



Thermal decomposition and oxidation of β -caryophyllene in black pepper during subcritical water extraction

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Abstract Subcritical water extraction is an efficient technique for extracting components from various plants by changing the polarity of water. β -caryophyllene is a natural bicyclic sesquiterpene with the highest content found among black pepper essential oils. In this study, the efficiency of extraction and yield of β -caryophyllene from black pepper were investigated using a subcritical water extraction technique. The optimal conditions of β -caryophyllene (1.19 ± 0.38 mg/g), and caryophyllene oxide (0.82 ± 0.38 mg/g) were obtained from black pepper under extraction conditions of 170 °C/10 min, and 200 °C/15 min, respectively. As the extraction temperature was increased, β -caryophyllene oxidation proceeded and the extraction content of caryophyllene oxide increased. It is anticipated that both β -caryophyllene and caryophyllene oxide with high biological activity can be used to selectively extract compounds using subcritical water extraction, which will be helpful in industrial applications.

Keywords Subcritical water extraction · Black pepper · Caryophyllene · Thermal decomposition · Oxidation

Introduction

Black pepper (*Piper nigrum* L.) is a very common and important flavoring agent of the Piperaceae family (Bastos et al., 2020). It is often called “the king of spice” in tropical countries and is one of the most widely used spices because of its characteristic aromatic odor and pungency (Joshi et al., 2018; Kapoor et al., 2009). Black pepper is grown by harvesting mature *P. nigrum* fruits and drying them in the sun. The essential oil of black pepper includes monoterpenes and sesquiterpenes, which are composed of terpenoids. This indicates anticancer, antifungal, and immunomodulatory activities (Collings et al., 2018; Myszka et al., 2017). In pepper oils, 94 monoterpenoids and sesquiterpene hydrocarbons have been identified, and only formed 13% of caryophyllene oxide (Gopalakrishnan et al., 1993; Ozel et al., 2003). The main aromatic compound in pepper berries is piperine (Wang et al., 2018). Recent studies have shown that piperine, an alkaloid amine component, has excellent anticancer and antioxidant properties and sterilization effects (Shityakov et al., 2019; Zarai et al., 2013). However, other terpenic compounds besides piperine affect health and biological activities. Representative terpene compounds include β -caryophyllene, α -pinene, β -pinene, limonene, sabinene, and linalool (Zachariah et al., 2010).

β -caryophyllene is the most abundant component in black pepper oil (Tran et al., 2019). It is a natural bicyclic sesquiterpene, the most ubiquitous sesquiterpene hydrocarbon in the plant kingdom. β -caryophyllene is known to have many biological activities. A recent review showed that its anti-cancer and analgesic properties have been widely reported (Nguyen et al., 2017). Caryophyllene oxide is derived from β -caryophyllene found in many species and food plants. It is a major oxidation product that

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derives from the alkene olefin of caryophyllene, which becomes an epoxide (Fidyt et al., 2016). β -caryophyllene produces oxidized derivatives via autoxidation (Sköld et al., 2006). Medically, its main functions include strong anti-inflammatory, antioxidant, antimicrobial, and insecticidal activities. Both β -caryophyllene and caryophyllene oxide have considerable anticancer activities, influencing growth and a rapid increase in the number of cancer cells. In addition, it has been reported that these compounds disturb aqueous media, such as biological fluids, in cells (Sarpietro et al., 2015). This is because of their low water solubility. However, these molecules can interact with the lipid bilayer comprising cell membranes.

In general, oil-bearing plants are extracted using an organic solvent such as hexane. However, solvents are highly volatile and poses a risk of poisoning. Therefore, efficient extraction methods have been developed by many researchers. Among many extraction methods, for the experiments described here, subcritical water extraction (SWE) was used. This technique has been used to isolate and analyze the essential oils of medicinal plants and spices (Ayala and De Castro, 2001). SWE is based on the environmental use of water as an extractant (Herrero et al., 2006). During SWE, the polarity of water changes significantly and increases in ionic products, making the water reactive without the need to add other substances (Pedras et al., 2020). This knowledge of changes in water with temperature and pressure is important for understanding SWE (Akiya and Savage, 2002; Ozel et al., 2003). The utilization of the SWE method is advantageous for the selective extraction of high-quality compounds with the lowest environmental impact (Essien et al., 2020).

