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Determination of Peroxide Values of Edible Oils by Ultraviolet Spectrometric Method

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Abstract A novel and rapid method for measuring the peroxide value (PV) in edible oils by using ultraviolet (UV) spectrometric method was developed. The technique was based on spectrophotometric monitoring at 266 nm of triphenylphosphine oxide (TPPO). TPPO is formed from stoichiometric reaction of hydroperoxides present in oil with triphenylphosphine (TPP), which exhibits a measurable absorption band at 264 nm. Calibration standards, prepared by the gravimetric addition of peroxide-free oil, were used to develop a linear calibration equation that relates the concentration of TPPO (expressed as equivalent PV) to the absorption value of TPPO in the UV spectra. Various PV standards and common edible oils were taken to validate the calibration. Using the linear regression, the proposed method was correlated with the standard American Oil Chemists' Society titration method. Best correlation coefficients were achieved, proving that no significant difference exists ($p<0.05$) in the results. The improved UV approach was not affected by the oil types and can be successfully applied to a variety of edible oils. The proposed method is accurate and simple and could be used to serve as an alternative to the conventional method for both qualitative and quantitative analyses of PV in edible oils.

Keywords Edible oils · Peroxide value · Ultraviolet spectroscopy · Determination

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Introduction

Fats and oils are recognized as essential nutrients in both human and animal diets. Edible oils are susceptible to oxygen in air and produce hydroperoxide as primary oxidation products. The subsequent decomposition reactions generate various low-molecular-weight secondary products, including free fatty acids, ketones, aldehydes, and alcohols (Köckritz and Martin 2008). The autoxidation of oil is an important deteriorative reaction that has significant commercial implication in terms of product value in particular relative to off-flavors. Peroxide value (PV), a common parameter in the chemistry of fats, is an indicator of the initial stages of oxidation and measures the amount of total peroxides in a substance. Although PV is not associated with rancidity in lipid-containing food products per se, it is considered one of the most frequently determined quality parameters during oil production, storage, and marketing (Saad et al. 2006). PV is also a useful indicator to control food quality and safety (Shiozawa et al. 2007).

The most reliable and widely used method for determination of PV is the well-established American Oil Chemists' Society (AOCS) iodometric titration method, which is based on the stoichiometric conversion of potassium iodide to molecular iodine by hydroperoxides present in oil (AOCS 1997). The liberated iodine in the acidic environment is titrated with standardized sodium thiosulfate (Ruíz et al. 2001). Besides their adequate sensitivity and simplicity, titrimetric methods are highly empirical and labor-intensive, with the organic solvents (Guillén and Cabo 2002). In addition, titrimetric methods involve the oxidation of excess iodide by atmospheric oxygen. Thus, titration must be required immediately (Dhaouadi et al. 2006). When exposed to sunlight or in acidic solutions, sodium thiosulfate titrant will decompose, thereby, causing erroneous results. Another well-recognized problem

