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Research article

Evaluation of Peppercorn Quality from Ba Ria - Vung Tau, Viet Nam

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Abstract

In this study, the quality of some pepper species, collected in Ba Ria – Vung Tau, Vietnam in March 2022, was investigated. The content of oleoresin and piperine was evaluated based on the species, size, ripeness, and density of the peppercorn. Piperine content in the peppers was determined by spectrophotometry (UV-Vis) and high-performance liquid chromatography (HPLC). In addition, the study also examined the quality of white pepper. The results showed the Vinh Linh pepper had high oleoresin content (23.72±0.01%, second only to Sri Lankan pepper) but there were no outstanding records in piperine content among all investigated pepper species. For the size parameter, the piperine content on the dry material declined from the smallest peppercorn size of 2.50-4.00 mm (4.94±0.32%) to the largest size > 5 mm (2.66±0.09%). The oleoresin and piperine recovery efficiency of Vinh Linh pepper tended to stop or decrease when entering the ripening stage. Specifically, ripe peppercorns had low oleoresin content (15.92±0.51%). Similarly, from density of 600 to 300 g/L, the lower the density of pepper, the higher the piperine content (3.11±0.09% to 5.04±0.05%) was found. Furthermore, black pepper obtained 2 times higher oleoresin content than white pepper, but the piperine content was lower by many times. This research shredded light on the classification of the Vietnamese pepper for suitable applications in the commercial market.

Keywords: piperine, oleoresin, black pepper, white pepper

1. Introduction

Pepper (*Piper nigrum* L.) is a major agricultural exported commodity in all pepper-producing countries, and it is also the most widely traded spice (Ravindran & Kallupurackal, 2012). Normally, 80% of the crop is processed into black pepper, while the remainder is processed into white pepper. Black and white pepper are different in their harvest and

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progressing methods. White pepper is the dried seeds that are produced from ripened berries after the removal of the pericarp (Aziz et al., 2019), while black pepper is processed by drying the immature fruit until the wrinkled skin is formed. Both pepper types are widely used in the food industry, and also in the pharmaceuticals and cosmetics industries (Li et al., 2020). Elements affecting pepper quality include differences in species, climate and geology conditions (Oanh et al., 2021), post-harvest handling, as well as storage treatments (Shango et al., 2021).

Oleoresin is known as the spice flavor representative of black pepper (Mathai, 1981), accounts for about 3.9-11.5% of the raw material weight (Dhas & Korikanthimath, 2003). It is extracted by organic solvents such as acetone, ethanol, ethyl acetate, or ethylene dichloride. Today, oleoresin is commonly marketed as spice drops and contains the total pungency ingredients such as piperine and pepper aroma (Ravindran & Kallupurackal, 2012), with a specification of 40:20, meaning there are 40% of piperine and 20% of volatile essential oils (Attokaran, 2017).

Piperine is an alkaloid compound that characterizes the spicy taste of pepper, accounting for about 98% of the total alkaloids (ISO,1981). The piperine concentration varies from 2% to as high as 9%, depending on environmental factors such as climate and/or place of origin, as well as growing conditions. Investigations of piperine bioactivities have reported a very high spectrum of physiological effects, including antihypertensive, antiaggregant, antioxidant, and many others. Along with an array of biological activities, piperine is known for its ability to increase the bioavailability of drugs, and thus enhance their therapeutic potential (Stojanović-Radić et al., 2019). The piperine content in oleoresin is the most important factor from the aspect of commercial utilization (Mathai, 1981).

Presently, in Viet Nam, there have not been many studies on oleoresin and piperine content for different pepper varieties and factors affecting the content of these active chemicals. The aim of the present study is to select the suitable pepper for the utilization purpose, quality requirements, and product cost, regarding the economic value of the pepper production industry in Viet Nam. The evaluation of the oleoresin and piperine content in different types of pepper in Ba Ria – Vung Tau was also evaluated.

