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Influence of the parameters of the Rancimat test on the determination of the oxidative stability index of cod liver oil

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ABSTRACT

The operational parameters of the Rancimat method (airflow rate, sample weight and temperature) were studied to determine their effects on the oxidative stability index (OSI) of cod liver oil. To this end, experimental data were firstly fitted to a complete quadratic model and an ANOVA analysis was performed, which concluded that airflow rate and temperature were significant ($p < 0.05$). By means of this model and using response surface methodology in order to minimize the OSI, the optimal conditions for the three parameters of the Rancimat method were found to be $Q = 25$ L/h, $M = 6.91$ g and $T = 88.26$ °C. The different trend obtained for the OSI at increasing temperatures supports that the oxidation mechanism of fish oil at the conditions studied may differ from the lipoperoxidation mechanism at room temperature. Secondly, a simplified linear model was assayed, obtaining also that the influences of airflow rate and temperature were significant ($p < 0.05$). Moreover, the temperature contribution resulted to have the most important effect on the OSI, obtaining a temperature coefficient of -3.29×10^{-2}.

1. Introduction

At present, the production of high quality fish oil has gained a great importance since it is considered the main natural source of omega-3 polyunsaturated fatty acids (Rubio-Rodríguez et al., 2012). Among these PUFAs, eicosapentaenoic acid (C20:5 n-3, commonly called EPA) and docosahexaneoic acid (C22:6 n-3, commonly called DHA) are particularly important since they have been reported to promote several benefits on human health (Lees & Karel, 1990, chap. 2; Uauy & Valenzuela, 2000).

As a consequence of its high degree of unsaturation, fish oil is very susceptible to oxidation. Due to the fact that the oxidation process causes the development of undesirable fishy off-flavours, which deteriorate the overall quality of the oil reducing considerably its use in both pharmaceutical and food applications (Jacobsen, Let, Nielsen, & Meyer, 2008; Jacobsen & Nielsen, 2007), fish oil should be optimally produced, stored and packed. Moreover, addition of different types of antioxidants is carried out in order to protect fish oil against oxidation (Rubio-Rodríguez et al., 2010). Therefore, the measurement of the oxidative stability of fish oil is of great importance for researchers and manufacturers in order to control and optimise the production process and to predict the shelf-life of the final oil.

Determining the resistance of oils and fats to oxidation is a tedious and time-consuming analysis when carried out at room temperature. Thus, several accelerated methods employing high temperatures and airflow supply have been developed to assess oxidative stability in a relatively short period (Reynhout, 1991). Among these techniques, the Active Oxygen Method (AOM) (AOCS, 1980) has traditionally been used for such determinations. However, this method is labour-intensive, non reproducible and involves the use of toxic chemicals (Mateos, Uceda, Aguilera, Escuderos, & Maza, 2006). A superior accelerated analysis, the Rancimat test, was developed by Hadorn and Zurcher (1974), becoming the AOCS standard method Cd 12b-92 (AOCS, 1992) after the study carried out in 1991 by Jebe, Matlock, and Sleeter (1993). As a result of this fact and due to its ease of use and reproducibility, the Rancimat test has been widely employed over the past two decades. Furthermore, the Rancimat method has been specially validated for oxidative stability determination on both bulk (Méndez, Sanhueza, Speisky, & Valenzuela, 1996) and micro-encapsulated fish oils (Bustos, Romo, Yáñez, Díaz, & Romo, 2003; Velasco, Dobarganes, Holgado, & Márquez-Ruiz, 2009).

The Rancimat method is based on the conductivity changes experienced by deionised water after collecting the volatile organic acids produced in the final steps of the accelerated oil oxidation

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process (Jebe et al., 1993; de Man, Tie, & de Man, 1987; Méndez et al., 1996). The time required to produce a sudden increase of the conductivity due to volatile acids formation, mainly formic acid, determines the oxidative stability index (OSI), which can be defined as a measure of the resistance to oxidation of a fat or oil. In addition, previous studies have demonstrated the correlation between the stability data obtained by the Rancimat test and those determined by other sensory and/or analytical methods (Anwar, Bhanger, & Kazi, 2003; Coppin & Pike, 2001; Gordon & Mursi, 1994).

Airflow rate, oil sample weight and temperature are the operational parameters that can be adjusted easily in the Rancimat method and may affect the determination of the OSI number (Farhoosh, 2007a, 2007b). Although a number of studies (Farhoosh, 2007a; Hasenhuettl & Wan, 1992; Hill & Perkins, 1995; Kochhar & Henry, 2009; Reynhout, 1991) have investigated the individual or simultaneous effect of the parameters of the Rancimat test on the oxidative stability of vegetable oils, no comprehensive studies have been reported so far about the influence of these three parameters on the OSI evaluation of fish oil.

