Physicochemical properties of products and waste of black seed produced by cold press method

Nisa Nurmilati Barkah¹, Komang Gede Wiryawan²*, Yuli Retnani², Wayan Teguh Wibawan³, and Elizabeth Wina⁴

¹Program Study of Nutrition and Feed Science, Postgraduate School, Bogor Agricultural University, Bogor, West Java, Indonesia
²Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor, West Java, Indonesia
³Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, West Java, Indonesia
⁴Livestock Research Institute, Ciawi, Bogor, West Java, Indonesia

*E-mail: kgwiryawan61@gmail.com

Abstract. Black seed or black cumin has the scientific name Nigella sativa (NS) is an herbal plant that is widely used to treat various diseases. The extraction of oil from NS seeds produces a byproduct called NS waste. This study aims to evaluate the physicochemical properties of products and NS waste produced by the cold press method. Analysis of physical properties was carried out using a completely randomized design (CRD) with 3 treatments and 5 replications. The thymoquinone test was carried out using high-performance liquid chromatography (HPLC). The moisture content of seed and NS waste were 6.21 and 7.91%, respectively. The crude protein content in NS seeds and waste reached 19.34 and 27.78%, and crude fat contents were 23.99 and 16.93%. The TQ content in NS seeds, oil, and waste were 1680, 2701, and 350 ppm, respectively. Other active compounds in NS seeds are saponins, tannins, flavonoids, triterpenoids, and glycosides. Extraction rate of NS using cold press method is 34.46% and produces NS oil products and waste with the desired physicochemical properties, so the product can be used as material for the pharmaceutical purposes.

1. Introduction
Antibiotic growth promoters (AGP) are drugs that can kill or inhibit bacterial growth and are given with low sub-therapeutic doses for animal. The use of antibiotics to promote growth has emerged with the intensification of animal. The presence of pathogens can reduce animal productivity. Administration of sub-therapeutic doses of antibiotics and antimicrobial agents has been shown to be effective in controlling pathogens [1]. The use of AGP as a feed additive provides many economic benefits, but can also have negative impacts. Excessive use of antibiotics over a period of time can cause local bacterial populations become resistant to antibiotics [2] and the presence of antibiotic residues in products and the environment [3,4] which can endanger human and animal health [5]. For this reason, the World Health Organization concluded that the use of antimicrobials in animal feed is a public health problem that has led to the banning of AGP in various countries. Therefore, an effective alternative is needed to control infectious diseases and limit the spread of resistant bacteria.
Some alternatives to AGP include probiotics, prebiotics, synbiotics, organic acids, enzymes, hyperimmune IgY, antimicrobial peptides, and phytobiotics [6]. Phytobiotics are naturally occurring bioactive compounds derived from plants and incorporated into animal feed to increase productivity [7]. Black seed has scientific name *Nigella sativa* (NS) or commonly called black cumin is a herbal plant that has long been used to treat various types of diseases. The use of NS in large and medium industries in Indonesia reaches 144,817 kg year\(^{-1}\) [8]. The potential for NS waste produced is as much as 101,372 kg year\(^{-1}\) or 70% of the total raw material [9]. NS contains 0.5-1.6% volatile oil, 35.6-41.6% fixed oil, and 22.7% protein [10]. One of the active compounds in NS is thymoquinone (TQ) which is part of the volatile oil. TQ content up to 60% of total volatile oil [11]. TQ shows antibacterial activity [12], anti-fungal [13], antioxidant [14], anti-inflammatory [15], and anticancer [16].

Despite its many beneficial properties, the use of TQ therapy is limited due to its hydrophobic properties, poor solubility in aqueous media, sensitivity to light, temperature, and pH [17]. Until now, various NS oil extraction methods have been developed that can minimize the loss of TQ, one of which is the cold pressing method. NS oil processing by cold press method carried out at a temperature of 30-35 °C requires less energy and can maintain phytobiotic compounds which are thermolable. Therefore, this study aims to identify physicochemical characteristics of product and waste of NS, identification of phytobiotic compounds in products and waste of NS, and TQ content in NS oil extraction which was processed by cold press method.