2. Materials and Methods

2.1 Chemical

Ethanol 96% from Chemsol, Vietnam was used to extract the oleoresin in pepper samples. Meanwhile, piperine 98% from Sigma Chemical Co., USA was utilized to make the standard solution for the UV-VIS and HPLC measurement. Acetic acid and acetonitrile from Merck Co., Germany was used as the eluting solvents for the HPLC analysis.

2.2 Equipment

A moisture meter (MA35, Sartorius, Göttingen, Germany) was used to determine the moisture content of the pepper samples and the oleoresin. A UV-VIS spectrophotometer (Thermo Genesys 10S UV-Vis, Waltham, MA, USA) and an HPLC Aligent 1100 (Agilent Scientific Instruments, USA) were used in the process of quantifying piperine.

2.3 Sample preparation

Fresh pepper seeds were collected in March 2022 in Ba Ria - Vung Tau, Viet Nam. The spiked berries were dried in the sun for 4 to 8 days on a tarp until they turned black, and the moisture content dropped to less than 12%. Next, the studied samples were sieved and impurities were blown. Subsequently, cleaned peppercorns were classified according to pepper species, size, ripeness, and density (Table 1). For white pepper, the berries were peeled from ripe Vinh Linh peppers by fermentation in the water. After this process, peppers were dried and stored tightly to avoid re-absorption of moisture. For the experimentation, the pepper seeds were ground carefully and gently with a grinder. The milled sample must have a relatively uniform size of less than or equal to 1 mm (ISO 2825:1981 standard) (ISO, 1981).

Table 1. The characteristics of peppers

Group	Classification
Species	Sri Lankan pepper
	Se pepper
	Malaysia pepper
	Vinh Linh pepper
	Sri Lankan pepper
Ripeness	Ripe Vinh Linh pepper
	Old Vinh Linh pepper
	Mature Vinh Linh pepper
Size	Vinh Linh pepper, larger than 5.00 mm
	Vinh Linh pepper, from 4.75 to 5.00 mm
	Vinh Linh pepper, from 4.00 to 4.75 mm
	Vinh Linh pepper, from 2.50 to 4.00 mm
Density	Vinh Linh pepper, larger than 600 g/L
	Vinh Linh pepper, from 500 to 600 g/L
	Vinh Linh pepper, from 400 to 500 g/L
	Vinh Linh pepper, from 300 to 400 g/L
	Vinh Linh pepper, smaller than 300 g/L
White pepper	Ripe Vinh Linh pepper

2.4 Process of extracting sample and quantification of oleoresin content

The process of sample extraction was based on ISO 11027:1993 standard (ISO, 1993). In this study, extraction conditions with the parameters such as ethanol solvent concentration (96% v/v), solid/solvent ratio (1/100 of g solid per mL of solvent), temperature (boiling point of ethanol), and extraction time (180 minutes) were selected. The extraction was performed with a reflux extractor (Figure 1). First, 5 g portions of the ground pepper (weighed to the nearest 0.0001 g) were extracted by refluxing for 3 h with 500 mL of ethanol. After cooling, the extracts were filtered with a vacuum filter to remove solid particles. Finally, each solution was evaporated with a rotary evaporator to obtain oleoresin. The oleoresin content was determined by the difference in mass of the flask after and before evaporation.

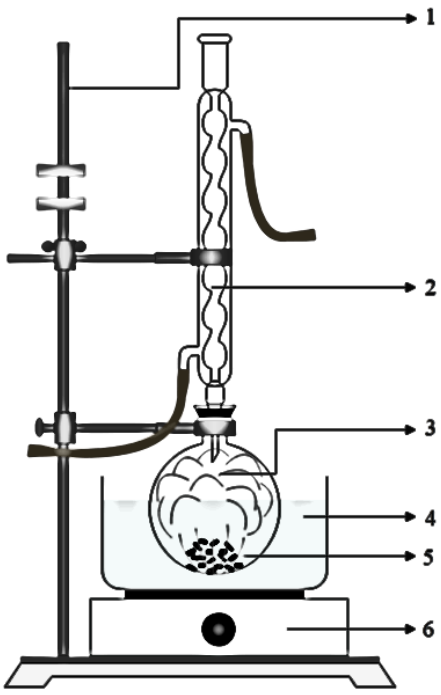

Figure 1. Reflux extraction system
1. Retort stand 2. Condenser 3. Flask
4. Water bath 5. Boiling stone 6. Heat source

