Korean J Food Preserv, 30(5), 758-769 (2023)
https://doi.org/10.11002/kjfp.2023.30.5.758
pISSN: 1738-7248, eISSN: 2287-7428
Korean Journal of Food Preservation

Research Article

Evaluation of physicochemical and biological properties of python fat (*Python bivittatus*)

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Abstract The main aim of this study was to determine python fat's several physicochemical properties, including dimensions, color, structure, acid value (AV), saponification value (SV), density, and recovery efficiency. The optimum yield obtained was approximately 80.40% at 180℃ for 60 min with an AV of 0.3366 and SV of 179.56 mg KOH/g. Fatty acids, comprising oleic acid (72.462%), palmitic acid (26.243%), linolenic acid (0.835%), and myristic acid (0.459%), were identified using gas chromatography-mass spectrometry (GC-MS). The python fat had a very weak antioxidant capacity and almost no antibacterial ability with gram-positive (*Staphylococcus aureus* - ATCC 25923 and *Bacillus cereus* - ATCC 10876) and gram-negative (*Escherichia coli* - ATCC 25922 and *Salmonella enterica* - ATCC 35664) bacteria (used the paper disc diffusion method for antibiotic susceptibility testing). Moreover, python fat is considered to be very resistant to high temperatures.

Keywords antimicrobial activity, antioxidant capacity, GC-MS, *Python bivittatus*, python fat

Citation: Quyen PT, Quoc LPT. Evaluation of physicochemical and biological properties of python fat (*Python bivittatus*). Korean J Food Preserv, 30(5), 758-769 (2023)

Received: August 10, 2023
Revised: October 05, 2023
Accepted: October 06, 2023

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1. Introduction

The Burmese python (*Python bivittatus*) is a warm and humid animal often found near water, semi-aquatic, or in trees. The length is 5-7 m, and the female is larger than the male. They have fine scales with large scales on top, small limbs on the tail, and two developed lungs (Giao, 2009). *P. bivittatus* can be found in various regions worldwide, particularly Southeast Asia. This animal is promoted as livestock production in Vietnam because of its high economic value. People often use python skin for export or processing in the tanning industry for purses, bags, belts, etc., while meat and fat are considered by-products. Nevertheless, these materials hold significant potential for future applications, particularly in the medical and food industries.

Today, many scientists have studied python fat. The results of earlier works indicate that the physicochemical composition of the fat extracted from pythons depends on material sources (Offurum et al., 2019; Wang and Li, 1953). Python fat is very useful for treating burns and preventing infection. In addition, it also

increases the ability to heal wounds extremely effectively (Thuy et al., 2006). The body's cholesterol could be reduced thanks to the fatty acid components extracted from python fat. In addition, because python fat is rich in fatty acids, it can regenerate cells, helping to improve scars, soothe, and heal wounds (Wang and Li, 1953). On the other hand, the African *Python sebae* fat possesses a lot of fatty acids such as oleic acid (49.47%), palmitic acid (28.51%), stearic acid (9.38%), linoleic acid (5.85%), palmitoleic acid (3.80%), myristic acid (1.30%), gadoleic acid (0.72%), α-linolenic acid (0.36%), arachidonic acid (0.25%), γ-linolenic acid (0.19%), and vaccenic acid (0.15%). In addition, the fat is used to prepare python extract to effectively treat diseases related to bones and joints (Offurum et al., 2019).

Moreover, oleic acid found in plant oil, particularly olive oil, has many significant beneficial effects on human health, for instance, anticancer, autoimmune and anti-inflammatory diseases, and wound healing (Sales-Campos et al., 2013). Python fat also has a high proportion of oleic acid. This suggests that python fat might replace some plant oil and be used in food processing or medicine. Python fat accounts for 10% of the total weight, but it is considered a waste product of the skin harvesting process, although it may have great potential when applied in cosmetics and medicine.

Solid python fat is unusable and easily damaged at room temperature. Therefore, it must be converted into liquid form for easy storage and convenient use. However, the quality and yield of liquid fat obtained can vary depending on the extraction method. Besides, liquid and solid fat states have entirely different physicochemical properties. They will affect treatment methods in food technology and medicine. Therefore, evaluating the quality and characteristics of python fat in both states is necessary. However, until now, there have been no studies on the chemical components, physicochemical properties, and bioactivities of *P. bivittatus* fat in both liquid and solid forms. It shows that this is a gap in food science that needs to be filled. Python fat promises many exciting discoveries, and the primary purpose of this study is to clarify and determine the chemical profile, antibacterial activity, physical properties, thermal stability, and antioxidant capacity of this material.