2. Materials and methods
This research was conducted at the Feed Industry Laboratory, Faculty of Animal Science, Bogor Agricultural University, West Java, Indonesia on June until November 2020. NS seeds was purchased from a distributor in Surabaya, East Java, Indonesia.

2.1. Physicochemical properties of *Nigella sativa* product and waste
The physicochemical properties of NS products and waste were observed through analysis of its physical properties and also chemical component content in it. NS seeds and waste were ground using a blender and then observed their physical properties, including particle size, moisture content and water activity. Particle size was measured using the Ro-tap Sieve Shaker [18]. The water activity analysis was carried out using the thread hygrometer (Aw-Wert-Messer Lufft, 5803.00) which has been extensively tested by Rodel and Leistner [19]. Moisture content was carried out using grain moisture meter (Kett PM-650). Phytobiotic analysis was performed qualitatively for tannins, saponin, steroid, and triterpenoid [20], flavonoid [21], alkaloid and glycosides [22]. Proximate analysis was performed using the AOAC method [23] and carbohydrate content was calculated from the percent of dry matter which has been reduced by the percent of ash, crude fat, crude protein, and crude fiber. The determination of thymoquinone components in NS was carried out by HPLC [24].

2.2. Extraction of *Nigella sativa* oil
Extraction of NS oil using the pressing method was carried out by inserting the whole NS seeds with a known weight into a cold press machine (Type SG30-1).

2.3. Data analysis
Analysis of physical properties was carried out using a completely randomized design (CRD) with 3 treatments (NS whole seeds; NS fine seeds; NS waste) and 5 replications. Data are presented by taking into consideration the standard deviation. Results were analyzed by one-way analysis of variance to identify significant differences and compare means test analyzed by the Duncan test at 5%.

3. Results and Discussion
3.1. Physicochemical properties of *Nigella sativa* seeds, oil, and waste
3.1.1. Moisture content and water activity of Nigella sativa seeds and waste. Moisture content and water activity are the two main variables used to evaluate the shelf life. The results of water content analysis showed that there was a significant difference between moisture content NS whole seeds, fine seeds, and waste. Whole seeds NS have the lowest water content compared to other ingredients. This water content was included in the range of water content of NS seeds from previous studies, which was between 4.99 and 7.00% [25,26]. Exposure of high humidity sample to a dry atmosphere can cause moisture loss, resulting a drop in water content. Conversely, dry samples exposed to a humid atmosphere can cause the sample to absorb water, so that the water content increases. NS whole seeds have low water content after the grinding process, these fine seeds have the potential to absorb water from the atmosphere, consequently its water content is higher than NS whole seeds. The NS waste moisture content in this study was higher than the NS waste water content reported by Gharby et al. [27] who extracted NS oil using the cold press method is 8.1%. The high moisture content in NS waste related with the NS waste particle size which is smaller compared with others (see Table 2). This results in a higher surface area of NS waste which will increase its ability to bind water. Moreover, higher moisture content increases plasticity and thereby reduces compression rates and contributes to low oil recovery [28]. The moisture content also functions as a lubricant for the engine, so the higher moisture content results in insufficient friction during pressing [29] which ultimately lowers the oil extraction yield. The results of measuring water activity showed that water activity was significantly different in NS whole seeds, fine seeds, and waste. NS fine seeds have the lowest water activity value compared to other ingredients. However, the water activity value of the materials used in this study was more than 0.7. This can cause the material to become microbiologically unstable. Drying and storing the material in a room that is not humid can minimize material damage. The value of water activity in this study was higher than the water activity value of NS beverage products with was added Arabic gum which was in the range 0.138 to 0.336 [30]. This difference in water activity can be caused by the addition of gum arabic as an encapsulation material that can protect the core material. In addition, the differences in the analytical methods used can affect the accuracy of the resulting data.