The objective of this study was to determine the optimum temperatures and extraction times to analysis thermal decomposition mechanism of β -caryophyllene from black pepper during SWE. SWE yields were compared to conventional organic solvent extraction involving hexane, methanol, and hot water. It was confirmed that SWE was effective in selectively extracting β -caryophyllene and caryophyllene oxide from black pepper.

Materials and methods

Sample preparation and chemicals

Black pepper (BP) was provided by Ottogi Vietnam Co., Ltd. and obtained from Shinyoung (Gwangju, Gyeonggi, Korea). These BP (5 mm, about 0.02 g) were harvested in 2020. They were dried to a moisture content of < 17%. The samples were stored in a shaded place until analysis. To obtain uniform-sized samples prior to analysis, the BP was ground using a high-speed blender for approximately

1 min. At this time, it was carefully and smoothly crushed to prevent any temperature increase. This is because, as the temperature increases, the quantity and quality of the components extracted from samples decrease (Liu et al., 2018). The crushed BP was sieved with a particle size of 425 μm to obtain a fine powder. It was used immediately to minimize the loss of the components in the sample.

Standard chemicals, including trans-caryophyllene (β -caryophyllene) and β -caryophyllene oxide, were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and solvents were graded as gas chromatography. The solvent was mainly used from methanol (99.9% purity) and n-hexane (95% purity).

SWE

SWE was conducted using an accelerated solvent extractor (Model 350, Dionex, Sunnyvale, CA, USA) using only purified water. 0.5 g of BP powder was mixed with 1.5 g of diatomaceous earth to make an extraction sample. The extraction procedure is shown in Fig. 1. The cellulose filter was filled in each of the bottoms of a stainless-steel extraction cell with a volume of 22 mL. Then, the sample cell was placed in an oven where a pump injected water into the cell for approximately 30 s. At this time, the cell was heated by setting the temperature and time under high pressure to proceed with the extraction. Extraction was carried out at temperatures of 110, 130, 150, 170, 190, and 200 °C for 5, 10, 15, and 20 min. When the extraction was complete, the solvent was purged with nitrogen gas and the extract was placed in a collection bottle. Liquid–liquid extraction was performed using the collected extract and 20 mL of n-hexane. The supernatant was then separated and stored in a glass vial. The vial was stored in a frozen state and analyzed gas chromatography (GC) and gas chromatography with mass spectrometry (GC–MS).

Conventional extraction methods

For analysis of terpenes, conventional extraction methods using solvents of different polarities including hexane, methanol, and hot water were performed. Conventional extraction was performed for comparison with the yield of SWE. The extraction solvents were 99.8% methanol and 95% n-hexane. Ground BP powder (0.5 g) was extracted using 22 mL of solvent. The extractions were performed at 60 °C for 2 h in a water bath. After extraction, the solution was filtered using filter paper, and liquid–liquid extraction was performed using hexane. The supernatant was then collected and placed into vials for GC analysis.