2. Materials and methods

2.1. Fat material

P. bivittatus fat sacs were provided by the Minh Quan farm (Ho Chi Minh City, Vietnam). They were small pieces of fat from the fat bags extracted from the belly of the python, about a year old, with a length of 2 m and weight of 10 kg. After slaughter, the samples were immediately transported to the laboratory and stored at -18℃. The fat color was white and characteristically fishy, had no rancid smell, and was tasteless. For liquid python fat, the initial materials were treated using the heating method from 160 to 200℃ for 30-90 min. Then, the obtained oil was collected after final filtration (diameter of hole sieve of 0.1 mm) and centrifugation (5,000 rpm) and was stored at 28℃ until further analyses.

2.2. Bacteria strains

The bacteria used in this study included four strains: two gram-positive bacteria (*Staphylococcus aureus* - ATCC 25923 and *Bacillus cereus* - ATCC 10876) and two gram-negative bacteria (*Salmonella enterica* - ATCC 35664 and *Escherichia coli* - ATCC 25922). These bacteria were provided by the Institute

of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City.

2.3. Chemicals

This study uses 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma, St. Louis, Missouri, USA) and dimethyl sulphoxide (DMSO, Nanjing, China). The culture and antibacterial media included Mueller-Hinton agar (HiMedia, Thane, India), nutrient broth (HiMedia), and other chemicals of analytical reagent grade.

2.4. Measurement of dimensions of python fat in solid state

A sample was selected by a random method; then a digital caliper (model 500-182-30, Mitutoyo, Aurora, Illinois, USA) was used to measure the length (L), width (W), and height (H) of the pieces of fat.

2.5. Measurement of color of python fat in solid and liquid states

The color of the surface of the fat was measured directly with the colorimeter (model CR-400, Konika Minolta, Osaka, Japan). The CIE color space model was applied to interpret the meaningful parameters: L*: lightness, a*: green/red value, and b*: blue/yellow value. The sample was measured in quartz cuvettes for python fat in liquid state.

2.6. Measurement of structure of python fat in solid state

A texture profile analysis (TPA) was performed with a texture analyzer (model CT3, Brookfield Ametek, Middleborough, Massachusetts, USA). The sample was compressed twice in a row using a TP4/1000 probe with a diameter of 38.1 mm and thickness of 20 mm at a velocity of 2 mm/sec; and for the second cycle, compressing 50% of the thickness of a piece of fat.

2.7. Determination of acid value (AV), saponification value (SV), peroxide value (PV), density, and boiling point of python fat

The AV is a measure of the free fatty acids present in fats, and it was determined by the titrimetric method. The sample (10 g) is dissolved in 100 mL of the alcohol/toluene (ratio of 1/1, v/v), using 0.1 M potassium hydroxide (KOH) solution to neutralize the free fatty acids in fat and phenolphthalein indicator solution (1%). AV is expressed in milligrams of KOH per gram of the product tested (mg KOH/g) (ISO 660, 2009). While the SV is a measure of the free and esterified acids present in fats and fatty acids, the sample (5 g) is saponified by boiling under reflux with an excess of ethanolic KOH (0.5 M, 25 mL), followed by titration of the excess KOH with 0.5 M hydrochloric acid (HCl) solution, SV is also expressed in milligrams of KOH required for the saponification of one gram of the product tested (mg KOH/g) (ISO 3657, 2013).

For the PV, the sample (5 g) is dissolved in 50 mL of the isooctane/glacial acetic acid (ratio of 1/1, v/v), and 0.5 mL of a saturated solution of potassium iodide (KI) is added. The iodine liberated by the peroxides is determined iodometrically (visually) with a starch indicator (1%) and a 0.01 M sodium thiosulphate ($Na_2S_2O_3$) standard solution. PV is expressed in milliequivalent (meq) of active oxygen per kilogram (meq/kg) (ISO 3960, 2007).

Fat density means the quotient of the mass in air of fat to its volume (g/mL) at a given temperature (25℃). It was determined by the Gay-Lussac pycnometer (50 mL) (ISO 6883, 2007). The boiling point (BP) was determined with a thermometer when the fat began to boil.

