# សាកលវិទ្យាល័យភូមិន្ទភ្នំពេញ

**ROYAL UNIVERSITY OF PHNOM PENH**


# ការសិក្សាពីខ្សែសង្វាក់ផលិតកម្មនិងការពិនិត្យទៅលើគុណភាពនៃម្រេចខ្មៅសរីរាង្គនៅក្នុងក្រុមហ៊ុន ខនហ្វីរេល ខូអិលអឺឌី

**Study of production line and quality control in organic black pepper at CONFIREL Co., Ltd.**

A Final Report
In Partial Fulfilment of the Requirement for the Degree of
Bachelor of Engineering in Bio-Engineering


**សុខនី វ៉ារី**

**SOKNY VARY**


**July 2024**

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**ROYAL UNIVERSITY OF PHNOM PENH**

# មហាវិទ្យាល័យវិស្វកម្ម

**FACULTY OF ENGINEERING**

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**SOKNY VARY**


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| | Mr. YEN Chamroeun | (Member) |

**July 2024**

Report

Entitle

**Study of production line and quality control in organic black pepper at CONFIREL Co., Ltd.**

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# មូលន័យសង្ខេប

ម្រេចខ្មៅសរីរាង្គគឺជាគ្រឿងទេសមួយប្រភេទដែលប្រើយ៉ាងទូលំទូលាយបំផុតនៅជុំវិញពិភពលោក។ វាដើរតួជាគ្រឿងទេសដ៏ល្អមួយ ដោយសារតែវាមានរសជាតិហិរផ្លែក ដែលគេយកទៅប្រើជាគ្រឿងផ្សំធ្វើម្ហូប ជាច្រើនមុខ។ ក្រុមហ៊ុន CONFIREL Co Ltd,. បានធ្វើការនាំចូលនូវម្រេចខ្មៅសរីរាង្គពីខេត្តកំពត។ចំពោះការដាំដុះដំណាំម្រេចសរីរាង្គគឺយកចិត្តទុកដាក់ និងថែទាំយ៉ាងខ្លាំង ដោយគេដាំដោយមិនប្រើនូវសារធាតុគីមី ថ្នាំសម្លាប់សត្វល្អិត ឬជីគីមី។ សម្រាប់ការប្រមូលផលម្រេចគេត្រូវចំណាយពេល២ទៅ៣ឆ្នាំ។ មួយវិញទៀតម្រេចខ្មៅសរីរាង្គគឺបានមកពីគេប្រមូលយកម្រេចបៃតងទៅហាល ហើយនៅពេលវាស្ងួតវាហ្នឹងប្តូរពណ៌ទៅជាពណ៌ខ្មៅ។ គោលបំណងនៃការចុះកម្មសិក្សា នៅក្រុមហ៊ុន ខនហ្វឺរេលគឺដើម្បីសក្សាពីខ្សែសង្វាក់ផលិតកម្ម និង ការពិនិត្យទៅលើគុណភាពផលិតផលម្រេចខ្មៅសរីរាង្គលើប៉ារ៉ាម៉ែត្រ រូបនិង មីក្រូជីវសាស្ត្រ។ ចំពោះដំណើរការម្រេច គឺយើងសិក្សាពី ផលិតផលពាក់កណ្តាលសម្រេចដល់ផលិតផលសម្រេច ដោយមើលលំអិតពីជំហាននីមួយៗ។ សម្រាប់ប៉ារ៉ាម៉ែត្ររូប គឺធ្វើតេស្តទៅប៉ារ៉ាម៉ែត្រចំនួនបួនមានសំណើម ដង់ស៊ីតេ អង្កត់ផ្ចិត និង ភាពហិរ។ លទ្ធផលក្នុងរយៈពេល3ខែ ម្រេចខ្មៅសរីរាង្គមានសំណើមទាបជាង12% ដង់ស៊ីតេធំជាង 570g/l អង្កត់ផ្ចិត ធំ

ជាង4mm និង កម្រិតភាពហិរខ្ពស់ជាង3.5%។ មួយវិញទៀតចំពោះ ប៉ារ៉ាម៉ែត្រមីក្រូជីវសាស្ត្រ
យើញថា ជាលទ្ធផល គ្មានវត្តមានបាក់តេរី*salmonella* និង *E-coli* តែចំពោះ total mold and yeast
និង total aerobic microbial count 30 °C ផ្តល់ជាលទ្ធផលវិជ្ជមានតែស្ថិតក្នុងបរិមាណតិចតួច ប៉ុន្តែ
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MANUFACTURING។សម្រាប់បុគ្គលិកដែលធ្វើការនៅទីនោះគឺបណ្តុះបណ្តាលត្រឹមត្រូវពីអនា
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ផ្សារទាំងក្នុងស្រុក និងក្រៅស្រុក។

## ABSTRACT

Organic black pepper is one of the most widely used spices around the world. It acts as a good spice because of its spicy taste, which is used in many foods. CONFIREL Co. Ltd. imported organic black pepper from Kampot province. The cultivation of organic pepper is very careful and caring; it is grown without the use of chemicals, pesticides, or chemical fertilizers. It takes two to three years to get peppercorns. Organic black pepper, on the other hand, is obtained by collecting green pepper and drying it, and when it dries, it changes color to black. Organic black pepper is one of the most widely used spices around the world. It acts as a good spice because of its spicy taste, which is used in many foods. The purpose of the internship at CONFIREL Co Ltd. is to study the production line and check the quality of organic black pepper products on physical and microbiological parameters. For the pepper process, we study from semi-raw material to the finished product by looking at the details of each step. For the physical parameters are tested to four parameters: moisture content, density, diameter and piperine. Results in 3 months of organic black pepper has a moisture content of less than 12%, a density greater than 570g / l, a diameter greater than 4mm and a higher piperine level of 3.5%. On the other hand, the microbiological parameters were found to be free of *salmonella* and E. coli, but for total mold and yeast and total aerobic microbial count 30 °C, the results were positive but in small quantities. For hygiene, clean the material before and after use, wear gloves before handling the product, and have clothes to work in production. After checking the quality of organic black pepper products, it can be declared that the pepper is of good quality and does not worry about health hazards. We then take it to the sales market in both domestic and foreign markets.

## SUPERVISOR'S RESEARCH SUPERVISION STATEMENT

TO WHOM IT MAY CONCERN

Name of program: Bachelor of Engineering in Bioengineering
Name of candidate: Sokny Vary

Title of research report: Study of production line and quality control in organic black pepper at CONFIREL Co., Ltd.

This is to certify that the research carried out for the above titled Bachelor's research report was completed by the above-named candidate under my direct supervision. This thesis material has not been used for any other degree. I played the following part in the preparation of this research report: Study of processing and quality assessment in organic black pepper (The whole pepper)

Supervisor's name: Ms.UNG Sivlin, Msc., Ph.D. candidate

Supervisor's signature:

Date

## CANDIDATE'S STATEMENT

TO WHOM IT MAY CONCERN

This is to certify that the research report that I (SOKNY Vary) hereby present entitled for the degree of Bachelor of Engineering at the Royal University of Phnom Penh is entirely "Study of production line and quality control in organic black pepper at CONFIREL Co., Ltd.''

For the degree of Bachelor of Engineering at the Royal University of Phnom Penh is entirely my own work and, furthermore, that it has not been used to fulfil the requirements of any other qualification in whole or in part, at this or any other University or equivalent institution.

No reference to, or quotation from, this document may be made without the written approval of the author.

Signed by (SOKNY Vary):

Date:

Sign by Supervisor: Ms.UNG Sivlin, Msc., Ph.D. candidate

Supervisor's signature:

Date

## ACKNOWLEDGEMENT

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Finally, I would like to apologize for any misunderstanding or error that occurred accidentally. I will commit to putting this knowledge to good use in our professional life. On this occasion as well, I wish the supervisor, teachers, my parents, and all staffs of CONFIREL Co., Ltd have a happy and healthy life full of joy, good luck, and success in your lives.

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## LIST OF APPREVIATIONS

| | | |
|---|---|---|
| % | = | Percentage |
| °C | = | Degree Celsius |
| g | = | Gram |
| L | = | Liter |
| mm | = | Millimeter |
| QC | = | Quality Control |
| PPM | = | PHARMAPRODUCT MANUFACTURING |
| M | = | Moisture |
| d | = | Density |
| Dia | = | Diameter |
| cfu/g | = | Colony forming unit per gram |

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# CHAPTER 1
# INTRODUCTION

## 1.1. Background organic black pepper

Black pepper (*Piper nigrum*) is an annual pepper possesses antioxidant, antipyretic, antimicrobial potential, gastro-protective modules and anti-inflammatory property. The most significant bioactive component of black pepper, piperine, has health advantages. With piperine as its primary constituent, black pepper has a rich phytochemistry that also includes oleoresins, volatile oils, and alkaloids. In addition, piperine, one of *Piper Nigrum*'s main alkaloid components, enhances food absorption, improves gastrointestinal health, and aids in cognitive brain function.

Black pepper is a good source of manganese, a mineral that can help with bone health, wound healing, and metabolism. Organic black pepper is grown without the use of harmful chemicals and pesticides and is guaranteed to be free from harmful chemicals and pesticides. They are grown with utmost care and dedication; this exquisite spice carries flavor and health benefits within its tiny grains. Simply put, our gluten-free black pepper is nurtured in harmony with the environment, free from synthetic pesticides and chemicals. The processing of organic black pepper is considered quite simple and is mainly performed in order to protect this seasoning from contamination, besides increasing its conservation period and adding value to the products. Because it is consumed in human feeding, some hygienic cares should be taken during its harvest, processing and management in order to avoid macro- and microbiological contamination (Butt et al., 2013). Between 2013 and 2017, the world's pepper production increased significantly, rising from 379,300 tons to 501,495 tons at an annual rate of 12%. With the majority of pepper farmers, Vietnam is a major contributor to the growth in production. But during this time, other nations—India, Brazil, Indonesia, Malaysia, Sri Lanka, China, and Cambodia—also had notable increases. According to the Value Chain Unit of the Ministry of Commerce (MOC) of Cambodia's 2010 pepper sector profile, the country's overall pepper production increased from 60 tons in 2000 to 861 tons in 2010. Furthermore, in terms of productivity and output, Cambodia is quickly rising in the worldwide market. From 1,000 tons in 2011 to 21,000 tons in 2017, there was a 31% yearly growth in production. Between 4.5 and 7.2 tons were produced per acre. From 2011 to 2017, there was an average of 40% of new planting. In 2023, Cambodian exported 103 tons of the renowned Kampot pepper where Vietnam exported over 30,914 tons of pepper. The complex issue of

Cambodia's low export numbers has sparked worries about a number of things, including price manipulation, the poor quality of black pepper in particular, which does not comply with the standard of European requirements, and finding innovative methods to increase productivity and combating plant-killing illnesses are two other issues(SOK, 2021).

