@unibz.it)

Faculty of Agricultural, Environmental and Food Sciences, Free University of Bolzano, Italy

Tutor: Prof. Matteo Scampicchio

The effect of vegetable oils on the resulting rate of autoxidation in mayonnaise samples was studied by oximetry and isothermal calorimetry at 60°C. The two methods were highly correlated (R2 = 0.99), showing similar onset times (i.e., antioxidant capacity, *τ*), and rates of inhibited (*Rinh*) and uninhibited (*Runi*) periods. The mayonnaise samples showing the highest resistance against peroxidation (i.e., highest τ and lowest *Rinh*) were those prepared with extra virgin olive, followed by corn > grapeseed > sunflower > apple seed oil, whereas the *A.E.* was maximum for sunflower oil. Most importantly, isothermal calorimetry allowed the simultaneous measurement of up to 24 samples, with minimal experimental effort.

Efficienza antiossidante e ossidabilità della maionese mediante ossimetria e calorimetria isotermica

L'effetto degli oli vegetali sulla risultante velocità di autossidazione nei campioni di maionese è stato studiato mediante ossimetria e calorimetria isotermica a 60°C. I due metodi erano altamente correlati (R2 = 0,99), mostrando tempi di insorgenza simili (cioè capacità antiossidante, τ) e tassi di periodi inibiti (Rinh) e non inibiti (Runi). I campioni di maionese che hanno mostrato la più alta resistenza alla perossidazione (cioè, τ più alto e Rinh più basso) sono stati quelli preparati con olio extravergine di oliva, seguiti da mais > vinaccioli > girasole > olio di semi di mela, mentre l'A.E. è stato massimo per l'olio di girasole. Ancora più importante, la calorimetria isotermica ha consentito la misurazione simultanea di un massimo di 24 campioni, con uno sforzo sperimentale minimo.

**Keywords**: Induction time; Fatty acids; Antioxidant activity; Lipid oxidation; Oxidation rate.

# **1. Introduction**

In accordance with the PhD thesis project, this poster reports the main results of the first activity concerning: A1 - the oxidation kinetics of mayonnaise by oximetry and isothermal calorimetric analysis.

# **2. Materials and Methods**

Mayonnaise samples were prepared by mixing oil (80%), egg yolk (10%) and vinegar (10%). Sodium azide (0.05% w/w) was added as microbial inhibitor. To control the rate of free radical formation a lipid soluble radical initiator AIBN was added in the oil phase to reach a final concentration of 25 mM. Samples were labelled according to the oil type used, as MSO, MCO, MEVOO, MGO and MAO, respectively, for sunflower, corn, extra virgin olive, grapeseed, and apple seed oils. To determine the oxidative stability, mayonnaise samples (200±5 mg) were kept in hermetically sealed glass ampoules (4.0 cm3) and heat flow over time was recorded using an isothermal calorimeter (Thermal Activity Monitor, Model 421 TAM III, TA Instruments) at 60°C. In addition, the concentration of oxygen inside the glass ampoules was monitored with an oxygen meter (Fibox 4, PreSens GmbH, Germany) at 60°C. A typical workflow was used to transform the isothermal calorimetric heat flow to oxygen concentration, which was then used to determine the onset time (*t*, antioxidant capacity), rate of inhibited period (*Rinh*), and rate of uninhibited period (*Runi*). The oxidizability of mayonnaise was determined based on *Runi*. Additionally, antioxidant efficiency (*A.E.*) was calculated using *t* and *Rinh*.

# **3. Results and Discussion**

Figure 1 (A) shows the calorimetric traces obtained for the analysis of five different mayonnaise samples, each prepared with the same water phase, but with different plant-based oils. Figure 1 (B) shows the transformation of the calorimetric trace into the corresponding oxygen consumptions. From Figure 1 (B), it was possible to determine the induction time (τ) for the oxidation of each mayonnaise samples, as well as the rates of oxygen consumption during the inhibited (*Rinh*) and uninhibited (*Runi*) periods.

## **3.1 Oxidizability**

Oxidizability index *(O.I.)* of mayonnaise samples was determined based on eq. (1).

|  |  |  |
| --- | --- | --- |
|   | $$O.I.=\frac{k\_{p}}{\sqrt{2k\_{t}}}=\frac{R\_{uni}}{\left[RH\right]\_{0}∙\sqrt{R\_{i}}}$$ |  (1) |

Where, *Runi* is the rate of oxidation during uninhibited period, [RH]0 represent the molar concentration of lipid substrate and *Ri* is the rate of initiation.