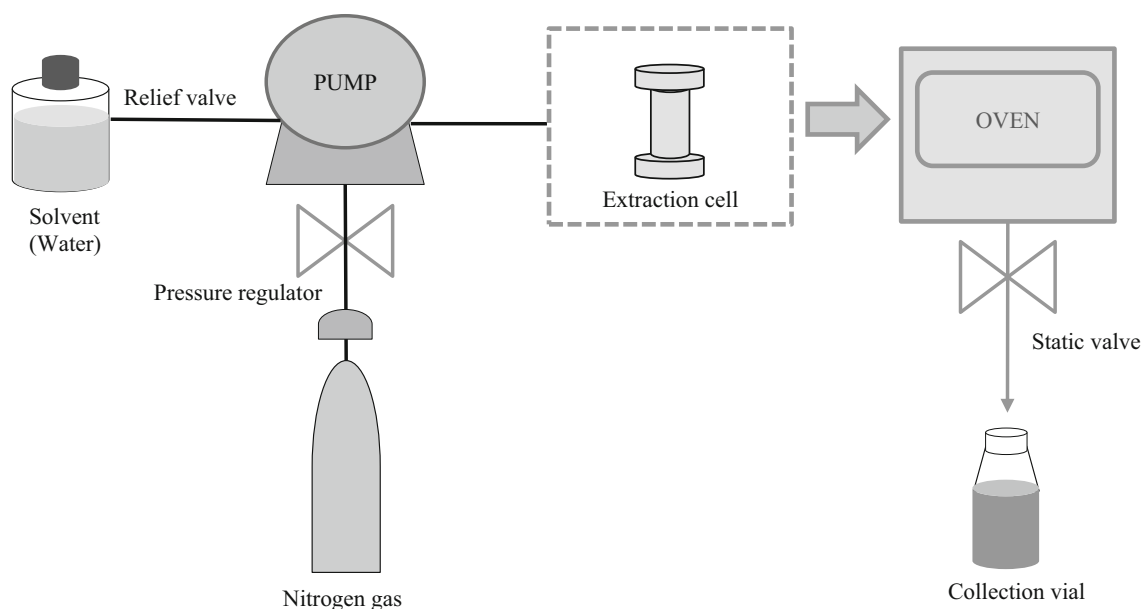


Fig. 1 Schematic diagram of SWE system

GC and GC–MS analysis

Analyses were performed on an HP 6890 GC system (Agilent Technologies, Santa Clara, CA, USA) with flame ionization detection. An HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) was used to separate the terpenes. The injection and detector temperatures were maintained at 250 °C for the BP extract. The injection volume was 1 μL with a split ratio (1:10). Helium, hydrogen, and air were used as carrier gases. The oven temperature was maintained at 50 °C for 2 min and then increased at a heating rate of 4 °C for 5 min until a final temperature 250 °C of was reached.

GC–MS analysis was performed on a 7890B GC system (Agilent Technologies, Santa Clara, CA, USA) with a 5977A mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). An HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) was used to separate the terpenes. The injection and detector temperature were 250 °C for BP extracts. The injection volume was 1 μL with a split ratio (1:10). Helium, hydrogen, and air were used as carrier gases. The oven temperature was maintained at 60 °C for 2 min and then increased at a heating rate of 20 °C/min until it reached a final temperature of 280 °C.

Statistical analysis

Results were obtained from three replicate experiments. Values are expressed as means ± standard deviation. In addition, through graphing, the approximate extraction efficiency that varied depending on the extraction

conditions was estimated. One-way analysis of variance with Duncan's significant difference test ($p < 0.05$) was performed using SPSS Statistics software (version 21, IBM, Chicago, IL, USA).

Results and discussion

GC analysis of caryophyllene and caryophyllene oxide from black pepper

Caryophyllene and caryophyllene oxide standards were diluted in n-hexane to obtain a standard curve for each terpene. Figure 2 shows chromatograms for caryophyllene and caryophyllene oxide from BP. These results indicated that each compound had a specific retention time (RT) of 27.03 min for caryophyllene and 32.46 min for caryophyllene oxide. The standard curves of caryophyllene and caryophyllene oxide were $y = 1429.9x + 0.7$ ($R^2 = 1$), $y = 1474.4x + 5.2$ ($R^2 = 0.999$), respectively. The terpenes of the BP extract were exactly identified using GG-MS analysis.

Optimization of SWE conditions for extraction of caryophyllene and caryophyllene oxide

These experiments were conducted to investigate the effect of SWE temperature conditions on the terpenes of BP extracts. The extraction temperature was determined under extraction conditions (110, 130, 150, 170, 190, and 200 °C). As shown in Fig. 3, the extraction content of caryophyllene was maximal at 170 °C. The caryophyllene