of this method is that its accuracy depends on a variety of experimental parameters, such as temperature, accurate timing, and protection of the reaction mixture from light. For colored samples, given their inherent color, oil particles that persist in the aqueous phase after shaking can be misinterpreted as triiodide residue, with the reproducibility of the results affected (Nouros et al. 1999). Therefore, in the literature, alternative approaches have been reported for PV determinations that were based on either direct or indirect measurement of hydroperoxides. A successful PV approach based on the stoichiometric reaction of hydroperoxides with triphenylphosphine (TPP) to produce triphenylphosphine oxide (TPPO) and corresponding hydroperoxide alcohols was developed (Stein and Slawson 1963; Porter et al. 1979; Yu et al. 2007). Ma et al. (1997) overcame the limitations of original approach and used Fourier transform infrared (FTIR) spectroscopy to measure the hydroperoxide-related functional groups to quantify the PV in various edible oils based on this well-known stoichiometric reaction. The TPPO formed produces an intense band at 542 cm^{-1} in the mid-IR spectrum. Based on the stoichiometric reaction, Dong et al. (1997) predicted PV by using the near infrared reflection (NIR) spectral region from 4,710 to 4,540 cm^{-1} where TPP and TPPO both absorb. Ma et al. (2000) developed a mid-FTIR spectroscopic method specifically suited to monitoring oils with high PV. Li et al. (2000) upgraded the NIR PV method for the determination of PV over the range of 0–10 mmol/kg, while Ruíz et al. (2001) employed continuous flow system and FTIR spectroscopic detection to determine PV in edible oils. Yu et al. (2009) described an FTIR spectrometer coupled to an autosampler and some attendant methodologies for a TPP-based method for determination of PV of edible oils by FTIR spectroscopy using spectral reconstitution. Although workable, such spectrophotometry requires infrared spectrophotometers which are expensive and less common than ultraviolet (UV)-visible spectrometers which are ubiquitous in most laboratories. Talpur et al. (2010) developed an effective UV spectrometric method for the determination of PV of the frying oil based on the TPP-TPPO stoichiometric reaction. Quantification of TPPO produced exhibits a readily measurable absorption band at 240 nm in the UV portion of the spectrum. However, this method could only be used in measuring PV values in rapeseed oil, limiting the method. The application of UV spectrometry to oils and fats is a common quality in composition studies (Gotoh et al. 2011; Bezzi et al. 2008; Kardash-Strochkova et al. 2001; Moh et al. 1999). The objective of this work was to develop a general UV method for the determination of PV according to the well-recognized TPP-TPPO reaction. As illustrated by PV analysis, the oil dependency limitations continue to impede the exploitation of the benefits associated with UV methodology for edible oil analysis.

Material and Methods

Materials and Reagents

Analytical grade chemicals and distilled deionized water were used in the experiment. TPP (>99 %) and TPPO (>99 %) were obtained from Sigma-Aldrich, and separated concentrated stock solution (40 %, *w/w*) of TPP and TPPO was prepared in chloroform. Both TPP and TPPO were ground in a mortar and pestle and passed through a 60-mesh sieve to facilitate their dissolution. Potassium iodide, sodium thiosulfate, and acetic acid were obtained from Bodi Chemical Company (Tianjin, China).

Various edible oils including corn oil, rapeseed oil, soybean oil, blend oil, deep sea fish oil, camellia oil, palm oil, peanut oil, and olive oil (including virgin olive oil and refined olive oil) were purchased from Xi'an Yihai Kerry Oils and Fats Industry Co. Among these, refined rapeseed oil was used as the base oil for the preparation of calibration standards; the other oils were blended with a high PV rapeseed oil to produce samples of varying PV for methodology evaluation. Refined rapeseed oil was passed through a column of microwave-activated silica gel to remove partially polar oxygenated molecules, particularly any residual hydroperoxides. The rapeseed oil was verified to be peroxide-free by AOCS Cd 8b-90 titrimetric PV procedure.

Instrumentation

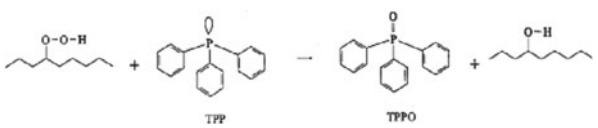
For this work, a UV-2550 double-beam UV spectrophotometer (Shimadzu, Japan) was employed, using standard 1.00 cm quartz cells, scanning from 230 to 290 nm range.

Stoichiometric Analysis

PV is determined by the reference method according to the reactions given below.

$$ROOH + 2KI \rightarrow K_2O + I_2 + ROH$$
$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$$

On the basis of the stoichiometry of the two reactions, the AOCS method uses mixture of oil and chloroform/acetic acid, which was left to react with a solution of potassium iodide in darkness; the free iodine then titrated with a sodium thiosulfate solution.



A schematic of the stoichiometric reaction of TPP with hydroperoxides to produce TPPO is shown. In the presence of an excess of TPP, hydroperoxides are rapidly and completely converted into alcohols while oxidizing a stoichiometric amount of TPP to TPPO (Talpur et al. 2010).