Table 1. Average moisture and water activity of Nigella sativa whole seeds, fine seeds, and waste.

| Variable          | NS whole seeds | NS fine seeds | NS waste   |
|-------------------|----------------|---------------|------------|
| Moisture content (%) | 6.04 ± 0.09a   | 6.92 ± 0.04b  | 9.24 ± 0.11c |
| Water activity     | 0.847 ± 0.001b | 0.783 ± 0.007a| 0.851 ± 0.006b |  

3.1.2. Particle size of seed and waste of Nigella sativa. NS milling process significantly reduced its particle size by 67% from the original particle size (P <0.05). The smaller the particle size of the material, the shorter the pathway that the solvent must travel, which reduces the extraction time of phytobiotic compounds [31]. Moreover, the milling process can damage the grain cell structure and reduce resistance in oil extraction [32]. NS seeds with a particle size of 60 mesh (equivalent to 250 μm) are recommended as the ideal particle size for producing high extraction rates using n-hexane solvent [33]. However, oil extraction using the cold press method does not require a reducing in the particle size of the material before it is extracted. In a study conducted by Teh [34] the best extraction results are achieved in the processing of whole seeds. Oil extraction by the press method is a mechanical solid-liquid phase separation system that is widely used to extract oil from seeds with oil extraction yields below 20% [35]. Pressure is used in the separation of these phases, so the large particle size helps facilitate the process of pressing the grain and the oil can be produced.

3.2. Phytobiotic and thymoquinone content on products and waste of Nigella sativa.
The data in Table 3 shows that NS seeds contain saponins, tannins, flavonoids, triterpenoids, and glycosides. According to Farag et al. [36] showed that NS oil contains more than 52 metabolites including 8 saponins, 10 flavonoids, 6 phenolics, 10 alkaloids, and 18 fatty acids which can be utilized for health. Saponins function as guards against microbial attack on plants and treat yeast and fungal infections [37]. Tannins have antiviral, antibacterial, and anticancer activities [38,39,40]. Flavonoids
are widely used as antioxidants [41]. In general, the chemical compounds contained in this plant can be useful as natural antibiotics that can fight microbial invasion and infection [42]. Moreover, much of the plant's therapeutic properties of NS seeds are due to the presence of thymoquinone (TQ) which is the main active chemical component of essential oils.

### Table 2. Average particle size distribution of *Nigella sativa* whole seeds, fine seeds, and waste.

| Sample                  | Particle size (μm) |
|-------------------------|--------------------|
| *Nigella sativa* whole seeds | 1179.67 ± 3.78b     |
| *Nigella sativa* fine seeds       | 392.93 ± 12.19a    |
| *Nigella sativa* waste         | 394.66 ± 27.66a    |

### Table 3. Phytobiotic contents in *Nigella sativa* seeds.

| Sample | Variables       | Results | Method |
|--------|-----------------|---------|--------|
| *Nigella sativa* seeds | Alkaloid       | -       | Qualitative |
|         | Saponin         | +       |        |
|         | Tannins         | +       |        |
|         | Phenolic        | -       |        |
|         | Flavonoid       | +       |        |
|         | Triterpenoid    | +       |        |
|         | Steroid         | -       |        |
|         | Glycosides      | +       |        |

The data in Table 4 showed that the highest TQ content was found in NS oil, which was 37.8% higher than the TQ content in the seeds. One of the benefits of thymoquinone is that it acts as an antibacterial. TQ has a wide spectrum as antibacterial [43]. The lowest TQ content found in NS due to TQ is in the volatile oil component (essential oil) in NS oil. This volatile oil component is an unstable component, so it is easily lost during the production process. NS contain volatile oil as much as 0.4-0.45% [44]. The TQ content in NS waste in this study was 57% higher than the TQ content in NS waste reported by Retnani et al. [45]. This is because the NS waste in this study was produced from the cold press method which can minimize the loss of TQ as a thermolable substance.

