Studies On the Kinetics of Oil Oxidation Using Benzoyl Peroxide and Its Synergistic Effect in Fish Oil Tanning

by

Bindia Sahu,[a]* Gladstone Christopher Jayakumar[a] and Suresh Kumar Anandasadagopan[b]

[a] *Centre for Academic and Research Excellence*

[b] *Biochemistry & Biotechnology Lab*

Central Leather Research Institute, Council of Scientific and Research, Adyar, Chennai 600020, India

Abstract

The oxidation of oil is an unavoidable process during oil tanning which aids in the forming of aldehydes that subsequently interact and react with amino groups in collagen. Oxidizing agents are commonly used to catalyze a faster reaction, thereby reducing days required for oil tanning. Fish oils are widely and regularly used for oil tanning. The oxidation of fish oil depends on its content of unsaturated fatty acids. In the current research, we focused on the kinetics of fish oil oxidation using Benzoyl Peroxide [BPO]. To assess the catalytic behavior of BPO, the kinetic study of the oil oxidation was carried out by determining the peroxide and p-Anisidine values. The maximum peroxide and p-Anisidine values were obtained on the 4th day. This is in accordance with our earlier studies.

Introduction

Fish oil is highly unsaturated, which makes it readily susceptible to the various chemical reactions associated with double bonds, especially addition reactions of oxidation. The oxidation of unsaturated oil is one of the most important and studied reactions where its double bonds play an essential role. The two most common methods associated with the oxidation of oils are autoxidation and photo-oxidation of fatty acids present in fish oil. Catalysts such as metals, oxidizing agents, and enzymes accelerate the rate of oxidation.[1-3]

The high unsaturation content in fish oil makes it vulnerable to the various chemical reactions associated with double bonds, especially addition reactions. After that, the final product of oxidation reactions can be utilized in different applications.[4,5]

Several studies describe how oil oxidation depends on its fatty acid composition. The autoxidation of monounsaturated acid (oleic acid) can be achieved at high temperatures, while polyunsaturated fatty acids such as linolenic and linoleic acids undergo rapid oxidation even at room temperature.[5] Due to the free radical chain reaction, the primary oxidized products, such as allyl hydroperoxides and hydroperoxide, would be further oxidized to secondary products such as saturated and unsaturated aldehydes, short-chain ketones, alcohols, acids, esters, ethers, and hydrocarbons.[6] The use of oxidizing agents is to accelerate the rate of production of primary and secondary products.[7,8]

In principle, the standard oxidative quality parameters are based on measurements of primary and secondary oxidation products which are determined by Peroxide Value (PV) and p-Anisidine value (AV) respectively by Fourier transform infrared (FTIR) Spectroscopy,[9] 1H Nuclear Magnetic Resonance (NMR) Spectroscopy[10] and Dynamic Headspace Gas Chromatography-Mass Spectroscopy (GC-MS) techniques.[11]

In the present study, the effect of different percentages of BPO on the rate of oxidation of fish oil has been investigated. The current research predominantly focuses on reducing the oxidation period to understand fish oil oxidation. The effect of oxidants and the kinetics of the fish oil oxidation have been evaluated by its Peroxide and p- Anisidine values. Further, this can be confirmed by the previous study[14] where fish oil tanned leathers made within four days by using BPO (1%).

Materials and Methods

Materials

Fish oil was purchased from a local supplier, Chennai. BPO was procured from Sigma-Aldrich, Chennai. All the other chemicals were obtained commercially and of analytical grade.

PV measurements by Iodometric titration: Acetic acid (glacial) 99.7%, Isooctane, Potassium Iodide, Distilled water. AV measurements: p-Anisidine reagent, Acetic acid (glacial) 100%, Iso-octane

Methods

In order to determine the chemical kinetics of fish oil oxidation, the first set of experiments corresponds to the determination of Peroxide and p-Anisidine values of fish oil (2g) without an oxidizing agent [blank]. Another set of experiments was carried out in the presence of 1 % of BPO (0.02g) for the determination of the experimental Peroxide and p-Anisidine values.

