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Efficacy of Different Solvent for Oleoresin Extraction and Physicochemical Properties of White Pepper Produced *Via* Water Retting

(Keberkesanan Pelarut Berbeza untuk Pengekstrakan Oleoresin dan Pencirian Fizikokimia Lada Putih yang Terhasil Melalui Pengeretan Air)

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ABSTRACT

Initially, four different solvents were compared to determine the best solvent for white pepper oleoresin extraction. Methanol was chosen as it resulted in the highest yield and total phenolic content (TPC) of oleoresin extract from white pepper. This study was also carried out to determine the functional and physicochemical properties of white pepper produced via *water retting. Fresh pepper berries were soaked in distilled water (1:2, w/w) at four different temperatures of 28, 35, 42, and 49 °C for 16 days. The yield, oleoresin content, colour, fracturability, free radical scavenging activity, and TPC of white pepper produced were determined on the 4th, 8th, 12th, and 16th day of the retting process. Results showed that 8 days of retting at 28 °C was sufficient to produce a significantly ($p < 0.05$) high yield and most fractured white pepper. For other properties, it was suggested that retting time of 8 days was sufficient to produce satisfactory whiteness value, piperine content, free radical scavenging activity, and TPC. Therefore, it can be concluded that the quality of white pepper very much depended on retting temperature compared to the duration of the retting process.*

Keywords: Oleoresin extraction; phenolic content; physicochemical; water retting; white pepper

ABSTRAK

Kajian awal melibatkan perbandingan empat pelarut berbeza untuk menentukan pelarut terbaik bagi pengekstrakan oleoresin lada putih. Metanol telah dipilih kerana memberikan hasil dan jumlah kandungan fenolik (TPC) tertinggi daripada ekstrak oleoresin lada putih. Kajian ini juga dijalankan untuk menentukan ciri-ciri berfungsi dan fizikokimia lada putih yang dihasilkan melalui pengeretan air. Beri lada segar direndam dalam air suling (1: 2, w/w) pada empat suhu berbeza iaitu 28, 35, 42 dan 49 °C selama 16 hari. Jumlah hasil, kandungan oleoresin, warna, kebolehretakan, aktiviti perencatan radikal bebas dan TPC lada putih yang dihasilkan ditentukan pada hari ke-4, ke-8, ke-12 dan ke-16 proses pengeretan. Hasil menunjukkan bahawa pengeretan selama 8 hari pada suhu 28 °C adalah mencukupi untuk menghasilkan jumlah hasil lada putih dan kebolehretakan yang tinggi ($p <0.05$). Bagi ciri-ciri lain, dicadangkan bahawa tempoh masa lapan hari pengeretan adalah mencukupi untuk menghasilkan nilai kecerahan, kandungan piperina, aktiviti perencatan radikal bebas dan TPC yang memuaskan. Oleh itu, dapat disimpulkan bahawa kualiti lada putih adalah sangat bergantung kepada suhu berbanding dengan tempoh masa proses pengeretan.

Kata kunci: Fizikokimia; kandungan fenolik; lada putih; pengekstrakan oleoresin; pengeretan air

INTRODUCTION

Water retting is a traditional practice that has been widely used worldwide to produce white pepper. During the water retting technique, ripe berries of pepper (*Piper nigrum* L.) are immersed for up to two weeks in flowing water such as the river or in static water tanks to allow the decaying of the outer layer of the pepper pericarp (Nair 2011; Tainter & Grenis 2001). During this soaking period, the pepper pericarp begins to decay slowly. The naturally existing microorganisms in the water help to loosen and degrade the pericarp skin from the core (Vinod et al. 2014). After sufficient retting, pepper berries are threshed to remove the outer skin and finally sun dried (Paulus 2009). Water retting usually recovers about 25% or 25-28 kg of white pepper from 100 kg retted ripe berries, with an

average loss of 8-9 kg during the production process (Aziz et al. 2019). Besides water retting, dried pepper berries or black pepper can also be used to produce white pepper (Sons et al. 2007). White pepper has been reported to be used in various food, added in meats, seafood, snacks, and in vegetable preparations (Attokaran 2011). The quality of white pepper is often characterised by its functional and physicochemical properties (Vinod et al. 2014). Therefore, it is essential to study these properties to determine the quality of white pepper.

Oleoresin, piperine, and essential oil are the major constituents that are responsible for the peppery sensation of white and black pepper (Tainter & Grenis 2001). Piperine is a major bioactive component of pepper, characterised by nitrogen-containing compound contributing to the pungency of pepper (Ravindran & Kallupurackal 2001) and can be sensitive to light and oxygen (Shaikh et al. 2006). The concentration of piperine in the Piperaceae family can be varied; for example, it has been reported to constitute about 2.0 and 7.4% in black pepper and white pepper, respectively, while in some reports the piperine content was found to be much higher in black pepper at up to 9.0% (Parthasarathy et al. 2008; Peter 2006; Ravindran 2003). Overall, the value of pepper depends on its pungency and flavour, which is commonly attributed by the presence of piperine alkaloid as well as oleoresin and essential oils (Gorgani et al. 2017). These beneficial compounds can be extracted using the selected method and solvent.