The oleoresin yield from the evaporated process with respect to the dry material is calculated by the formula:

$$H(\%) = \frac{m_{oleoresin} \times (1 - W\%)}{m_{dry\ material}} \times 100 \quad (1)$$

With

H (%):	Oleoresin extraction yield with respect to the dry material (%)
$m_{oleoresin}$:	Oleoresin weight (g)
W (%):	The moisture content of oleoresin
$m_{dry\ material}$:	Input dry material weight (g)

2.5 Quantification of piperine content

Recently, spectrophotometry (UV-Vis) and high-performance liquid chromatography (HPLC) are the two optimal methods that have been used to determine piperine. The analysis is based on the absorption of piperine in the UV region at 343 nm (ISO 5564:1982 standard) (ISO, 1982). Both methods are fast and simple and have high specificity for piperine (ISO,1981).

2.5.1 Uv-vis spectrometric method

Primarily, 0.5 g portions of the ground material were extracted by refluxing for 3 h with 50 mL of ethanol (96% v/v) and were carried out in the same procedure for the quantification of oleoresin. The extracts were filtered and made up to volume with ethanol rinsings in 100 mL volumetric flasks. Next, the solution was then further diluted 50-fold with ethanol (96% v/v) and the absorbance (A) of each solution in the UV-VIS spectrophotometer at 343 nm was measured. The experiments were performed three times and repeated if the values deviated by over 5%. The piperine content was calculated as a percentage by mass of the dry material by the formula:

$$\mathrm{PC} = \frac{\mathrm{A}}{1238} \times 50 \times \frac{100}{\mathrm{m}} \times \frac{100}{100 - \mathrm{W}\%} \quad (2)$$

With:

PC:	Piperine content (%)
m:	Material weight (g)
W%:	The moisture content of material
A:	The absorbance of the final solution
1238:	The absorbance of piperine 1% solution in 1 cm cuvette at 343 nm
50:	Diluted coefficient
100:	The volume of the volumetric flask (mL)

2.5.2 High-performance liquid chromatography

1) Chromatographic analytical conditions

HPLC (Agilent 1100, USA) with an autosampler and a photodiode-array detector was used for the analysis of piperine. Analyses were carried out on a ready-packed column (150 x 4.6 mm) of 5 µm Spherisorb ODS-2 (a fully capped C18 bonded-phase packing from Phase Separations Ltd, UK) without the use of a guard column. The flow rate was 1.0 $mL.min^{-1}$ and the injection volume was 10 µL (ISO,1981).

2) Determination of piperine calibration curve

A piperine standard solution was prepared from a 1 g/L piperine stock solution in ethanol 96% solvent. The mobile phase solvent was a solution of 52% acetic acid (99 % v/v) and 48% acetonitrile. Meanwhile, HPLC-grade water was mixed with glacial acetic acid in the proportion 1:99 by volume. The standard equation collected from the calibration curve was $y = 20887x - 20.635$ at $R^2 = 0.9992$. The piperine concentration standard curve was determined according to the peak area (ISO 11027:1993 standard) (ISO, 1993).