Auto oxidation of fish oil

Fish oil [20mL sample] was added in a beaker and exposed to air for ten days. The resultant autoxidized oil was sampled at predetermined intervals to assess its oxidation state.

*Corresponding author email: bindiya1480@gmail.com

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Peroxide value determination

Peroxide Value [PV] measures peroxides contained in the oil and is determined by measuring iodine released from the oxidation of potassium iodide. The determination of PV was performed as per standard method.[12]

A fish oil sample (2 ±0.01 g) was dissolved in 30 mL iso-octane/acetic acid solution (2:3). The saturated Potassium Iodide [KI] solution and distilled water were added, followed by vigorous shaking to liberate iodine into the extracting chloroform layer. The mixture was titrated with 0.1 N sodium thiosulphate using starch solution as an indicator until the dark blue color disappeared. As per the kinetic studies of the auto-oxidation of oil, it is indicated that the rate of formation of the fatty acid hydroperoxides and the rate of degradation of polyunsaturated fatty acids is low at the start of the process.

The peroxide value was calculated from the following formula:

$$\text{Peroxide Value} = (\text{mEq peroxide kg–1 oil}) = [C(Va–Vb)/m] \times 1000$$

Where C represents the concentration of Titrant (mol/L), Va is the volume of Titrant (mL), Vb is the volume of blank, m is the mass of the oil sample (g), and 1000 is the unit conversion factor.

The analysis was performed in triplicate, and the experiments were carried out again in the presence of 1% of BPO. The results were reported in mEq peroxide kg^{-1} oil in Table I.

p-Anisidine value determination

The Anisidine value is defined as the spectroscopic absorbance of a solution of 1g of oil dissolved in 100mL of solvent (iso-octane) and added reagent (0.25% p-Anisidine, dissolved in glacial acetic acid). Aldehydes derived from the secondary oxidation of the oil matrix react with p-Anisidine, determining a variation in the absorbance measured at 350 nm. The p-Anisidine value was determined according to the protocol from AOCS Official Method.[13]

The 0.25% p-Anisidine reagent was prepared every working day. p-Anisidine [0.25 grams] was dissolved in 100 mL of 100% acetic acid, and the absorbance was measured to ensure a value below 0.2. To analyze the samples, about 0.1 grams of oil were weighed directly in test tubes and dissolved in 5 mL of iso-octane. An aliquot of 2.5mL of this sample was transferred to a cuvette, and the absorbance was measured at 350 nm against pure iso-octane as blank. Then, 0.5 mL of p-Anisidine reagent was added, and the cuvette was shaken by hand. The cuvette was kept in the dark for 10 minutes before the second absorbance measurement was made. The measurements were performed in triplicate. The analysis was performed again in the presence of 1% of BPO and the results are reported in the Table II.

Peroxide value (PV) and p-Anisidine value (p-AV) were used to measure the level of peroxide/hydroperoxide and secondary by-products formed during the oil oxidation. To measure the peroxide value (PV) and p-Anisidine value (p-AV) of the oil with 1% of BPO, the oxidation property of fish oil is studied and compared with that of fish oil without the oxidizing agent.

The p-AV was calculated using the following formula:

$$\text{p-Anisidine Value (p-AV)} = V \times \{[1.2 \times (ES2–B2) – (ES1–B1)]/m\}$$

Where V is the volume (mL) of iso-octane used to dissolve the oil sample, ES1 is the first spectrophotometric reading of the experimental sample, ES2 is the second spectrophotometric reading of the experimental sample, B1 is the first spectrophotometric reading of blank, B2 is second spectrophotometric reading of blank and m is mass (g) of oil sample.