Various techniques and solvent extractions have been conducted on pepper to obtain its valuable chemical constituents such as oleoresins, piperine, as well as essential and volatile oils. These constituents have been widely reported to be beneficial not only as a flavouring agent but also as a food supplement and in pharmaceutical (Shanmugapriya et al. 2012) due to the medicinal and preservative properties (Meghwal & Goswami 2013; Zarai et al. 2013). Due to the bioactive compound present, pepper is found to exhibit numerous health and therapeutic effects to humans in both traditional and modern practices (Meghwal & Goswami 2013; Ravindran & Kallupurackal 2001).

In the determination of good solvents for extraction, a solvent with a high extraction rate and good reproducibility should be chosen (Qi et al. 2014). The solubility of compounds in solvents can vary considerably. For instance, a more polar compound such as phytosteryl glycosides is greatly soluble in polar solvents (Dutta 2003). In terms of sample preparation, a homogenous and high surface area of the sample is able to enhance the extraction efficiency. Dry sample must be ground into a fine size and sieved to get a homogenous size to enhance extraction reproducibility (Mothersill & Austin 2003). The nature of the sample also plays a significant role in extraction. It is relatively easy to liberate most phytosterols from oil grains and nuts through solvent extraction compared to hard cell walls or from complex polysaccharide-protein matrices in wet vegetable tissues and cereals (Dutta 2003).

Among the different extraction techniques that have been previously reported are subcritical water extraction (Hassas Roudsari 2007), continuous subcritical water extraction (Gámiz-Gracia & Luque de Castro 2000; Jiménez-Carmona, et al. 1999; Soto Ayala & Luque de Castro 2001), classic solvent extraction (Shaikh et al. 2006; Shingate et al. 2013; Zarai et al. 2013), supercritical fluid extraction (Hamrapurkar et al. 2011; Lenucci et al. 2015), and hydrotropes extraction (Shingate et al. 2013). Other studies mentioned that the conventional Soxhlet method yields the best overall results among five different extraction procedures (Prytula & Pavolostathis 1996). Therefore, Soxhlet extraction using dry and ground sediment is recommended in this study.

It is known that different types of extractants provide different responses and ability to extract bioactive compounds present in the food sample. Previous works have shown that pepper can be extracted repeatedly with organic solvents such as acetone, ethanol, or chlorinated hydrocarbons (Marion 1960), dichloromethane (Raman & Gaikar 2002), petroleum ether and chloroform (Kolhe et al. 2011), a mixture of solvents such as n-hexane, ethyl acetate, and glacial acetic acid (Hamrapurkar et al. 2011), as well as ethanol and water (Santamaría et al. 2000). The findings from these studies vary differently as the solvents have different effects thus, yielding different results. This study was conducted to determine the antioxidant potential as well as the total phenolic content of commercial white pepper extracted using four different solvents, namely n-hexane, dichloromethane, methanol, and ethanol. Each of the solvents was chosen due to the different polarity and ability to extract active compounds from the sample.

Materials and Methods

Pre-determination solvent for white pepper extraction

Five gram of white pepper powder (Peace Brand, Chiapheng Chng Sdn Bhd) was purchased from local supermarket Jaya Grocer, Bangi and was treated with four different solvents (200 mL) namely n-hexane, dichloromethane, methanol, and ethanol (analytical grade, Merck Sdn Bhd) for three hours. All extracts were then subjected to the determination of oleoresin, DPPH activity and TPC for comparison to choose the best solvent prior to extract the white pepper from water retting.

DETERMINATION OF OLEORESIN YIELD OF WHITE PEPPER FROM WATER RETTING

Five grams of fine ground white pepper powder was sieved and subjected to controlled Soxhlet extraction. Each extraction used 200 mL of methanol and extracted for 3 h. The extracted methanol was collected in empty round bottom flask, W_i (g) and then removed using rotatory evaporator consistently for 24-25 min at 60 rpm on 50 °C until thick and green liquid was obtained. The mass of flask after solvent removal W_f (g) deduce W_i (g) and recorded as mass of oleoresin extracted W_o (g). The extracted yield was recorded (%) and stored at a chill temperature below 4 °C.

DETERMINATION OF FUNCTIONAL AND PHYSICOCHEMICAL PROPERTIES OF WHITE PEPPER PRODUCED BY WATER RETTING PROXIMATE ANALYSIS OF FRESH PEPPER BERRIES

Three batches of pepper berries were analysed to determine the ash content, moisture, total protein, total fat, crude fibre, and total carbohydrates. All sample were done in quadruplicate (n = 4) using the AOAC (1975) analytical method.

WATER RETTING PROCESS

Fresh pepper berries were obtained from Herba Bagus farm in Kluang, Johor. Fresh pepper was immersed in water with a 1:2 ratio (pepper berries:water, w/w) (Aziz et al. 2018). All flasks (n = 3) were then placed in a temperature-controlled Orbital water bath shaker (Gyromax 929) at temperature of 28, 35, 42, and 49 °C for 4, 8, 12 and 16 days. The temperature of the water bath was monitored regularly using a thermometer. Retting water was changed daily to avoid bacterial contamination and to mimic the conventional retting method.