Furthermore, in considering this problem, the biggest company in Cambodia, CONFIREL Co., Ltd., developed higher quality goods and is a producer and exporter of pepper products to the European market. Confirel is a groundbreaking agrobiological company in Cambodia, founded in 2001 by Dr. Hay Ly Eang. It is located on 138 Bis, 110, Prey Chisak, Chom Chao, Por Sen Chey, Phnom Penh, Cambodia. Confirel is a company that promotes sustainable rural development. Its product lines and brands include Kampot pepper, palm sugar, herbal teas, mango (dry, pureed, chips, and more), vinegar, palm wines and spirits, candies, and more. Kampot pepper has a complex flavor, both sweet and spicy, and a fruity aroma with hints of citrus. Since 2011, Confirel has started manufacturing black, red, and white Kampot pepper, with the PGI mark ensuring its traceability. Confirel is able to provide high-quality products that meet consumer demands. We lead the market in Cambodia for natural and organic products because of this. In order to preserve quality and guarantee consumer safety, the manufacture adheres to GMP (Good Manufacturing Process) guidelines. The company also has Hazard Analysis Critical Control Points (HACCP) certification. This is a system which provides the framework for monitoring the total food system, from harvesting to consumption, to reduce the risk of foodborne illness.

The contamination in black pepper is caused by physical material such as the presence of stones, seeds of other vegetal, insects (alive, died, in whole or fragments) and microbiological such as the presence of moldy grains. The microbiological aspects that should be avoided are the presence of Salmonella, *Escherichia coli*, yeasts, molds, aerobic microbial and residues of pesticides.

### 1.2. Problem statement

Organic black pepper, derived from *Piper nigrum* dried berries, is a spice cultivated using organic farming methods. The main challenge is ensuring sustainable production, maintaining quality, and preserving health benefits. Organic farming methods aim to maintain soil fertility, minimize synthetic fertilizers and pesticides, and protect the environment. Quality maintenance is

crucial, ensuring the pepper is free from contaminants and maintains nutritional value. Preserving health benefits requires optimal growing conditions, harvesting, and processing.

### 1.3. Aim and Objectives of the Study

To study production line and quality control on organic black pepper from semi-raw materials to finished product.

### 1.4. Rationale of the Study

The main problem with organic black pepper is that it can contain small stones, iron, and especially fungi, insects, and worms, which means that the pepper cannot be stored for a long time and is harmful to the health of consumers. Therefore, the reason for the study is that we remove them by using some methods and treatments during processing to make sure that the pepper is not infected with microorganisms or physical materials.

### 1.5. Scope and Limitation of the study

The scope of this experiment focuses on the processing and quality of organic black pepper from semi-raw material to the finished product at CONFIREL Co. Ltd. We detail each step of processing and identify some parameters for organic black pepper, such as moisture, peperine, density, and diameter for confirming quality. Data is obtained from testing and is compared with previous literature and specifications in the company.

### 1.6. Structure of Study

Figure 1 shows the flow of the production line for organic black pepper from semi-raw material until finished product.

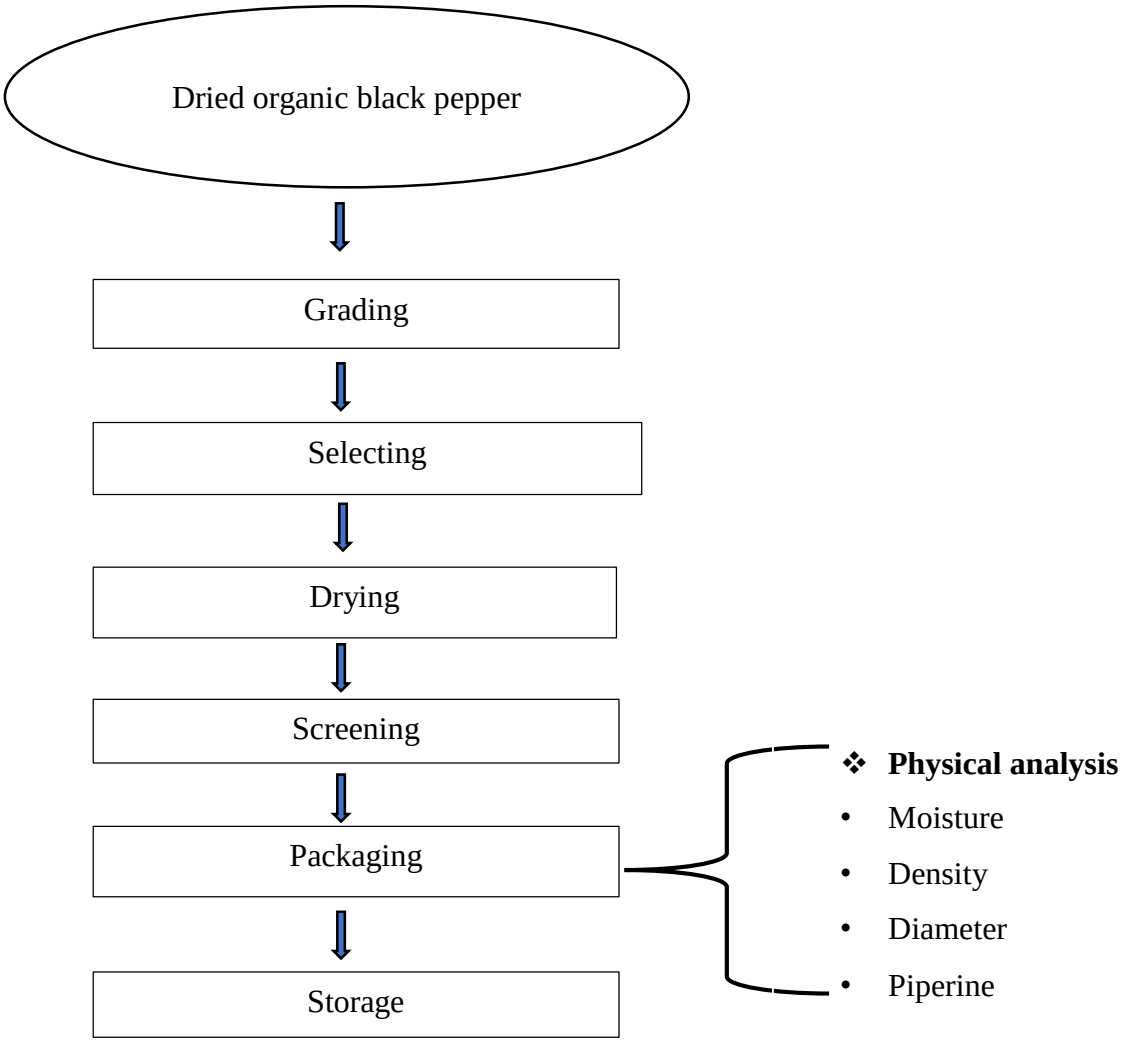


**Figure 1.** Structure of study

# CHAPTER 2
# LITURATURE REVIEW

## 2.1. History of peppercorn in Cambodia

Although peppercorns, often known as black pepper, are native to southern India and have been cultivated there since 2000 BC, the exact date of pepper cultivation in Cambodia is unknown. The earliest known record of pepper in Cambodia dates from the 13th century, when Chinese ambassador Zhou Dougan observed pepper growing near ancient Angkor city. The history of pepper in Cambodia was mostly shaped by French and Indonesian traders. In the 20th century, the cultivar of the Cambodian pepper gained popularity thanks to the French colony in Cambodia and the culinary establishment. Located on the foothills of the Dam rei Mountains, Kampot offers ideal pepper growing conditions because to its quartz-rich soil, warm, humid climate, and elevation. In this kind of environment, peppercorn vines thrive and produce a spice with an exceptional flavor and perfume. Given their name and the place in which they are grown, these peppers are regarded as the best in the world. The French appellation, which protects the trade names of special food items, was initially applied to this pepper, making it the first product from Cambodia to do so. The farmer's protection status, which replaced the geographical indication status in 2016, guarantees that pepper is supplied to farmers and that the quality is maintained at an elevated level. Red, black, and white Kampot peppercorns are the three varieties. Because so few berries become red when they ripen, the red Kampot peppercorns are exceptional and rare. At this point, the peppers taste sweet and delicious. The sun dries the perfectly ripe berries after they have been manually removed from the remainder. The pepper releases a unique scent with captivating notes of pine, eucalyptus, and crisp citrus peel. Although they have a distinctly earthier and piney flavor, the Black Kampot peppercorns are nevertheless wonderful. Most people in Europe, especially the French, adore kampot pepper. The Kampot region of Cambodia is where the pepper is grown. The majority of well-known chefs worldwide favor utilizing the Kampot pepper. Southern Cambodia is home to the province of Kampot; Phnom Bokor is bordered by the Gulf of Thailand in the South China Sea, and Kampot National Park rises like a folding screen to the west of Kampot. The region experiences good annual precipitation and a brief dry season every year. The area's humid tropical climate and Kampot soil combine to create the perfect environment for producing high-quality Kampot pepper, which makes the pepper distinctive. Due to the fact that farms that grow Kampot pepper are home to cafes and restaurants that employ the pepper in their main courses, the pepper

has grown in popularity. Additionally, the restaurants offer cooking schools when chefs learn how to prepare traditional Khmer cuisine with pepper. Although Kampot has always been a pepper producer, its notoriety really took off when the French colonized the area. The flavor and taste of the Kampot pepper was recognized by the French colonists as valuable. Its popularity stems from the fact that eight thousand peppers are exported to France annually by French consumers who enjoy it. This pepper is the best in the world because of its flavor and taste, which have sustained its reputation. The pepper is distinct because it is grown entirely organically. Only after becoming a member of the Kampot Pepper Promotion Association (KPPA), a grouping of numerous regional pepper growers, may the pepper be marketed or exported under the trade name "Kampot pepper." The pepper must pass quality screening, oversight, and verification before it can be sold under the Kampot pepper brand. Prior to receiving certification markings via geographic identification, farmers must undergo a rigorous verification process that looks into the use of chemical fertilizers. Kampot is the source of the product's certification markings and origin assurance. Because of the pepper's somewhat spicy flavor and enduring flavor, several chefs and Kampot pepper fans pair it with wine (IntoCambodia.com, 2024).