Results and Discussion

Peroxide value determination

The official method for Peroxide value determination is based on an iodometric titration technique. During the process, the peroxides in the oil sample oxidize Iodide ion (I^-) to Iodine (I_2). Iodine then complexes with starch, resulting in the development of a dark blue color. Iodine is then reduced by thiosulfate, as indicated by the disappearance of the dark blue color. Therefore, it is concluded that the amount consumed of sodium thiosulfate solution can be directly related to the milliequivalents of hydroperoxides present in the oil sample.

During the course of the oxidation of fish oil with and without an oxidizing agent, the peroxide value (PV) was steadily increasing for three days. The actual rate of accumulation of peroxides was uneven over time and during storage, there were a series of maxima and minima. The presence of maxima at the beginning of oxidation (the 1st day) indicates the free-radical nature of oil oxidation. The highest rate of accumulation of peroxides was observed for the fish oil due to the presence of high unsaturation therein. Peroxides being unstable, primary products of fat oxidation quickly form new radicals or stable secondary products.

From Figures 1 and 2 it is concluded that, the PV values for fish oil with and without oxidizing agents shows appreciable changes. The PV values are measured with a time interval of one day. The fish oil without an oxidizing agent shows a gradual increase in PV values in the initial stage, reaching to maximum by the 6th day. The PV values start decreasing from the 7th day till the 10th day. This is due to the non-availability of driving force for further oxidation of oil where Peroxide stopped oxidizing iodide ion (I^-) to Iodine (I_2). But in the case of oil with an oxidizing agent, the initial 1st day gives a too high value of PV compared with oil without an oxidizing agent. This indicates the formation and enhancement of peroxide molecules in the oil to undergo an accelerated oxidation

Table I

PV values with and without oxidizing agents

SI. No	Time in days	PV value without Oxidizing Agent (meq/kg)	PV value with Oxidizing Agent (meq/kg)
1	1	1.573 ± 0.05	3.081± 0.05
2	2	1.886± 0.05	4.491± 0.05
3	3	2.221± 0.05	4.575± 0.05
4	4	3.123± 0.05	1.002± 0.05
5	5	4.266± 0.05	
6	6	4.133± 0.05	
7	7	3.699± 0.05	
8	8	2.418± 0.05	
9	9	1.054± 0.05	
10	10	1.005± 0.05	