In the light of the above, the purposes of this work were mainly two: a) to assess, by means of experimental design and analysis of variance (ANOVA) techniques, the influence of each operational parameter upon the oxidative stability index of cod liver oil; and b) to develop, by means of response surface methodology (RSM), empirical models in order to correlate the oxidative stability index with the operational parameters involved in the Rancimat test. These models were then optimised to determine the combination of experimental factors which minimise the OSI.

2. Materials and methods

2.1. Materials

Partially refined cod liver oil employed was purchased from Acofarma (Barcelona, Spain), with the following declared properties: density 923 kg/m^3, refractive index 1.477, peroxide value 3.12, acidity value 0.16, mean iodine value (Hanus) 165, EPA 8.18% (w/w), DHA 11.02% (w/w) and non-saponifiable content 0.39%. The oil was kept in a bottle covered with aluminium foil at 4 °C under nitrogen atmosphere. Antioxidants were not added during storage.

2.2. Apparatus

A Metrohm Rancimat model 743 (Metrohm Instruments, Herisau, Switzerland) was employed for OSI determination. Before each run, the sample tubes were rigorously cleaned and immersed overnight in a hot solution of an alkaline detergent (3%), rinsed off with distilled water and acetone and then dried in oven at 80 °C. This procedure was carried out in order to avoid any contamination which could catalyse the autooxidation process. Also, electrodes, connecting tubes and measuring vessels were cleaned with alcohol and distilled water and were blown out with nitrogen.

2.3. OSI determination

A stream of filtered, cleaned and dried air was bubbled into the oil sample contained in a reaction vessel. This vessel was placed in an electric heating block, which was set at the desired temperature for each experimental run. Effluent air containing volatile organic acids from the oil sample were collected in a measuring vessel with 60 mL of distilled water. The conductivity of the water was continuously recorded and the OSI was automatically determined by the apparatus. Eight oil samples were analysed in the equipment at the same time.

2.4. Experimental design

Three experimental factors were considered for this study: airflow rate (Q), sample weight (M) and temperature (T). They were varied according to a $4 \times 4 \times 4$ full factorial design. To this end, each input factor was set at four levels, as follows: airflow rate (10, 15, 20 and 25 L/h), sample weight (3, 5, 7 and 9 g) and temperature (60, 70, 80 and 90 °C). The stability oxidative index (OSI) was evaluated for a total of 64 determinations, as shown in Table 1.

2.5. Statistical analysis

The Statgraphics software (version 5.1) was used to generate the statistical analysis and the regression models. Firstly, the OSI was related to the input variables (airflow rate, sample weight and temperature) by a second degree polynomial according to Eq. (1):

$$\mathrm{OSI} = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i<j}^{3} b_{ij} X_i X_j \quad (1)$$

where the coefficients b_i and b_{ii} are related to the linear and quadratic effects, respectively, of each input factor on the OSI and the cross-product coefficients b_{ij} represent the interactions between two input variables.

Secondly, the modified variable log (OSI) was chosen to obtain an alternative correlation when fitting the data to a linear regression model, Eq. (2):

$$\log(\mathrm{OSI}) = b_0 + \sum_{i=1}^{3} b_i X_i \quad (2)$$

Finally, the analysis of variance (ANOVA) tables were generated. The significance of all terms in the models was judged statistically by computing the p-value at a confidence level of 95%. The regression coefficients were then used to generate contour maps and find the optimal Rancimat parameters values which minimize the OSI. For this final purpose, response surface methodology approach was employed as described by Ba and Boyaci (2007).

3. Results and discussion

The OSI results for the chosen experimental design are presented in Table 1. It can be seen that, at constant values of airflow rate and sample weight, the OSI values significantly decreased with

Table 1
Experimental design and measured values for the oxidative stability index.

Run	Airflow rate, L/h	Sample weight, g	Temperature, °C			
			60	70	80	90
1–4	10	3	18.03	8.12	4.22	1.97
5–8	10	5	17.91	8.03	3.95	2.00
9–12	10	7	17.76	8.09	3.97	2.04
13–16	10	9	17.98	8.15	4.06	2.08
17–20	15	3	17.71	8.10	3.85	1.93
21–24	15	5	17.51	8.01	3.76	1.93
25–28	15	7	17.55	7.90	3.81	1.93
29–32	15	9	17.72	8.08	3.89	1.96
33–36	20	3	17.67	8.01	3.47	1.80
37–40	20	5	17.56	7.97	3.38	1.67
41–44	20	7	17.34	7.85	3.33	1.72
45–48	20	9	17.63	7.87	3.42	1.84
49–52	25	3	18.12	8.21	3.31	1.78
53–56	25	5	17.35	8.04	3.42	1.74
57–60	25	7	17.38	7.78	3.11	1.69
61–64	25	9	17.46	8.03	3.26	1.52