DETERMINATION OF YIELD

White pepper yield obtained after the retting process was air dried for a day and dried in an oven for 5 h at 60 °C. The weight of white pepper after oven drying was recorded as the final yield.

DETERMINATION OF WHITENESS

White pepper seed was ground into fine powder to assess the colour of the pepper produced. Colour was measured using a Minolta colourimeter (Chromameter CR 400, Japan) with a Hunter Lab colour system (L *, a *, and b *). The whiteness value was calculated using the Color iMatch Color Calculations Guide formula, WI*hunter* = L - 3b.

DETERMINATION OF FRACTURABILITY

The fracturability test of white pepper was carried out using Shimadzu Twin-Column Texture Analyzer 500 N (AGS-Japan). White pepper seeds (n = 3) within the size range of 4.0-4.2 mm were compressed using a 20 mm diameter cylindrical probe at a rate of 25 mm min^{-1} and load of 0.5 kN. The value of force (N) required to break the white pepper seed was recorded.

DETERMINATION OF PIPERINE CONTENT

White pepper was extracted for 3 h with 95% methanol using the Soxhlet extraction technique. The liquid extract was diluted 1000x, and 20.00 µL of the diluent was injected into High-Performance Liquid Chromatography (HPLC) equipped with *Photodiode Array Detector* (PDA). The column used was Water XBridge (C18 4.6 × 150 mm, 4.5 µm) with a flow rate of 1 mL min^{-1} and mobile phase of methanol:deionized water (2.3:1). Piperine analytical standard (Sigma Aldrich) of 1, 2, 4, 6, 8, and 10 ppm were prepared as a reference.

DETERMINATION OF RADICAL SCAVENGING ACTIVITY (DPPH)

A diluted volume of 1 mL sample (0.5 mg/mL) was added to 2.9 mL methanolic DPPH solution (0.15 mM) in the test tube. After storing for 30 min in the dark, 200 µL of mixture reaction was pipetted into 96 well plates and measured at 517 nm by using a microplate spectrophotometer (BioTek Epoch). The percentage of radical activity was measured according to (1).

$$\% \text{ Free radical scavenging activity} = (100\,[Ab1 - As2])/Ab1 \quad (1)$$

where Ab1 was the absorbance of DPPH blank and As2 was the absorbance of sample. Ascorbic acid standard calibration was prepared for 0, 10, 20, 30, 40, and 50 ppm.

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

Phenolic content of white pepper was measured using the Folin-Ciocalteu method. Sample extract of 100 µL (0.5 mg/mL) was added to 5 mL of Folin-Ciocalteu reagent (1:10) and 4 mL solution of 7.5% Na_2CO_3. The mixture was then stored in the dark for 90 min. Afterwards, 200 µL of the mixture was pipetted into 96 well plates and measured at 760 nm absorbance by the microplate spectrophotometer BioTek Epoch. A standard curve Gallic acid was prepared at a concentration of 1-100 ppm and phenolic activity was determined for per g sample (mg GAE/g).

STATISTICAL ANALYSIS

All the measurements were carried out in triplicates, and the values obtained were expressed as mean ± standard deviation (SD). The data obtained from the experiments were subjected to the two-way analysis of variance (ANOVA) followed by the Duncan test to determine the means and significant difference. IBM Statistical Package for the Social Sciences (Version 22) software was used for conducting the statistical analysis.

RESULTS AND DISCUSSION

EFFICACY OF DIFFERENT SOLVENT FOR EXTRACTION OF WHITE PEPPER OLEORESIN

From the study, there is significant different (p<0.05) for the extracted yield obtained using different solvents (Table 1). The most significantly (p<0.05) highest yield was obtained by methanolic extracts at 13.89 ± 1.28% and the lowest was by acetic acid at 3.84 ± 3.30%. The extracted yield collected from solvent extraction from white pepper powder is the oleoresin as mentioned in many references (Borges & Pino 1993; Premi 2000; Ravindran & Kallupurackal 2012). Oleoresin is the thick and greenish liquid extract mainly composed of oils, resin, piperine, and volatile oils (Srinivasan 2007). With respect to hygienic considerations, spice oils and oleoresins prove to be a suitable alternative for food flavouring (Schweiggert et al. 2007). Besides white pepper, oleoresin can also be found in other types of plants such as cardamom, ginger, turmeric, and garlic.

From the study, a significant difference ($p < 0.05$) was observed for the oleoresin yield obtained using different solvents. Table 1 shows the most significant highest ($p <$ 0.05) yield was obtained by methanolic extracts at 13.89 ± 1.28% and the lowest was by acetic acid at 3.84 ± 3.30%. Acetic acid extracted yield was not significantly different ($p > 0.05$) from n-hexane and ethanol. Oleoresin in pepper has been reported to be extractable using dipolar aprotic solvents (acetone and ethyl acetate), halogenated solvent (ethylene dichloride), and polar protic solvent (ethanol) (Balasubramanian et al. 2016; Ravindran & Kallupurackal 2001).