2.8. Determination of chemical composition of python fat

Sample (5 g) was extracted by Soxhlet siphoning-

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type extractor with *n*-hexane (100 mL) as solvent at 50℃. Then, the solvent is removed from the extract, and the extract is weighed (ISO 659, 2009). On the other hand, protein content was evaluated based on the Kjeldahl method (Kirk, 1950), and it was indirectly determined through the nitrogen content of the sample (both nitrogen in organic substances and inorganic compounds).

2.9. Determination of recovery efficiency (RE) of python fat in liquid state

The RE was determined by the difference in weight of initial solid python fat and liquid fat obtained (%).

2.10. Determination of viscosity of python fat in liquid state

Viscosity was determined by a viscometer (model LVDV-E, Brookfield Ametek) with an S62 probe calibrated at 100 rpm at 25℃ (unit of measure in centipoise, cp).

2.11. Gas chromatography-mass spectrometry (GC-MS) analysis of fatty acids of python fat

Samples were methylated by the rapid method according to ISO 12966-2 (2011), and the ester conversion of neutral lipids occurred by alkaline catalysis in the presence of anhydrous methanol (methyl conversion) with the reagent KOH. The chemical composition of fat was determined by the procedure described by ISO 12966-4 (2015). The fatty acid compositions were determined using the Agilent Technology 5977E MSD with an auto-sampler and the Agilent 7820A GC system (Santa Clara, California, USA). The chromatographic separation was performed on a Carbowax 20M^{TM} column (30 m×0.25 mm×0.25 μm). A sample of 0.2 μL was filtered, and then a sample volume injection of 0.1 μL was pumped into a capillary gas chromatograph equipped with a split/nonlinear injection and a fused silica capillary column coated with different stationary phases. The temperature was kept at 165℃ for 3 min, ramped at a rate of 4℃/min to 195℃, and then held at this point for 23 min. The total analysis time was 30 min. The fatty acids were identified by comparing the retention times of a standard mixture to the retention times of the fatty acids and by comparing with the NIST spectral library.

2.12. Determination of antioxidant capacity (AC) of python fat

The procedure to determine the AC of the python fat was described according to (Akmal and Roy, 2017) with some minor changes. The *P. bivittatus* fat (PbF) was dissolved in ethanol (96%, v/v) to obtain different concentrations (10, 20, 40, 75, 150, and 300 μg/mL); 0.2 mL of the obtained solution and 3.8 mL DPPH 0.1 mM in ethanol solution were mixed. The mixture was kept in the dark for 30 min at room temperature. The DPPH radical scavenging capacity ($DPPH_{RSC}$) is expressed in terms of the degree of color reduction of the DPPH solution, as determined by measuring the optical absorbance at 517 nm using a spectrophotometer (model Genesys 20, Thermo Scientific, Waltham, Massachusetts, USA). Butylated hydroxytoluene (BHT) was used as a standard to compare with the AC of the PbF. The percent inhibition was plotted against the PbF concentrations to estimate the concentration providing 50% inhibition (IC_{50}). The AC was calculated using the following expression:

$$DPPH_{RSC}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$ is the absorbance of a solution containing only DPPH solution, and A_{sample} is the absorbance of the sample in the presence of DPPH.

2.13. Determination of rancidity of python fat

Three samples, including the control, added BHT (200 ppm), and butylated hydroxyanisole (BHA) samples (200 ppm) were heated at 180℃. The PVs were evaluated according to ISO 3960 (2007) after 0, 5, 10, and 20 min.

2.14. Determination of antibacterial activity (AA) of python fat in liquid state

AA was determined using the paper disc diffusion method for antibiotic susceptibility testing according to the procedure of Nguyen et al. (2017) with some slight modifications. Firstly, 100 μL of bacteria suspension (0.5 McFarland standard, approximately 1.5×10^8 CFU/mL) were spread on MHA media (Mueller-Hinton agar) by a sterile hockey stick. Then, the sterile paper discs 6 mm in diameter were impregnated with the selected PbF (5 μL). Gentamicin (10 μg/disc) and dimethyl sulfoxide (DMSO) solution (5%, v/v) were used as a positive and negative control to compare the AA of the PbF. Finally, all dishes were incubated for 24 h at 37℃, and the AA was assessed by an inhibitory zone with a paper disc of 6 mm in diameter.