### Table 4. Thymoquinone content on *Nigella sativa* seeds, oil, and waste.

| Sample          | Sample conditions | Result | Unit | Methods |
|-----------------|-------------------|--------|------|---------|
| *Nigella sativa* seeds | Solid            | 1680   | ppm  | HPLC    |
| *Nigella sativa* oil       | Fluid            | 2701   | ppm  | HPLC    |
| *Nigella sativa* waste     | Solid            | 350    | ppm  | HPLC    |

### 3.3. Nutrient content of *Nigella sativa* seeds and waste

Proximate analysis of NS seeds and NS waste is shown in Table 5. A significant difference in nutrients in NS seeds and NS waste is found in the content of crude protein, crude fat, crude fiber, and carbohydrates. The crude protein content in NS waste was 31.6% higher than NS seeds. The process of extracting oil from NS seeds causes a decrease in crude fat content and an increase in the proportion of crude protein in NS waste. Crude fat content in NS waste was 28% lower than NS seeds. The TQ content in NS waste in this study was 57% higher than the TQ content in NS waste reported by Retnani et al. [45]. This is because the NS waste in this study was produced from the cold press method which can minimize the loss of TQ as a thermolable substance.
content in this study was lower than the results presented by Iqbal et al. [46] which reached 31% due to differences in the origin of the NS seeds used which could affect the nutritional content in it.

| Sample          | Dry matter (%) | Ash (%) | Crude protein (%) | Crude fat (%) | Crude fiber (%) | Carbohydrate (%) |
|-----------------|----------------|---------|-------------------|---------------|----------------|------------------|
| Nigella sativa  | 93.79          | 5.09    | 20.62             | 25.57         | 19.62          | 29.10            |
| seeds           |                |         |                   |               |                |                  |
| Nigella sativa  | 92.10          | 7.20    | 30.16             | 18.38         | 4.71           | 39.55            |
| waste           |                |         |                   |               |                |                  |

3.4. Nigella sativa oil extraction
The results of NS oil extraction using the cold press method are listed in Table 6. The NS oil extraction using the cold press method which is carried out at a temperature of 31-38 °C have extraction rate of up to 34%. This contradicts the opinion of Zuorro et al. [35] who stated that the extraction yield by mechanical method is generally below 20%. However, this amount has not been able to extract the whole fixed oil in NS seeds, so the extraction by cold press method has not been able to extract all the oil components in NS seeds. To overcome this, some industries usually do the extraction twice, so that no oil is wasted. According to Teh [34], most of the oil cannot come out of the seeds without preheating and heating the device and makes it easier to release the oil while waiting for a certain period of time. In this study, the extraction was started when the engine temperature reached 31 °C. Cold press method is able to retain phytobiotic compounds, especially those substances that are thermolabile. Cold press method is an extraction method that is environmentally friendly because it does not require a solvent.

| Variables                  | Results             |
|----------------------------|---------------------|
| Time (seconds)             | 1369.67±132.54      |
| Waste (%)                  | 60.87±3.42          |
| Extraction rate (%)        | 34.46±1.12          |

4. Conclusion
The extraction rate of Nigella sativa using cold press method is 34.46%. The extraction of Nigella sativa oil using the cold press method produces Nigella sativa oil products and waste with the desired physicochemical properties, so the product can be used as material for pharmaceutical purposes.