Report stated that yields of extractable oleoresin were in the range of 5-15% showing methanol (13%) is a great solvent to extract oleoresins in white pepper due to its high extraction yield while n-hexane and acetic acid are the opposite. This may be due to the higher polarity of methanol that enables it to give a better yield extract. Polar lipids such as phospholipid may be contained in the oleoresin extract as it dissolves easily in methanol. Meanwhile, lipids such as triacylglycerides or sterol esters with a functional group of low polarity are soluble in non-polar hydrocarbon solvents like hexene (Meullemiestre 2015). From the study, the low polarity solvent (e.g. hexane) results in a lower oleoresin yield, but it shows the amount of non-polar lipid extractable from the sample. However, the concentration in the extract may be reduced if a non-polar solvent is used because methanol dissolves a larger portion of polar compounds.

As shown in Table 1, all extracts from different solvents resulted in significantly different ($p < 0.05$) free radical scavenging activities. The results showed that each solvent has a different ability to extract the component that exhibits the free radical scavenging capability. The lowest activity was recorded with acetic acid extract at 42.95 ± 1.52%, followed by n-hexane at 51.89 ± 0.09%, methanol at 83.16 ± 0.38%, and the highest was ethanol at 87.51 ± 0.38% (Borges & Pino 1993; Kanaki et al. 2008; Sadasivam & Manickam 1992; Sruthi et al. 2013). This shows that ethanol is a great solvent to extract antioxidant compounds from white pepper powder, followed by methanol, n-hexane, and lastly acetic acid. Many researchers have previously used ethanol to extract the chemical content of pepper oleoresin.

Oleoresin in white pepper may be the compound that contributes to the DPPH activity and TPC. However, the solubility of oleoresin in each solvent seems to be different, hence giving the different results of DPPH activity. Misharina et al. (2009) demonstrated a significant influence of the concentration of white pepper essential oils on their antioxidant properties. Singh et al. (2013) stated that piperine, flavonol glycosides, and phenolic amides are responsible for the antioxidant activity of oleoresins. Piperine in oleoresins extracted using an organic solvent such as ethanol is able to exhibit an antioxidant effect (Choi et al. 2007; Upadhyay et al. 2013).

Zarai et al. (2013) stated that piperic acid, the base-hydrolysis of the alkaloid piperine, is able to give a higher radical scavenging effect due to the presence of the -COOH radical after the transformation of piperine into piperic acid. Other than that, Steinhaus and Schieberle (2005) reported that a-pinene, linalool, b-damascenone, eugenol, skatole, m-cresol, guaiacol, and piperonal are among the main chemical constituents with antioxidant capacity in white pepper. Piperine compound has different solubility in different solvents; as shown in the work of Shingate et al. (2013), ethanol, dichloromethane, and glacial acetic acid gave 3.2, 5.0, and 4.6% yield of piperine, respectively. Piperine is generally soluble in petroleum ether, chloroform, ethanol, and methanol but has poor solubility in water (Kolhe et al. 2011). Because of the different degree of solubility of piperine in different polarities of solvent, some studies have worked on optimising the solvent extraction and the use of a mixture solvent such as n-hexane: ethyl acetate: glacial acetic

acid (3:1:0.1) in HPTLC (Hamrapurkar et al. 2011) and double by-pass Soxhlet extraction by using methanol (Subramanian et al. 2016).

TPC was conducted to determine the polyphenols of the sample. Plant polyphenols are found to be an effective oxygen scavenger, reducing agent, and hydrogen atom donator (Karaman et al. 2010; Nurhanan et al. 2012). According to Hatami et al. (2014), plant phenolics are able to function as primary antioxidants or free radical scavengers. There is a significantly different ($p < 0.05$) effect of different solvents on the TPC, in which methanol gave significantly ($p < 0.05$) the highest TPC compared to the other solvents with 49.96 ± 4.73 mg GAE/g (Table 1). This indicates that methanol solvent is able to extract more yield as well as phenolic content of white pepper whilst acetic acid solvent extract has the lowest results on TPC. This is similar to Butsat and Siriamornpun (2016), where the treatment of *Amomum chinense* leaves with 80% methanol for 12 h gave a higher amount of phenolics and antioxidant activity compared to the other conditions tested.

This shows that the most appropriate solvent to give a high percentage of free radical scavenging activity is ethanol whilst methanol is best used to obtain a high total phenolic content from white pepper. The high free radical scavenging activity exhibited by the results may also be contributed by α-tocopherol and polyphenol contents (Ravindran & Kallupurackal 2001) and also the alkaloid piperine present in white pepper (Andrade & Ferreira 2013; Upadhyay et al. 2013). This polyphenol content subsequently gives the effect of high antioxidant activity in white pepper but often lower than in black pepper (Agbor et al. 2006). Table 2 shows the correlation between oleoresin yield, TPC and antioxidant. It can be observed that there is a positive and significant ($p<0.05$) correlation between the three variables. Similar results of a highly positive correlation between TPC and antioxidant activity has been reported in various plant species and plant-based food products (Esmaeili et al. 2016; Wern et al. 2016). This study chooses methanol as the solvent for extracting the sample as it is able to give a significantly high extraction yield and extract the active phenolic compound from white pepper.