2.15. Statistical data analysis

All the experimental results were analyzed using the Statgraphics Centurion software (version 15.1.02). Every assay was done in triplicate. Analysis of variance (ANOVA) with Fisher's least significant difference procedure was used to determine the significant differences ($p<0.05$) between means.

3. Results and discussion

3.1. Physicochemical properties of python fat in solid state

P. bivittatus fat (PbF) sacs are not spherical at all; most fat sac sizes are completely uneven and vary greatly. The length fluctuates at 13.90±1.77 mm, the width at 10.27±1.52 mm, and the height at 4.80±0.84 mm (Table 1). The external appearance of the pieces of PbF is the same as that of *P. sebae* originating from the Congo (Moussounga et al., 2018). The color parameters of the PbF sacs are L* of 64.49±1.31, a* value of 6.28±1.65, and b* value of 10.11±0.83 (Table 1). However, when seen with the naked eye, the fat sacs vary from ivory white to milky white or pale pink. Besides, PbF sacs have a fishy smell, a characteristic pungent smell that is completely different from other fats such as pork fat, beef fat, chicken fat, etc.

PbF sacs have relatively poor elasticity because the difference between cycle 1 (13.50±0.86 N) and cycle 2 (8.49±1.15 N) is up to 1.59 times. However, after compressing twice, the fat sample was completely unbroken, which is explained by the fact that the adipose tissue cells are covered by a

Table 1. Physical properties of the pieces of python fat in solid state

Physical properties		Value
Dimension	Length (mm)	13.90±1.77[1)]
	Width (mm)	10.27±1.52
	Height (mm)	4.80±0.84
Color parameters	L*	64.49±1.31
	a*	6.28±1.65
	b*	10.11±0.83
Structure	Hardness cycle 1 (N)	13.5±0.86
	Hardness cycle 2 (N)	8.49±1.15
	Adhesiveness (mJ)	0.13±0.06
	Chewiness (mJ)	0.87±0.37
	Gumminess (N)	1.08±0.36
	Cohesiveness	0.08±0.03
	Resilience (mJ)	0.08±0.05

[1)]Values are mean±SD (n=3).

https://doi.org/10.11002/kjfp.2023.30.5.758

membrane that makes them more closely linked. Furthermore, when other factors of adhesiveness are taken into account, a minimum of 0.13±0.06 mJ is required to sever the surface bonds of PbF sacs; in terms of chewiness, 0.87±0.37 mJ is required to grind the PbF sacs; in terms of gumminess, 1.08±0.36 N is required to crush the PbF sacs; in terms of cohesiveness, the PbF sacs will deform at 0.08±0.03; and 0.08±0.05 mJ energy is required for the PbF sacs to recover to its original state (Table 1). Considering that published scientific works on the physicochemical characteristics of this material are non-existent, it is not easy to make any inference concerning the results obtained. However, these results are noteworthy and can be used as a reference for further studies.

3.2. Yields and chemical properties of python fat in liquid state

After heating treatment, we found that as the temperature and time increased, L* of the liquid fat decreased significantly from 64.49±1.31 to the lowest level of approximately 1.5 times (44.93±0.22) at 160℃ and to the highest level of approximately 3 times (23.72±0.07) at 200℃; the color of the fat has darkened about 1.5-3 times after processing (Table 2). The majority of a* and b* values tended to increase after increasing time and temperature but decreased significantly compared to a* and b* values of the PbF sacs. This is explained by removing the protective film of the sample and the significant variation from processing that affected the above results, especially at high temperatures. These values are similar to those of plant oils, for example, refined camellia seed oil (L*: 47.02±0.17, a*: -0.57±0.12, and b*: 14.27±0.38) (Zhong et al., 2023).

Table 2 shows that the AV is very low, ranging from 0.28 to 0.48 mg KOH/g, which proves that there are very few free fatty acids in PbF. Compared with the African *P. sebae*, their acid index ranges from 2.38 to 127.59 mg KOH/g (Moussounga et al., 2018). It is much higher than our study's, while the AV of *Python molurus bivittatus Schlegel* is only 0.16 mg KOH/g (Wang and Li, 1953). The SV achieved in our study was very high, ranging from 168.4 to 213.25 mg KOH/g. This value is similar to the fat of African *P. sebae*, whose SV ranged from 157.99 to 178.73 mg KOH/g (Moussounga et al., 2018), similar to the SV of *Python molurus bivittatus Schlegel* at 191.3 mg KOH/g (Wang and Li, 1953). Various python sources may explain this difference.