The mayonnaise sample with the highest *O.I.* – i.e., the ones with the highest susceptibility toward oxidation were apple seed oil and sunflower oil mayonnaise with no statistical significant difference (*p* < 0.05), followed by grape seed oil > corn oil > extra virgin olive oil (Table 1). The *O.I.* values were correlated with the content of unsaturated fatty acids (R2 = 0.99). This correlation can be expected considering that the rate constant for a termination reaction, 2*kt*, is similar among different oxidizable substrates (~107 M-1s-1) (Baschieri *et al.*, 2019), whereas the values for the propagation rate constant *k*p is greatly dependent on the degree of unsaturation in the fatty acids (Xu *et al.,* 2009). Moreover, the *kp* values that can be calculated from *O.I.* and a 2*kt* value of 107 M-1s-1 is of the same order of magnitude (Table 1) as that determined in homogeneous system by oximetry technique, as *kp* of pure methyl linoleate is 62 M-1s-1.



**Figure 1** (A) Isothermal calorimetry trace of mayonnaise at 60°C, (B) oxygen consumption derived from heat flow data. (1) MAO, (2) MSO, (3) MGO, (4) MCO and (5) MEVOO.

## **3.2 Antioxidant Efficiency**

The concept of "antioxidant efficiency" is far more helpful and practical for quantitatively describing the effects of antioxidants in inhibiting lipid peroxidation (Bravo-Díaz, 2022). The *A.E.* of mayonnaise samples was expressed with eq. (2) (Pryor *et al.*, 1993). As *Ri* was constant for all the mayonnaise samples, *A.E.* can be simply expressed using the onset time (τ) and *Rinh*.

|  |  |  |
| --- | --- | --- |
|  | $$A.E.=\frac{k\_{inh}}{k\_{p}}=\frac{R\_{i}}{n∙\left[AH\right]\_{0}}∙\frac{\left[RH\right]\_{0}}{R\_{inh}}=\frac{\left[RH\right]\_{0}}{τ∙R\_{inh}}$$ | (2) |

Based on the results reported in Table 1, mayonnaise prepared using sunflower oil showed the highest *A.E.* value, followed by apple seed oil > grapeseed oil > extra virgin olive oil > corn oil with no significant difference (*p* < 0.05) between extra virgin olive oil and corn oil. The *A.E*. of sunflower oil antioxidants in mayonnaise was found to be approximately 2.5 times that of extra virgin olive oil and corn oil, and 1.8 and 1.5 times that of grapeseed and apple seed oils, respectively.

***Table 1*** *Kinetic parameters derived using the isothermal calorimetry trace of mayonnaise samples at 60°C.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample | *Rinh* | *Runi* | *O.I.* | *kp* | *τ* | *A.E.* |
| **10-7 mol/L.s** | **10- 7 mol/L.s** | **10-3 (mol/L)-1/2s-1/2** | **(mol/L)-1 s-1** | **104 s** | **-** |
| MSO | 2.3±0.1c | 14.2±1.1a | 8.7±0.6a | 29 | 5.4±0.1d | 198.1±6.6a |
| MCO | 2.8±0.1b | 5.2±0.2c | 3.2±0.1c | 11 | 11.8±0.9b | 74.8±4.4d |
| MEVOO | 1.6±0.1d | 2.5±0.1d | 1.7±0.1d | 5.7 | 18.8±0.4a | 77.4±2.9d |
| MGO | 2.9±0.1b | 8.8±1.5b | 5.4±0.9b | 18.3 | 7.8±0.1c | 108±4.6c |
| MAO | 4.1±0.3a | 14.8±0.2a | 8.9±0.1a | 30 | 4.8±0.2d | 128.6±10.5b |

In a column means±SD that do not share a superscript letter are significantly different (*p* < 0.05).

# **4. References**

Baschieri, A., Pizzol, R., Guo, Y., Amorati, R., & Valgimigli, L. (2019) Calibration of Squalene, p -Cymene, and Sunflower Oil as Standard Oxidizable Substrates for Quantitative Antioxidant Testing. *Journal of Agricultural and Food Chemistry*, **67**: 6902–6910.

Bravo-Díaz, C. (2022) Advances in the control of lipid peroxidation in oil-in-water emulsions: kinetic approaches *†. Critical Reviews in Food Science and Nutrition*, 1–33.

Pryor, W. A., Cornicelli, J. A., Devall, L. J., Tait, B., Trivedi, B. K., Witiak, D. T., & Wu, M. (1993) A rapid screening test to determine the antioxidant potencies of natural and synthetic antioxidants. *The Journal of Organic Chemistry*, **58**: 3521–3532.

Xu, L., Davis, T. A., & Porter, N. A. (2009) Rate Constants for Peroxidation of Polyunsaturated Fatty Acids and Sterols in Solution and in Liposomes. *Journal of the American Chemical Society*, **131**: 13037–13044.