TABLE 1. Effect of different extraction solvent on the oleoresin yield, DPPH, and TPC

Types of solvent	Polarity Index*	Oleoresin (%)	DPPH activity (%)	TPC (mg GAE/g)
Acetic acid	6.0	3.84 ± 3.30 ^{b}	42.95 ± 1.52 ^{d}	19.56 ± 0.37 ^{c}
Methanol	5.1	13.89 ± 1.28 ^{a}	83.16 ± 0.38 ^{b}	49.96 ± 4.73 ^{a}
Ethanol	4.3	7.75 ± 1.07 ^{b}	87.51 ± 0.38 ^{a}	42.97 ± 6.14 ^{ab}
N-hexane	0.1	4.28 ± 0.36 ^{b}	51.89 ± 0.10 ^{c}	33.19 ± 6.09 ^{bc}

*As stated in Lough and Wainer (1995)

TABLE 2. Correlation between oleoresin yield, TPC, and antioxidant

	Factor	Oleoresin	TPC	Antioxidant
Oleoresin	Pearson Correlation	1	0.771*	0.760*
	Sig. (2-tailed)		0.025	0.029
	N	8	8	8
TPC	Pearson Correlation	0.771*	1	0.875**
	Sig. (2-tailed)	0.025		0.004
	N	8	8	8
Antioxidant	Pearson Correlation	0.760*	0.875**	1
	Sig. (2-tailed)	0.029	0.004	
	N	8	8	8

*significant at $p < 0.05$ **significant at $p < 0.01$

PROXIMATE ANALYSIS OF FRESH PEPPER BERRIES

Many studies have shown the proximates of white pepper and black pepper but they are lacking on fresh pepper berries. From Figure 1, it can be seen that pepper berries have high moisture content (67.26%), followed by total carbohydrate (17.44%), crude fibre (8.75%), total fat (4.4%), ash (1.43%), and total protein (0.72%). Dry recovery percentage varies among cultivars and growing conditions, but usually, the recovery range is 28-38% for black pepper (Ravindran & Kallupurackal 2012) and 20-25% for white pepper (Paulus 2009). This indicates more than half of the mass of pepper berries belongs to the non-recovery matters mainly from their disposed skin and flesh. It has been reported that the proportion of starch increases as the berries reach maturity for green pepper. From the age of 4.0 to 5.5 months, the starch content of green pepper can range from 15.66 to 46.62% according to respective cultivars (Ravindran & Kallupurackal 2001). Ravindran (2003) found that the bulk density has a positive correlation with the starch of black pepper. A positive correlation was also observed between crude fibre and essential oil, crude fibre and total fat, and essential oil and total fat. Total fat (4.4%) obtained from pepper berries may represent the oleoresin and essential oils content in pepper berries. Crude fibre found in pepper berries was 8.75%. Fibres from different sources of the plant contain different amounts of cellulose, hemicellulose and lignin. The lignocellulosic material in the plant fibres degrades during storage, but the ash content containing inorganic materials increases with storage period (Ramawat & Ahuja 2016). Ash provides a measure of inorganic minerals in pepper berries which is 1.43%. Plant leaves commonly have a higher ash content compared to other parts of the plant (Goss 2013). The total protein content from this study was found lower at 0.72% probably due to the young pepper berries used, as protein is usually higher in mature seeds or plants. Samaj and Thelen (2007) stated that plant cells generally contain low amounts of protein protected by the cell walls; hence, the extraction of protein from plant samples is a bit challenging and requires extreme measures.

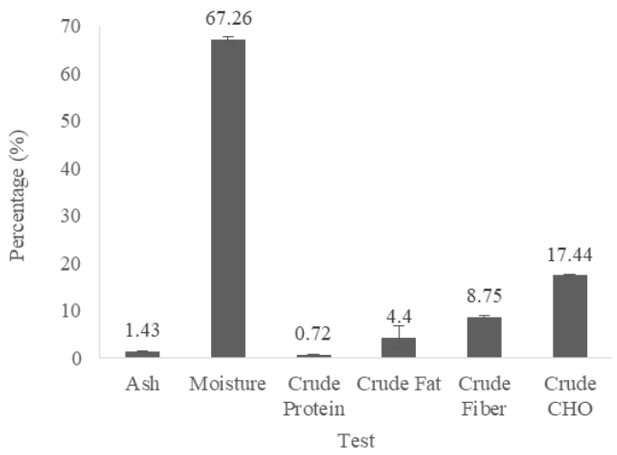

FIGURE 1. Proximate analysis of fresh pepper berries

PHYSICOCHEMICAL PROPERTIES OF WHITE PEPPER PRODUCED *VIA* WATER RETTING WHITE PEPPER YIELD

Figure 2 shows the white pepper yield produced after the water retting process at different temperatures and days. It can be observed that the retting temperature of 28 °C produced a significantly higher yield by mass ($p < 0.05$) compared to other retting temperatures tested. The highest yield (19.19 ± 4.35%) was obtained after 12 days of water retting at 28 °C but was not significantly different from the yield retted on day 8 at 28 °C. Other previous works have reported variations in the maximum number of days ranging from 9 to 15 days for the soaking of pepper berries (Ibrahim et al. 2014). According to Paulus (2009), after 10 days of retting, the recovery rate for white pepper is about

