Application of Essential Oils to Prolong the Shelf-life of the Pre-cooked Asian Noodle – Hokkien Noodle: A Case Study of the Systematic Design Approach

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Abstract

Asian noodle is one of the traditional staple foods with a long history in the Asian regions. There are several varieties of Asian noodles which are now consumed worldwide by different ethnic groups. One of the popular noodle products commonly found in New Zealand (NZ) is the pre-cooked Asian noodle, such as the Hokkien noodle. Typically, the Hokkien noodle is yellowish with a chewy texture and characteristic taste. The shelf-life of the pre-cooked Hokkien noodle ranges from a few days to less than a fortnight at 4°C, but this depends on the efficiency of packaging and storage conditions as well as the manufacturing environment. The main challenge affecting the shelf-life of pre-cooked noodles is microbial spoilage.

The current study investigated the potential of using essential oils (EOs) to improve the shelf-life of the pre-cooked Hokkien noodles sold in NZ. The study comprised of three stages: the selection of EOs with antimicrobial properties; modelling the ratio of two selected EOs for formulation design; and evaluating the effect of the optimum formulation of the EOs on the storage stability of the Hokkien noodle. In stage one, the broth micro-dilution and the agar disc diffusion methods were used to select potential EOs with high antibacterial and/or antifungal properties. Clove and oregano essential oil showed the best inhibitory effects against fungi and bacteria, respectively.

In stage two, the experimental mixture design was used to determine the optimum combination of the two EOs for developing the final formulations. The designed model included the regulated national limits for the standard plate count (SPC) and yeasts and moulds count (YMC). Overall consumer sensory acceptance of the products was also evaluated by the 9-point hedonic scale. The model predicted that 2.72% of oregano EO combined with 10.91% of clove EO (in the presence of 86.37% soybean oil) could provide 1.72 and 0.9 log CFU/g reductions on bacteria and fungi counts compared with the control sample, respectively. The overall consumer acceptability of EOs-added Hokkien noodle was predicted at 68.03%.

The final phase investigated the shelf stability of the Hokkien noodle treated with the

optimum combination of clove and oregano EOs for 65 days. The prepared noodles were packaged under MAP condition (N_2:CO_2 = 70:30) and storage at 4˚C. Samples of Hokkien noodle treated with the essential oils were analysed for SPC and YMC. Water activity, pH, colour and texture were also measured.

The microbial counts (SPC and YMC) of the experimental samples and the control did not exceed the regulated national limits (6 log CFU/g & 4 log CFU/g, respectively) throughout the experimental period. Control samples contained 4.16 log CFU/g of SPC and 1.96 log CFU/g of YMC by the end of the study. By using the Baranyi-Robert predictive model, the shelf-life of the control samples were estimated to be around 81 days/4°C. For the EO-coated samples, SPC decreased to ≈1 log CFU/g and stabilised until the end of the experiment, while fungi were recorded at <1 log CFU/g. The shelf-life of the EOs-treated Hokkien noodle was calculated to be least 22 days more than the control, thus was achieving over 100-days. It was also shown that additional EOs did not affect other parameters (pH, Aw, colour, texture) of the Hokkien noodle ($p \geq 0.05$). For future studies, using active packaging technology to deliver EOs to the product is recommended, which may increase consumer acceptability.

Keywords: Asian noodle, Shelf-life, Oregano, Clove, Essential oil, Antimicrobial, Mixture Design, Modelling

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Abbreviations and Symbols

AACC	American Association for Clinical Chemistry
ANOVA	Analysis of variance
A_w	Water activity
CFU	Colony forming unit
CIE	*Commission Internationale de I'Eclariage's*
CLSI	Clinical and Laboratory Standards Institute
DFARI	Drug and Food Administration Republic of Indonesia
DOE	Design of Experiment
EO(s)	Essential oil(s)
E-sample	Essential oil coated sample
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization
FSANZ	Food Standards Australia New Zealand
IFST	Institute of Food Science and Technology
ISO	International Organization for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
MAP	Modified atmosphere packaging
MBC	Minimum bactericidal concentration
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimum inhibitory concentration
MPINZ	Ministry for Primary Industries New Zealand
N/A	Not available
NC	Negative control
NOS	Nitric oxide synthases
NZD	New Zealand Dollar
OA%	Overall acceptability percentage
OPP	Oriented polypropylene
PC	Positive control
PP	Polypropylene
PE	Polyethylene
PPO	Polyphenolase
PVdC	Polyvinylidene chloride
ROS	Reactive oxygen species
RPMI 1640	Roswell Park Memorial Institute 1640 media
SPC	Standard Aerobic Count
SPCA	Standard Plate Count Agar
S-sample	Soybean oil coated sample
TEA	Texture extensibility analysis
TFA	Texture firmness analysis
TLTB	The larger the better

TPA	Texture profile analysis
TSB	Tryptic Soy Broth
TSTB	The smaller the better
YMC	Yeasts and Moulds Count
YGCA	Yeast Extract Glucose Chloramphenicol Agar
h_0	Initial physiological state
N	Number of microorganisms
N_0	Number of initial organisms
Sig.	Significant level
ΔBI%	Browning index percentage difference
ΔC	Chroma difference
ΔE	Overall colour difference
λ	Lag phase
μ_{max}	Maximum growth rate
˚C	Degree Celsius
R^2	Determination coefficient

1. Introduction

Asian noodle, along with rice and bread, are the daily staple foods in oriental cultures (Bin, 2007). Asian people have been consuming noodles for thousands of years and now they are popular around the world (Lu et al., 2005). Asian noodles have been predominantly made from wheat, but buckwheat, rice and potato starch are also being used (Gary, 2010). According to the Food and Agriculture Organisation (FAO) Agricultural Statistics Databases (2012), more than twelve percent of wheat was used to produce Asian noodles in 2005 and this number has been increasing in recent years.

According to Euromonitor International (2019), the retail sales value of Asian noodles increased from about 68 million New Zealand Dollar (NZD) to about 76 million NZD from 2013 to 2018, and it is expected to reach about 92 million NZD in 2022. As the demand for commercial Asian noodles increased, one of the main problems that the industry facing is almost all the kinds of Asian noodles have relatively short shelf-life that varies from a few days to less than a fortnight (Guoquan & Mark, 1998). The short shelf-life of the noodle products not only results in a high level of wastage but also limits its sales scope, followed by the potential risk of foodborne poisoning. According to FAO (2018), nearly 300 million tonnes of cereals food were lost as food waste around the world due to food safety problems, which caused nearly US $700 million loss in developed countries and about US $300 million in the developing countries. Meanwhile, nearly 10% of the world population (approximately 700 million people) are suffering from severe "food insecurity", which is defined by FAO as insufficient food supply or undernourished health caused by non-nutritive food. Therefore, it is necessary to find suitable approaches to prolong the shelf-life of food including Asian noodles. The preservation of food will contribute towards the development of the economy and the livelihood of the people.

There are three types of commercial Asian noodles that are found in the New Zealand

market, which are raw, parboiled and fully-cooked noodle, respectively. The noodle products are mainly supplied through the local supermarket and Asian cuisine restaurants. In terms of the pre-cooked (parboiled and fully-cooked) noodles, even though each of them has their own formulation and production process, they share the similar intrinsic parameters which characterised by high water activity, moisture content, and high content carbohydrates. Therefore, these products are susceptible to microbial spoilage. On the other hand, the shelf-life of the noodle products can also be limited by extrinsic parameters, such as poor plant hygiene environment, improper handling, deficient packing or inadequate storage conditions (Jay, Loessner, & Golden, 2005).

To overcome some of the challenges associated with the production of raw and pre-cooked noodles, the (noodles) industry has been investigating the treatments to prolong the shelf-life of the products. It has been explored that Asian noodle products are commonly spoiled by the growth of microorganisms and relative biochemical changes. Lipid oxidation also contributes to the degradation of the products which is mainly induced by light. These changes result in the production of unpleasant odour and/or appearance, thereby lowering the acceptability of the products, as well as raising the food safety concern.

Currently, one of the matured strategies to achieve long shelf-life of pre-cooked Asian noodles is to pasteurise the products at high temperature (95˚C) for a long time (>4 minutes) in combined with acidification and vacuum packaging. Udon is the only Asian noodle that using this strategy which can provide a six-month shelf-life at room temperature (Hou, 2010). However, this method is not suitable for most of the Asian noodles, especially *kan-sui* noodles, since *kan-sui* noodles should have a relatively high pH (9-11) to perform unique sensory properties. Therefore, the acidification process would damage the desired properties of *kan-sui*. For instance, the characteristic yellow colour at alkaline pH would be neutralised by the acid. In addition, vacuum packaging has very limited application in raw noodles and oiled noodle products, since those noodles

easily stick together. The soft, flexible packaging material would be squeezed once vacuum applied, resulting in the severe caking of the products.

Without the long-time high-temperature pasteurisation nor vacuum packaging, one of the most common and economical methods applied in the noodle industry is to add preservatives in pre-cooked noodles to inhibit product quality depletion that is due to the growth of microorganisms. However, with the increasing demand for “green food” or “natural food”, it is urgent for noodles industry to explore new methods that use natural preservatives to prolong the shelf-life of pre-cooked noodles instead of synthetic chemical preservatives, such as sorbate.

In recent years, essential oils extracted from aromatic plants have been studied not only as flavouring agents but also as preservatives (Fisher & Phillips, 2008). Essential oils and other plant metabolite components are considered as natural phytochemicals with antimicrobials effects (Brenes & Roura, 2010). The original ingredients of food along with the small amount of essential oils have bactericidal or bacteriostatic effects on the growth of microbes (Callaway, Carroll, Arthington, Edrington, Anderson, & Ricke, 2011). Hence, essential oils could be good alternatives as natural antimicrobial agents instead of artificial chemicals (Fisher & Phillips, 2008; Solorzano & Miranda, 2012).

In previous studies, essential oils as antimicrobial additives have been studied mostly in raw meat, such as chicken and beef, or fruits and vegetables, but there are no investigations reported in noodle products. Therefore, this project focused on the application of essential oils to extend the shelf-life of a pre-cooked Asian noodle, Hokkien noodle, by applying a systematic design approach.

Aim and objectives of the study

The aim of the project was to use natural preservative (essential oil in this case) to prolong the shelf-life of pre-cooked Asian noodle (Hokkien noodle), from currently 45 days

(labelled) to over 100 days. The specific objectives were to:

A. Investigate the antimicrobial activity of the selected essential oils by minimum inhibitory concentration and inhibitory zone.
B. Select one or two essential oils that have strong antibacterial and antifungal effect for inhibiting the growth of microorganisms on Hokkien noodle.
C. Investigate the best ratio of EOs and soybean oil to achieve long shelf-life and high customers' acceptability.
D. Specify if EOs cause any changes in noodle's characteristics during the storage period compared with the original one.
E. Determine the original shelf-life (tested) of pre-cooked Hokkien noodle.
F. Determine the shelf-life of EOs added pre-cooked Hokkien noodle.

2. Literature Review

2.1. Asian Noodles

Asian noodles have existed for thousands of years (Hatcher, 2001). The cereal products originated from China, then modified by Japan and other Southeast Asia countries. Now Asian noodles have spread all over the world. Adapted to different cultures and cuisines, Asian noodles have been developed in different ingredients and production methods. Asian noodles can be placed into two groups based on their major ingredients. There is regular, salted white noodles, containing 2-8% of sodium chloride added by the weight of the flour used (Bin, 2007). These salted noodles are usually bright white, soft, smooth and less tensile, represented by *Udon*. The other is alkaline yellow noodles, with 0.3-1.5% of sodium carbonate and/or potassium carbonate added in their formulations (Bin, 2007). The alkaline noodles have a particular brown-yellow colour with a relatively firm, elastic and high tensile profile, with a pH range of 9-11, represented by *kan-sui* noodles and Hokkien noodles. When classified by processing methods, Asian noodles can be separated into three different categories: raw, parboiled and fully-cooked noodles. Each of these products has different characteristics, processing procedure and packaging technology, which result in variable shelf-life. To extend the shelf-life of the Asian noodles, it is necessary to study the factors that limit the shelf-life, the processing procedure of different Asian noodles, and packaging technology.

2.1.1. Factors that Affect the Shelf-Life of Asian Noodles

Since the quality of food decreases during storage, food products have shelf-life limits. According to the Institute of Food Science and Technology (IFST) of the United Kingdom (n.d.), the shelf-life of a food product has been defined as:

> "The period between food product manufacture and retail purchase, which the food product has maintained safe, remain a certain level of sensory, chemical, physical, microbiological and functional characteristics, along with any

nutritional functions that have been claimed on the label declaration."

In general, in terms of the shelf-life of noodle products, there are three aspects that should be considered (Hatcher, 2001). The first aspect is the microbiological regulation administrated by government authority. As Asian noodles are not a kind of traditional western foods, in New Zealand there is no national food safety standard for any kind of noodle products regulated by Food Standards Australia New Zealand (FSANZ). However, the Ministry for Primary Industries New Zealand (MPINZ) have provided a microbiological criterion referring to Drug and Food Administration Republic of Indonesia (DFARI). According to that criterion, noodles that undergone heat treatment (pre-cooked noodles) require the standard plate count (SPC) does not excess 10^6 colony formatting unit per gram (CFU/g) sample and total yeasts and moulds number (YMC) does not excess 10^4 CFU/g sample. Secondly, by considering the characteristics of noodles such as colour, Hou et al., (1979) reported that Hokkien noodles should be bright yellow with a little colour change over the storage period. However, the Maillard reaction is inevitable on noodle product (Li et al., 2011), resulting in the browning of noodle strings, thereby losing their bright-yellow colour. Also, the texture requirements of Hokkien noodle are a good bite, chewy, elastic and non-sticky. Considering that the Hokkien noodle requires second-time cooking, the tension of the string should be strong enough to undergo the cooking process to prevent strings from breaking (Karim & Sultan, 2015). Lastly, for the nutritional aspect, the main purpose of consuming Hokkien noodle is to provide energy. The Hokkien noodle consists of the main carbohydrate with little vitamins and minerals, while most of the protein is utilised in gelatinisation, forming a network structure and capturing moisture (Hou, 2010). These network structures are stable and not easily broken (Damodaran, Parkin, & Fennema, 2008). As such, the nutrition loss in Hokkien noodles is negligible. Thus, the shelf-life of the Hokkien noodles are mainly limited by microbial growth.

In terms of the factors that affect the growth of microorganisms, several parameters have

been concluded by James, Martin and David (2005). Those factors that could indicate the microbial environment of the food products can be divided into two categories including 6 intrinsic parameters and 4 extrinsic parameters. The details are shown in the following Table 2-1.

Table 2- 1. The parameters that effecting the microbial environment.

Intrinsic Factors	Extrinsic Factors
pH	Temperature of storage
Water Activity	Relative humidity within package
Oxidation-reduction potential	Presence and concentration of gases
Nutrient content	Presence and activities of other microorganisms
Antimicrobial constituents	
Biological structures	

Within all those factors, extrinsic factors could be controlled by packaging technology and storage condition, which will be discussed later. With regards to intrinsic factors, redox potential, nutrition content, and biological structures are determined by the material of the product. As a result, the pH and water activity (A_w) are two factors that crucially relative to the product's shelf-life.

pH

With regards to pH, it has been concluded that most of the microorganisms have the maximum growth rate at pH around neutral (7.0). Overall, the fungi are more sustainable to pH than bacteria, few bacteria could resist when pH drops below 4.0, details are shown in Figure 2-1.

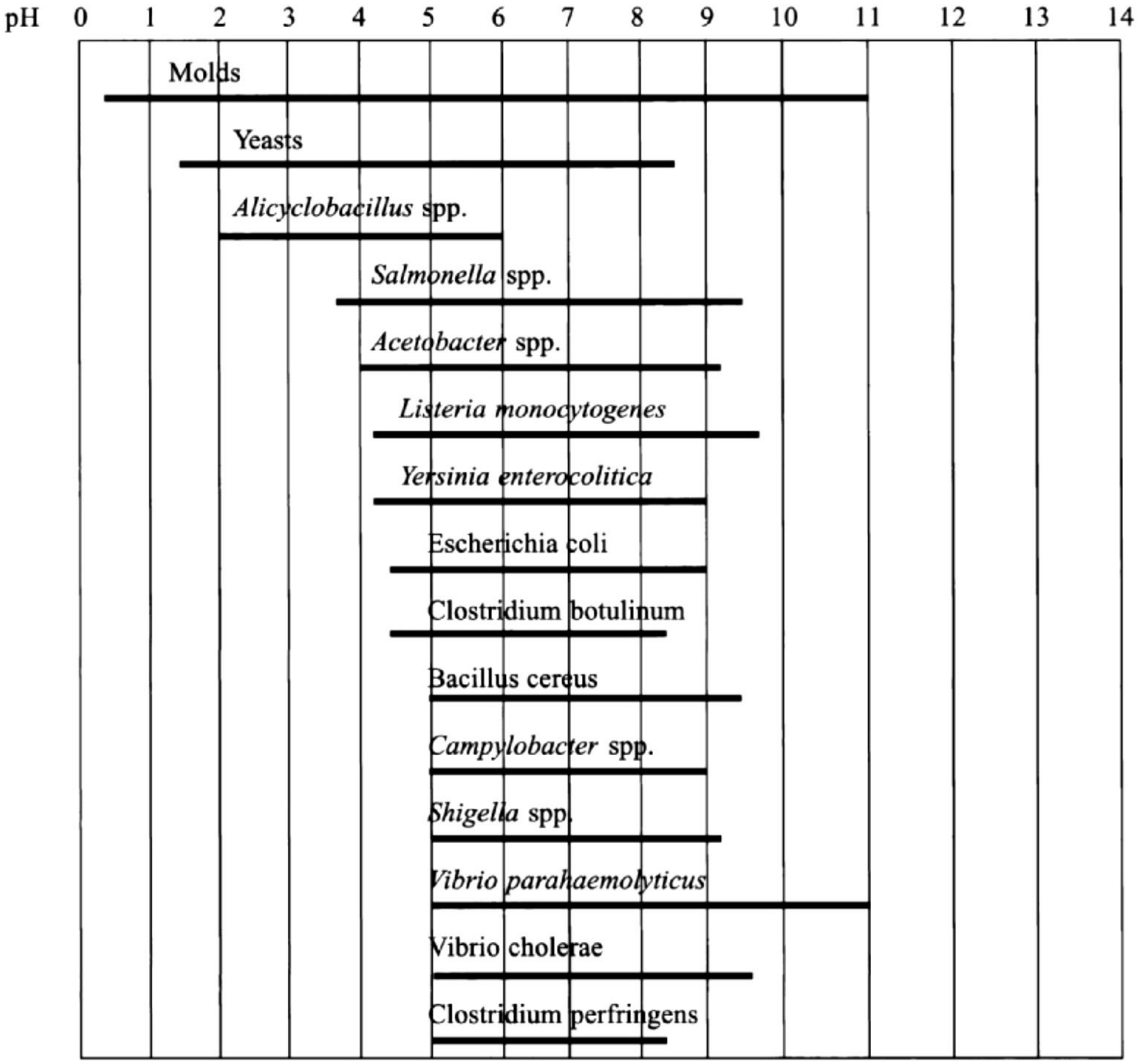

Figure 2- 1. The pH growth ranges for typical foodborne pathogens (Jay, Loessner, & Golden, 2005).

As can be seen, the moulds could grow from extreme acidity (even pH lower than 1) environment to moderate alkaline environment (pH 11), whose ranges are fully cover the growth ranges of both yeasts and bacteria. On the other hand, yeasts are more likely to grow in acidic to neutral pH, whereas most of the bacteria could not grow when pH is lower than 4 except *Alicyclobacillus* spp. As for commercial pre-cooked Hokkien noodle, the pH is adjusted to 3-5 by acidity regulator, where the pH still supports the growth moulds, indicating that extending the shelf-life of pre-cooked Asian noodles by only controlling the pH only is not available. However, pH could significantly affect the growth rate of the microorganisms, exampled by the growth rate of *A. faecalis* incubating from pH 5-9, shown in Figure 2-2.

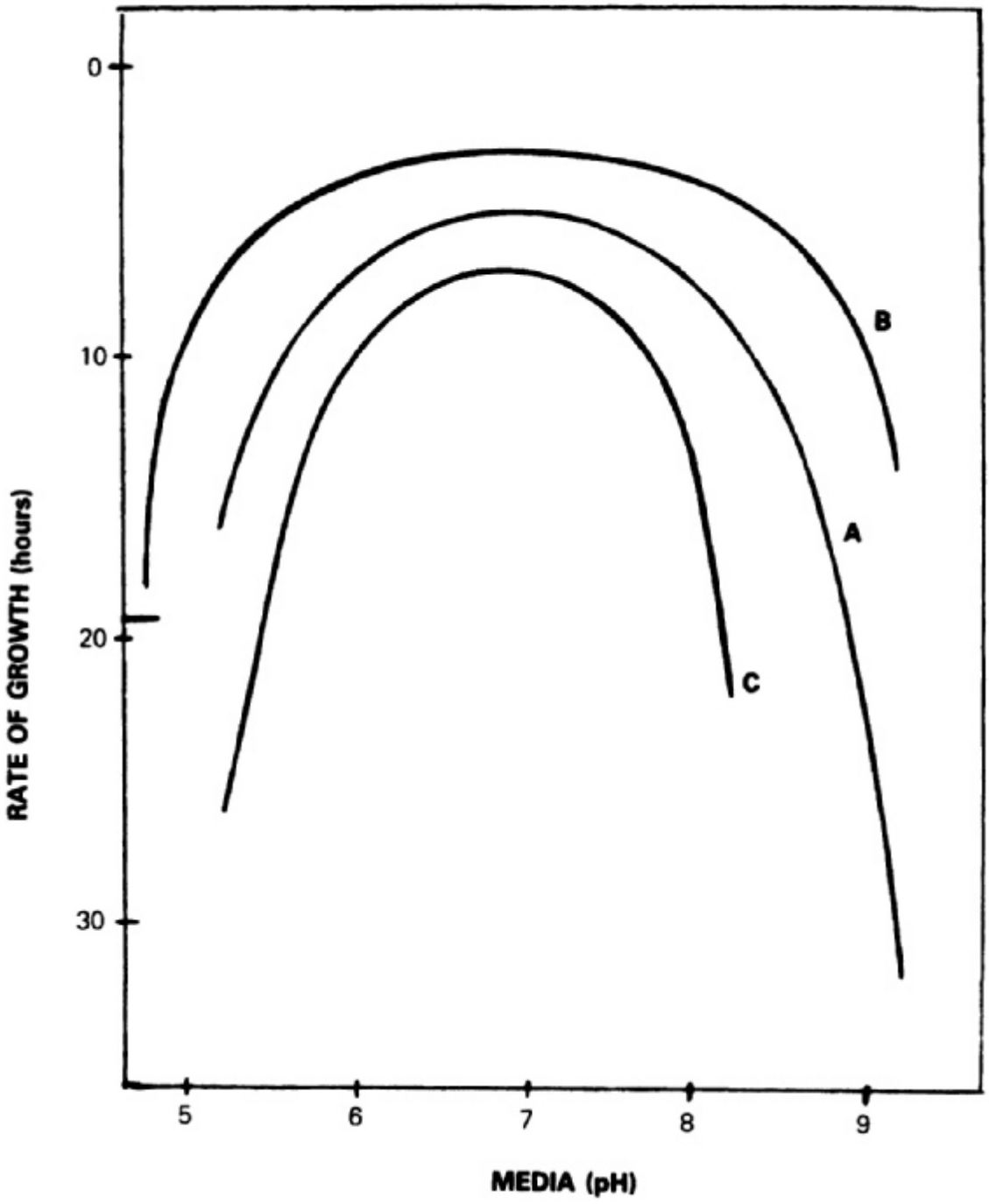

Figure 2- 2. The growth rate of *A. faecalis* in three media of different pH (Jay, Loessner and Golden, 2005).
Note: A: peptone water 1%; B: NaCl 0.2M; C: peptone water 1% & Na citrate 0.2M

As could be seen, the slope of the growth curve alters rapidly indicating that pH could affect the microorganism's growth rate effectively in all media. Base on that, the growth of microorganisms could be retarded by adjusting the pH of the Hokkien noodle, as a

result, the shelf-life could be prolonged.

A_w

Water is essential to the growth of microorganisms (Jay et al, 2005). There are two parameters that describe the water in the food product, which are moisture content and water activity. However, the moisture content is not suitable for indicating the growing environment for microorganisms, since not all the water can be utilised. Therefore, water activity is more appropriate to represent how water supports the growth of microbes on food (Damodaran et al., 2008). Water activity also relative to water potential, which is a measurable chemical parameter in vapour phases. When water potential is the same within the food itself and between food and environment, equilibrium occurs. Yet, when the gradient of water potential occurs, it determines the moisture movement direction, where water tends to move from high water potential (high water activity) area to low water potential (low water activity) area to reach equilibrium and the lowest Gibbs free energy (Damodaran et al., 2008). Therefore, water activity could be an indicator of moisture migration instead of water content.

Basically, there are three states of water presents in a food product (Figure 2-3), high water activity (Region III) supports not only chemical reactions but also the growth of microorganisms. The growth of bacteria required highest water activity, followed by yeast, where moulds have the widest growth range on water activity. The minimum A_w for common foodborne microorganisms were shown in Figure 2-4.

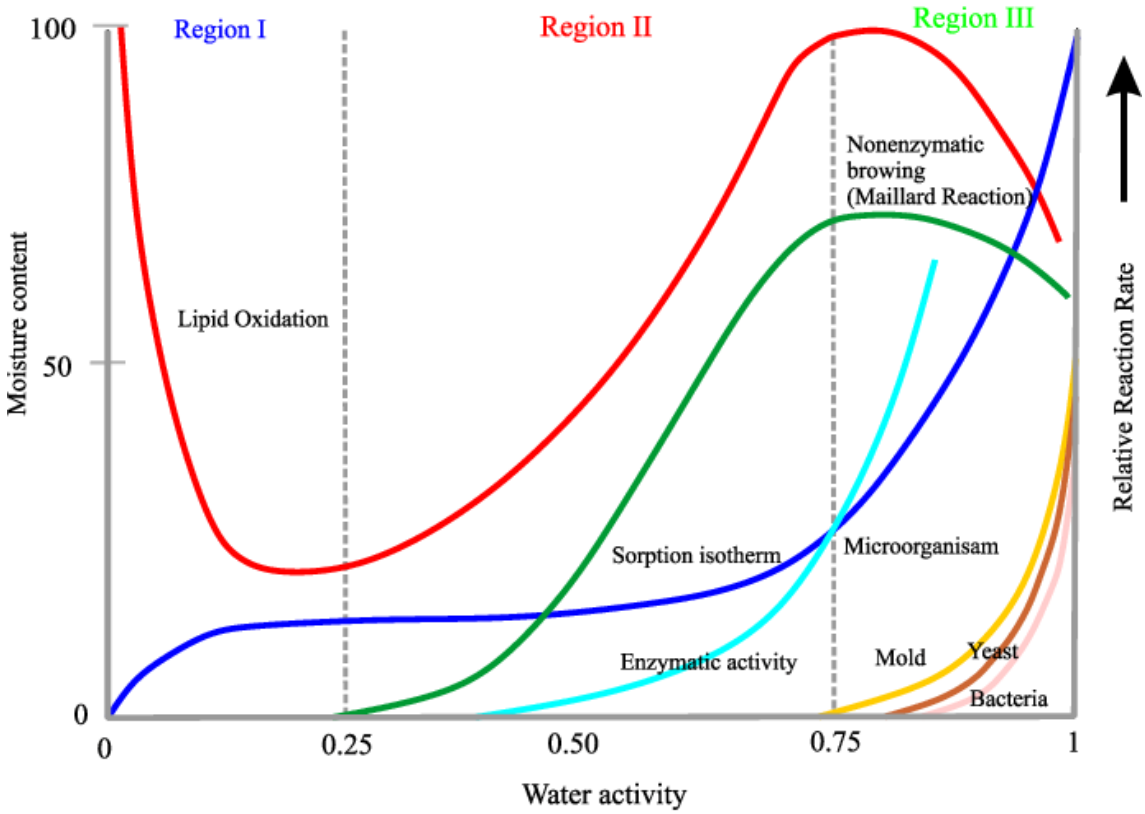

Figure 2- 3. The water state in food product (Labuza, 1972).

Organisms	a_w	*Organisms*	a_w
Groups		**Groups**	
Most spoilage bacteria	0.9	Halophilic bacteria	0.75
Most spoilage yeasts	0.88	Xerophilic molds	0.61
Most spoilage molds	0.80	Osmophilic yeasts	0.61
Specific Organisms		**Specific Organisms**	
Clostridium botulinum, type E	0.97	*Candida scottii*	0.92
Pseudomonas spp.	0.97	*Trichosporon pullulans*	0.91
Acinetobacter spp.	0.96	*Candida zeylanoides*	0.90
Escherichia coli	0.96	*Geotrichum candidum*	ca. 0.9
Enterobacter aerogenes	0.95	*Trichothecium* spp.	ca. 0.90
Bacillus subtilis	0.95	*Byssochlamys nivea*	ca. 0.87
Clostridium botulinum, types A and B	0.94	*Staphylococcus aureus*	0.86
Candida utilis	0.94	*Alternaria citri*	0.84
Vibrio parahaemolyticus	0.94	*Penicillium patulum*	0.81
Botrytis cinerea	0.93	*Eurotium repens*	0.72
Rhizopus stolonifer	0.93	*Aspergillus glaucus**	0.70
Mucor spinosus	0.93	*Aspergillus conicus*	0.70
		Aspergillus echinulatus	0.64
		Zygosaccharomyces rouxii	0.62
		Xeromyces bisporus	0.61

*Perfect stages of the *A. glaucus* group are found in the genus *Eurotium*.

Figure 2- 4. The minimum A_w for the growth of foodborne microorganisms (Jay, Loessner, & Golden, 2005).

In general, bacteria could grow from water activity 0.87 to 1, while the fungi could growth from 0.61 to 1. Lowering the A_w to below 0.90 could effectively inhibit the growth of most of the bacteria. If the A_w of the food product drop below 0.80, there is barely microorganism could grow on it. As a result, the shelf-life could be extended. Li, Zhu, Guo, Peng and Zhou (2011) used glycerol, propylene glycol, compound phosphate and salt as water activity lowering agent, to decrease the water activity of raw noodle from 0.979 to 0.900. And the shelf-life of raw noodle was extended from 2-day to 14-day.

2.1.2. Ingredients and Production of Asian Noodles

To extend the shelf-life of the pre-cooked Asian noodles, three aspects that impact shelf-life of Asian noodles should be considered including the ingredients, processing steps and product characteristics.

Ingredients

In terms of ingredient, using Hokkien noodles as an example in line with the focus of this study. The original ingredients for the Hokkien noodle contain *kan-sui* (which means alkaline solution in Cantonese) that are usually comprised of sodium carbonate and potassium carbonate, sometimes sodium hydroxide is also used. The original purpose of using alkaline salts was to increase the shelf-life of the noodles by limiting mould growth instead of achieving the unique flavour and texture (Fu, 2008). The pH of alkaline noodles ranges from 9-11 depending on the ratio of the salts used and ionic strengths. At this pH range, it could effectively preserve the noodles from microbial growth (Miskelly, 1996). However, Gray (2010) reported that even the alkaline noodles have relatively high pH, it did not achieve the desired long shelf-life due to the high moisture content, and should be consumed within a day if stored at room temperature. Therefore, alkaline salts are mainly used in homemade Hokkien noodle to produce a firm, elastic texture and typical yellow colour (Fu, 2008).

Current ingredients used in noodle industries have been slightly modified from the

traditional formulations, where the alkaline solution has been abandoned to adapt to the mass production and achieve longer shelf-life. The ingredients for Hokkien noodle have been modified by noodle industries, which include wheat flour, modified starch, soybean oil, salt, Tartrazine (E102), potassium sorbate (E202), lactic acid (E270) and citric acid (E330). In terms of the functionality of the ingredients, wheat flour and modified starch are mixed with water to form flour dough, where glutenin and gliadin in wheat flour are formed gluten which creates a strong, elastic network structure to capture water molecules (Damodaran et al., 2008). Also, the modified starch, which contains a high percentage of amylose is responsible for the dough gelatinisation process; intermolecular bonds are broken and form hydrogen bonding when water and heat are engaged (Damodaran et al., 2008). Starch and wheat flour work together to form the base of the noodles. As the absence of alkaline salts, a water-soluble artificial yellow dye, tartrazine (also called brilliant yellow), which brings lemon-yellow colour to food or beverages, is introduced to reproduce the typical colour of Hokkien noodle. Tartrazine is an azo dye which is stable during heating, exposure to lighting and pH variations (Choi & Emerton, 2008). Hence, the pH of the Hokkien noodles products can be adjusted without affecting the typical yellow colour. Moreover, lowering the pH could retard the growth of microorganisms (Hou, 2010), also, the browning reaction on noodles can be mitigated. Potassium sorbate, mainly used as food preservative, shows a strong and wide range of antimicrobial effect against foodborne pathogens, particularly yeasts and moulds, affecting neither the taste nor the flavour (Emerton & Choi, 2008), which is mostly applied in dairy products, meat products and fungistatic packing material (Arvanitoyannis & Sun, 2012).

Furthermore, lactic acid and citric acid are used as pH regulators to lower the pH of products. Both are weak acids and can reach a dynamic equilibrium when they are hydrolysed in water. The carboxylic functional group (R-COOH) is partially hydrolysed in $R\text{-}COO^-$ and H^+ to reduce pH. The ionization constant for lactic acid and citric acid are 3.86 and 3.31 respectively, giving a buffer solution with pH ranges from 3-4.6 depending on the temperature of solutions (Damodaran et al., 2008).

Processing Procedure

Although the process of noodle production varies from products to products, when comparing with fresh raw noodles processing (Figure 2-5, left), the parboiled noodles like Hokkien noodles (Figure 2-5, right), have similar production procedures until the (noodle) cutting step. According to Gary (2010), the fresh raw noodles have very limited shelf-life, which normally is no more than a few days depending on the packaging and the storage conditions. On the contrary, the pre-cooked noodles have relatively longer shelf-life than raw noodles, since the microorganisms and enzymes are inactivated during the boiling stage.

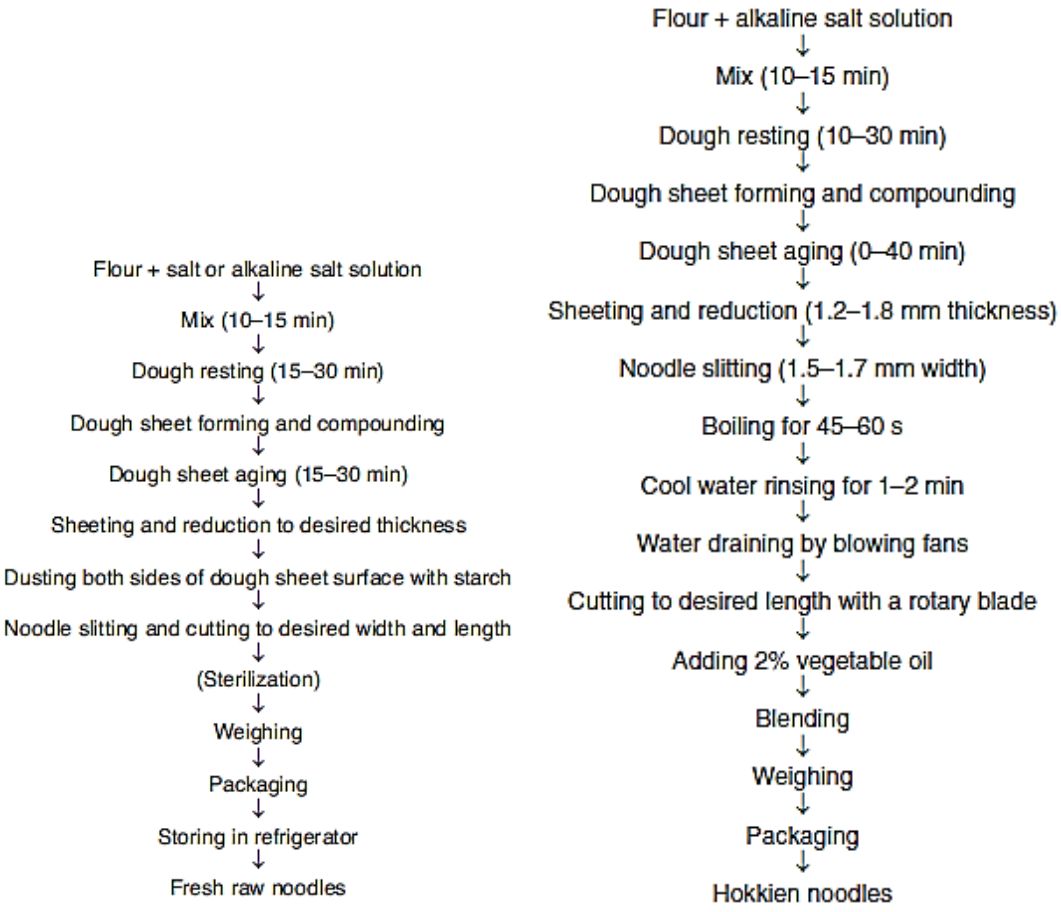

Figure 2- 5. The production process of raw noodles (left) and parboiled Hokkien noodle (right) (Gary, 2010).

For Hokkien noodle (Figure 2-5 right), the cooking time should be strictly controlled

within 45-60 seconds depending on the thickness and width of the strips to achieve 70-80% of the desired gelatinisation of the starch. A white core could be visible in the centre of the noodle strings. This is because the core of the noodles remains raw and the starch gelatinisation is not complete. Following cooking, the noodle strings are immediately steeped into cold water to remove the heat and prevent further gelatinisation. Then the excess water on the surface of the noodles is removed by blowing air. After that, the noodles are cut into desired lengths (some plants could cut the noodles before boiling) before coating with 1-2% of vegetable oil to prevent sticking together, followed by packaging, storage and dispatch.

The processing steps of boiling and steeping are mostly responsible for the increase of the water activity of pre-cooked noodles. Even though the moisture could migrate from the surface to the centre of the noodles, the water activity of the surface of the noodles is still very high (around 0.99), combined with a high content of carbohydrate, the pre-cooked noodles are suitable for the growth of microorganisms (Gary, 2010). In terms of food safety, the cooling processes (cold water bath and air fans) can be considered as high-risk steps that cause contamination, if the cold water or the air fans are not clean enough. Consequently, this results in an increase in the initial microorganism population of the final product (Gary, 2010).

As shown in Figure 2-6, the process of producing standard fully-cooked noodles share the same processing steps with the parboiled noodles until the boiling step. To achieve a stable long shelf-life, the fully-cooked noodles are not only required a longer boiling time (10-15 minutes for *Udon* noodles), but also needed acidification to adjust the pH to 4-5 and steam-pasteurisation of more than half-hour at 95-98˚C. The acidification during washing and cooling steps aims to limit the growth of the heat-resistance microorganisms, as a lower pH of the noodle would provide better inhibition results. However, as reported by Gary (2010), the product flavour might be affected if the pH is lower than 5. Also, some of the acidulants, like acetic acid, have excellent preservation effect but have a

strong odour (Shiau and Yeh, 2001).

Although sterilised fully-cooked noodles could achieve more than 6 months of shelf-life at room temperature, it is inevitable that their quality tends to degrade as the storage time increases. That is because the noodles are treated with acid and severe pasteurisation (high-temperature and long-time), which result in deteriorated texture and odour of the products (Shiau and Yeh, 2001). Furthermore, these processes are not suitable for producing *chukamen* noodles nor *kan-sui* noodles, as these noodles are critical to maintain the relatively firm, elastic texture, the pasteurisation cause to severe overcooked and results in product rejected by customers.

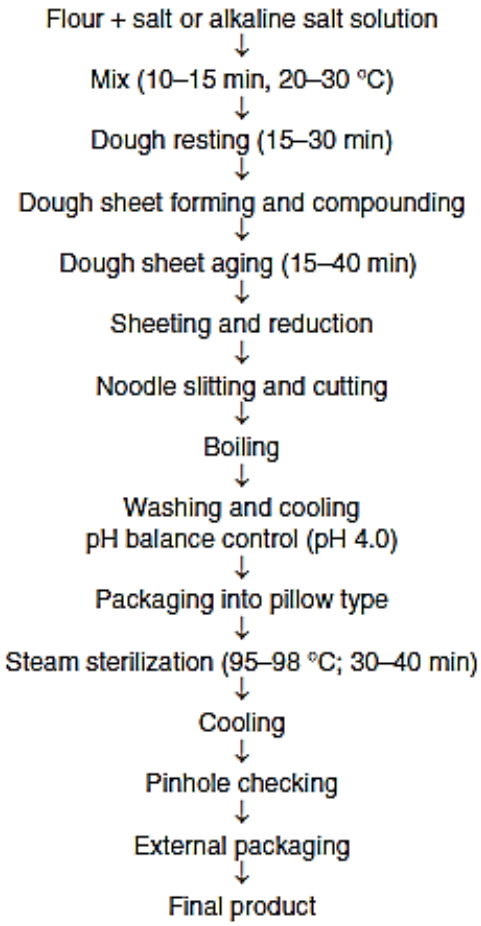

Figure 2- 6. Processing of standard of fully boiled noodles (Gary, 2010).

Except for high-temperature long-time pasteurisation, some other processing procedures

have been investigated to achieve longer shelf-life. Li et al. (2011) used 4kGy of radiation to extend the shelf-life of raw noodle from 2-day to over 16-day. Maria, Layal, Mohamed, Abiad and Hany (2017) dipped the Phyllo (a dough-based wheat flour product) into chitosan and natamycin, which doubled the shelf-life of Phyllo, from originally 5-day to 10-day. Bai, Guo, Zhu and Zhou (2017) used the aqueous ozone water to replace the distilled water for producing buckwheat noodle, which reduced 1.8 log CFU/g of the initial standard plate count number (day 0).

2.1.3. Packaging Technologies for Asian noodles

Except for the ingredients and production process, packaging materials and/or storage condition are the alternative approach to extend the shelf-life of the product. According to Ling (2010), there are five functions of the packaging of the noodles, which include containing the noodles, protecting the noodles, providing convenience, traceability and enhancing marketability. In this study, the packaging functionality of protecting the noodles and extending its shelf-life is focused.

According to Troller and Christian (1978), there are three most important properties affecting the shelf-life of the noodles after packaging, which include water activity, pH and fat content. The water activity and pH determine the type of microorganisms that can grow on the noodles (Hocking and Christian, 1995), whereas the fat content directly affects the rancidity and off-flavours caused by lipid oxidation. Hence, the packaging technology should properly control or maintain these three properties of pre-cooked noodles, along with proper storage condition to achieve the maximum shelf-life and minimum deterioration.

The water activities of several commercial noodles have been reported by Ling (2010). Pre-cooked noodles have the highest water activity compared with dried noodles or instant noodles. The average water activity of pre-cooked noodles is 0.998, which means the pre-cooked noodles can support the growth of most microorganisms in water activity

aspect (Beuchat, 1981). These results are agreed with a study by Gary (2010) who concluded that pre-cooked noodles have relatively high water activity and limited shelf-life since the boiling and rinsing steps significantly increase the water activity of the pre-cooked noodles. Nonetheless, the packaging of the noodles aims to maintain the moisture instead of lowering it, because the moisture is an important quality indicator of pre-cooked Asian noodles. When moisture migrates from the noodle surface to the headspace of the package, then penetrating through package bag and releasing in air, noodle tends to dry up, stiff and break down (Okafor & Omodamiro, 2006). On the contrary, if the packaging material has relatively low water permeability, the moisture equilibrium occurs between noodles surface and headspace of the package, then moisture can be retained.

According to Miskelly and Gore (1991), the pH for white salted noodles is around neutral (6.5-7.0), while the *kan-sui* salts noodles could reach pH 9-11 depending on the composition and the amount of the (*kan-sui*) salts. For the *kan-sui* noodle, if the pH can be maintained, the microorganisms are not likely to outbreak during the storage period. However, carbon dioxide can react with free water within the product to form carbonic acid, which lowers the pH of alkaline noodles (Okafor & Omodamiro, 2006). As the pH drops to near neutral, the multiplication of microorganisms could be accelerated, along with the biochemical process, then the pre-cooked noodles would become unacceptable (Ling, 2010). Hence, the package should resist the carbon dioxide penetration for any alkaline food product.

Ling (2010) reported that parboiled, oiled noodles have an average fat content of 5%, which is higher than dried noodles but much lower than instant noodles. Pre-cooked noodles have higher fat content than dried noodles due to the oiling process applied to prevent the noodles from sticking together. However, the oil basically forms a layer on the surface of the noodles, which means if the packaging condition fails to separate the product from oxygen, the coating oil would directly react with the oxygen from the air, leading to rancidity and off-flavour thereby reducing the shelf-life of the noodles before

microbial spoilage steps in. Hence, removing oxygen from the product packaging container is recommended for pre-cooked noodles coated with oil. Vacuum packaging, nitrogen flushing, and oxygen scavengers are the three most common strategies used to eliminate oxygen from noodles packaging. Furthermore, low light transmission package is another approach to limit the lipid oxidative on noodle product. Due to free radical could easily be excited by light, the alkyl group of unsaturated lipid acid tend to react with oxygen, results in an unpleasant flavour in the product.

In industry, the noodles are packaged in paper-based, polymers or metallic containers. According to Ling (2010), metal-based packaging is the most suitable material, due to its ability to block any light from activating lipid oxidation and prevent browning. Also, metal has great barrier properties that prevent oxygen, water vapour or any other gas passing through the package. However, metal is not the most common material used in noodle industries, since Asian noodles are usually not sold at a high price. Therefore, the metal package results in an increased selling price and lost competitiveness. Instead, polymers seem to be the best choice for the noodle industries, not only for the price, but also the lightweight, flexibility, easy sealing and providing the possibility of vacuum packaging (Hanlon et al., 1998). Nevertheless, the negative aspects of polymers are obvious, which include relatively high permeability of water vapour, air and light (Parry, 1993). A single layer of the polymer is barely suitable for Asian pre-cooked noodles packaging to achieve long shelf-life due to its high permeability of oxygen and water steam, but multiple layers of polymers along with aluminium foil layer and laminating technologies would largely improve the properties of polymers. This kind of multiple layered-polymers has excellent abilities for blocking gas, water and light as well as the heat-bonding property that allows the package to be sealed in a short time (Parry, 1993). The noodle packing bag is made by laminating of PE (polyethylene), PP (polypropylene) and OPP (oriented polypropylene) polymers.

Except for vacuum packaging, some novel packaging technologies have been introduced

to noodle industries. One of the new packaging technologies is active antimicrobial packaging, where the antimicrobial agents are previously added to the packaging materials, and then are released to the headspace inside the package after sealing. Three types of the antimicrobial agents have been introduced in active packaging, which includes chemical antimicrobial agents such as acetic acid and sorbic acid, neutral antimicrobial agents such as extractive of herbs or spices, nisin, and lastly, probiotics (Han, 2005). In addition, some natural antimicrobial agents may be also used as antioxidative agents, such as essential oils (Ruberto & Baratta, 2000). The main components of essential oil such as monoterpene hydrocarbons can effectively react with oxygen to prevent oxidation of the noodles. Still, there are some obstacles to applying active packaging together with antimicrobial agents, such as strong odour that might affect customer acceptability, and the high cost of the packaging materials (Ioannis, 2012).

Alternatively, modified atmosphere packaging (MAP) is another approach to extend the shelf-life of noodle products. There are two types of packaging processing for modified atmosphere packaging in noodle industries, one is the vacuum-flush heat seal process, another is overload down-flush heat seal process (Ling, 2010). For vacuum-flush processing, the noodle is dropped to the packaging bag, then the air is sucked out before the modified gases are flushed into the package to designed pressure. The bright side of the vacuum-flush processing are: i) oxygen could be furthest expelled from package, ii) the usage of modified gas is controlled, iii) high pressure could be achieved if necessary. However, the dark side is also obvious, i) noodle is squeezed during vacuuming, which causes noodle caking, ii) relatively required longer processing time. Alternatively, over-flush packaging requires overloaded modified gases to flush the package after noodle is loaded. The air in the packaging bag is replaced by the mixed gases because of the density difference. Keeping the gas flowing when the packaging bag is heat-sealed. The advantages of this process are i) avoided squeezing, ii) quick process. Nevertheless, the oxygen level might different within packages, also, only natural pressure can be achieved.

A previous study conducted by Zardetto (2005) reported that the shelf-life of fresh pasta was less than 3 days when packaged by 100% of nitrogen, extended to 11 days when 50% of nitrogen and 50% of carbon dioxide was applied, and peaking at 100% of carbon dioxide where the shelf-life could achieve 61 days. Also, a study performed by Bai et al. (2017) stated that the buckwheat noodles could achieve the longest shelf-life when preserved in 70/30 CO_2/N_2 modified atmosphere packaging. The shelf-life was prolonged from 3-day to 9-day.