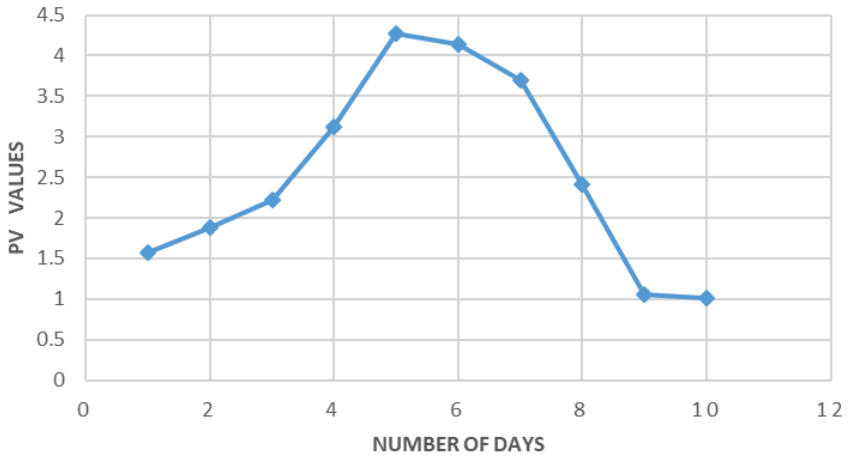

Figure 1. Peroxide values without oxidizing agent

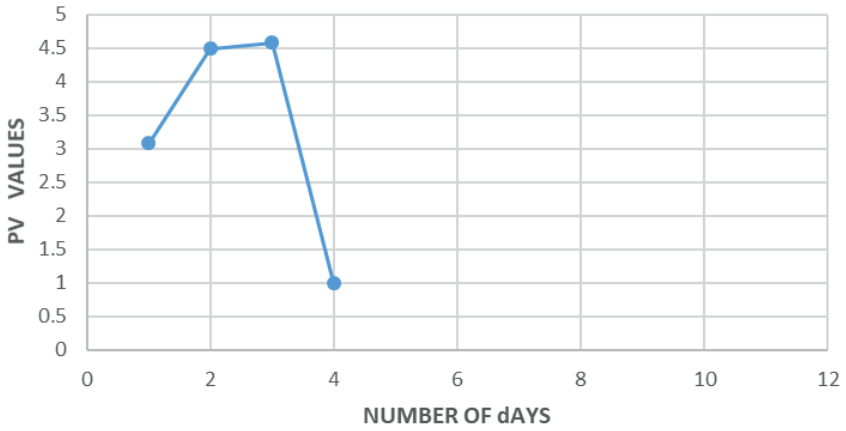

Figure 2. Peroxide values with oxidizing agent

Table II

p- Anisidine values with and without oxidizing agents

Sl. No	Time in days	p-AV value without oxidizing agent	p-AV value with oxidizing agent
1	1	0.11± 0.05	2.081± 0.05
2	2	0.23± 0.05	19.491± 0.05
3	3	1.52± 0.05	19.575± 0.05
4	4	2.52± 0.05	19.175± 0.05
5	5	4.69± 0.05	
6	6	19.235± 0.05	
7	7	19.089± 0.05	
8	8	18.135± 0.05	
9	9	17.175± 0.05	
10	10	17.063± 0.05	