3) Measuring the piperine sample

After filtering, the sample extracts were put into 100 mL volumetric flasks and made up to volume with ethanol rinsings. Each solution was diluted 2.5-fold and was then filtered through a 0.22 µm membrane filter with a HPLC syringe filter. The quantification was achieved by comparison of the piperine peak area for each sample with the mean peak area for the standard. The average value was determined from three experiments and

repeated when the standard deviation between values exceeded 5%. The piperine content in each sample was calculated by the formula:

$$PC = C \times 2.5 \times \frac{100}{m_x} \times 100 \quad (3)$$

$$C = \frac{A + 20.635}{20887} \quad (4)$$

With:

PC: Piperine content (%)

A: Piperine peak area (mAU.s)

C: The concentration determined from the piperine peak area standard curve (ppm)

m_x: Sample weight (mg)

2.5: Diluted coefficient

3. Results and Discussion

In this research, four main types of pepper grown in Ba Ria - Vung Tau province, including Sri Lankan pepper, Se pepper, Malaysia pepper, and Vinh Linh pepper were investigated. The quality differences among the peppers depended on the parameters of density, oleoresin, and piperine concentration.

3.1 Quality evaluation of peppercorns according to the density

Nowadays, density is an important element used to evaluate pepper quality and the larger the density, the higher the price of pepper. As shown in Table 2, Vinh Linh pepper had a higher average density (585.00±3.25 g/L) than other species and was much higher than Se pepper (426.33±1.2 g/L). Vinh Linh pepper was a suitable pepper that was planted and grew well in the geological conditions of Ba Ria – Vung Tau province, giving high yields (the Department of Agriculture and Rural Development of Ba Ria - Vung Tau Province, Vietnam, 2014).

Table 2. Pepper density (g/L) according to classification of species, ripeness and size

Species		Ripeness		Size	
Sri Lankan	503.00±20.1	Mature	562.00±5.62	2.5-4 mm	390.33±13.4
Se	426.33±1.20	Old	638.00±3.52	4-4.75 mm	627.67±8.41
Malaysia	570.00±7.80	Ripe	642.00±3.00	4.75-5 mm	597.00±3.60
Vinh Linh	585.00±3.25			> 5mm	568.00±2.60

In addition to the dissimilarity in color, ripe peppercorns are usually larger in size and density than peppers at other stages (Figure 2). The result from the investigation of pepper density correlation with maturity shows that ripe Vinh Linh pepper had a density of 642.00±3.00 g/L, which was much higher than that of mature Vinh Linh pepper, which was 562.00±4.64 g/L (Table 2). Therefore, the ripened pepper was appropriate for producing white pepper due to the large peppercorn size, skull, and density. For the black pepper production process, farmers in Ba Ria - Vung Tau province typically harvest pepper clusters at the mature stage with 10% ripe red fruits. This finding was similar to that obtained by Deans *et al.* (2001) who described the black pepper manufacturing procedures.

(a) Mature pepper (b) Old pepper (c) Ripe pepper

Figure 2. Pepper bunches according to maturity

Consequently, peppercorns in the size of 2.50-4.00 mm were rather slight (390.33±3.43 g/L) because the kernel growth was not complete. On the contrary, the density of pepper was immensely increased in the size of 4.00-4.75 mm and gradually decreased as size further increased. The dimension influenced the dispensation of peppercorns in a pepper-liter density apparatus. The larger the size, the more the space among the peppers increased, so the density of pepper tended to diminish.

3.2 Quality evaluation of Vinh Linh peppercorns according to the oleoresin content

In this experiment, the oleoresin content per dry material weight of pepper species was high, ranging from 21 to 25%, while Anith *et al*. (2018) investigated reflux extraction with 95% ethanol solvent for 24 h at a lower temperature (40^{o}C), and found the content of 10.79%. Nhật et al. (2020) reported that temperature was also an important factor affecting process efficiency, leading to the difference in oleoresin content.

Regarding the ripeness factor, mature Vinh Linh pepper oleoresin content was 21.08±0.15%, rising gradually until the older stage (23.49±0.13%) and then slightly improving when red pepper ripened (23.82±0.87%) (Figure 3). The maturity was also closely related to the size of the pepper. The riper the peppers, the larger the dimension of the seed, and specifically, the oleoresin content gradually decreases with size, from

21.13±0.48% (2.50-4.00 mm) to 15.92±0.51 % (> 5mm). The percentage of oleoresin by peppercorn size showing a tendency to decline after the mature stage was also reported by Mathai (1980).