2.2. Essential Oils

The use of essential oils (EOs) in food as an additive has occurred for centuries, not as antimicrobial agents but flavouring agents (Burt, 2004). Most of the essential oils contain small and volatile compounds which bring the unique flavour of their original plant to the food, with a pleasant odour and desirable taste. In recent years, the demand to reduce the use of synthetic chemicals as antimicrobial agents have increased, since more foodborne microorganisms have developed resistant ability against synthetic chemicals. Also, carcinogenicity, toxicity and teratogenicity of the synthetic chemicals have generated negative responses by customers (Faleiro, 2011). Thus, natural antimicrobial agents have drawn the attention of the food industry, imposing pressure on the industry to search for natural materials or compounds against foodborne pathogens, without or low negative impacts (Fisher & Phillips, 2008).

Essential oils have been found as alternative antimicrobial agents (Burt, 2004). Among over 3000 known essential oils, about one-tenth of these are commercially effective in flavours and fragrances field (Burt & Reinders, 2003). The essential oils studied for antimicrobial activity in food industries are only selected from herbs and spices that are commonly used in foods (Cueva et al., 2010), since they do not raise any public safety concern.

2.2.1. Characteristics of Essential Oils

Extracted from aromatic plants, essential oils are not pure substances but a group of hydrophobic volatile compounds with good solubility in ethanol or propylene glycol and low molecules weight (Burt, 2004). The composition of EOs can be determined by the particular plant and the parts of the plant used. Flowers, leaves, fruits and even the roots of plants can provide different amounts and various chemical compositions of EOs (Novak, Draxler, Gohler, & Franz, 2005). For most of the plants, high levels of mono-terpenes can be found in EOs extracted from flowers, while low levels are extracted from leaves or roots.

There are many different types of chemicals in EOs, most of them are terpenes/terpenoids $(C_5H_8)_n$, alcohols (-OH), acids (-COOH), esters (-COOC-), aldehydes (-CHO) and other small amounts of components (Bakkali, Averbeck, & Idaomar, 2008). Based on the properties of the components in EOs, they can be classified into two groups: bioactive compounds and aroma compounds (Pichersky, Noel, & Duareva, 2006; Bakkali et al., 2008). The antimicrobial activity of EOs only corresponds with the content of their bioactive compounds (Mahmoud & Croteau, 2002). Another opinion was raised by Bakkali, Averbeck and Idaomar (2008), they believed the antimicrobial characteristics of the EO are determined by the types and amount of the volatile components, including terpenes, alcohols, esters and epoxides. However, Bajpai, Beak and Kang (2012) studied the antimicrobial effect of terpene compounds extracted from aromatic plants, none of them could achieve the antimicrobial activity as the whole EO did at the same dosage, indicating that the aroma compounds somehow have synergic effect with the terpene attributing to the antimicrobial activity.

Badi, Yazdani, Ali and Nazari (2004) reported that the content of EOs is mainly influenced by the harvesting time. Plants harvested at the initial blooming stage can achieve higher EO yield than at any other stages. Also, the yield and chemical

composition of the EOs is affected by the drying methods as well as the distillation methods (Fathi & Sefidkon, 2012). The combination of shade-drying along with hydro-distillation provides the highest EOs yield. After distillation, the hydrophobic mixture (upper layer) called essential oil, the hydrophilic mixture (aqueous phase) called hydrolat. The planting conditions, harvesting time and processing methods (drying and distillation) together affect the yield and the proportion of each component of EOs (Burt, 2004).

2.2.2. Antimicrobial Mechanisms of Essential Oils

On the whole, the mechanisms of the EOs against microorganisms are not fully understood. However, the basic antimicrobial ability of EOs is attributed to their hydrophobic and low molecular weight properties, which allow them to penetrate through the bacterial/fungal membranes and disrupt the membranes and cell organelle functions (Fisher & Phillips, 2009), leaking the internal contents out of the cell (Bajpai et al., 2012), resulting in cell death (Friedly, Crandall, Ricke, Roman, O'Bryan, & Chalova, 2009). In general, four main mechanisms of the anti-fungal activity of EOs have been recognised: (i) cell membrane disruption, (ii) mitochondria dysfunction, (iii) H^+-ATPase inhibition and (iv) reduction of nitric oxide synthases (NOS) level.

Cell membrane disruption

Chitin is one of the most indispensable elements in the construction of the fungal cell membrane. However, chitin, along with glucan and mannan, are the main therapeutic targets for essential oils. EOs can inhibit the polymerisation of chitin during cell division, leading to the disruption of the cell membrane, septum and bud ring. Thus, the multiplication of microorganisms is inhibited (Wu, Cheng, Sun, & Lou, 2008). Besides, the components from some essential oils (like Tea-tree EO) have the ability to alter permeability and/or fluidity of the microbial cell membrane, as well as leading a thinning and distortion hyphal wall. As a result, flatten and empty hyphal tips indicate the death of the cell (Hammer, Carson, & Riley, 2004). Moreover, some EOs like *Litsea cubeba* can damage the microbial cell membrane, resulting in the leakage of the materials used for

the biosynthesis of DNA, RNA or protein from the cell, sometimes even leading cytoplasm leakage (Hammer et al., 2004). The synthesis of ergosterol is also inhibited by EOs (Kerekes et al., 2013). The absence of ergosterol in microbial cell membranes can cause osmotic disorder and the malfunction of metabolic activity, and accelerate the death of the cell (Rajput & Karuppayil, 2013).

Mitochondria dysfunction

Some EOs have shown an ability to limit the mitochondrial effectiveness via decreasing the dehydrogenases activity in mitochondria, which results in breaking the ATP synthesis cycle and limiting the energy supply in the cell (Chen et al., 2013). The essential oil extracted from *Anethum graveolens* is one of the typical EOs that can cause dysfunction of mitochondria in microorganisms. Furthermore, terpenoids in essential oils can significantly diminish the amount of mitochondrial, which further limit ATP-generation in the cell (Haque et al; 2016).

H^+-ATPase inhibition Effect

H^+-ATPase plays an important role in maintaining regular transmembrane activity, such as regulating the electrochemical proton gradient, supporting the large molecules crossing the cell membrane, and keeping the intracellular pH within a normal level (Set-Young, Monk, Mason, & Perlin, 1997). Also, the antimicrobial activity of eugenol and thymol might partially relate to their ability on inhibiting the H^+-ATPase activity, which caused acidification within the microbial cell and eventually, cell death (Ahamd, Khan, & Manzoor, 2013). When associated with the azole, thymol exhibited an inhibition on efflux-pump, which significantly prevented the elimination of azole by efflux-pump, as a result, the effect of azole on antimycotic could be increased (Ahmad et al., 2013).

Nitric Oxide Synthases (NOS) level reduction effect

Produced by bacterial nitric oxide synthases (NOS), intracellular nitric oxide (NO, with strong reducibility) can protect bacteria from very wide range of antibiotics, allowing

microorganisms to resist the chemical toxic compounds (strong oxidability) from antibiotics, thus, the effectiveness of antibiotics can be enhanced by limiting the NOS activity (Belenky & Collins, 2011). EOs can restrain the NO level by reducing the NOS activity and limit the generation of H_2O_2 by producing the reactive oxygen species (ROS) (Cotoras et al, 2013). The synergy of these functions provided by EOs can cause oxidative damage to microorganisms. Thymol, one of the bioactive compounds in thyme essential oil can effectively eliminate the growth of *Aspergillus* spp. via causing oxidative damage by generating the relatively high level of ROS (Shen et al., 2016).

Antibacterial effect

In terms of the antibacterial characteristics of EOs, these compounds a similar pattern with their antifungal mechanism on the bacterial cell membrane. Rather than target the chitin, the lipophilic property of EOs allows them to isolate the lipids from the bacterial cell membrane, resulting in a high permeability of the membrane (Burt, 2004; Friedly et al., 2009). In general, bacteria can be divided into two groups: (i) Gram-positive bacteria with a high level of peptidoglycan in the cell wall and, (ii) Gram-negative bacteria with a high level of lipid in the cell wall. Thus, it was considered that Gram-positive bacteria are more susceptible to EOs because the protection from cell wall is far weaker than Gram-negative bacteria (Smith-Palmer et al., 1998; Cimanga et al., 2002; Sokovic et al., 2010). Whereas, Gram-negative bacteria are believed to be more resistant to EOs since their cell wall are hydrophilic (Kim et al., 2011), and help the bacteria to reduce the penetrating force from the lipophilic compounds (Clasamiglia et al., 2007; Ravichandran et al., 2011). However, there are some compounds in EOs that show unexpected ability against Gram-negative bacteria, such as carvacrol and thymol. A report by Dorman and Deans (2000), showed that carvacrol and thymol had high antibacterial effectiveness against Gram-negative bacteria. Further results showed that those compounds caused the decomposition of the outer cell membrane and increased cell membrane permeability (Burt, 2004).

2.2.3. Antimicrobial Effectiveness of Essential Oils

Every microorganism has different susceptibility to different essential oils, but several essential oils have shown strong effectiveness against a broad spectrum of foodborne pathogens. Friedman, Henika and Mandrell (2002) tested hundreds of EOs against 4 most common bacterial genera in food. The results found that *Campylobacter jejuni* was the most sensitive to the essential oils extracted from ginger root, jasmine, celery and orange. Oregano, thyme, bay leaf, clove and allspice oils showed high activity against *Escherichia coli*, while *Listeria monocytogenes* and *Salmonella enterica* was susceptible to oregano, thyme, bay leaf, clove and allspice essential oils. This conclusion was consistent with similar research conducted by Moreira, Ponce, de Valle and Roura (2005), which also confirmed clove essential oil could significantly limit the growth of *E. coli*. In addition, Singh, Marimuthu, Murali and Bawa (2005) found that *Bacillus* spp. was highly susceptible to black pepper oil. Further research by Karsha and Lakshmi (2010) indicated that the minimal inhibitory concentration (MIC) of black pepper oil against *Bacillus* spp. was 250 ppm. Overall, oregano, thyme, and clove essential oils have remarkable results on inhibiting broad-spectrum of the foodborne bacteria, while bay leaf, allspice and black pepper oil showed outstanding results on inhibiting specific bacteria.

Tatjana et al. (2014) investigated several essential oils against the most common fungi present in the air, which is one of the most common sources contaminating the unpackaged food. Oregano, thyme and savory EOs had relatively low MICs on a wide-range of fungi ranging from 0.62-0.14 mg/mL for savory, 0.28-0.07 mg/mL for oregano and 1.18-0.14 mg/mL for thyme, respectively. It is apparent that these essential oils showed broad-spectrum antifungal activity. However, *Fusarium subglutinans* was found to be the most resistant fungus to all EOs tested. Oregano, thyme and savory EOs not only have a great inhibitory action on bacteria but can also suppress the growth of fungi. The explanation for this phenomenon might be the antibacterial mechanisms of EO are similar to their antifungal mechanisms. For a food product that is susceptible to fungal and

bacterial contamination, broad-spectrum inhibition EOs should be considered.

To understand why savory, oregano and thyme have a relatively strong antimicrobial effect, the composition of those EOs were analysed (Carmo et al., 2008; Gallucci et al., 2014). The main compound for thyme EO is thymol (43.7%), while savory and oregano EOs contain 50% and 75.8% of carvacrol, respectively. These phenolic compounds are mainly responsible for their strong antifungal ability. EOs with high antimicrobial effectiveness contain relatively high phenolics (thyme 73%; savory 78%; oregano 84%). Sokovic et al. (2010) reported similar findings on EOs from common herbs used in food. Oregano oil from *Origanum vulgare* showed the best results against most of the microorganisms. The bioactive substances in oregano EO were over 75% (w/w, bioactive substances/whole EO). The results could further explain why savory, thyme and oregano have such an outstanding antimicrobial activity. A higher percentage of bioactivity compounds leads to better results of inhibition of fungi and bacteria.

Clove and eucalyptus EOs were studied by Suman, Stuti, James, Apekshita and Anjana (2014) against two Gram-negative bacteria (*Sphingobium indicum*, *Escherichia coli*), and two Gram-positive bacteria, (*Staphylococcus aureus*, *Bacillus subtilis*) using the disc diffusion method. The results showed that clove essential oil had a larger inhibition area across all tested bacteria than eucalyptus essential oil. The eucalyptus EO failed to inhibit the growth of Gram-positive bacteria (*S. aureus* and *B. subtilis*) with no inhibitory zone at all, while showing a minute antibacterial effect against *S. indicum* and *E. coli*. In contrast, clove EO showed relatively strong antibacterial effect with lager inhibition area on both Gram-positive and Gram-negative bacteria. Overall, Gram-positive bacteria were more susceptible to clove EO than Gram-negative bacteria, where *B. subtilis* was the most susceptible (7.543 cm^2) and *E. coli* was the most resistant (5.144 cm^2). Omidbeygi, Barzegar, Hamidi and Naghdibadi (2007) used both laboratory media (Sabouraud Dextrose Broth) and food matrix (tomato paste) to test the antifungal activity of savory and clove EOs. Savory EO completely inhibited (100%) the survival of *A. flavus* while

clove essential oil (500 ppm) only inhibited 87.5% at the same dosage. When in food matrix media, about 59% of *A. flavus* was inhibited by 500 ppm of savory EO, while 48% for clove oil. It may be concluded that savory EO was more effective on inhibiting the *A. flavus* than clove EO. Also, the antifungal activity of EOs was limited when applying EOs in real food. To achieve a similar inhibitory effect to the laboratory test, the dosage of EOs should be increased during food production. The possible reason could be related to the complexity of the food matrix than laboratory media, where certain chemical components of the paste might raise a protective effect on the pathogens.

Generally, foodborne pathogens can barely generate resistance against two different essential oils with multiple components (Tatjana et al., 2014; Filomena, Florinda, Raffaele, & Vincenzo, 2017). Thus, the use of the combination of two or more essential oils can show a significant advantage in inhibiting the growth of microorganisms, with a low concentration of each EO used. It is beneficial to use of a combination of EOs to extend the shelf-life of food product to minimize the impact on product odour. Typically, the minimum bactericidal concentration (MBC) of thyme oil and oregano oil against *L. innocua* are 250 and 150 ppm, respectively (Garcia, Lopez, & Palou, 2011), however, it only requires 137 ppm of mixed oil (62 from thyme and 75 from oregano) to completely limit the growth of *L. innocua*. Besides, some synergistic effects of two essential oils have been found against specific pathogens, the combination of rose & lavender oil have a synergistic effect against *F. subglutinans*, *F. equiseti* and *F. sporotrichioides*, while the combination of thyme and oregano oil has a synergistic effect against *A. flavus, F. solani, F. semitectum and Penicillium* spp. (Tatjana et al., 2014). In general, the synergistic effect of thyme & oregano oils (or thymol & carvacrol) is the most outstanding combination against the widest-range of bacteria and fungi (Gutierrez, Barry-Ryan, & Bourke, 2009; Tatjana et al., 2014; Thanissery & Smith, 2014). It is predictable that lower MIC/MBC can be obtained by two or more combined EOs against specific microorganism on a food product. Using low EOs dosage can achieve longer shelf-life of food but also have better customer acceptability.

The synergistic antimicrobial effect of organic acids and EOs have been reported by Friedly et al. (2009). Citrus EOs is more effective against Gram-positive bacteria in the presence of organic acids, especially *Listeria* spp. Only one-tenth of the EOs dosage would be required to inhibit the growth of *Listeria* spp. when combining with citric or ascorbic acid, compared with using EOs alone. A similar study by Zhou et al. (2007) reported that *S. typhimurium* was inhibited by acetic acid or citric acid with a relatively low concentration of thyme or oregano oils. Organic acids and EOs have some overlap on antimicrobial mechanisms, such as disordering the intracellular pH, altering the permeability of cell membrane, or increasing the osmotic stress around the cell (Russell, 1992; Ricke, 2003). Thus, the two types of compounds (EOs and organic acids) have synergistic antimicrobial effects. The synergistic antimicrobial effect is commonly found between traditional chemical food preservative (such as organic acid, trisodium phosphate) and one EO, rather than a combination of two or more EOs (Juliany, Philip, Corliss, & Steven, 2015). However, a product containing chemical preservative can be not classified as "natural preservation food", which can lead to the loss of potential customers who prefer natural food.

2.2.4. Essential Oils in Food Systems

Many factors in a real food product can affect the antimicrobial activity of essential oils, from the food composition (protein, carbohydrate, fat, salt, water) to physical parameters (pH, water activity, moisture) (Burt, 2004; Friedly et al., 2009). Thus, the MIC in typical food media is usually higher than standard laboratory media. The limitation of essential oils used as preservatives in food is the undesired flavour or the too-strong odour that can completely overpower any other odour of the food products (Friedly et al., 2009; Tiwari et al., 2009; Bajpai et al., 2012). In general, about 1-3% (w/w, EO/food product) of essential oil is required to extend the shelf-life, which is normally higher than organoleptic acceptability (<0.5% w/w) (Firouzi et al; 2007). For a commercial product in the food industry, the minimum inhibitory concentration (MIC) of essential oils rather

than the minimum bactericidal concentration (MBC) is usually applied to extend the product shelf-life, whereas sterilisation should be handled by physical methods during the production process to provide a low initial microorganisms population (Li et al., 2011).

Since the structure and composition of the food product is complex, and the antimicrobial mechanisms of EOs are not completely known, therefore, the antimicrobial effect of EOs influenced by certain food constituents is not fully understood. However, several influences have been reviewed.

Studies by Smith-Palmer et al (1998) and Burt (2004), reported that higher lipid content required a higher concentration of EOs to limit the growth of microorganisms. According to Gutierrez et al., (2008), high content of protein could promote the growth of *L. monocytogenes*, however, the antimicrobial efficacy of EOs (such as oregano or thyme) was also increased by protein. More importantly, the flavour of the EOs could be better bounded by a higher content of proteins (Baranauskien et al., 2006), as a result, the acceptability was higher on high protein content food product than low protein one at the same dosage of EOs. Moreover, Shelef et al. (1984) reported that the content of carbohydrates could affect neither the growth of bacteria nor the antimicrobial efficiency of EOs. This statement was later agreed by Gutierrez et al. (2009), who concluded that the presence of carbohydrates (up to 11.6%) did not show any impact on EO efficiency.

Lastly, it was generally considered that higher salt content or lower pH could enhance the antimicrobial activity of EOs (Burt, 2004; Friedly et al., 2009). Higher acidity could increase the hydrophobicity of EOs, as well as the solubility and stability, those phenomena result in the greater ability of EOs to perturb the cell membrane and inhibit the growth of bacteria (Hsieh et al., 2001).

3. General Description of the Methodology

3.1. Background

The study used systematic design methods to investigate and select a suitable combination of essential oils that may prolong the microbial quality of pre-cooked Asian noodle during storage at refrigeration temperature (4˚C). To achieve this goal, the project was conducted in three phases. Phase one investigated the antimicrobial effects of selected essential oils against the pure cultures of bacteria and fungi that are commonly found in food. During this stage, the broth micro-dilution method and agar disc diffusion tests were used to test the antimicrobial effects of EOs on *Escherichia coli* NCTC 8196, *Staphylococcus aureus* NCTC 4163, *Aspergillus brasiliensis* NZRM 2578 and *Penicillium chrysogenum* NZRM 2999. The promising antimicrobials were selected for optimisation of preservation technique in phase two.

In phase two, the experimental design was applied to establish a mathematical model to determine the optimum combinations of two EOs. There were three parameters (oregano EO, clove EO & soybean oil) that used in the mixture design to develop the response surface models, while Standard Plate Counts (SPC), Yeasts and Moulds Counts (YMC) and Overall Acceptability percentage (OA%) were set as the response variables for the modelling. For microbiological analyses of the samples, the standard procedures are provided by International Organization for Standardisation, SPC analysis method is described in ISO 4833-1 (ISO, 2013) whilst YMC analysis method is described in ISO 21527-2 (ISO, 2012) respectively. SPC and YMC results were used to assess the antimicrobial effect of the EOs combinations, whereas the overall acceptability of the consumer sensory test was applied to evaluate customers preferences to modified Hokkien noodle with added EOs. Hence, the best combination of essential oils was estimated, and used in phase three to determine the shelf-life of the modified Hokkien noodle.

Phase three focused on the shelf stability of the EOs applied Hokkien noodle. The experiment in the stage lasted 65 days. During the period, SPC and YMC were determined to estimate the shelf-life in the microbial aspect. Water activity, pH, colour profile and texture profile were measured to assess the shelf stability. Also, gaseous composition in the modified atmosphere packaging was measured for packaging stability. The microbiological results of the samples were also applied to Baranyi-Roberts model for predicting the growth curves of microorganisms on Hokkien noodle after the experimental period.

3.2. Determination of the Effectiveness of Antimicrobial EOs

The antimicrobial effectiveness of the essential oils has been previously studied using different techniques, such as the agar diffusion, agar dilution and broth dilution methods (Burt, 2004). The agar diffusion methods consist of the agar disc diffusion and the agar well diffusion methods. The broth dilution methods include the broth macro-dilution and broth micro-dilution method. The agar disc diffusion method is mostly used to compare the antimicrobial effect of EOs whilst broth micro-dilution method is used to investigate the MIC (Balouiri, Sadiki, & Ibnsouda, 2016). Hence, those two methods were applied to evaluate the antimicrobial capability of the selected EOs for further investigation in phase two.

3.2.1. Agar Disc Diffusion Method

The agar disc diffusion method is a standard method that is used to investigate the antimicrobial effect of tested materials against specific strains, which standard procedures are described in M02-A11 (CLSI, 2012) and E.DEF 9.3 (EUCAST, 2015). The method is easy to perform, and is suitable for testing both bacteria and fungi. The antimicrobial effects of the tested material are expressed by the diameter of the inhibition zone around the paper discs. Firstly, the 0.5 MacFarland inoculum is prepared, which gives $1\text{-}2*10^8$

CFU/ml of bacteria in Tryptic Soy Broth (TSB) or 1-5*10^6 CFU/ml of moulds in 0.1% peptone water. The procedure for preparing the standard microbial suspension is shown in Figure 3-1. Then suitable solidified laboratory media (e.g. Mueller-Hinton agar for bacteria) is evenly inoculated by the standardised inoculum. After that, filter paper discs (Ø=6 mm) containing the tested material at desired concentrations are placed on the surface of the laboratory media. After incubation, the diameter of the inhibitory zone is measured (Figure 3-2).

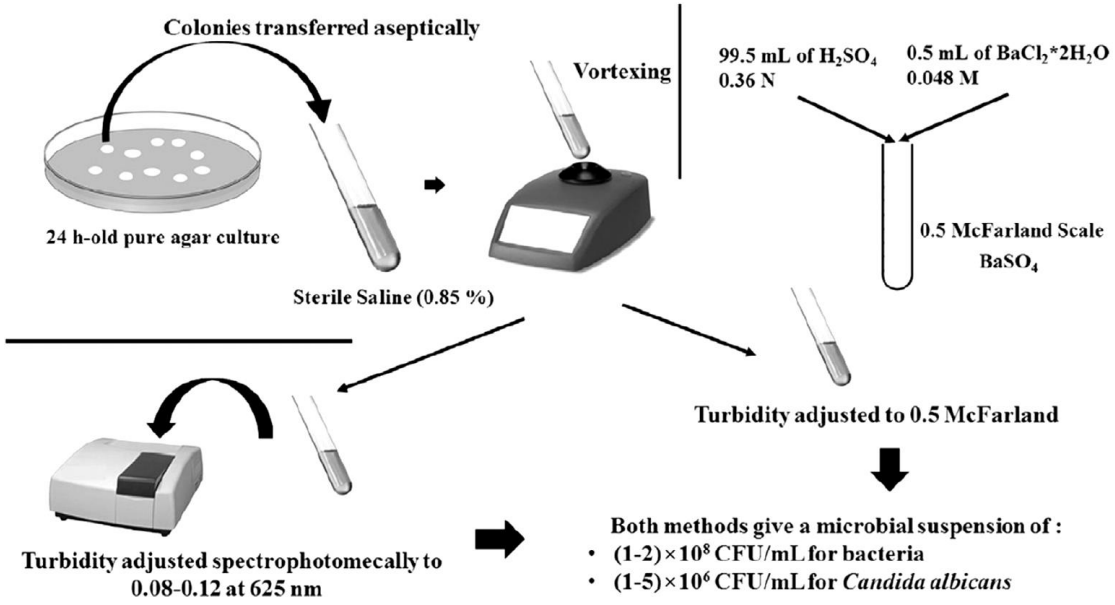

Figure 3- 1. Standard procedure for preparing 0.5 McFarland bacteria suspension (Balouiri et al., 2016).
Note: In the latest CLSI documents (CLSI, 2012), the absorbance ranged from 0.08 to 0.13 at 625 nm.

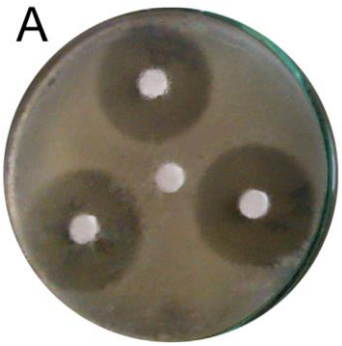

Figure 3- 2. An example of the inhibition zone in agar disc diffusion method (Balouiri et al., 2016).

3.2.2. Broth Micro-dilution Method

The broth micro-dilution method is a standard assay that used to investigate the antimicrobial ability of chemicals. The method is described in M07-A9 (CLSI, 2008) and E.DEF 9.3 (EUCAST, 2015). The results are commonly presented as the minimum inhibitory concentration (MIC) of the tested materials against specific microorganisms. The broth micro-dilution assay is performed on a 96-well microtitration plate. First, suitable concentrations of selected test materials, along with relevant laboratory media (MHB or RPMI), are added to the 96-well plate. Then the serial two-fold dilutions are performed before inoculating the standardised inocula (1/150 of 0.5 McFarland for bacteria and 1/10 of 0.5 McFarland for moulds) are inoculated to the plate, as shown in Figure 3-3. The MIC is obtained from the lowest concentration well with a positive result.

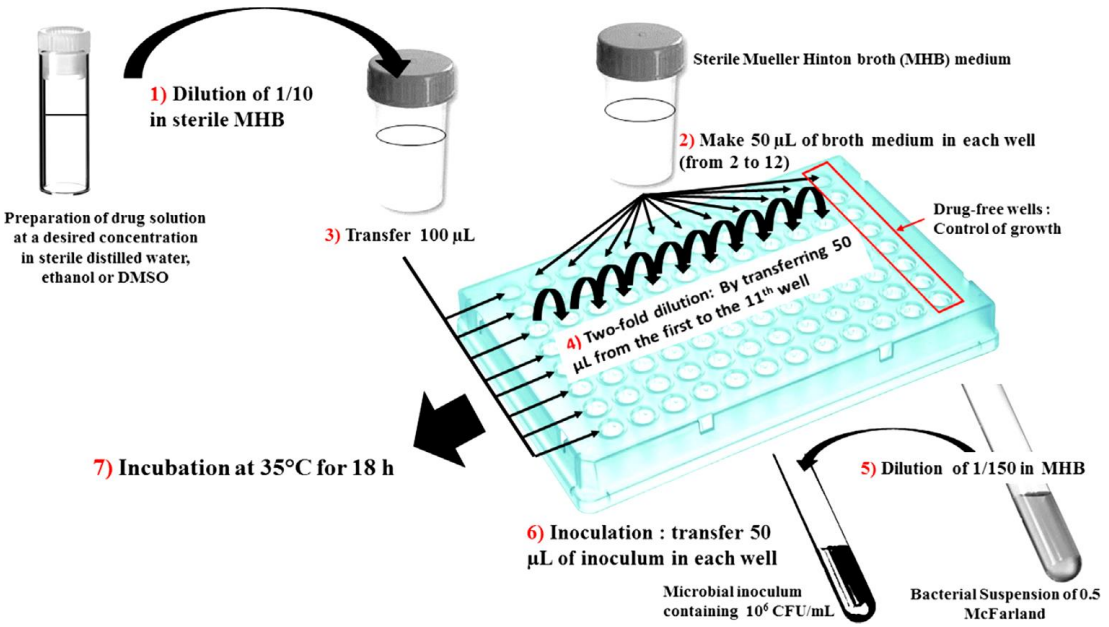

Figure 3- 3. The general procedure of the broth micro-dilution method for antimicrobial test recommended by CLSI (Balouiri et al., 2016).

Since the essential oils were hydrophobic, 0.1% tween 80 (v/v) was added to the laboratory media as an emulsifier (Gutierrez et al., 2008). In some cases, where the fungi might not be suitable to grow at 35˚C for 48 hours (CLSI, 2008), the incubating condition is adjusted to 28˚C for 72 hours to achieve better growth of the fungi (Tatjana et al. 2014).

3.3. Design of Experiment: Mixture design

The design of the experiments (DOE) is a scientific, systematic approach to investigate the effect of independent factors on the responses, by conducting trials within a feasible scale (Ezgi Aktar, Yeliz, Nimetullah, & Mustafa Tamer, 2015). Among the available of DOEs, the response surface design is applied when the independents or the interaction of the independents show a complex effect on the results. The mixture design is a branch of response surface design, which is widely used in optimising the formulations in the food industry. Hence, the ingredients in mixture design are usually shown in percentages (Yeliz et al., 2015). In the mixture design, the number of factors and permissible range of those factors must be given. Also, the summation of the proportions for each component must equal to 1, expressed by equations (1) and (2).

$$0 \leq X_i \leq 1 \qquad i = 1, 2, 3, \ldots, q. \tag{1}$$

$$\sum_{i=1}^{q} X_i = 1 \tag{2}$$

where X_i represents the proportions of the i^{th} component

q represents the total number of the component

In terms of investigating the food ingredients in the food industry, X_i represents the proportions of the i^{th} ingredient, q represents the total number of the ingredients considered in the mixture design. The remaining ingredients are neither considered nor restricted by equation (2). For the model output, one or more responses can be considered. Each response can be set for the given most desired value, maximisation or minimisation. The best result can be adjusted by optimising the balance between the responses.

There are several types of mixture designs, such as Simplex Lattice Design, Simplex Centroid Design, Simplex Axial Design and Extreme Vertex Design etc. In food experiments, if there are both upper and lower limit for the content of one or more

ingredients, the Extreme Vertex Design should be used. The bounds for the ingredients can be a specific percentage (such as the flour content in noodle ingredients) or equation (such as the dosage of several acid regulators that should reach a certain amount of the total food weight). In general, the number of total combinations (N) that need to be conducted in the experiment is determined by the number of the vertices (V) of the feasible region by default (Snee, 1979), the relationship between N and V is shown in equation (3).

$$N = 2 * V + 1 \quad (3)$$

For example, a three-ingredients Extreme Vertex Design with three different bounds can be expressed by Figure 3-4.

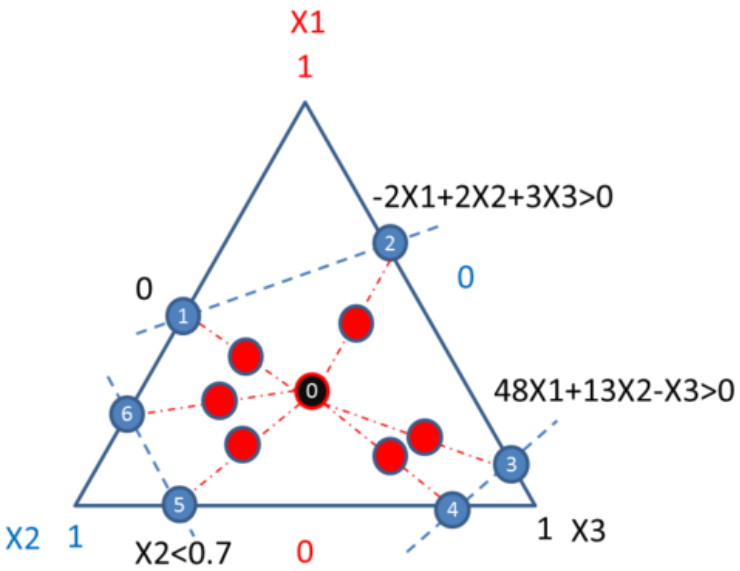

Figure 3- 4. Three ingredients three bounds Extreme Vertex Design (Snee, 1979)
Note: Blue = vertices; Red = axial point; Black = overall centroid.

Figure 3-4 shows an example of the available area of a 6-vertice (number 1-6) and 1 overall centroid (number 0) Extreme Vertex Design. The red dots are axial points, which are located in the middle of the vertices and the overall centroid. Therefore, the total number of the combinations that need to be conducted in the experiment are 2*6 +1, 13

combinations in this case.

Once the model is built and trials are conducted, the optimisation of the model can be performed by adjusting significant affecting factors, statistical weights between responses and the target values for each response. Then, a polynomial equation will be developed to indicate the coefficient of each factor effected on the response.

Model verification then could be performed if necessary, which required an extra confirmation experiment. The most promising predicted combination is selected to perform an extra experiment with any other conditions kept constant, to verify if the result will fall into the prediction interval.

3.4. Food Characterisation

The addition of EOs to the Hokkien noodle might alter its food profile. To ensure a stable shelf-life of the Hokkien noodle, meanwhile, the main characteristics of the product are still acceptable for customers, the sensory characterisation should be determined. Therefore, the colour and texture profiles of the samples were measured to compare the differences between the experimental noodles and the control, as well as the differences between experimental samples during storage and the fresh (day-0) samples.

3.4.1. Colour

Appearance is the first sensory attribute presented to the customers, which is affected by the packaging, size, colour, gloss etc. (Costa et al., 2011). Among the parameters, colour is one of the most important evaluating indicators that represent the quality and the freshness of the product, and it impacts consumers' acceptance (Leon et al., 2006), due to consumer expectation.

The detection of the colour profile of the Hokkien noodle samples follows the instruction

provided by the instrument provider user manual. To describe the colour difference between two different samples, chroma difference (ΔC), browning index difference (ΔBI) and the total colour difference (ΔE) are calculated (Adekunte et al., 2010).

Chroma or saturation (C*) is calculated by following equation (4), a* and b* stand for greenness and yellowness respectively, given by the colour profile assessment. The colour chroma difference between two samples is calculated by equation (5).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (4)$$

$$\Delta C = \sqrt{\Delta a^{*2} + \Delta b^{*2}} \quad (5)$$

where

$$\Delta a^* = (a_1^*) - (a_2^*) \quad (6)$$

$$\Delta b^* = (b_1^*) - (b_2^*) \quad (7)$$

Additionally, the browning index (BI%) could be used to characterise the degree of the browning reaction, BI% can be calculated by equations (8) and (9), L stands for the lightness given by colour profile measurement. The difference of BI% between two samples is calculated by equation (10)

$$BI\% = 100 * \left(\frac{X - 0.31}{0.17}\right) \quad (8)$$

where

$$X = \frac{(a^* + 1.75L)}{5.645L + (a^*) - 3.012(b^*)} \quad (9)$$

$$\Delta BI = BI_1\% - BI_2\% \quad (10)$$

Furthermore, the total colour difference is calculated by equation (11). According to Adekunte et al. (2010), if the $\Delta E > 3$, the difference between samples is distinct, Non-trained normal customers may distinguish the difference. For $1.5 < \Delta E < 3$, the difference can only be found by the trained technicians. When the difference decreases to below 1.5,

it is barely detectable by the human eyes.

$$\Delta E = \sqrt{\Delta L *^2 + \Delta a *^2 + \Delta b *^2} \quad (11)$$

Hence, in this study, the chroma difference (ΔC), the browning index difference (ΔBI) and the overall colour difference (ΔE) were detected as parameters to show colour changes of the Hokkien noodle samples during storage.

3.4.2. Texture

Except for colour, the texture is another most important factor that affects customer acceptability (Guoquan et al., 1998). Both the production procedure and storage condition will directly or indirectly affect the texture of the noodles. Chinese Hokkien noodle products should have a good bite, chewy and elastic texture. Hence, the texture should be considered in any noodles-related study.

There are several kinds of texture analyses for noodles, such as the texture profile analysis (TPA) is used for ready to eat noodles, noodles extensibility analysis (TEA) is applied mainly for pre-cooked noodles, and noodles firmness analysis (TFA) is most common for raw fresh noodles (Wang, Lu, & Yuan, 2003). As a branch of pre-cooked Asian noodles, Hokkien noodle is usually analysed by the noodles extensibility analysis (Li, 2008), since the noodle requires tensile stability during the storage period and should withstand the re-cooking process before consumption. The extensibility analysis is performed by two plastic L-shape tensile rigs (A/SPR). One is located on the platform and the other is attached below the moving parts. One string of sample is attached on both L-shape rigs and no dragging force should be applied. The distance between two rigs at the original position is pre-set and recorded. When the upper rig moves up at a pre-set speed to the final position, the string is expected to break before the rig return to starting position. The force required for the rig movement is recorded, as shown in Figure 3-5:

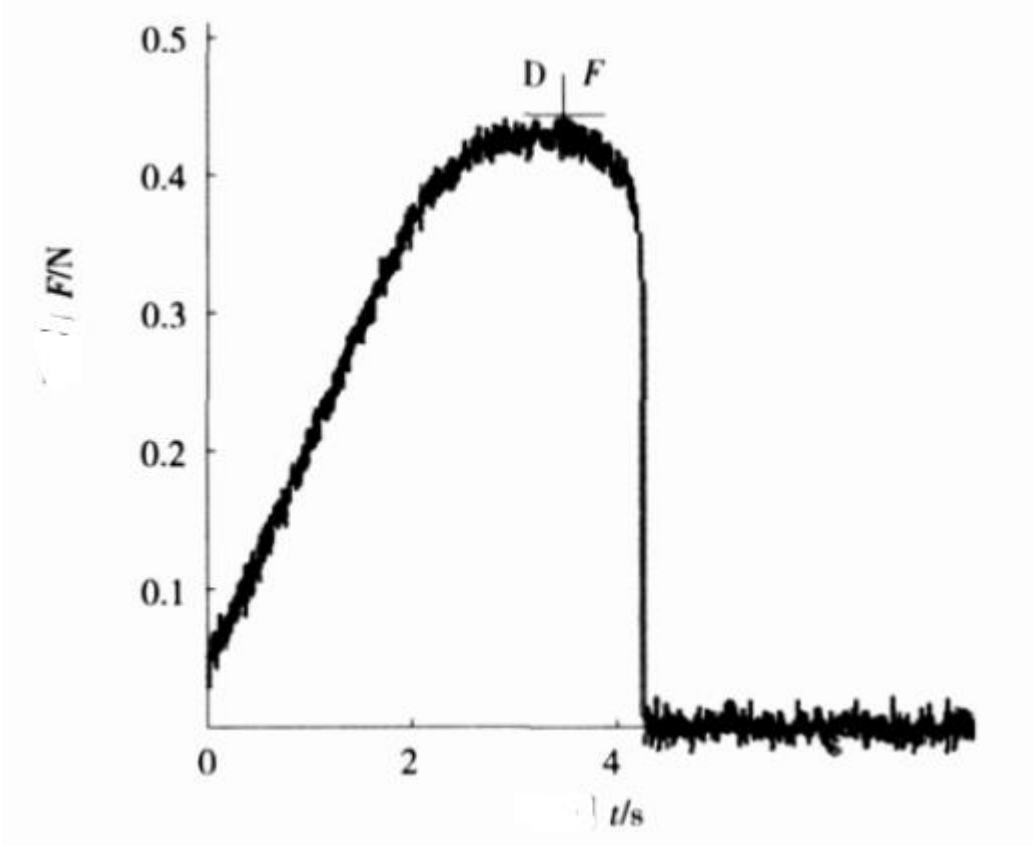

Figure 3- 5. The feature pattern for Texture Extensibility Analysis (Li, 2008)

During the measurement of the TEA, the required force reaches the peak value right before the string is broken. The force required to reach the peak (F) and the time required to reach the peak (D) are recorded. D stands for distance, which is automatically calculated by the following equation (12):

$$\text{Distance} = \text{testing speed} * \text{peaking time} \quad (12)$$

Hence, in this project, extensibility analysis would be conducted as a profile to exhibit the texture change of the Hokkien noodle samples during the storage period.

4. Screening of Essential Oils

The antimicrobial properties of essential oils (EOs) are variable due to their chemical composition. *In vitro* tests are commonly used to determine the antimicrobial effects of EOs. The main objective of this stage of the study was to determine four food-grade essential oils with the strongest antibacterial and/or antifungal effect against four selected foodborne pathogens, to evaluate their antimicrobial properties. The assays consisted of antimicrobial effectiveness test and the minimum inhibitory concentration (MIC) test. MICs of EOs were obtained by conducting the broth micro-dilution assay following the standard protocol. Then the antimicrobial effects of EOs in the aqueous phase were determined, since the laboratory media used were water-soluble. Also, the EOs were diluted in appropriate concentration with soybean oil to perform the disc diffusion assay, in order to investigate the antimicrobial effect of EOs in the lipid phase, which simulated the real applying situation.

In this study, four EOs were selected for the tests at this stage, which included wild oregano, thyme thymol, savory and clove bud. The broth micro-dilution and agar disk-diffusion methods were used for the antimicrobial efficiency test and effectiveness test. Four foodborne microorganisms were chosen for the assays which included Gram-negative rod *Escherichia coli* (*E. coli*), Gram-positive coccus *Staphylococcus aureus* (*S. aureus*), *Aspergillus brasiliensis* (*A. brasiliensis*) and *Penicillium chrysogenum* (*P. chrysogenum*).

4.1. Materials and Methods

4.1.1. Essential Oils

All the essential oils (5.0 g per bottle) used in the project were purchased from Florihana Ltd (Riviera, France). The specifications of the EOs are listed in Table 4-1 and the most abundant compounds in the EOs are listed in Table 4-2. Detailed information about the

EOs provided by the supplier are shown in Appendix A.

4.1.2. Microbial Cultures

All the microbial strains including *Escherichia coli* NCTC 8196, *Staphylococcus aureus* NCTC 4163, *Aspergillus brasiliensis* NZRM 2578 and *Penicillium chrysogenum* NZRM 2999 were obtained from the Food Microbiology Culture Bank of the School of Food and Advanced Technology, Massey University, Auckland, New Zealand. The cultures were stored at -80˚C until required for the experiments.

4.1.3. Media and Reagents

Tryptic Soy Broth (TSB), Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) were purchased from Difco™ Ltd (Oxford, the United Kingdom). Yeast extract Glucose Chloramphenicol Agar (YGCA) and Universal peptone M66 were obtained from Microbiology Ltd (Auckland, New Zealand). Roswell Park Memorial Institute (RPMI 1640) Part A and B were collected from Himedia Ltd (Auckland, New Zealand). Tween 80, Trigene and paper disk (6mm diameter, 20 pieces/bottle) were purchased from Thermo Fisher Scientific Ltd (Auckland, New Zealand). Resazurin was provided by ACROS Organics (Geel, Belgium). DAHUAT Soybean oil (Auckland, New Zealand) was obtained from the local market.

Table 4- 1. Specifications of selected essential oils (Florihana Ltd, 2019).

Essential oils	Plant Latin Name	Geographic origin (Culture mode)		Parts for extraction	Extraction mode	Density, g/cm^3@20˚C
Clove	*Eugenia Caryophyllus*	Madagascar	(Cultivated)	Buds	Steam distillation	1.0601
Oregano	*Origanum Compactum*	Morocco	(Wild)	Flowering Tops	Steam distillation	0.9363
Savory	*Satureja Montana*	Spain	(Cultivated)	Flowering Tops	Steam distillation	0.9260
Thyme	*Thymus vulgaris*	Spain	(Wild)	Flowering Tops	Steam distillation	0.9150

Table 4- 2. Top 5 most abundant compounds of selected essential oils (Florihana Ltd, 2019).

Essential oils	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5
Clove	Eugenol (78.872%)	Acetate D'Eugenyle (14.005%)	β-Caryophyllene (5.761%)	α-Humulene (0.661%)	Salicylate De Methyle (0.136%)
Oregano	Carvacrol (53.553%)	γ-Terpinene (17.426%)	Thymol (9.455%)	p-Cymene (7.861%)	α-Terpinene (1.971%)
Savory	Carvacrol (43.606%)	p-Cymene (17.135%)	γ-Terpinene (13.106%)	Thymol (4.954%)	β-Caryophyllene (4.073%)
Thyme	Thymol (37.270%)	p-Cymene (18.520%)	γ-Terpinene (13.090%)	Linalol (4.860%)	Carvacrol (3.220%)

4.1.4. Apparatus

Equipment used during this stage of the study are shown in Table 4-3.

Table 4- 3. Instruments used *in vitro* antimicrobial effect tests.

Instrument	**Model (Brand)**
Spectrophotometer	Novaspec III (Amersham Biosciences)
Vortex Mixer	VM-10 (WiseMix)
Electric Microscope	Axiostar plus (Carl Zeiss AG)
Microscope Camera	AxioCam MRc (Carl Zeiss AG)

4.1.5. Preparation of Standard Culture Suspensions

The standard culture suspensions for this stage of study were prepared to meet the requirement of 0.5 McFarland culture solutions. The standard 0.5 McFarland culture suspensions for bacteria and fungi were prepared following the steps as described. First, one colony of *S. aureus* or *E. coli* was collected by a sterilised wire loop and transferred into 9 mL of sterile TSB. Then the TSB bottles were incubated at 35˚C for 24 hours to obtain the stock solutions for *S. aureus* and *E. coli*. Second, the spectrophotometer (Novaspec III, Amersham Biosciences, the United Kingdom), was calibrated using clean TSB to give a zero absorbance. Then a certain amount of stock inoculum solutions was gradually transferred to sterile 9 mL of TSB to achieve absorbance within 0.080 – 0.130 at 625 nm. The absorbance values within this range indicated that the 0.5 McFarland standard culture suspensions were prepared as required. The concentrations of the standardised culture were around 1-2 $*10^8$ CFU/mL for *E. coli* and *S. aureus*.

For fungi, one colony of *A. brasiliensis* and *P. chrysogenum* were inoculated to sterile 9 mL of 0.1% peptone water respectively, with adequate mixing using a vortex mixer (VM-10, WiseMix) to obtain stock solutions for *A. brasiliensis* and *P. chrysogenum*. Clean peptone water (0.1%) was used to calibrate the spectrophotometer (Novaspec III, Amersham Biosciences, the United Kingdom) to give zero absorbance. The stock

solutions of *A. brasiliensis* and *P. chrysogenum* were gradually transferred to 9 mL sterile peptone water to give absorbance within 0.080-0.100 at 530 nm. The 0.5 McFarland turbidity gave the concentration of *A. brasiliensis* or *P. chrysogenum* at around 1-5 * 10^6 CFU/mL.

4.1.6. Broth Micro-dilution Assay

The tested EOs were diluted in relevant laboratory media to maintain the consistency of the solvent during the assay, which provided the antimicrobial effect of the tested EOs in the aqueous base system. The broth micro-dilution tests were conducted following the standard procedure (CLSI, 2012) with some modifications. The final inocula sizes for micro-dilution assays were around 1 *10^6 CFU/mL for bacteria and 1-5 *10^5 CFU/mL for fungi. To obtain that concentrations, 0.1 mL of 0.5 McFarland suspensions of bacteria were transferred to 14.9 mL of sterile MHB or 1 mL of 0.5 McFarland suspensions of mould were transferred into 9 mL of 0.22 nm filtered RPMI 1640A (for fungi). Each essential oil at three different initial concentrations was used to assay the minimum inhibitory concentration against two bacteria and two fungi using the 96-well microtitration plate. A full description of the 96-well microtitration plate setup is shown in Table 4-4, 4-5 and 4-6. Both MHA and RPMI 1640A contained 0.2% (v/v) of tween 80 as the emulsifier. The microtitration plates were incubated at 35°C for 20-hour for bacteria and 48-hour for fungi respectively. Additionally, 40 µL of 0.1 mg/mL of resazurin solution was added 2 hours before each result inspection.

4.1.7. Agar Disc Diffusion Assay

The agar disc diffusion assay was conducted on the solidified laboratory media, which allowed the tested EOs to be diluted in the lipid phase without affecting the consistency of the media. Hence, the agar disc diffusion assay was employed to determine the antimicrobial effect of EOs in the lipid phase situation. The standard agar disk diffusion tests were conducted following the standard procedure (CLSI, 2008; EUCAST, 2017)

with some modifications. Each 0.5 McFarland culture suspension was evenly inoculated on solidified MHA plates (for bacteria) or YGCA plates (for fungi) using sterilised cotton swabs. The essential oils were diluted into soybean oil to obtain 9%, 12% and 15% (w/w) of concentrations respectively to obtain different EO solutions. Sterile 6-mm paper discs were immersed in each prepared EO solution and placed onto the surface of inoculated agar plates. Paper discs immersed in soybean oil (100%) or Trigene (2%) were set as negative or positive controls, respectively. The MHA plates were incubated at 35˚C for 18 hours and YGCA plates at 25˚C for 72 hours. The diameter of the inhibitory zone around the paper discs was measured by a vernier calliper (701-2701, Fuller, New Zealand). All assays were conducted in triplicates.

4.1.8. Statistical Analysis of Data

The mean value and standard deviation of the diameter of the inhibitory zone were expressed in bar charts via Microsoft Office Excel 2016 (Microsoft, USA). The results of the MIC were expressed as mean ± standard deviation. All data were analysed by the SPSS Statistic Version 24.0 (IBM^{TM}, USA) with Shapiro-Wilk for normality test at 95% confidence level for normal distribution analyses. Normally distributed data were conducted the analysis of variance test (ANOVA). Non-normally distributed data were analysed by the Non-parametric Wilcoxon test.