As the heating time and temperature increase, the quality of the fat sample decreases. Since the AV indicates the freshness of the fat, the higher this index, the more the fat has been decomposed or oxidized. The culpable part of the process is the exposure to higher temperatures for a long time, which causes the AV to increase, thereby reducing the quality of the sample.

At the same time, when changing the temperature, the recovery efficiency will change; the higher the temperature, the higher the recovery efficiency. The highest efficiency is 90.93% at 200℃ for 90 min, while the lowest is 39.05% at 160℃ for 30 min (Table 2). However, in terms of color, the PbF samples collected at 200℃ will be the same color as those of the damaged grease samples when exposed to too high temperatures. In addition, when the PbF solidifies when exposed to low temperatures, it becomes opaque yellow, the same result when tested with lard.

When PbF in solid state is processed at different temperatures and times, the physicochemical parameters of PbF will also be markedly different. However, 180℃ for 60 min will yield suitable recovery

Table 2. Physicochemical properties of the python fat in liquid state

Time (min)	Temperature (°C)		
	160	180	200
L* (lightness)			
30	44.93±0.22$^{Cc1)}$	44.64±0.10^{Ca}	44.66±0.01^{Cb}
60	41.81±1.45^{Bb}	43.46±0.05^{Bc}	25.26±0.08^{Ba}
90	41.46±0.22^{Ac}	31.95±0.12^{Ab}	23.72±0.07^{Aa}
a* (green/red value)			
30	−1.36±0.03^{Ac}	−1.89±0.01^{Aa}	−1.54±0.02^{Ab}
60	−1.22±0.17^{Ba}	−1.22±0.01^{Ba}	0.94±0.07^{Bb}
90	−0.98±0.02^{Cb}	2.37±0.06^{Cc}	1.12±0.02^{Ca}
b* (blue/yellow value)			
30	5.09±0.06^{Aa}	9.61±0.04^{Bb}	11.91±0.01^{Cc}
60	9.76±0.02^{Bc}	5.22±0.02^{Aa}	5.55±0.12^{Ab}
90	14.08±0.02^{Cb}	16.82±0.19^{Cc}	5.81±0.08^{Ba}
Acid value (mg KOH/g)			
30	0.2806±0.00021^{Ab}	0.2526±0.0002^{Aa}	0.3366±0.0001^{Ac}
60	0.3086±0.00021^{Ba}	0.3366±0.0000^{Bb}	0.4206±0.0004^{Bc}
90	0.3646±0.00015^{Ca}	0.3892±0.0057^{Cb}	0.4766±0.0003^{Cc}
Saponification value (mg KOH/g)			
30	190.74^{Ab}±0.31	168.4±0.40^{Aa}	196.37±0.36^{Cc}
60	193.54^{Bc}±0.47	179.56±0.26^{Ca}	182.30±0.15^{Bb}
90	213.25^{Cc}±0.23	171.18±0.15^{Ba}	171.21±0.27^{Ab}
Recovery efficiency (%)			
30	39.05±0.93^{Aa}	72.22±0.56^{Ab}	82.12±0.46^{Ac}
60	80.12±0.58^{Ba}	80.40±0.54^{Bb}	87.18±0.35^{Bc}
90	85.53±0.50^{Ca}	88.1±0.66^{Cb}	90.93±0.35^{Cc}

[1)]Values are mean±SD (n=3). Different superscript capital letters (A–C) in the same column indicate significant differences ($p<0.05$), while different superscript lowercase letters (a–c) in the same row indicate significant differences ($p<0.05$).

performance and superior quality indicators.

3.3. Physicochemical properties of python fat in liquid state

Table 3 illustrates that the viscosity of PbF in the liquid state reaches 254.0±0.30 cp, which is considered quite high. The high viscosity of liquid PbF revealed that this material could be applied to manufacture lubricating greases and fuels (Adebanjo et al., 2005). According to Zahir et al. (2017), the viscosity changed because of the different arrangements of the fatty acids on the glycerol backbone of the triglyceride molecule. Therefore, viscosity is related to the chemical properties of the fats, such as chain

https://doi.org/10.11002/kjfp.2023.30.5.758

Table 3. Physicochemical properties of python fat in liquid state

Physicochemical properties	Value
The viscosity (cp)	254.0±0.30[1)]
The density (g/mL)	0.85±0.00
The boiling point (℃)	140.5±2.78

[1)]Values are mean±SD (n=3).

length and saturation/unsaturation.