### 2.2. Taxonomical classification of *Piper nigrum*

**Table 1.** Classification of Piper nigrum (Srivastava & Singh, 2017)

| **Groups** | **Name** |
|---|---|
| Kingdom | Planate |
| Class | Equisetopsida |
| Sup class | Magnoliidae |
| Super order | Magnolianae |
| Order | Piperales |
| Family | Piperaceae |
| Genus | *Piper* |
| Species | *nigrum* |

### 2.3. The black pepper plants

Mature fruits of the perennial woody evergreen climber *Piper nigrum L.* are used to make black pepper. Pepper vines are grown under cultivation in columns that are 1.0–2.0 meters in diameter and 5–6 meters tall, supported by supports. The vine can grow up to 20 meters or more in height when it is trailed on big trees (Figs 2). When the pepper vine flowers, it develops pendent

spikes that resemble leaves and emerge on side branches. These spikes hold many tiny flowers, which are just bract-subtended ovary and stamens. The fruit is a sessile, tiny, globular drupe with a single seed; however, it is also sometimes referred to as a berry (Ravindran & Kallupurackal, 2012) .Black pepper takes about 7-8 months after flowering to reach full maturity. In India the crop is harvested during December –January in plains and January-April in the high ranges of Western Ghats as in Cambodia, Pepper is picked and harvested just before the rainy season begins. If left for longer periods of time, pepper does not enjoy too much water and it can destroy the harvest. Fresh green pepper can only be best appreciated in Cambodia itself – ideally right at the farm, but you can take home the flavor of the dried pepper. For a dried product to have a nice color and look, pepper must be harvested at the right stage of maturity. When one or two berries turn yellow, harvest season begins. Spikes that fall to the ground can be gathered individually, cleaned, and then combined with other materials to form a general lot. When pepper is harvested, its moisture content ranges from 65% to 70%; sufficient drying will reduce this to a safety level of 10%.

Table 2. Optimum maturity at harvest for different pepper products (Ravindran & Kallupurackal, 2012)

| Product | Stage maturity at harvest |
|---|---|
| Canned pepper | 4-5 months |
| Dehydrated green pepper | 10-15 days before maturity |
| Oleoresin and essential oil | 15-20 days before maturity |
| Black pepper | Fully mature and 1-2 berries start turning from yellow to red in each spike |
| Pepper powder | Fully mature with maximum starch |
| White pepper | Fully ripe |

**Figure 2.** Portion of a pepper vine climbing on a large tree

### 2.4. Chemical composition in black pepper

Black pepper fruit is ball shaped, 8 mm in diameter, brown with a mesh-wrinkled surface. It has a spicy taste, a specific smell. Chemical constituents of black pepper fruit are given in Table 3.

**Table 3**. Chemical constituents of black peppper dried fruit(in percentage) (Ravindran & Kallupurackal, 2012)

| Constituent | Percentage rang, % |
|---|---|
| Moisture | 8.7-14.0 |
| Total nitrogen | 1.5-2.6 |
| Volatile ether extract | 0.3-4.2 |
| Nonvolatile ether extract | 3.9-11.5 |
| Alcohol extract | 4.4-12.0 |
| Crude fiber | 8.7-18.0 |
| Total ash | 3.6-5.7 |
| Acid insoluble ash | 0.03-0.55 |

As shown in Table 4, the chemical components of pepper can be divided into three main categories: a) compounds that contribute to the spice (spicy taste); b) compounds that give the pepper a distinctive aroma; and c) compounds that form the main element (starch). The fruit's essential oil gives pepper its scent, while the presence of alkaloids—primarily piperine—contributes to the spice of the fruit. Commercially available pepper extracts come in the form of oleoresins, which also contain phenolic chemicals. Black peppers' cooking and nutritional value

may be influenced by starch. For pepper as well as other spices, plant quality is the most crucial factor in cultivation. The concentrations of oleoresin, essential oil, and piperine in pepper are what primarily influence its quality (Ravindran & Kallupurackal, 2012).

**Table 4**. The main chemical constituents of black pepper fruit (Ravindran & Kallupurackal, 2012)

| Chemical | Percentage (%) |
|---|---|
| Piperine | 2-9% |
| Oleoresin | 4.4-12% |
| Essential oil | 0.4-7% |
| Starch | 28-49% |
| Fatty oils (acids) | 1.9-9% |

- Piperine

The primary aromatic component of pepper is piperine. Peppercorns' primary alkaloid, piperine (Figure 3), is responsible for the distinctive scent and fiery flavor of black pepper. Changes in the growth environment, such as the climate, drying conditions, and the origin, can have an impact on the amount of piperine. Piperine is present in black pepper berries in 2-9% of cases. There are various techniques for piperine isolation. Transtrans isomer (piperine), cis-trans isomer (isopiperine), cis-cis isomer (chavicine), and trans-cis isomer (isochavicine) are the four isomeric forms of black pepper. In contrast to piperine, isomers of piperine don't taste as strong or spicy. Additional alkaloids found in the black pepper fruit, including piperanine, piperettine, piperylin A, piperylin B, and pipericine, have also been identified through piperine investigations. Many biological activities may be lost as a result of the intensifying isomerization process that occurs when exposed to varying weather conditions and rising light intensity (Ravindran & Kallupurackal, 2012).

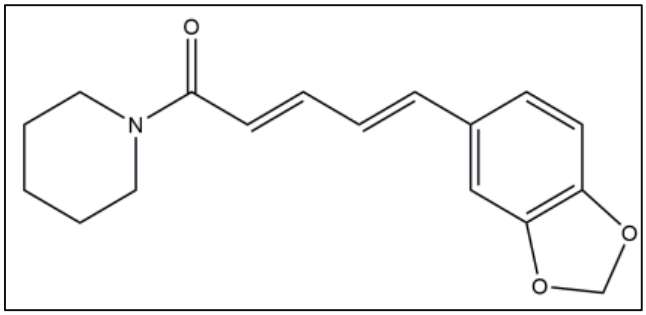


**Figure 3.** Structure of piperine

- Black pepper oleoresin

Spice oleoresins can be thick, sticky pastes or viscous oils. They are made up of a variety of ingredients, including pigments, fixed oils, pungent ingredients like alkaloids, essential oils, etc. It is not possible to add oleoresin's constituent parts directly to meals. Because of their great stability, oleoresins can be kept in storage for up to a year without losing any of their quality. Controlled storage conditions, including humidity and temperature, are not necessary. Black pepper contains between 6% and 13% oleoresin. The volatile oil and piperine components of black pepper oleoresin, the amount of which varies depending on the type of pepper and its maturation stage, the extraction solvent used, and the extraction conditions, affect the organoleptic qualities of the substance. 15% to 20% volatile oil and 35% to 55% piperine are often found in oleoresin (Ravindran & Kallupurackal, 2012).

- Essential oil

The essential oil content of black pepper ranges from 1% to 3%, while some research has found values as high as 9%. Black pepper essential oil has a distinct smell and is colorless to yellow-greenish in liquid form. It smells peppery. The dried, immature fruits are distilled to extract the essential oil. This essential oil is classified as levorotatory because of its specific gravity of 0.860–0.884, refractive index of 1.478–1.488 at 20 °C, and optical rotation range of - 1 ° C to - 23 °C. Monoterpene hydrocarbons are the most prevalent components in black pepper essential oil, followed by sesquiterpene hydrocarbons (Ravindran & Kallupurackal, 2012).

- Starch

About half of the dry weight of black pepper fruit is made up of starch. The nutritional characteristics of food can be altered by the interactions between starch and other ingredients, such

as polyphenols or piperine. In any case, not much is known about the molecular makeup of black pepper starch. However, another study supports the idea of piperine being released under controlled conditions from starch nanoparticles, which may serve as piperine's potential nanocarriers. Since pepper is mostly used as a spice to enhance food's aroma rather than adding nutritional value, its protein content has not been thoroughly studied (Ravindran & Kallupurackal, 2012).

- Fats

Black pepper contains fats in the range of 1.9-9.0%, sometimes about 15%. The most common fatty acids are: palmitic (16-30%), oleic (18-29%), linoleic (25-35%), and linolenic acid (8-19%) (Ravindran & Kallupurackal, 2012).

- Phenols

Phenols in black pepper are a mixture of glycosides of phenolic acids and flavanol glycosides. Some of the most abundant phenolic compounds are phenolic acids containing hydroxybenzoic and hydroxycinnamic acids along with significant amounts of quercetin and kaempferol (Ravindran & Kallupurackal, 2012).

### 2.5. Biological activities of black pepper

- Antioxidant activity

Black pepper has a strong potential for antioxidants. The primary alkaloid found in black pepper, piperine, has the ability to lessen the oxidative stress that high-fat diets cause to cells while preserving levels of several enzymes, including glutathione peroxidase, catalase, glutathione-s-transferase, and glutathione, that are comparable to those found in control groups. A few studies have demonstrated the strong antioxidant activity of black pepper extracts made using various solvent systems. When compared to aqueous and methanolic extracts, black pepper ethanolic extract showed the strongest antioxidant activity in all examined doses, according to a study by Nahak and Sahu. The ethanolic extract of black pepper had a nearly two-fold lower total phenol concentration than the ethanolic extract of cubeb pepper. Black pepper has a higher antioxidant activity than cubeb pepper, although coming from the same plant family. This suggests that other bioactive substances that have been isolated, such as some of the alkaloids, are also accountable for the antioxidant activity of black pepper in addition to phenolic components. It is not impossible

that the synergistic interaction between phenolic compounds and other biomolecules extracted from the plant material might account for the antioxidant activity of the ethanolic extract of black pepper(Ravindran & Kallupurackal, 2012).