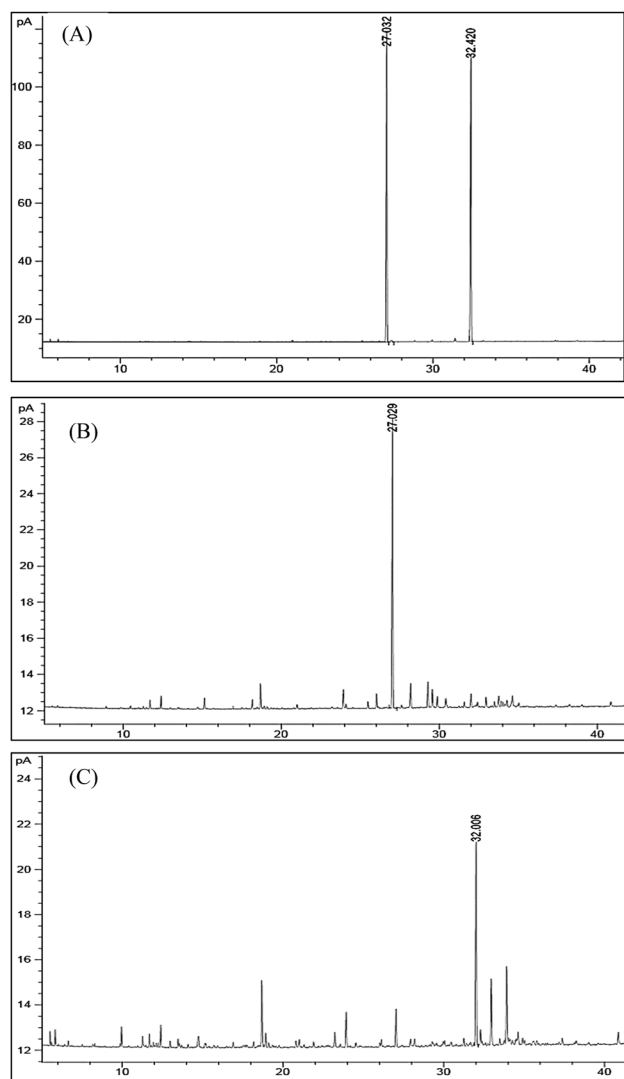


Fig. 2 GC chromatograms of standard β -caryophyllene, and caryophyllene oxide (A), and β -caryophyllene (under temperature/time conditions of 170 °C/20 min) (B), caryophyllene oxide (under temperature/time conditions of 200 °C/20 min) (C) extracted from black pepper

concentration tended to increase as the temperature increased from 110 to 170 °C. Subsequently, it was hardly extracted at temperatures as high as 190–200 °C. Caryophyllene oxide, the oxidized form of caryophyllene, was not extracted at temperatures ranging from 110 to 170 °C. This showed that caryophyllene oxide is more difficult to extract at lower temperatures than caryophyllene. Caryophyllene oxide was extracted at a high temperature of 190–200 °C, illustrating the possibility of selective extraction at high temperatures. The extraction content of caryophyllene oxide was maximal at 200 °C, which indicated the need for further research through extraction at temperatures > 200 °C. The amount of caryophyllene extracted by SWE decreased at > 170 °C,

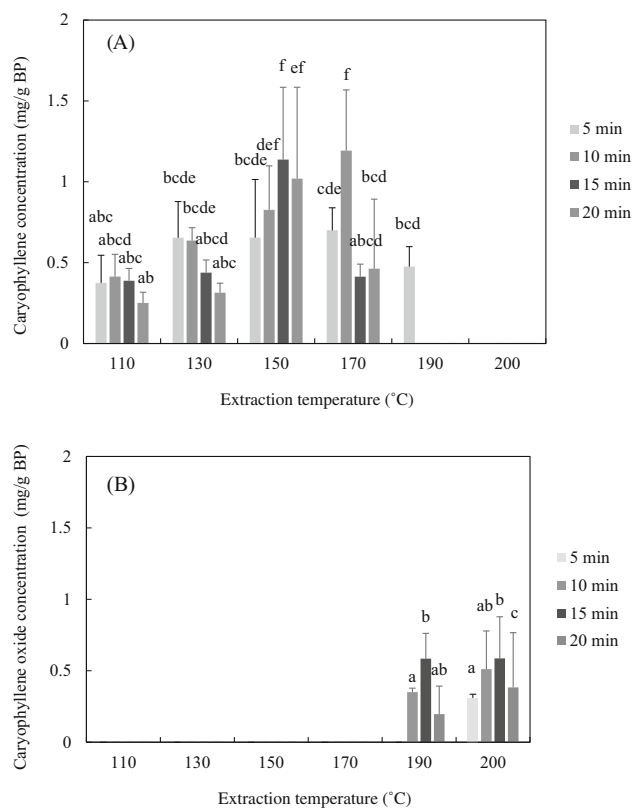


Fig. 3 Effect of extraction temperature and time on the SWE of β -caryophyllene (A), and caryophyllene oxide (B) concentration from black pepper. Means in bars with same letters are not significantly different according to Duncan's test at $p < 0.05$