Preparation of Calibration Standards and Validation Samples

A series of TPPO standard samples covering the range 0–14 mmol/kg was prepared by mixing 4 g of zero-PV rapeseed oil sample with the stock solution of TPPO in a 25-mL vial and heating them in a microwave oven for ~30 s to facilitate the dissolution of TPPO. Chloroform was then added up to 25 mL, and the mixture was shaken thoroughly. Chloroform and the stock solution of TPPO (40 %) were added to labeled blank reagent (BR) and reactive reagent (RR), respectively. All the samples were diluted to appropriate concentration, and the UV spectra of RR with BR as reference were collected. The series of TPP standard samples covering the range 0–14 mmol/kg was prepared in the same manner. The net effect is to vary TPP-TPPO ratio in the presence of oil based on the stoichiometry of reaction.

Validation samples were prepared by gravimetrically mixing oxidized oil and zero-PV rapeseed oil to produce samples having PV values over a range of 0–14 mmol/kg. Two additional sets of samples were prepared in the same manner utilizing peanut (PV ~14 mmol/kg) or camellia oil (PV ~6 mmol/kg) instead of oxidized rapeseed oil. The samples involved the mixing of oil (4.000 g) with TPP (40 % in chloroform) shaken on a vortex mixer for 30 s to complete the reaction of ROOH with TPP to form TPPO; chloroform only was then added and diluted similarly to the sample. Subsequently, 50 μL of chloroform and 50 μL of the stock solution of TPP (40 %) were added to labeled BR and RR, respectively. All the samples were diluted to appropriate concentration, and the UV spectra of RR with BR as reference were collected.

PV Determinations

The efficacy of the calibration to predict PV was assessed by comparing the predicted PV by the UV method for validation samples with the values obtained by the AOCS method.

Analytical Protocol

Approximately 4 g of the oil sample was mixed with the stock solution of TPP (50 μL) in a 25-mL vial shaken on a vortex mixer for 30 s to complete the reaction of peroxide of oil with TPP to form TPPO. Chloroform was then added up to 25 mL until it was well mixed. Approximately 50 μL of chloroform and 50 μL of the stock solution of TPP (40 %) were added to labeled BR and RR, respectively. All the samples were diluted to appropriate concentration, and the UV spectra of RR with BR as reference were collected.

Calculations and Statistical Analyses

Each standard and sample was analyzed three times. The data obtained were entered into OMNIC and TQ Analyst program.

Results

General Spectroscopy

This study aimed to determine the feasibility of spectroscopic determination of PV. Figure 1 illustrates the UV spectra of the chloroform diluent spiked with TPP or TPPO and that of the diluent containing TPP and TPPO with chloroform as reference.

The differential spectra clearly show that TPPO has a characteristic absorbance at 238 and 266 nm, and the corresponding TPP band exhibits a measurable absorption at 264 nm. The diluents containing TPP and TPPO with the same concentrations reveal an absorption band at 266 nm, whereas TPP and TPPO in UV spectroscopy influenced each other. Therefore, based on the stoichiometric reaction of TPP with the ROOH present in oil to produce TPPO, the UV method can take advantage of the absorbance of TPP and TPPO at specific wavelengths to figure out TPPO formation and to further determine the hydroperoxide content.

Quantitative Chemical Analysis

The TPP mole number is equal to the generated TPPO mole number and the amount of hydroperoxide. A test set of samples was prepared by serial of TPP and TPPO peroxide-free

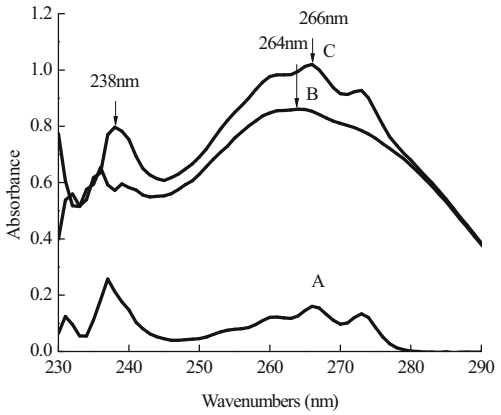


Fig. 1 Differential spectra of TPPO (**a**), TPP (**b**), and TPP + TPPO (**c**) in chloroform with the same concentration

oil with fresh peroxide-free oil to simulate chemical reaction process (Table 1).