- Antimicrobial activity

Black pepper has been shown to have antimicrobial properties against a variety of fungus (*F. oxysporum, A. niger, Candida spp*.) and bacteria (*E. coli, S. typhi, S. typhimurium, S. aureus, P. aeruginosa, B. subtilis*). It was discovered that the different solvents employed to extract black pepper had an impact on the microorganisms' varying susceptibilities. Black pepper fruit extracts (hot, cold, and methanol) have been shown to exhibit antibacterial activity against a variety of pathogens in a study by Khan et al. The maximum inhibition zone (23 mm) was observed in the cold-water extract against *E. coli*, whereas the maximum inhibition zone (22 mm) was observed in the hot-water extract against *S. typhi* and *S. aureus. S. aureus* was unaffected by the methanolic extract; however, it did exhibit a 21 mm inhibitory zone against *E. Coli, S. typhi, and P. aeruginosa*. Black pepper, 3,4-dihydroxyphenyl ethanol glucoside, and 3,4-dihydroxy-6-(N-ethylamino) benzamide have been shown to have chemicals that suppress the growth of bacteria that are associated with foodborne illnesses, such as *S. aureus, B. cereus, E. coli, and S. typhimurium* (Ravindran & Kallupurackal, 2012).

- Anticancer activity

Pepper significantly boosts other anti-tumor spices' efficacy and effectiveness against cancer by increasing their absorption. Black pepper directly prevents the development of cancer. Piperine, the primary phytochemical found in it, suppresses some pro-inflammatory cytokines generated by tumor cells. By disrupting the signaling pathways among cancerous cells, it lowers the likelihood of tumor advancement. By promoting the xenobiotic biotransformation enzymes, pepper inhibits the development of chemical carcinogenesis. Piperine and related unsaturated amides' antioxidant qualities help to stop the development of cancer. An essential part of the body's defense against the deteriorative alterations brought on by mutagens may be the dietary consumption of natural antioxidants. Substances included in essential oils prevent xenobiotics from forming DNA adducts (Meghwal & Goswami, 2012). A substance found in black pepper fruit called piperlonguminine demonstrated cytotoxic properties against colorectal and breast cancer cells. It has been discovered that using piperine orally greatly reduces the risk of various

types of gastrointestinal cancer. By changing the reaction of lipid peroxidation, the black pepper ethanolic extract, which contains piperine, successfully combats lung cancer. Furthermore, piperine has the ability to restrict the G1/S phase of the cell cycle, which prevents HUVEC (human umbilical vein endothelial cells) from proliferating and migrating (Ravindran & Kallupurackal, 2012).

- Anti-inflammatory activity

Black pepper is one of the substances that can reduce inflammation in the human body. Inflammation is a complex biological reaction of vascular tissues to damaging stimuli, such as pathogens, damaged cells, or irritants. It is also known as an anti-inflammatory. About 50% of analgesics are anti-inflammatory medications, which relieve pain by lowering inflammation (Meghwal M, 2012). Piperine inhibited LPS-stimulated endotoxins (lipopolysaccharides) by activating interleukin (IL) in synoviocytes. Additionally, it was shown that in the dosage range of 10-100 µg/ml, piperine decreases the synthesis of IL-6, MMP (matrix metallopeptidase)-13, and prostaglandin E. Furthermore, piperine's anti-inflammatory properties were studied in colorectal locations, where it was found to suppress ulcerative colitis brought on by acetic acid and FFA (free fatty acid)-induced inflammation, which is mediated by TLR4 (the Toll receptor). Other black pepper alkaloids also show anti-inflammatory properties in mouse macrophages through preventing the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells, or NF-kB pathway (Ravindran & Kallupurackal, 2012).

### 2.6. The effect of drying method

Since drying reduces the moisture content of the product, it is a traditional method for extending the shelf life of spices. Drying in the open sun is a climate-dependent traditional method that is unregulated, labor-intensive, time-consuming, and unsanitary. Apart from this, an extended drying period could raise the possibility of microbial infection, which could result in unsightly grayish goods. Thus, shorter drying times could lead to fewer microbial infections. However, drying times can be shortened using solar and mechanical dryers. A solar dryer that uses sunshine and a few other suitable technologies was utilized to create pepper with a high microbiological standard, a deep black color, and low humidity in order to increase the pepper's overall quality (Ravindran & Kallupurackal, 2012). Nonetheless, the air temperature surrounding the product in a solar dryer is higher than the surrounding air temperature. The product's temperature may also

be increased by direct sun radiation absorption, depending on the kind of solar dryer. A solar dryer's temperature is higher than that of sun drying, which shortens the drying process and typically enhances the quality of the finished product. The produce is shielded inside the solar drier from animals and weather, preventing crop losses and spoiling. The principle of solar dryer is the air inside the collecting plate warms as solar radiation strikes it. The warm air rises and discharges into the collector. Therefore, natural convection is used to circulate the air. The material to be dried is placed on a vertical stack of trays that make up the drying chamber. Common commodities like black pepper, wheat, and rice require a drying temperature of 45°C to 60°C. Each commodity has a certain fixed range of temperatures that it can tolerate since they dry at various speeds. It is suggested that spoiling bacteria are still active in drying items at any temperature lower than 45 °C. Conversely, a temperature greater than 75 °C might harm vital biological components and structural elements of items (Rizalman, Moung, Dargham, Jamain, & Farzamnia, 2023). The disadvantages in solar dryer are that the products cannot be dried after the sunshine and during cloudy weather conditions.

Besides using solar dryer, we have another method that is available all the season. It is black pepper drying machine (tray dryer). Tray dryer is the most extensively used because of its simple and economic design. The food is spread out on trays at an acceptable thickness so that the product can be dried uniformly. Heating may be produced by hot air stream across the trays, conduction from heated trays, or radiation from heated surfaces. In a tray dryer, more products can be loaded as the trays are arranged at different levels. The key to the successful operation of the tray dryer is uniform airflow distribution over the trays. Any traditional dryer that runs on electricity or fossil fuels can utilize the tray dryer. It can also be used with solar dryers. The final moisture content of the dried goods on the trays will be consistent if there is adequate airflow distribution. Typically, an electronic balance is used to calculate the moisture content by comparing the product's starting and final masses. A tray dryer typically comprises of multiple trays stacked inside an enclosed chamber with hot air circulated via a fan or natural flow. A portion of the expelled air is occasionally circulated again inside the drying chamber. The chamber's tray may move or remain stationary. The stationary tray dryer is widely used because of its simple design. The stationary tray dryer is widely used because of its simple design. The trays in this arrangement are fastened in place. To achieve consistent product quality, the airflow distribution across the trays must be uniform. Inadequate airflow dispersion frequently results in variations in

the dried product's final moisture content at various tray positions. The amount of product that may be placed into the dryer system is likewise restricted by this issue. The uneven drying of the drying chamber is reduced or eliminated by a well-designed tray dryer system (Misha, Mat, Ruslan, Sopian, & Salleh, 2013).

### 2.7. Parameter analysis

#### 2.7.1. Physicochemical analysis

##### 2.7.1.1. Moisture content

When pepper is harvested, its moisture content ranges from 65% to 70%; sufficient drying will reduce this to a safety level of 10%. The pigment chlorophyll is what gives mature peppers their green color. Black pepper with a high moisture content (>12%) is vulnerable to fungal attack as enzymatic browning occurs during drying and the phenolic components are oxidized by ambient oxygen under the catalytic activity of the enzyme phenolase. A pepper's high moisture content leaves it open to fungal attack. Fungal attacks on peppers can be dangerous because the fungi release compounds that can harm plants. Poisonous compounds released by the fungi render the pepper unsafe for human eating. The fungal attack produces mycotoxins, which make the pepper unsafe for human consumption. Pepper berries are spread out on a clean, dry concrete floor, bamboo mats, or PVC sheets, and they are sun-dried for four to six days to produce a high-quality dry product. Black pepper is also dried with the aid of mechanical driers created by different organizations. There are models with different capacities that can be used to dry black pepper by keeping the temperature below 55°C, either by burning agricultural waste or by using electricity. The traditional technique for drying black pepper is sun drying. To reduce the moisture content below 10%, the despiked berries are spread out on a concrete floor and dried in the sun for three to five days. (Devasahayam et al., 2014).

##### 2.7.1.1. Piperine

The main pungent alkaloid in black peppercorns, piperine (1-peperoylpiperidine), is obtained from the fruit bodies of *Piper nigrum* and is widely used in many diets worldwide. Apart from its widespread use as a flavor enhancer, it has seen numerous and varied uses, such as flavoring brandy or acting as an insecticide Additionally, it has been reported to have antioxidant

activity, which may provide protection against the harmful effects of a high-fat diet (McNamara, Randall, & Gunthorpe, 2005).

#### 2.7.1.2. Standard for pepper in the EU

The EU does not have a formal pepper quality standard. As a result, European pepper actors frequently adhere to guidelines established by associations like the European Spice Association (ESA), the International Pepper Community (IPC) standard specifications for black, white, and green pepper, the Codex Alimentarius Standard for peppers, and the ISO specifications for peppers.

**Table 5**. Standard in black pepper

| **The whole dried black pepper** | | **Standard** |
|---|---|---|
| Moisture | ≤12% | European Spice Association (ESA) |
| Density | >550 (g/l) | European Spice Association (ESA) |
| Diameter | 2.5-7 mm | Codex Alimentarius Standard for Black |
| Piperine | 3.5 % | Codex Alimentarius Standard for Black |

### 2.7.2. Microbiology analysis

The contamination may occur from the soil, air, and water during collection and drying or due to animal waste in the environment, as well as using contaminated equipment during production. These can lead to contamination of spices with microorganisms like *Salmonella sp.*, *Escherichia coli*, mold and yeast. Especially some types of molds and yeast can produce mycotoxins that cannot be degraded by cooking and threaten human health (İslamoğlu & Korkmaz, 2021). Current practices of harvesting, handling, and production often cause additional contamination and microbial growth. Many spices are grown, harvested, processed, and stored in poor sanitary conditions, which increase the risk of contamination even with pathogens.

#### 2.7.2.1. *Salmonella*

*Salmonella* is a genus of rod-shaped (bacillus) Gram-negative bacteria of the family Enterobacteriaceae. *Samonella spp* is a bacterium that can cause an illness called salmonellosis in human. *Salmonella sp*. is an important foodborne pathogen and most of the regulations suggest its absence in a food sample. According to the current microbiological criteria for spices in Turkish Food Codex, *Salmonella* should not exist in 25 g of sample, as well as in the International Food Standards, Codex Alimentarius Commission suggests the absence of *Salmonella* in spice. Although dry pepper by itself is a shelf-stable product, the microorganisms in it have the potential to shorten the product's shelf life or cause foodborne illnesses when added to high moisture, minimally processed food. If the food is dehydrated and there are enough nutrients available, dry stress resistant microorganisms, such as bacterial spores or some forms of Salmonella, are still viable and have the potential to grow. Because they do not undergo additional heat treatments, ready-to-eat food products are especially concerning (İslamoğlu & Korkmaz, 2021).