20-25% of the weight of mature berries. In comparison, this study shows that it is sufficient to ret the pepper berries for only 8 days at 28 °C (19.02 ± 3.48%) to produce a significant yield. Retting conducted in natural water sources such as the river and pond may be subjected to inconsistent temperatures according to the weather. Thus, it may require a longer time to ret the pepper berries fully. The presence of microorganisms at optimum temperature is able to produce sufficient enzymes that can breakdown the cell wall of the pepper thus aiding the decortication process (Akin 2010; Tahir et al. 2011). The lowest yield obtained was 2.11 ± 0.94% on the 4th day at 42 °C. Yields from all other temperatures tested (35, 42 and 49 °C) regardless of the number of retting days had no significant difference ($p > 0.05$) between each other suggesting that a temperature higher than 28 °C is not favourable for pepper berries decortication.

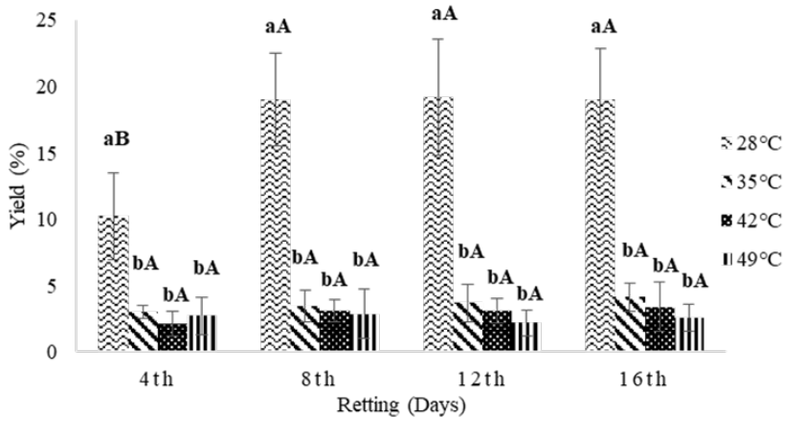

FIGURE 2. Yields of white pepper produced after the water retting process at different temperatures and days. [a-b] Means ± standard error with different retting temperatures are significantly different ($p < 0.05$). [A-B] Means ± standard error with different retting times are significantly different ($p < 0.05$)

WHITENESS OF WHITE PEPPER

According to Zachariah (2008), a bright creamy white colour is one of the properties that contribute to the high value and acceptance of white pepper produced. It was observed that prolonged immersion in low temperature (28 °C) particularly after 12 to 16 days lowered the whiteness of white pepper. Figure 3 shows that retting at 42 °C and after 8 days gave a significantly ($p < 0.05$) high whiteness level of 31.17 ± 0.91. Meanwhile, a lower whiteness level of 27.25 ± 1.22 and 27.27 ± 1.55 was obtained at 28 °C after 12 and 16 days, respectively.

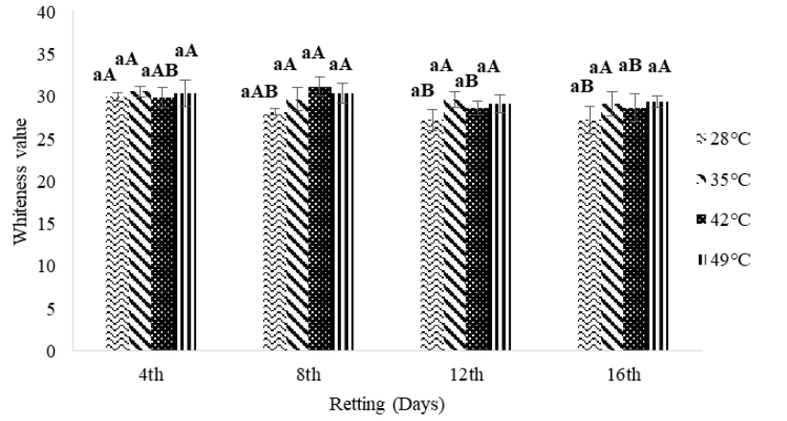

FIGURE 3. Whiteness of ground white pepper produced after the water retting process at different temperatures and days. [a-b] Means ± standard error with different retting temperatures are significantly different ($p < 0.05$). [A-B] Means ± standard error with different retting times are significantly different ($p < 0.05$)

FRACTURABILITY OF WHITE PEPPER

Figure 4 shows the fracturability of white pepper produced. The result shows that a lower retting temperature at 28 °C recorded a significantly ($p < 0.05$) lower mean force compared to other temperatures (at the same day) showing the ease of breaking the white pepper produced. A low force was obtained at 28 °C on day 4 followed by day 8, and the lowest is on day 16 with 22.67 ± 0.19 N for the same temperature (28 °C). The longer time and low temperature of retting seem to allow more uptake of water that would soften the pericarp and also the internal seed. This result was also reported by Rosnah and Chan (2014), describing the efficiency of the retting process was very much influenced by the uptake of water into the pericarp during the soaking period that softened the pericarp. Their findings show that at the fifteenth soaking day, the fracture force becomes constant at 21.89 N for 10 samples tested. The lower force to crack white peppercorn can help to ease milling process to get pepper powder.