In addition, the density of liquid PbF is only 0.85±0.00 g/mL, which is lighter than water and lower than that of some vegetable oils such as pumpkin seed oil (0.91 g/mL) (Hagos et al., 2023), mustard oil (0.9694 g/mL), and corn oil (0.9223 g/mL) (Zahir et al., 2017). The boiling point (BP) is relatively low at just 140.5±2.78℃ but higher than that of lard (86-113℃) (Sudjana et al., 2020). Compared to plant oils, the BP of PbF is significantly lower than that of coconut oil (260-262℃) (Mena et al., 2020) and mustard oil (170℃) (Zahir et al., 2017). In general, the different physicochemical properties between various materials are due to the different chemical compositions, and BP depends upon the degree of unsaturation of fatty acids.

3.4. Chemical composition of python fat

GC-MS analysis was used to determine the chemical composition of PbF. The results indicate that PbF comprised approximately 92.75% of the total chemical constituents (Table 4). Protein was not detected in this material. The compounds occupying the highest content in PbF included oleic acid (72.462%), palmitic acid (23.243%), linolenic acid (0.835%), and myristic acid (0.459%). The ratio of unsaturated fatty acids to saturated fatty acids is 3:1. When compared with the African *P. sebae* fat, the proportion of unsaturated fatty acids is high, accounting for 60.80%, of which the most active

Table 4. The major chemical component and the fatty acid composition of python fat

The major chemical component of python fat	Value
Lipid (%)	92.75
Protein (%)	0
The fatty acid composition of python fat	**Value**
Oleic acid (%)	72.462
Palmitic acid (%)	23.243
Linolenic acid (%)	0.835
Myristic acid (%)	0.459

ingredient is oleic acid, accounting for 49.47%; saturated fatty acids account for only 39.19% of the total fatty acids with a significant proportion of 28.51% palmitic acid (Moussounga et al., 2018). With a high oleic acid content, PbF may have many health benefits and be a potential material that prevents infections, treats inflammatory and cardiovascular diseases, improves the immune system, or repairs skin based on this fatty acid. Although linolenic acid only accounts for a very small amount, this is also a good compound for human health, especially heart health (Sales-Campos et al., 2013). In addition, we especially note the presence of myristic acid, a saturated fatty acid. Based on the above, we can conclude that PbF has very good potential for medicine in the future.

3.5. Antioxidant capacity (AC) of python fat in liquid state

With a significant increase in the concentration of liquid PbF, the AC peaked at 12.27% for a fat concentration of 300 μg/mL, while the initial AC was 7.2% at 10 μg/mL. This demonstrates that IC_{50} was not detected in the tested concentration range, while the control (BHT) exhibited IC_{50} at a concentration of 200 μg/mL (Fig. 1). These results show that the AC of liquid PbF is many times lower than that of

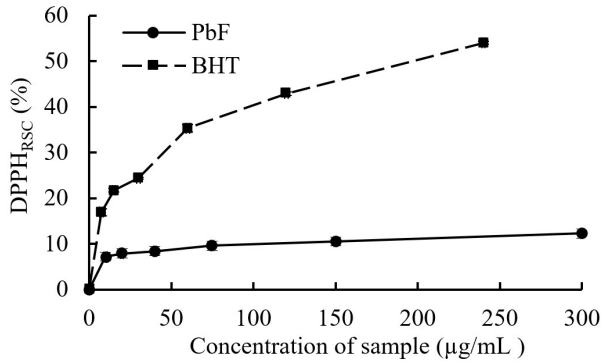

Fig. 1. **Antioxidant capacity of python fat in liquid state.**

BHT and *Terminalia catappa* seed oils (950-2,529 μg/mL) (Nguy et al., 2023). The low AC can be explained by the fact that liquid PbF did not contain some bioactive compounds, especially antioxidants. There are no published studies on the AC of python fat in the literature, so it was impossible to compare our results.