Table 4- 4. Reference table for 96-well plate with the highest essential oil concentration at 9.6%.

Row	Strains/Column	1	2	3	4	5	6	7	8	9	10	11	12
1	*E. coli* NCTC8196	PC	B	9.6%	4.8%	2.4%	1.2%	0.6%	0.3%	0.15%	0.075%	0.0375%	NCa
2													
3	*S. aureus* NCTC4163												
4													
5	*P. chrysogenum* NZRM2999												NCb
6													
7	*A. brasiliensis* NZRM2578												
8													

Note: PC = Positive Control (2% Trigene); B = Blank (Empty well); NCa = Negative Control (MHA for bacteria); NCb = Negative Control (RPMI 1640A for fungi); The percentage from columns 3 to 11 represents the final concentrations of essential oil in each column

.

Table 4- 5. Reference table for 96-well plate with the highest essential oil concentration at 7.68%.

Row	Strains/Column	1	2	3	4	5	6	7	8	9	10	11	12
1	*E. coli* NCTC8196	PC	B	7.68%	3.84%	1.92%	0.96%	0.48%	0.24%	0.12%	0.06%	0.03%	NCa
2													
3	*S. aureus* NCTC4163												
4													
5	*P. chrysogenum* NZRM2999												NCb
6													
7	*A. brasiliensis* NZRM2578												
8													

Note: PC = Positive Control (2% Trigene); B = Blank (Empty well); NCa = Negative Control (MHA for bacteria); NCb = Negative Control (RPMI 1640A for fungi); The percentage from columns 3 to 11 represents the final concentrations of essential oil in each column.

Table 4- 6. Reference table for 96-well plate with the highest essential oil concentration at 5.76%.

Row	Strains/Column	1	2	3	4	5	6	7	8	9	10	11	12
1	*E. coli* NCTC8196	PC	B	5.76%	2.88%	1.44%	0.72%	0.36%	0.18%	0.09%	0.045%	0.0225%	NCa
2													
3	*S. aureus* NCTC4163												
4													
5	*P. chrysogenum* NZRM2999												NCb
6													
7	*A. brasiliensis* NZRM2578												
8													

Note: PC = Positive Control (2% Trigene); B = Blank (Empty well); NCa = Negative Control (MHA for bacteria); NCb = Negative Control (RPMI 1640A for fungi); The percentage from columns 3 to 11 represents the final concentrations of essential oil in each column.

4.2. Results and Discussion

4.2.1. Broth Micro-dilution

The broth micro-dilution results for the four essential oils (oregano, clove, savory, thyme) against two bacteria (*S. aureus* NCTC4163 and *E. coli* NCTC8196) and two fungi (*A. brasiliensis* NZRM2578 and *P. chrysogenum* NZRM2999) are shown in Appendix C1-I. Oregano EO started at 7.68% (highest concentration) (Figure 4-1) and clove EO started at 9.6% (highest concentration) (Figure 4-2) were shown as the example for MIC tests. Full mean results are shown in Table 4-7.

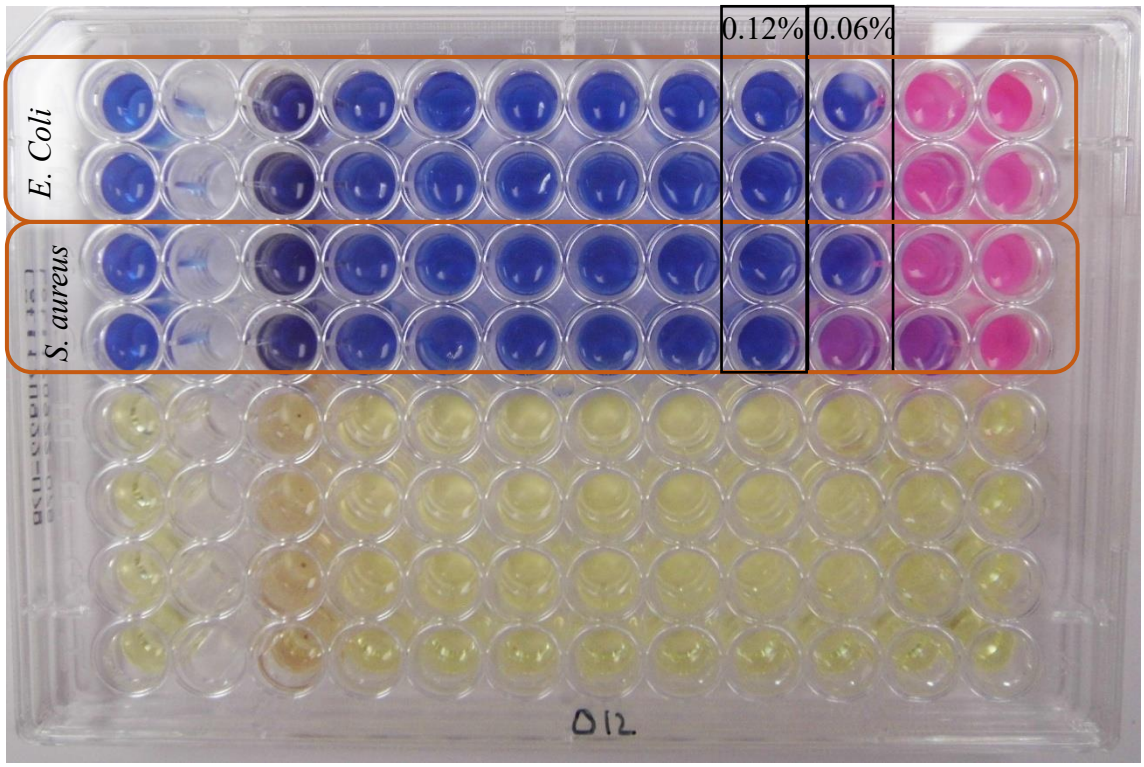

Figure 4- 1. The 96-well plate of oregano EO starting at 7.68% on 3rd column.
Note: Reference Table 4-5; Colour changes were found from column 10th to 11th for *E. coli*, the MIC for oregano against *E. coli* was 0.06%; Colour changes were found from column 9th to 11th for *S. aureus*, the MICs for oregano against *S. aureus* were 0.06% & 0.12%.

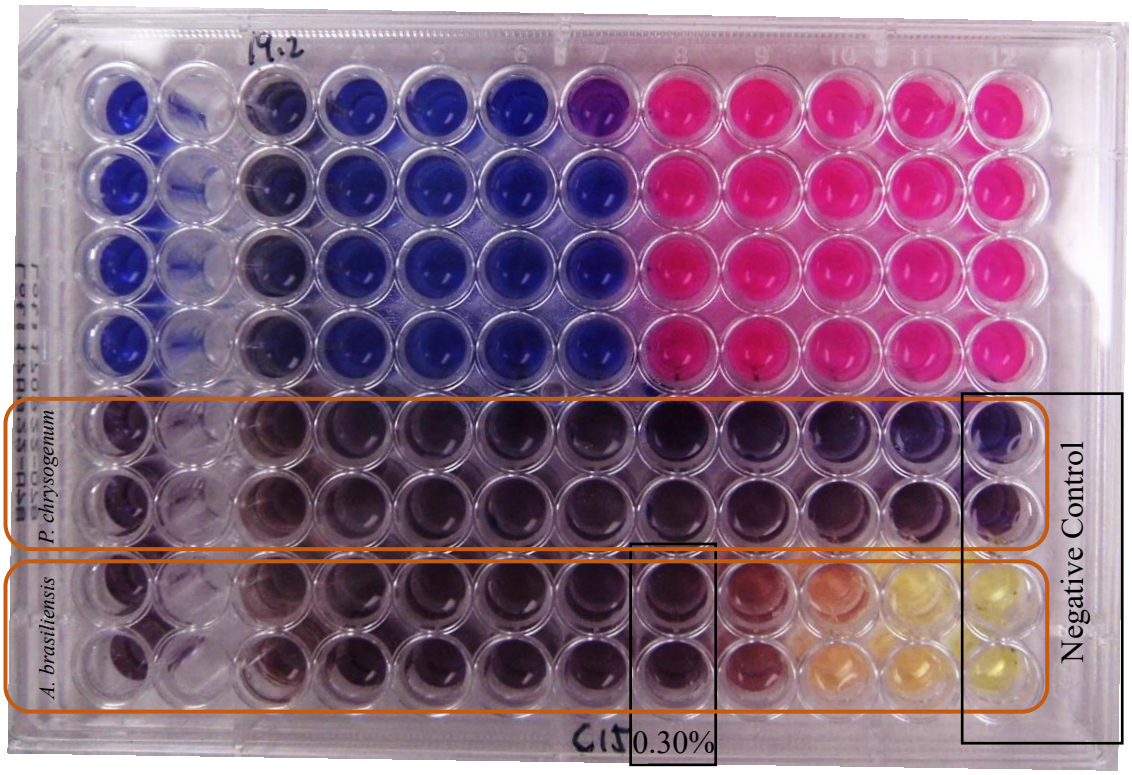

Figure 4- 2. The 96-well plate of clove EO Starting at 9.60% on 3rd column.

Note: Reference Table 4-4; Positive results were found in negative control column for *P. chrysogenum*, results were invalid; Colour changes were found from column 8th to 9th for *A. brasiliensis*, the MIC for clove against *A. brasiliensis* was 0.30%

For all the tested *P. chrysogenum* in 96-well plates, positive results were found in negative control column (column 12th), indicating that the *P. chrysogenum* was not suitable to grow under the standard incubating conditions recommended by CLSI 2012 (48 hours at 35˚C). Similar phenomena have been reported by Tatjana et al (2014) that *P. chrysogenum* was not suitable to grow under those incubation conditions. Hence, the assays for *P. chrysogenum* have been repeated using the recommended condition given by Tatjana et al., which was incubated for 72 hours at 28˚C. The results are shown in Figure 4-3.

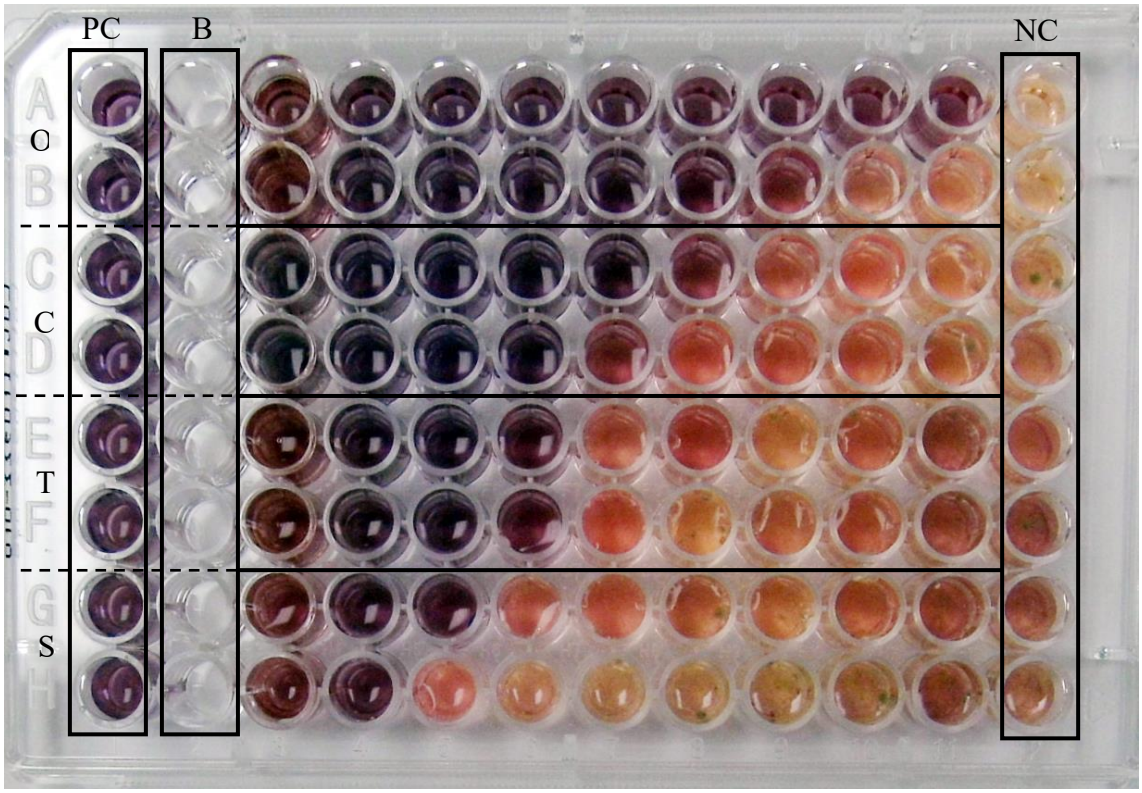

Figure 4- 3. Four EOs against *P. chrysogenum* NZRM2999 started at 5.76% at 3rd column. Note: Incubating condition: 28˚C for 72 hour; PC = positive control column (Trigene 2%); NC = negative control column (RPMI 1640A); B = blank column (empty well); O = oregano EO; C = clove EO; T = thyme EO; S = savory EO.

Figure 4-3 shows the growth of *P. chrysogenum* in the control columns as purple in the positive control and orange in the negative control, indicating that the adjusted conditions were suitable for the growth the *P. chrysogenum*. Results showed that Oregano EO (0.135%) was the most effective against *P. chrysogenum* followed by Clove EO (0.27%), Thyme EO required 0.27% of total volume to inhibit the growth of *P. chrysogenum*. *P. chrysogenum* was most resistant against savory EO, which could survive until the concentration of savory EO reached 2.16%.

Table 4- 7. The MICs (%) of EOs against four different strains of microorganisms*.

EOs	*E. coli* NCTC8196	*S. aureus* NCTC4163	*A. brasiliensis* NZRM2578	*P. chrysogenum* NZRM2999
Thyme	0.13 ± 0.08	0.20 ± 0.08	0.54 ± 0.07	0.72 ± 0.00
Savory	0.38 ± 0.24	0.51 ± 0.24	0.60 ± 0.14	2.16 ± 0.83
Oregano	0.04 ± 0.01	0.06 ± 0.01	0.14 ± 0.02	0.14 ± 0.05
Clove	0.39 ± 0.10	0.21 ± 0.10	0.33 ± 0.03	0.27 ± 0.10

*Freshly grown cultures were used.

The MICs of the four selected EOs against four different tested strains of microorganisms are shown in Table 4-7. A lower MIC indicated a stronger antimicrobial property of EOs, hence, the oregano EO was the most effective antimicrobials against both bacteria and fungi, which had the lowest MICs on all four strains tested (0.04% on *E. coli*, 0.06% on *S. aureus*, 0.14% on both fungi). The results were similar to the study by Friedman et al. (2002) and Sokovic et al. (2010), who also reported the effectiveness of oregano oil against a broad-spectrum of microbes. Thyme EO also produced a reasonable antimicrobial effect on bacteria, but was not effective against fungi. Savory oil showed the highest MICs on the Gram-positive *S. aureus* (0.51%) and the fungi (0.60% on *A. brasiliensis* and 2.16% on *P. chrysogenum*), which represented the lowest antimicrobial effectiveness. The results on savory EO were contrary to the finding of Tatjana et al. (2014), who reported a relatively strong antifungal effect of savory that similar to oregano and thyme EOs. The discrepancies in the results might be attributed to the differences in the concentrations of bioactive compounds in EOs, such as phenolics. Tatjana et al. (2014) reported that the total amount of phenolic compounds was 73% in thyme EO and 78% in savory EO. However, the total amount of phenolics in the tested savory and thyme EOs were only about 49% and 45 %, respectively. The difference in the concentrations of the major compounds could directly affect the antimicrobial effect of the same kind of essential oil, since phenolics were one of the most effective antimicrobial compounds (Carmo et al., 2008). Furthermore, although clove EO did not show strong bacteriostatic effect, it had better antifungal activity than thyme and savory EOs. This result was contrary to Moreira et al. (2005), who reported a strong inhibition of clove EO on Gram-negative bacteria, such as *E. coli*. However, the antifungal results were in line with

Maryam et al. (2006), who confirmed an effective inhibition of clove EO on fungi such as *Aspergillus flavus*.

For thyme, oregano and savory EOs, which have similar major compounds (carvacrol, γ-Terpinene, p-Cymene and thymol), it was unexcepted that Gram-negative bacterium (*E. coli*) was more susceptible than Gram-positive bacterium (*S. aureus*). For instance, it needed 0.38% of savory EO to inhibit the growth of *E. coli*, but it required 0.51% against *S. aureus*. It has been reported that EO should have a better inhibitory effect on Gram-positive bacteria than Gram-negative bacteria (Burt, 2004; Friedly et al., 2009). Repeated experiments were showing the same results. The unexpected results needed to be confirmed on the agar disc diffusion assay, to verify if the antimicrobial effects of tested EOs were affected by the aqueous base solution. While *P. chrysogenum* was more resistant than *A. brasiliensis* when subjected to thyme, savory and oregano EOs. Moreover, clove EO had relative similar MICs (0.21%-0.39%) against either bacteria or fungi.

Normality tests of the MIC results have been conducted. As can be seen from Appendix D1-I, most of the MIC values did not obey normal distribution at 95% confidence level, as the p-values (0.024) were less than 0.05. Hence, the non-parametric Kruskal-Wallis Test was deployed instead of ANOVA test to determine whether there was a significant difference in the MICs of different EOs against different cultures.

As could be seen in Appendix D1-II (a), the significant level was $0.006<0.05$, implying at least one of the MIC mean values was not equal to others. Oregano EO had the lowest MIC (0.04±0.01%) on inhibiting *E. coli* and distinctively different with other oils. Thyme EO also had a high inhibitory effect against *E. coli* (0.13±0.08%), but it has a similar range on MICs when the standard deviation was considered. There was almost no difference on MIC between savory and clove EOs against *E. coli*. It could be concluded that oregano had the best inhibitory effect on the growth of *E. coli*.

According to Appendix D1-II(b), oregano EO had the lowest MIC on inhibiting *S. aureus* (0.06±0.01%) and differed from other essential oils. Thyme and clove EOs had similar MICs on *S. aureus* (0.20% and 0.21% respectively), which were different from *E. coli.* The least inhibitory effect was savory EO, which required a concentration of 0.51±0.24% to suppress the growth of *S. aureus*. However, it still shared a similar range with thyme and clove EOs. It could be concluded that oregano had the best inhibitory effect on the growth of *S. aureus*.

With regards to Appendix D1-II(c), the results showed that oregano EO had the lowest MIC on the growth of *A. brasiliensis* (0.14±0.02%), followed by clove EO (0.33±0.03%) that needed double concentration of oregano EO against *A. brasiliensis*. Those two EOs were markedly different from other EOs. Meanwhile, thyme and savory EOs had every similar MICs and relatively poor inhibitory effects on *A. brasiliensis* (>0.40%).

As the results showed in Appendix D1-II(d), both oregano and clove EOs have shown excellent effects (0.14% & 0.27% respectively) on the inhibition of *P. chrysogenum*, followed by thyme EO (0.72%). Savory EO had the highest MIC (2.16%) that required around a triplicate concentration of thyme EO to inhibit *P. chrysogenum*, and it had a remarkable difference with any other EOs.

Overall, it was obvious that the oregano EO was the most effective to inhibit the growth of the bacteria tested. With respect to fungal inhibition, oregano and clove EOs showed high efficiency at relatively low MICs. These results needed to be confirmed on agar disc diffusion assay to verify if the EOs could maintain their antimicrobial effects when being dissolved in the lipid phase (soybean oil), where would fit the real applying circumstances.

4.2.2. Agar Disc Diffusion

The Agar Disc diffusion results for the four essential oils (oregano, clove, savory, thyme)

dissolved in soybean oil at different concentrations (9%, 12% and 15%) against the growth of two bacteria (*S. aureus* NCTC4163 and *E. coli* NCTC8196) and two fungi (*A. brasiliensis* NZRM2578 and *P. chrysogenum* NZRM2999) are shown in Figures 4-4, 4-5, 4-6 and 4-7. The results of statistical analysis ($p<0.05$) are shown in Table 4-8 and Table 4-9.

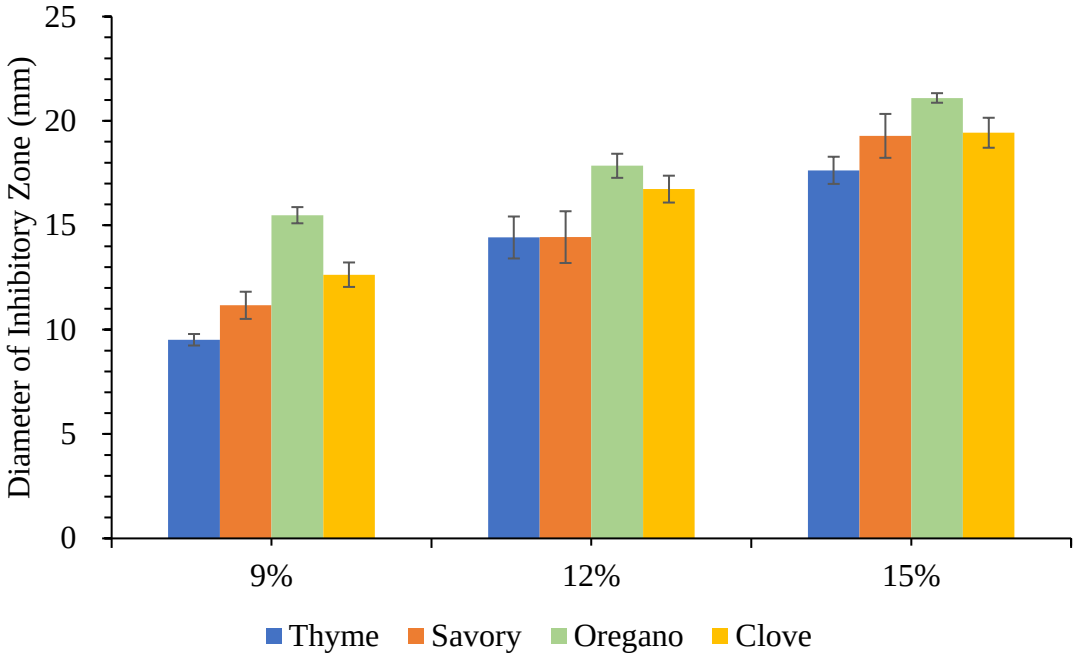

Figure 4- 4. The diameter of inhibitory zone (mm) for different EOs against *E. coli* NCTC8196*.

*Freshly grown cultures were used.

As shown in Figure 4-4, in general, the antimicrobial effect of EOs increased with concentration. Oregano EO achieved better inhibitory effect against the growth of *E. coli* than the other EOs regardless of which concentration applied, which obtained a diameter of over 15 mm at 9%, then increased to 17.85 mm at 12% and over 21 mm at 15%. The second best inhibitory effect on *E. coli* was found with clove EO instead of thyme EO, thus deviating from the broth micro-dilution results. Among the EOs, thyme EO had the lowest inhibitory diameter (9.52 mm at 9%; 17.63 mm at 15%) against *E. coli*, indicating for the worst effects on suppressing *E. coli*. The possible reason for this might be that the diffusion of thyme EO was ineffective in lipid phase, or the soybean oil somehow

protected the cell membrane of *E. coli* from the bioactive compounds of thyme EO (Smith-Palmer et al., 1998), resulting in decreased antibacterial effect of thyme EO.

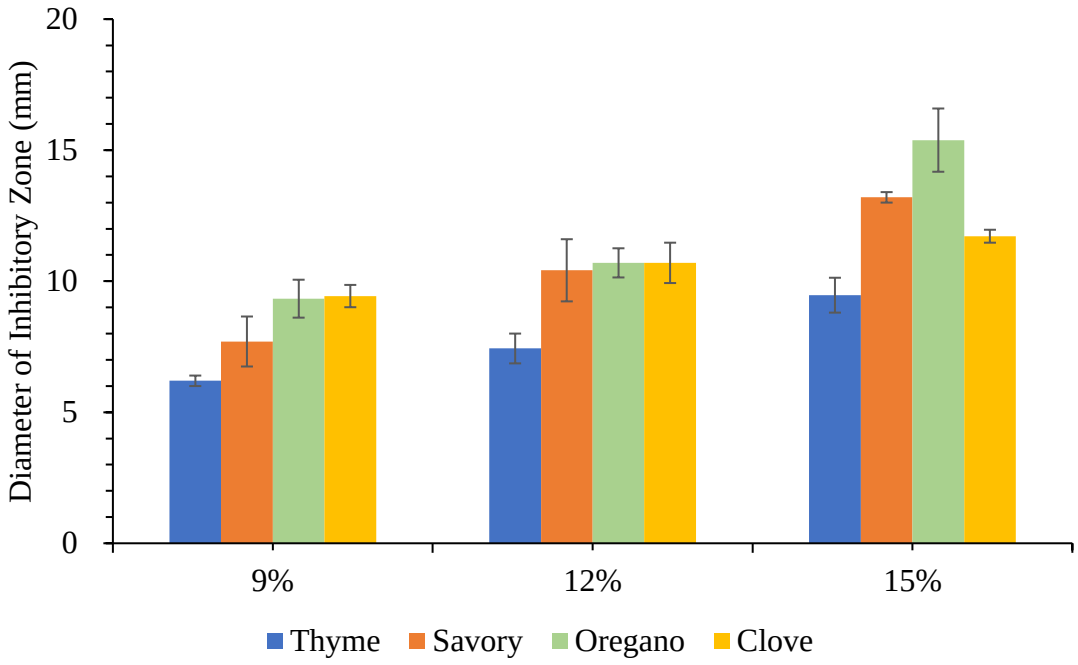

Figure 4- 5. The diameter (mm) of inhibitory zone for different EOs against *S. aureus* NCTC4163*

*Freshly grown cultures were used.

With respect to the Gram-positive bacterium (*S. aureus*) shown in Figure 4-5, overall, all the EOs had similar antibacterial effects (inhibition diameter = 10.00 ± 1.19 mm) when the EO concentrations were lower than 12% except for thyme EO. Thyme EO achieved very minimal antibacterial effect against *S. aureus* when the concentration was lower than 12%, with the diameters of the inhibitory zone at 6.2 mm at 9% & 7.4 mm at 12%, which were nearly the same with the diameter of the paper disc (6 mm). When the concentration of EOs increased to 15%, the disparity in the antimicrobial effect of different EOs was apparent. Oregano EO showed the best inhibitory effect (15.38 mm) against *S. aureus*, which was consistent with the MIC results, followed by savory EO (13.20 mm). Thyme EO still showed the least inhibitory effect against *S. aureus* (lower than 10 mm). The results about thyme EO against *S. aureus* were divided compared to Henika and Mandrell

(2002) and Moreira et al. (2005), who reported thyme EO had relatively strong antimicrobial, which was similar to oregano EO. The reasons for this outcome against *S. aureus* may be similar to those reported for *E. coli*.

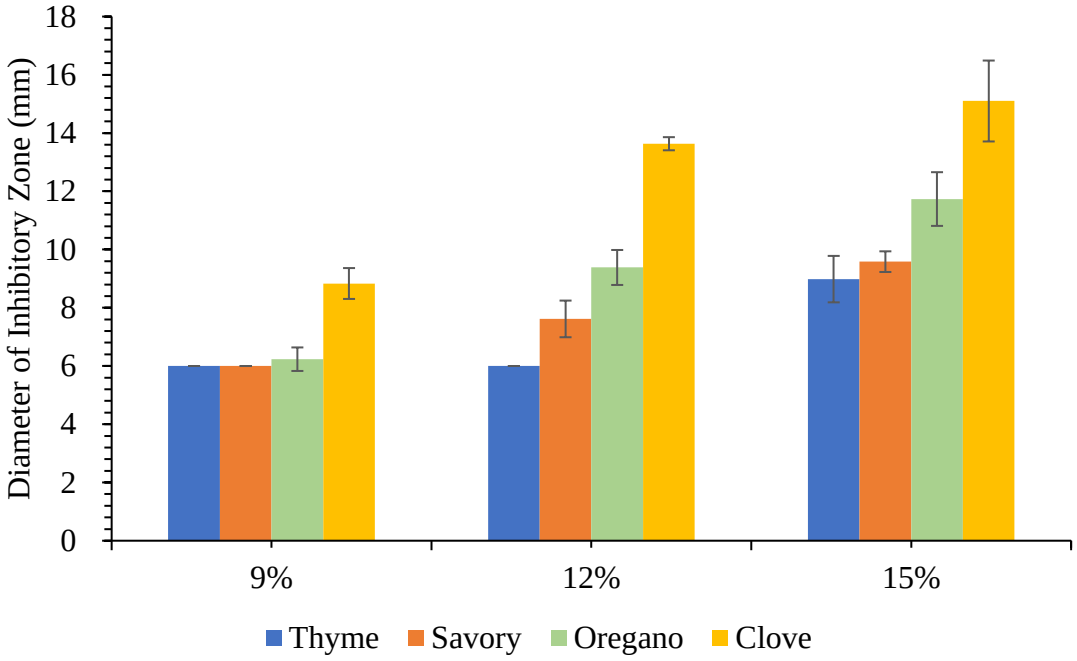

Figure 4- 6**.** The diameter (mm) of inhibitory zone for different EOs against *A. brasiliensis* (NZRM2578)*.
*Freshly grown cultures were used.

With regards to *A. brasiliensis* (Figure 4-6), clove EO had the highest antifungal effect against the fungus for all the tested concentrations ranges (8.83, 13.63 & 15.10 mm at 9, 12 & 15% respectively), followed by oregano EO. Neither thyme nor savory EO could show any inhibitory effect against *A. brasiliensis* at 9% (6 mm), with a minute inhibitory effect for oregano EO (6.23 mm). When the concentration of EOs increased to 12%, the differences among EOs were apparent. Clove EO achieved better inhibition against growth of *A. brasiliensis* than oregano EO, followed by savory EO. These results divided from the founding conducted by Maryam et al. (2006), who believed savory EO should have a better inhibitory effect on *Aspergillus* spp. than clove EO. A possible explanation might relative to the bioactive compounds in clove EO had better diffusion in the lipid phase and reached the fungus cell. Alternatively, the subpopulation of *Aspergillus* spp.

had different resistances against the same EOs.

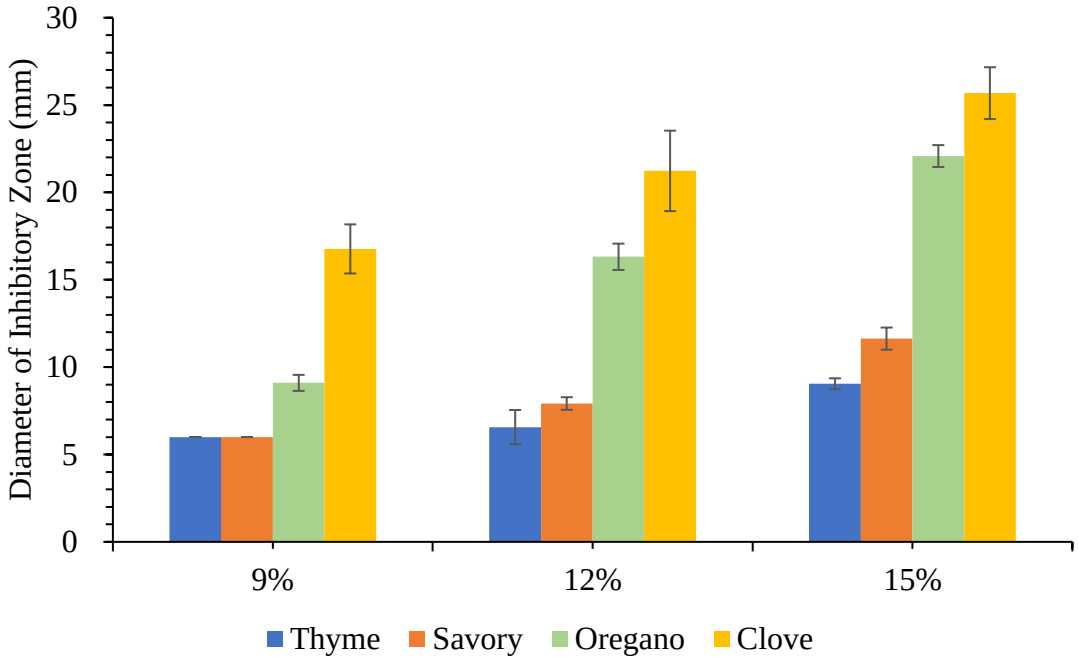

Figure 4- 7. The diameter (mm) of inhibitory zone for different EOs against *P. chrysogenum* NZRM2999*
*Freshly grown cultures were used.

With regard to *P. chrysogenum* (Figure 4-7), similar to *A. brasiliensis* results, thyme and savory did not achieve any inhibitory effect at concentration 9% (diameter around 6mm) and tiny suppressing effect at 12% (lower than 8 mm). On the contrary, both clove and oregano EOs achieved excellent antifungal effects against *P. chrysogenum*. Clove EO produced a large inhibitory zone (16.77 mm) at the low concentration (9%), which was even larger than oregano EO at 12% (16.32 mm), indicating clove EO was effective on inhibiting *P. chrysogenum*. Meanwhile, the inhibitory zone of oregano EO against *P. chrysogenum* tremendously increased (9.10 – 22.1 mm) as the concentration of the oregano EO increased from 9% to 15%, suggesting that the suppression effect of oregano EO against *P. chrysogenum* was highly depended on its concentration.

Overall, oregano EO had the highest antibacterial effect among all the EOs tested using the agar disc diffusion method. Thus, these results were consistent with the broth micro-

dilution assays. However, the highest antifungal effect was obtained with clove EO rather than oregano EO, indicating that the bioactive compounds in clove EO might have better diffusion rates in the lipid phase. Hence, clove EO achieved the best antifungal effects using the agar disc diffusion method (Figure 4-8). When 9% of oregano was used against *A. brasiliensis*, only a tiny inhibition was found. Considering that food matrices are more complex than laboratory media, the dosage of EO should be higher than 9%.

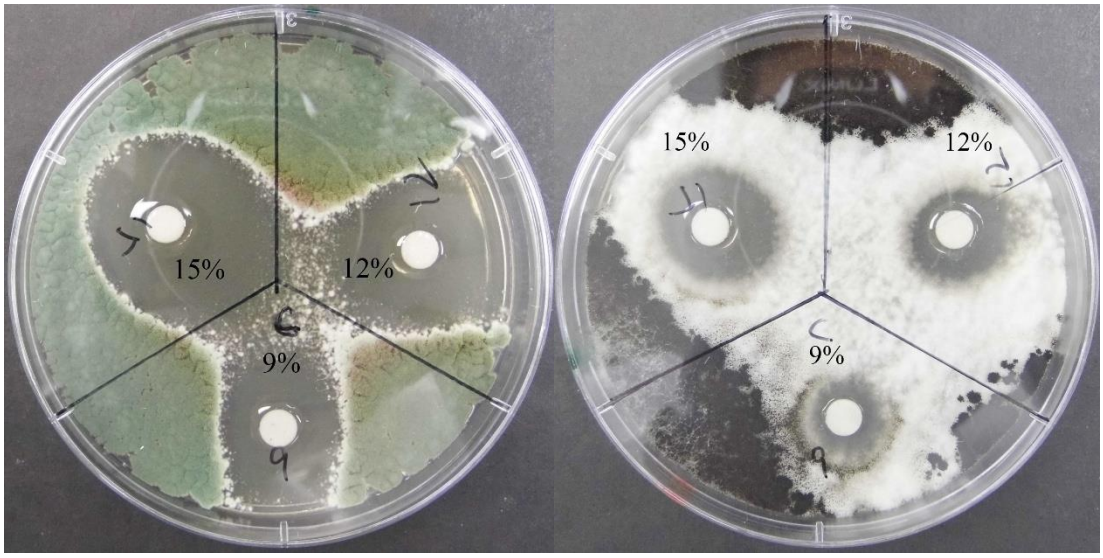

Figure 4- 8. Inhibitory zones of clove EO at different concentrations against *P. chrysogenum* NZRM2999 (Left) and *A. brasiliensis* NZRM2578 (Right).

The normality test (Appendix D1-III) showed that most of the p-values were higher than 0.05. Hence, the null hypophysis was accepted that the results from agar disc diffusion assay obey normal distribution except thyme EO against fungal, which was reasonable that it did not show any antifungal effect at 9%. As a result, those data were deployed in ANOVA analysis.

Table 4- 8. The mean value of the diameter of the inhibitory zone sorted by concentration.

Concentration %	*E. coli* NCTC8196 (mm)	*S. aureus* NCTC4163 (mm)	*A. brasiliensis* NZRM2578 (mm)	*P. chrysogenum* NZRM2999 (mm)
9	12.20^{c}	8.17^{c}	6.77^{c}	9.47^{c}
12	15.86^{b}	9.81^{b}	9.16^{b}	13.01^{b}
15	19.36^{a}	12.44^{a}	11.35^{a}	17.11^{a}

Note: Means with different superscripts within the same column are significantly different at $p<0.05$.

Table 4-8 shows that increases in the concentration of EO produced larger inhibitory zones regardless of the types of oil or strains used ($p<0.05$). Larger inhibitory zone stands for better inhibitory effect. The antimicrobial effect of the EOs could be significantly strengthened by increasing the percentage of EO in soybean oil.

Table 4- 9. The mean values of the diameter of the inhibitory zones (mm) of the Essential Oils.

Essential Oils	*E. coli* NCTC8196	*S. aureus* NCTC4163	*A. brasiliensis* NZRM2578	*P. chrysogenum* NZRM2999
Thyme	$13.86^{d,A}$	7.70^{cB}	6.99^{dB}	7.21^{dB}
Savory	$14.96^{c,A}$	10.44^{bB}	7.73^{cB}	8.51^{cB}
Oregano	$18.14^{a,A}$	11.81^{aBC}	9.12^{bC}	15.83^{bAB}
Clove	$16.27^{b,B}$	10.62^{bC}	12.52^{aBC}	21.23^{aA}

Note: Means with different superscripts (lowercase) within the same column are significantly different at $p<0.05$; Means with different superscripts (capital) within the same rows are significantly different at $p<0.05$.

Overall (Table 4-9), the two bacteria (*E. coli* and *S. aureus*) were significantly more susceptible to oregano EO than the other three EOs ($p<0.05$) at the tested concentrations, followed by clove EO. Bacteria were more resistant to thyme EO ($p<0.05$), shown by the smallest inhibitory halos; 13.86 mm for *E. coli* and 7.70 mm for *S. aureus*. On the other hand, both fungi (*A. brasiliensis* and *P. chrysogenum*) were significantly susceptible to clove EO, with an average inhibitory zone diameter at 12.52 mm for *A. brasiliensis* and 21.23 mm for *P. chrysogenum* respectively, which were larger than the other tested EOs

($p<0.05$). The second best antifungal effect was found with oregano EO. Similar to the bacterial results, the fungi were also most resistant to thyme EO, with a hardly antifungal effect observed, as only 1 mm of clearance was found excluding the diameter of the paper disc. Furthermore, Gram-negative bacterium (*E. coli*) was more susceptible than the Gram-positive bacterium (*S. aureus*) irrespective of the EO used. Previous studies showed that clove EO had a relatively high inhibitory effect on the growth of the Gram-negative bacteria (Moreira et al., 2005). However, the results obtained with the other three EOs, might be relative to the occurrence of unexpected phenomena during the assay (Figure 4-9), which are discussed later. Lastly, *A. brasiliensis* was significantly resistant to clove and oregano than *P. chrysogenum*, the dosage of EO should be increased if the food product is mainly contaminated by *A. brasiliensis*.

4.2.3. Unexpected Phenomena

During this stage of the study, it was unexpected that the Gram-negative bacterium (*E. coli*) would be more susceptible to EOs than the Gram-positive bacterium (*S. aureus*) in both aqueous phase (broth micro-dilution) and lipid phase (agar disc diffusion) assays shown in Tables 4-7 and Table 4-9, since previous studies reported that Gram-negative bacteria should be more resistant against essential oils than Gram-positive bacteria (Smith-Palmer et al., 1998; Cimanga et al., 2002; Burt, 2004; Friedly et al., 2009; Sokovic et al., 2010). Furthermore, different growth morphologies occurred when *S. aureus* was inhibited by oregano, savory and thyme EO from clove EO shown in Figure 4-9.

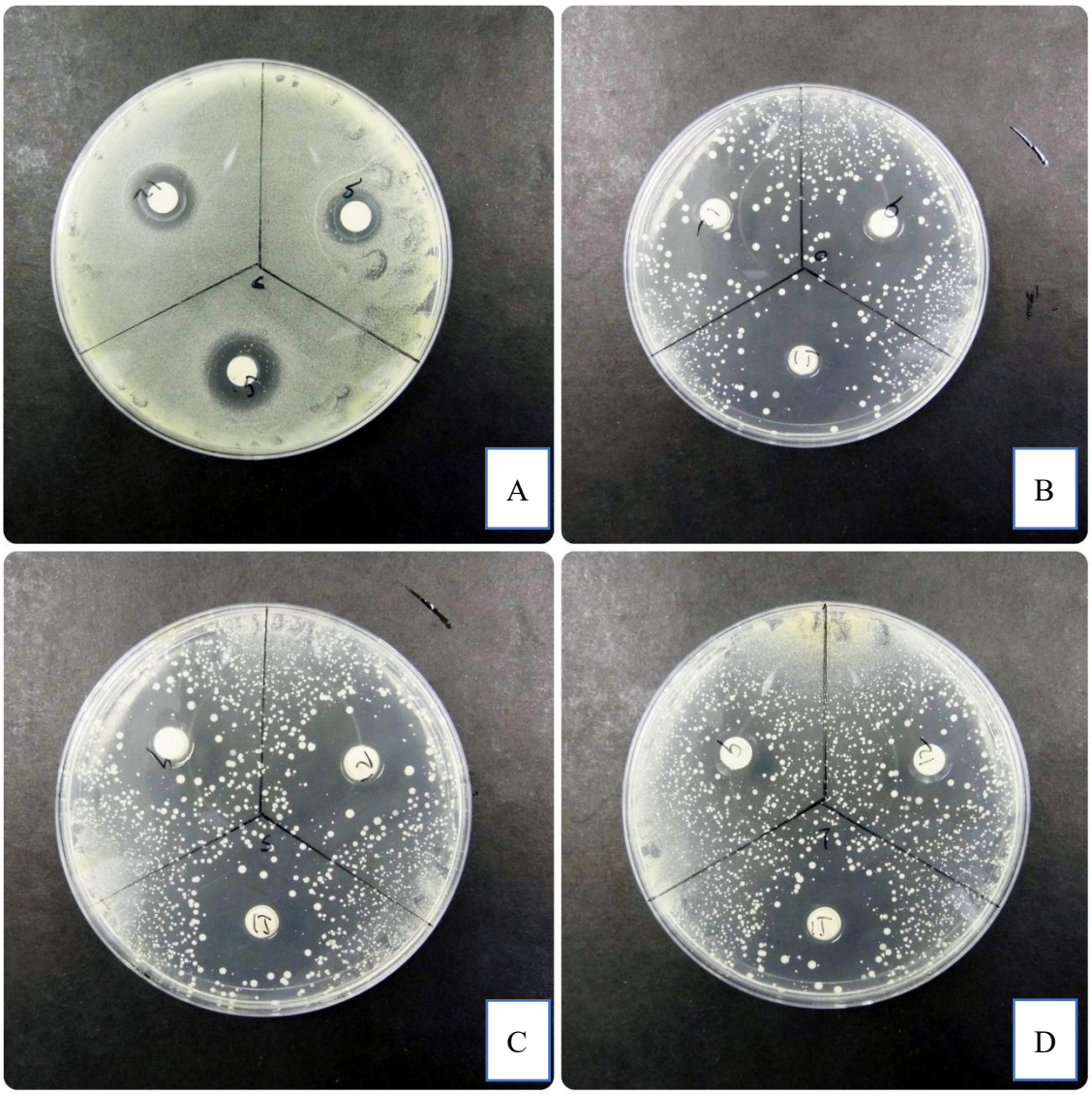

Figure 4- 9. The different phenomena of *S. aureus* NCTC4163 inhibited by different essential oils.

Note: A = Clove EO; B = Oregano EO; C = Savory EO; D = Thyme EO

To confirm whether the unexpected phenomena shown in Figure 4-9 were caused by contamination of the cultures, repeated assays and Gram-stain were conducted. After repeating the experiments, the same results were obtained. In Figure 4-10, the morphologies of the bacteria were identical, which were Gram-positive, cocci-like and clustered, which agreed with published data (Bergey et al., 2012). Consequently, the bacteria could be confirmed as *S. aureus* and therefore, the possibility of contamination was eliminated.

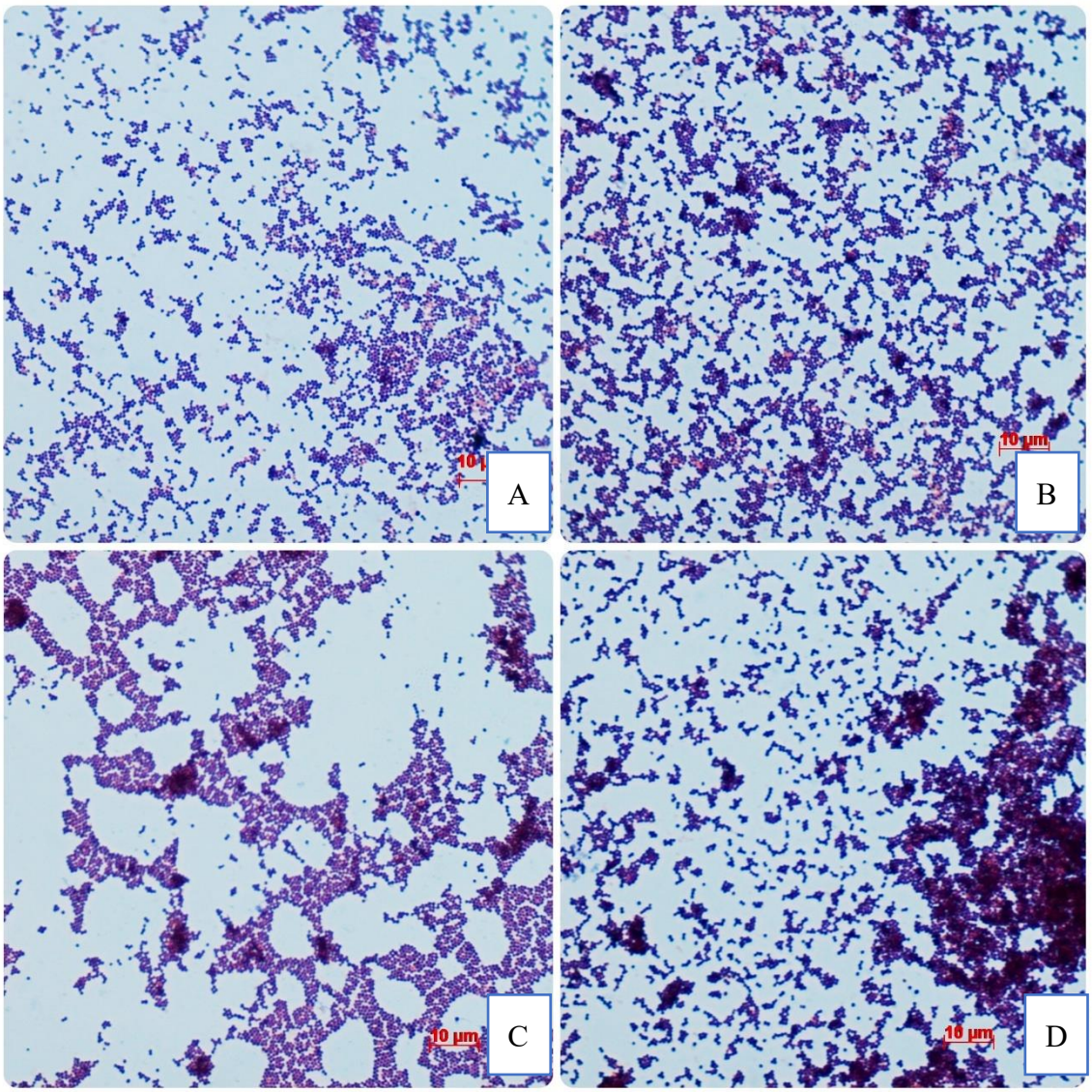

Figure 4- 10. The microscopic image of *S. aureus* NCTC4163 affected by EOs.
Note: Image obtained by Microscope camera; Amplification details: 100x /1 25 Oil; Essential Oils: A = Clove; B = Oregano; C = Savory; D = Thyme.

Currently, there are no previous studies which have reported the grown colonies of *S. aureus* showed relevant morphology when they affected by the essential oil. However, Omar and Miguel (n.d.) described this phenomenon as “Heteroresistance”, which means the subpopulations of a bacteria behave a level of susceptibilities against one or several specific antibiotics. A standard method to determine the presence of Heteroresistance has not been reported. The phenomenon would affect both the agar diffusion method and MIC

test results. Several bacteria were reported to have Heteroresistance subpopulations against traditional antibiotics, including *S. aureus* (Band & Weiss, 2019). According to Omar and Miguel (n.d.), a treatment was failed when vancomycin was used to inhibit *S. aureus* as certain subcultures of *S. aureus* could not be suppressed by the antibiotic.