Along with the decrease of PV_{TPP} and the increase of PV_{TPPO}, the absorbencies of samples show a corresponding reduction with range from 264 to 266 nm (Fig. 2). Experimentation shows that the characteristic absorption peak of samples is 264 nm. However, when PV_{TPPO} becomes larger to some given extent, the characteristic absorption peak of samples is 266 nm. Given that both the TPP and TPPO characteristic absorption influenced the absorption peak of samples, we propose that they be combined.

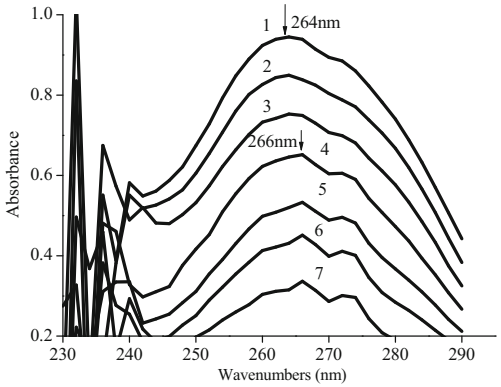


Fig. 2 UV spectra of TPP and TPPO in peroxide-free rapeseed oil

Calibration

Analysis of Standard Samples

The UV spectra of TPPO and TPP standards were collected with BR as reference. The relationship between the absorbance and PV_{TPP} or PV_{TPPO} at 264 or 266 nm was established. The equations of absorbance vs. concentration of TPP were $PV_{TPP/264\ nm}=0.141+24.038Abs$ ($R=0.9987$) and $PV_{TPP/266\ nm}=0.124+24.356Abs$ ($R=0.9986$). The equations of absorbance vs. concentration of TPPO were $PV_{TPPO/264\ nm}=5.629+247.414Abs$ ($R=0.9992$) and $PV_{TPPO/266\ nm}=3.931+186.452Abs$ ($R=0.9986$).

The plot of the absorbance vs. the concentration of TPP or TPPO has a good linear relationship ($R\geq0.99$). The sum of PV_{TPPO} and PV_{TPP} is specified as a parameter to M, which does not change with the stoichiometric reaction of TPP with hydroperoxides to produce TPPO according to TPP and TPPO equimolar transformation. These relation analyses led to the mathematical relationship of the absorbance of the TPP and TPPO mixed system at 264 or 266 nm vs. PV_{TPPO}.

$$PV_{TPPO/264\ nm} = 1.107M-0.762-26.620Abs \quad (1)$$

$$PV_{TPPO/266\ nm} = 1.150M-0.733-28.015Abs \quad (2)$$

Simulation of Stoichiometric Reaction

In this experiment, 50 μL of the stock solution of TPP (40 %) mixed with 4.000 g oil showed a PV_{TPP} of 25.02 mmol/kg. Thus, M in Eqs. (1) and (2) is evaluated to be 25.02 mmol/kg, which is used to calculate Eqs. (3) and (4).

$$PV_{TPPO/264\ nm} = 26.935-26.620Abs \quad (3)$$

$$PV_{TPPO/266\ nm} = 28.040-28.015Abs \quad (4)$$

The TPP/TPPO mixed zero-PV oils were prepared with various ratios of TPP and TPPO, ensuring that sum of PVTPP and PV_{TPPO} is 25.02 mmol/kg to reflect the stoichiometric reaction. The peak absorbance of the TPP/TPPO blended oils was tested. PV_{TPPO} results obtained by Eq. (3) or Eq. (4) depended on the wavelength of maximum absorption, corresponding to 264 nm in Eq. (3) and 266 nm in Eq. (4). Equations (5) and (6) obtained the predicted PV_{TPPO} based on the above relation at a certain corresponding wavelength vs. true PV_{TPPO} converted by actual addition.

$$PV_{Predicted/264\ nm} = 0.0755 + 0.985PV_{true},\ (R = 0.9995) \quad (5)$$

$$PV_{Predicted/266\ nm} = 0.0220 + 0.999PV_{true},\ (R = 0.9998) \quad (6)$$

Table 1 Calibration matrix of oil containing serial stock solution of TPP and TPPO