References
[1] Hughes P 2001 Antibiotic growth promoters in food animals: Assessing quality and safety of animal feeds, Food and Agriculture Organization of The United Nations, Rome
[2] Diarra M S, Silversides F G, Diarrassouba F, Pritchard J, Masson L and Brousseau R 2007 Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, clostridium perfringens and enterococcus counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in Escherichia Coli isolates Appl. Environ. Microbiol. 73 6566-76
[3] Carvalho I T and Santos L 2016 Antibiotics in the aquatic environments: a review of the European scenario Environ. Int. 94 736-57
[4] Ronquillo G M and Angeles H J C 2017 Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods Food. Contr. 72 255-67
[5] Diarra M S, Rempel H, Champagne J, Masson L, Pritchard J and Topp E 2010 Distribution of antimicrobial resistance and virulence genes in Enterococcus spp. and characterization of isolates from broiler chickens Appl. Environ. Microbiol. 76 8033-43
[6] Gadde U, Kim W H, Oh S T and Lillehoj H S 2017 Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review Anim. Health. Res. Rev. 18 26-45
[7] Windisch W, Schedle K, Plitzner C and Kroismayr A 2008 Use of phytogenic products as feed additives for swine and poultry J. Anim. Sci. 86 140-8
[8] Wahyuni S 2009 Black cumin cultivation opportunities and benefits (Nigella sativa L.) Puslitbangbun. 15 23-5
[9] Gokdogan O, Eryilmaz T and Yesilyurt M K 2015 Determination of energy use efficiency of Nigella sativa L. (Black seed) oil production. Am-Euras. J. Agric. and Environ. Sci. 15 1-7
[10] Al-Gaby AM 1988 Amino acid composition and biological effects of supplementing broad bean and corn proteins with Nigella sativa (Black cumin) cake protein. Nahrung. 42 290-4
[11] Guler T, Dalkılıç B, Ertas O N and Çiftçi M 2006 The effect of dietary black cumin seeds (Nigella sativa L.) on the performance of broilers. Asian-Aust. J. Anim. Sci. 19 425-30
[12] Halawani E 2009 Antibacterial activity of thymoquinone and thymohydroquinone of Nigella sativa L. and their interaction with some antibiotics Adv. Biol. Res. 3 148-52
[13] Abdel Azeiz A, Saad A H and Darweesh M F 2013 Efficacy of thymoquinone against vaginal candidiasis in prednisolone-induced immunosuppressed mice J. Am. Sci. 9 155-9
[14] Hosseinzadeh H, Taiai S and Nassiri-Asl M 2012 Effect of thymoquinone, a constituent of Nigella sativa L. on ischemia-reperfusion in rat skeletal muscle N-S. Arch. Pharmacol. 385 503-8
[15] El Gazzar M, El Mezayen R, Marecki J C, Nicolls M R, Canastar A and Dreskin SC 2006 Anti-inflammatory effect of thymoquinone in a mouse model of allergic lung inflammation Int. Immunopharmacol. 6 1135-42
[16] Gali-Muhtasib H, Ocker M, Kuester D, Krueger S, El Doush II 2006 Level of selenium, tocopherol, thymoquinone and thymol of Nigella sativa seed J. Food. Compos. Anal. 19 167-75
[17] Younus H 2018 Molecular and Therapeutic Actions of Thymoquinone, Springer, Nature Singapore Pte Ltd
[18] ASAE American Society of Agricultural Engineers 2003 Method of Determining and Expressing Fineness of Feed Materials by Sieving ANSI/ASAE S319, American Society of Agricultural Engineers, Michigan
[19] Rodel W and Leistner L 1971 Ein einfacher Aw-wert-messer fur die praxis die Fleischwirtschhaft 51 1800-2
[20] Eijkeme C M, Ezeonu C S and Eboatu A N 2014 Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria Eur. Sci. J. 10 247-70
[21] Sofowara A 1993 Medicinal Plants and Traditional Medicine in Africa, Spectrum Books, Nigeria
[22] Hikino H, Kiso Y, Wagner H and Fiebig M 1984 Antihepatotoxic actions of flavonolignans from Silybum marianum fruits Plant. Med. 50 248-50
[23] AOAC. 2005. Official Methods of Analysis of The Association of Analytical Chemist, Association of Official Analytical Chemist Inc, Virginia
[24] Al-Saleh IA, Billedo G and El-Doush II 2006 Level of selenium, tocopherol, thymoquinone and thymol of Nigella sativa seed J. Food. Compos. Anal. 19 167-75