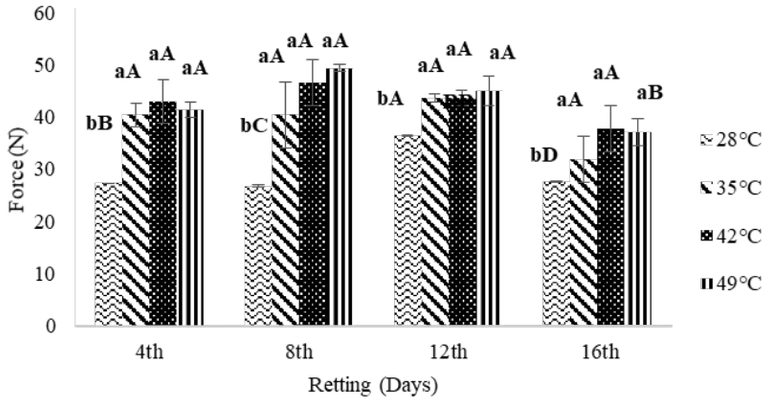

FIGURE 4. Fracturability of white pepper produced after water retting process at different temperature and days. [a-b] Means ± standard error with different retting temperature are significantly different ($p<0.05$). [A-B] Means ± standard error with different retting time are significantly different ($p < 0.05$)

FUNCTIONAL PROPERTIES OF WHITE PEPPER PRODUCED *VIA* WATER RETTING FREE RADICAL SCAVENGING ACTIVITY OF WHITE PEPPER

Table 3 shows that at 28 °C, the free radical scavenging activity of white pepper is significantly lower ($p < 0.05$) compared to all other temperatures (35, 42, and 49 °C). High antioxidant properties of white pepper were observed at a higher temperature of 35 °C on day 8 with 92.09 ± 0.19% while a lower temperature of 28 °C and day 4 produced the lowest antioxidant properties with 85.07 ± 5.60%. Agbor et al. (2006) reported lower concentrations of the non-hydrolysed extract of white pepper had significantly higher ($p < 0.001$) DPPH radical scavenging compared to the non-hydrolysed extract of black pepper. The study suggests that the principal components in white pepper belong to the phenolic compounds that exhibit antioxidant activity.

The antioxidant activity in pepper is attributed by the tocopherol and polyphenol contents in it (Andrade & Ferreira 2013). The result of DPPH is consistent with the total phenolic content (TPC) in which 35, 42, and 49 °C resulted in higher TPC compared to retting at 28 °C. Besides the polyphenol compound, Zarai et al. (2013) also stated that piperic acid is able to give a higher radical scavenging activity than piperine. At a final concentration of 50 mg/mL, the percentage of radical scavenging activity of piperine and piperic acid was 10.28% and 29.5%, respectively. The scavenging activity after transformation of piperine into piperic acid can be related to the presence of the -COOH radical.

TABLE 3. Free radical scavenging activity (%) of white pepper produced after the water retting process at different temperatures and days

	28 °C	35 °C	42 °C	49 °C
Day 4	85.07 ± 5.60 ^{bA}	92.03 ± 0.47 ^{aA}	91.76 ± 0.31 ^{aA}	91.71 ± 0.19 ^{aA}
Day 8	87.76 ± 0.82 ^{bA}	92.09 ± 0.19 ^{aA}	92.07 ± 0.22 ^{aA}	91.61 ± 0.29 ^{aA}
Day 12	87.87 ± 0.29 ^{bA}	91.68 ± 0.19 ^{aA}	91.95 ± 0.15 ^{aA}	91.87 ± 0.15 ^{aA}
Day 16	89.32 ± 1.27 ^{bA}	91.38 ± 0.15 ^{aA}	91.95 ± 0.13 ^{aA}	91.64 ± 0.43 ^{aA}

$^{a-b}$ Means ± standard error with different retting temperatures within the same row are significantly different ($p < 0.05$). $^{A-B}$ Means ± standard error with different retting times within the same column are significantly different ($p < 0.05$)