The density had a negligible effect on oleoresin extraction yield. Specifically, the oleoresin content of Vinh Linh pepper according to density did not differ much (ranging form 20.39 to 23.13%) and did not follow any specific direction (Figure 3).

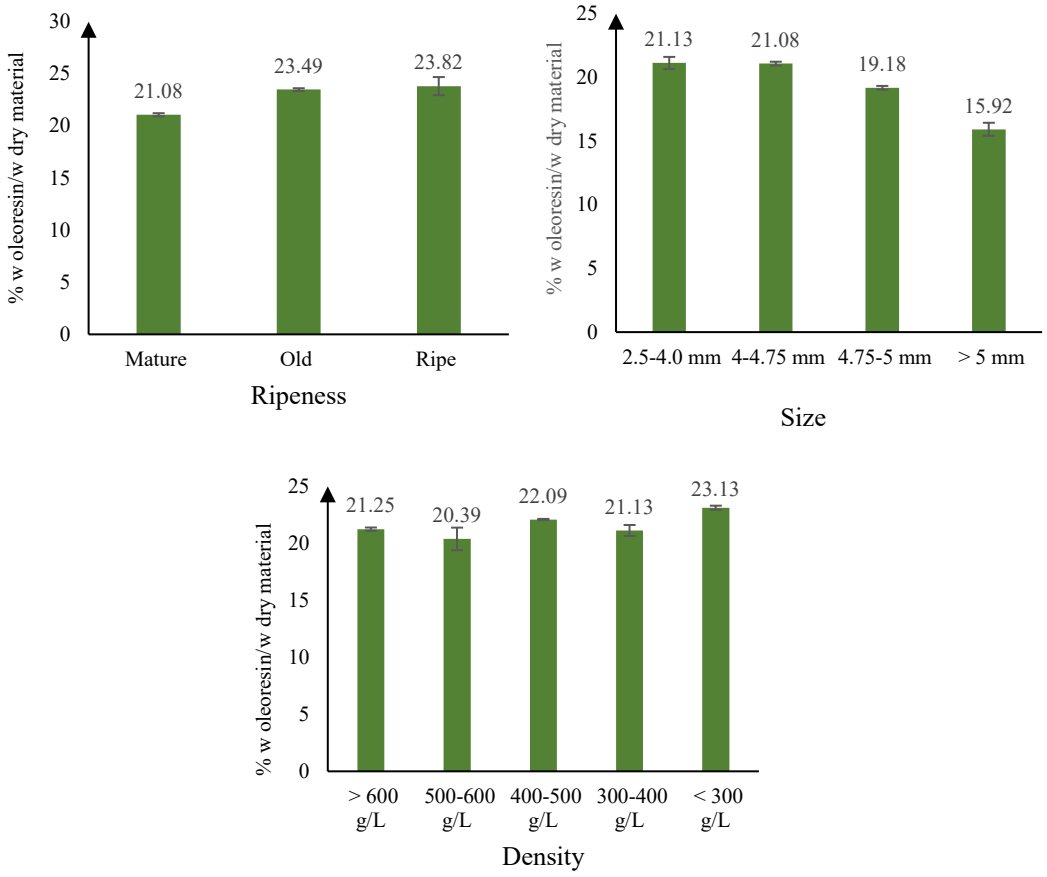

Figure 3. Oleoresin content of Vinh Linh pepper according to ripeness, size and density

3.3 Quality evaluation of Vinh Linh peppercorns according to piperine content

The isomers of piperine (isopiperine, chavicine, isochavicine) and other alkaloids (piperettine, piperanine, piperyline, piperolenine) present in the pepper extract were also detected at 343 nm – which was the piperine maximum absorption wavelength (Gorgani et al., 2017). Typically, the UV-Vis method shows the total content of substances structurally similar to piperine. The piperine content determined by the UV-Vis method was about 2.08-3.00% higher than that of the HPLC method, shown in Figure 4. Therefore, the study also determined the piperine content by the HPLC method, which was more accurate.