Thus, it was possible that the clear zone between isolated large colonies of *S. aureus* was caused by the inhibitory effect given by essential oils. The isolated white spots were probably the subpopulations of *S. aureus* that had strong resistance against EOs of savory, oregano and thyme. Comparing the composition of the EOs, it shows that thyme, oregano and savory EOs have very similar major components, which are dominated by the high levels of carvacrol, p-Cymene, γ-Terpinene and thymol, shown in Table 4-2. Meanwhile, the tested clove EO did not contain these compounds as its main constituent, but consisted of a high level of Eugenol (78.87%) and Acetate D'Eugenyle (14.01%). The differences in the major compounds might cause the variations in the suppression of *S. aureus*.

It should be pointed out that at the very edge of the plates of thyme, savory and oregano, the density of the cultures was similar to the one on clove EO plate in Figure 4-9, indicating that thyme, savory and oregano EO would affect a relatively large area of the *S. aureus*. If the plate had been large enough, the morphology on clove EO plate could have reappeared on the other three EO plates. Nonetheless, the reference information on the disc diffusion method provided by EUCAST (2017) stated that for *S. aureus*, the inhibitory zone is determined by the closest colony to the paper disc containing the tested material and no noise could be exempted, where noise was defined as isolated colony found around the paper disc (EUCAST, 2017). Hence, the inhibitory effects indicated by agar disc diffusion assays for the four EOs against *S. aureus* (Gram-positive) were pooper than *E. coli* (Gram-negative). Similarly, that could be the reason that the viable subcultures of *S. aureus* in the broth micro-dilution assay could have produced a negative result at relatively high concentration wells. Hence, *S. aureus* was more resistant than *E. coli* against thyme, savory and oregano EOs in broth micro-dilution assays.

4.3. Conclusion

Overall, oregano EO achieved the best antimicrobial effects with the lowest MICs on both bacteria (0.04%-0.06%) and fungi (0.14%) in the aqueous phase assays using the broth micro-dilution method. Meanwhile, in the lipid phase, oregano EO also achieved the best antibacterial effect. However, the best antifungal effect was found with clove EO. Therefore, both oregano and clove EOs would be further studied and carried on to the next phase of the experiment.

5. Determination of Optimum Concentrations of Clove and Oregano Essentials Oils on their Antimicrobial Effect

Results from the previous section (chapter 4) showed that oregano essential oil to be the most effective bioactive against bacteria, whereas fungi were the most susceptible to clove essential oil. Hence, clove and oregano EOs were included in the lipid phase formulation design. This stage focused on establishing optimum formulations of the EOs that would not only provide considerable microbial inhibitory effect, but also meet customer acceptability of the pre-cooked Hokkien noodles.

In terms of formulation design, especially in investigating the ratio between different components, the mixture design was used to determine the optimum proportions of clove, oregano EO and soybean oil in the final formulation. The total amount of added lipid was kept constant to maintain the consistency between noodle products; therefore, the soybean oil was partially replaced by clove and/or oregano EOs. Soybean oil was added onto the noodle surface during production to prevent strings from sticking together.

Therefore, the objectives of this stage were to:

A. Use mixture design to set up the DOE that evaluated the antimicrobial effect of EOs within feasible trials.
B. Evaluate the acceptability of EOs added Hokkien noodle in customers.
C. Determine the antibacterial effect of the combinations of the EOs.
D. Determine the antifungal effect of the combinations of the EOs.
E. Generate the response surfaces to predict the optimum combination in antimicrobial aspects and customer sensory acceptance aspect.

5.1. Material and Methodology

5.1.1. Material

Oregano and clove EOs were purchased from Florihana Ltd (Riviera, France). Soybean oil and Hokkien noodle samples were supplied by LIANHUAT Ltd (Auckland, New Zealand). Standard Plate Count Agar (SPCA) was purchased from Oxoid™ Ltd (Auckland, New Zealand). Yeast extract Glucose Chloramphenicol Agar (YGCA) was obtained from Microbiology Ltd (Auckland, New Zealand). Sensory cups (20 mL) with lids were purchased from Thermo Fisher Scientific (Auckland, New Zealand).

5.1.2. Mixture Design

Mixture design was used to investigate the optimum proportions of the three different oils comprising of oregano EO (A), clove EO (B) and soybean oil (C, control). The total amount of lipid added in the oiling step was kept constant at 1% (w/w, oil/noodle). The lower limit of essential oils (A + B) was set as not lower than 9% (w/w, EO/oil) to ensure the presence of the antimicrobial effect, which was determined by the results of phase one. Also, high concentration of EOs might bring an unpleasant flavour leading to reduced consumer acceptability, hence, the upper limit of total EOs was set at 15% (w/w, EO/oil) (Firouzi et al., 2007). Samples collected from two different days were designated as two blocks. The purpose of the ‘blocking’ was to manage any uncontrollable factors in the experiment. Microbial results and overall acceptability were set as the responses for the mixture design model. This setting resulted in the generation of a mixture design shown in Table 5-1.

Table 5- 1. The limitation conditions of mixture design.

Type	Parameter	Range
Independent	Oregano EO (A)	0 - 15 %
Independent	Clove EO (B)	0 - 15 %
Independent	Soybean Oil (C)	85 - 91%
Dependent	Total plate count	TSTB
Dependent	Yeasts and moulds count	TSTB
Dependent	Overall acceptability	TLTB

Note: TSTB = the smaller the better, TLTB = the larger the better

The mixture design was set up by applying the condition listed in Table 5-1. There were 9 combinations generated by the mixture designs (Minitab 18, Minitab, USA) listed in Table 5-2.

Table 5- 2. The proportions of 9 combinations of oils generated by mixture design.

Order	Oregano %	Clove %	Soybean oil %
1	10.5	3.0	86.5
2	7.5	3.0	89.5
3	0.0	9.0	91.0
4	6.0	6.0	88.0
5	15.0	0.0	85.0
6	9.0	0.0	91.0
7	0.0	15.0	85.0
8	3.0	7.5	89.5
9	3.0	10.5	86.5

In addition, the original sample (S0) which did not contain any EO was also prepared as the control to provide the baseline in microbial test and sensory evaluation. To eliminate any uncontrollable variants, the experiment was repeated by collecting the samples on two-different batches. The first batch trial was set as block 1 and the second batch was set as block 2. The difference occurs between manufactured batch and uncontrollable factors were covered by block.

5.1.3. EOs Added Hokkien Noodle Samples Preparation

The EOs and soybean oil were pre-mixed following the mixture design (Table 5-2), then

were sealed in brown bottles before they were used. The samples were prepared and packaged at the local Hokkien noodle factory following the commercial standard production procedure, except for the oiling step. For oiling, 100 g of Hokkien noodles were dispensed into a sterile four-layered (Figure 5-1; Table 5-3) plastic bag made of PVdC-coated OPP (Trias Sentosa, Australia), and then 1 g of the mixed oils was applied (1% w/w, oils/noodles). The samples were flushed with premixed modified atmosphere gas 30:70 (CO_2: N_2) (ALIGAL 13, Air liquide, New Zealand) before heat-sealing using a sterile vertical packaging machine (AS 520Y, ArrowSystems SDN BHD, USA). The details of the supplier of material and apparatus were shown in Table 5-4.

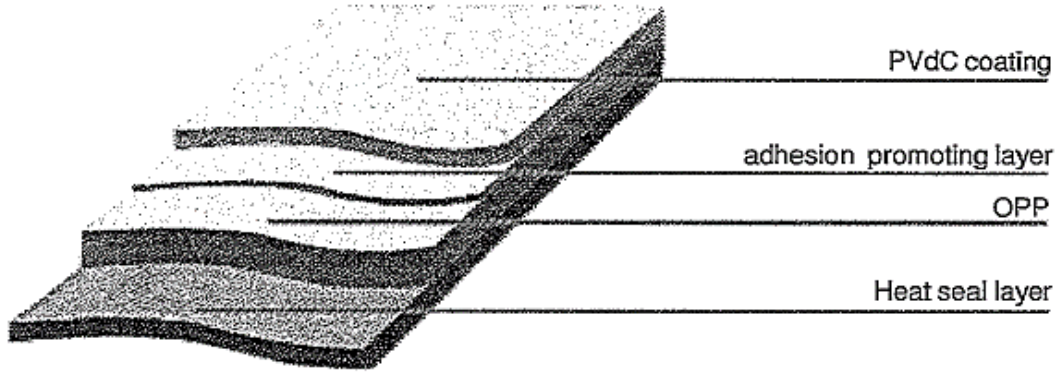

Figure 5- 1. The multiple-layered packaging material for Hokkien noodles (Trias Sentosa, 2019)

Table 5- 3. The permeability of OPP, PVdC and PVdC coated OPP

Type of Film	O_2	CO_2	N_2	Water vapour
	mL/m2 day atm			g/m^2 day atmosphere
OPP	2000	8000	400	6-7
PVdC	2-4	20-30	35-50	0.5-1
PVdC coated OPP	10-20	35-50	8-13	4-5

Source: Parry, (2012); McMillin, (2008); Aspen et al., (2001).

Table 5- 4. The supplier of material and apparatus for noodle packaging

Material or Apparatus	Model (Provider)
Packaging bag	PVdC Coated OPP Film FOS (Trias Sentosa)
Pre-mix modified gas	ALIGAL 13 (Air liquide)
Stomacher	1669/500 (IUL Instruments)
Vertical Packaging System	AS 520Y (ArrowSystems SDN BHD)

The prepared samples were immediately transported to Massey University, Auckland Campus under cold chain (4°C) and were stored in lightproof 4˚C walk-in refrigerator until required for analysis. The experiments were repeated twice (block-2).

5.1.4. Sensory Test

The sensory test was conducted with the approval given by the Massey University Human Ethics Committee (Application ID: 4000020116). For sensory evaluation, the samples were prepared by transferring 1-2 noodle strings to a sensory cup and then sealed with a lid. The sealed cups were stored at 4˚C for 2 hours before sensory evaluation. All sensory samples were coded by 3-digital random numbers (Cochran and Snedecor, 1981) as shown in Table 5-5.

Table 5- 5. Random digital code for noodle samples during sensory test.

Sample	Random Code	Oregano EO %	Clove EO%	Soybean oil %
0	289	0.0	0.0	100.0
1	341	10.5	3.0	86.5
2	744	7.5	3.0	89.5
3	812	0.0	9.0	91.0
4	200	6.0	6.0	88.0
5	804	15.0	0.0	85.0
6	935	9.0	0.0	91.0
7	636	0.0	15.0	85.0
8	273	3.0	7.5	89.5
9	318	3.0	10.5	86.5

Sixty-five sensory participants ageing from 18-60 were randomly invited to the sensory test. Participants were required to evaluate the product for appearance and odour through

observation and smelling, then gave the score for the overall acceptability (OA) using the 9-point hedonic scale, with 1 as dislike extremely and 9 as like extremely (Appendix B). Score higher than 5 (including 5) were considered as the sample was accepted.

5.1.5. Microbiological Analysis

The microbiological tests consisted of Standard Plate Count (SPC) and Yeasts and Moulds Count (YMC) following the standard procedures (ISO, 2012; ISO, 2013), respectively. For each sample, 25 g of noodle strings were transferred into sterile stomacher bag (5.5" x 9", Fisherbrand, New Zealand), followed by adding 225 g of 0.1% peptone water, then mixed for 90 seconds in the stomacher (1669/500, IUL Instruments, Germany). Afterwards, 1 mL of the sample solution was added to 9 mL of sterile 0.1% peptone water, and then suitable serial dilutions were prepared. For plating, 15 mL of molten SPCA or YGCA were mixed with 1 mL diluted samples in Petri dishes except for 10^0 plates. For 10^0 plates, 3.333 mL of 10^{-1} sample solution was transferred to a sterile petri dish followed by pouring 20 mL of molten SPCA or YGCA, three plates were conducted for one sample ($9.999 \approx 10$ mL of 10^{-1} sample solution in total). The plates were allowed to solidify at ambient temperature (20°C) before being flipped over and incubated at 35˚C for 48 hours (SPCA plates) and 25 ˚C for 120 hours (YGCA plates). The microbiological tests were duplicated.

5.1.6. Statistical Analysis of Data

The sensory results were analysed by SPSS Statistics 24.0 (IBM^{TM}, USA) for Frequencies summary of acceptability percentage. Also, the paired T-Tests were conducted for comparing treatment samples (EOs added) and the control sample (289, no EO added). The null hypothesis was that the mean score of EO added sample was not significantly different to the control sample; the alternative hypothesis was that the mean score of EO added sample was significantly different to the control sample (two-tailed). The results of standard plate count (SPC), yeasts and moulds count (YMC), and overall acceptability

rates (OA%) were processed by Minitab 18.0 (Minitab Ltd, USA) for establishing regression models of the mixture design. Three response surfaces were generated to predict the optimum formulation for the oil coating of the noodle product.

5.2. Results and Discussion

5.2.1. Sensory Evaluation

The sensory test results are shown in Table 5-6. The control sample (289) (original formulation) obtained the highest scores for odour and overall acceptability at 75.4% and 76.9%, respectively, although its appearance was the lowest (73.7%). This might because the OA was dominated by the odour, while the appearance had little impact on the OA, indicating the main obstacle of applying EOs on any food product was its strong odour (Shiau & Yeh, 2001; Ioannis, 2012). This dominant phenomenon also can be seen from such as sample 812, even it obtained the highest score of appearance (89.2%), however, the OA of it was lower than the Sample 289 (73.8%). The result could be attributed to the OA of samples was mainly dominated by their odour.

Table 5- 6. The sensory results of Hokkien noodles-coated with essential oils.

Sample	Code	Oregano EO %	Clove EO%	Soybean oil %	Acceptability %		
					Odour	Appearance	Overall
0	289	0.0	0.0	100.0	75.4	73.8	76.9
1	341	10.5	3.0	86.5	49.2	83.1	61.5
2	744	7.5	3.0	89.5	53.8	84.6	63.1
3	812	0.0	9.0	91.0	73.8	89.2	75.4
4	200	6.0	6.0	88.0	52.3	81.5	64.6
5	804	15.0	0.0	85.0	36.9	80.0	53.8
6	935	9.0	0.0	91.0	41.5	80.0	58.5
7	636	0.0	15.0	85.0	52.3	86.2	70.8
8	273	3.0	7.5	89.5	49.2	83.1	66.2
9	318	3.0	10.5	86.5	44.6	87.7	67.7

Note: A 9-point hedonic scale was used with 1 as lowest score, and 9 as highest; scores above 5 (including 5) were considered acceptable; The total frequencies of the accepted

score (5-9) were expressed in acceptability percentage.

The OA peaked for the original sample, and then decreased as the concentration of either EO increased (Table 5-6). For oregano EO, the OA dropped from 76.9% (0% oregano EO, sample 289) to 58.5% (9% oregano EO) and bottoming at 53.8% (15% oregano EO). However, noodles coated with clove oil was more acceptable to the sensory participants. The OA was only decreased by 1.5% (to 75.4%, sample 812) when 9% of clove EO was added, even 15% of clove EO (sample 636) was able to maintain over 70% of OA (70.8%). Moreover, in terms of appearance, all EO(s) added samples had higher acceptability than the original sample (73.8%, sample 289). It might relate to the EO(s) could exhibit the antioxidant effect to some extent that maintained the bright-yellow colour of the Hokkien noodle by preventing it from the browning reaction.

The paired sample T-Tests were conducted between noodles with added EOs and the control sample (289) to verify whether those EOs combinations could significantly affect the overall acceptability. The results are shown in Table 5-7.

Table 5- 7. Paired T-Test results of overall acceptability of EOs coated samples to the original sample (289).

Pair number	Paired Samples	Mean	Std. Deviation	Sig. (2-tailed)
Pair 1	318 - 289	-0.492	2.359	0.097
Pair 2	273 - 289	-0.708	2.213	0.012
Pair 3	636 - 289	-0.462	1.993	0.066
Pair 4	955 - 289	-0.923	2.426	0.003
Pair 5	804 - 289	-1.077	2.287	0.000
Pair 6	200 - 289	-0.538	2.144	0.047
Pair 7	812 - 289	-0.292	1.800	0.195
Pair 8	744 - 289	-0.692	2.143	0.011
Pair 9	341 - 289	-0.769	2.178	0.006

Notes: The proportion of oils of each sample was shown in Table 5-5; Sample 289 (control) did not contain any EOs.

Table 5-7 shows that there were three pairs that had a p-value higher than 0.05, which included 318-289 (0.097), 636-289 (0.066) and 812-289 (0.195) (highlighted in the red). It indicated that for those three samples, their OA were not significantly different comparing to the original sample. Detailly, samples 636 & 812 contained clove EO only, while 318 contained 3.0% oregano and a high concentration of clove EO (10.5%). These results indicated that although the odour of high concentration (>9%) of clove EO was noticeable, it did not raise significant aversion within the participants at 15% dosage or below. When comparing pairs (273-289) and (318-289), both 273 and 318 contained 3% of oregano EO, but 318 contained 3% more clove EO resulting in higher OA%, implying that certain amount of clove EO could weaken the undesirable odour of oregano EO, and increased the OA%.

5.2.2. Standard Plate Counts

As shown in Figure 5-2, the original sample (S0) had the highest aerobic plate counts throughout the period, which increased around 10 times from 3.31 log CFU/g on day-0 to 4.26 log CFU/g after 21 days of storage. Also, the control was the only sample that contained more than 4.5 log CFU/g at the end of the storage period, day-35.

For most of the EO(s) added samples except S1, the plate counts decreased within the first week, then remained stable until the end of the experimental period. The MAP and low-temperature storage conditions might be the reasons that led to the decrease (*ca* 1 log) of SPC on day 7. Further, S3 and S7 (without oregano EO), had higher viable aerobic cell counts than other samples containing EOs from day-21 to the end of the experiment. The cell counts at the end of the experiment were >4 log CFU/g. The results indicated that oregano EO had a relatively strong antibacterial effect, which in lined with Carmo et al. (2008) and Gallucci et al. (2014), and could better inhibit the growth of bacteria than clover EO at the same dosage. This result was agreed with the key finding in phase one.

However, the best antibacterial results were achieved not only on S5 (oregano EO at 15%),

but also on S8 and S9, which were containing a low concentration of oregano EO (3%) that exhibited the same level of antibacterial effect. There was a possible synergetic antimicrobial effect in samples S8 and S9 which had lower total EOs concentrations (10.5% and 13.5%, respectively) than S5 (15%).

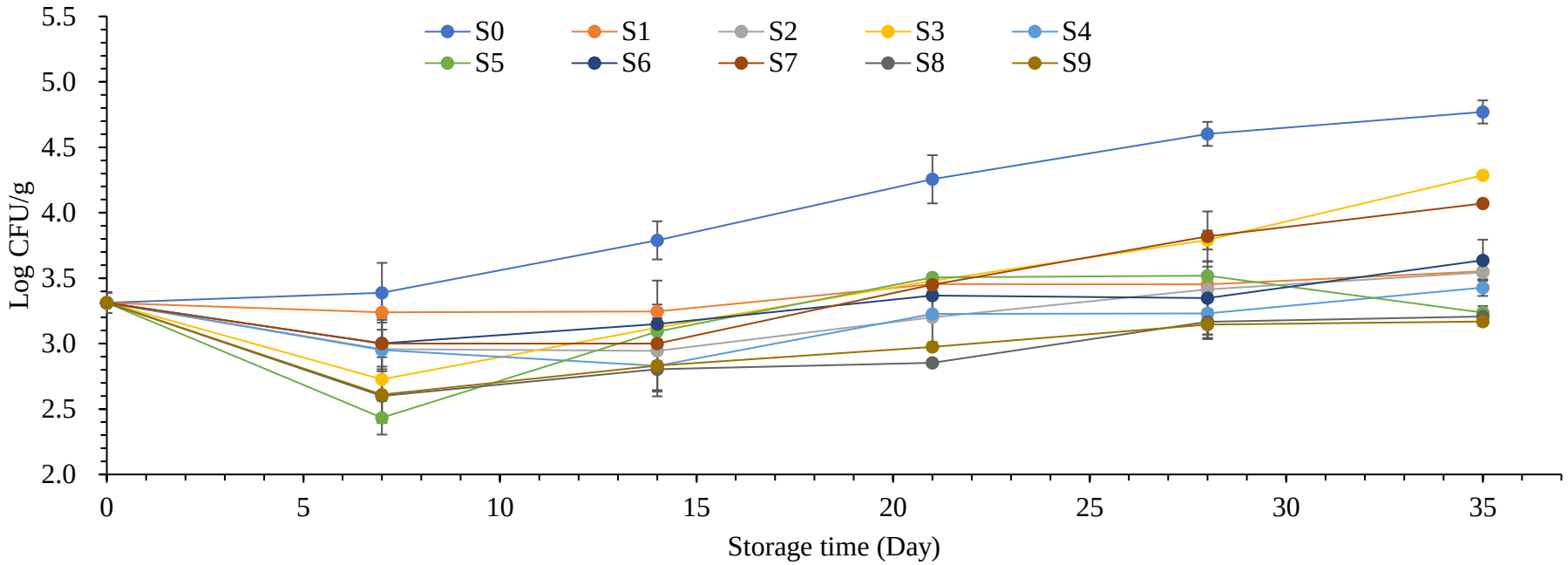

Figure 5- 2. Aerobic bacteria growth cell counts (log CFU/g) on Hokkien noodle samples.

Note: The ratio of 3 components A:B:C (A = Oregano EO %; B = Clove EO %; C = Soybean oil %) in each sample were: S0 = 0:0:100; S1 = 10.5:3:86.5; S2 = 7.5:3:89.5; S3 = 0:9:91; S4 = 6:6:88; S5 = 15:0:85; S6 = 9:0:91; S7 = 0:15:85; S8 = 3:7.5:89.5; S9 = 3:10.5:86.5; Standard deviation obtained from two independent experiments; n = 2.

5.2.3. Yeast and Mould Counts

The results for yeasts and moulds were different from the bacterial results (Figure 5-3). YMC were gradually decreased for all samples with added EOs. None of them could achieve over 1.6 log CFU/g of fungi at the end of the period. On the contrary, for the control (S0), YMC increased to 2.2 log CFU/g in the first week, then gradually decreased for the next three weeks before bottomed at 1.59 log CFU/g by day-28, then started to ascend at the end of the period (1.89 log CFU/g). The possible reasons could be due to the combined effect of low-temperature storage and MAP which suppressed the growth of yeasts and moulds for about a month. After that, the growth of fungi on the control sample (S0) overwhelmed the treatments, and started to multiply under chill and anaerobic conditions. Meanwhile, EOs were able to continuously restrain the growth of fungi after day-28, as they provided a complex chemical environment containing bioactive compounds that behaved antifungal effect. In terms of EO(s) added samples, the best results were obtained from S8 and S9, which the YMC values were gradually decreased throughout the period. Both of S8 and S9 have been recorded at 0.86 log CFU/g on the day-35, and were expected to keep decreasing if storage period had extended.

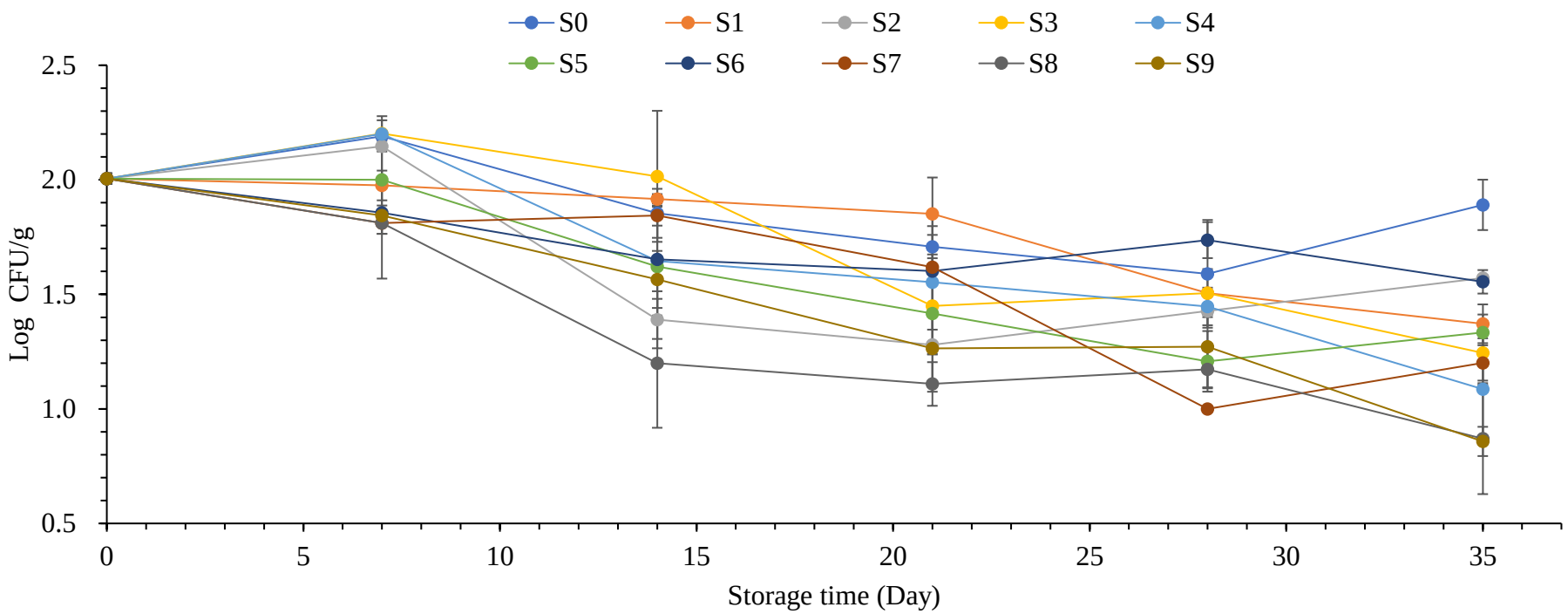

Figure 5- 3. Yeasts and Moulds Growth cell counts (log CFU/g) in Hokkien noodle samples.

Note: The ratio of 3 components A:B:C (A = Oregano EO %; B = Clove EO %; C = Soybean oil %) in each sample were: S0 = 0:0:100; S1 = 10.5:3:86.5; S2 = 7.5:3:89.5; S3 = 0:9:91; S4 = 6:6:88; S5 = 15:0:85; S6 = 9:0:91; S7 = 0:15:85; S8 = 3:7.5:89.5; S9 = 3:10.5:86.5; Standard deviation obtained from two independent experiments; n = 2.

Comparing the YMC results with the SPC results, it was noted that sample 5 (one of the best samples in antibacterial aspects) was no longer achieved the same level of antifungal effect as S8 or S9 did. High level of oregano EO failed to achieve the antifungal effect as the combinations of EOs did. It could be concluded that the synergetic antimicrobial effects found in S8 and S9 were valid for both bacteria and fungi, and could better inhibit the growth of microbes than single EO. This result was in line with Tatjana et al. (2014) and Filomena et al. (2017), who reported that foodborne pathogens were susceptible to two or more mixed EOs. Also, if there is a synergetic effect on inhibiting bacteria, it should be able to inhibit the growth of fungi to a certain extent. Table 5-8 shows the statistical analysis on both standard plate count and yeasts and moulds counts results obtained at the end of the period.

Table 5- 8. The mean value of microbial results of two round at day-35.

Code	Oregano EO %	Clove EO %	Mean value (CFU/g)	
			SPC	YMC
S0	0.0	0.0	51000^{d}	93^{g}
S1	10.5:	3.0	3220^{a}	27^{e}
S2	7.5:	3.0	3600^{a}	37^{f}
S3	0.0	9.0	18750^{c}	22^{cd}
S4	6.0	6.0	2970^{a}	18^{b}
S5	15.0	0.0	1870^{a}	25^{de}
S6	9.0	0.0	3350^{a}	33^{f}
S7	0.0	15.0	11450^{b}	18^{bc}
S8	3.0	7.5	1575^{a}	5^{a}
S9	3.0	10.5	1400^{a}	8^{a}

Note: Means with different superscripts within the same column are significantly different at $p<0.05$

Table 5-8 shows that the addition of EOs on Hokkien noodles suppressed the growth of bacteria, yeasts and moulds ($p<0.05$) and should be able to extend the shelf-life of Hokkien noodle in some extent. Furthermore, in terms of standard plate count, the samples that only contained clove EO (such as S7 & S3) had significantly higher SPC (11450 CFU/g for S7; 18750 CFU/g for S3) than the other EOs added candidates.

Correspondingly, no significant difference was found within the rest of the samples containing oregano EO ($p>0.05$), regardless of which concentration of oregano EO was applied. With regards to yeasts and moulds count, S8 (5 CFU/g) and S9 (8 CFU/g) were significantly lower than any others ($p<0.05$), and even significantly better than S5 (15% oregano) and S7 (15% clove) at 95% confidence level. The lower dosage of EOs with better antifungal effect might indicate that the synergetic effect occurred in S8 and S9, resulting in the best antimicrobial effect on both bacterial and fungal assays.

5.2.4. Mixture Design Modelling

Three response surfaces (SPC, YMC and OA%) were generated for the mixture design model, to predict the optimum combination of EOs to exhibit desired antimicrobial effect and customers satisfaction. Figure 5-4 shows the available range of factors in the mixture design. The range area is shown as an isosceles trapezoid, indicating a three-component design with two ingredients sharing the same range. Also, EOs have shown to behave the antimicrobial effect, hence, the combination without any EO was not considered (soybean oil = 100%). The microbial results and OA% were set as the responses for the mixture design.

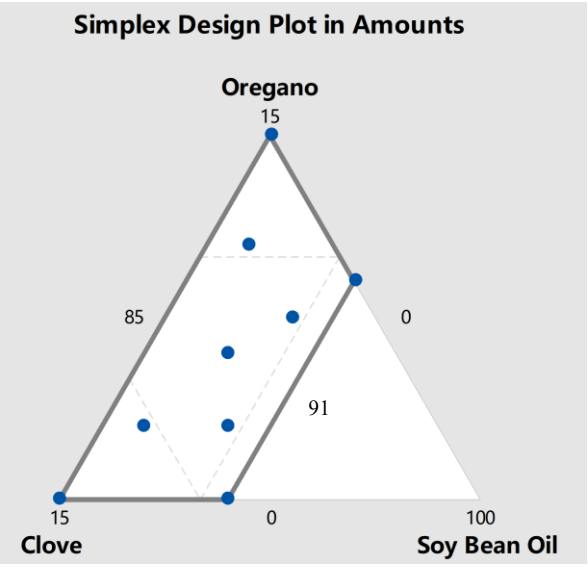

Figure 5- 4. The output of the available range for 3 components in mixture design.

Before generating the response surface model, SPC, YMC and OA% were conducted for the residual plots to examine their normality by the Normal Probability Plot and Histogram by Minitab 18 (Minitab, USA). Furthermore, the homogeneity of variance was determined by the Versus Fits plots. Lastly, the randomness of observation is shown by the Versus order graph. The results are shown in Figures 5-5, 5-6 and 5-7, respectively.

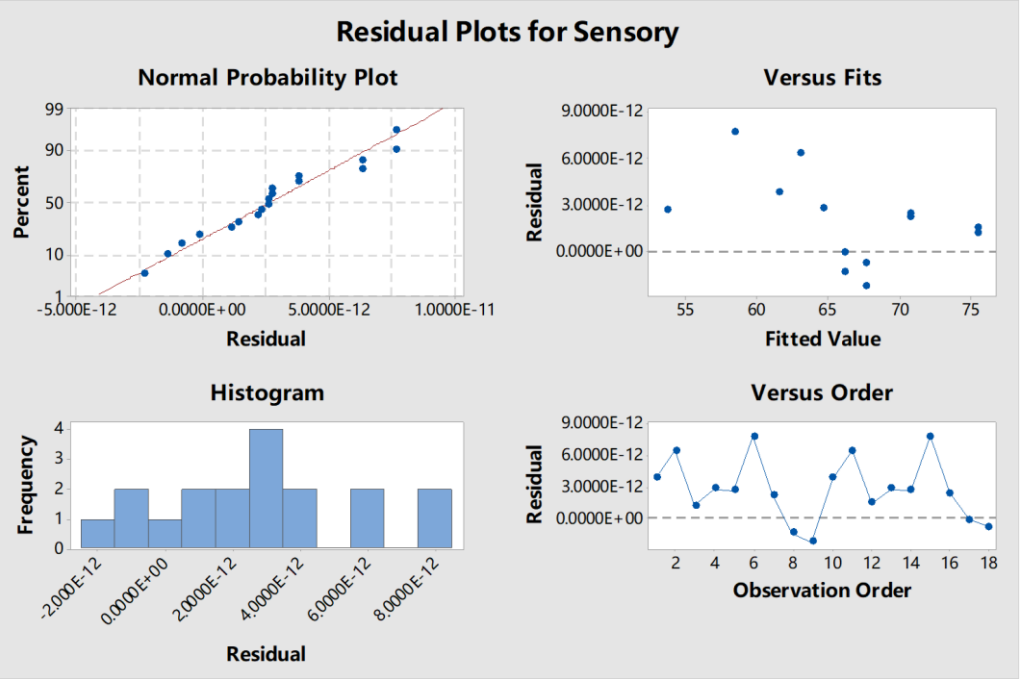

Figure 5- 5. The output of the residual plots of overall acceptability percentage in the mixture design modelling.
Notes: The normal probability plot verify if residuals were normally distributed; Histogram was used to show any outliers or skewness of data; versus fits show the homogeneity of variance of residual; versus order was used to verify the independence of the observations.

For overall acceptability (Figure 5-5), although the histogram did not show a perfectly normally distributed shape (bell shape), there was no obvious skewness. Also, most of the data points in normal probability plot were aligned along the line of best fit, implying the residuals fitted the normal distribution.

The ‘Versus fit graph” showed that even the variances were not perfectly distributed on either side of the zero-line, but neither the fanning effect nor funnelling effect was found.

Lastly, there was no successive ascend or descend points in the observation order graph, which means the observations were independent. The model for OA, therefore, was acceptable.

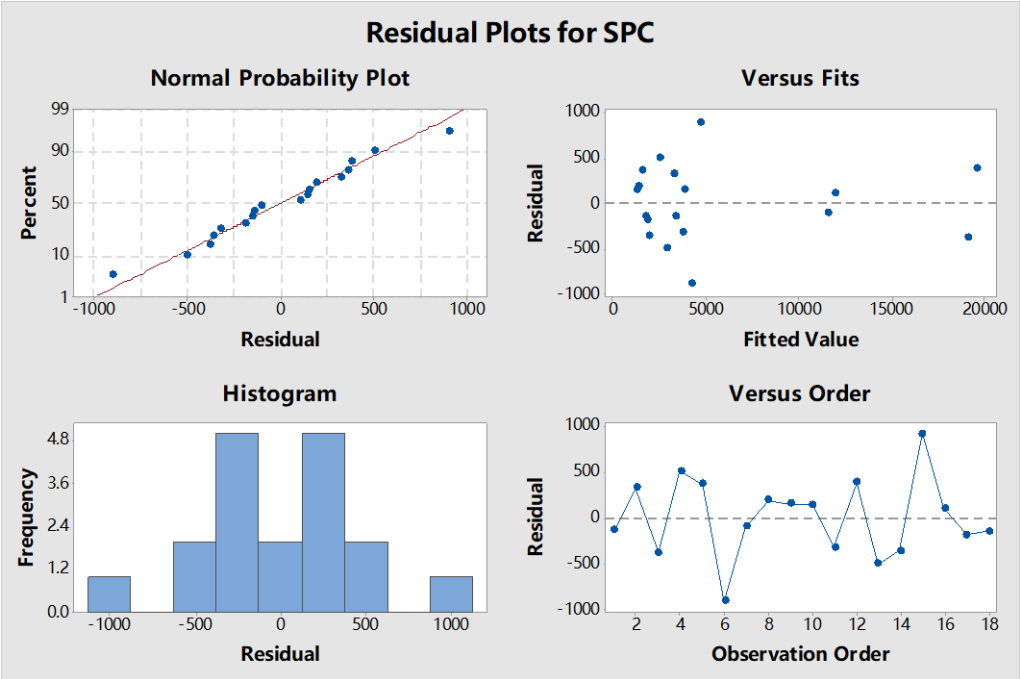

Figure 5- 6. The output of the residual plots of standard plate counts in the mixture design modelling.
Notes: The normal probability plot verify if residuals were normally distributed; Histogram was used to show any outliers or skewness of data; versus fits show the homogeneity of variance of residual; versus order was used to verify the independence of the observations.

In terms of the aerobic bacteria counts (Figure 5-6), a rough bell shape was found in the histogram graph and most of the data points were lined up in the normal probability plot, which implied that the SPC results were normally distributed. Furthermore, there were no successive ascend or descend point in the Versus order, indicating the observation was independent.

In Versus fit graph, the data points were evenly located on either side of the zero-line, it was observed that four points were located away from other points, hence, those points were considered as influential points. The reason for this phenomenon was that the EO-

treatment combinations containing oregano had relatively low SPCs and differences were found when comparing with the sample that contained clove only ($p<0.05$). Alternatively, the diversity of the growth of the bacteria might also cause the dispersion of the data plots in the versus fit graph (Jay et al., 2005). Nonetheless, there was no funnel-shaped or fan-shaped occurred, therefore the SPC data were acceptable.

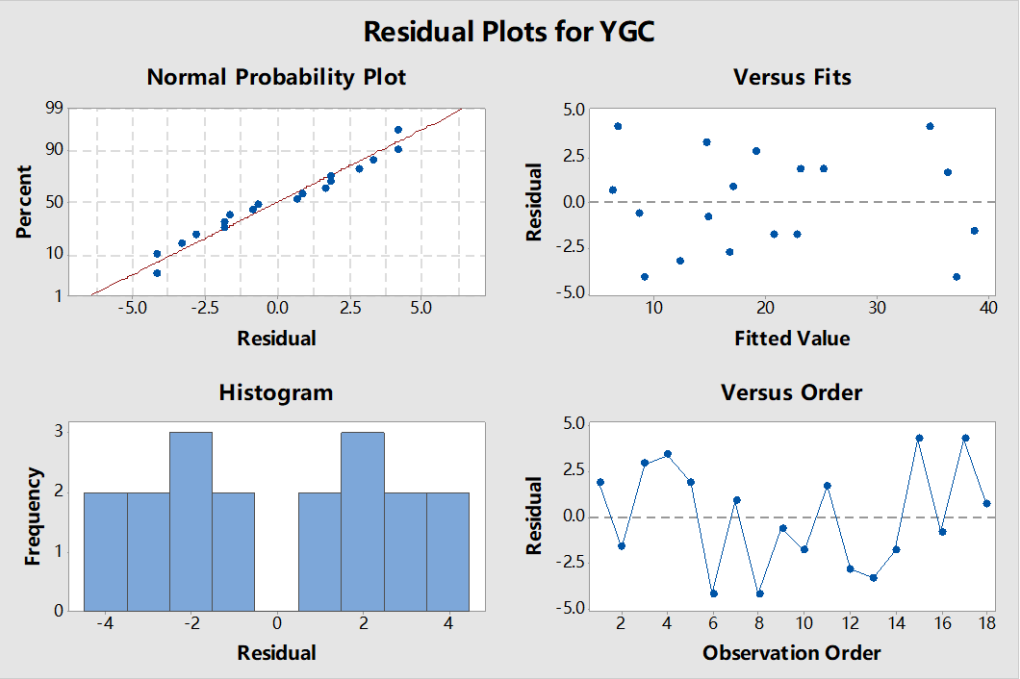

Figure 5- 7. The output of the residual plots of yeasts and moulds counts in the mixture design modelling.
Notes: The normal probability plot verify if residuals were normally distributed; Histogram was used to show any outliers or skewness of data; versus fits show the homogeneity of variance of residual; versus order was used to verify the independence of the observations.

With regard to the yeasts and moulds counts (Figure 5-7), the histogram and normal probability plot showed that the counts on fungi were normally distributed. The 'Versus fit graph' showed that all data points were evenly distributed, indicating that the homogeneity was found in the variance. Lastly, no successive ascend or descend point in the versus order graph implying that the observations were independent.

For the results, three response surfaces have been generated, expressed by three equations.

The regression formulation of SPC, YMC and OA% are shown in equations (13) (14) and (15).

$$SPC = -2945070A+943166B+16785C-21476AB+51184AC-12206BC+520ABC-35AB(A-B)+252\ AC(A-C)-223 \quad (13)$$

$R^2 = 99.48\%$

$$YMC = -17052.6A-1165.6B-20.1C+36.9AB+273.8AC+15.1BC+0.5ABC-0.2AB(A-B)+1.0AC(A-C)+1.2 \quad (14)$$

$R^2 = 93.68\%$

$$OA\% = -2495.65A+10.1B+2.66C+0.09AB+41.04AC-1.36BC+0.17ABC-0.03AB(A-B)+0.17AC(A-C) \quad (15)$$

$R^2 = 100.00\%$

Where A stands for oregano EO (%)

B stands for clove EO (%)

C stands for soybean oil (%)

Based on equations (13), (14) and (15), the R^2 values for all three equations were higher than 90%, indicating that the models would be able to accurately predict the SPC, YMC and OA%. As for results, a response surface graph was generated (Figure 5-8). The OA% required as high as possible while YMC and SPC required as low as possible. The optimum point was indicated within the response surfaces (red dot in Figure 5-8).

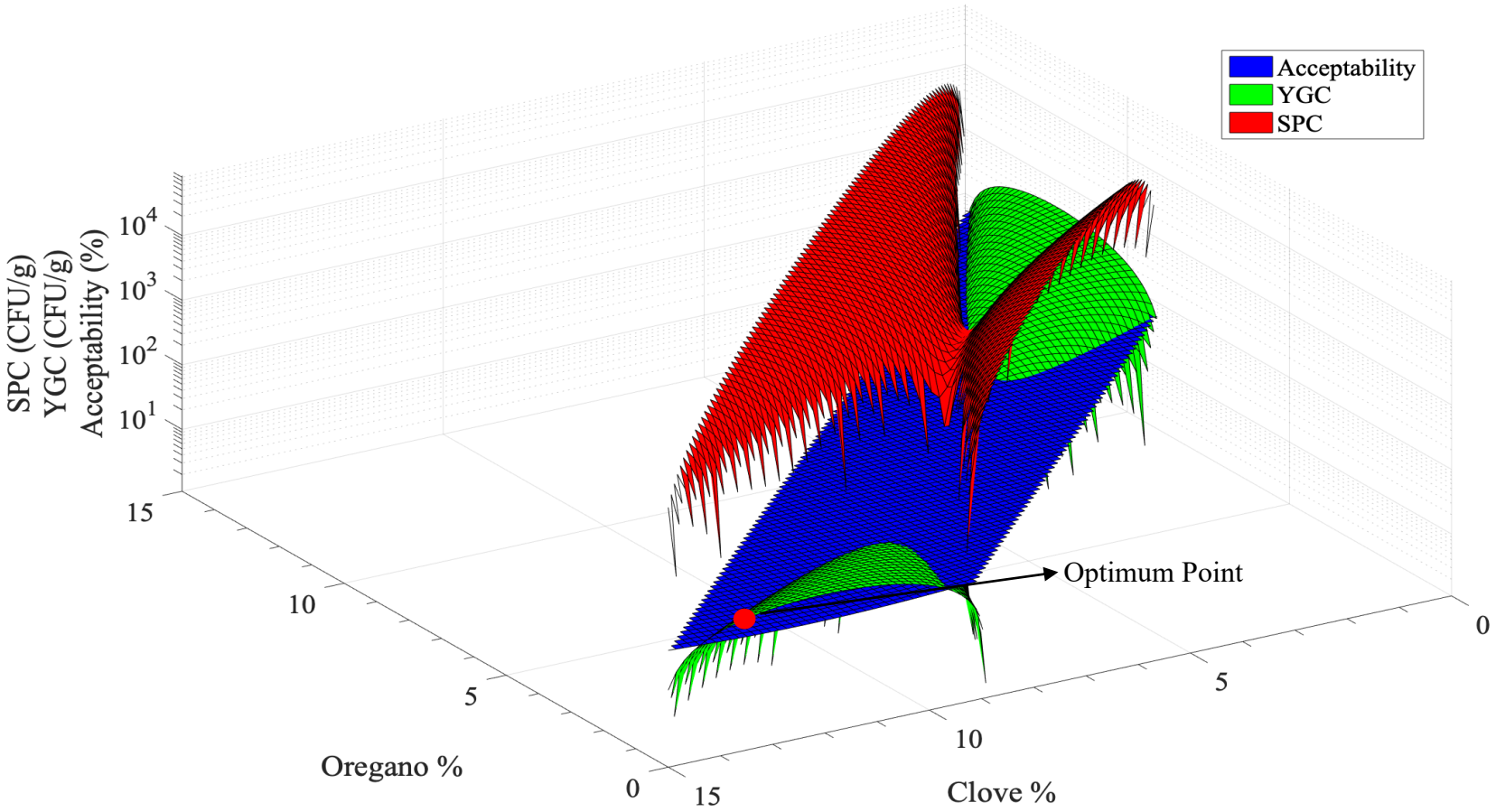

Figure 5- 8. Response surface of standard plate count (Red), yeasts and moulds count (Green) and overall acceptability percentage (Blue) affected by the concentration of oregano and clove EO.

Note: YGC = Yeasts and moulds counts; Acceptability = overall acceptability percentage.

The best combination was predicted as 2.72:10.91:86.37 (Oregano EO: Clove EO: Soybean oil), which gave 3.05 log CFU/g on standard plate count (1.72 log CFU/g less than control), 1 log CFU/g on yeasts and moulds count (0.9 log CFU/g reduction from control) and about 68.03% of the overall acceptability. At this level, the increased percentage of oregano EO could lead to higher SPC level, which deviates from results of phase one that a higher concentration of essential oil would have a greater antimicrobial effect. The synergetic effect between oregano and clove EOs were highly depended on their proportions. Thus, increasing the level of either EO may affect the antimicrobial effect of the combined essential oils. Furthermore, the results showed that oregano EO reduced the overall acceptability of the product while clove EO increased the sensory acceptability at around 11%. Figure 5-9 shows the effect of narrow changes of EOs proportion around the optimum combination on standard plate count, yeasts and moulds count and overall acceptability percentage.

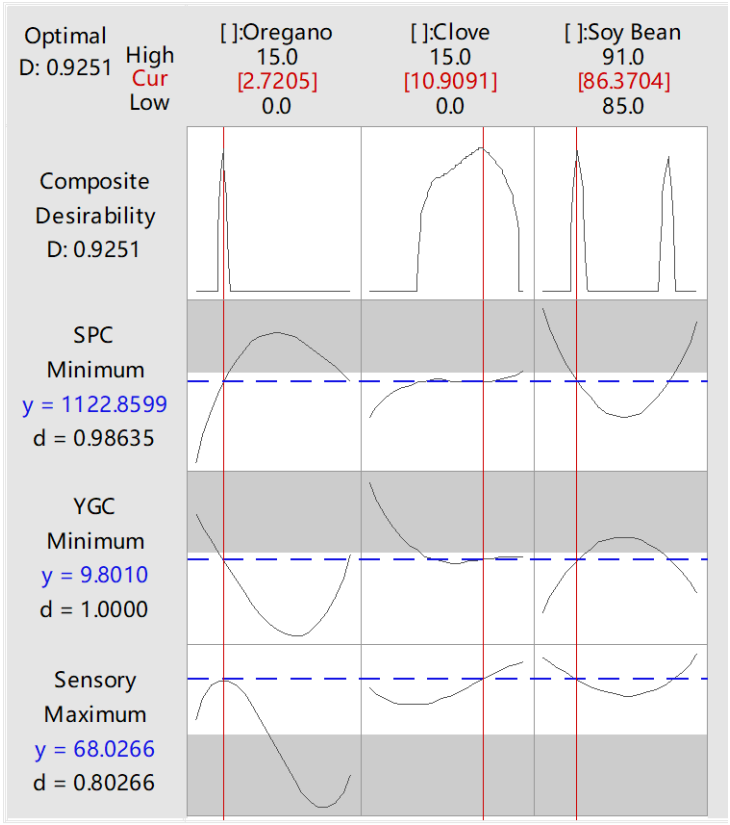

Figure 5- 9. The optimised combination indicated by mixture design modelling.
Note: YGC = Yeasts and moulds counts; Sensory = overall acceptability percentage.

5.3. Conclusion

The application of oregano EO in Hokkien noodle significantly affected the overall consumer sensory acceptability of the products ($p<0.05$). The effect of two mixed EOs showed better microbial inhibition than any single EO. The results of the mixture design showed that the combination of 2.72%:10.91%:86.37% (oregano EO: clove EO: soybean oil) in the coating oil could reduce 1.72 and 0.9 log CFU/g on bacteria and fungi than control, respectively. The overall sensory acceptability for EOs-treated Hokkien noodles was predicted at about 68% by the mixture design.