Samples	1	2	3	4	5	6	7
25 PV TPP oil (g)	3.23	2.84	2.46	2.01	1.48	1.25	0.81
23.6 PV TPPO oil (g)	0.31	0.73	1.12	1.60	2.16	2.41	2.87
Pure oil (g)	0.43	0.43	0.42	0.43	0.43	0.43	0.43
PV_{TPP}	20.19	17.75	15.38	12.56	9.25	7.81	5.06
PV_{TPPO}	1.83	4.31	6.61	9.44	12.74	14.22	16.93
$PV_{TPP\ +\ TPPO}$	22.02	22.06	21.98	22.00	21.99	22.03	22.00

PV peroxide value, PV_{TPP} PV_{TPPO} the amounts of triphenylphosphine oxide (molecular weight=262.28) and triphenylphosphine (molecular weight=278.29) added to peroxide-free rapeseed oil are expressed in terms of PV equivalents, based on the stoichiometry of the reaction between TPP and hydroperoxides to form TPPO, *TPP* triphenylphosphine, *TPPO* triphenylphosphine oxide

The equations indicate that the true PV_{TPPO} correlates well with the predicted value what is obtained by the standard AOCS titration method. The experimental results suggest that the wavelength of maximum absorption is 266 nm when PV_{TPPO} exceeds 5.95 (±0.5)mmol/kg. However, the wavelength becomes 264 nm as PV_{TPPO} decreases to 5.95 (±0.5)mmol/kg. Analyses of Eqs. (3) and (4) show that the absorbance of blended oil is equal to 266 nm, corresponding to PV_{TPPO} of 5.849 mmol/kg, with the experimental results being identical. Taken together, our results reveal that the wavelength of maximum absorption changed due to the concentration of the TPP-to-TPPO ratio.

Validation

The UV method was validated using two sets of oils, one spanning a PV range of 0–6 mmol/kg and the other 6–15 mmol/kg. The sets of oil were prepared by gravimetrically mixing oxidized oil and zero-PV rapeseed oil. The camellia oil (PV ~15 mmol/kg) and zero-PV rapeseed oil produced a set of samples with PV of 0–6 mmol/kg, accordingly. The other set of samples with PV of 6–15 mmol/kg was prepared by mixing peanut oil (PV ~14 mmol/kg) and zero-PV rapeseed oil. Figure 3 shows the results of the determination of PV by UV and iodometry.

As seen in Fig. 3, two methods for PV vs. oxidized oil (camellia oil or peanut oil) were measured and were linearly related. The equations show that they have similar slopes and the results of the two methods being identical. The standard deviation (SD) of the UV method is less that of AOCS iodometry, as is repeatability. Meanwhile, the experiments show that the samples of 0–6 mmol/kg have characteristic absorption peak at 264 nm, while the samples of 6–15 mmol/kg have characteristic absorption peak at 266 nm.

Investigation of Oil Dependency

As seen in the plot of TPPO concentration (expressed as PV) vs. absorbance, a non-zero intercept exists at 266 or 264 nm, which is due to the minor contribution of oil to the absorbance. This non-zero intercept raised the possibility that PV values obtained by the UV procedure would be affected by the type of oil being analyzed. To investigate this possibility, various oils were analyzed by both UV and AOCS iodometry.

Figure 4 illustrates that the PV results by two methods are in good linear correlation ($R \geq 0.99$), with a slope close to 1 and the intercept close to 0. Thus, the two methods track each other well, and no effect is associated with oil type.