TOTAL PHENOLIC CONTENT OF WHITE PEPPER

For the total phenolic content (TPC), all temperatures employed produced significantly different ($p < 0.05$) amounts of TPC regardless of days of retting. Table 4 shows that the lowest TPC was obtained at 28 °C on day 4 (12.21 ± 0.40 mg GAE/g), followed by 42 and 49 °C. The highest TPC was at 35 °C and day 4 (33.05 ± 1.47 mg GAE/g) but was not significantly different from day 8. According to Lu et al. (2011), there is a strong relationship between phenolics concentration in spices and their free radical scavenging. Phenolic compounds in spices may be the major contributor to their antioxidant capacity. Zarai et al. (2013) reported the highest amount of total phenolics in black pepper ethanolic extracts gave a high antioxidant activity and was higher than BHT standard antioxidant. Similarly, the lower polyphenolics compound present at 28 °C, day 4 retting resulted in a lower DPPH activity compared to another sample tested. Variyar and Bandyopadhyay (1994) earlier work reported syringic, vanillic, and ferulic acid in the phenolic acid fraction. Pepper also has been reported to contain 3,4-dihydroxyphenyl ethanol glucoside, 3,4-dihydroxy-6-(N-ethylamino) benzamide, and phenolic acid glycosides as the main phenolic compounds (Chatterjee et al. 2007). Other than that, Agbor et al. (2006) explained that the effects of maturity or processing treatment may also reduce the polyphenol content in white pepper eventually.

TABLE 4. Values of the total phenolic content (mg GAE/g) of white pepper produced after the water retting process at different temperatures and days

	28 °C	35 °C	42 °C	49 °C
Day 4	12.21 ± 0.40 ^{dC}	33.05 ± 1.47 ^{aA}	28.23 ± 1.06 ^{bA}	24.54 ± 0.13 ^{cA}
Day 8	12.66 ± 0.31 ^{cC}	32.95 ± 0.18 ^{aA}	24.51 ± 0.72 ^{bB}	23.99 ± 0.82 ^{bA}
Day 12	14.80 ± 0.15 ^{dA}	31.09 ± 0.34 ^{aA}	27.57 ± 0.37 ^{bA}	24.85 ± 1.32 ^{cA}
Day 16	13.89 ± 0.10 ^{dB}	30.95 ± 0.51 ^{aA}	27.43 ± 0.43 ^{bA}	21.74 ± 1.11 ^{cA}

$^{a-b}$ Means ± standard error with different retting temperatures within the same row are significantly different ($p < 0.05$). $^{A-B}$ Means ± standard error with different retting times within the same column are significantly different ($p < 0.05$)

PIPERINE CONTENT

Table 5 shows the highest piperine content was obtained from white pepper retted at 35 °C on day 8 (2.70 ± 0.25%). The study shows that the piperine content in the *Piperaceae* family is different according to the species (Raman & Gaikar 2002). In *Piper nigrum* L. fruit, the range of piperine is 2.0 to 7.4% (Peter 2006; Risfaheri & Nurdjannah 2003) suggesting that the yield of piperine is within an acceptable range at temperatures of 28, 35, and 42 °C. An increase in the retting period shows no effect at all temperatures except at 49 °C with decreasing piperine as the retting time increased. Due to longer retting time, piperine was prone to be released and lost to the surrounding by the heat treatment. As for the temperature effect on day 8 and day 16 at a higher retting temperature (49 °C), it caused the piperine content to reduce significantly. Another study showed that as the temperature increased when subjected to boiling or pressure cooking treatment, piperine content in *Piper nigrum* L. gradually decreased (Attokaran 2011; Meghwal & Goswami 2013). The study of Dang and Phan (2014) showed a poor correlation ($R^2 = 0.32$) of the piperine content with black pepper oleoresin yield. Extraction using supercritical CO_2 at different pressures, temperatures, and durations were able to yield a higher range of piperine from 25.74 to 48.32%. Piperine and piperic acid could be used as a natural antioxidant and antibacterial agents in both food preservation and human health (Zarai et al. 2013).

TABLE 5. Piperine content (%) of white pepper produced after the water retting process at different temperatures and days

	28 °C	35 °C	42 °C	49 °C
Day 4	1.76 ± 0.08 ^{aA}	2.21 ± 0.30 ^{aA}	2.10 ± 0.09 ^{aA}	2.06 ± 0.03 ^{aA}
Day 8	2.59 ± 0.20 ^{abA}	2.70 ± 0.25 ^{aA}	2.14 ± 0.04 ^{bcA}	1.80 ± 0.10 ^{cB}
Day 12	2.35 ± 0.11 ^{aA}	1.97 ± 0.28 ^{aA}	2.11 ± 0.08 ^{aA}	1.92 ± 0.00 ^{aAB}
Day 16	2.06 ± 0.31 ^{aA}	1.81 ± 0.27 ^{abA}	2.27 ± 0.03 ^{aA}	1.54 ± 0.13 ^{bC}

$^{a-b}$ Means ± standard error with different retting temperatures within the same row are significantly different ($p < 0.05$). $^{A-B}$ Means ± standard error with different retting times within the same column are significantly different ($p < 0.05$)

CONCLUSION

The study shows that the best solvent is methanol due to its ability to recover highest yield and total phenolic content of oleoresin extract. In addition, there are effects of temperature and day on the physicochemical and functional properties of white pepper produced, with the temperature factor being more pronounced on the properties tested. Retting temperature at 28 °C and 8 days was sufficient to produce a significantly higher yield and most fractured white pepper. Meanwhile, white pepper retted at higher temperatures for 8 days was sufficient to produce better colour (42 °C) as well as the highest piperine content, highest free scavenging activity, and TPC value (35 °C).

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