6. Characterisation of Hokkien Noodle Treated with Mixed Essential Oils and Shelf-life Determination

Results from the previous section (chapter 5) showed that the mathematical predicted optimum combination of oregano EO: clove EO: soybean oil was 2.72%: 10.91%: 86.37% for preserving the best balance between microbial quality and overall sensory acceptability of the product. However, the shelf-life of pre-cooked Asian Hokkien noodles coated with the mixed essential oils needed to be validated with empirical data. Therefore, the parboiled Hokkien noodle was prepared, coated with mixed EOs, packaged under modified atmosphere condition, storage under 4˚C and analysed during a 65 days period. The samples coated with EOs (E-sample) and control which coated with soybean oil (S-sample) were analysed every 8 days for the first 40 days, and inspected every 5 days until the end of the experimental period. For each sampling day, 2 bags of E samples and 2 bags of S samples were used as duplicated tests. According to the results on the concentrations of the oregano and clove EOs, the cost of Hokkien could increase ≈ 0.103 NZD/bag (data calculated base on Florihana Ltd EOs retail selling price; currency exchange rate: 1.000 EUR ≈ 1.792 NZD, 20 April, 2020)

Therefore, the objectives of this stage were to:

A. Compare the gas composition of S-sample and E-sample within MAP.
B. Determine if added EOs could raise pH difference in noodle products.
C. Determine if added EOs could raise A_w difference in noodle products.
D. Verify if EOs could bring a colour difference to the Hokkien noodle.
E. Verify if EOs could bring a texture difference to the Hokkien noodle.
F. Determine the shelf-life of S-sample and E-sample in microbial aspect.

6.1. Materials and Methodology

6.1.1. Material

The essential oil, soybean oil, SPCA and YGCA were obtained from the same provider mentioned in Chapter 5.1.1. Septum (Ø = 15 mm) and sterile single-use needle (21G * 1 1/2" 0.8 * 40 mm) were obtained from Matt solution Ltd (Auckland, New Zealand). Sterile cylindrical container with lid (150 mL) was purchased from Thermo Fisher Scientific (Auckland, New Zealand). Petri dishes with lids for water activity meter were supplied by Aqualab Ltd (Auckland, New Zealand).

6.1.2. Apparatus

The Apparatus used in stage 3 were shown in Table 6-1.

Table 6- 1. Apparatus used in stage three.

Instruments	**Model (Brand)**
Gas Analyser	Check Mate 3 (Matt Solutions Ltd)
Chroma Meter	CR-300 (Konica Minolta)
Chroma Data processor	DP-301 (Konica Minolta)
Texture Analyser	TA. XT. Plus (Stable Micro System Ltd)
Tensile Probe	A/SPR (Stable Micro System Ltd)
pH Meter	PB - 20 (Sartorius Ltd)
Mettler Toledo	Pro-ISM (Sartorius Ltd)
Water Activity Meter	4TEV (Aqua LAB Ltd)

6.1.3. Sample Preparation

All samples were produced at a local noodle manufactory using the same procedures mentioned in Chapter 5.1.3 except for the formulation of the essential oils. For E-sample, a mixture of essential oils comprising of oregano (2.72%), clove (10.91%) and soybean oil (86.37%) was pre-mixed and homogenized before applying 1% oil mixture (w/w) onto the pre-cooked Hokkien noodles. The S-sample was coated with 100% soybean (1% w/w) set as control. Both samples were packed under the modified atmosphere of 30:70

(CO_2:N_2) (ALIGAL 13, Air liquid, New Zealand) and then heat-sealed (AS 520Y, ArrowSystems SDN BHD, USA). The packaged samples were transported to Massey University (Albany, Auckland, New Zealand) under cold chain and storage in a light-proof walk-in refrigerator (4˚C) until required for further study.

6.1.4. Analysis of Gas Composition for Modified Atmosphere Packaging

The composition of the MAP mixed gas was analysed using the Check Mate 3 (Matt Solutions Ltd, New Zealand). The gas analyser was allowed to warm for 10 minutes and the settings of the gas analyser were adjusted as shown in Table 6-2. The septum (Ø = 15 mm) was attached onto the middle of each sample package before testing. A single-use needle was used to pierce the package through the septum for sampling the gases (Figure 6-1). The needle should avoid attaching the noodle strings or another side of the package. The needle was removed and septum was left on the package for preventing gas leaking. Each sample was measured twice.

Table 6- 2. Settings for the gas analyser.

Setting	**Parameter**
Detecting Oxygen	Positive
Oxygen Sensor Temperature	28.8˚C
Detecting Carbon Dioxide	Positive
Carbon Dioxide Sensor Temperature	60.0˚C
Balance Gas	Nitrogen
Cold-junction Temperature	36.9˚C
Equilibrium Pressure	m Bar
Gas Intaking Time	10 second
Returning Gas	Negative
Advanced settings	Not available

Source: Gas analyser user manual, Matt Solutions, 2019.

Figure 6- 1. Analysis of gas component with the gas analyser.
Notes: Image captured by One plus 5t.

6.1.5. Microbiological Analysis

The microbial analysis was similar to that described in Chapter 5.1.5.

6.1.6. Physicochemical Characteristics Analysis

Measurement of pH

The pH of the noodle samples was measured using the standard method 02-52.01 provided by AACC (1999) with some modifications. Noodle strings (10 g) were cut into

pieces and transferred to a 150-mL dry container. Then deionized water (100 mL, 20˚C) was added to the container. The suspension was mixed manually for 10 minutes and then settled for 10 minutes. The pH of the supernatant was measured using a pre-calibrated pH meter (PB-20; Pro-ISM, Sartorius Ltd, Germany) following the manufacturer's instructions. The measurements of pH were triplicated for each sample.

Measurement of Water activity

The water activity of the Hokkien noodle was measured using water activity meter (4TEV, Aqua LAB ltd, New Zealand) following the manufacturer's instructions. Noodle strings were cut into pieces and transferred to the supplied disposal petri dish to achieve a single-layer of sample loading. The petri dish was loaded into the water activity meter vessel and water activity was measured automatically. The water activity and corresponded temperature were recorded. The measurements were triplicated for each sample. Fresh samples and new Petri dishes were used between measurements.

6.1.7. Food Characteristic Test

Measurement of colour profile

The Chrome meter (CR-300, Konica Minolta, Japan) was used to objectively measure the colour profile of pre-cooked Hokkien noodle in the CIE colour system following the manufacturer's instructions and previous studies (Morris, Jeffers, & Engle, 2000). The noodle strings were cut in small pieces and transferred into a glass petri dish. The noodles were lightly compressed to prevent illuminating light from scattering. The colour sensor (DP-301, Konica Minolta, Japan) was calibrated using the CRL standard calibrating pad under Illuminant-C light and the calibration data are shown in Table 6-3. The petri dish was turned for 120˚ for second measuring and 240˚ for third measuring, the average of the three measurements was considered as the result of the sample. The overall colour difference, chroma difference and the browning index were calculated using the recorded data.

Table 6- 3. The technical details for Chroma Meter for colour profile measuring.

Items	**Values**
Chroma Meter and Senor Head	CR – 300
Receptors	Six silicon photocells filtered
Spectral Response	CIE 1973 standard observe curves
Light Source	Pulsed Xenon are lamp
Light Temperature	Illuminant C (6770K)
Measurement System	Diffuse illumination
View angle	0˚ included specular component
Measuring Area	8 mm in diameter
Calibrating standard	CRL ch00
Calibrating data for Illuminant C	Y=92.40 x=0.3138 y=0.3192

Measurement of the texture profile

Texture profile measurement of the Hokkien noodle was conducted as described by Li (2008). The tensile force and elasticity of the noodle string were selected as the texture parameters for the pre-cooked Hokkien noodle. The noodle strings without visible physical scars or wounds caused by cutting or packaging processes were selected for texture measurement. The noodle string was attached to two L-shape probes and slightly tightened the string to avoid sagging (Figure 6-2). The upper L-shape probe was pulled up by the texture analyser until the string broke before the probe returned to the original position. The force required to complete the movement was recorded, as well as the distance from the starting position until the breaking point. Every sample required 4-9 noodle strings for the measurement to reduce variations. The details and settings for texture analyser are shown in Table 6-4.

Table 6- 4. The details and settings for Texture analyser for texture profile measuring.

Items	Values
Texture analyser	TA. XT. Plus
Probe	A/SPR set
Load Cell	5 kg
T.A. Sequence	Return to start
Original Probe Distance	30.000 mm
Test mode	Tension
Pre-test Speed	1.00 mm/sec
Test Speed	2.00 mm/sec
Post-test Speed	10.00 mm/sec
Target Mode	Distance
Distance	50.000 mm
Trigger Type	Auto by Force
Trigger Force	5.0 g
Tare Mode	Auto
Break Mode	Off

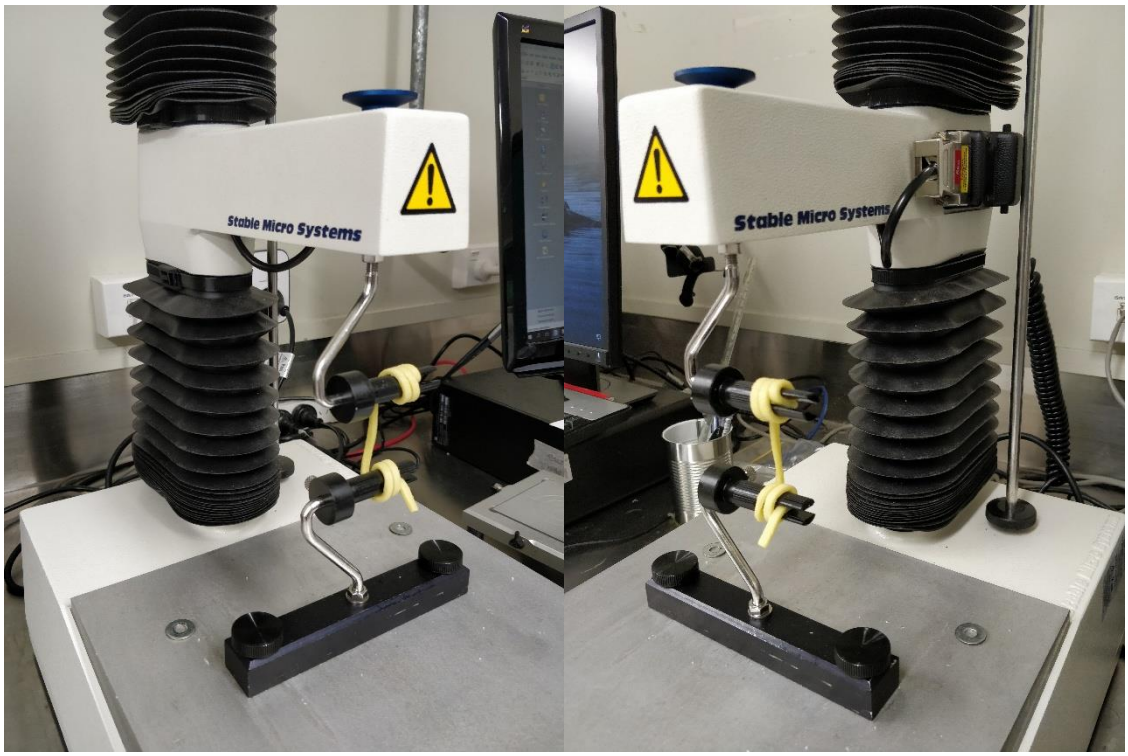

Figure 6- 2. Measuring the noodle string texture using the texture analyser (TA. XT. Plus, Stable Micro System Ltd, the United Kingdom)

Notes: Image captured by One plus 5t.

6.1.8. Statistical Analysis of Data

The changes in MAP gas composition, pH and A_w are shown in graphs over the period. The noodle strings colour profile was used to calculate the browning index difference (ΔBI%), chroma difference (ΔC) and overall colour difference (ΔE) based on equations (10), (5) and (11), respectively. The data of the texture profile (force & extended distance) were analysed by the SPSS Statistics Version 24.0 (IBM™, USA) with independent sample T-Test and ANOVA at 95% of confidence level. The microbial results (SPC & YMC) were used to generate the Baranyi-Robert model and predicted the shelf-life of the original sample and EO-coated samples, respectively.

6.2. Results and Discussion

6.2.1. Gas Composition in MAP

The levels (%) of oxygen, carbon dioxide and nitrogen in the MAP for E-sample and S-sample were monitored during the experimental period (Figure 6-6 to Figure 6-8), respectively. Also, an empty bag produced by the same condition except loading with noodle was used to represent the gas permeability of the packaging bag, which was not affected by the growth of the microorganisms.

As could be seen in Figure 6-3, at the beginning of the period, the empty bag had a lower oxygen level (0.17%) than both S-sample and E-sample (0.55%). The noodle strings may have absorbed oxygen, nitrogen, moisture and other gases in air in their network structure during production procedures. Once the packaging of noodle sample was filled with modified gases and sealed, the differences in the gas concentrations led the gases within the strings released into the headspace of the package, resulting in a higher oxygen level on both noodle-contained packaged bags initially.

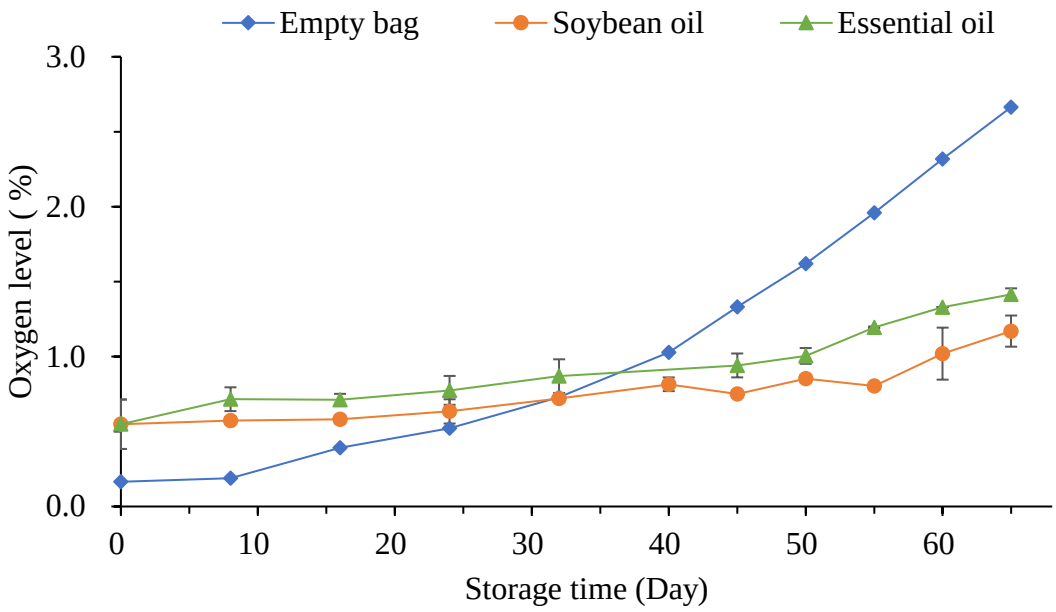

Figure 6- 3. The oxygen level of essential oil-coated sample, soybean oil-coated sample and empty bag in the MAP for a 65-day period.

During storage, the permeability of the packaging material allowed the oxygen to be transmitted through the bag, expressed by the gradually increased oxygen level within the empty bag, which reached 2.66% at the end of the period. Additionally, after 40-day, the empty bag had a higher oxygen level (1.03%) than noodle containing bag (0.95/0.75, E/S sample), which might be led by the microbial activities and chemical reactions had consumed the oxygen in noodle containing package. On the other hand, although having a higher initial oxygen level, E-sample package had a relatively stable oxygen level with slightly increased by 0.87% during the storage period (from 0.55% to 1.42%). Also, E-sample bags had a higher oxygen level than S-sample bags throughout the period, which might relative to the growth of microorganisms, as the microbes in the S-sample were more active than the E-sample. As a result, oxygen consumption within S-sample was higher than the E-sample. Furthermore, the oxygen level difference between S-sample and E-sample was increased to 0.4% from day-55, expecting the growth rate of microorganisms in S-sample had increased.

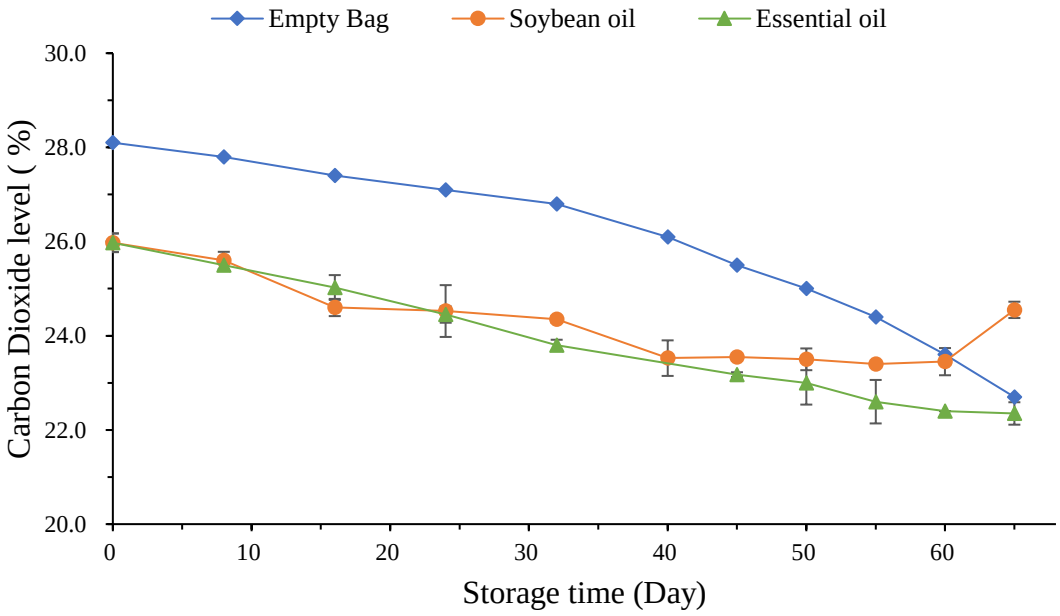

Figure 6- 4. The carbon dioxide level of essential oil-coated sample, soybean oil-coated sample and empty bag in the MAP for a 65-day period.

As could be seen in Figure 6-4, the changes in the carbon dioxide level showed an opposite pattern with oxygen level. Since the concentration of carbon dioxide in the air (0.03%) was lower than its in modified gases (30%), also the permeability of carbon dioxide of the packaging material, the carbon dioxide was gradually released into the air over time, resulted in descended carbon dioxide level. The carbon dioxide level of S-sample shared the same pattern with E-sample until day-45, where the carbon dioxide in S-sample was started to maintain stable at around 23% and significantly increased to 24.55% at the end of the period. However, the carbon dioxide level of the E-sample gradually decreased from 23.18% on day-45 to 22.35% on day-65. There was highly possible that the microorganisms in S-sample were multiplied and released carbon dioxide as the metabolite. Meanwhile, the growth of the microbe in the E-sample was not obvious.

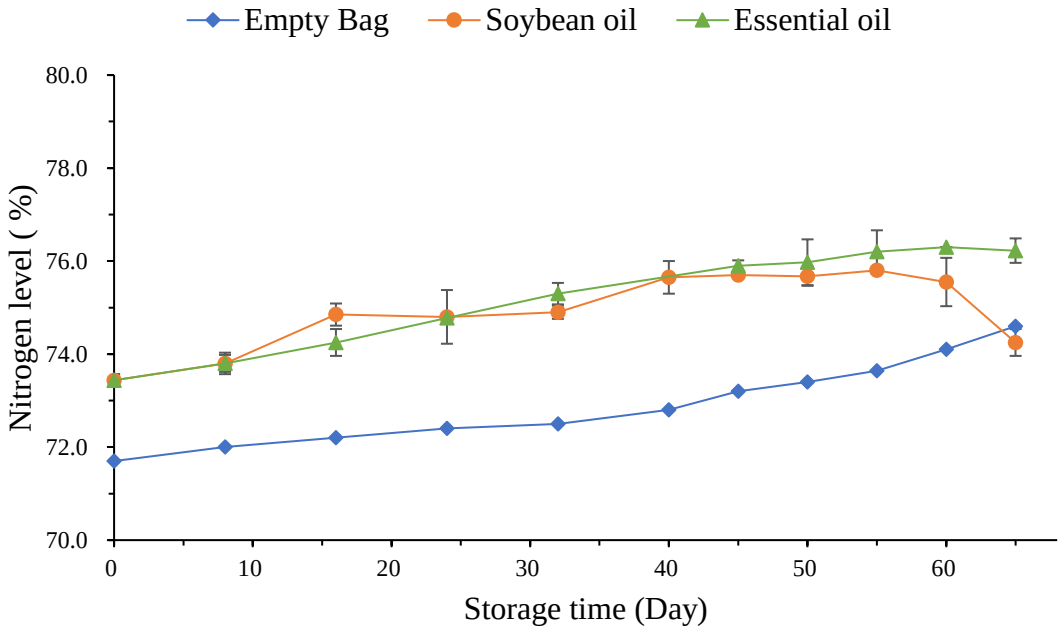

Figure 6- 5. The nitrogen level of essential oil-coated sample, soybean oil-coated sample and empty bag in the MAP for a 65-day period.

The nitrogen level was corresponded with oxygen and carbon dioxide level, since it was calculated as balance gas during detection. Because the carbon dioxide levels in S-sample significantly increased by around 1% at the end of the experimental period, the nitrogen level in S-sample correspondingly decreased around 1%. Whilst, the nitrogen level of E-sample and the empty bag was steadily ascended over time, from 73.44% at the beginning and ascended to 76.23% on day-65 for E-sample, from 71.70% increased to 74.60% for the empty bag (Figure 6-5).

6.2.2. Physicochemical Parameters

pH

The pH of the Hokkien noodle was acidic at the beginning due to the presence of acids in the ingredients (Figure 6-6). Starting from near 4.6, the pH of the EO-coated samples and the control gradually increased from 4.65 to 4.9 in the first month (day-32) of the experiment, then stabilised at pH around 5.0 until the end of the period. During the study, the pH of both samples did not get below 4.6, indicating that neither of the products could

be classified as acid food (Jay et al., 2005). The increase of the pH in the first month might attribute to the decrease in the carbon dioxide level in the package. According to equation (16), as carbon dioxide decreases, the carbonic acid formed on the noodle was probably transformed into carbon dioxide and water to achieve an equilibrium of the reaction. After 45 days, the fluctuations of the pH might be caused by microbial activities and their metabolites (Jay et al., 2005). In general, there were no obvious differences in the pH between S-sample and E-sample ($p>0.05$). Also, the pH of both samples was not low enough to prevent the growth of bacteria or fungi (Jay et al., 2005). The acids in the ingredients did not prevent the growth of microorganisms, thus, other treatments are required to achieve longer shelf-life of the product.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-} \quad (16)$$

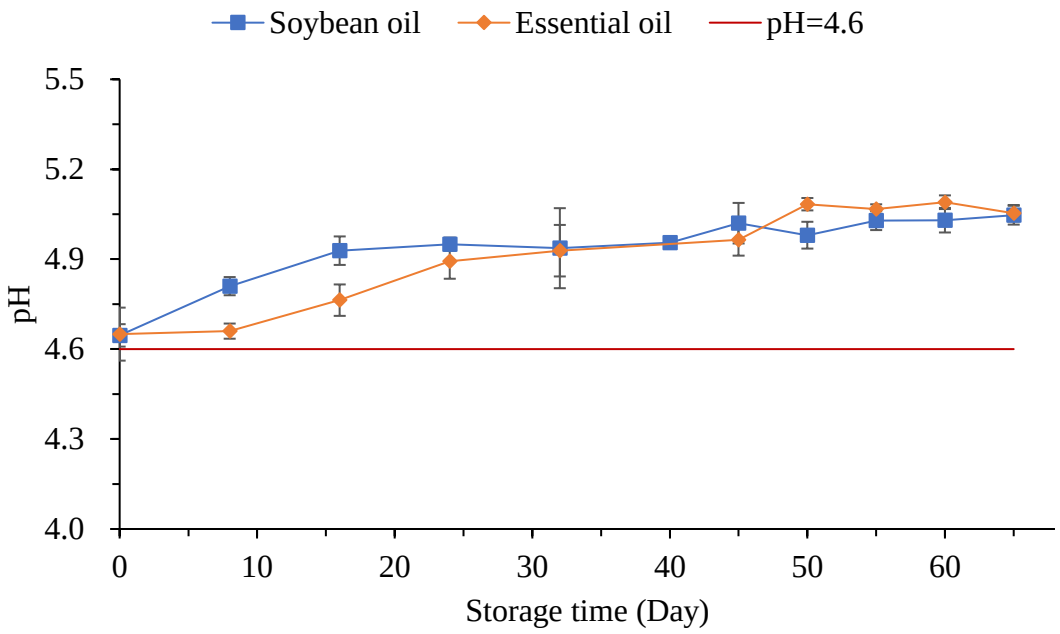

Figure 6- 6. The pH of different samples during a 65-day storage at 4˚C.
Note: solid red line = the threshold of acid food, pH < 4.6; Essential oil = E-sample; Soybean oil = S-sample.

A_w

The A_w of the fresh Hokkien noodle samples (day-0) was around 0.995, which agreed with the study by Ling (2010) who reported that the water activity of the pre-cooked Asian noodle was around 1. As the storage time increased, the A_w of the Hokkien noodle samples slightly decreased to 0.987, then stabilised with minimal fluctuations. Figure 6-7 shows that the differences in A_w between the S-sample and E-sample were small and continued to decrease with increase in storage time. The results suggest that the additional EOs (clove and oregano) did not affect the A_w of the Hokkien noodle, especially after day-40 ($p>0.05$). Beyond day 40, the water activity of both samples was around 0.990. According to Labuza (1972), the A_w of the Hokkien noodle falls in region III (A_w = 0.75-1.00), indicating it could support the growth of different types of bacteria and fungi at this water activity. This further supports the need to search for new treatments to improve the product's shelf-life.

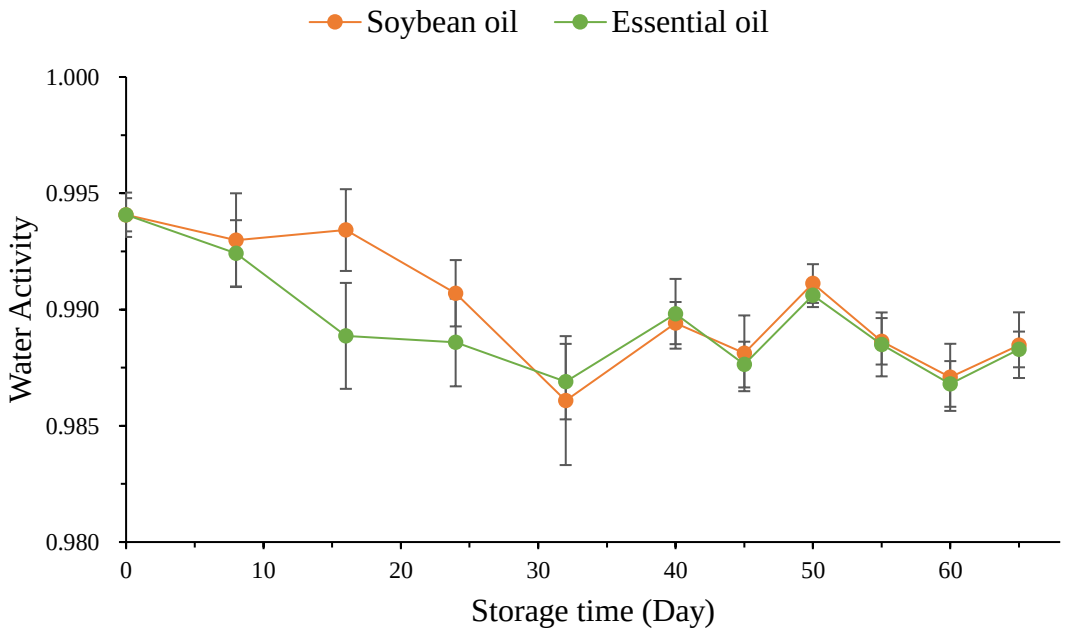

Figure 6- 7. The water activity of different samples during a 65-day storage at 4˚C.
Note: Essential oil = E-sample; Soybean oil = S-sample.

6.2.3. Sensory Parameters

Colour profile

The colour profiles of two Hokkien noodle samples (S-sample and E-sample) were analysed by two approaches. First, S-sample and E-sample were compared with Fresh Hokkien noodle (Day-0) independently (Table 6-5), to verify whether soybean oil or EOs was able to maintain the colour of the noodle strings during the storage. Second, the S-sample was compared with the E-sample that collected on the same day, to determine if there was any effect on the noodle string's colour caused by the addition of the EOs (Table 6-6).

Table 6- 5. Colour parameter differences between fresh sample (Day-0) and experimental examples within 65-day period.

Sample	Parameter /Day	0	8	16	24	32	40	45	50	55	60	65
S-Samples	ΔBI %	N/A	3	4	7	6	5	7	8	5	6	4
	ΔC	N/A	0.33	1.22	2.87	2.23	1.43	3.55	2.64	1.68	1.67	0.48
	ΔE	N/A	1.76	1.63	3.23	2.81	2.90	3.75	3.64	2.78	2.90	2.51
E-Samples	ΔBI %	N/A	4	4	8	7	7	7	8	6	8	5
	ΔC	N/A	0.65	0.99	2.73	3.14	2.64	2.69	3.12	1.37	2.24	1.98
	ΔE	N/A	2.10	2.20	3.87	3.20	3.24	3.55	3.75	3.00	3.74	2.25

Note: N/A= Not available.

Table 6- 6. Colour parameter differences between S-samples and E-samples within 65-day period.

Parameter/Day	0	8	16	24	32	40	45	50	55	60	65
ΔBI %	0	1	0	1	1	2	0	0	1	2	1
ΔC	0.00	-0.32	0.23	0.13	-0.91	-1.21	0.85	-0.48	0.31	-0.57	-1.50
ΔE	0.00	0.42	0.94	1.44	1.42	1.39	2.16	0.27	0.83	0.85	2.07

Browning index (ΔBI%) is a colour parameter that mainly determined by the lightness, it shows the level of the browning reaction of the food product. The colour change occurred on Hokkien noodle during storage was supposed to cause by the browning reaction. When comparing with fresh sample (day-0), the ΔBI% of the S-sample increased from 3 on day-8, peaked to 8 on day-50, but unexpectedly decreased to 4 at the end of the period (Day-65). It was clear that ΔBI% did not correspondingly change with time ($p>0.05$). However, the browning reaction is a time-depended chemical reaction (Damodaran et al., 2008), which means the level of the browning should increase with storage time. The results suggested that the lightness difference on the S-sample was not caused by the browning reaction. Hou et al. (1979) reported that the brightness of the noodle product was depended on water content, ingredients, chemical reactions and coating material. Since the change of BI% for the S-sample was not time-depended and the samples were coated with the same kind of oil, the only possible explanation might be the inadequate consistency of the product. Similar phenomena were found in E-sample comparing with fresh sample (Table 6-5), the ΔBI% increased from 4 on day-0 to 8 on day-50, then decreased to 5 at the end of the experimental period, which further confirmed the inconsistency of the product.

The chroma, or so-called saturation of the commercial Hokkien noodle is dominated by the food dye (E102) added in ingredients (Francis, 1995). The chroma difference (ΔC) of S-sample comparing with fresh sample showed a similar pattern with ΔBI%, with a few fluctuations in the middle of the experimental period and decreased at the end (Table 6-5). The chroma differences between samples might cause by unevenly mixed ingredients or variations in cooking time (Pek et al., 2010). For the overall colour difference (ΔE), the S-sample examined on day-24 (3.23), day-45 (3.55) and day-50 (3.64) had apparent colour differences that could be perceived by untrained sensory panellists because the ΔE was higher than 3 (Adekunte et al., 2010). However, untrained panellists were not expected to find colour difference on Day-65 sample with fresh sample since ΔE was $2.51<3$.

The E-sample had a similar colour profile with the S-sample when was compared with the fresh sample. The ΔE between E-samples and fresh sample were varied from 3.00 to 3.87 within Day-24 to Day-60, which was slightly higher than 3 and might be noticeable by consumers. Since the total content of coating oils was kept constant, therefore, the level of soybean oil was reduced for the additional EOs. Soybean oil was used as a coating material that contributed to the consistency of the appearance of the product (Fu, 2008). This suggested that the lower concentration of soybean oil in EO-treated samples might intensify the inconsistent of colour between samples. Hence, E-samples showed a lightly higher amount than S-samples that exhibited noticeable colour difference during storage. Nevertheless, on Day-65, customers were not expected to find out the colour difference on E-sample, comparing with fresh Hokkien noodle ($\Delta E=2.25<3$).

However, when E-samples were compared with the S-sample with the same storage period (Table 6-6), they had very minimal differences on ΔBI% ($\leqslant$ 2) and ΔC ($\leqslant$ 1.5), which resulted in a low level of ΔE ($\leqslant$ 2.16). According to Table 6-6, there were no any E-sample that showed visible colour difference with S-sample that with the same storage time, which indicated that the additional EOs could not cause a noticeable difference in the colour parameters of the products.

Texture

The difference in texture profile data of the S-sample and E-sample are shown in Table 6-7. As can be seen, the tensile force decreased during storage from 41.81g to around 30 g for both S and E-samples. Also, the results show that there was no significant difference in the tensile force between the S-sample and E-sample in identical sampling day as all p-values were greater than 0.05. Although in day 50, the E-sample (34.88g) had a relatively higher tensile force difference comparing with S-sample (31.53g), statistically, the difference was still not significant as $p = 0.051 > 0.05$. By day-65. The level of deterioration for S-sample on the tensile force was still the same with E-sample. Hence,

it can be concluded that there was no significant difference in tensile force occurred throughout the experimental period between samples.

The extended distance showed a similar pattern with tensile force except for Day-65. There was no significant difference between the S-sample and E-sample on elasticity until the last day of the period ($p \geq 0.096$). The E-sample (34.60 mm) achieved a longer distance before it broke than the S-sample (33.27 mm) since Day-8. The extended distance on the S-sample gradually descended to 25.14 mm while the E-sample was relatively stable at 32.00 mm on Day-65, where the significant difference on elasticity between S-sample and E-sample was shown ($p = 0.008$).

Table 6- 7. Texture profile independent sample T-Test between Soybean oil-coated samples and EO-coated samples.

Day	Force (g)			Distance (mm)		
	S-Sample Mean	E-Sample Mean	T-test Sig. (2-tailed)	S-Sample Mean	E-Sample Mean	T-test Sig. (2-tailed)
0	41.81	41.81	N/A	36.70	36.70	N/A
8	38.23	38.17	0.981	33.27	34.60	0.657
16	37.64	37.58	0.967	33.76	37.07	0.203
24	36.75	37.57	0.563	35.27	37.08	0.647
32	34.69	37.16	0.232	30.21	30.47	0.932
40	34.18	36.84	0.080	26.08	31.29	0.096
45	32.93	35.47	0.067	28.18	32.18	0.269
50	31.53	34.88	0.051	30.85	28.16	0.299
55	32.02	33.64	0.230	30.50	29.19	0.694
60	30.92	32.73	0.337	29.10	32.42	0.403
65	29.10	31.83	0.057	25.14	32.00	0.008

Note: N/A = Not available; p-value lower than 0.05 was considered as significant difference.

Even though there was no difference between S-sample and E-sample on either tensile force or extended distance during storage for two months (60-day). It was found that the tensile force and extended distance of both samples were decreased over time. Therefore, the data of the texture of the noodles were compared using ANOVA ($p>0.05$) to determine

the effect of storage (Table 6-8).

Table 6- 8. The mean values of tensile force and extended distance of Hokkien noodle sorted by day.

Day	Force (g)	Distance (mm)
0	41.81^{f}	-36.70^{b}
8	38.20^{ef}	-33.96^{ab}
16	37.64^{de}	-35.49^{ab}
24	37.21^{de}	-36.29^{b}
32	35.81^{cde}	-30.32^{ab}
40	35.56^{bcde}	-28.77^{a}
45	34.09^{abcd}	-30.02^{ab}
50	33.21^{abc}	-29.51^{ab}
55	32.76^{abc}	-29.90^{ab}
60	31.73^{ab}	-30.69^{ab}
65	30.46^{a}	-28.57^{a}

Note: Means with different superscripts within the same column are significantly different at $p<0.05$

As the results showed in Table 6-8, it could be seen the tensile force dropped as storage time increase. It only took 16 days (37.64 g) to find the significant difference in force comparing with fresh Hokkien noodle (Day-0, 41.81 g). The tensile force had been weakening until the end of the experimental period at 95% confidence level. Combining the finding in Table 6-7, it could be concluded that the tensile force of the Hokkien noodle was descended over time, neither soybean oil nor essential oil was able to retain the force ($p<0.05$). Deteriorate tensile force in Hokkien noodle should be tackled by other approaches.

On the other hand, the extended distance was much more consistent. No significant difference was found during the period when compared with fresh Hokkien noodle, except Day-40 (28.77 mm) and Day-65 (28.57 mm) (highlighted in red). Since there were significant differences found between S-sample and E-sample on extended distance aspect (Table 6-7), further T-tests were conducted on S-sample and E-sample (Day40 & Day65), respectively, with fresh Hokkien noodle (Day-0). The results were shown in Table 6-9.

Table 6- 9. Extended distance independent sample T-Test between fresh noodle and Day-40 and Day-65 data sorted by Soybean oil and EO-coated samples.

Sample	Mean value (mm)	Sig. (2-tailed)
Fresh Noodle (Day0)	36.70	N/A
S-sample Day 40	26.08	0.001
S-sample Day 65	25.14	0.000
E-sample Day 40	31.29	0.104
E-sample Day 65	32.00	0.105

Note: N/A = Not available.

The results showed that the extended distance data collected from S-sample on both Day-40 (26.08 mm) and Day-65 (25.14 mm) had significant differences with Day-0 sample ($p<0.05$). Whilst the E-sample analysed on Day-40 (31.29 mm) and Day-65 (32.00 mm) had no significant difference with fresh Hokkien noodle. These results confirmed that the EOs were able to remain the ductility of Hokkien noodle within a 65-day period at 95% confidence level. This phenomenon might relate to the antimicrobial effect of EOs. According to Li et al. (2017), the deterioration in the texture of the noodle product was mainly attributed to the microbial activity. The inhibitory of the growth of microorganisms was able to reduce the textural change during the storage period, resulting in higher acceptability and longer shelf-life (Li et al., 2011; Bai et al., 2017).

6.2.4. Microbiological Modelling

The standard plate count (SPC) and yeasts and moulds count (YMC) for both samples are shown in Appendix C3 -I and -II. Since neither S-sample nor E-sample reached the national legal limits for SPC at 6 log CFU/g or YMC at 4 log CFU/g during the 65-day storage period, the shelf-life of both samples were uncertain. Hence, the mean values of SPC and YMC were applied to Baranyi-Roberts equations to generate the predictive model, to estimate the shelf-life of both samples (Baranyi & Roberts, 1995). The number of microorganisms (N) and the storage time (t) obtained from experiment were applied to Baranyi-Roberts model, while initial organisms number (N_0), maximum growth rate

(μ_{max}), initial physiological state (h_0) and maximum organisms number (N_{max}) were determined by the highest determination coefficient (R^2) of the model with the data obtained from the experiment for 65 days. The length of the lag phase (λ) was calculated by the ratio of h_0 and μ_{max}. The parameters of the Baranyi-Roberts model are shown in equations (17) (18) and (19) (Yimenu et al., 2019).

$$\lambda = \frac{h_0}{\mu_{max}} \tag{17}$$

$$ln\frac{N}{N_0} = \mu_{max}A_B(t) - \ln\left(1 + \frac{\exp(\mu_{max}A_B(t)) - 1}{\exp\left(\ln\left(\frac{N_{max}}{N_0}\right)\right)}\right) \tag{18}$$

$$A_B(t) = t + \frac{1}{\mu_{max}}\ln\left(\exp(-\mu_{max}t) + \exp(-h_0) - \exp(-\mu_{max}t - h_0)\right) \tag{19}$$

The results of the Baranyi-Roberts models are shown in Table 6-10.

Table 6- 10. Data for the Baranyi-Roberts models for the S-sample and E-sample.

Baranyi-Roberts model Parameters	SPC		YMC	
	S-sample	E-sample	S-sample	E-sample
λ (Day)	43.76		55.88	
N_0 (CFU/g)	71.10		31.80	
μ_{max} (Day^{-1})	0.257		0.084	
h_0	11.25		4.69	
N_{max} (CFU/g)	5739162		599916	
Average log CFU/g	1.486	N/A	0.396	N/A
Sum of squared residuals (Average)	153.00		12.39	
Sum of squared residuals (Model)	6.34		5.82	
R^2	0.959		0.530	

Note: N/A = data not available to generate the model.

The data collected from E-sample were not fitted into Baranyi-Roberts model, since neither the SPC nor YMC of the E-sample had reached the exponential phase within 65 days/4˚C. A longer experimental period for the E-sample was required for collecting data in the exponential phase and predicting its shelf-life.

The Baranyi-Roberts models were built on both the SPC and YMC for the S-sample. The determination coefficient (R^2) for SPC of the S-sample was 0.959, suggesting that the model could predict the SPC on S-sample precisely. Moreover, the R^2 for YMC of S-sample was just 0.530. This might because the experimental period was dominated by the lag phase (55 days), thus, only a few data were collected from the log phase. Hence, the prediction from the YMC model was not as accurate as the SPC model.

During the storage period, the bacteria (SPC) had a shorter lag phase compared to the YMC (43 < 55 days) and a larger maximum growth rate (0.257 > 0.084) than fungi (YMC). The results suggested that the growth of bacteria on Hokkien noodle was earlier than the fungi. Based on the Baranyi-Roberts models built for the S-sample, the shelf-life of this sample was calculated from the SPC and YMC respectively. The shelf-life was determined by the shorter time required to reach the national regulated limits. Calculated shelf-life for the S-sample based on SPC and YMC are shown in Table 6-11, respectively.

Table 6- 11. The predicted shelf-life of S-sample based on SPC and YMC

Models Parameters	SPC	YMC
Lag Phase (Day)	43	55
Legal Limit (log CFU/g)	6	4
$A_B(t)$	37.915	68.588
Time to reach the limit (calculated, Day)	81.7	126.5
Time to reach the limit (adjusted, Day)	81	126
Time between lag phase end to reach the limit (Day)	38	71

Using the predicted data shown in Table in 6-11, the S-sample reached the legal limit of SPC (81 days) before YMC did (126 days). Hence, the shelf-life of S-sample was predicted at 81 days/4˚C, which also indicated that the shelf-life of the original Hokkien noodle was limited by the growth of aerobic bacteria. As a result, the accuracy of the model on predicting the shelf-life of Hokkien noodle was determined by the R^2 of the SPC model, which was reasonably precise. The growth curves of SPC on E-sample and S-sample (including the prediction) are shown in Figure 6-8, whereas the growth curves for YMC are shown in Figure 6-9.

Standard Plate Counts

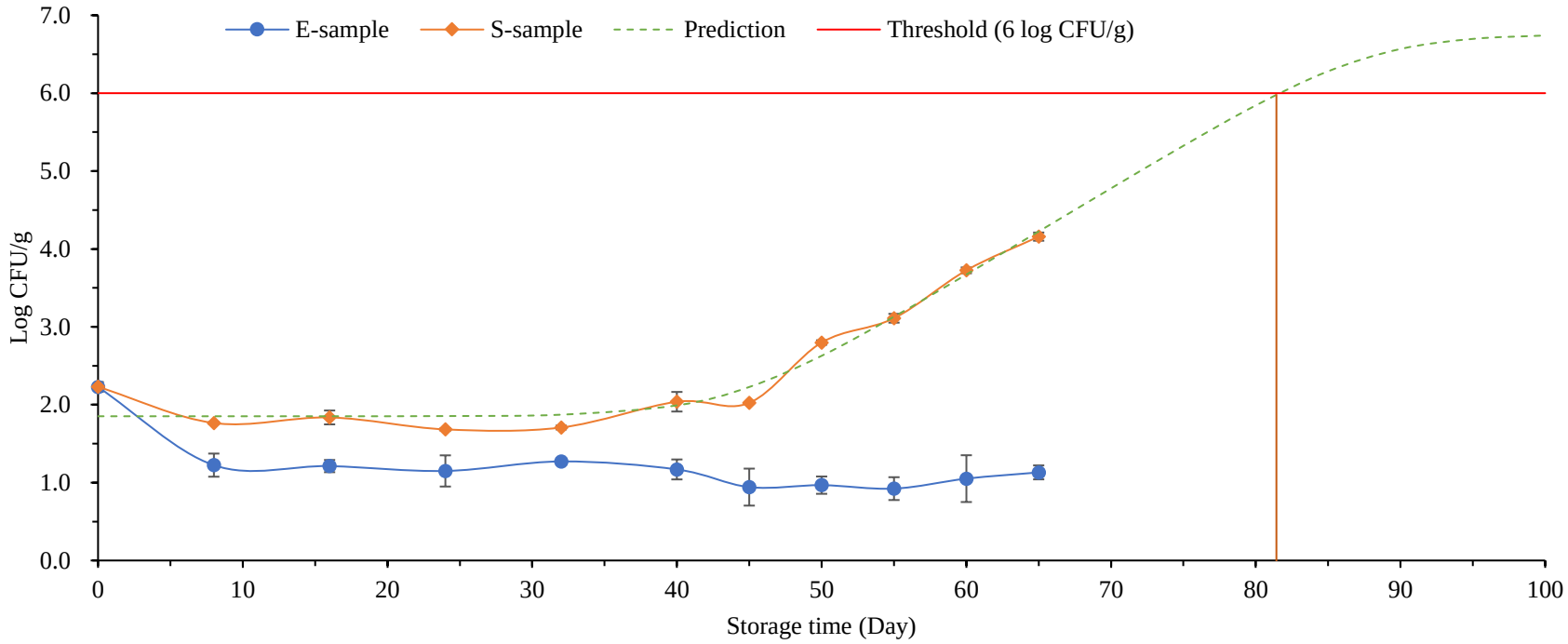

Figure 6- 8. SPC (log CFU/g) curves of soybean oil-coated (S-sample) and EO-coated (E-sample) Hokkien noodle during storage for 65 days
Note: Threshold (Horizontal red line) = National regulated limits 6 log CFU/g; the shelf-life of EO-coated sample on SPC was not predicted due to insufficient data; vertical continuous red line = predicted shelf-life of S-sample in SPC aspect.

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Yeasts and Moulds Counts

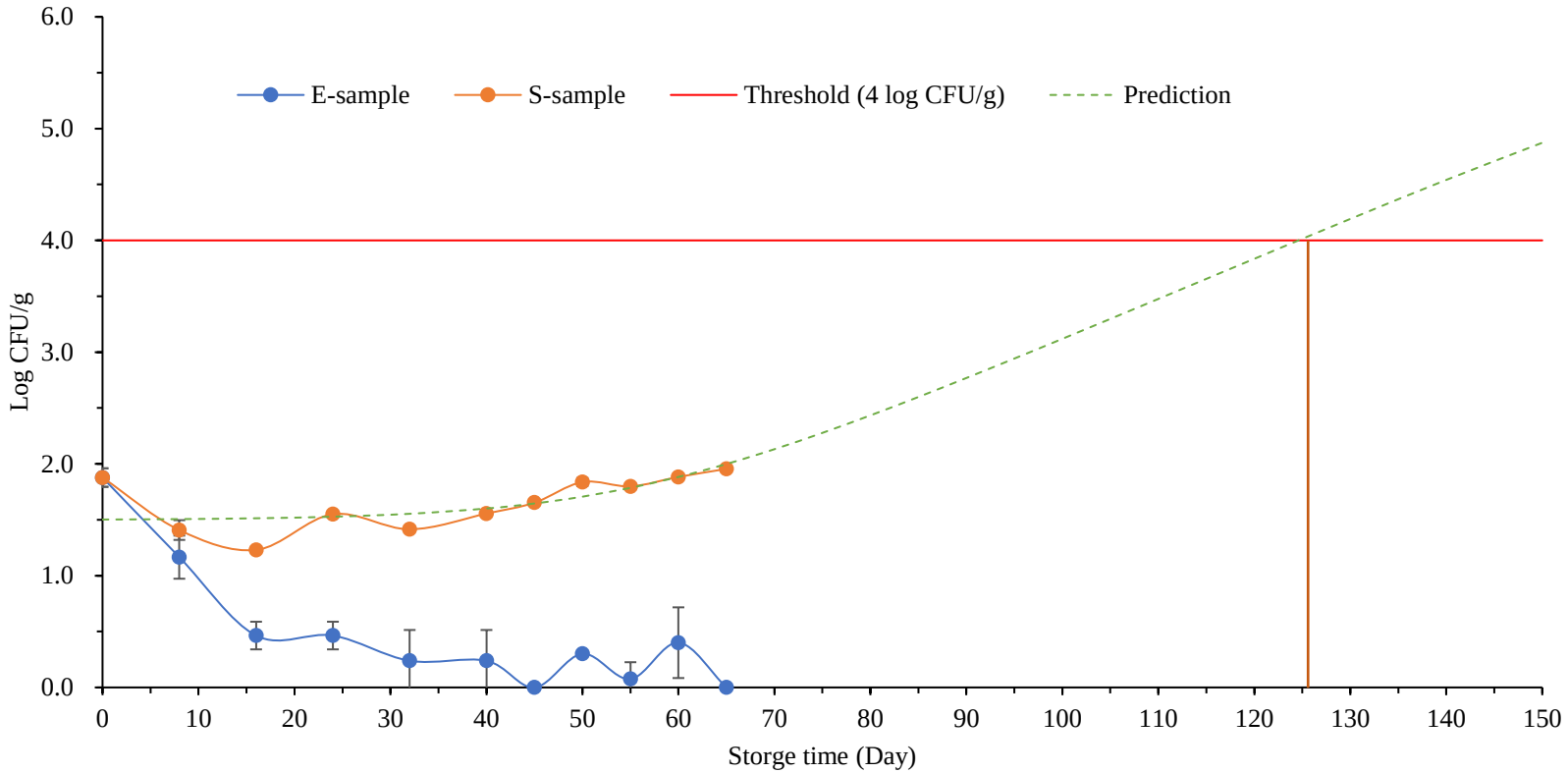

Figure 6- 9. YMC (log CFU/g) curves of soybean oil-coated (S-sample) and EO-coated (E-sample) Hokkien noodle during storage for 65 days
Note: Threshold (Horizontal red line) = National regulated limits 4 log CFU/g; the shelf-life of EO-coated sample on YMC was not predicted due to insufficient data; vertical continuous red line = predicted shelf-life of S-sample in growth of yeasts and mould counts.