#### 2.7.2.2. *Escherichia coli*

*Escherichia coli* is one of the world's best-characterized organisms, as it has been extensively studied for over a century. *Escherichia coli*, a gram-negative bacillus found in normal intestinal flora, can cause intestinal and extraintestinal illness in humans and is commonly found in hospitals and long-term care facilities. Since it naturally exists in both human and animal intestines, it is regarded as the most significant sign of fecal contamination. The presence of *E. Coli* in food is a sign of poor distribution, processing, or operational cleanliness. Certain *E. Coli* strains have the potential to seriously harm both humans and animals (Mueller & Tainter, 2023).

#### 2.7.2.3. Total aerobic microbial count

Other names for the aerobic plate count (APC) are total plate count, aerobic colony count, aerobic mesophilic count, and standard plate count. A food sample's bacterial population can be estimated using the APC. It does not assess the diversity of bacterial species present in a food product or evaluate the overall bacterial population. The number of microorganisms that can thrive aerobically at mesophilic temperatures is estimated by this method. Sanitary quality, sensory acceptability, and compliance with good manufacturing practices (GMPs) can all be evaluated using the APC. A food processor can learn about raw material quality or handling history, food

processing and storage conditions, and management of the final product from the APC results. It can also be used to estimate a food product's shelf life or impending sensory change. The synthesis of enzymes and microbial growth often cause detectable changes in food quality features when the APC climbs to approximately $10^6$–$10^7$ per g or ml. The absence of a direct correlation between the APC and the presence of toxins or pathogens renders it an untrustworthy indicator of microbiological food safety. A low APC does not indicate that a product or any of its constituents are free of pathogens. Though pathogen testing results are still awaiting, it might be presumed that a public health danger exists if an abnormally high APC is found in a food product or ingredient. One must take into account the type of food product and whether or not a high APC is characteristic of that product when evaluating APC data (Mendonca, Thomas-Popo, & Gordon, 2020).

#### 2.7.2.4. Total Mold- Yeast count

Molds are capable to produce mycotoxins easily at room temperatures ranging from 25°C to 30°C and moisture contents of above 16%. These toxins, which are generally effective even in trace amounts, can cause chronic diseases or acute deaths. Inappropriate storage directly affects the risk of mycotoxin proliferation, particularly, if spices were formerly contaminated with molds. The International Food Standards of Codex Alimentarius Commission still suggests a maximum limit of $1x10^3$ cfu/g for mold-yeast in spices. Three mechanisms—direct mold infection, immune system suppression, and toxic byproducts—are how molds infect humans and cause illness. Yeasts are unicellular fungi that reproduce mostly asexually. They grow quickly on simple carbohydrates and frequently use both respiratory and fermentative pathways (İslamoğlu & Korkmaz, 2021).

### 2.8. Application and benefit of organic black pepper

In home cooking, gastronomy, and food technology, herbs and spices play a major role. The most common definition of spices is "products of plant origin." At first, their primary applications were in food flavoring and presentation. Then, it was mentioned that the shelf life and nutritional content of food are greatly impacted by the preservative, antioxidant, and antibacterial qualities of spices. While humans have been aware of the preservation qualities of spices since ancient times, we are now discovering the specific components that carry out this function because of extremely sophisticated analytical techniques. The utilization of spices also has health-promoting qualities. Many chemical compounds with health-improving qualities can be found in

herbs and spices, which may offer protection against cancer, diabetes type 2, neurological illnesses, cardiovascular disease, and other conditions. But we also need to be aware of the nomenclature discrepancies, especially when it comes to distinguishing between therapeutic herbs—which are categorized as medicinal products—and herbs—which are categorized as food products (Newerli-Guz & Śmiechowska, 2022).

# CHAPTER 3
# METHOLOGY

## 3.1. Materials and equipment

**Table 6.** Some materials and its source

| **Material** | **Source** |
|---|---|
| Organic black pepper | Kompot province |
| Grinder | NA |
| Sola room | NA |
| **Machine** | **Model** |
| Grading machine | TGI - 500- Grader |
| Screening machine | CE-PM-001-009 |

**Table 7.** Chemical and equipment using in laboratory at CONFIREL Company

| **Chemical** | **Equipment** |
|---|---|
| Ethanol 95.5% | Blender |
| Distilled water | Digital vernier calipers |
| | Aluminum paper |
| | Hot plate |
| | Magnetic stirrer |
| | Cylinder (250ml) |
| | Beaker (100ml) |
| | Pipette |
| | UV Spectrometry |
| | Moisture analyzer (MA160) |
| | Electronic balance |

### 3.2. Method

The processing operation of organic black pepper involves grading, selecting, drying, screening, packaging and storage. Processing care should be taken to maintain the quality at each step of operation.

#### 3.2.1. Post-harvest handling

Post-harvest handling of organic black pepper should be manually accomplished. When we want to get black pepper, the harvest should be accomplished when the fruits are completely developed. Organic black pepper is grown and harvest from Kompot province. Handling crops after harvest is essential to producing a high-quality product. After harvesting, the spikes are either heaped or covered for an overnight fermentation that facilitates fruit separation, or they are stored in bags for 12 to 24 hours. After that, the spikes are manually threshed by rubbing or trampling underfoot or by employing a variety of automated threshers. Large growers are the only ones who employ mechanical threshers. Fruits that have been threshed are evaluated before being sun-dried. The best way to grade is to separate the berries based on size and use a mesh to get rid of the light berries and pinheads. Before drying, fruits should be blanched by dipping them (in a wire basket) in boiling water for two minutes. That way the berries are cleaned and skin gets black during the drying. After that, the fruits are laid out on the ground to dry. Blanching improves color and also removes dust and adhering microbial contamination. Drying is done in the open sun in most cases. A black topped cement floor is the best for sun-drying. Mechanical, electrical and solar dryers are also used for rapid drying. After proper drying, the moisture content should be around 10 % only. Dried black pepper with high moisture content (>12%) is susceptible to fungal attack. Mycotoxins produced by the fungal attack render the pepper unfit for human consumption. The dried peppers are import to industry and they become semi-raw materials. Diameter and moisture are checked and done some test to confirm that dried peppers can process continuedly (Ravindran & Kallupurackal, 2012).

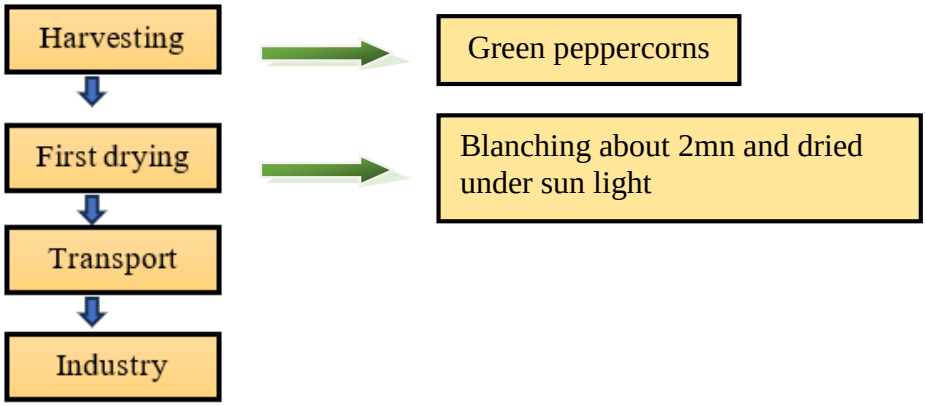


**Figure 4.** Process during harvesting

**Table 8.** Standard of checking on moisture, density, and diameter of dried organic black pepper in semi-raw material

| **Parameters** | | **Specification** |
|---|---|---|
| Moisture | Moisture analyzer (%) | M≤10% |
| Density | d= m/v (g/l) | d ≥570g/l |
| Diameter | Dial= (Dial1+Dial2+Dial3+Dial4+Dial5)/5 (mm) | Dia≥4mm |

### 3.2.2. Processing

The dried peppers are imported into industry, and they become raw materials. Density, diameter, and moisture are determined, and it is checked on peppers to see if they contain insects or not. If dried peppers contain insects, blanching treatment is used, whereas if they don't have insects or mold, they are just dried and packaged.

1. **Grading**

This grading method is to remove the iron, broken black pepper, and insects. The grading of black pepper is done by using sieves and sifting black pepper into different grades based on size. The first method used when processing organic black pepper is screening. They are screened using a sieve machine. The new high-efficiency screening equipment has a screen-moving trajectory that is approximately straight. This sieve machine has a simple structure, a large screening capacity, low energy consumption, and easy maintenance. The stainless steel linear vibrating screen is

widely used in mining, coal, electric power, metallurgy, building materials, and other industries for large block materials, medium block materials, and small granular materials. The screen box relies on the vibrating motor to rotate in the opposite direction simultaneously so that the whole screen machine supported by the damper vibrates in a straight line. After the material falls into the screen box from the feeding end, it quickly moves, loosens, and sifts through the screen to complete the screening operation. This machine is composed of a sieve box, a vibration motor, a vibration damping system, and a chassis. The sieve box is composed of a sieve frame, a sieve plate, and a lining plate. Before we use a sieve machine, we have to wash thoroughly, wipe with alcohol, and dry well to avoid infection by other microorganisms. The characteristics of the screening machine are three parts; each part has a different hole or shield. The first part has four shock irons, and each hole has a length of bigger than 4 mm. The second part has three shock irons, and the hole's length is bigger than or equal to 3 mm. The last part has a length under 3mm and is not taken. The first and second parts of screening pepper are taken to process continuously.

**2. Selecting**

After sifting, dried black pepper has extraneous matter like spent spikes, pinheads, stones, the bad peppers, broken peppers, stones, soil particles, etc. mixed with it. The selection method is to pick out extraneous matter, such as bad pepper, and check for worms or fungi. Selecting is done by handpicking, which removes most of the impurities. Before selecting, clean the room with alcohol. Before handling the pepper, wear gloves to avoid infection with the pepper.

**3. Drying**

After organic black pepper is selected, we have to dry it in the solar room using sunlight until the moisture content drops below 5%, taking 6 hours. Characteristics of a solar room: solar panels can make your room hotter. This is because they absorb some of the heat from the sun and act like a greenhouse, trapping the heat inside your room. However, it’s important to note that this additional heat isn’t necessarily a bad thing. In fact, depending on the climate, this extra warmth could be beneficial and help reduce your energy bills in colder climates. When installing solar panels, it’s important to consider their position carefully. Directly facing south is preferred so that they get as much sunlight as possible during peak hours (usually between 10 a.m. and 4 p.m.).