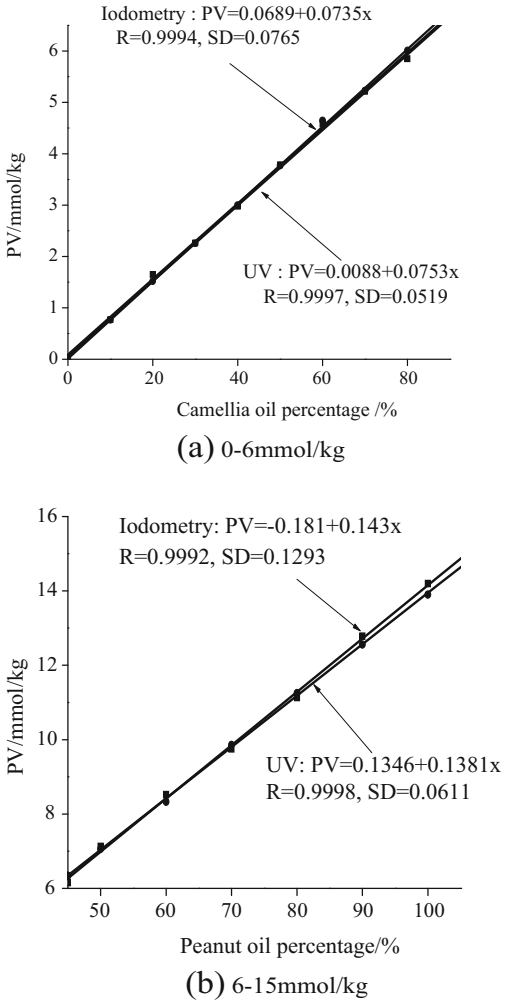


Fig. 3 Plots of PV results obtained from the valiation samples by UV and AOCS iodometry. **a** 0–6 mmol/kg. **b** 6–15 mmol/kg

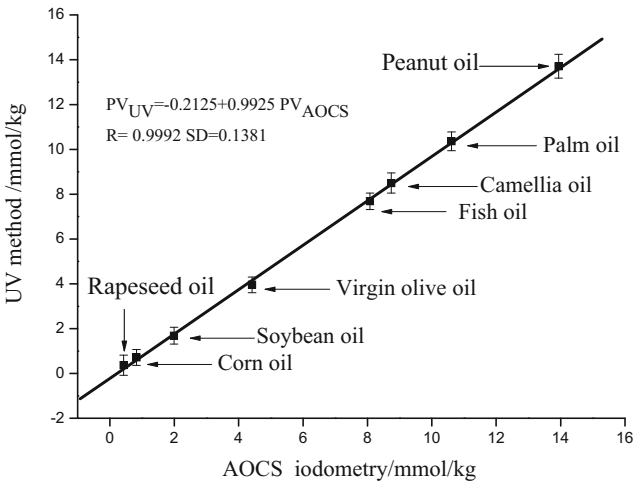


Fig. 4 Plot of PV determined by UV and AOCS iodometry for different types of oil

Discussion and Conclusion

The results obtained using the new UV and AOCS method were highly consistent, with the repeatability of the UV method being better than that of the AOCS method because the SD of the UV method is lower than that of AOCS iodometry. The characteristic absorption peak of TPPO and TPP is close to each other. The UV method was based on spectrophotometric monitoring at 266 nm of TPPO. TPPO is generated from the stoichiometric reaction of hydroperoxides present in oil with TPP, which exhibits a measurable absorption band at 264 nm. The sum of the molality of TPPO and TPP is 25.02 mmol/kg when the characteristic absorption appeared at 264 nm. This finding proved that PV was not above 6 mmol/kg, and the equation was $PV_{TPPO/264\ nm}$=26.935–26.620Abs. In addition, when the characteristic absorption appeared at 266 nm, the PV was above 6 mmol/kg, and the equation was $PV_{TPPO/266\ nm}$= 28.040–28.015Abs. As stated in the provisions of CODEX STAN 210-1999 Codex standard for named vegetable oils, the PV of non-oxidized oil should be below 5 mmol/kg. Therefore, to some extent, qualitative analysis of the degree of oxidation of edible oil could be achieved using the characteristic absorption peaks.

By contrast, complicated steps are involved, and large testing agent consumption is made in the traditional AOCS titration method. Thus, the proposed UV method not only eliminates the use of polluting reagents but also has better stability compared with AOCS titration method, and UV spectrophotometer is much cheaper and has the same role of measuring PV in edible oils compared with infrared spectrophotometer, it is easy to use in quality control laboratory. UV spectroscopy has the unique ability of simultaneously determining multiple indicators. Additional research is currently under way to further research should constructing involve a comprehensive index to fully reflect the degree of oxidation. This degree shall be expressed through commonly used indicators of oxidation, such as AV, CV, and their weights.

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Compliance with Ethics Standards

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Conflict of Interest Ning Wang, Tian Ma, Xiuzhu Yu, Lirong Xu, and Rui Zhang declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Not applicable.

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