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Analytical Methods for Determining the Peroxide Value of Hemp Seed (Cannabis Sativa L.) Oil

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ABSTRACT

The peroxide Value is the number that expresses, in milli equivalents of active oxygen, the quantity of peroxide contained in 1000 g of the substance. Hemp seed oil containing essential omega fatty acids and proteins. This organic hemp seed oil has a pleasant nutty smell, deep green hue, and absorbs well into the skin. Hemp-seed oil has several positive effects on skin problems such as dryness and those related to the aging process. Hemp seed oil sample were peroxide value (POV) assayed using a standard starch solution, American Oil Chemist's Society (AOCS) Method Cd 8-53, and used as a verified reference method for peroxide determination. Peroxide values (POV) is a standard test in the food and cosmetic industry to ensure product quality and self-life. In this study, peroxide value in Hemp Seed (Cannabis Sativa L.) oil has been determined by iodometric titration method using acetic acid-chloroform reagent. The average result is less than 4.0 meq O_2 / Kg. Relative repeatability Standard Deviation %RSD is 2.64 %. The average result meets the specification.

Keywords: Peroxide Value, Cannabis Sativa, Hemp Seed Oil, Sodium thiosulphate.

INTRODUCTION

Hydrogen peroxide has been used widely as an oxidant [1] for many years in numerous products as well as in technical and chemical process. The main field of application is cellulose and paper bleaching followed by chemical industry [2]. Hydrogen peroxide, H_2O_2 may cause long-term adverse effects in the environment. Not expected to be harmful to aquatic organisms. Causes eye burns, harmful if inhaled. May cause irritation of respiratory tract. Keep away from heat and sources of ignition. Store in a well-ventilated place [3].

The peroxide value (POV) of an edible oil is an indicator of freshness as viewed through oxidative degradation. Chemically, peroxide value is measurement of the primary oxidation of hydroxyl groups of unsaturated fats in oils by molecular oxygen into hydro peroxides and peroxides [4]. This measurement is often presented in mill equivalents O_2/Kg (meq H_2O_2/Kg)

of oil. Full auto-oxidation of oils further converts the created peroxides and hydro peroxides into alcohols, aldehydes and ketones, which are directly responsible for the rancidity of the oil [5,6]. Fresh oils have peroxide values below approximately 10 meq O_2/Kg [5]. Furthermore, peroxide values as high as 100 meq O_2 /Kg have been linked to cases of food poisoning [7]. The American Oil Chemists Society (AOCS) Official Method Cd-8-53 [8] and the Commission Regulation (EEC) No: 2568/91 of 11 July 1991 [9] have established standard Iodometric titrations for the determination of edible oil peroxide value (POV) in an attempt to maintain product quality control [10].

Hemp (Cannabis Sativa) Seed Oil thought to be of Asian origin, the hemp plant has reached around the globe. Cannabis Sativa L. Oil also known as-hemp seed oil, hemp oil and hempoil. Oils from hemp seeds contain valuable cis-polyunsaturated fatty acids(PUFAs) (such as oleic acid) [11]. Adding food made with hemp seed oil to the diet seem to lower risk of heart attacks because of omega-3 fatty acids reduce the clotting tendency of the blood and improve cholesterol profiles, blood circulation and brain health. They also have a natural anti-inflammatory effect that makes them useful for people with arthritis and autoimmune disorders [11].

Hemp seed oil is easily digestible in its raw state. It contains less than 10% saturated fats, and 70-80% polysaturated fatty acids. It contains 55% Linoleic (omega-6) and 20% alpha-linoleic (omega-3) acids and antioxidants like vitamin E and carotene. Hemp seed oil should not be fried, and should preferable be used cold. Hemp seed oil may be added to body care or cosmetic products, including anti-aging, acne, sun care, shaving products, lotions, facial or body oils, massage oils, shampoo, conditioner, lip balm, soap, hair care products, and any other products. Hemp seed oil can cure skin issues such as psoriasis and eczema. Hemp seeds oil also high in magnesium, phosphorus, potassium and zinc.

MATERIALS AND METHOD

Reagents and Solutions

All reagents are analytical reagent grade and only deionized water should be used.