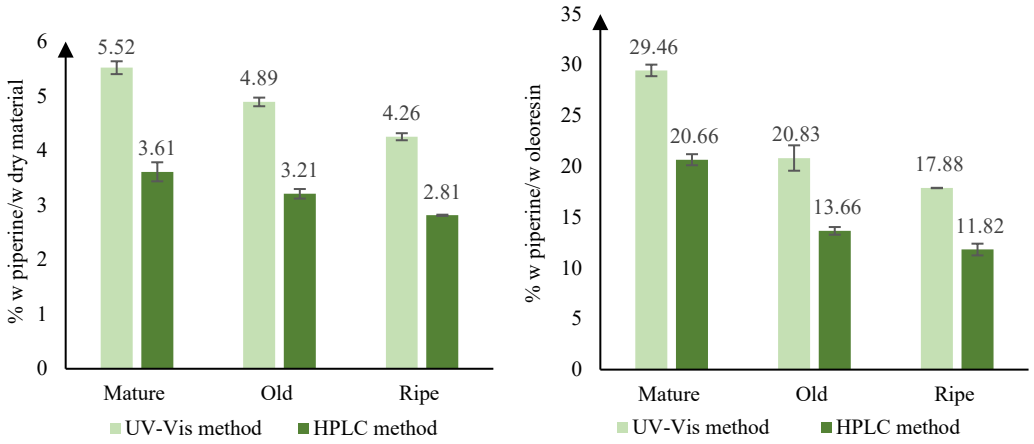

Figure 4. Piperine content of Vinh Linh pepper according to maturity

3.3.1 On peppercorns ripeness

It is clear that the percentage of piperine declined significantly from the mature to the ripe period. The piperine content per mass of the oleoresin changed from 20.66±0.58% at mature stage to 11.82±0.48% at ripe stage (Figure 4). This observation corresponded with that for Sri Lankan pepper reported by Jansz et al. (1984). In accordance with the biochemical description reported by Arianne, piperine is formed by piperine synthase activity, which is abundant in the early phase of pepper growth (Schnabel et al., 2021). Thus, piperine synthesis is not dominant in the second half of development, resulting in a gradual decrease in the concentration of this alkaloid.

3.3.2 On peppercorns size

As reported by Jansz et al. (1984), starch synthesis dominates the second half of pepper development, greatly influencing piperine formation. This finding led to a gradual decrease in piperine content with size as well as maturity. Regarding the result in Figure 5, the alkaloid content per mass of dry material consistently dropped off from the smallest size (4.94±0.32%) to the largest size (2.66±0.09%). The optimal period for harvesting pepper depends on the geological features in the growing location (Mathai, 1980). From a commercial perspective, mature seeds of large size and density were collected to achieve high profits. On the other hand, for extraction purposes, the purchase of small peppercorns both minimizes the cost of raw materials and achieves a high piperine content.

3.3.3 On peppercorns density

Based on the HPLC method, the piperine concentration gradually increased by the decreasing shift from the largest density of > 600 g/L (3.38±0.15%) to the smallest density of < 300 g/L (5.04±0.05%) (Figure 6). Peppercorns size (mentioned in Section 3.1) is related to their density. High-density peppers are referred to as large size but low piperine content. Meanwhile, the peppercorns at a density lower than 300 g/L had kernels that were not yet fully developed. After drying, the peppers mostly accommodated the space of outer