[25] Simonen HS, Baharin BS and Bagheri H 2014 Antioxidant property, thymoquinone content and chemical characteristics of different extracts from Nigella sativa L. seeds. J. Am. Oil Chem. Soc. 91 295-301
[26] Cheikh-Rouhou S, Besbes S, Hentati B, Blecker C, Deroanne C and Attia H 2007 Nigella sativa L.: chemical composition and physicochemical characteristics of lipid fraction J. Food. Chem. 101 673-81
[27] Gharby S, Harhar H, Guillame D, Roudani A, BoulbaroudS, Ibrahimi M, Ahmad M, Sultana S,
Hadda TB, Chafchaouni-Moussaoui I and Charrouf Z 2015 Chemical investigation of *Nigella sativa* L. seed oil produced in Morocco *J. Saud. Soc. of Agric. Sci.* **14** 172-7
[28] Singh J and Bargale PC 1990 Mechanical expression of oil from linseed (*Linum usitatissimum* L.) *J. Oilseeds. Res.* **7** 106-10
[29] Reuber M 1992 New Technologies for Processing *Crambe abyssinica* Thesis, Iowa State University, Ames
[30] Varastegani B, Lubowa M, Wann TS, Yang TA and Easa AM 2018 Production of *Nigella sativa* beverage powder by drum drying using arabic gum as adjunct *Ital. J. Food. Sci.* **30** 583-601
[31] Shi J, Nawaz H, Pohorly J, Mittal G, Kakuda Y and Jiang Y 2005 Extraction of Polyphenols from plant material for functional foods engineering and technology *Food Rev. Inter.* **21** 139-66
[32] Li Y, Liu M and Zhang H 2016 Study on the extraction process of oils from almond skin and physicochemical property of almond skin oil *Cereals & Oils* **29** 24-6
[33] Ma C, Liu C, Ahmed AF, Niu Y and Kang W 2019 Optimum extraction technology for the seed oil of *Nigella sativa* L. *Hind. J. of Food Qual.* 1-6
[34] Teh HE 2016 Extraction and characterization of functional components from fruit and vegetable processing waste Thesis, University of California, Davis
[35] Zuorro A, Lavecchia R, Medici F and Piga L 2014 Use of cell wall degrading enzymes for the production of high-quality functional products from tomato processing waste *Chem. Eng. Trans.* **38** 355-60
[36] Farag M A, Gad H A, Heiss A G and Wessjohann L A 2014 Metabolomics driven analysis of six *Nigella* species seeds via UPLC-qTOF-MS and GC-MS coupled to chemometrics *Food Chem.* **151** 333-42
[37] Sheikh N, Kumar Y, Misra A K and Pfoze L Phytochemical screening to validate the ethnobotanical importance of root tubers of *Dioscorea* species of Meghalaya, North East India *J. of Med. Plants Stud.* **1** 62-9
[38] Haslam E 1996 Natural polyphenols (vegetable tannins) as drugs: possible modes of action *J. of Nat. Prod.* **59** 205-15
[39] Khanbabaee K and van Ree T. 2001. Tannins: classification and definition. *Natural Product Reports.* **18** 641–49
[40] Kakiuchi M H, Nishizawa M, Yamagishi T, Okuda T and Namba T. 1986. Studies on dental caries prevention by traditional medicines. VIII. Inhibitory effect of various tannins on glucan synthesis by glucosyltransferase from Streptococcus mutans,” *Chem. and Pharm. Bulletin.* **34** 720–25
[41] Kim S Y, Kim J H, Kim S K, Oh M J and Jung M Y 1994 Antioxidant activities of selected oriental herb extracts *J. Am. Oil Chem. Soc.* **71** 633–40
[42] Sodipo O A, Akiniyi J A and Ogubano U S 2000 Studies on certain characteristics of extracts of bark of *Pausinystalia johimbe* and *Pausinystalia macroceras* (K.Schum.) Pierre ex Beille. *Global J. of Pure and Appl. Sci.* **6** 83–7
[43] Forouzanfar F, Fazly Bazzaz BS and Hosseinzadeh H 2014 Black cumin (*Nigella sativa*) and its constituent (thymoquinone): a review on antimicrobial effects *Iran J. Basic Med. Sci.* **17** 929-38
[44] Thembhurne S V, Feroz S and Sakarkar D M 2014 A review on therapeutic potential of *Nigella sativa* (kalonji) seeds *J. Med. Plants. Res.* **8** 167-77
[45] Retnani Y, K G Wiryawan, Khotijah L, Barkah N N, Gustian R A and Dermawan I R 2019. Growth performance, blood metabolites, and nitrogen utilization of lambs fed with *Nigella sativa* meal *Pak. J. Nutr.* **18** 247-53
[46] Iqbal M S, Ghafoor A, Inamullah and Ahmad H 2013 Genetic variation in yield performance for three years in *Nigella sativa* L. germplasm and its association with morpho-physiological traits and biochemical composition *Pak. J. Bot.* **45** 2065-70.