- Carbon dioxide free D.I. Water, on the day of use. Water was purified using a Millipore Milly-Q system via a pure water device marked Purelab Option-Q7BP.
- Chloroform, Pharmco-Aaper, HPLC grade, 99.96%, Lot # PB004029CHL
- 3.5 M KCl Electrode Fill Solution- HI7082
- Hemp Seed Oil CAS No: 8016-24-8
- Acid Mixture: Dissolve 0.18 g ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$) in 750 mL water. While stirring, slowly add 300 mL of H_2SO_4 (conc.). Wear safety googles and gloves when handling concentrated H_2SO_4.
- Acetic Acid-Chloroform solution- Mix 3 volumes CH_3COOH with 2 volumes CH_3Cl_3 [Caution: Acetic Acid-Chloroform solvent mixture is toxic by ingestion and inhalation. It is a Strong irritant to skin and tissue. A properly operating fume hood should be used when working with this solvent mixture.
- Acetic Acid, Sigma-Aldrich >99%, Lot # SHBG5375V
- Potassium Iodide, KI (10%) Test Solution (TS). Dawn Scientific, Inc. lot # 215801. Dissolve 100 mg potassium Iodide (KI) in 1,000 mL of water.

- Starch Indicator Solution, 0.5%. (5.0 g/L); Weigh 0.5 g of soluble starch into a 150-mL beaker. While stirring, gradually add about 5 mL of water until a paste is formed. Add the paste to 100 mL of boiling water. Cool and add 5g of potassium iodide. Stir until dissolution is complete and transfer to a plastic bottle.
- Potassium Iodate Solution (KIO_3) (0.1N): Weigh (to the nearest 0.1 mg) about 3.57 g dried primary standard potassium iodate and transfer to a 1-liter volumetric flask. Add 400 mL of H_2O, 2.0 g of sodium hydroxide (NaOH), and dilute to volume and mix well.
- Dilute sulfuric acid H_2SO_4
- Preparation of 0.1N Sodium Thiosulfate Dissolve 24.8g of sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$) in 800 ml of freshly boiled and cooled water and mix thoroughly by shaking for approximately 15 minutes. Make up the volume to 1000 ml. Preparation of 0.1N

Equipment

- Timer
- Electrodes: Platinum-reference combination electrode (long type)
- 50 mL burette (Class A) for titration.
- Iodine Flask, borosilicate glass, 250 mL capacity, glass-stoppered.
- Erlenmeyer flask, glass- stoppered, 125-mL and 250-mL.
- Pipette, Mohr, measuring type, 1-mL capacity and, 20 mL
- Magnetic stirrer
- Analytical balance, Class B (± 0.1 mg/L)
- Medical dropper.

EXPERIMENTAL

Standardization of Sodium Thiosulfate ($Na_2S_2O_3$) (0.1N)

Sodium thiosulfate ($Na_2S_2O_3$) is commonly standardized using potassium iodate (KIO_3) or potassium dichromate ($K_2Cr_2O_7$) in the presence of excess potassium iodide (KI), which liberates iodine (I_2) for titration. Here's the process using potassium iodate, which is the most common and accurate method.

Standardization Procedure:

Pipet 20.0 mL of KIO_3 solution into a 125 mL Erlenmeyer flask and stir in about 1 g KI. Add 20 mL D.I. water. Add about 1 mL H_2SO_4. Immediately titrate the dark brown solution with $Na_2S_2O_3$ to a straw yellow color. Add 2 pipets full (about 4 mL) of starch solution. Continue the titration until the purple color disappears and solution becomes colorless. Calculate the $Na_2S_2O_3$ concentration using the titration equations.

$$IO_3^- + 5I^- \rightarrow 3I_2$$

$$3I_2 + 6S_2O_3^{2-} \rightarrow 6I^- + 3S_4O_6^{2-}$$

(i.e. 6 mmol $S_2O_3^{2-}$ per mmol IO_3^-).

Record the titration volume and calculate the normality of the sodium thiosulfate solution as shown below. Re-standardize every few days, or as often as the solution is needed.

Repeat procedure two additional times (3 total) and take the three values as the standardized normality.

$$\text{Normality of sodium thiosulfate solution} = \frac{(\text{g KIO3})(mL\ KIO3)}{(\text{mL } Na_2S_2O_3)(35.67\ g.\frac{L}{eq})}$$

858.0 mg KIO_3	20 mL KIO_3	mmol KIO_3	6 mmol S_2O_3	
	100 mL	214.001 mg	mmol KIO_3	47.96 mL S_2O_3

= 0.10032 N

858.0 mg KIO_3	20 mL KIO_3	mmol KIO_3	6 mmol S_2O_3	
	100 mL	214.001 mg	mmol KIO_3	47.95 mL S_2O_3