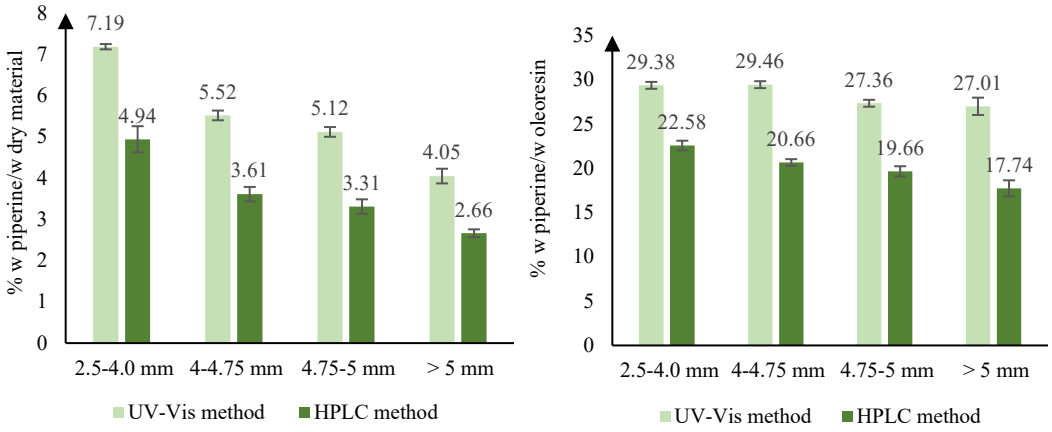

Figure 5. Piperine content of Vinh Linh pepper according to peppercorns size

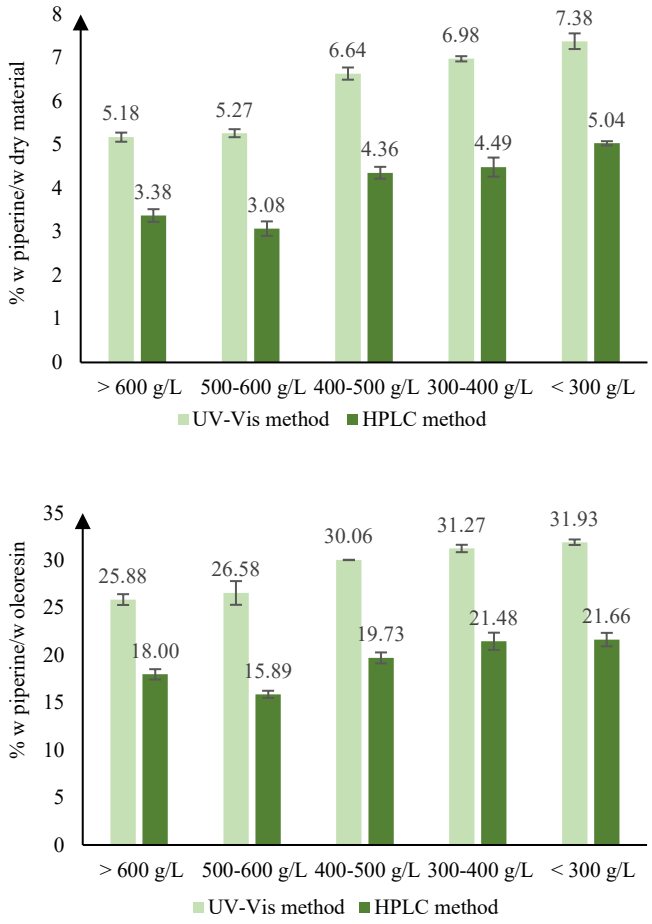

Figure 6. Piperine content of Vinh Linh pepper according to density

shell (Nhật et al., 2020), so the market price was much lower than those of larger density. Despite the low price, this type of pepper contained a high piperine concentration. The reason for this was that for peppers harvested at the old stage, a part of the piperine was still present in the pericarp, leading to high alkaloid content. On account of these advantages, the low-density seeds were considered suitable peppercorns for the purpose of obtaining piperine extracts.