**4. Screening**

After drying, black pepper is sifted again using a rotary vibrating screen with a small hole to filter out small pieces of debris before packing. The working principle of the rotary vibrating screen: the vertical vibrating motor is used as the excitation source, and the upper and lower ends of the motor are equipped with eccentric weights, which convert the rotational motion of the motor into a three-dimensional motion of horizontal, vertical, and inclined. Transfer to the sieve surface for sieving. Adjusting the phase angle of the upper and lower ends can change the movement track of the material on the screen surface. By changing the angle and mass of the upper and lower eccentric blocks of the vibrating motor, the vibrating motor can drive the equipment to generate vibration forces of different sizes, thereby changing the vibration amplitude in the horizontal or vertical direction, and then changing the flow rate and efficiency of the screening.

**5. Packaging and storage**

Organic black pepper should be packed. The materials used inside the package ought to be fresh, hygienic, of food-grade quality, and they should not harm the fruit internally or externally. Black pepper is hygroscopic in nature and absorption of moisture from air, during rainy season when there is high humidity may result in mold and insect infestation. Before storage it is to be dried to less than 10 per cent moisture. During storage and transportation, it must safeguard the product's safety and quality. Bulk material must be packed and stored in an air-permeable container to prevent condensation. Bulk materials need to be kept out of the direct sun, in a dry, clean space with good ventilation and no pests.

### 3.3. Sampling

- **Sampling sample of organic black pepper receiving**

Organic black pepper cannot test on 100% of Organic black pepper product therefore we determine the sampling size. This sampling size varies according to type of size in one production lot.

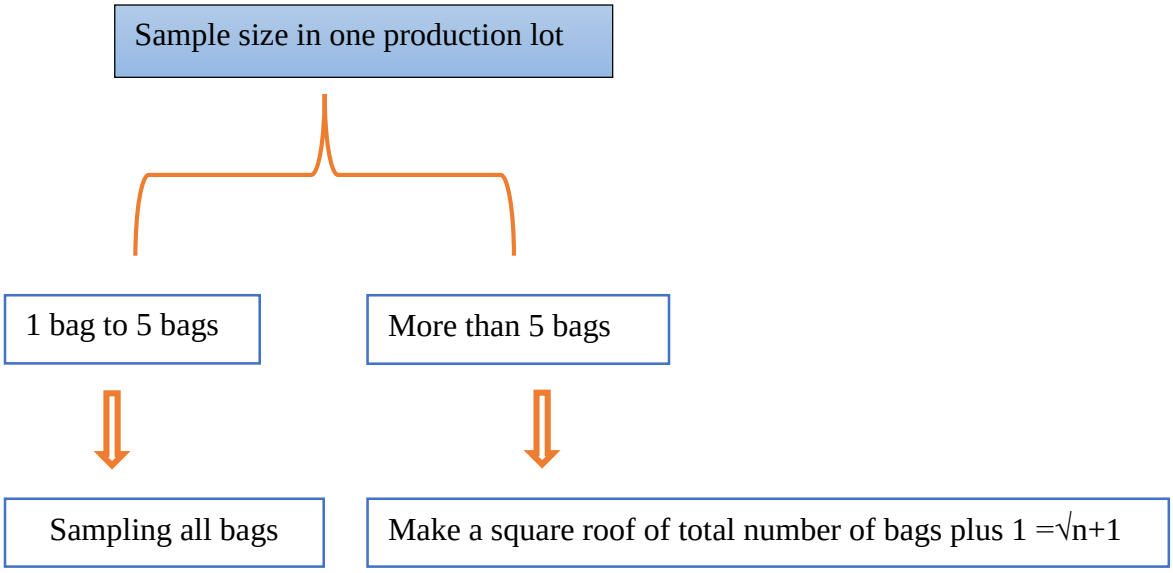


**Figure 5.** Flow chat of sampling sample in one product lot

In the flow chart above, it is shown that when organic black pepper is incoming in one product lot, the QC department makes the sampling size of organic black pepper into bags. For 1 to 5 bags, the QC checks all bags of black pepper. As well as, for more than 5 bags, the QC must make a square roof of the total number of bags in one product plus one. After the bags are checked, they should be stored in a dry place to maintain their quality.

Noted: 1 bag < 50kg

- **Sampling sample in production (finished product)**

All finished products have to check quality. After black pepper are packed already, QC have selected 2 samples randomly amount of finished product. One sample is transferred to physical laboratory to check physical parameter at CONFIREL Company whereas another is sent to test microbiological parameter at PPM.

### 3.4. Sensory parameters

Sensory evaluation is a scientific discipline used to measures, analyzes, and interprets responses to food material's properties as they are perceived by the senses of taste, smell, touch, and hearing (sound). Measuring the sensory qualities of food quality allows manufacturers to assess consumer preference and acceptance while producing a product that is both affective and acceptable at the highest possible economy of production. The evaluation is done to determine quality raw materials and finished products. standards Food scientists may determine whether a

food conforms with established government or industry standards, food grades, and product development while maintaining desired sensory characteristics with the aid of sensory procedures (V. Jain, 2005). Sensory parameters in dried organic black pepper have three parameters such as aroma, color, flavor. Flavor is the experience derived from food or anything else on your tongue, experienced with the help of the cells on the inside of mouth above the tongue. It is like a taste plus experience. Aroma is what the cells in the nostril convey to the brain when you about to eat. All sensory parameters are evaluation by human sensation (confirm or non-confirm)

**Table 9.** Specification of sensory parameters in Confirel Company

| **Sensory parameters** | **Specification** |
|---|---|
| **Aroma** | Distinctive black pepper |
| **Color** | Black to dark-grey, black to brown |
| **flavor** | Intensely spicy |

### 3.5. Physicochemical analysis

#### 3.5.1. Moisture content

Moisture analysis is a parameter used to measure the value of water content in a sample, such as organic black pepper. Maximum of moisture machine is 15g and model (MA160, Germany). Firstly, an aluminum pan was prepared in the moisture machine, and then the method (raw for raw material and tea for semi- and final product) was set on the screen of the machine. Organic Black peppers are ground by using blander. 5 g of ground black peppers are weighted and put on aluminum peppers, and the moisture content is analyzed by a moisture analyzer. It will show the percent of moisture contained in the sample.

Black pepper was grade into small pieces and weight 5g to measure moisture

Measure by using Electromatic Moisture

**Figure 6.** Method of testing moisture

### 3.5.2. Diameter

5 black peppers are picked out from 50 g of black pepper and measured diameter by diameter analyzer. The value of each pepper is recorded to find average values.

We calculate using formula:

Average diameter = (Dia1+Dia 2+Dia 3+Dia 4+Dia 5)/5

Weigh pepper 50g and 5 black peppers are picked out.

Measured diameter by Digital vernier calipers.

**Figure 7.** Method of measuring diameter

### 3.5.3. Density (g/l)

50 g of black pepper is weighted and filled to cylinder 250ml to get volume of pepper.

Formula density

d (g/l) =m/v

m=mass of sample (g)

v= volume of sample in cylinder (l)

Weigh pepper 50g

Fill to cylinder 250ml to get volume

**Figure 8.** Method to measuring density

### 3.5.4. Piperine

1 g of ground black pepper is diluted with 50 ml of 99.5% ethanol for 3 h on a hot plate that heats to 50 °C. After heating for 3 hours, keep it cool down and dilute with the same solvent (ethanol, 99.5%) to reach 100 ml. The solution is filtered by filtration paper. 1 ml of solution is diluted with 99 ml of ethanol and mixed well. Piperine of black pepper is measured by using the spectrum at a wavelength 343 nm, and ethanol is used as a blank.

First turn on the machine (UV spectrophotometer) and wait for the machine to run, then click on ‘the ‘spectrum’ column and change the wavelength to 343 nm, and then click ‘ok’. Then, we need to calibrate the machine by using a blank (ethanol). We click on spectrum again to check our changing and saving parameters (piperine, blank, and black pepper). After running the machine, prepare the blank, which is ethanol in the curvet glass, and measure it in the machine (UV spectrophotometer), then click Auto Zero and press start to calibrate the machine. Then the machine will run, and it will show the value of absorbance and concentration on the computer screen. Then prepare samples (the first curve is ethanol and the second curve is black pepper solution) to measure piperine. We have to press the unknown sample and wait until the output value is displayed on the screen, then save. Peperine testing is twice.

**Formula calculation: P%= (A x 100 x 100 x 100)/ [1238 x 1 x m x (100-H)]**

A: Absorbent

m: Weigh of organic black pepper (g)

H: Moisrue content (%)

P (%): Percentage of piperine (%)

