ANOVA ANALYSIS UPON THE INFLUENCE OF HYDROGEN PEROXIDE USED IN SUNFLOWER OIL EPOXIDATION

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Abstract

In this paper it is presented the Anova analysis regarding the different amounts of hydrogen peroxide used in epoxidation reaction of sunflower oil. All the fields for the peroxy acids applications are presented. The easiest and better method is epoxidation in the presence of an immobilized lipase when mono and di-carboxylic acids are converted to peroxy acids by reaction with hydrogen peroxide. This parameter was optimized for the high conversions of chemo-enzymatic epoxidation of sunflower oil.
***Keywords**: kinetic parameters, statistical methods, epoxidation reaction, sunflower oil.*

Introduction

The epoxidized oils are used in many fields. They are used in PVC plasticizers and stabilizers industry, due to their ability to slowing degradation and as reactive diluents for paints.

For the plasticizers industry the epoxidized oil must be have a higher epoxy group value. But, if it will be used as diluents for paints, lower viscosity is the important characteristics (Klaas, 1999, Godtfredsen, 1991, Kirk, 1994).

The enzymatic reaction of triglycerols with hydrogen peroxide form as products peroxy fatty acids, epoxidized mono- and diglyceride and a small amount of epoxidized free fatty acids.

Some researchers consider that separation of these compounds is hardly possible (Klaas, 1998).

Klaas and Warwel (1996, 1997, 1999) have been proposed a new method for epoxidation of triacilglycerols.

This method use 5 % of free fatty acids and in this case the hydrolysis occur again, but all the hydroxyl groups are re-esterified immediately by the excess of free fatty acids.

So, the reaction products are epoxidixed triacilglycerols and epoxidized free fatty acids, which can be easily removed by alkaline washing (Klaas and Warwel, 1999).

Experimental

Novozym 435 that contain immobilized lipase was kindly donated by Novo Nordisk A/S (Copenhagen, Denmark). Sunflower oil and starch were supplied by supermarket Mercator (Slovenia). Oleic acid with 99% purity and sodium hydroxide solution 0,1 mol /L were supplied by Merck.

Thiosulphate solution 0.25 mol/L, chloroform, iodine monochloride for synthesis, acetic acid 100 % anhydre, toluene pa, hydrogen peroxide 35 % solution in water, hydrochloric acid fuming 37 % were purchased from Merck KDaA (Darmstadt, Germany). Potassium iodine powder pa was supplied by Riedel-de Haen (Seelze, Gemany). Piridyn, phenophthalein and sodium hydrogen carbonate powder were obtained from Kemika (Zagreb, Croatia) and Merck (Darmstadt, Germany). Ethanol was supplied by Aldrich Chemical Co. (Diesenhofen, Germany).

The epoxidation of sunflower oil, catalyzed by immobilized lipase was performed in a batch stirred tank reactor at atmospheric pressure according to Klaas and Warwel (1999) and Klaas *et al*., (2002).

For all the samples the iodine values (Wijs method) and epoksi group according to AOCS procedures (1997, 1992) were determined.

Results and Discussion

The optimal volume of hydrogen peroxide for epoxidation reaction of sunflower oil was established in this work. All the experiments were performed at atmospheric pressure and 40°C using 0.8 g enzyme.

The amount of H_2O_2 effects

The volume of H_2O_2 solution is a very important parameter for optimization of epoxidation reaction.

Warwel *et al.* (1996) have used the same amount of H_2O_2 for all epoxidation reactions. They considered that the concentration of solution is very important.

In a preliminary study the efficiency of H_2O_2 with 35 % concentration for epoxidation of oleic acid was demonstrated. This concentration was used because we consider that is a standard oxidant and higher concentration solutions are very difficult to manipulate (Gîtin *et al.*, 2005).

The effects of the amount of H_2O_2 upon epoxidation reaction were considered for 20 μL, 30 μL, 54 μL, 64 μL and 74 μL which were added slowly during6 hours, from 7.5 mL to 7.5 minutes.

The effects of H_2O_2 amount on iodine and peroxy group values are presented in figure 1.