3.4 Quality evaluation of white pepper and black pepper

White pepper is produced by removing the peel of ripe Vinh Linh pepper, the more ripened the pepper, the harder the endocarp and the heavier the peppercorns. White pepper has a much higher density (759.5±0.92 g/L) than black pepper (585.0±0.03 g/L). As reported by Aziz et al. (2019) who declared that oleoresin was a mixture of resin, essential oil, and piperine alkaloid found in the peppers. They reported that the essential oil was present mainly in the pericarp, so the oleoresin content in black pepper (23.72±0.09%) was 2 times higher than that of white pepper (11.04±0.05%). On the other hand, the piperine was mostly formed in the pepper core (90%) (Somashekar et al., 2021). Consequently, in our work, the removal of the outer shell significantly increased the average piperine concentration in white pepper (31.35±0.38%) which was much greater than in black pepper (19.02±0.03%) (Figure 7). Today, the production and consumption of white pepper is filling the demand gap in the current pepper market. White pepper is widely used in food as well as in medicine because of its high piperine content.

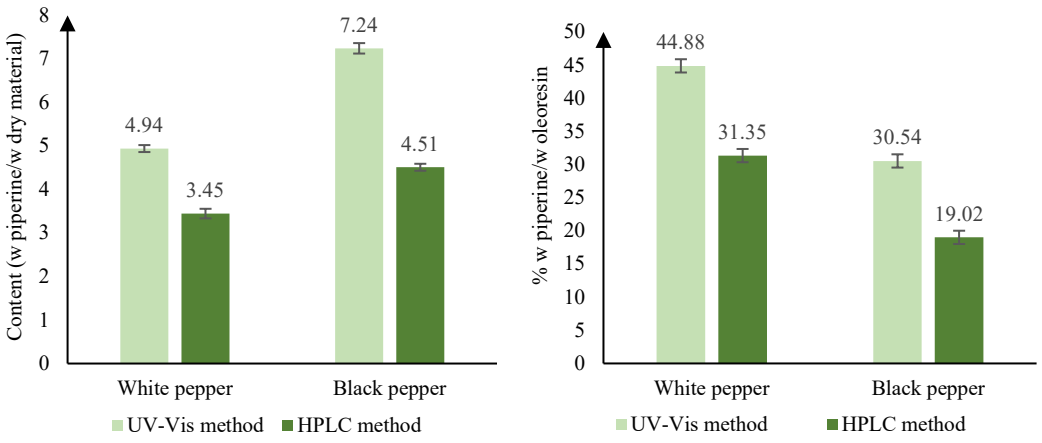

Figure 7. Piperine content of white pepper and black pepper

4. Conclusions

This study evaluated the quality of peppercorns in Ba Ria - Vung Tau, Vietnam based on the classification of species, maturity, size, density, and quality of white pepper. Vinh Linh pepper is a commonly used pepper because of its relatively high piperine and oleoresin content and its high density, and because it grows well in the area. For economic purposes and the requirements of the utilization, the determination of density, oleoresin, and piperine content has brought momentous significance to the pepper market in Vietnam. Specifically, suppliers want to sell high-weight and good-quality peppers to achieve profits. On the other

hand, low-density peppercorns but high piperine content save costs for the purchaser. With the object of evaluating peppercorn quality more completely, it is necessary to conduct further quantification of essential oils in black pepper and determine the composition of essential oils by gas chromatography-mass spectrometry (GC-MS). Besides, the process of piperine crystallization in white pepper needs to be studied further to more efficiently produce piperine crystals of higher purity. The crystallization of piperine in coarsely ground black pepper after partial removal of the essential oil is also considered necessary because of its lower cost than white pepper.

5. Acknowledgements

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6. Conflicts of Interest

We have no conflicts of interest to disclose. All authors made significant contributions to this study and approved the final version of the manuscript. Specifically, Xuan-Tien Le and Hong-Nhung Duong-Nguyen administered the project, completed the manuscript; Ai-Man Tran performed the experiments; and Minh-Chau Pham-Vu edited and finalized the article.

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