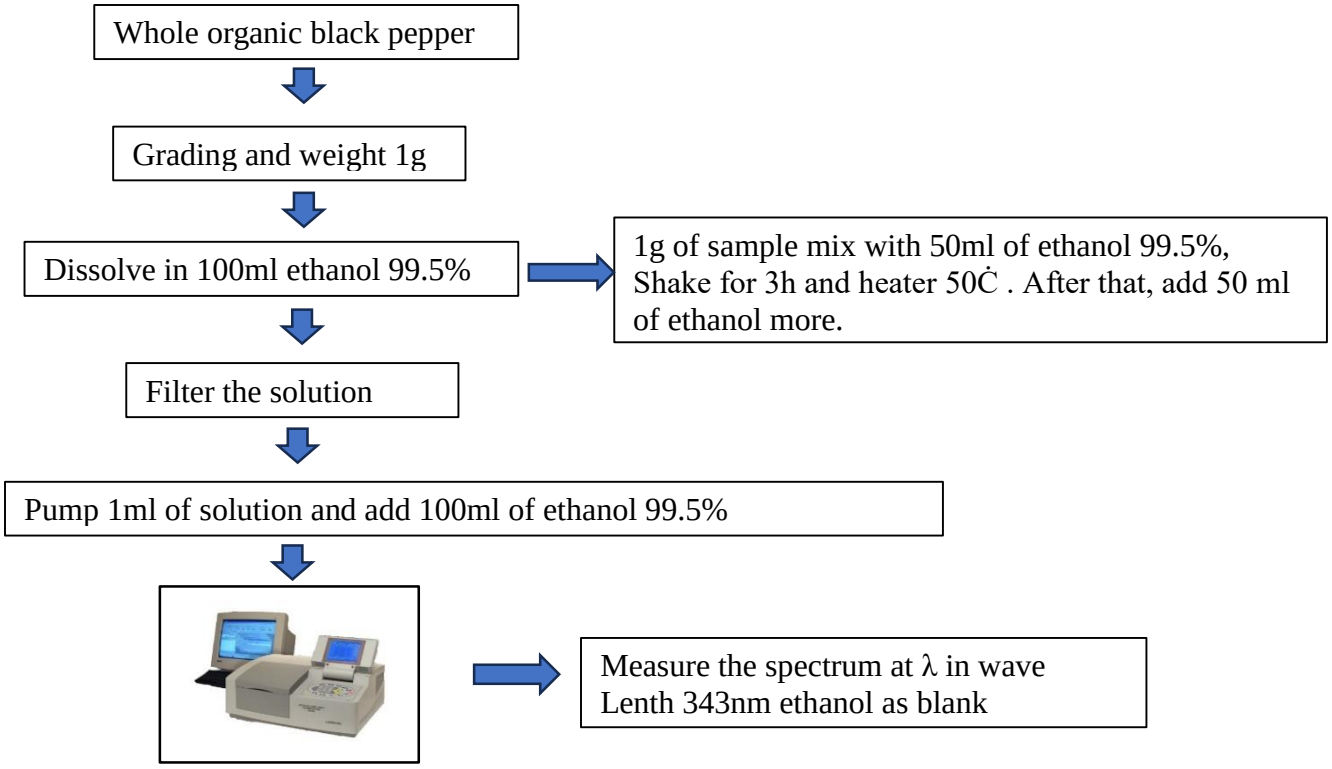


**Figure 9.** Flowchart of testing piperine in organic black pepper

# CHAPTER 4
# RESULTS AND DISCUSSION

## 4.1. Specification in Confirel Company

In table 10 below are shown about specification in company which is the results of testing on finished product both physical parameters to do comparison with standard.

**Table 10.** Specification of physicochemical analysis

| Moisture | Density | Diameter | Piperine |
|---|---|---|---|
| M≤12% | d ≥570g/l | Dia≥4mm | P≥3.50% |

## 4.2. Result of semi-raw material (Dried organic black pepper

The quality of organic black pepper is analyzed in table 13 and figure 10, 11, 12, and 13 based on physical data provided by CONFIREL Company. According to Table 12 shows the value of chemical parameter that tested at CONFIREL Company. Every parameter was measured every week. First week, moisture, density, and diameter were detected by 8.03±0.77%, 593.6±2.3g/l, and 4.41±0.18mm, respectively. For another week has described in table 12 below. Depending on specification of raw material of dried organic black pepper in table 3.2 that are compared with the results for each week, the value of moisture is lower than 10%. Density has value bigger than 570g/l and value of diameter is bigger than 4mm. The results that have tested on these parameters are in rang of standard, so the dried organic black pepper could process continually. Moreover, if we look at differentiation in density of dried organic black paper every week, it could course from collection in different place and characteristic inside of each organic black pepper.

**Table 11.** Result physicochemical test semi-raw materials

| Parameters | Moistures (%) | Density(g/l) | Diameter(mm) | Number of sample (n) |
|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD | |
| **Week1** | 8.03±0.77 | 593.6±2.3 | 4.41±0.18 | n = 5 |
| **Week2** | 8.05±1.08 | 577.2±1.79 | 4.55±0.19 | n = 4 |
| **Week3** | 8.03±0.78 | 588.6±1.14 | 4.49±0.15 | n = 4 |
| **Week4** | 8.05±1.09 | 592.2±2.59 | 4.59±0.25 | n = 6 |
| **Week5** | 7.88±1.02 | 595±1.58 | 4.43±0.13 | n = 3 |
| **Week6** | 8.34±1.13 | 578.6±1.14 | 4.59±0.15 | n = 5 |
| **Week7** | 7.6±1.19 | 588.2±1.64 | 4.53±0.25 | n = 4 |
| **Week8** | 7.68±0.69 | 592.8±1.92 | 4.56±0.18 | n = 2 |
| **Week9** | 7.69±0.97 | 591.6±2.07 | 4.46±0.21 | n = 5 |
| **Week10** | 8.56±0.45 | 594.8±1.3 | 4.41±0.18 | n = 4 |
| **Week11** | 8.24±0.89 | 586.2±1.92 | 4.48±0.23 | n = 7 |
| **Week12** | 7.2±0.79 | 584±1.58 | 4.59±0.25 | n = 6 |

### 4.3. Result of finished product

In table 4.4. is illustrated the results of sensory test by human sensation in finished product, not only aroma but also color and flavor of organic black pepper are conformed with specification.

**Table 12.** Result of sensory test

| Sensory parameters | Specification | Human sensation |
|---|---|---|
| **Aroma** | Distinctive black pepper | conform |
| **Color** | Black to dark-grey, black to brown | conform |
| **flavor** | Intensely spicy | conform |

Figures 10, 11, 12, and 13 show parameters for testing on the finished product, which is already packed. These figures show four parameters. The importance of these parameters is that if the organic black pepper's moisture is over range and its density, diameter, or piperine are low range, it is unacceptable to consumers and may lead to rejection of the black pepper. These results of the parameters are measured over 12 weeks and one randomly selected amount of finished product in one day.

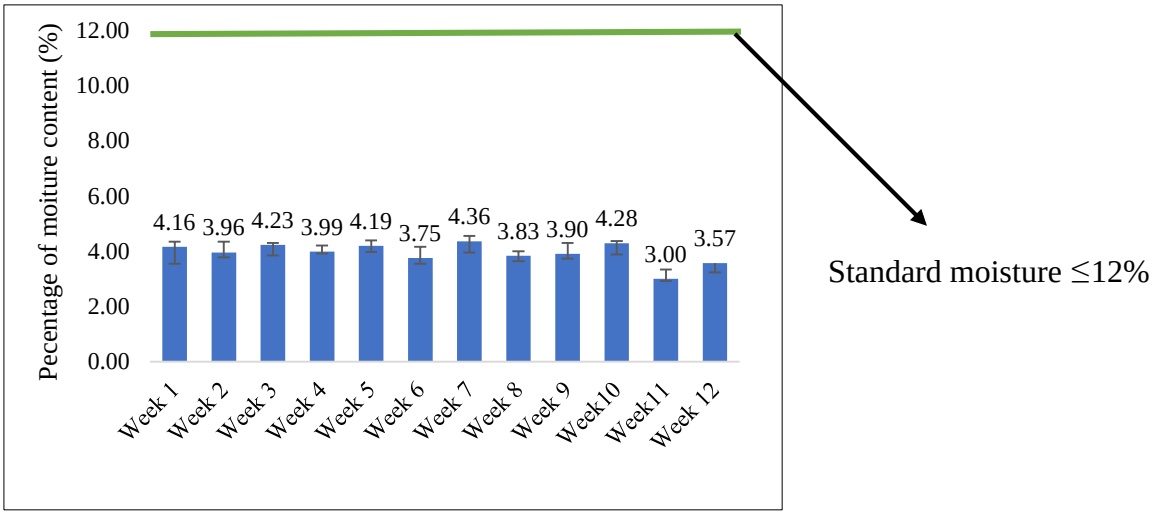


**Figure 10.** Moisture of dried organic black pepper for 12 weeks

For result of moisture in figure 10, provide valuable moisture by week such as 4.16±0.62, 3.96±0.18, 4.23±0.39, 3.99±0.07, 4.19±0.21, 3.75±0.2, 4.36±0.41, 3.83±0.19, 3.9±0.17, 4.28±0.4, 3±0.09, 3.57±0.34, respectively. If we compare with the value of the company's specification in Table 10, we are notified that the results of the moisture parameter stay within the range of the standard, where moisture is lower than 12%. The moisture content of sundried pepper was not safe

for storage, as moisture content over 12% can increase the chance of insect pest attack. (Dhas & Korikanthimath, 2003) reported that moisture content should be less than 10% to store the product safely after drying. The mechanical dryer decreased the black peppers' moisture content to the safer limit (<10%). As peppercorns are hygroscopic and so during storage, peppercorns absorb moisture from the weather. That is why moisture levels to 10-11% may stop mold growth (Thangaselvabal, Gailce Leo Justin, & Leelamathi, 2008). Lower moisture content makes organic black pepper have a long shelf life and no contamination from mold, yeast, *salmonella*, E. coli, and so on. In the hand, we have seen that the moisture content is reduce below 5% because in the processing organic black pepper is using drying method. Moisture must be removed from a product throughout the drying process, and thermal drying uses heat to do this. The product's moisture vapor pressure rises above that of the surrounding air due to the heat. Moisture, both liquid and vapor, migrates to the product's surface due to pressure and temperature gradients. Water vapor is released into the surrounding air by evaporation. There is a combination of bound and free moisture in a crop. Water that is able to pass freely through a substance is known as free moisture. Its movement is independent of the crop's internal structure. When there is enough free water in the product to replace the water that evaporated at its surface, the vaporization-evaporation process reaches its peak. The product's moisture content decreases as the moisture evaporates, and its temperature approaches the drying air's wet bulb temperature.