Figure 6-8 shows that the SPC of the S-sample had around 2.2 log CFU/g at the start, then slightly decreased to 2 log CFU/g then stabilised until day 45. After that, the exponential increase was found in SPC, which reached 4.22 log CFU/g on day 65. The suppression between day 8 to day 45 might be related to the scarcity of oxygen and low temperature (4˚C) storage. Thereafter, the growth transformed into the log phase after day 45, which was also in line with the shelf-life of current retail products. The lag phase of mesophilic aerobic bacteria of the S-sample agreed with the model (44/45 days respectively). Furthermore, for S-sample, the level of carbon dioxide in the package was found to increase at the beginning of the exponential phase. This, therefore, confirmed the suggestion that the microbial activities in the packaged sample could have produced the carbon dioxide.

On the contrary, the SPC of E-sample started at 2.23 log CFU/g, then decreased to around 1 log CFU/g for the rest of the storage time of 65 days. E-sample (1.13 log CFU/g) was found more than 3 log reduction when comparing with S-sample (4.22 log CFU/g) at the end of the period. This result showed that the presence of EOs was not only able to suppress the growth of bacteria on Hokkien (bacteriostatic effect), but also reduce its population (bactericidal effect). These results confirmed that the combination of EOs has achieved an outstanding antibacterial effect. The shelf-life of the product could be prolonged by restraining the growth of bacteria and extending the lag phase. For S-sample, the lag phase was estimated as 43 days, while E-sample was still in lag phase after 65 days, it was promising that the shelf-life of E-sample could be prolonged at least 22 days more, achieving over 100-day of shelf-life (103 days).

In terms of fungi, the number of YMC count on S-sample decreased for the first two-week, from 1.88 to 1.23 log CFU/g on day-14, and experienced a few fluctuations, then gradually increased to 2.0 log CFU/g at Day-65. The decrease of YMC on S-sample in the first fortnight and its long lag phase led to a low R^2 of the YMC predicting model. The reducing population of fungi might relate to the storage condition that was not suitable for the growth of fungi. For E-sample that containing EOs, the number of fungi

was radically descended from 1.88 on day-0 to 0.46 log CFU/g on day-14. Also, differed from S-sample, as storage time increased, the population of fungi kept reducing during the 65-day period, and it never grew above 0.5 log CFU/g, even 0.00 CFU/g of fungi was detected on Day-45 and Day-65. There was 2 log reduction for E-sample comparing with S-sample at the end of the period. These results indicated that the combination of the EOs achieved an effective and efficient antifungal effect on Hokkien noodle, most of the fungi were inhibited.

6.3. Conclusion

During a 65-day period, there was no significant difference in pH, water activity and strings tensile force between E-sample and S-sample ($p>0.05$). The overall colour difference between S-sample and E-sample was not detectable for untrained sensory panellists ($\Delta E \leq 2.16$). However, it was found that the inconsistency within samples led to detectable colour difference with fresh Hokkien noodle ($\Delta E > 3$), regardless of coated by soybean oil or essential oils. EOs was found to be able to retain the elasticity of the strings during the study where soybean oil did not ($p > 0.05$). Neither S-sample nor E-sample has excessed the national regulated microbial limit within 65-day. The Baranyi-Roberts models predicted that the shelf-life of S-sample has around 81 days. It was calculated that the shelf-life of the E-sample could be at least 20 days longer than S-sample, hence achieving over 100-day. Lastly, the carbon dioxide level within the MAP might be an alternative indicator of the microbial condition for noodle product, it responsibly increased as the population of microorganisms increased.

7. Overall Conclusions

Within the tested essential oils, oregano had the highest antimicrobial activity against bacteria and fungi using the aqueous base broth micro-dilution assay; whilst using the agar disc diffusion method in the lipid phase, oregano EO was still the most effective against bacteria, while fungi were the most susceptible to clove EO. The sensory test revealed that the application of oregano EO on pre-cooked Hokkien noodle could significantly affect the overall consumer sensory acceptability ($p<0.05$), whereas clove EO achieved relatively higher tolerance level. The coating oil consisting of oregano EO (2.72%) and clove EO (10.91%) on pre-cooked noodle provided an optimum balance between microbial inhibitory and acceptance by the consumers. The optimum combination of EOs neither affected the physicochemical characteristics (pH & A_w) nor the sensory parameters (Colour & tensile force) of the noodle. Results from this study suggested that the application of essential oils on pre-cooked Hokkien noodle could extend its shelf-life (tested) from current 81 days to over 100 days (4˚C; 30:70 CO_2:N_2).

8. Recommendations

It is recommended to test the antimicrobial effects of the essential oils used in this study on more Gram-positive bacteria to limit the impact of Heteroresistance effect. Also, a longer experimental period is recommended to allow the microorganisms on EOs-coated sample to show the exponential phase, which could provide more precise information on the EOs-coated product. For further research, it is recommended to apply the essential oils from multiple approaches (such as active antimicrobial packaging) to achieve higher customer acceptability (Arvanitoyannis & Sun, 2012). Alternatively, it is recommended to apply EOs in various MAP conditions (such as 70:30; CO_2:N_2) (Bai et al., 2017), to achieve longer products shelf-life. Moreover, the synergistic effect within the combinations of multiple treatments, including MAP, EOs and physicochemical-control agent (such as water activity lowering agent) are recommended to investigate, which allow the shelf-life of flour-base products to be further prolonged with limited sensory impact (Li et al., 2011). Lastly, it is recommended to apply essential oils on other production lines to extend their shelf-life.

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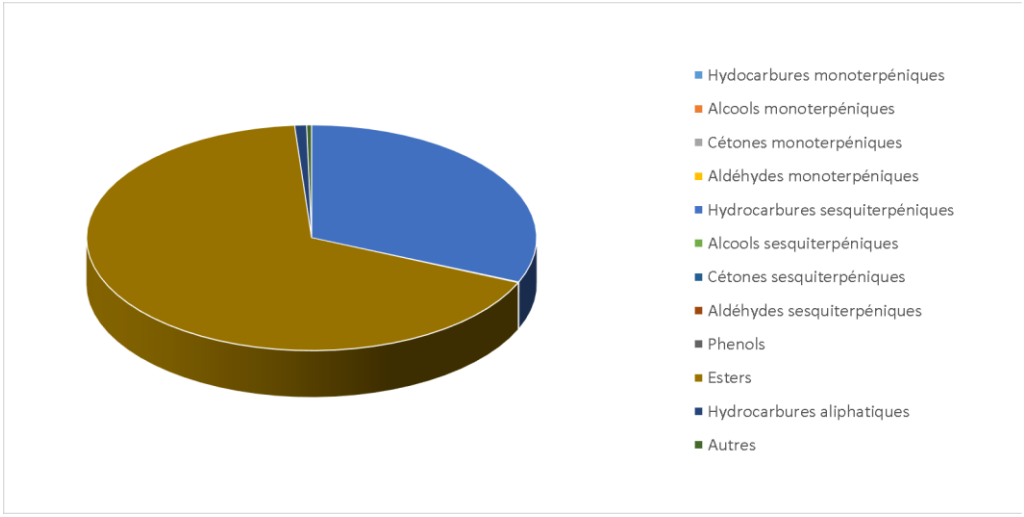
Appendixes

Appendixes A: Essential Oil Spec Sheet

A1. Clove Bud Essential Oil

Florihana JE INTERNATIONAL	ENREGISTREMENT DES BULLETINS ANALYTIQUES : CHROMATOGRAPHIE ESSENTIAL OIL CHROMATOGRAPHY SHEET RECORDS	FORM-LAB005-B	Page 1 sur 2
		Date d'entrée en vigueur / taking effect : 10/06/2011	

Date	:	16/04/2018
Référence produit / Product reference	:	FLE042
Huile essentielle de / Essential oil of	:	Clou de Girofle / Cloves bud
Numéro de lot / Lot Number	:	B160418MG
Densité à 20°C (g/cm³) / Density to 20°C (g/cm³)	:	1.0601
Indice de réfraction / Refractive index	:	1.53489
Pouvoir rotatoire à 20°C / Optical rotation to 20°C	:	-0.43
Mode de culture / Culture mode	:	Cultivé / Cultivated
Pays / Country	:	Madagascar
Date de production / Production date	:	03/2018
D.L.U. / Shelf life	:	04/2023
Mode d'extraction / Extraction mode	:	Distillation à la vapeur / Steam distillation
% Bio / % Organic	:	100%
Nom Latin / Latin Name	:	Eugenia Caryophyllus
Parties utilisées / Used Parts	:	Bourgeons / Buds



Molécule	%
SALICYLATE DE METHYLE	0.136
CHAVICOL	0.089
EUGENOL *	78.872
ALPHA-COPAENE	0.06
BETA-CARYOPHYLLENE	5.761
ALPHA-HUMULENE	0.661
ACETATE D'EUGENYLE	14.005
DELTA-CADINENE	0.061
SPATHULENOL	0.024
OXYDE DE CARYOPHYLLENE	0.074
Total	**99.743**

* = Substance(s) allergène(s) / allergen(s)
** = Substance(s) classée(s) CMR / Substance(s) classified as CMR

HUILE ESSENTIELLE / ESSENTIAL OIL

CLOU DE GIROFLE BIO /

ORGANIC CLOVE BUD

Référence produit / Product reference: FLE042

Number of pages: **4** VERSION 11/2017

1. IDENTIFICATION DE LA SOCIETE / IDENTIFICATION OF THE COMPANY

JE INTERNATIONAL / DISTILLERIE FLORIHANA
Les Grands Prés - 06460 Caussols - France
Tel : 04 93 09 06 09 - Fax : 04 93 09 86 85
E-mail : qualite@florihana.com

2. IDENTIFICATION DE LA SUBSTANCE / IDENTIFICATION OF THE SUBSTANCE

Nom du produit / Product's name: HUILE ESSENTIELLE DE CLOU DE GIROFLE BIO / ESSENTIAL OIL OF ORGANIC CLOVE BUD
Référence interne / Internal reference: FLE042
Législation : Substance 100% pure et naturelle / Matter 100% pure and natural
Nom INCI / INCI name: EUGENIA CARYOPHYLLUS BUD OIL
Nom botanique / Botanical name: *Eugenia caryophyllus*

N°CAS EINECS	**:** 84961-50-2
N°EINECS	**:** 284-638-7

3. MODE D'OBTENTION / PRODUCTION MODE

Huile essentielle obtenue par distillation à la vapeur d'eau des clous de Eugenia caryophyllus
Origine de la plante : Madagascar, Sri Lanka

Essential oil obtained by water steam distillation from bud of Eugenia caryophyllus
Origin of plant: Madagascar, Sri Lanka

4. CARACTERISTIQUES ORGANOLEPTIQUES ET PHYSIQUES / PHYSICAL AND ORGANOLEPTIC CHARACTERISTIC

Couleur : Jaune à jaune clair
Odeur : Epicée, typique de l'eugénol

Color: Yellow to light Yellow
Odor: Spicy, typical of eugenol

Densité à 20°C	**:** [1.042 – 1.065]	**Density at 20°C**	**:** [1.042 – 1.065]
Indice de réfraction à 20°C	**:** [1.528 – 1.538]	**Refractive index at 20°C**	**:** [1.528 – 1.538]
Indice de rotation à 20°C	**:** [-2° ; 0°]	**Optical rotation at 20°C**	**:** [-2° ; 0°]
Point éclair	**:** +120°C	**Flash point**	**:** +120°C
pH à 20°C	**:** Non applicable	**pH at 20°C**	**:** Not applicable

5. PRINCIPAUX INGREDIENTS / MAIN INGREDIENTS

Eugénol	(72,00 - 88,00%)
Eugényl acétate	(4,00 - 22,00%)
Béta caryophyllène	(2,00 - 14,00%)

L'origine naturelle des produits ne permet pas d'obtenir une composition identique pour chaque production. Ces valeurs sont indicatives et n'excluent pas la possibilité de légères variations.

Products from natural origin do not provide identical composition for each production. These values are indicative and do not exclude the possibility of slight variations.

6. INFORMATIONS REGLEMENTAIRES / REGULATORY INFORMATIONS

Règlement CLP (CE n°1272/2008)

 DANGERS	H304	Peut être mortel en cas d'ingestion et de pénétration dans les voies respiratoires	May be fatal if swallowed and enters airways.
 ATTENTION	H317	Peut provoquer une allergie cutanée	May cause an allergic skin reaction.
	H319	Provoque une sévère irritation des yeux	Causes serious eye irritation

Classification substance CMR (cancerogene, mutagene, toxique pour la reproduction) / CMR classification (cancerigen, mutagen, toxic for reproduction) : Not regulated

MENTION D'AVERTISSEMENT / WARNINGS

Danger / Danger

CONSEIL(S) DE PRUDENCE / PRECAUTION ADVISES

P280	Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/du visage	Wear protective gloves/protective clothing/eye protection/face protection.
P301/310	En cas d'ingestion: appeler immédiatement un CENTRE ANTIPOISON ou un médecin.	IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
P302/352	En cas de contact avec la peau: laver abondamment à l'eau et au savon.	IF ON SKIN: Wash with plenty of soap and water.
P305/351/338	En cas de contact avec les yeux : rincer soigneusement avec de l'eau pendant plusieurs minutes. Retirer les lentilles de contact si vous en portez et qu'elles sont simples à enlever. Continuer à rincer	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P501	Éliminer le contenu/récipient conformément aux législations en vigueur	Dispose of contents/container according to regulation in force

7. STOCKAGE ET CONSERVATION / STORAGE ET PRESERVATION

La conservation des produits se fait dans les containers d'origine, fermés, à l'abri de l'air, de la lumière, à une température modérée (max. 15°C) et stable.

Keep the product in original containers, well closed, and protected from air, light, and at moderate temperatures (max. 15 ° C) in a cool room.

Au-delà de 5 ans, dans les conditions de conservations décrites ci-dessus, il peut se produire une diminution de la teneur en substances aromatiques ou une légère coloration du produit. De même, pour les eaux non stabilisées, des modifications bactériologiques peuvent survenir.

Beyond 5 years, in storage conditions described above, there may be a decline in flavoring or a slight coloration. Idem for the floral waters not stabilized, biological changes may occur.

8. TRANSPORT

NON REGLEMENTE / NOT REGULATED
Code douanier / Customs rate code **3301.29.511300**

9. INFORMATIONS ADDITIONNELLES / SPECIAL INDICATIONS

La présence de substances allergènes dans un produit fini doit être indiqué par voie d'étiquetage si leurs concentrations respectives dépassent 100 ppm dans un produit rincé et 10 ppm dans un produit non rincé (7ème amendement Directive cosmétique européenne 2003/15/CE)

The presence of the following allergen in a finished product must be indicated by way of labelling if their respective concentration exceeds 100 ppm in a rinsed product and 10 ppn in a product not rinsed. (7th amendment of Cosmetic Directive European 2003/15/EC).

Allergenes presents :		Present allergens :	
Eugénol	(72,00 à 88,00%)	Eugénol	(72,00 à 88,00%)
Benzyl benzoate	(≤ 0,20%)	Benzyl benzoate	(≤ 0,20%)
Isoeugénol	(≤ 0,10%)	Isoeugénol	(≤ 0,10%)

Restrictions IFRA: Cette substance et/ou certains de ses composants sont concernés par le Code of Practice de l'IFRA, 48ème amendement du 9 juillet 2015, consultable sur le site internet www.ifraorg.org

IFRA restrictions: This substance and/or some of its components are covered by the Code of Practise of the IFRA, the 48h Amendment of July 9th 2015, available on the internet website www.ifraorg.org

L'origine naturelle des produits ne permet pas d'obtenir une composition identique pour chaque production. Ces valeurs sont indicatives et n'excluent pas la possibilité de légères variations.

Products from natural origin do not provide identical composition for each production. These values are indicative and do not exclude the possibility of slight variations.

Biologique : produit issus de l'agriculture biologique certifiés par Ecocert FR-BIO-01, NOP/USDA certifié par Control Union BV.
Matière première certifiée par ECOCERT FR-BIO-01
100% des ingrédients sont d'origine naturelle
100% du total des ingrédients sont issus de l'Agriculture Biologique

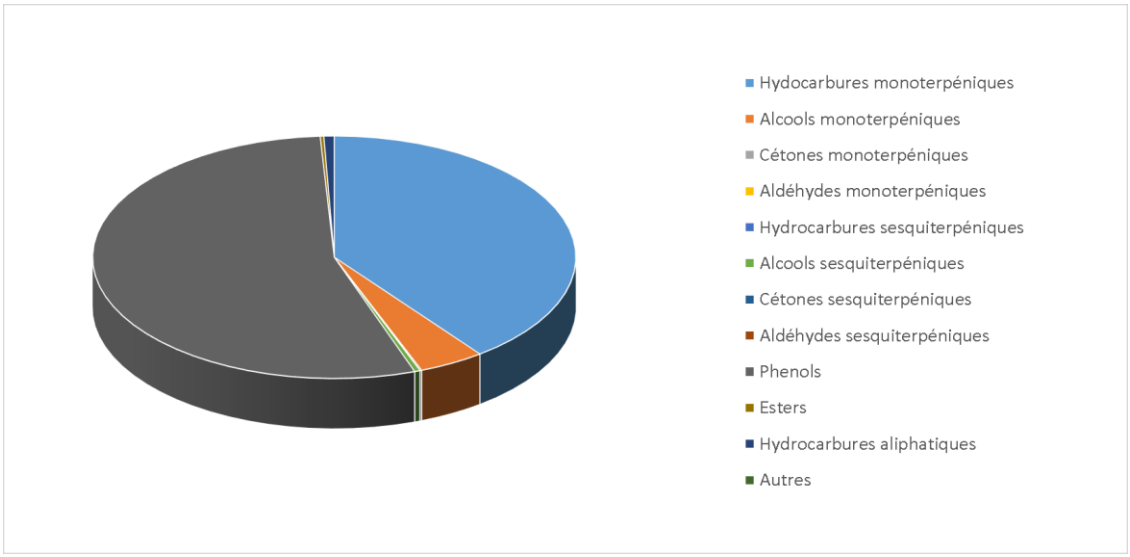
Organic: agro-food products from organic farming certified by Ecocert FR-BIO-01, NOP/USDA certified by Control Union BV.
Raw materials certified by Ecocert FR-BIO-01
100% ingredients from natural origin
100% of the total ingredients are from organic farming

NOMBRE DE PAGES : 4 **FIN DU DOCUMENT / END**

A2. Savory Essentia oil

FLORIHANA	ENREGISTREMENT DES BULLETINS ANALYTIQUES : CHROMATOGRAPHIE ESSENTIAL OIL CHROMATOGRAPHY SHEET RECORDS	FORM-LAB005-B	Page 1 sur 3
JE INTERNATIONAL		Date d'entrée en vigueur / taking effect : 10/06/2011	

Date	:	03/04/2019
Référence produit / Product reference	:	FLE082
Huile essentielle de / Essential oil of	:	Sarriette / Savory
Numéro de lot / Lot Number	:	B030419ES
Densité à 20°C (g/cm^3) / Density to 20°C (g/cm^3)	:	0.926
Indice de réfraction / Refractive index	:	1.50141
Pouvoir rotatoire à 20°C / Optical rotation to 20°C	:	-1.03°
Mode de culture / Culture mode	:	Cultivé / Cultivated
Pays / Country	:	Espagne / Spain
Date de production / Production date	:	08/2018
D.L.U. / Shelf life	:	09/2023
Mode d'extraction / Extraction mode	:	Distillation à la vapeur / Steam distillation
% Bio / % Organic	:	100%
Nom Latin / Latin Name	:	Satureja montana
Parties utilisées / Used Parts	:	Sommités Fleuries / Flowering Tops



Molécule	%
TRICYCLENE	0.012
ALPHA-THUJENE	0.875
ALPHA-PINENE	1.205
THUJA-2,4(10)-DIENE	0.015
FENCHENE	0.032
CAMPHENE	0.365
SABINENE	0.009
BETA-PINENE	0.268
1-OCTEN-3-OL	0.493
3-OCTANONE	0.165
MYRCENE	1.406
PARA-MENTHA-1(7)8-DIENE	0.019
ALPHA-PHELLANDRENE	0.133
DELTA-3-CARENE	0.067
ALPHA-TERPINENE	0.952
PARA-CYMENE	17.135
LIMONENE *	0.484
BETA-PHELLANDRENE	0.277
1,8-CINEOLE (EUCALYPTOL)	0.152
(Z)-BETA-OCIMENE	0.162
(E)-BETA-OCIMENE	0.134
GAMMA-TERPINENE	13.106
CIS-HYDRATE DE SABINENE	0.169
TERPINOLENE	0.109
PARA-CYMENENE	0.037
LINALOL *	1.44
TRANS-HYDRATE DE SABINENE	0.099
CIS-THUJONE	0.014
CAMPHRE	0.182
BORNEOL	1.486
TERPINENE-4-OL	0.565
PARA-CYMENE-8-OL	0.029
ALPHA-TERPINEOL	0.247
THYMOL METHYL ETHER	0.148
THYMOL	4.954
CARVACROL METHYL-ETHER	3.083

CARVACROL	43.606
ACETATE DE LINALYLE	0.015
CARVONE	0.093
ACETATE DE CARVACRYLE	0.017
ACETATE DE NERYLE	0.069
ACETATE DE GERANYLE	0.072
ACETATE DE THYMYLE	0.055
ALPHA-COPAENE	0.026
BETA-BOURBONENE	0.015
BETA-CARYOPHYLLENE	4.073
ALPHA-HUMULENE	0.14
BICYCLOGERMACRENE	0.016
BETA-BISABOLENE	0.549
GAMMA-CADINENE	0.022
DELTA-CADINENE	0.052
CIS-CALAMENENE	0.022
SPATHULENOL	0.033
OXYDE DE CARYOPHYLLENE	0.323
Total	**99.226**

* = Substance(s) allergène(s) / allergen(s)
** = Substance(s) classée(s) CMR / Substance(s) classified as CMR

HUILE ESSENTIELLE / ESSENTIAL OIL

SARRIETTE BIO /

ORGANIC SAVORY

Référence produit / Reference product : FLE082

Number of pages: **3** VERSION 05/2018

1. IDENTIFICATION DE LA SOCIETE / IDENTIFICATION OF THE COMPANY

JE INTERNATIONAL / DISTILLERIE FLORIHANA
Les Grands Prés
06460 Caussols
France
Tel : 04 93 09 06 09
Fax : 04 93 09 86 85
E-mail : laboqualite@florihana.com

2. IDENTIFICATION DE LA SUBSTANCE / IDENTIFICATION OF THE SUBSTANCE

Nom du produit / product's name: HUILE ESSENTIELLE BIO - SARRIETTE BIO / ORGANIC SAVORY
Référence interne / Internal reference: FLE082
Législation : substance 100% pure et naturelle / Matter 100% pure and natural
Nom INCI / INCI name: SATUREJA MONTANA OIL
Nom botanique / Botanical name: *Satureja montana L.*
Substance : Arôme Naturel / Natural flavour

N°CAS EINECS	: 90106-57-3
N°EINECS	: 290-280-2
N° CoE	: 426n
N° FEMA	: 3016

3. MODE D'OBTENTION / PRODUCTION MODE

Huile essentielle obtenue par distillation à la vapeur des sommités fleuries de *Satureja montana L.*
Origine de la plante : Albanie, Espagne

Essential oil obtained by steam distillation of the Flowering top of Satureja montana L.
Origin of plant: Albania, Spain

4. CARACTERISTIQUES ORGANOLEPTIQUES ET PHYSIQUES / PHYSICAL AND ORGANOLEPTIC CHARACTERISTIC

Couleur : Jaune pâle à brun
Odeur : Caractéristique
Utilisation : Alimentaire

Color: *Pale yellow to brun*
Odor: *caracteristic*
Using : Food

Densité à 20°C	: 0,910 à 0,939	**Density at 20°C**	: *0,910 to 0,939*
Indice de réfraction à 20°C	: 1,490 à 1,510	**Refractive index at 20°C**	: *1,490 to 1,510*
Indice de rotation à 20°C	: -5 ° à +5 °	**Optical rotation at 20°C**	: *-5 ° to +5 °*
Point éclair	: +63°C	**Flash point**	: +63°C
pH à 20°C	: Non applicable	**pH at 20°C**	: Not applicable

5. PRINCIPAUX INGREDIENTS / MAIN INGREDIENTS

Carvacrol (25,00 à 50,00%)
Gamma terpinène (5,00 à 25,00%)
Para cymène (5,00 à 25,00%)
Thymol (<= 15,00%)

6. INFORMATIONS REGLEMENTAIRES / REGULATORY INFORMATIONS

Règlement CLP (CE n°1272/2008)

PICTOGRAMME(S) DE DANGER /
SYMBOL (S) OF DANGER


SGH05


SGH07


SGH08


SGH09


SGH02

MENTION(S) DE DANGER */ REFERENCE (S) OF DANGER*

H302	Nocif en cas d'ingestion.	*Harmful if inhalated*
H304	Peut être mortel en cas d'ingestion et de pénétration dans les voies respiratoires	*May be fatal if swallowed and enters airways.*
H314	Provoque des brûlures de la peau et des lésions oculaires graves	*Causes severe skin burns and eye damage*
H317	Peut provoquer une allergie cutanée	*May cause an allergic skin reaction.*
H411	Toxique pour les organismes aquatiques, entraîne des effets néfastes à long terme.	*Toxic to aquatic life with long lasting effects*
H226	Liquide et vapeurs inflammables	*Flammable liquid and vapour.*

MENTION D'AVERTISSEMENT / WARNINGS

Danger / Danger

CONSEIL(S) DE PRUDENCE / PRECAUTION ADVISES

P273	Éviter le rejet dans l'environnement.	*Avoid release to the environment.*
P280	Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/du visage	*Wear protective gloves/protective clothing/eye protection/face protection.*
P301/310	En cas d'ingestion: appeler immédiatement un CENTRE ANTIPOISON ou un médecin.	*IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.*
P302/352	En cas de contact avec la peau: laver abondamment à l'eau et au savon.	*IF ON SKIN: Wash with plenty of soap and water.*
P305/351/338	EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer.	*EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer.*
P501	Éliminer le contenu/récipient conformément aux législations en vigueur	*Dispose of contents/container according to regulation in force*

Classification substance CMR (cancerogene, mutagene, toxique pour la reproduction) /
CMR classification (cancerigen, mutagen, toxic for reproduction) :

Composants CMR* n'entrainant pas de classification	**Methyl eugénol (< 0,50%)**	*CMR data without classification*	*Methyl eugénol (< 0,50%)*

7. STOCKAGE ET CONSERVATION / STORAGE ET PRESERVATION

La conservation des produits se fait dans les containers d'origine, fermés, à l'abri de l'air, de la lumière, à une température modérée (max. 15°C) et stable.
Au-delà de 5 ans, dans les conditions de conservations décrites ci-dessus, il peut se produire une diminution de la teneur en substances aromatiques ou une légère coloration du produit. De même, pour les eaux non stabilisées, des modifications bactériologiques peuvent survenir.

Keep the product in original containers, well closed, and protected from air, light, and at moderate temperatures (max. 15 ° C) in a cool room.

Beyond 5 years, in storage conditions described above, there may be a decline in flavoring or a slight coloration. Idem for the floral waters not stabilized, biological changes may occur.

8. TRANSPORT

Non reglementé / Not regulated
Code douanier / Customs rate code **3301 29 41 00**

9. INFORMATIONS ADDITIONNELLES / SPECIAL INDICATIONS

La présence de substances allergènes dans un produit fini doit être indiqué par voie d'étiquetage si leurs concentrations respectives dépassent 100 ppm dans un produit rincé et 10 ppm dans un produit non rincé (7ème amaendement Directive cosmétique européenne 2003/15/CE)

Allergenes presents :
D-Limonène (<= 3,00%)
Linalol (<= 3,00%)
Géraniol (<= 1,00%)

Restrictions IFRA: Cette substance et/ou certains de ses composants sont concernés par le Code of Practice de l'IFRA, 48ème amendement du 9 juillet 2015, consultable sur le site internet www.ifraorg.org

The presence of the following allergen in a finished product must be indicated by way of labelling if their respective concentration exceeds 100 ppm in a rinsed product and 10 ppn in a product not rinsed. (7th amendment of Cosmetic Directive European 2003/15/EC).

Present allergens :
D-Limonène (<= 3,00%)
Linalol (<= 3,00%)
Géraniol (<= 1,00%)

IFRA restrictions: This substance and/or some of its components are covered by the Code of Practise of the IFRA, the 48th Amendment of July 9th 2015, available on the internet website www.ifraorg.org

Biologique : produit issus de l'agriculture biologique certifiés par Ecocert FR-BIO-01, JAS certifié par Control Union BV.
Matière première certifiée par ECOCERT FR-BIO-01
100% des ingrédients sont d'origine naturelle
100% du total des ingrédients sont issus de l'Agriculture Biologique

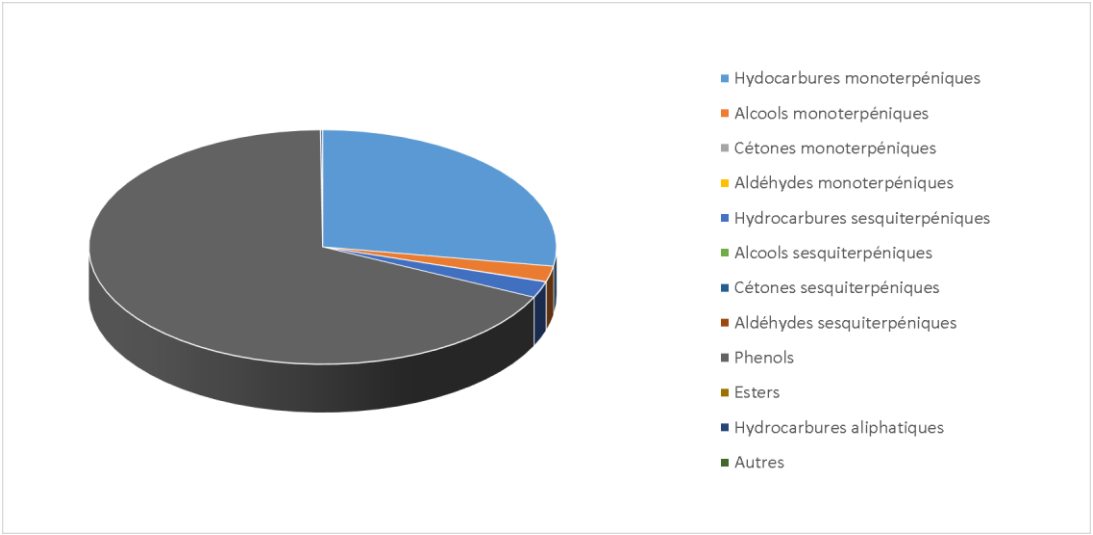
Organic: agro-food products from organic farming certified by Ecocert FR-BIO-01, JAS certified by Control Union BV.
Raw materials certified by Ecocert FR-BIO-01
100% ingredients from natural origin
100% of the total ingredients are from organic farming

NOMBRE DE PAGES : 3 **FIN DU DOCUMENT / END**

A3. Wild Oregano Essential Oil

FLORIHANA	ENREGISTREMENT DES BULLETINS ANALYTIQUES : CHROMATOGRAPHIE ESSENTIAL OIL CHROMATOGRAPHY SHEET RECORDS	FORM-LAB005-B	Page 1 sur 3
JE INTERNATIONAL		Date d'entrée en vigueur / taking effect : 10/06/2011	

Date	:	12/03/2019
Référence produit / Product reference	:	FLE126
Huile essentielle de / Essential oil of	:	Origan Sauvage / Wild Oregano
Numéro de lot / Lot Number	:	H120319MA
Densité à 20°C (g/cm^3) / Density to 20°C (g/cm^3)	:	0.9363
Indice de réfraction / Refractive index	:	1.50392
Pouvoir rotatoire à 20°C / Optical rotation to 20°C	:	0°
Mode de culture / Culture mode	:	Sauvage / Wild
Pays / Country	:	Maroc / Morocco
Date de production / Production date	:	08/2018
D.L.U. / Shelf life	:	09/2023
Mode d'extraction / Extraction mode	:	Distillation à la vapeur / Steam distillation
% Bio / % Organic	:	100%
Nom Latin / Latin Name	:	Origanum Compactum
Parties utilisées / Used Parts	:	Sommités Fleuries / Flowering Tops



Molécule	%
2-METHYL-BUTANOATE DE METHYLE	0.018
ALPHA-THUJENE	1.206
ALPHA-PINENE	0.757
THUJA-2,4(10)-DIENE	0.025
CAMPHENE	0.107
SABINENE	0.014
BETA-PINENE	0.174
OCTEN-3-OL	0.048
3-OCTANONE	0.076
MYRCENE	1.906
ALPHA-PHELLANDRENE	0.223
DELTA-3-CARENE	0.088
ALPHA-TERPINENE	1.971
PARA-CYMENE	7.861
LIMONENE *	0.276
BETA-PHELLANDRENE	0.187
1,8-CINEOLE (EUCALYPTOL)	0.266
(E)-BETA-OCIMENE	0.069
CIS-HYDRATE DE SABINENE	0.14
TERPINOLENE	0.085
PARA-CYMENENE	0.029
LINALOL *	1.154
CAMPHRE	0.053
TERPINENE-4-OL	0.378
ALPHA-TERPINEOL	0.07
TRANS-DIHYDROCARVONE	0.052
CARVACROL METHYL-ETHER	0.202
THYMOL	9.455
CARVACROL	53.553
BETA-CARYOPHYLLENE	1.828
ALPHA-HUMULENE	0.088
BICYCLOGERMACRENE	0.014
BETA-BISABOLENE	0.05
GAMMA-CADINENE	0.07
GAMMA-TERPINENE	17.426
Total	**99.919**

* = Substance(s) allergène(s) / allergen(s)
** = Substance(s) classée(s) CMR / Substance(s) classified as CMR

HUILE ESSENTIELLE / ESSENTIAL OIL

ORIGAN SAUVAGE BIO /

ORGANIC WILD OREGANO

Référence produit / Product reference: FLE126

Number of pages: **4** VERSION 11/2018

1. IDENTIFICATION DE LA SOCIETE / IDENTIFICATION OF THE COMPANY

JE INTERNATIONAL / DISTILLERIE FLORIHANA
Les Grands Prés
06460 Caussols
France
Tel : 04 93 09 06 09
Fax : 04 93 09 86 85
E-mail : qualite@florihana.com

2. IDENTIFICATION DE LA SUBSTANCE / IDENTIFICATION OF THE SUBSTANCE

Nom du produit / Product's name: Huile essentielle ORIGAN SAUVAGE BIO / Essential oil of ORGANIC WILD OREGANO
Référence interne / Internal reference: FLE126
Législation : Substance 100% pure et naturelle / Matter 100% pure and natural
Nom INCI / INCI name: ORIGANUM COMPACTUM OIL
Nom botanique / Botanical name: *Origanum compactum* L.

N°CAS TSCA	: -
N°CAS EINECS	: 90082-26-1
N°EINECS	: 290-114-9
N°FEMA	: -

3. MODE D'OBTENTION / PRODUCTION MODE

Huile essentielle obtenue par distillation à la vapeur des sommités fleuries de Origanum compactum Benth.
Origine de la plante : Maroc

Essential Oil obtained by steam distillation from the flowering top of Origanum compactum Benth.
Origin of plant: Morocco

4. CARACTERISTIQUES ORGANOLEPTIQUES ET PHYSIQUES / PHYSICAL AND ORGANOLEPTIC CHARACTERISTIC

Couleur : Jaune clair à jaune brun
Odeur : Note phénolée, caractéristique du carvacrol

Densité à 20°C	: [0.905 – 0.950]
Indice de réfraction à 20°C	: [1.495 – 1.514]
Indice de rotation à 20°C	: -5° à +1°
Point éclair	: +62°C
pH à 20°C	: Non applicable

Color: Clear yellow to brownish yellow
Odor: Note of phenol, characteristic of carvacrol

Density at 20°C	: [0.905 – 0.950]
Refractive index at 20°C	: [1.495 – 1.514]
Optical rotation at 20°C	: -5° to +1°
Flash point	: +62°C
pH at 20°C	: Not applicable

5. PRINCIPAUX INGREDIENTS / MAIN INGREDIENTS

Carvacrol	(21,00 to 57,00%)
Thymol	(8,00 to 28,00%)
Gamma terpinene	(9,00 to 26,00%)
Para cymene	(6,00 to 20,00%)

6. INFORMATIONS REGLEMENTAIRES / REGULATORY INFORMATIONS

Règlement CLP (CE n°1272/2008)

 DANGER	H304	Peut être mortel en cas d'ingestion et de pénétration dans les voies respiratoires	May be fatal if swallowed and enters airways.
	H317	Peut provoquer une allergie cutanée	May cause an allergic skin reaction.
	H371	Peut causer des dommages aux organes	May cause damage to organs.
	H302	Nocif en cas d'ingestion	Harmful if swallowed
	H314	Provoque des brulures de la peau et des dommages aux yeux	Causes severe skin burns and eye damage.
 ATTENTION	H411	Toxique pour les organismes aquatiques, entraîne des effets néfastes à long terme	Toxic to aquatic life with long lasting effects.

Classification substance CMR (cancerogene, mutagene, toxique pour la reproduction) / CMR classification (cancerigen, mutagen, toxic for reproduction) : Not regulated

MENTION D'AVERTISSEMENT / WARNINGS

Danger / Danger

CONSEIL(S) DE PRUDENCE / PRECAUTION ADVISES

P273	Éviter le rejet dans l'environnement.	Avoid release to the environment.
P280	Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/du visage	Wear protective gloves/protective clothing/eye protection/face protection.
P301/310	En cas d'ingestion: appeler immédiatement un CENTRE ANTIPOISON ou un médecin.	IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
P302/350	En cas de contact avec la peau: lavez soigneusement avec beaucoup de savon et d'eau	IF ON SKIN: Gently wash with plenty of soap and water
P303/P361/P353	En cas de contact avec la peau (ou les cheveux) : Enlevez immédiatement tous les vêtements contaminés. Rincez la peau avec de l'eau puis douchez vous	IF ON SKIN (or hair): Remove, Take off immediately all contaminated clothing. Rinse skin with water, shower.
P305/361/353	En cas de contact avec les yeux : Rincez soigneusement avec de l'eau pendant plusieurs minutes. Retirez vos lentilles de contact si vous en portez puis continuer de rincer	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P501	Éliminer le contenu/récipient conformément aux législations en vigueur	Dispose of contents/container according to regulation in force

7. STOCKAGE ET CONSERVATION / STORAGE ET PRESERVATION

La conservation des produits se fait dans les containers d'origine, fermés, à l'abri de l'air, de la lumière, à une température modérée (max. 15°C) et stable.
Au-delà de 5 ans, dans les conditions de conservations décrites ci-dessus, il peut se produire une diminution de la teneur en substances aromatiques ou une légère coloration du produit. De même, pour les eaux non stabilisées, des modifications bactériologiques peuvent survenir.

Keep the product in original containers, well closed, and protected from air, light, and at moderate temperatures (max. 15 ° C) in a cool room.

Beyond 5 years, in storage conditions described above, there may be a decline in flavoring or a slight coloration. Idem for the floral waters not stabilized, biological changes may occur.

8. TRANSPORT

Classe 6.1 + 8, Groupe d'emballage II, UN n° 1169 / category 6.1 + 8, PG II, UN n° 2927
Code douanier / Customs rate code **3301.29.515000**

9. INFORMATIONS ADDITIONNELLES / SPECIAL INDICATIONS

La présence de substances allergènes dans un produit fini doit être indiqué par voie d'étiquetage si leurs concentrations respectives dépassent 100 ppm dans un produit rincé et 10 ppm dans un produit non rincé (7ème amendement Directive cosmétique européenne 2003/15/CE)

The presence of the following allergen in a finished product must be indicated by way of labelling if their respective concentration exceeds 100 ppm in a rinsed product and 10 ppn in a product not rinsed. (7th amendment of Cosmetic Directive European 2003/15/EC).

Allergenes presents :
Linalol (<= 2,00%), D-Limonene (<= 1,00%)

Present allergens :
Linalool (<= 2,00%), D-Limonene (<= 1,00%)

Restrictions IFRA: Cette substance et/ou certains de ses composants sont concernés par le Code of Practice de l'IFRA, 48ème amendement du 9 juillet 2015, consultable sur le site internet www.ifraorg.org

IFRA restrictions: This substance and/or some of its components are covered by the Code of Practise of the IFRA, the 48th Amendment of July 9th 2015, available on the internet website www.ifraorg.org

Biologique : produit issus de l'agriculture biologique certifiés par Ecocert FR-BIO-01, JAS certifié par Control Union BV.
Matière première certifiée par ECOCERT FR-BIO-01
100% des ingrédients sont d'origine naturelle
100% du total des ingrédients sont issus de l'Agriculture Biologique

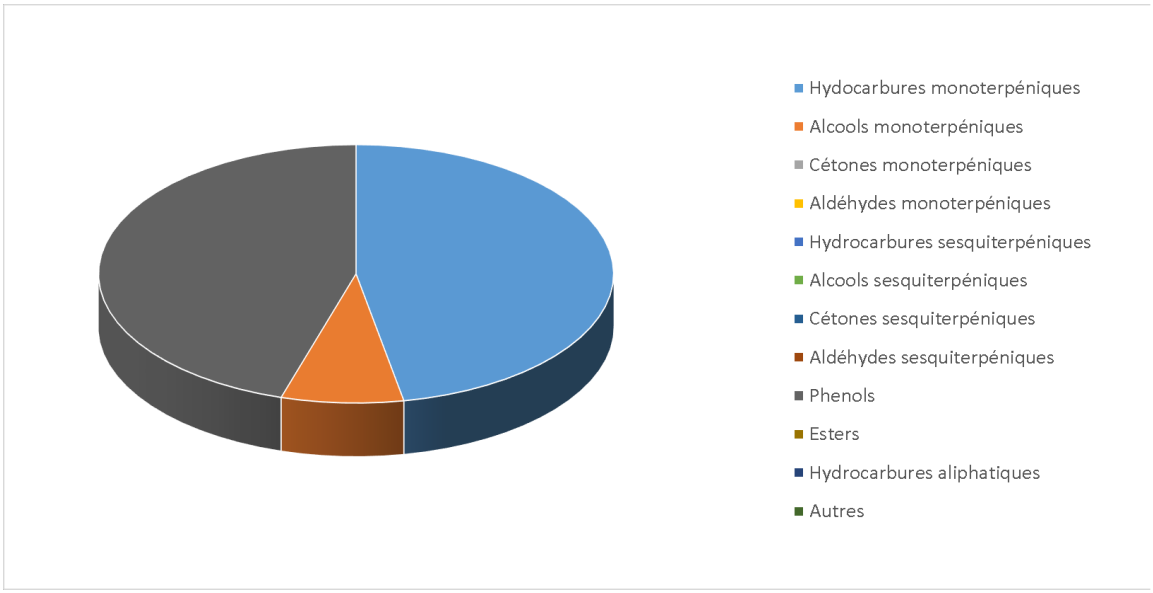
Organic: agro-food products from organic farming certified by Ecocert FR-BIO-01, JAS certified by Control Union BV.
Raw materials certified by Ecocert FR-BIO-01
100% ingredients from natural origin
100% of the total ingredients are from organic farming

NOMBRE DE PAGES : 4 **FIN DU DOCUMENT / END**

A4. Thyme Thymol Essential Oil

Florihana JE INTERNATIONAL	ENREGISTREMENT DES BULLETINS ANALYTIQUES : CHROMATOGRAPHIE ESSENTIAL OIL CHROMATOGRAPHY SHEET RECORDS	FORM-LAB005-B	Page 1 sur 2
		Date d'entrée en vigueur / taking effect : 10/06/2011	

Date	:	16/10/2018
Référence produit / Product reference	:	FLE091
Huile essentielle de / Essential oil of	:	Thym Thymol / Thyme Thymol
Numéro de lot / Lot Number	:	L121018ES
Densité à 20°C (g/cm^3) / Density to 20°C (g/cm^3)	:	0.915
Indice de réfraction / Refractive index	:	1.498
Pouvoir rotatoire à 20°C / Optical rotation to 20°C	:	- 3°
Mode de culture / Culture mode	:	Sauvage / Wild
Pays / Country	:	Espagne / Spain
Date de production / Production date	:	06/2018
D.L.U. / Shelf life	:	07/2023
Mode d'extraction / Extraction mode	:	Distillation à la vapeur / Steam distillation
% Bio / % Organic	:	100%
Nom Latin / Latin Name	:	Thymus vulgaris thymoliferum
Parties utilisées / Used Parts	:	Sommités Fleuries / Flowering Tops



Molécule	%
ALPHA-PINENE	1.65
ALPHA-THUJENE	1.43
CAMPHENE	1.28
BETA-MYRCENE	2.17
ALPHA-TERPINENE	1.89
LIMONENE *	0.57
GAMMA-TERPINENE	13.09
PARA-CYMENE	18.52
LINALOL *	4.86
BORNEOL	1.99
THYMOL	37.27
CARVACROL	3.22
Total	**87.94**

* = Substance(s) allergène(s) / allergen(s)
** = Substance(s) classée(s) CMR / Substance(s) classified as CMR

HUILE ESSENTIELLE / ESSENTIAL OIL

THYM THYMOL BIO /

ORGANIC THYME THYMOL

Référence produit / Product reference: FLE091

Number of pages: **4** VERSION 10/2018

1. IDENTIFICATION DE LA SOCIETE / IDENTIFICATION OF THE COMPANY

JE INTERNATIONAL / DISTILLERIE FLORIHANA
Les Grands Prés
06460 Caussols
France
Tel : 04 93 09 06 09
Fax : 04 93 09 86 85
E-mail : qualite@florihana.com

2. IDENTIFICATION DE LA SUBSTANCE / IDENTIFICATION OF THE SUBSTANCE

Nom du produit / Product's name: Huile essentielle de THYM THYMOL BIO / Essential oil of ORGANIC THYME THYMOL
Référence interne / Internal reference : FLE091
Législation : Substance 100% pure et naturelle / Matter 100% pure and natural
Nom INCI / INCI name: THYMUS VULGARIS FLOWER/LEAF OIL (SYM : THYMUS ZYGIS HERB OIL)
Nom botanique / Botanical name: Thymus vulgaris thymoliferum (sym : zygis)

N°CAS TSCA	: 8007-46-3
N°CAS EINECS	: 85085-75-2
N°EINECS	: 285-397-0
N°FEMA	: -
N°FDA	: 182.20
N°CoE:	: 457n
FCC	: -
RIFM	: -
FMA	: -
AFNOR	: NF T 75-349

3. MODE D'OBTENTION / PRODUCTION MODE

Huile essentielle obtenue par distillation à la vapeur d'eau des sommités fleuries de Thymus vulgaris thymoliferum
Origine de la plante : Espagne

Essential oil obtained by water steam distillation from flowering tops of Thymus vulgaris thymoliferum
Origin of plant: Spain

4. CARACTERISTIQUES ORGANOLEPTIQUES ET PHYSIQUES / PHYSICAL AND ORGANOLEPTIC CHARACTERISTIC

Couleur : Jaune à rouge brun
Odeur : Agreste, aromatique, forte et épicée

Color: Yellow to red brown
Odor: Rural, aromatic, strong and spicy

Densité à 20°C	: [0.915 – 0.937]	**Density at 20°C**	: [0.915 – 0.937]
Indice de réfraction à 20°C	: [1.490 – 1.505]	**Refractive index at 20°C**	: [1.490 – 1.505]
Indice de rotation à 20°C	: [-10° ; +10°]	**Optical rotation at 20°C**	: [-10° ; +10°]
Point éclair	: +60°C	**Flash point**	: +60°C
pH à 20°C	: Non applicable	**pH at 20°C**	: Not applicable

5. PRINCIPAUX INGREDIENTS / MAIN INGREDIENTS

Thymol	37 – 55 %
Para cymène	8 - 28 %
Gamma terpinène	6 - 14 %
Carvacrol	<= 14,00%

L'origine naturelle des produits ne permet pas d'obtenir une composition identique pour chaque production. Ces valeurs sont indicatives et n'excluent pas la possibilité de légères variations.