whereas caryophyllene oxide yield increased. The structure of caryophyllene appears to have been converted into oxidized caryophyllene with stability at high temperatures. According to previous studies, the caryophyllene molecule is highly modified and undergoes various alterations according to different environmental conditions (Da Silva Rocha et al., 2010).

To determine the effects of extraction time on SWE, it was conducted for 5, 10, 15, and 20 min at temperatures of 110, 130, 150, 170, 190, and 200 °C. Both caryophyllene and caryophyllene oxide concentrations tended to decrease when the extraction time exceeded 15 min. There were no significant changes in the contents of caryophyllene and caryophyllene oxide with time.

The optimal conditions of β -caryophyllene (1.19 ± 0.38 mg/g), and caryophyllene oxide (0.82 ± 0.38 mg/g) were obtained from black pepper under extraction conditions of 170 °C/10 min, and 200 °C/15 min, respectively.

Thermal decomposition and oxidation of caryophyllene during SWE

Caryophyllene appears to be structurally transformed owing to the influence of the extraction temperature during SWE. According to previous studies, the higher the temperature of the essential oil of BP, the lower the extraction yield (Maschietti, 2011). Therefore, the lower the temperature, the higher the caryophyllene concentration in the essential oil (Rouatbi et al., 2007). In this study, when the extraction temperature was increased above 170 °C, caryophyllene was not extracted. In addition, new compounds are produced by thermal decomposition of essential oils (Chiou et al., 2020; Jakab et al., 2018). It was considered that caryophyllene oxide decomposed once it was generated from caryophyllene under subcritical water at 190 °C. This is because the decomposition of fragrances begins at high temperatures. It appears that caryophyllene decomposed at extraction temperatures > 170 °C and was oxidized and converted into oxidized caryophyllene oxide. In previous studies, oxidation was induced by temperature, UV irradiation, and metals (Nguyen et al., 2009; Sinki et al., 1997).

Thus, it was confirmed that the molecular structure of caryophyllene, which is thermally unstable, was oxidized and converted into caryophyllene oxide due to the influence of high temperature. As shown in Fig. 4, caryophyllene extracted at high temperatures was oxidized to the primary hydroperoxides 2, 3, and 4. The structures of hydroperoxides 2 and 3 are similar to that of

caryophyllene, but unlike caryophyllene, which has exocyclic double bonds, hydroperoxides have endocyclic double bonds and define as methine-carbon-containing carbon (C-3). Hydroperoxide 2 has a second exocyclic double bond and two methyl groups. In contrast, hydroperoxide 3 has both primary exocyclic and endocyclic double bonds, and one methyl group is also present. The resulting primary oxides differ individually in terms of structural stability, and the most unstable hydroperoxide, 5, forms a stable structure through secondary oxidation. In the event of secondary oxidation, hydroperoxides 2 and 3 are not further decomposed into secondary hydroperoxide, and caryophyllene oxide is not formed. On the other hand, hydroperoxide 5 rapidly decomposes, and homolysis occurs. As a result, electrons forming oxygen–oxygen bonds are transferred one by one to achieve a balance. Subsequently, an epoxide-type caryophyllene oxide structure is formed by the decomposition of the alkyl radical in hydroperoxide 6. The final oxide, caryophyllene oxide, is formed and has a stable structure in which no further oxidation occurs.