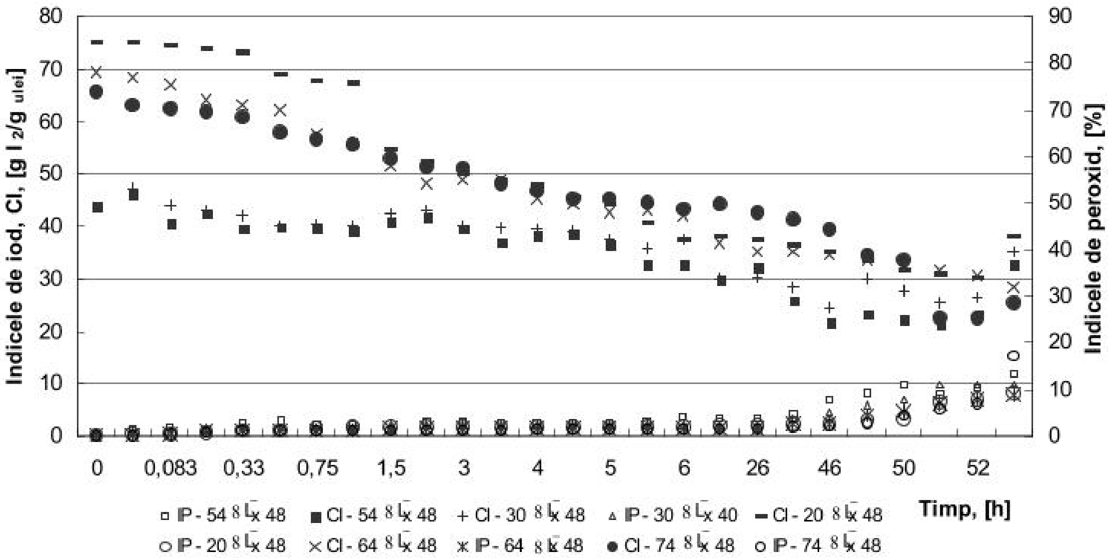

Fig.1: The amount of H_2O_2 35% influence on the IV and EP values in epoxidation reaction of sunflower oil

It can be noticed, from figure 1, that after 1.5 hours, the iodine values decrease by 27.43%, 25% and 16%, respectively for 20 μL, 64 μL and 74 μL H_2O_2. For 54 μL and 30 μL a slow increase of iodine values during a period of 3 hours (from 1.5 hours to 4.5 hours) can be observed. However, the lower iodine values are obtained for 54 μL hydrogen peroxide solution, so these values, after 6 hours and 50 hours, are with 12.32 % and, respectively with 19 % much lower than the values achieved with 30 μL.

During 24 hours the best epoxy group values are obtained with 54 μL and 30 μL, but after 28 hours and 50 hours the epoxy group values are 25.23% and 32% much higher than the values obtained with 30 μL.

Higher volume values of H_2O_2 than 54 μL are not recommended due to a possible inhibition of a biocatalyst. Thus, it could be considered that the best results for epoxidation of sunflower seed oil are achieved when was used 2592 μL H_2O_2, so 54 μL in 48 portions at 7.5 minutes.

Analysis of H_2O_2 35% variation of the solution volume and the time on kinetic parameters

The H_2O_2 35% solution volume and time influence on the IV and EP values was analyzed by using the ANOVA two-way method.
In this regard two hypotheses are presented: a null hypothesis H_0, according to which the EP and IV values are not significantly influenced by the factors and the alternative hypothesis H_1, which presents the significant influence of the values by the tested variables.

In table 1 are presented the results of Anova two way methods for the study of time and H_2O_2 35 % solution volume influence on EP values.

Table 1: Anova two-way for statistical study of the time and H_2O_2 35% solution volume on EP values

Sources of variation	*Variance*	*df*	*F*	$F_{critical}$
Time	887.2497	25	35.53127	1.616349721
Volume of H_2O_2	43.2228	4	10.81827	2.462613224
Total	1030.356	129		

From the table 1, it is to be noticed a certain influence of the two variables, time and H_2O_2 35% volume, on the EP values. Thus, in the case of time influence on EP values the Fischer test show that F (35,531) >> $F_{critical}$ (1,616). In the same way, Fischer test for H_2O_2 volume influence on EP values prove that F (10,818) >> $F_{critical}$ (2,462)

The analysis of variance of the time and H_2O_2 volume influence on IV values is similar; the results are presented in table 2.

Table 2: Dispersional analysis of statistical study of the time and H_2O_2 volume on IV values

Sources of variance	*Variance*	*df*	*F*	$F_{critical}$
Time	16808.25	25	21.82803	1.61635
Volume of H_2O_2	8002.131	4	64.94978	2.462613
Total	27890.5	129		

Because in statistical method was used a significance factor $\alpha = 0.05$, was accepted with a probability of 95 % the alternative hypothesis H_1 which present the time and H_2O_2 volume influence on EP values.

Also, in this case, the same influence of these two factors could be observed. Thus, an alternative hypothesis H_1 is taken into consideration, with a probability of 95 %, as the $F_{calculated} >> F_{critic}$.

Conclusions

The Anova methods were used to present the influence of different cause factors on the EP and IV values.

The comparative analysis showed that the solvent volume have significantly influence on IV values, thus, an alternative H_1 have been accepted. But, the time did not influence the IV values, so a null hypothesis H_0 is accepted with a 95 % of probability.

The Anova two-way method present a significantly influence of all variables on the EP values, so the alternative hypothesis it is accepted.

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