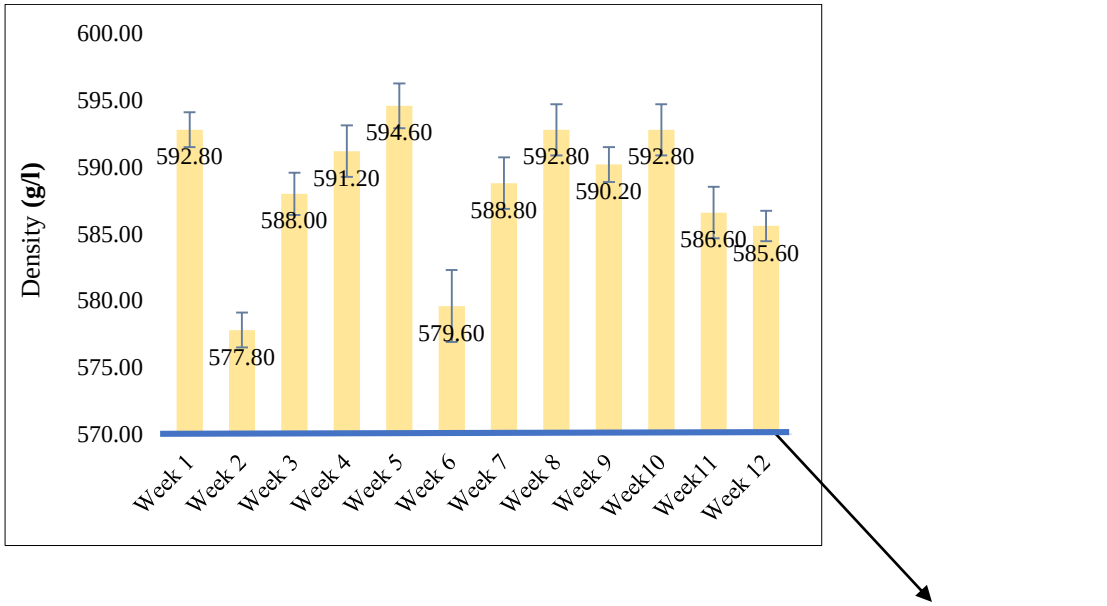


**Figure 11.** Density of dried organic black pepper

The density of black pepper is an important factor that affects its quality and functionality. High-density black pepper is typically considered to be of better quality because it is more compact and has less empty space between the black peppers. The results of density indicated in Figure 11 have values by week 592.8±1.3, 577.8±1.3, 588±1.58, 591.2±1.67, 594.6±2.7, 579.6±1.92, 588.8±1.92, 592.8±1.3, 590.2±1.92, 592.8±1.92, 586.6±1.92, and 585.6±1.14, respectively. Moreover, if we compare the value of density with the standard in the company, it stays in the range of 570 g/l. In figure above, density of some weeks is lower than others, it causes from increase in volumetric expansion of the sample was greater than the mass of the sample.

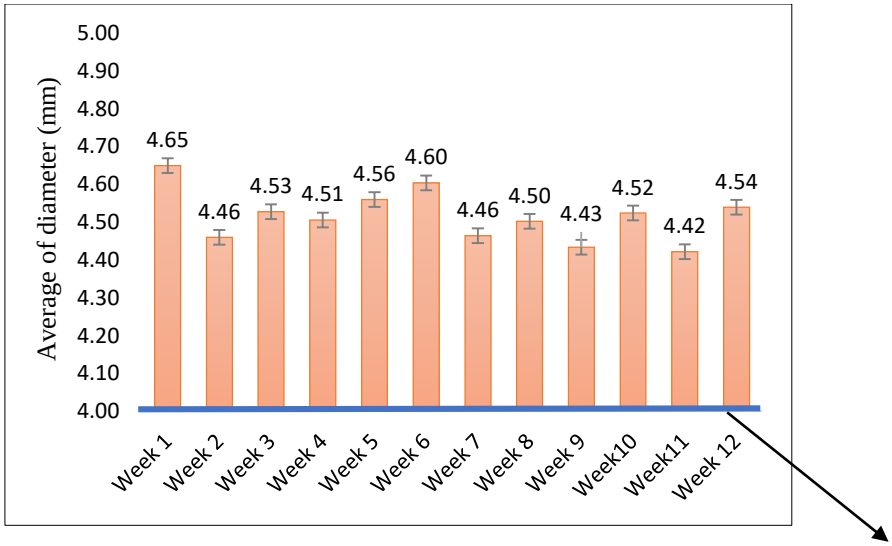


**Figure 12.** Diameter of dried organic black pepper

The diameter of black pepper is also an important factor that affects its quality and functionality. Black peppers with a larger diameter tend to have a more robust flavor and aroma, while smaller-diameter peppercorns may have a milder flavor. The diameter of black pepper is typically measured by digital venire calipers, as illustrated in Figure 12. Figure 12 shows the value of a diameter bigger than 4 mm that is in standard.

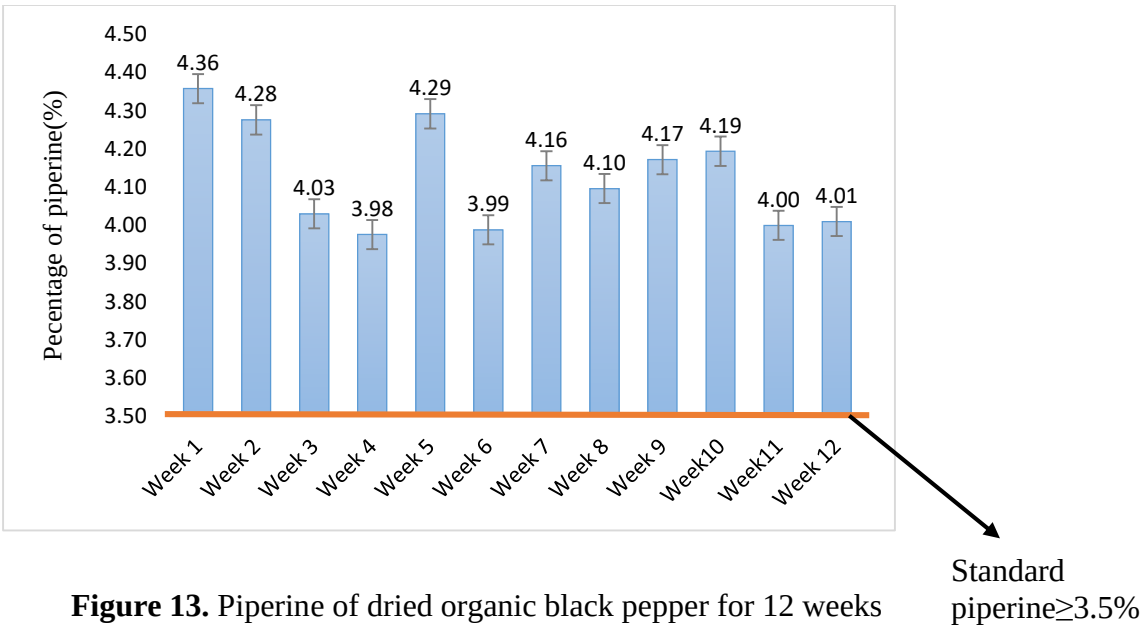


**Figure 13.** Piperine of dried organic black pepper for 12 weeks

At last, piperine is the most important bioactive compound in black pepper, responsible for its characteristic pungent flavor and aroma. Piperine is an alkaloid that has been shown to have numerous health benefits, including anti-inflammatory, antioxidant, and antimicrobial properties. The piperine content of black pepper varies depending on factors such as the variety, growing conditions, place of origin and processing methods (Chopra, Dhingra, Kapoor, & Prasad, 2016). The content of piperine varies from plant to plant belonging to the *Piperaceae* family and varies from 2% to 9% in black pepper (Tiwari, Mahadik, & Gabhe, 2020). The result of piperine also stays in the range of specifications in the company, which is a maximum 3.5%, as illustrated in Figure 13. It has values such as 4.36±0.46, 4.28±0.53, 4.03±0.34, 3.98±0.26, 4.29±0.42, 3.99±0.11, 4.16±0.38, 4.1±0.12, 4.17±0.58, 4.19±0.4, 4±0.18, and 4.01±0.51 following from week 1 to week 12 in orderly.

One more thing: if we look at the results of all the samples, they are not the same; they differ depending on the whole organic black pepper, with some being compact, some larger, and some more spicy. On the other hand, the tested parameters are within the standard and within the range of the guidelines of the standard in Europe market. Therefore, the organic black pepper can be assumed to be good for consumption and does not contain any chemicals that can affect physical health.

# CHAPTER 5
# CONCLUSION

After three months of internship at CONFIREL Co., Ltd., work as production line and quality control on organic black paper product including semi-raw material to finish products. This study is constrained to physicochemical analysis because biological analysis, we do not test in company. Instead, they are tested at PPM and provide only data to the company. In physicochemical analysis we have 4 parameters such as moisture content, density, diameter, and piperine. Organic black pepper processing reduced the amount of metal (stone, Fe), broken pepper and foreign seeds that reduced by grading and selecting process before drying and packaging. The specification of organic black pepper should follow the standard of the company to keep with good quality. After the result shows physical, and biological parameters, we observe that black pepper products at CONFIREL Co., Ltd., from Kompot province after re-processing, the value of all parameters follow the standard of a company if compared to the specification. So, the quality control of finished products at CONFIREL Co., Ltd., on physical are acceptable to eat and export.

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Cambodia Kampot pepper,nd. Intocambodia.com. https://intocambodia.com/learn/cambodia-kampot-pepper/index.html

# APPENDIX

**Appendix A:** Picture of instrument use in production line

Grading pepper machine

Screening pepper machine

Solar room using sun light

Tray Tryer


Electronic balance


Finished product

**Appendix B:** Photos of equipment in laboratory

Hot Plate

Earlenmeyer flask

Blender

Volumetric cylinder


Beaker

Pipette

Magnetic bar

Filter paper

Aluminum plate

**Appendix C:** Results physical testing in organic black pepper for finished products

| Parameters | Moistures (%) | Density(g/l) | Diameter(mm) | Piperine (%) |
|---|---|---|---|---|
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **Week1** | 4.16±0.62 | 592.8±1.3 | 4.65±0.15 | 4.36±0.46 |
| **Week2** | 3.96±0.18 | 577.8±1.3 | 4.46±0.31 | 4.28±0.53 |
| **Week3** | 4.23±0.39 | 588±1.58 | 4.53±0.25 | 4.03±0.41 |
| **Week4** | 3.99±0.07 | 591.2±1.67 | 4.51±0.13 | 3.98±0.26 |
| **Week5** | 4.19±0.21 | 594.6±2.7 | 4.56±0.05 | 4.29±0.42 |
| **Week6** | 3.75±0.2 | 579.6±1.92 | 4.6±0.17 | 3.99±0.52 |
| **Week7** | 4.36±0.41 | 588.8±1.92 | 4.46±0.32 | 4.16±0.38 |
| **Week8** | 3.83±0.19 | 592.8±1.3 | 4.5±0.23 | 4.1±0.6 |
| **Week9** | 3.9±0.17 | 590.2±1.92 | 4.43±0.17 | 4.17±0.58 |
| **Week10** | 4.28±0.4 | 592.8±1.92 | 4.52±0.29 | 4.19±0.4 |
| **Week11** | 3±0.09 | 586.6±1.92 | 4.42±0.15 | 4±0.18 |
| **Week12** | 3.57±0.34 | 585.6±1.14 | 4.54±0.23 | 4.01±0.51 |