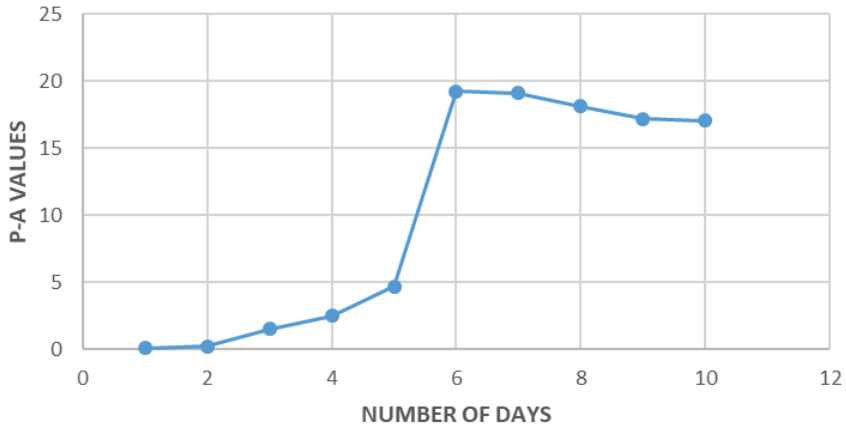

Figure 3. p- Anisidine values without oxidizing agent

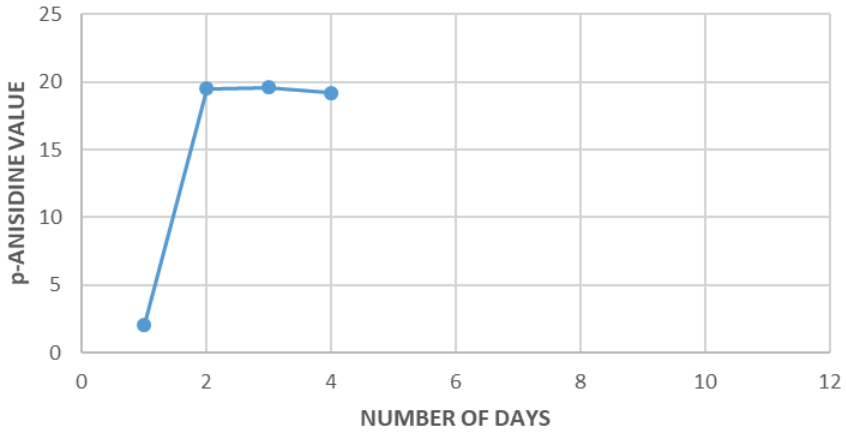

Figure 4. p- Anisidine values with oxidizing agent

process by the oxidizing agent. In this case, the oxidizing agent drives peroxide/hydroperoxide molecules to participate in the reaction with KI actively. For the subsequent days, the PV value for the sample with oxidizing agent increases gradually. In contast the 4th-day sample shows less PV due to the lower concentration of peroxide/hydroperoxide molecules. Therefore, the oxidizing agent in the initial stage speed up the reaction rate for the formation of required peroxide/hydroperoxide for the tanning process. It indicates that the oxidizing agent enhances the rate of peroxide formation and enables the long-term stable intermediate for higher PV values. It suggests that the sample without oxidizing agent will take up to 6 days to complete the oxidization reaction. In contrast, the sample with an oxidizing agent takes just 3 days.

p-Anisidine values determination

p-Anisidine values are essential for the complete study of secondary oxidation products of oxidized fish oil with and without an oxidizing agent.

The secondary oxidized products of fish oil without an oxidizing agent (BPO) were reported in less concentration initially from day one to day four and only increased from day 5 to day 7. The concentration of the same starts decreasing from day 8 till day 10 and stands nearly constant. This may be due to the slow and steady production of secondary by-products initially. After reaching maxima again, the production starts decreasing.

In contrast, fish oil with an oxidizing agent (BPO) exhibited high p-Anisidine values from day 2 to day 4. It is presumably due to the reaction of p- Anisidine reagent with the secondary by-products of fish oil oxidation such as aldehyde, acetone, and their derivatives.

Significance of the rate of oil oxidation

The present research establishes the synergistic effect rate of oil oxidation has in oil tanning using fish oil. The core objective was to explore the catalytic behavior of oxidizing agent towards accelerating the rate of oxidation of fish oil which in turn speeds up the oil tanning processes. Conventional oil tanning takes about 10-12 days and is labor intensive depending on environmental condtions. To accelerate the oil tanning process, oxidizing agents are preferred due to the reduction in manufacturing days. Several oxidizing agents have reported to achieve the completion of tanning in shorter duration. In our earlier studies,[14] we have reported the use of benzoyl peroxide as an oxidizing agent for chamois leather manufacture and documented that the fish oil tanning can be completed within 4 days and physical strength characteristics of the experimental leathers found to be as comparable to the conventionally processed chamois leather. The mechanism of oil tanning is based on the oxidation of oil leading to carbonyl groups that interact/react with the Ɛ-amino groups of collagen to form covalent bonds. The interaction of oil with collagen primarily depends on the rate of oil oxidation. To understand the behavior of benzoyl peroxide on fish oil, kinetics studies have been carried out in the present research work and discussed the peroxide and p-anisidine values. Peroxide and p-anisidine values are the tangible measurements to ascertain the rate of oil oxidation and the values are directly correspondent. Concentration of oil and benzoyl peroxide are simulated as mentioned in our earlier research article with 1% concentration of oxidizing agent and the values are calculated to understand the oil tanning mechanism. It has been found that the oxidation of oil completed within 4-5 days as inferred from peroxide and p-anisidine values which is in accordance with the leather manufacture, as reported in our prior research article. Comprehensively the study provides an insight on the importance of the kinetics of oil oxidation and its relevance in leather manufacture. The exploration also provides a tool to identify and screen new or other oxidizing agent for leather manufacture with p-anisidine and peroxide values, which is a direct indictor for the completion of oil tanning based on the choice of oxidizing agent and oil substrate.

Conclusion

The oxidation pattern of fish oil is confirmed by the study[14] whereas 1% of BPO was used to make fish oil-tanned leather (chamois). According to our studies, the use of 1% of BPO completed the fish oil tanning within four days with improved water absorption and physical properties of the experimental leathers. The chemical kinetics also confirms the completion of fish oil oxidation within four days.

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