Products from natural origin do not provide identical composition for each production. These values are indicative and do not exclude the possibility of slight variations.

6. INFORMATIONS REGLEMENTAIRES / REGULATORY INFORMATIONS

Règlement CLP (CE n°1272/2008)

 ATTENTION	H226	Liquide et vapeurs inflammables	Flammable liquid and vapour.
 ATTENTION	H302	Nocif en cas d'ingestion	Harmful if swallowed
	H317	Peut provoquer une allergie cutanée	May cause an allergic skin reaction.
 DANGERS	H304	Peut être mortel en cas d'ingestion et de pénétration dans les voies respiratoires	May be fatal if swallowed and enters airways.
 DANGERS	H314	Provoque de graves brûlures de la peau et des lésions oculaires	Causes severe skin burns and eye damage
	H411	Toxique pour les organismes aquatiques, entraîne des effets néfastes à long terme.	Toxic to aquatic life with long lasting effects

Classification substance CMR (cancerogene, mutagene, toxique pour la reproduction) / CMR classification (cancerigen, mutagen, toxic for reproduction) : Non réglementée / not regulated

MENTION D'AVERTISSEMENT / WARNINGS

Danger / Danger

CONSEIL(S) DE PRUDENCE / PRECAUTION ADVISES

P210	Tenir à l'écart de la chaleur/des étincelles/des flammes nues/des surfaces chaudes. Ne pas fumer	*Keep away from heat/sparks/open flames/hot surfaces. - No smoking.*
P233	Maintenir le récipient fermé de manière étanche.	*Keep container tightly closed.*
P240	Mise à la terre/liaison équipotentielle du récipient et du matériel de réception	*Ground/bond container and receiving equipment.*
P241	Utiliser du matériel électrique/de ventilation/d'éclairage/antidéflagrant	*Use explosion-proof electrical/ventilating/lighting/.../equipment.*
P242	Ne pas utiliser d'outils produisant des étincelles	*Use only non-sparking tools.*

P243	Prendre des mesures de précaution contre les décharges électrostatiques	*Take precautionary measures against static discharge.*
P260	Ne pas respirer les poussières/fumées/gaz/brouillards/vapeurs/aérosols	*Do not breathe dust/fume/gas/mist/vapours/spray.*
P264	Se laver les mains soigneusement après manipulation	*Wash...thoroughly after handling*
P270	Ne pas manger, boire ou fumer en manipulant ce produit	*Do not eat, drink or smoke when using this product.*
P272	Les vêtements de travail contaminés ne devraient pas sortir du lieu de travail	*Contaminated work clothing should not be allowed out of the workplace.*
P273	Éviter le rejet dans l'environnement.	*Avoid release to the environment.*
P280	Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/du visage	*Wear protective gloves/protective clothing/eye protection/face protection.*
P301/310	En cas d'ingestion: appeler immédiatement un CENTRE ANTIPOISON ou un médecin.	*IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.*
P301/330/331	EN CAS D'INGESTION: rincer la bouche. NE PAS faire vomir	*IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.*
P303/361/353	EN CAS DE CONTACT AVEC LA PEAU (ou les cheveux): enlever immédiatement les vêtements contaminés. Rincer la peau à l'eau, se doucher.	*IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinseskin with water/shower.*
P304/340	EN CAS D'INHALATION: transporter la victime à l'extérieur et la maintenir au repos dans une position où elle peut confortablement respirer	*IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing*
P305/351/338	EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer.	*IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.*
P333/313	En cas d'irritation ou d'éruption cutanée: consulter un médecin	*If skin irritation or rash occurs: Get medical advice/attention.*
P363	Laver les vêtements contaminés avant réutilisation	*Wash contaminated clothing before reuse.*
P370/378	En cas d'incendie: utiliser l'extincteur adapté pour l'extinction.	*In case of fire: Use ... for extinction.*
P391	Recueillir le produit répandu	*Collect spillage.*
P403/235	Stocker dans un endroit bien ventilé. Tenir au frais	*Store in a well-ventilated place. Keep cool.*
P405	Garder sous clef	*Store locked up.*
P501	Éliminer le contenu/récipient conformément aux législations en vigueur	*Dispose of contents/container according to regulation in force*

7. STOCKAGE ET CONSERVATION / STORAGE ET PRESERVATION

La conservation des produits se fait dans les containers d'origine, fermés, à l'abri de l'air, de la lumière, à une température modérée (max. 15°C) et stable.

Keep the product in original containers, well closed, and protected from air, light, and at moderate temperatures (max. 15 ° C) in a cool room.

Au-delà de 5 ans, dans les conditions de conservations décrites ci-dessus, il peut se produire une diminution de la teneur en substances aromatiques ou une légère coloration du produit. De même, pour les eaux non stabilisées, des modifications bactériologiques peuvent survenir.

Beyond 5 years, in storage conditions described above, there may be a decline in flavoring or a slight coloration. Idem for the floral waters not stabilized, biological changes may occur.

8. TRANSPORT

Classe 3 + 8, Groupe d'emballage III, UN n° 2924 / category 3 + 8, PG III, UN n° 2924
Code douanier / Customs rate code **3301.29.515000**

9. INFORMATIONS ADDITIONNELLES / SPECIAL INDICATIONS

La présence de substances allergènes dans un produit fini doit être indiqué par voie d'étiquetage si leurs concentrations respectives dépassent 100 ppm dans un produit rincé et 10 ppm dans un produit non rincé (7ème amendement Directive cosmétique européenne 2003/15/CE)

The presence of the following allergen in a finished product must be indicated by way of labelling if their respective concentration exceeds 100 ppm in a rinsed product and 10 ppn in a product not rinsed. (7th amendment of Cosmetic Directive European 2003/15/EC).

Allergenes presents :
Linalol (2 à 7%), Géraniol (≤ 1,50%), D-Limonène (≤ 2,00%)

Present allergens :
Linalol (2 to 7%), Géraniol (≤ 1,50%), D-Limonène (≤ 2,00%)

Restrictions IFRA: Cette substance et/ou certains de ses composants sont concernés par le Code of Practice de l'IFRA, 48ème amendement du 9 juillet 2015, consultable sur le site internet www.ifraorg.org

IFRA restrictions: This substance and/or some of its components are covered by the Code of Practise of the IFRA, the 48th Amendment of July 9th 2015, available on the internet website www.ifraorg.org

L'origine naturelle des produits ne permet pas d'obtenir une composition identique pour chaque production. Ces valeurs sont indicatives et n'excluent pas la possibilité de légères variations.

Products from natural origin do not provide identical composition for each production. These values are indicative and do not exclude the possibility of slight variations.

Biologique : produit issus de l'agriculture biologique certifiés par Ecocert FR-BIO-01, NOP/USDA certifié par Control Union BV.
Matière première certifiée par ECOCERT FR-BIO-01
100% des ingrédients sont d'origine naturelle
100% du total des ingrédients sont issus de l'Agriculture Biologique

Organic: agro-food products from organic farming certified by Ecocert FR-BIO-01, NOP/USDA certified by Control Union BV.
Raw materials certified by Ecocert FR-BIO-01
100% ingredients from natural origin
100% of the total ingredients are from organic farming

NOMBRE DE PAGES : 4 **FIN DU DOCUMENT / END**

Appendix B: Sensory Form

Title of Work: Attitudes to, and preferences for Pre-cooked Asian noodles.

ETHICS CONSENT FORM

THIS CONSENT FORM WILL BE HELD FOR 12 MONTHS FROM DATE OF SIGNING

The information collected in this study will be used to complete an assignment in partial fulfilment of the Master of Technology in Food Technology. Non-participation will not affect your academic performance. No data linked to an individual's identity will be collected. You are under no obligation to accept this invitation. If you decide to participate, please read below statement and sign:

- I have read and understood the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.
- I agree to voluntarily participate in this study under the conditions set out in the Information Sheet.
- I understand I have the right to withdraw from the study at any time and to decline to answer any particular questions.
- I have advised and discussed with the Researcher any potentially relevant cultural, religious or ethical beliefs that may prevent me from consuming the Foods under consideration.
- I agree to be videotaped, but understand that I have the right to ask for the tape to be turned off at any time during the study.

Participants

Signature: .....**Date:** .....

Full Name –

printed: .....

Information Sheet

Researcher(s) Introduction:

Researchers Name:	Jiajun Chen	Supervisors Name:	Tony Mutukumira
Contact Details:	j.chen1@massey.ac.nz	Contact Details:	A.N.Mutukumira@massey.ac.nz

Welcome to the sensory evaluation of a food technology project from MIFST Massey University. We are now developing a new approach to extend the shelf-life of the pre-cooked Asian noodles, several extractives from natural herb were added to the noodles to bring the specific flavour and inhibit the growth of microorganisms on it. The scenario you currently facing is that: you are about to consume the pre-cooked Asian noodles which you bought from the supermarket, and now you just open the package of the pre-cooked Asian noodles, then you see the appearance and smell the odour of it. What do you think about it? How do you feel about the flavour and are you willing to process the cooking before you consume it? Please notice that this kind of noodles requires a short boiling before consuming.

During the session, you will **smell** and **observe** the products with different formulations, and then give your final acceptability of each product. Since these are **semi-cooked** products, **please do not eat it**. There is no same product coded with a different digital number. You will be excluded from taking part if you are allergic to any **pollen, herb, spice** or any cause of **allergic via smell**. You have the right to question the crew whether the products contain the specific material that you allergic to.

All information obtained during this session will be kept confidential and in accordance with the Human Ethics code of Massey University.

Your participation in this study will take a maximum of **10 minutes**.

“This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, HEC Application 13/05. If you have any concerns about the ethics of this research, please contact, Dr Brian Finch Chair, Massey University Human Ethics Committee: Southern A telephone 06 350 5799 x 2541,

Email: humanethicsoutha@massey.ac.nz.”

Thank you for your participation

Sample No: 318

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample No: 273

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample No: 636

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample No: 955

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample No: 804

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample No: 200

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample No: 812

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample No: 744

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample No: 289

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Sample No: 341

How much do you like the odour of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you like the appearance of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

What is your overall opinion of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Appendix C: Raw Data

C1. Phase One Raw Data

C1-I. Broth micro-dilution assay results expressed in minimum inhibitory concentrations.

Essential Oils	*E. coli* NCTC8196 %		*S. aureus* NCTC4163 %		*A. brasiliensis* NZRM2578 %		*P. chrysogenum* NZRM2999 %	
Thyme	0.24	0.12	0.12	0.24	0.48	0.48	0.72	0.72
	0.075	0.075	0.15	0.3	0.60	0.60	0.72	0.72
Savory	0.36	0.72	0.72	0.72	0.72	0.72	1.44	2.88
	0.3	0.15	0.3	0.3	0.48	0.48	1.44	2.88
Oregano	0.0225	0.045	0.045	0.045	0.12	0.12	0.09	0.18
	0.0375	0.0375	0.06	0.075	0.15	0.15	0.09	0.18
Clove	0.48	0.3	0.18	0.36	0.36	0.36	0.18	0.36
	0.48	0.3	0.15	0.15	0.3	0.3	0.18	0.36

C1-II. Agar disc diffusion assay results express in inhibitory area diameter.

Essential oils	Concentration %	E. coli NCTC8196 (mm)	*S. aureus* NCTC4163 (mm)	*A. brasiliensis* NZRM2578 (mm)	*P. chrysogenum* NZRM2999 (mm)
Thyme	9	9.52	6.20	6.00	6.00
	12	14.42	7.43	6.00	6.57
	15	17.63	9.47	8.98	9.05
Savory	9	11.17	7.70	6.00	6.00
	12	14.43	10.42	7.62	7.92
	15	19.28	13.20	9.58	11.63
Oregano	9	15.48	9.33	6.23	9.10
	12	17.85	10.70	9.38	16.32
	15	21.10	15.38	11.73	22.08
Clove	9	12.63	9.43	8.83	16.77
	12	16.73	10.70	13.63	21.23
	15	19.43	11.72	15.10	25.68

C2. Phase Two Raw Data

C2-I. Round 1 Standard plate count results.

Day	Dilution	Initial number	
		CFU/g	
0	10^{-1}	267	217
	10^{-1}	256	192
	10^{-2}	24	30
	10^{-2}	23	22

Day	Dilution	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9
		CFU/g									
7	10^{-1}	175	177	134	67	62	36	59	85	24	32
	10^{-1}	163	180	127	58	58	31	47	84	33	29
	10^{-2}	18	18	8	8	6	2	7	9	2	3
	10^{-2}	16	19	9	6	6	1	5	8	3	3
14	10^{-1}		173	169	112	46	142	85	86	35	64
	10^{-1}		151	123	95	54	131	80	91	56	65
	10^{-2}	46	17	8	10	7	14	9	7	5	8
	10^{-2}	51	14	16	8	4	15	7	10	8	6
21	10^{-1}		285	226	315	164		175	257	66	94
	10^{-1}		254	208	297	170		180	265	71	91
	10^{-2}	258	29	15	32	18	35	19	26	7	11
	10^{-2}	228	21	17	28	16	32	17	26	8	8
28	10^{-1}		276	283		248	315	144		121	120
	10^{-1}		269	277		222	280	139		129	117
	10^{-2}		29	26	58	25	31	15	50	14	14
	10^{-2}		26	28	52	24	29	14	47	12	13
	10^{-3}	36	4	5	7	2	7	4	10	3	2
	10^{-3}	33	3	4	7	2	3	3	9	1	1
35	10^{-1}		307			299	194			166	142
	10^{-1}		294			295	180			149	138
	10^{-2}		35	37	190	26	20	36	118	18	14
	10^{-2}		28	35	185	28	11	31	111	19	16
	10^{-3}	53	4	4	20	3	3	4	13	2	2
	10^{-3}	49	4	2	21	1	2	3	11	1	1

C2-II. Round 2 Standard plate count results.

Day	Dilution	Initial number	
		CFU/g	
0	10^{-1}	184	185
	10^{-1}	177	176
	10^{-2}	18	19
	10^{-2}	17	18

Day	Dilution	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9
		CFU/g									
7	10^{-1}		175	65	47	138	25	208	130	59	63
	10^{-1}		162	61	44	129	19	172	108	52	46
	10^{-2}	37	14	4	5	16	3	23	8	7	5
	10^{-2}	34	12	4	5	12	1	15	8	5	5
14	10^{-1}	329	196	57	171	93	113	261	119	91	72
	10^{-1}	298	188	49	167	90	111	224	107	88	71
	10^{-2}	80	29	12	13	9	8	31	12	10	7
	10^{-2}	76	13	11	6	9	8	29	11	7	6
21	10^{-1}		301	118	300	173	317	307	298	78	98
	10^{-1}		298	115	292	165	295	303	305	70	95
	10^{-2}	136	31	12	47	17	34	40	31	9	27
	10^{-2}	131	29	11	46	17	32	22	29	8	26
28	10^{-1}			311		135				174	168
	10^{-1}			309		112				169	165
	10^{-2}		29	26	71	14	40	39	93	19	14
	10^{-2}		30	22	68	13	34	31	87	17	13
	10^{-3}	50	4	1	7	2	7	4	10	3	2
	10^{-3}	43	3	1	7	2	3	3	9	1	1
35	10^{-1}					235	192			172	157
	10^{-1}					248	127			156	153
	10^{-2}		43	30	222	25	16	59	136	19	19
	10^{-2}		36	38	177	22	15	53	106	16	14
	10^{-3}	58	4	3	25	2	2	3	14	1	2
	10^{-3}	78	3	4	18	0	0	2	12	0	1

C2-III. Round 1 Yeasts and Moulds count results.

Day	Dilution	Initial number	
		CFU/g	
0	10^{-1}	8	12
	10^{-1}	6	13

Day	Dilution	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9
		CFU/g									
7	10^{-1}	16	14	15	15	21	10	4	5	7	7
	10^{-1}	14	7	13	14	15	6	5	7	7	6
14	10^{-1}	10	9	2	6	9	6	6	8	3	3
	10^{-1}	7	7	2	7	4	8	3	7	2	3
21	10^{-1}	7	6	4	4	6	4	6	4	2	4
	10^{-1}	6	5	1	0	4	3	5	4	1	1
28	10^{-0}	24	18	11	12	14	9	22	3	6	9
	10^{-0}	22	8	10	7	11	6	21	3	6	9
	10^{-0}	13	6	4	9	6	6	20	3	5	7
	10^{-0}	22	12	9	11	15	7	26	4	7	9
	10^{-0}	20	10	9	10	10	6	18	4	5	8
	10^{-0}	13	10	9	5	8	6	17	3	5	8
35	10^{-0}	41	15	16	9	8	11	14	8	2	4
	10^{-0}	29	7	11	8	5	6	10	7	2	2
	10^{-0}	21	5	9	6	4	8	9	3	1	2
	10^{-0}	32	11	14	8	9	10	14	9	3	4
	10^{-0}	32	9	12	5	4	11	10	6	2	3
	10^{-0}	31	7	11	8	5	3	9	3	0	1

C2-IV. Round 2 Yeasts and Moulds count results.

Day	Dilution	Initial number	
		CFU/g	
0	10^{-1}	8	12
	10^{-1}	7	15

Day	Dilution	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9
		CFU/g									
7	10^{-1}	19	8	15	18	16	14	16	8	8	8
	10^{-1}	13	9	13	17	12	11	7	6	4	7
14	10^{-1}	6	8	4	17	5	4	6	7	2	6
	10^{-1}	6	9	2	16	1	1	3	6	0	3
21	10^{-0}	17	38	6	17	10	10	14	24	5	6
	10^{-0}	14	31	6	13	9	6	8	13	3	5
	10^{-0}	9	25	4	9	7	5	8	10	3	4
	10^{-0}	17	31	7	15	9	8	10	15	4	5
	10^{-0}	16	30	4	14	8	6	9	15	4	5
	10^{-0}	7	29	2	11	8	4	9	14	3	2
28	10^{-0}	11	18	13	16	13	6	18	3	5	6
	10^{-0}	10	8	11	11	8	4	16	3	5	6
	10^{-0}	5	6	4	9	2	4	15	3	4	2
	10^{-0}	10	12	9	15	12	6	22	4	4	5
	10^{-0}	9	10	9	14	7	5	13	4	4	4
	10^{-0}	8	10	9	11	7	1	12	3	4	5
35	10^{-0}	25	8	16	10	5	8	22	9	8	5
	10^{-0}	21	8	12	4	2	7	10	3	2	2
	10^{-0}	18	4	10	2	1	3	9	2	2	0
	10^{-0}	24	9	14	4	4	7	14	6	4	3
	10^{-0}	21	6	13	4	4	7	12	4	3	2
	10^{-0}	21	6	11	4	1	6	11	4	3	1

C3. Phase Three Raw Data

C3-I. Phase three standard plate count data.

Day	Dilution	Initial number CFU/g			
0	10^{-1}	20	15	18	15
	10^{-2}	4	2	3	2

Sample	Dilution	D8		D16		D24		D32		D40		D45		D50		D55		D60		D65	
		CFU/g																			
S1	10^{0}	58	56	81	83	51	47			87	84	105	105								
	10^{-1}	9	5	10	10	5	5			7	6	13	11	69	62	117	121				
	10^{-2}	1	0	1	0	0	0	154	159	1	0	1	0	8	6	11	12	58	56	168	149
	10^{-3}							20	18									7	7	18	16
S2	10^{0}	59	60	57	58	47	48	53	49	130	150	108	105								
	10^{-1}	8	5	8	5	4	4	5	5	12	13	11	10	62	59	125	156				
	10^{-2}	1	0	0	0	0	0	1	0	1	2	1	1			14	18	48	51	134	128
	10^{-3}															2	3	5	5	16	15
E1	10^{0}	22	23	20	18	21	21	20	20	12	11	6	5	7	8	13	9	20	20	13	11
	10^{-1}	6	6	5	3	2	2	1	1	1	1	1	0	2	1	1	1	2	3	1	1
E2	10^{0}	12	13	14	14	10	9	17	18	20	18	14	14	12	11	7	6	5	8	18	13
	10^{-1}	1	1	1	1	2	1	1	1	2	2	1	1	2	1	1	0	0	1	2	2

Note: Yellow background stands for data were invalid because of package problem, data were excluded from the calculation.

C3-II. Phase three yeasts and moulds count data.

Day	Dilution	Initial number					
		CFU/g					
0	10^{-0}	33	28	28	30	30	29
	10^{-0}	27	22	19	22	17	21

Sample	Dilution	D8		D16		D24		D32		D40		D45		D50		D55		D60		D65	
		CFU/g																			
S1	10^{-0}	8	12	8	7	16	13			13	12			23	21			28	28	33	32
	10^{-0}	7	6	5	7	13	13			11	12			21	21			25	27	31	31
	10^{-0}	6	4	4	3	7	9			10	11			21	21			24	23	30	29
	10^{-1}	3	3	2	2	4	4			5	4	55	33	8	8	63	61	8	8	10	11
	10^{-2}	1	1	1	1	1	1	185	183	1	1	7	5	1	0	8	9	1	0	1	1
	10^{-3}							21	20												
S2	10^{-0}	14	12	7	6	14	12	10	12	13	13	16	17	28	25	22	25	28	26	31	30
	10^{-0}	8	10	6	6	12	12	9	8	12	13	15	16	24	25	22	21	24	25	28	29
	10^{-0}	9	8	4	5	10	11	7	6	12	12	14	12	22	24	19	17	24	24	28	29
E1	10^{-0}	10	12	4	2	4	2	2	2	1	1	1	1	2	0	1	1	3	2	0	0
	10^{-0}	6	6	0	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0
	10^{-0}	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
E2	10^{-0}	5	4	3	2	3	2	1	1	2	1	1	1	0	0	2	1	1	1	1	1
	10^{-0}	3	4	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0
	10^{-0}	2	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

Note: Yellow background stands for data were invalid because of package problem, data were excluded from the calculation.

C3-III. Modified atmosphere conditions within noodle sample package during phase three.

Sample	Gases	D0		D8		D16		D24		D32		D40	
S1	O_2%	0.411	0.411	0.559	0.556	0.558	0.558	0.565	0.564	21.000	20.900	0.855	0.855
	CO_2%	26.200	26.300	25.700	25.800	24.400	24.500	24.000	24.100	0.400	0.100	23.200	23.200
	N_2%	73.300	73.200	73.700	73.600	75.100	75.000	75.300	75.300	78.600	79.000	76.000	75.900
	mBar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
S2	O_2%	0.412	0.410	0.589	0.590	0.603	0.604	0.704	0.705	0.720	0.722	0.776	0.776
	CO_2%	26.000	26.000	25.400	25.500	24.700	24.800	25.000	25.000	24.300	24.400	23.900	23.800
	N_2%	73.500	73.600	74.000	73.900	74.700	74.600	74.300	74.300	75.000	74.800	75.300	75.400
	mBar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E1	O_2%	0.784	0.785	0.784	0.785	0.745	0.747	0.857	0.859	0.967	0.967	3.830	3.840
	CO_2%	25.700	25.800	25.600	25.600	25.200	25.300	24.300	24.300	23.900	23.900	20.100	20.200
	N_2%	73.500	73.400	73.600	73.600	74.000	74.000	74.900	74.800	75.100	75.100	76.000	76.000
	mBar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E2	O_2%	0.588	0.589	0.647	0.647	0.677	0.675	0.691	0.692	0.773	0.772	1.560	1.560
	CO_2%	25.900	25.900	25.400	25.400	24.800	24.800	24.600	24.600	23.700	23.700	23.100	23.000
	N_2%	73.500	73.500	74.000	74.000	74.500	74.500	74.700	74.700	75.500	75.500	75.400	75.400
	mBar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

(continue by the following table)

Note: Highlighted in yellow background stands for significant leaking were found in that package, data were excluded from the calculation.

(continue upper table)

Sample	Gases	D45		D50		D55		D60		D65	
S1	O_2%	20.500	20.500	0.873	0.875	18.700	18.700	1.170	1.170	1.260	1.260
	CO_2%	0.300	0.300	23.300	23.300	4.300	4.300	23.700	23.700	24.700	24.700
	N_2%	79.200	79.200	75.900	75.800	77.000	77.100	75.100	75.100	74.000	74.000
	mBar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
S2	O_2%	0.752	0.749	0.830	0.831	0.804	0.805	0.869	0.870	1.080	1.080
	CO_2%	23.500	23.600	23.700	23.700	23.400	23.400	23.200	23.200	24.400	24.400
	N_2%	75.700	75.700	75.500	75.500	75.800	75.800	76.000	76.000	74.500	74.500
	mBar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E1	O_2%	1.010	1.010	1.050	1.050	1.200	1.200	1.330	1.330	1.450	1.450
	CO_2%	23.200	23.200	23.400	23.400	23.000	23.000	22.400	22.400	22.500	22.600
	N_2%	75.800	75.800	75.600	75.500	75.800	75.800	76.300	76.300	76.000	76.000
	mBar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E2	O_2%	0.871	0.873	0.958	0.960	1.190	1.190	2.320	2.320	1.380	1.380
	CO_2%	23.200	23.100	22.600	22.600	22.200	22.200	21.400	21.500	22.200	22.100
	N_2%	76.000	76.000	76.400	76.400	76.600	76.600	76.200	76.200	76.400	76.500
	mBar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: Highlighted in yellow background stands for significant leaking were found in that package, data were excluded from the calculation.

C3-IV. pH value of Hokkien noodle samples during phase three.

Sample/Day	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
S1	4.73	4.78	4.89	4.97	5.18	4.93	5.15	4.94	5.00	4.99	5.04
	4.75	4.79	4.89	4.97	5.19	4.94	5.14	4.95	5.00	5.00	5.01
	4.75	4.78	4.88	4.97	5.17	4.95	5.14	4.93	5.00	4.99	5.01
S2	4.68	4.85	4.95	4.93	4.94	4.97	5.02	5.03	5.06	5.08	5.08
	4.67	4.83	4.97	4.93	4.94	4.97	5.02	5.02	5.06	5.06	5.07
	4.68	4.83	4.99	4.93	4.93	4.97	5.02	5.01	5.05	5.06	5.07
E1	4.67	4.69	4.73	4.84	4.85	5.13	4.91	5.09	5.06	5.09	5.09
	4.66	4.68	4.70	4.84	4.85	5.15	4.92	5.10	5.05	5.09	5.07
	4.65	4.67	4.72	4.84	4.85	5.15	4.92	5.11	5.05	5.09	5.07
E2	4.54	4.62	4.82	4.95	5.00	5.12	5.01	5.08	5.09	5.12	5.04
	4.49	4.65	4.81	4.95	5.01	5.13	5.02	5.06	5.07	5.13	5.03
	4.48	4.65	4.80	4.94	5.01	5.13	5.01	5.06	5.08	5.14	5.02

C3-IV. A_w value of Hokkien noodle samples during phase three.

Sample	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
S1	0.9942	0.9966	0.9904	0.9907	0.9806	0.9908	0.9885	0.9910	0.9897	0.9889	0.9897
	0.9939	0.9935	0.9937	0.9921	0.9881	0.9893	0.9868	0.9909	0.9876	0.9882	0.9881
	0.9957	0.9919	0.9925	0.9899	0.9864	0.9881	0.9901	0.9910	0.9881	0.9879	0.9907
S2	0.9948	0.9933	0.9953	0.9922	0.9873	0.9898	0.9890	0.9909	0.9899	0.9853	0.9872
	0.9939	0.9914	0.9947	0.9884	0.9864	0.9889	0.9856	0.9927	0.9877	0.9859	0.9877
	0.9940	0.9912	0.9939	0.9909	0.9877	0.9896	0.9887	0.9902	0.9888	0.9863	0.9874
E1	0.9952	0.9925	0.9921	0.9922	0.9888	0.9898	0.9866	0.9908	0.9895	0.9864	0.9881
	0.9941	0.9917	0.9900	0.9883	0.9875	0.9892	0.9880	0.9909	0.9862	0.9870	0.9881
	0.9940	0.9909	0.9886	0.9886	0.9847	0.9896	0.9874	0.9898	0.9884	0.9852	0.9878
E2	0.9931	0.9948	0.9898	0.9867	0.9877	0.9901	0.9874	0.9904	0.9898	0.9879	0.9877
	0.9936	0.9932	0.9869	0.9878	0.9876	0.9924	0.9894	0.9913	0.9877	0.9866	0.9882
	0.9924	0.9914	0.9858	0.9879	0.9851	0.9878	0.9870	0.9905	0.9894	0.9877	0.9898

C3-V. The tensile force of Hokkien noodle samples during phase three.

Sample	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
						(g)					
S1	54.088	35.775	37.909	37.464	44.554	38.472	29.867	29.885	28.886	36.275	32.739
	45.141	49.729	42.511	31.097	31.024	24.106	34.234	32.265	29.623	36.756	31.444
	39.477	47.629	42.634	36.917	36.465	41.354	37.295	34.309	31.03	31.583	26.976
	34.752	29.82	39.987	38.648	29.807	30.82	35.05	24.12	34.728	28.053	36.884
		36.043	34.849	41.262	34.767	35.97	31.922	37.839	33.197	26.399	27.825
			33.587	43.519		29.904	35.441	32.913	35.186	24.042	29.344
						39.779		32.142	33.108		22.787
S2	42.121	44.024	35.966	34.086	41.095	29.125	32.896	26.422	29.376	31.501	30.472
	37.707	22.799	36.982	34.086	35.688	35.437	30.963	35.571	36.792	32.417	33.588
	36.512	45.018	39.261	36.253	27.948	36.499	32.505	29.438	27.901	34.551	23.379
	45.718	36.796	31.42	34.153	28.796	37.75	33.142	26.064	34.257	31.58	35.119
	44.847	36.807	41.137		29.377	37.75	30.059	31.829	34.804	30.44	27.691
		36.115	35.407		42.089	30.064	34.158	37.113	27.343	27.435	23.122
					34.728	31.505	30.494				26.037

(continue by the following table)

(continue upper table)

Sample	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
						(g)					
E1	46.768	41.236	42.069	36.113	33.899	36.398	34.875	34.605	37.518	40.532	33.734
	40.087	30.712	36.651	38.459	38.132	37.594	35.925	39.687	28.996	25.952	28.405
	43.193	40.219	40.181	30.751	38.267	40.766	33.177	37.777	35.731	37.951	35.364
	42.31	36.499	30.217	37.721	42.299	39.035	38.852	38.682	33.799	28.645	33.421
	39.406	32.667	31.959	42.826	32.972	39.18	41.812	29.411	29.554	30.89	31.086
			36.874	37.409	34.368	36.622		29.009			33.499
			40.829	38.392		34.577		28.439			30.852
E2	42.523	40.337	38.51	38.646	40.154	35.677	34.091	40.163	35.947	34.465	34.605
	40.523	42.091	34.767	34.458	38.356	39.185	33.812	35.348	31.01	32.354	31.969
	39.618	44.225	38.7	37.809	39.473	33.823	31.98	33.449	38.684	30.365	28.852
	35.596	32.339	39.973	41.673	33.642	33.734	43.049	36.756	30.786	29.65	28.07
	43.953	38.572	38.175	37.954		37.073	31.857	37.728	34.886	28.991	33.052
		38.003	39.622	36.178		35.029	30.718	32.387	33.076	40.275	31.678
		41.119				37.464					30.986

Note: Every sample was detected at lease four-time, and kept detecting until the standard deviation lower than 5%.

C3-VI. The extended distance of Hokkien noodle samples during phase three.

Sample	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
						(-mm)					
S1	-40.024	-29.695	-31.085	-24.996	-25.935	-29.125	-28.465	-41.344	-42.113	-28.975	-18.946
	-33.714	-42.013	-34.924	-26.695	-18.846	-37.404	-37.744	-34.154	-30.015	-47.503	-29.795
	-49.563	-36.144	-35.994	-24.906	-33.724	-30.015	-27.235	-33.315	-23.446	-26.825	-16.087
	-36.994	-17.766	-30.185	-39.994	-35.184	-16.287	-22.926	-21.876	-31.785	-35.564	-31.805
		-27.605	-26.115	-45.263	-43.593	-28.895	-39.804	-30.915	-36.104	-31.375	-17.796
			-37.054	-49.003		-27.965	-29.325	-25.376	-21.396	-32.605	-33.445
						-24.736		-27.145	-19.976		-17.876
S2	-38.394	-45.393	-40.114	-33.245	-34.054	-18.046	-27.605	-25.616	-37.644	-27.905	-30.015
	-26.045	-35.884	-31.335	-41.564	-33.744	-33.355	-28.915	-37.524	-36.494	-14.207	-22.116
	-20.306	-34.004	-41.873	-29.075	-22.206	-24.436	-19.396	-31.685	-33.505	-22.896	-37.434
	-44.583	-35.714	-31.085	-37.964	-22.886	-30.985	-32.515	-31.615	-37.524	-33.325	-24.376
	-47.233	-19.946	-35.404		-20.126	-20.186	-20.456	-26.205	-26.005	-22.936	-26.095
		-41.763	-29.995		-36.334	-19.726	-30.655	-34.314	-20.476	-25.116	-24.756
					-35.834	-23.916	-21.356				-21.456

(continue by the following table)

Note: extended direction was considered as a negative value in software, hence, the unit was marked as -mm.

(continue upper table)

Sample	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
						(-mm)					
E1	-30.645	-39.664	-22.886	-33.505	-33.205	-52.112	-21.116	-21.666	-32.705	-35.844	-29.245
	-24.016	-32.005	-29.355	-34.044	-32.155	-39.284	-28.565	-34.834	-24.596	-20.646	-34.764
	-42.583	-38.484	-41.913	-33.704	-20.456	-23.706	-17.666	-29.645	-34.904	-24.516	-22.106
	-37.274	-32.235	-45.733	-52.202	-34.284	-43.323	-22.806	-26.105	-14.777	-38.544	-25.925
	-19.746	-35.954	-44.663	-20.586	-22.186	-35.124	-31.645	-21.266	-31.355	-20.076	-27.085
			-31.925	-35.534	-26.085	-44.273		-22.366			-39.614
			-41.753	-41.164		-23.166		-27.765			-35.604
E2	-44.353	-37.014	-32.565	-35.504	-32.345	-19.546	-34.594	-21.556	-32.545	-47.713	-22.706
	-39.774	-41.803	-36.824	-49.473	-38.854	-31.075	-30.475	-32.405	-23.236	-45.353	-39.334
	-46.213	-33.634	-28.925	-33.235	-33.555	-20.646	-52.562	-28.395	-20.926	-24.416	-37.184
	-31.495	-33.445	-33.874	-32.915	-31.525	-26.635	-39.214	-19.406	-44.003	-45.123	-30.575
	-44.403	-25.276	-45.633	-26.885		-28.945	-27.855	-36.324	-38.714	-24.726	-37.074
		-31.615	-45.923	-53.252		-25.915	-47.473	-44.373	-23.336	-29.795	-29.565
		-34.114				-30.835					-37.244

Note: extended direction was considered as a negative value in software, hence, the unit was marked as -mm.

C3-VII Colour profile of Hokkien noodle samples in phase three.

Sample	Parameter	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
	L	60.44	63.52	62.56	60.75	61.29	63.07	60.83	63.93	62.53	62.48	62.38
	a	-5.62	-5.75	-5.54	-3.75	-3.66	-4.73	-3.09	-4.63	-4	-5.19	-5.04
S1.1	b	26.35	26.6	25.45	22.78	23.3	24.99	23.16	24.27	24.4	24.02	25.85
	C	26.82	27.21	26.04	23.08	23.58	25.43	23.36	27.7	24.72	24.57	26.33
	h	100.7	102.1	102.2	99.3	98.8	100.6	97.5	100.7	99.2	102.1	101
	L	60.92	63.28	62.31	60.29	61.32	63.94	61.44	62.58	62.69	62.45	62.27
	a	-5.41	-5.59	-5.44	-3.88	-3.61	-4.92	-3.09	-4.65	-4.02	-5.11	-5.07
S1.2	b	26.71	26.11	24.97	22.75	23.46	25.19	23.42	23.69	24.36	24.27	25.71
	C	27.25	26.7	25.55	23.07	23.73	25.66	23.62	24.14	24.68	24.8	26.2
	h	101.4	102	102.2	99.6	98.7	101	97.4	101	99.3	101.8	101.1
	L	59.47	63.21	62.63	60.93	60.42	63.56	60.3	62.9	62.62	62.25	62.26
	a	-5.5	-5.65	-5.51	-3.66	-3.77	-4.85	-2.84	-4.75	-4.12	-5.18	-5.07
S1.3	b	26.64	26.19	26.13	22.62	22.74	25.21	22.8	23.79	24.53	24.04	25.78
	C	27.2	26.79	26.7	22.91	23.05	25.67	22.97	24.25	24.87	24.59	26.27
	h	101.6	102.1	101.8	99.1	99.3	100.8	97.1	101.3	99.5	102.1	101.1

Note: Every sample was detected three times, marked as X.1, X.2 and X.3.

(continue upper table)

Sample	Parameter	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
	L	61.41	62.45	60.19	63.32	64.14	63.3	61.43	62.3	63.67	63.91	64.26
	a	-5.55	-5.58	-4.86	-4.8	-5.3	-4.74	-4.36	-4.35	-5.36	-5.44	-5.7
S2.1	b	26.32	26.58	24.97	24.67	25.77	25.57	23.26	23.28	25.05	25.59	26.12
	C	26.89	27.15	25.43	25.13	26.3	26	23.66	23.68	25.61	26.16	26.73
	h	101.8	101.8	101	101	101.6	100.4	100.5	100.5	102	102	102.2
	L	60.78	60.83	61.87	64.17	64.72	63.22	62.15	61.42	62.98	63.53	64.22
	a	-5.19	-5.34	-5.41	-5.14	-5.37	-4.67	-4.7	-4.27	-5.24	-5.43	-5.61
S2.2	b	25.95	25.63	25	25.25	26.22	25.05	23.71	22.71	25.73	25.22	26.47
	C	26.46	26.18	25.57	25.76	26.76	25.48	24.17	23.1	26.25	25.79	27.05
	h	101.3	101.7	102.1	101.5	101.5	100.5	101.1	100.6	101.5	102.1	101.9
	L	56.81	61.83	61.52	63.47	62.48	62.64	61.04	62.4	63.31	64.28	64.16
	a	-4.43	-5.3	-5.45	-4.81	-5	-4.66	-4.22	-4.22	-5.14	-5.67	-5.66
S2.3	b	23.97	25.76	25.1	24.67	24.97	25.02	22.83	23.19	25.58	25.76	26.19
	C	24.37	26.29	25.68	25.13	25.46	25.45	23.21	23.57	26.09	26.37	26.79
	h	100.4	101.6	102.2	101	101.3	100.5	100.4	100.2	101.3	102.3	102.1

Note: Every sample was detected three times, marked as X.1, X.2 and X.3.

(continue upper table)

Sample	Parameter	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
	L	62.25	61.93	63.24	63.33	60.4	61.16	61.67	61.94	63.12	63.92	63.31
	a	-5.75	-5.28	-5.68	-4.62	-4.68	-4.04	-4.41	-4.55	-5.21	-5.27	-5.14
E1.1	b	27.96	25.16	25.54	23.5	23.16	23.25	23.1	23.68	24.32	24.1	25.58
	C	28.54	25.7	26.16	23.94	23.62	23.59	23.51	24.11	24.87	24.66	26.09
	h	101.6	101.8	102.4	101.1	101.4	99.8	100.7	100.8	102	102.3	101.3
	L	62.77	62.25	62.32	63.12	60.59	61.4	62.02	61.52	63.47	64.6	63.28
	a	-5.9	-5.43	-5.16	-4.7	-4.66	-4.08	-4.51	-4.41	-5.12	-5.39	-5.59
E1.2	b	28.1	25.46	25.21	23.39	23.31	23.06	23.06	24.15	24.8	24.11	26.11
	C	28.71	26.03	25.73	23.85	23.77	23.41	23.49	24.54	25.32	24.7	26.7
	h	101.8	102	101.5	101.3	101.3	100	101	100.3	101.6	102.5	102
	L	61.68	63.54	61.95	62.6	60.57	61.58	62.22	61.86	61.36	64.45	62.96
	a	-5.76	-5.84	-5.29	-4.46	-4.73	-4.13	-4.44	-4.45	-5.07	-5.42	-5.35
E1.3	b	26.66	25.69	24.8	22.88	23.17	23.19	23.25	23.63	25	24.25	25.88
	C	27.27	26.34	25.35	23.31	23.64	23.55	23.67	24.04	25.5	24.84	26.42
	h	102.1	102.7	102	101	101.5	100	100.7	100.6	101.4	102.5	101.6

Note: Every sample was detected three times, marked as X.1, X.2 and X.3.

(continue upper table)

Sample	Parameter	D0	D8	D16	D24	D32	D40	D45	D50	D55	D60	D65
	L	60.63	62.05	63.31	64.42	61.94	62.86	64.16	63.76	64.35	63.78	60.84
	a	-5.18	-5.68	-5.29	-5.01	-4.55	-4.61	-4.53	-4.46	-5.39	-5.24	-4.56
E2.1	b	26.37	25.59	25.98	24.38	23.68	24.35	24.88	23.43	25.5	24.18	23.27
	C	26.87	26.21	26.51	24.88	24.11	24.78	25.28	23.85	26.06	24.74	23.71
	h	101	102.4	101.5	101.5	100.8	100.6	100.2	100.7	101.8	102.2	101
	L	60.73	63.71	62.96	63.54	62.58	64.52	64.11	63.94	63.86	62.91	60.07
	a	-5.16	-5.71	-5.35	-4.86	-4.65	-5.07	-4.64	-4.87	-5.41	-5.08	-4.41
E2.2	b	26.41	26.02	25.88	24.68	23.69	25.04	24.65	23.05	25.56	24.86	23.34
	C	26.9	26.63	26.42	25.15	24.14	25.54	25.08	23.55	26.12	25.37	23.75
	h	101	102.3	101.6	101.1	101	101.4	100.6	101.8	101.9	101.5	100.6
	L	61.71	63.13	62.76	64.17	62.15	64.13	64.24	64.05	64.6	63	60.55
	a	-5.52	-5.49	-5.42	-4.97	-4.7	-4.81	-4.56	-4.61	-5.51	-5.07	-4.39
E2.3	b	26.71	26.93	25.6	24.24	23.71	25.09	24.67	22.99	25.63	24.02	23.32
	C	27.27	27.48	26.16	24.74	24.17	25.54	25.08	23.44	26.21	24.54	23.72
	h	101.6	101.5	101.9	101.5	101.1	100.8	100.4	101.3	102.1	101.8	100.6

Note: Every sample was detected three times, marked as X.1, X.2 and X.3.

Appendix D: Statistical analysis output.

D1. Phase one data statistical analysis.

D1-I. Normality test for broth micro-dilution assay results.

Strains	Eos	Shapiro-Wilk		
		Statistic	Df	Sig.
E. coli	Thyme	0.802	4	0.105
	Savory	0.921	4	0.541
	Oregano	0.895	4	0.406
	Clove	0.729	4	0.024
S. aureus	Thyme	0.939	4	0.650
	Savory	0.729	4	0.024
	Oregano	0.863	4	0.272
	Clove	0.729	4	0.024
A. brasiliensis	Thyme	0.729	4	0.024
	Savory	0.729	4	0.024
	Oregano	0.729	4	0.024
	Clove	0.729	4	0.024
P. chrysogenum	Thyme		N/A	
	Savory	0.729	4	0.024
	Oregano	0.729	4	0.024
	Clove	0.729	4	0.024

Note: the normality test for thyme EO against *P. chrysogenum* was not available since all the MIC reading were the same.

D1-II. Broth micro-dilution assay Nonparametric test: Independent Samples.

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
E. coli	16	0.2339	0.20240	0.02	0.72
S. aureus	16	0.2447	0.21034	0.05	0.72
Aspergillus	16	0.4013	0.20255	0.12	0.72
Penicillium	16	0.8213	0.91007	0.09	2.88
EOs	16	2.50	1.155	1	4

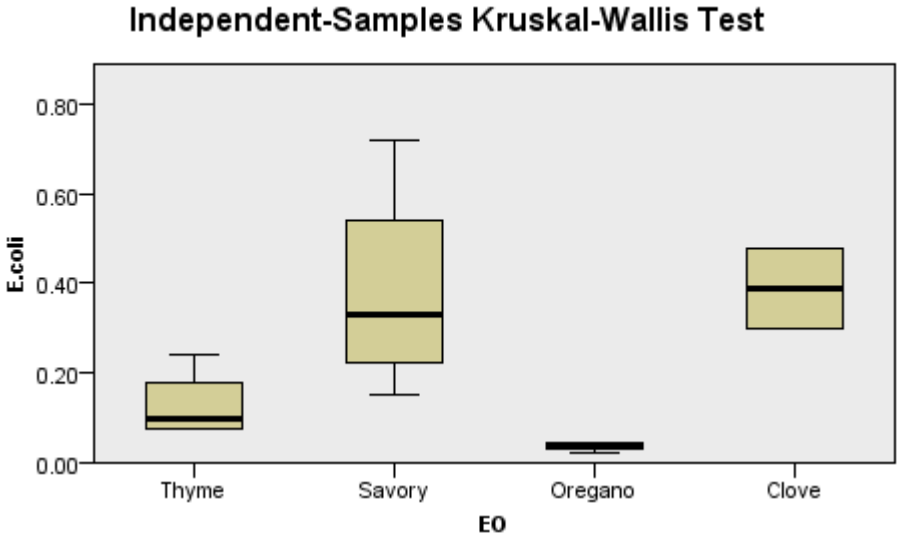
Nonparametric Tests

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of E.coli is the same across categories of EO.	Independent-Samples Kruskal-Wallis Test	.006	Reject the null hypothesis.
2	The distribution of S.aureus is the same across categories of EO.	Independent-Samples Kruskal-Wallis Test	.009	Reject the null hypothesis.
3	The distribution of Aspergillus is the same across categories of EO.	Independent-Samples Kruskal-Wallis Test	.004	Reject the null hypothesis.
4	The distribution of Penicillium is the same across categories of EO.	Independent-Samples Kruskal-Wallis Test	.003	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

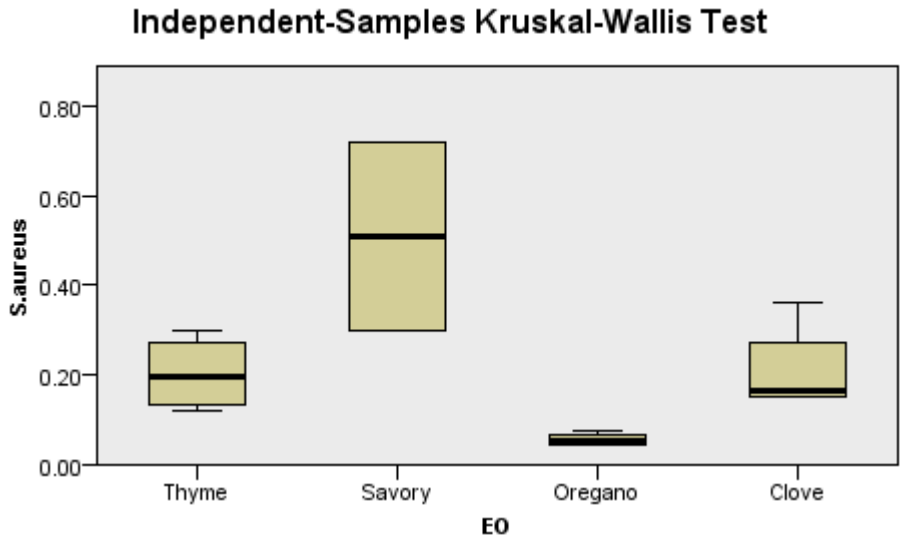
D1-II (a). Independent-Sample Kruskal-Wallis Test of *E. coli* (NCTC 8196).