= 0.10033 N, Average normality = 0.10030 N

Determination of Hydrogen Peroxide Procedure

Weigh 5.00 ± 0.05 g of sample [5.0921 g and, 4.965 g hemp seed oil were taken] into a 250-mL glass-stoppered Erlenmeyer flask and then add 30 mL of a mixture of glacial acetic acid-chloroform solution [3:2], swirl the flask until the sample is dissolved in the solution. Add 0.5 mL of saturated potassium iodide (KI) solution preferably Mohr type measuring pipette. Allow the solution to stand with occasional shaking for exactly 1 minute and then add 30 mL of distilled water. Titrate with 0.1 N sodium thiosulfate ($Na_2S_2O_3$) adding it gradually and with constant and vigorous shaking. Continue the titration until the yellow color has almost disappeared. Add 0.5 mL of starch indicator solution. Continue the titration, shaking the flask vigorously near the endpoint to liberate all the iodine from the chloroform layer. Add the thiosulfate dropwise until the blue color has just disappeared. Conduct a blank determination of the reagents daily. The blank titration must not exceed 0.1 mL of 0.1N sodium thiosulfate solution [8].

Table 1: Determination of Hydrogen Peroxide(H_2O_2)

Weight (mg)	Volume of Titrant mL			Corrected Volume V net (mL)	Peroxide Value	Average Peroxide Value	%RPD
	Initial reading	Final reading	Total, mL				
Blank	0.00	0.90	0.90			3.79	2.64
5.0921	0.90	1.99	1.09	0.19	3.74		
4.9650	2.00	3.09	1.09	0.19	3.84		

Specification is <4.0 meq O_2/Kg.

RESULTS AND DISCUSSION

The sample is treated in solution with a mixture of acetic acid and a suitable organic solvent and then with a solution of potassium iodide. The liberated iodine is titrated with a standard solution of sodium thiosulfate. Peroxide values are expressed either in milli-equivalents of

peroxide/kg or in milli-moles of peroxide/L. We measured peroxide value in hemp seed oil to monitor oxidative stability, detect early sign of rancidity, ensure product quality, and comply with safety regulations.

Reaction Scheme

The peroxide value is determined by measuring the iodine liberated from potassium iodide by a peroxide, using sodium thiosulfate solution as the titrant. In the presence of acetic acid, the reaction scheme for hydroperoxides is as follows. Generation of hydroperoxides:

$$R\text{–}H + O_2 \rightarrow ROOH \qquad \text{(Reaction I)}$$

Generation of iodine:

$$KI + CH_3COOH \rightarrow HI + CHCOO^-K^+ \qquad \text{(Reaction II)}$$

$$O_2^{2-} + I_2 + 4H^+ \rightarrow I_2 + 2H_2O$$

$$ROOH + 2HI \rightarrow ROH + H_2O + I_2 + \text{starch indicator} \qquad \text{(Reaction III)}$$

Titration step:

$$I_2 \text{ (purple)} + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI \text{ (colorless)} \qquad \text{(Reaction IV)}$$

$$I_2 + 2S_2O_3^{-2} \rightarrow 2I^- + S_4O_6^{2-}$$

The reaction of peroxides of the structures R-O-O-R' and follows an analogous pathway, whilst cyclic peroxides do not react quantitatively under the conditions described here [12].

CALCULATION

$$\text{Peroxide Value (POV)} = \frac{mL\ of\ titrant\ x\ N\ of\ Na2S2O3}{\text{weight of sample g}} \text{ x } \frac{1000\ g}{1\text{Kg}}$$

0.19 mL	0.1003 mmol S_2O_3	Meq H_2O_2		1000 g
	mL	1 mol S_2O3	5.0921 g	Kg

Peroxide Value (POV) = 3.74 meq H_2O_2 / Kg

0.19 mL	0.1003 mmol S_2O_3	Meq H_2O_2		1000 g
	mL	1 mol S_2O3	4.9650 g	Kg

Peroxide Value (POV) = 3.84 meq H_2O_2 / Kg

Average Peroxide Value (POV): 3.79 meq H_2O_2/Kg

% RPD = 2.64 %

CONCLUSION

The results for the determination of peroxide value (POV) using iodometric titration method is given Table1. Sample result and sample duplicate result have been 3.74 meq H_2O_2/Kg and 3.84 meq H_2O_2/ Kg respectively. The difference between two test results on the same Hemp Seed (Cannabis Sativa L.) Oil in the same laboratory under the same conditions, not exceed the repeatability value. Relative repeatability standard deviation, %RSD is 2.64%. Average test result meets the specification.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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