3.6. Rancidity of python fat in liquid state

Fig. 2 shows that the supplemented samples BHT and BHA increased more slowly than the control sample. Initially, for samples with an AV index of 0.61 meq/kg, after 20 min of exposure to a temperature of 180℃, the AV of the control sample increased 1.8 times (1.1 meq/kg); for samples supplemented with BHT, it was 1.44 times (0.88 meq/kg), while with BHA, it was 1.53 times (0.93 meq/kg). Rancidity is inevitable when heating the fat at high temperatures, so it is necessary to add antioxidant additives of natural or synthetic origin to prevent oxidation and restrain or limit the impact on consumers. The antioxidant mechanism explains the ability of the fat (or plant oils) to inhibit the oxidation process; BHA and BHT give an electron to the free radical in the fat sample, and then the free radicals become stable and no longer capable of causing damage. This makes oxygen unable to attack, making the oil more stable during heating (Milic et al., 2020). The results are quite reasonable; however, in the heating process, BHA and BHT do not have a high anti-oxidative effect, evaporate easily, and cannot protect against thermo-oxidative degradation (Augustin and Berry, 1983). In this study, liquid PbF can also be classified as an oil with relatively good heat resistance compared to adding BHA and BHT.

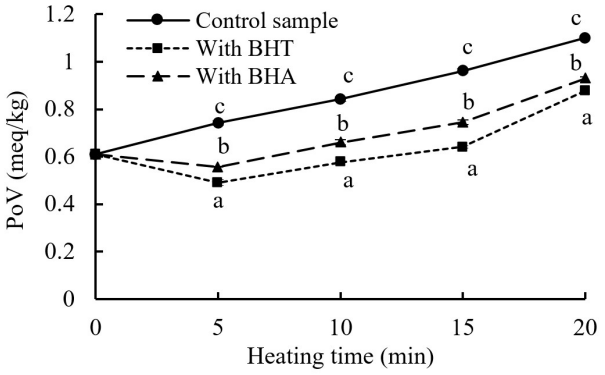

Fig. 2. **Peroxide value of liquid PbF with the control sample (non-additive) and adding BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) during the heating time.** Different superscript lower-case letters (a-c) in the same heating time indicate significant differences between samples ($p<0.05$).

3.7. Antibacterial activity (AA) of the python fat in liquid state

Table 5 shows that the positive control (Gentamicin) has a very strong antibacterial ability for four tested bacteria. Compared with liquid PbF, liquid PbF has almost no antibacterial ability, and these results are similar to those of the study about pure coconut oil (Nguyen et al., 2017). On the other hand, *T. catappa* seed oils inhibited the growth of five bacteria: *B. cereus*, *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Vibrio parahaemolyticus* (Nguy et al., 2023). The failure to show AA depends entirely on the chemical composition of the fat. However, no studies have been published on the antibacterial

https://doi.org/10.11002/kjfp.2023.30.5.758

Table 5. The antibacterial activity of python fat

No.	Microorganisms	Diameter of the inhibitory zones of positive control (gentamicin, mm)	Diameter of the inhibitory zones of PbF (mm)
1	*E. coli*	20.33±1.53[NS1)]	0
2	*S. enterica*	17.33±2.52[NS2)]	0
3	*B. cereus*	20.00±2.00[NS]	0
4	*S. aureus*	17.00±1.00[NS]	0

[1)]Values are mean±SD (n=3).
[2)]No significant difference between bacteria.

activity of liquid fats of python varieties to compare with our results.

4. Conclusions

In general, the physicochemical and biological properties of PbF were determined for both the solid and liquid states. PbF has a relatively high degree of unsaturation, which indicates that this type of fat is very sensitive to atmospheric oxygen and is prone to rancidity when storage conditions are not guaranteed, but they are quite stable to temperature. The liquid python fat recovery process can be more optimally completed when the heating process is fixed with the temperature parameter at 180℃ for 60 min. Four important fatty acid components were also found: oleic acid (72.462%), palmitic acid (23.243%), linolenic acid (0.835%), and myristic acid (0.459%). In addition, the antioxidant activities were quite weak (IC_{50} was not detected in the range of 0-300 μg/mL), and PbF did not exhibit antibacterial ability.

Acknowledgements
The authors would like to express their gratitude to the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City for supporting this research. The authors also thanks to Dinh Tran Minh Thu and Nguyen Hoang Thanh Truc for their helpful advice on various technical issues examined in this paper.

Conflict of interests
The authors declare no potential conflicts of interest.

Author contributions
Conceptualization: Quyen PT, Quoc LPT. Methodology: Quyen PT, Quoc LPT. Formal analysis: Quoc LPT. Validation: Quoc LPT. Writing - original draft: Quyen PT. Writing - review & editing: Quoc LPT.

Ethics approval
This article does not require IRB/IACUC approval because there are no human and animal participants.

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