Comparison with conventional extraction methods

The content of caryophyllene extracted by SWE was compared with conventional methods using methanol, ethanol, and water (Table 1). Differences in the concentration of the compound according to the extraction content and extraction time of the compound according to the extraction solvent were confirmed. The extraction yield of

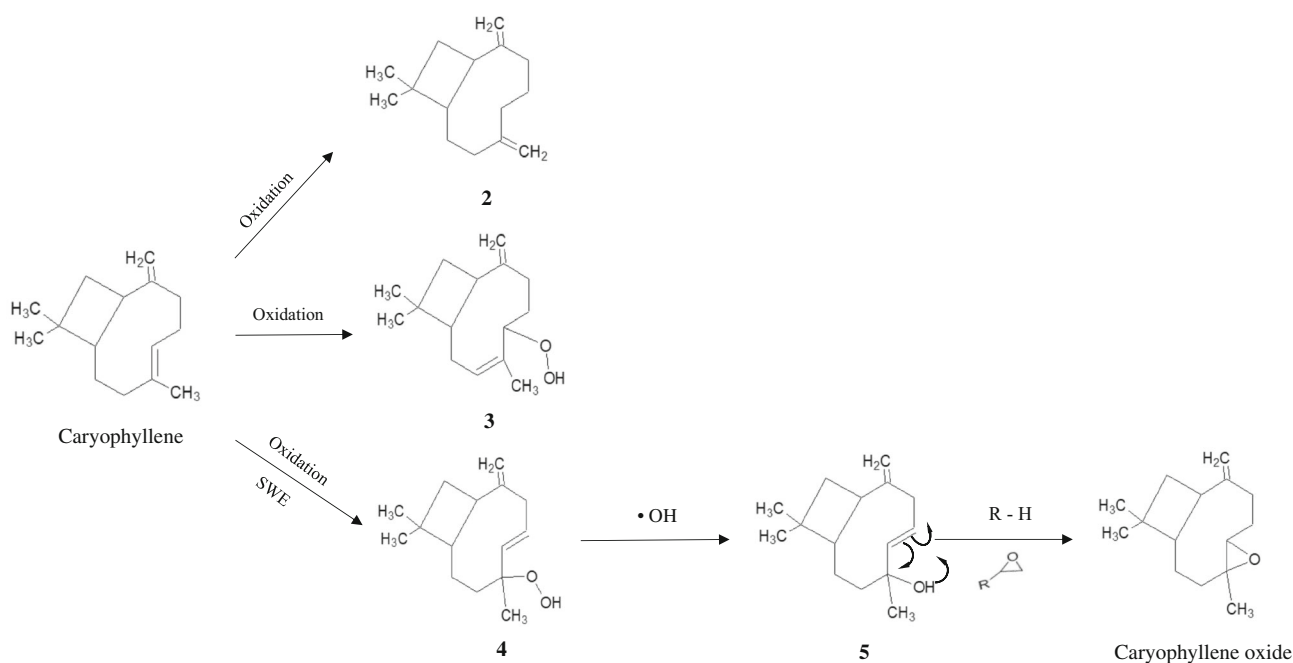


Fig. 4 Oxidation mechanism of β -caryophyllene in SWE

Table 1 Comparison of concentration of caryophyllene, and caryophyllene oxide obtained using conventional hexane, methanol, and hot water extraction methods (under temperature/time conditions of 60 °C/2 h) and SWE method (under temperature/time conditions of 170 °C/10 min) from black pepper

Method	Extraction conditions		Caryophyllene (mg/g BP)	Caryophyllene oxide (mg/g BP)
	Temperature (°C)	Time (min)		
Hexane	60	120	16.87 ± 0.32 ^d	ND
Methanol	60	120	13.61 ± 0.21 ^c	ND
Hot water	60	120	0.42 ± 0.26 ^a	ND
SWE	170	10	1.92 ± 0.38 ^b	0.88 ± 0.38

Means in a column followed by same superscript letters are not significantly different according to Duncan's test at $p < 0.05$

ND not detected

caryophyllene was lower in SWE than in conventional extraction methods using methanol and ethanol. This is because caryophyllene is a sensitive sesquiterpene and is hydrophobic and is consequently highly soluble in organic solvents. However, caryophyllene oxide, an oxidized form of caryophyllene, was only extracted by SWE. This forms an oxidized caryophyllene oxide which stabilize the otherwise unstable structure due to caryophyllene oxidation at high temperatures (Nguyen et al., 2009; Sinki et al., 1997). These results indicate that SWE is an efficient method for the extraction of caryophyllene oxide and caryophyllene with high physiological activity.

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Declarations

Conflict of interest The authors declare that they have no competing interest.

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