Total N	16
Test Statistic	12.370
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.006

1. The test statistic is adjusted for ties.

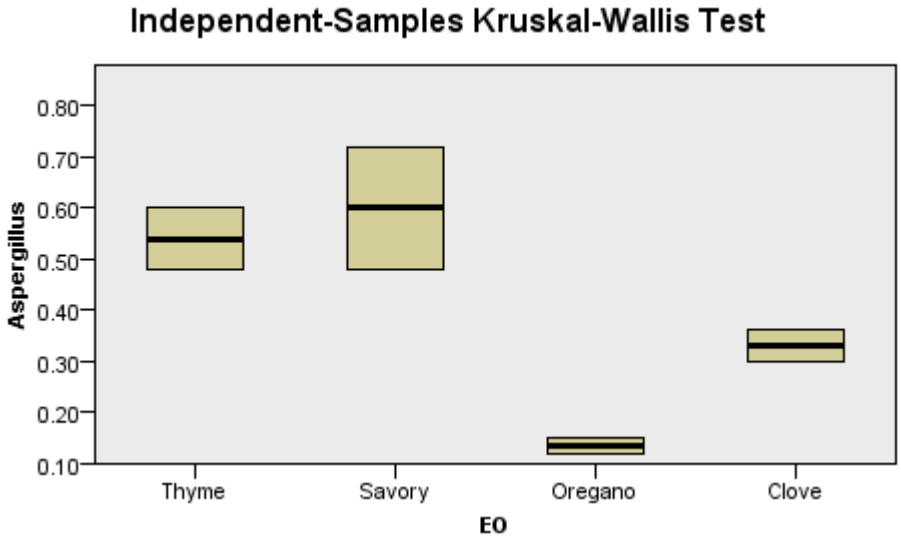
D1-II (b). Independent-Sample Kruskal-Wallis Test of *S. aureus* (NCTC4163).



Total N	16
Test Statistic	11.485
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.009

1. The test statistic is adjusted for ties.

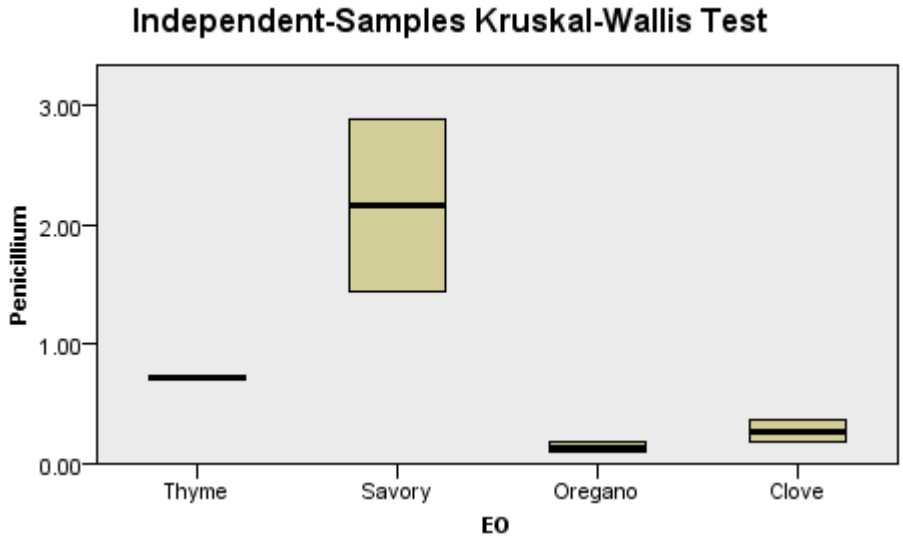
D1-II (c). Independent-Sample Kruskal-Wallis Test of *A. brasiliensis* (NZRM2578).



Total N	16
Test Statistic	13.102
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.004

1. The test statistic is adjusted for ties.

D1-II (d). Independent-Sample Kruskal-Wallis Test of *P. chrysogenum* (NZRM2999).



Total N	16
Test Statistic	13.994
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.003

1. The test statistic is adjusted for ties.

Descriptive statistics

	EO			Statistic	Std. Error
E. coli	Thyme	Mean		13.8556	1.19755
		95% Confidence Interval for Mean	Lower Bound	11.0940	
			Upper Bound	16.6171	
		5% Trimmed Mean		13.8673	
		Median		14.7000	
		Variance		12.907	
		Std. Deviation		3.59265	
		Minimum		9.20	
		Maximum		18.30	
		Range		9.10	
		Interquartile Range		7.63	
		Skewness		-.251	.717
		Kurtosis		-1.726	1.400
	Savory	Mean		14.9611	1.21452
		95% Confidence Interval for Mean	Lower Bound	12.1604	
			Upper Bound	17.7618	
		5% Trimmed Mean		14.9123	
		Median		14.6500	
		Variance		13.275	
		Std. Deviation		3.64355	
		Minimum		10.50	
		Maximum		20.30	
		Range		9.80	
		Interquartile Range		7.28	
		Skewness		.302	.717
		Kurtosis		-1.529	1.400
	Oregano	Mean		18.1444	.82312
		95% Confidence Interval for Mean	Lower Bound	16.2463	
			Upper Bound	20.0426	
		5% Trimmed Mean		18.1383	
		Median		18.0500	
		Variance		6.098	
		Std. Deviation		2.46937	
		Minimum		15.05	
		Maximum		21.35	
		Range		6.30	
		Interquartile Range		5.28	
		Skewness		.198	.717
		Kurtosis		-1.696	1.400
	Clove	Mean		16.2667	1.00613
		95% Confidence Interval for Mean	Lower Bound	13.9465	
			Upper Bound	18.5868	

		5% Trimmed Mean		16.2713	
		Median		17.0000	
		Variance		9.111	
		Std. Deviation		3.01838	
		Minimum		12.20	
		Maximum		20.25	
		Range		8.05	
		Interquartile Range		6.18	
		Skewness		-.260	.717
		Kurtosis		-1.523	1.400
S. aureus	Thyme	Mean		7.7000	.49917
		95% Confidence Interval for Mean	Lower Bound	6.5489	
			Upper Bound	8.8511	
		5% Trimmed Mean		7.6556	
		Median		7.6000	
		Variance		2.243	
		Std. Deviation		1.49750	
		Minimum		6.00	
		Maximum		10.20	
		Range		4.20	
		Interquartile Range		2.80	
		Skewness		.490	.717
		Kurtosis		-1.130	1.400
	Savory	Mean		10.4389	.83407
		95% Confidence Interval for Mean	Lower Bound	8.5155	
			Upper Bound	12.3623	
		5% Trimmed Mean		10.4599	
		Median		10.6000	
		Variance		6.261	
		Std. Deviation		2.50222	
		Minimum		7.10	
		Maximum		13.40	
		Range		6.30	
		Interquartile Range		5.10	
		Skewness		-.131	.717
		Kurtosis		-1.677	1.400
	Oregano	Mean		11.8056	.95002
		95% Confidence Interval for Mean	Lower Bound	9.6148	
			Upper Bound	13.9963	
		5% Trimmed Mean		11.7340	

		Median		10.8000	
		Variance		8.123	
		Std. Deviation		2.85005	
		Minimum		8.50	
		Maximum		16.40	
		Range		7.90	
		Interquartile Range		5.13	
		Skewness		.747	.717
		Kurtosis		-1.056	1.400
	Clove	Mean		10.6167	.36362
		95% Confidence Interval for Mean	Lower Bound	9.7781	
			Upper Bound	11.4552	
		5% Trimmed Mean		10.6324	
		Median		10.5000	
		Variance		1.190	
		Std. Deviation		1.09087	
		Minimum		8.95	
		Maximum		12.00	
		Range		3.05	
		Interquartile Range		1.90	
		Skewness		-.160	.717
		Kurtosis		-1.627	1.400
Aspergillus	Thyme	Mean		6.9944	.51468
		95% Confidence Interval for Mean	Lower Bound	5.8076	
			Upper Bound	8.1813	
		5% Trimmed Mean		6.9022	
		Median		6.0000	
		Variance		2.384	
		Std. Deviation		1.54403	
		Minimum		6.00	
		Maximum		9.65	
		Range		3.65	
		Interquartile Range		2.65	
		Skewness		1.087	.717
		Kurtosis		-.766	1.400
	Savory	Mean		7.7333	.53183
		95% Confidence Interval for Mean	Lower Bound	6.5069	
			Upper Bound	8.9597	
		5% Trimmed Mean		7.7093	

		Median		7.7000	
		Variance		2.546	
		Std. Deviation		1.59550	
		Minimum		6.00	
		Maximum		9.90	
		Range		3.90	
		Interquartile Range		3.43	
		Skewness		.173	.717
		Kurtosis		-1.775	1.400
	Oregano	Mean		9.1167	.82027
		95% Confidence Interval for Mean	Lower Bound	7.2251	
			Upper Bound	11.0082	
		5% Trimmed Mean		9.0880	
		Median		9.3500	
		Variance		6.056	
		Std. Deviation		2.46082	
		Minimum		6.00	
		Maximum		12.75	
		Range		6.75	
		Interquartile Range		4.88	
		Skewness		-.068	.717
		Kurtosis		-1.348	1.400
	Clove	Mean		12.5222	.97884
		95% Confidence Interval for Mean	Lower Bound	10.2650	
			Upper Bound	14.7794	
		5% Trimmed Mean		12.5219	
		Median		13.6500	
		Variance		8.623	
		Std. Deviation		2.93653	
		Minimum		8.35	
		Maximum		16.70	
		Range		8.35	
		Interquartile Range		5.23	
		Skewness		-.432	.717
		Kurtosis		-1.218	1.400
Penicillium	Thyme	Mean		7.2056	.49878
		95% Confidence Interval for Mean	Lower Bound	6.0554	
			Upper Bound	8.3557	
		5% Trimmed Mean		7.1506	
		Median		6.0000	

		Variance		2.239	
		Std. Deviation		1.49634	
		Minimum		6.00	
		Maximum		9.40	
		Range		3.40	
		Interquartile Range		2.88	
		Skewness		.542	.717
		Kurtosis		-1.925	1.400
	Savory	Mean		8.5167	.83570
		95% Confidence Interval for Mean	Lower Bound	6.5895	
			Upper Bound	10.4438	
		5% Trimmed Mean		8.4435	
		Median		8.1000	
		Variance		6.286	
		Std. Deviation		2.50711	
		Minimum		6.00	
		Maximum		12.35	
		Range		6.35	
		Interquartile Range		5.28	
		Skewness		.506	.717
		Kurtosis		-1.489	1.400
	Oregano	Mean		15.8333	1.88648
		95% Confidence Interval for Mean	Lower Bound	11.4831	
			Upper Bound	20.1836	
		5% Trimmed Mean		15.8481	
		Median		16.7000	
		Variance		32.029	
		Std. Deviation		5.65945	
		Minimum		8.60	
		Maximum		22.80	
		Range		14.20	
		Interquartile Range		12.38	
		Skewness		-.156	.717
		Kurtosis		-1.686	1.400
	Clove	Mean		21.2278	1.38552
		95% Confidence Interval for Mean	Lower Bound	18.0328	
			Upper Bound	24.4228	
		5% Trimmed Mean		21.2559	
		Median		20.5500	

	Variance	17.277	
	Std. Deviation	4.15655	
	Minimum	15.15	
	Maximum	26.80	
	Range	11.65	
	Interquartile Range	7.55	
	Skewness	.020	.717
	Kurtosis	-1.486	1.400

D1-III. Agar Disc Diffusion Assay Normality analysis.

Results output Sorted by EOs types.

Case Processing Summary

	EO	Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
E. coli	Thyme	9	100.0%	0	0.0%	9	100.0%
	Savory	9	100.0%	0	0.0%	9	100.0%
	Oregano	9	100.0%	0	0.0%	9	100.0%
	Clove	9	100.0%	0	0.0%	9	100.0%
S. aureus	Thyme	9	100.0%	0	0.0%	9	100.0%
	Savory	9	100.0%	0	0.0%	9	100.0%
	Oregano	9	100.0%	0	0.0%	9	100.0%
	Clove	9	100.0%	0	0.0%	9	100.0%
Aspergillus	Thyme	9	100.0%	0	0.0%	9	100.0%
	Savory	9	100.0%	0	0.0%	9	100.0%
	Oregano	9	100.0%	0	0.0%	9	100.0%
	Clove	9	100.0%	0	0.0%	9	100.0%
Penicillium	Thyme	9	100.0%	0	0.0%	9	100.0%
	Savory	9	100.0%	0	0.0%	9	100.0%
	Oregano	9	100.0%	0	0.0%	9	100.0%
	Clove	9	100.0%	0	0.0%	9	100.0%

Tests for Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	EO	Statistic	df	Sig.	Statistic	df	Sig.
E. coli	Thyme	.210	9	.200*	.886	9	.183
	Savory	.146	9	.200*	.925	9	.438

	Oregano	.201	9	.200*	.891	9	.202
	Clove	.170	9	.200*	.912	9	.328
S. aureus	Thyme	.171	9	.200*	.927	9	.452
	Savory	.180	9	.200*	.899	9	.246
	Oregano	.251	9	.108	.876	9	.141
	Clove	.248	9	.116	.910	9	.316
Aspergillus	Thyme	.407	9	.000	.680	9	.001
	Savory	.195	9	.200*	.880	9	.156
	Oregano	.170	9	.200*	.930	9	.484
	Clove	.284	9	.035	.869	9	.119
Penicillium	Thyme	.345	9	.003	.754	9	.006
	Savory	.225	9	.200*	.859	9	.093
	Oregano	.202	9	.200*	.876	9	.142
	Clove	.176	9	.200*	.938	9	.556

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Results output sorted by EOs concentrations.

Case Processing Summary

	Concentration	Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
E. coli	9%	12	100.0%	0	0.0%	12	100.0%
	12%	12	100.0%	0	0.0%	12	100.0%
	15%	12	100.0%	0	0.0%	12	100.0%
S. aureus	9%	12	100.0%	0	0.0%	12	100.0%
	12%	12	100.0%	0	0.0%	12	100.0%
	15%	12	100.0%	0	0.0%	12	100.0%
Aspergillus	9%	12	100.0%	0	0.0%	12	100.0%
	12%	12	100.0%	0	0.0%	12	100.0%
	15%	12	100.0%	0	0.0%	12	100.0%
Penicillium	9%	12	100.0%	0	0.0%	12	100.0%
	12%	12	100.0%	0	0.0%	12	100.0%
	15%	12	100.0%	0	0.0%	12	100.0%

Descriptive statistics

	Concentration			Statistic	Std. Error
E. coli	9%	Mean		12.2000	.67248
		95% Confidence Interval for Mean	Lower Bound	10.7199	
			Upper Bound	13.6801	
		5% Trimmed Mean		12.1667	
		Median		12.0000	
		Variance		5.427	
		Std. Deviation		2.32955	
		Minimum		9.20	
		Maximum		15.80	
		Range		6.60	
		Interquartile Range		4.71	
		Skewness		.391	.637
		Kurtosis		-1.140	1.232
	12%	Mean		15.8583	.50083
		95% Confidence Interval for Mean	Lower Bound	14.7560	
			Upper Bound	16.9606	
		5% Trimmed Mean		15.8759	
		Median		15.7750	

Descriptive statistics

	Concentration			Statistic	Std. Error
		Variance		3.010	
		Std. Deviation		1.73491	
		Minimum		13.10	
		Maximum		18.30	
		Range		5.20	
		Interquartile Range		2.54	
		Skewness		-.207	.637
		Kurtosis		-1.049	1.232
	15%	Mean		19.3625	.41061
		95% Confidence Interval for Mean	Lower Bound	18.4587	
			Upper Bound	20.2663	
		5% Trimmed Mean		19.3833	
		Median		19.2500	
		Variance		2.023	
		Std. Deviation		1.42241	
		Minimum		17.00	
		Maximum		21.35	
		Range		4.35	
		Interquartile Range		2.53	
		Skewness		-.141	.637
		Kurtosis		-1.160	1.232
S. aureus	9%	Mean		8.1667	.43048
		95% Confidence Interval for Mean	Lower Bound	7.2192	
			Upper Bound	9.1142	
		5% Trimmed Mean		8.1963	
		Median		8.6500	
		Variance		2.224	
		Std. Deviation		1.49124	
		Minimum		6.00	
		Maximum		9.80	
		Range		3.80	
		Interquartile Range		3.10	
		Skewness		-.321	.637
		Kurtosis		-1.720	1.232
	12%	Mean		9.8125	.46111
		95% Confidence Interval for Mean	Lower Bound	8.7976	
			Upper Bound	10.8274	

Descriptive statistics

	Concentration			Statistic	Std. Error
		5% Trimmed Mean		9.8833	
		Median		10.3000	
		Variance		2.551	
		Std. Deviation		1.59732	
		Minimum		6.80	
		Maximum		11.55	
		Range		4.75	
		Interquartile Range		2.89	
		Skewness		-.828	.637
		Kurtosis		-.587	1.232
	15%	Mean		12.4417	.67315
		95% Confidence Interval for Mean	Lower Bound	10.9601	
			Upper Bound	13.9233	
		5% Trimmed Mean		12.4185	
		Median		12.5000	
		Variance		5.438	
		Std. Deviation		2.33188	
		Minimum		8.90	
		Maximum		16.40	
		Range		7.50	
		Interquartile Range		3.35	
		Skewness		.122	.637
		Kurtosis		-.583	1.232
Aspergillus	9%	Mean		6.7667	.37011
		95% Confidence Interval for Mean	Lower Bound	5.9521	
			Upper Bound	7.5813	
		5% Trimmed Mean		6.6630	
		Median		6.0000	
		Variance		1.644	
		Std. Deviation		1.28210	
		Minimum		6.00	
		Maximum		9.40	
		Range		3.40	
		Interquartile Range		1.94	
		Skewness		1.368	.637
		Kurtosis		.201	1.232
	12%	Mean		9.1583	.86558
		95% Confidence Interval for	Lower Bound	7.2532	

Descriptive statistics

	Concentration			Statistic	Std. Error
		Mean	Upper Bound	11.0635	
		5% Trimmed Mean		9.0731	
		Median		8.5000	
		Variance		8.991	
		Std. Deviation		2.99847	
		Minimum		6.00	
		Maximum		13.85	
		Range		7.85	
		Interquartile Range		6.31	
		Skewness		.654	.637
		Kurtosis		-1.025	1.232
	15%	Mean		11.3500	.75819
		95% Confidence Interval for	Lower Bound	9.6812	
		Mean	Upper Bound	13.0188	
		5% Trimmed Mean		11.2333	
		Median		10.4250	
		Variance		6.898	
		Std. Deviation		2.62644	
		Minimum		8.10	
		Maximum		16.70	
		Range		8.60	
		Interquartile Range		4.53	
		Skewness		.845	.637
		Kurtosis		-.222	1.232
Penicillium	9%	Mean		9.4667	1.33924
		95% Confidence Interval for	Lower Bound	6.5190	
		Mean	Upper Bound	12.4143	
		5% Trimmed Mean		9.2019	
		Median		7.3000	
		Variance		21.523	
		Std. Deviation		4.63928	
		Minimum		6.00	
		Maximum		17.70	
		Range		11.70	
		Interquartile Range		7.74	
		Skewness		1.092	.637
		Kurtosis		-.439	1.232

Descriptive statistics

Concentration			Statistic	Std. Error
12%	Mean		13.0083	1.85041
	95% Confidence Interval for Mean	Lower Bound	8.9356	
		Upper Bound	17.0811	
	5% Trimmed Mean		12.7981	
	Median		11.8000	
	Variance		41.088	
	Std. Deviation		6.41000	
	Minimum		6.00	
	Maximum		23.80	
	Range		17.80	
	Interquartile Range		11.16	
	Skewness		.341	.637
	Kurtosis		-1.558	1.232
15%	Mean		17.1125	2.10656
	95% Confidence Interval for Mean	Lower Bound	12.4760	
		Upper Bound	21.7490	
	5% Trimmed Mean		17.0361	
	Median		17.0000	
	Variance		53.251	
	Std. Deviation		7.29733	
	Minimum		8.80	
	Maximum		26.80	
	Range		18.00	
	Interquartile Range		13.86	
	Skewness		.083	.637
	Kurtosis		-2.056	1.232

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Concentration	Statistic	df	Sig.	Statistic	df	Sig.
E. coli	9%	.139	12	.200*	.921	12	.294
	12%	.161	12	.200*	.946	12	.582
	15%	.150	12	.200*	.955	12	.713
S. aureus	9%	.172	12	.200*	.866	12	.058
	12%	.226	12	.092	.890	12	.116
	15%	.101	12	.200*	.968	12	.889
Aspergillus	9%	.392	12	.000	.654	12	.000
	12%	.171	12	.200*	.862	12	.052
	15%	.210	12	.152	.909	12	.207
Penicillium	9%	.273	12	.014	.746	12	.002
	12%	.276	12	.012	.870	12	.065
	15%	.243	12	.049	.836	12	.025

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Normality test of agar disc diffusion assay results.

Strains	Eos	Shapiro-Wilk		
		Statistic	Df	Sig.
E. coli	Thyme	0.886	9	0.183
	Savory	0.925	9	0.438
	Oregano	0.891	9	0.202
	Clove	0.912	9	0.328
S. aureus	Thyme	0.927	9	0.452
	Savory	0.899	9	0.246
	Oregano	0.876	9	0.141
	Clove	0.910	9	0.316
A. brasiliensis	Thyme	0.680	9	0.001
	Savory	0.880	9	0.156
	Oregano	0.930	9	0.484
	Clove	0.869	9	0.119
P. chrysogenum	Thyme	0.754	9	0.006
	Savory	0.859	9	0.093
	Oregano	0.876	9	0.142
	Clove	0.938	9	0.556

D1-IV. Agar Disc Diffusion Assay One-way ANOVA.

Results sorts by EOs types.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Thyme	Between Groups	292.444	3	97.481	19.720	.000
	Within Groups	158.182	32	4.943		
	Total	450.626	35			
Savory	Between Groups	283.164	3	94.388	13.309	.000
	Within Groups	226.943	32	7.092		
	Total	510.107	35			
Oregano	Between Groups	440.078	3	146.693	11.218	.000
	Within Groups	418.444	32	13.076		
	Total	858.522	35			
Clove	Between Groups	590.781	3	196.927	21.759	.000
	Within Groups	289.606	32	9.050		
	Total	880.387	35			

Thyme

Tukey HSD[a]

		Subset for alpha = 0.05	
Culture	N	1	2
Aspergillus	9	6.9944	
Penicillium	9	7.2056	
S. aureus	9	7.7000	
E. coli	9		13.8556
Sig.		.906	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Savory

Tukey HSD[a]

		Subset for alpha = 0.05	
Culture	N	1	2
Aspergillus	9	7.7333	
Penicillium	9	8.5167	
S. aureus	9	10.4389	
E. coli	9		14.9611
Sig.		.158	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Oregano

Tukey HSD[a]

Culture	N	Subset for alpha = 0.05		
		1	2	3
Aspergillus	9	9.1167		
S. aureus	9	11.8056	11.8056	
Penicillium	9		15.8333	15.8333
E. coli	9			18.1444
Sig.		.405	.105	.535

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Clove

Tukey HSD[a]

Culture	N	Subset for alpha = 0.05		
		1	2	3
S. aureus	9	10.6167		
Aspergillus	9	12.5222	12.5222	
E. coli	9		16.2667	
Penicillium	9			21.2278
Sig.		.543	.058	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Results sort by strains.

Tests of Between-Subjects Effects

Dependent Variable: E. coli

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	410.149[a]	11	37.286	70.094	.000
Intercept	8994.942	1	8994.942	16909.551	.000
EO	91.787	3	30.596	57.517	.000
Concentration	307.856	2	153.928	289.369	.000
EO * Concentration	10.506	6	1.751	3.292	.017
Error	12.767	24	.532		
Total	9417.858	36			
Corrected Total	422.916	35			

a. R Squared = .970 (Adjusted R Squared = .956)

E. coli

	EO	N	Subset 1	2	3	4
Tukey B[a,b]	Thyme	9	13.8556			
	Savory	9		14.9611		
	Clove	9			16.2667	
	Oregano	9				18.1444
Duncan[a,b]	Thyme	9	13.8556			
	Savory	9		14.9611		
	Clove	9			16.2667	
	Oregano	9				18.1444
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .532.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = 0.05.

E. coli

	Concentration	N	Subset 1	2	3
Tukey B[a,b]	9%	12	12.2000		
	12%	12		15.8583	
	15%	12			19.3625
Duncan[a,b]	9%	12	12.2000		
	12%	12		15.8583	
	15%	12			19.3625
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .532.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = 0.05.

Tests of Between-Subjects Effects

Dependent Variable: S. aureus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	211.357[a]	11	19.214	36.681	.000
Intercept	3701.708	1	3701.708	7066.764	.000
EO	81.398	3	27.133	51.798	.000
Concentration	111.588	2	55.794	106.513	.000
EO * Concentration	18.372	6	3.062	5.845	.001
Error	12.572	24	.524		
Total	3925.638	36			
Corrected Total	223.929	35			

a. R Squared = .944 (Adjusted R Squared = .918)

S. aureus

	EO	N	Subset 1	Subset 2	Subset 3
Tukey B[a,b]	Thyme	9	7.7000		
	Savory	9		10.4389	
	Clove	9		10.6167	
	Oregano	9			11.8056
Duncan[a,b]	Thyme	9	7.7000		
	Savory	9		10.4389	
	Clove	9		10.6167	
	Oregano	9			11.8056
	Sig.		1.000	.607	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .524.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = 0.05.

S. aureus

	Concentration	N	Subset 1	Subset 2	Subset 3
Tukey B[a,b]	9%	12	8.1667		
	12%	12		9.8125	
	15%	12			12.4417
Duncan[a,b]	9%	12	8.1667		
	12%	12		9.8125	
	15%	12			12.4417
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .524.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = 0.05.

Tests of Between-Subjects Effects

Dependent Variable: Aspergillus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	309.394[a]	11	28.127	70.402	.000
Intercept	2975.703	1	2975.703	7448.308	.000
EO	162.115	3	54.038	135.260	.000
Concentration	126.122	2	63.061	157.844	.000
EO * Concentration	21.158	6	3.526	8.826	.000
Error	9.588	24	.400		
Total	3294.685	36			
Corrected Total	318.982	35			

a. R Squared = .970 (Adjusted R Squared = .956)

***Aspergillus* spp.**

			Subset			
	EO	N	1	2	3	4
Tukey B[a,b]	Thyme	9	6.9944			
	Savory	9		7.7333		
	Oregano	9			9.1167	
	Clove	9				12.5222
Duncan[a,b]	Thyme	9	6.9944			
	Savory	9		7.7333		
	Oregano	9			9.1167	
	Clove	9				12.5222
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .400.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = 0.05.

***Aspergillus* spp.**

			Subset		
	Concentration	N	1	2	3
Tukey B[a,b]	9%	12	6.7667		
	12%	12		9.1583	
	15%	12			11.3500
Duncan[a,b]	9%	12	6.7667		
	12%	12		9.1583	
	15%	12			11.3500
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .400.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = 0.05.

Tests of Between-Subjects Effects

Dependent Variable: Penicillium

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1601.394[a]	11	145.581	142.766	.000
Intercept	6268.681	1	6268.681	6147.439	.000
EO	1163.219	3	387.740	380.241	.000
Concentration	351.385	2	175.693	172.295	.000
EO * Concentration	86.789	6	14.465	14.185	.000
Error	24.473	24	1.020		
Total	7894.548	36			
Corrected Total	1625.867	35			

a. R Squared = .985 (Adjusted R Squared = .978)

***Penicillium* spp.**

			Subset			
	EO	N	1	2	3	4
Tukey B[a,b]	Thyme	9	7.2056			
	Savory	9		8.5167		
	Oregano	9			15.8333	
	Clove	9				21.2278
Duncan[a,b]	Thyme	9	7.2056			
	Savory	9		8.5167		
	Oregano	9			15.8333	
	Clove	9				21.2278
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.020.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = 0.05.

***Penicillium* spp.**

			Subset		
	Concentration	N	1	2	3
Tukey B[a,b]	9%	12	9.4667		
	12%	12		13.0083	
	15%	12			17.1125
Duncan[a,b]	9%	12	9.4667		
	12%	12		13.0083	
	15%	12			17.1125
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.020.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = 0.05.

D2. Phase two data statistical analysis.

D2-I. Sensory data Frequencies analysis.

Overall

Overall_318

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.5	1.5	1.5
	2	3	4.6	4.6	6.2
	3	5	7.7	7.7	13.8
	4	12	18.5	18.5	32.3
	5	18	27.7	27.7	60.0
	6	8	12.3	12.3	72.3
	7	10	15.4	15.4	87.7
	8	6	9.2	9.2	96.9
	9	2	3.1	3.1	100.0
	Total	65	100.0	100.0	

Overall_273

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	5	7.7	7.7	7.7
	3	5	7.7	7.7	15.4
	4	12	18.5	18.5	33.8
	5	19	29.2	29.2	63.1
	6	12	18.5	18.5	81.5
	7	7	10.8	10.8	92.3
	8	4	6.2	6.2	98.5
	9	1	1.5	1.5	100.0
	Total	65	100.0	100.0	

Overall_636

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	1	1.5	1.5	1.5
	3	7	10.8	10.8	12.3
	4	11	16.9	16.9	29.2
	5	20	30.8	30.8	60.0
	6	14	21.5	21.5	81.5
	7	4	6.2	6.2	87.7
	8	5	7.7	7.7	95.4
	9	3	4.6	4.6	100.0
	Total	65	100.0	100.0	

Overall_341

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.5	1.5	1.5
	2	5	7.7	7.7	9.2
	3	3	4.6	4.6	13.8
	4	16	24.6	24.6	38.5
	5	19	29.2	29.2	67.7
	6	9	13.8	13.8	81.5
	7	6	9.2	9.2	90.8
	8	3	4.6	4.6	95.4
	9	3	4.6	4.6	100.0
	Total	65	100.0	100.0	

Overall_744

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	2	3.1	3.1	3.1
	3	9	13.8	13.8	16.9
	4	13	20.0	20.0	36.9
	5	13	20.0	20.0	56.9
	6	17	26.2	26.2	83.1
	7	7	10.8	10.8	93.8
	8	4	6.2	6.2	100.0
	Total	65	100.0	100.0	

Overall_289

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.5	1.5	1.5
	2	4	6.2	6.2	7.7
	3	3	4.6	4.6	12.3
	4	7	10.8	10.8	23.1
	5	12	18.5	18.5	41.5
	6	10	15.4	15.4	56.9
	7	17	26.2	26.2	83.1
	8	8	12.3	12.3	95.4
	9	3	4.6	4.6	100.0
	Total	65	100.0	100.0	

Overall_804

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.5	1.5	1.5
	2	3	4.6	4.6	6.2
	3	12	18.5	18.5	24.6
	4	14	21.5	21.5	46.2
	5	17	26.2	26.2	72.3
	6	8	12.3	12.3	84.6
	7	6	9.2	9.2	93.8
	8	4	6.2	6.2	100.0
	Total	65	100.0	100.0	

Overall_200

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.5	1.5	1.5
	2	1	1.5	1.5	3.1
	3	6	9.2	9.2	12.3
	4	15	23.1	23.1	35.4
	5	13	20.0	20.0	55.4
	6	15	23.1	23.1	78.5
	7	8	12.3	12.3	90.8
	8	5	7.7	7.7	98.5
	9	1	1.5	1.5	100.0
	Total	65	100.0	100.0	

Overall_955

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	2	3.1	3.1	3.1
	2	5	7.7	7.7	10.8
	3	8	12.3	12.3	23.1
	4	12	18.5	18.5	41.5
	5	13	20.0	20.0	61.5
	6	15	23.1	23.1	84.6
	7	5	7.7	7.7	92.3
	8	3	4.6	4.6	96.9
	9	2	3.1	3.1	100.0
	Total	65	100.0	100.0	

Overall_812

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	2	3.1	3.1	3.1
	3	3	4.6	4.6	7.7
	4	11	16.9	16.9	24.6
	5	17	26.2	26.2	50.8
	6	14	21.5	21.5	72.3
	7	13	20.0	20.0	92.3
	8	5	7.7	7.7	100.0
	Total	65	100.0	100.0	

Odour

Odour_318

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	3	4.6	4.6	4.6
	2	5	7.7	7.7	12.3
	3	11	16.9	16.9	29.2
	4	17	26.2	26.2	55.4
	5	6	9.2	9.2	64.6
	6	9	13.8	13.8	78.5
	7	7	10.8	10.8	89.2
	8	6	9.2	9.2	98.5
	9	1	1.5	1.5	100.0
	Total	65	100.0	100.0	

Odour_273

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	5	7.7	7.7	7.7
	3	10	15.4	15.4	23.1
	4	18	27.7	27.7	50.8
	5	10	15.4	15.4	66.2
	6	14	21.5	21.5	87.7
	7	6	9.2	9.2	96.9
	9	2	3.1	3.1	100.0
	Total	65	100.0	100.0	

Odour_636

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	3	4.6	4.6	4.6
	3	6	9.2	9.2	13.8
	4	22	33.8	33.8	47.7
	5	9	13.8	13.8	61.5
	6	14	21.5	21.5	83.1
	7	6	9.2	9.2	92.3
	8	3	4.6	4.6	96.9
	9	2	3.1	3.1	100.0
	Total	65	100.0	100.0	

Odour_955

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	3	4.6	4.6	4.6
	2	7	10.8	10.8	15.4
	3	10	15.4	15.4	30.8
	4	18	27.7	27.7	58.5
	5	5	7.7	7.7	66.2
	6	12	18.5	18.5	84.6
	7	5	7.7	7.7	92.3
	8	2	3.1	3.1	95.4
	9	3	4.6	4.6	100.0
	Total	65	100.0	100.0	

Odour_804

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	4	6.2	6.2	6.2
	2	10	15.4	15.4	21.5
	3	14	21.5	21.5	43.1
	4	13	20.0	20.0	63.1
	5	8	12.3	12.3	75.4
	6	7	10.8	10.8	86.2
	7	6	9.2	9.2	95.4
	8	3	4.6	4.6	100.0
	Total	65	100.0	100.0	

Odour_200

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	2	3.1	3.1	3.1
	2	3	4.6	4.6	7.7
	3	8	12.3	12.3	20.0
	4	18	27.7	27.7	47.7
	5	6	9.2	9.2	56.9
	6	10	15.4	15.4	72.3
	7	14	21.5	21.5	93.8
	8	4	6.2	6.2	100.0
	Total	65	100.0	100.0	

Odour_812

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	2	3.1	3.1	3.1
	3	7	10.8	10.8	13.8
	4	8	12.3	12.3	26.2
	5	13	20.0	20.0	46.2
	6	18	27.7	27.7	73.8
	7	12	18.5	18.5	92.3
	8	5	7.7	7.7	100.0
	Total	65	100.0	100.0	

Odour_744

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	3	4.6	4.6	4.6
	3	9	13.8	13.8	18.5
	4	18	27.7	27.7	46.2
	5	10	15.4	15.4	61.5
	6	14	21.5	21.5	83.1
	7	7	10.8	10.8	93.8
	8	4	6.2	6.2	100.0
	Total	65	100.0	100.0	

Odour_289

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	2	3.1	3.1	3.1
	2	5	7.7	7.7	10.8
	3	4	6.2	6.2	16.9
	4	5	7.7	7.7	24.6
	5	8	12.3	12.3	36.9
	6	11	16.9	16.9	53.8
	7	18	27.7	27.7	81.5
	8	7	10.8	10.8	92.3
	9	5	7.7	7.7	100.0
	Total	65	100.0	100.0	

Odour_341

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	2	3.1	3.1	3.1
	2	7	10.8	10.8	13.8
	3	9	13.8	13.8	27.7
	4	15	23.1	23.1	50.8
	5	10	15.4	15.4	66.2
	6	8	12.3	12.3	78.5
	7	8	12.3	12.3	90.8
	8	2	3.1	3.1	93.8
	9	4	6.2	6.2	100.0
	Total	65	100.0	100.0	

Appearance

Apperance_318

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	2	3.1	3.1	3.1
	3	3	4.6	4.6	7.7
	4	3	4.6	4.6	12.3
	5	23	35.4	35.4	47.7
	6	10	15.4	15.4	63.1
	7	14	21.5	21.5	84.6
	8	9	13.8	13.8	98.5
	9	1	1.5	1.5	100.0
	Total	65	100.0	100.0	

Apperance_273

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	1	1.5	1.5	1.5
	3	3	4.6	4.6	6.2
	4	7	10.8	10.8	16.9
	5	23	35.4	35.4	52.3
	6	14	21.5	21.5	73.8
	7	12	18.5	18.5	92.3
	8	4	6.2	6.2	98.5
	9	1	1.5	1.5	100.0
	Total	65	100.0	100.0	

Apperance_636

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	1	1.5	1.5	1.5
	3	3	4.6	4.6	6.2
	4	5	7.7	7.7	13.8
	5	25	38.5	38.5	52.3
	6	14	21.5	21.5	73.8
	7	11	16.9	16.9	90.8
	8	4	6.2	6.2	96.9
	9	2	3.1	3.1	100.0
	Total	65	100.0	100.0	

Apperance_955

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.5	1.5	1.5
	3	4	6.2	6.2	7.7
	4	8	12.3	12.3	20.0
	5	18	27.7	27.7	47.7
	6	20	30.8	30.8	78.5
	7	9	13.8	13.8	92.3
	8	3	4.6	4.6	96.9
	9	2	3.1	3.1	100.0
	Total	65	100.0	100.0	

Apperance_804

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3	6	9.2	9.2	9.2
	4	7	10.8	10.8	20.0
	5	20	30.8	30.8	50.8
	6	13	20.0	20.0	70.8
	7	14	21.5	21.5	92.3
	8	4	6.2	6.2	98.5
	9	1	1.5	1.5	100.0
	Total	65	100.0	100.0	

Apperance_200

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	1	1.5	1.5	1.5
	3	2	3.1	3.1	4.6
	4	9	13.8	13.8	18.5
	5	15	23.1	23.1	41.5
	6	17	26.2	26.2	67.7
	7	16	24.6	24.6	92.3
	8	5	7.7	7.7	100.0
	Total	65	100.0	100.0	

Apperance_812

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3	1	1.5	1.5	1.5
	4	6	9.2	9.2	10.8
	5	20	30.8	30.8	41.5
	6	15	23.1	23.1	64.6
	7	18	27.7	27.7	92.3
	8	4	6.2	6.2	98.5
	9	1	1.5	1.5	100.0
	Total	65	100.0	100.0	

Apperance_744

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3	1	1.5	1.5	1.5
	4	9	13.8	13.8	15.4
	5	21	32.3	32.3	47.7
	6	17	26.2	26.2	73.8
	7	13	20.0	20.0	93.8
	8	4	6.2	6.2	100.0
	Total	65	100.0	100.0	

Apperance_289

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.5	1.5	1.5
	2	3	4.6	4.6	6.2
	3	3	4.6	4.6	10.8
	4	10	15.4	15.4	26.2
	5	15	23.1	23.1	49.2
	6	11	16.9	16.9	66.2
	7	13	20.0	20.0	86.2
	8	7	10.8	10.8	96.9
	9	2	3.1	3.1	100.0
	Total	65	100.0	100.0	

Apperance_341

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	2	3.1	3.1	3.1
	3	2	3.1	3.1	6.2
	4	7	10.8	10.8	16.9
	5	23	35.4	35.4	52.3
	6	16	24.6	24.6	76.9
	7	7	10.8	10.8	87.7
	8	6	9.2	9.2	96.9
	9	2	3.1	3.1	100.0
	Total	65	100.0	100.0	

D2-II. Sensory data Pair sample T-test.

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Overall_318	5.29	65	1.783	.221
	Overall_289	5.78	65	1.892	.235
Pair 2	Overall_273	5.08	65	1.623	.201
	Overall_289	5.78	65	1.892	.235
Pair 3	Overall_636	5.32	65	1.602	.199
	Overall_289	5.78	65	1.892	.235
Pair 4	Overall_955	4.86	65	1.828	.227
	Overall_289	5.78	65	1.892	.235
Pair 5	Overall_804	4.71	65	1.618	.201
	Overall_289	5.78	65	1.892	.235
Pair 6	Overall_200	5.25	65	1.620	.201
	Overall_289	5.78	65	1.892	.235
Pair 7	Overall_812	5.49	65	1.459	.181
	Overall_289	5.78	65	1.892	.235
Pair 8	Overall_744	5.09	65	1.518	.188
	Overall_289	5.78	65	1.892	.235
Pair 9	Overall_341	5.02	65	1.754	.218
	Overall_289	5.78	65	1.892	.235

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Overall_318 & Overall_289	65	.176	.160
Pair 2	Overall_273 & Overall_289	65	.214	.087
Pair 3	Overall_636 & Overall_289	65	.359	.003
Pair 4	Overall_955 & Overall_289	65	.149	.235
Pair 5	Overall_804 & Overall_289	65	.158	.209
Pair 6	Overall_200 & Overall_289	65	.262	.035
Pair 7	Overall_812 & Overall_289	65	.447	.000
Pair 8	Overall_744 & Overall_289	65	.225	.072
Pair 9	Overall_341 & Overall_289	65	.288	.020

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Overall_318 - Overall_289	-.492	2.359	.293	-1.077	.092	-1.682	64	.097
Pair 2	Overall_273 - Overall_289	-.708	2.213	.274	-1.256	-.159	-2.578	64	.012
Pair 3	Overall_636 - Overall_289	-.462	1.993	.247	-.955	.032	-1.867	64	.066
Pair 4	Overall_955 - Overall_289	-.923	2.426	.301	-1.524	-.322	-3.068	64	.003
Pair 5	Overall_804 - Overall_289	-1.077	2.287	.284	-1.644	-.510	-3.797	64	.000
Pair 6	Overall_200 - Overall_289	-.538	2.144	.266	-1.070	-.007	-2.025	64	.047
Pair 7	Overall_812 - Overall_289	-.292	1.800	.223	-.738	.154	-1.309	64	.195
Pair 8	Overall_744 - Overall_289	-.692	2.143	.266	-1.223	-.161	-2.605	64	.011
Pair 9	Overall_341 - Overall_289	-.769	2.178	.270	-1.309	-.230	-2.848	64	.006

D2-III. Microbial data One-way ANOVA.

Descriptive statistics

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
SPC	0	2	51000	2828	2000	25588	76412	49000	53000
	1	2	3220	396	280	-338	6778	2940	3500
	2	2	3600	141	100	2329	4871	3500	3700
	3	2	18750	354	250	15573	21927	18500	19000
	4	2	2970	28	20	2716	3224	2950	2990
	5	2	1870	99	70	981	2759	1800	1940
	6	2	3350	354	250	173	6527	3100	3600
	7	2	11450	495	350	7003	15897	11100	11800
	8	2	1575	120	85	495	2655	1490	1660
	9	2	1400	28	20	1146	1654	1380	1420
	Total	20	9919	15063	3368	2869	16968	1380	53000
YGC	0	2	93	3	2	68	118	91	95
	1	2	27	0	0	27	27	27	27
	2	2	37	1	1	30	43	36	37
	3	2	22	1	1	9	35	21	23
	4	2	18	1	1	11	24	17	18
	5	2	25	1	1	18	31	24	25
	6	2	33	0	0	33	33	33	33
	7	2	18	0	0	18	18	18	18
	8	2	5	0	0	5	5	5	5
	9	2	8	0	0	8	8	8	8
	Total	20	28	24	5	17	40	5	95

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SPC	Between Groups	4302415805.000	9	478046200.600	549.627	.000
	Within Groups	8697650.000	10	869765.000		
	Total	4311113455.000	19			
YGC	Between Groups	11017.450	9	1224.161	1064.488	.000
	Within Groups	11.500	10	1.150		
	Total	11028.950	19			

SPC

Tukey HSD[a]

number	N	Subset for alpha = 0.05			
		1	2	3	4
9	2	1400			
8	2	1575			
5	2	1870			
4	2	2970			
1	2	3220			
6	2	3350			
2	2	3600			
7	2		11450		
3	2			18750	
0	2				51000
Sig.		.43	1.00	1.00	1.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

YGC

Tukey HSD[a]

number	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
8	2	5						
9	2	8						
4	2		18					
7	2		18	18				
3	2			22	22			
5	2				25	25		
1	2					27		
6	2						33	
2	2						37	
0	2							93
Sig.		.253	1.000	.069	.443	.443	.134	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

D2-IV. Mixture design modelling.

Regression for Mixtures: SPC, YGC, Sensory

Regression for Mixtures: SPC versus Block, Oregano, Clove, Soybean Oil

The following terms cannot be estimated and were removed:

Clove*Soybean Oil*(-)

Estimated Regression Coefficients for SPC (component proportions)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	-223	146	-1.53	0.164	1.00
Oregano	-294506970	63334960	*	*	1.10481E+09
Clove	94316583	16245846	*	*	72691676.08
Soy Bean Oil	1678500	283633	*	*	2934971.72
Oregano*Clove	-214763333	46578107	-4.61	0.002	487221.23
Oregano*Soy Bean Oil	511840648	102223707	5.01	0.001	2.18116E+09
Clove*Soy Bean Oil	-122058333	21003454	-5.81	0.000	92080263.78
Oregano*Clove*Soy Bean Oil	520461420	71340662	7.30	0.000	878467.53
Oregano*Clove*(-)	-35047840	9176205	-3.82	0.005	58.20
Oregano*Soy Bean Oil*(-)	252197531	40187497	6.28	0.000	1.95615E+08

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
618.321	99.48%	98.90%	15484008	97.38%

Analysis of Variance for SPC (component proportions)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	1	895568	895568	895568	2.34	0.164
Regression	8	586581969	586581969	73322746	191.78	0.000
Linear	2	219741494	26361369	13180685	34.48	0.000
Quadratic	3	242477829	51078216	17026072	44.53	0.000
Oregano*Clove	1	173110861	8128024	8128024	21.26	0.002
Oregano*Soy Bean Oil	1	38754084	9585056	9585056	25.07	0.001
Clove*Soy Bean Oil	1	30612884	12911651	12911651	33.77	0.000
Special Cubic	1	7918922	20348442	20348442	53.22	0.000
Oregano*Clove*Soy Bean Oil	1	7918922	20348442	20348442	53.22	0.000
Full Cubic	2	116443724	116443724	58221862	152.29	0.000
Oregano*Clove*(-)	1	101387083	5577309	5577309	14.59	0.005
Oregano*Soy Bean Oil*(-)	1	15056642	15056642	15056642	39.38	0.000
Residual Error	8	3058569	3058569	382321		
Total	17	590536107				

Estimated Regression Coefficients for SPC (component amounts)

Term	Coef
Blocks	-223
Oregano	-2945070
Clove	943166
Soy Bean Oil	16785
Oregano*Clove	-21476
Oregano*Soy Bean Oil	51184
Clove*Soy Bean Oil	-12206
Oregano*Clove*Soy Bean Oil	520
Oregano*Clove*(-)	-35
Oregano*Soy Bean Oil*(-)	252

Fits and Diagnostics for Unusual Observations

Obs	StdOrder	SPC	Fit	SE Fit	Resid	Std Resid	
13	4	3350	4252	461	-902	-2.19	R
14	13	5600	4698	461	902	2.19	R

R Large residual

Regression for Mixtures: YGC versus Block, Oregano, Clove, Soy Bean Oil

The following terms cannot be estimated and were removed:

Clove*Soy Bean Oil*(-)

Estimated Regression Coefficients for YGC (component proportions)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	1.167	0.946	1.23	0.253	1.00
Oregano	-1705260	411319	*	*	1.10481E+09
Clove	-116563	105506	*	*	72691676.08
Soy Bean Oil	-2013	1842	*	*	2934971.72
Oregano*Clove	368593	302495	1.22	0.258	487221.23
Oregano*Soy Bean Oil	2737642	663877	4.12	0.003	2.18116E+09
Clove*Soy Bean Oil	150679	136404	1.10	0.301	92080263.78
Oregano*Clove*Soy Bean Oil	525514	463311	1.13	0.290	878467.53
Oregano*Clove*(-)	-197531	59593	-3.31	0.011	58.20
Oregano*Soy Bean Oil*(-)	1025514	260992	3.93	0.004	1.95615E+08

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
4.01559	93.68%	86.57%	653.063	68.01%

Analysis of Variance for YGC (component proportions)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	1	24.50	24.50	24.50	1.52	0.253
Regression	8	1888.11	1888.11	236.01	14.64	0.001
Linear	2	887.61	278.40	139.20	8.63	0.010
Quadratic	3	478.59	278.89	92.96	5.77	0.021
Oregano*Clove	1	143.78	23.94	23.94	1.48	0.258
Oregano*Soy Bean Oil	1	333.56	274.21	274.21	17.01	0.003
Clove*Soy Bean Oil	1	1.25	19.68	19.68	1.22	0.301
Special Cubic	1	27.51	20.75	20.75	1.29	0.290
Oregano*Clove*Soy Bean Oil	1	27.51	20.75	20.75	1.29	0.290
Full Cubic	2	494.41	494.41	247.20	15.33	0.002
Oregano*Clove*(-)	1	245.45	177.16	177.16	10.99	0.011
Oregano*Soy Bean Oil*(-)	1	248.96	248.96	248.96	15.44	0.004
Residual Error	8	129.00	129.00	16.13		
Total	17	2041.61				

Estimated Regression Coefficients for YGC (component amounts)

Term	Coef
Blocks	1.2
Oregano	-17052.6
Clove	-1165.6
Soy Bean Oil	-20.1
Oregano*Clove	36.9
Oregano*Soy Bean Oil	273.8
Clove*Soy Bean Oil	15.1
Oregano*Clove*Soy Bean Oil	0.5
Oregano*Clove*(-)	-0.2
Oregano*Soy Bean Oil*(-)	1.0

Regression for Mixtures: Sensory versus Block, Oregano, Clove, Soy Bean Oil

The following terms cannot be estimated and were removed:

Clove*Soy Bean Oil*(-)

Estimated Regression Coefficients for Sensory (component proportions)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.000000	0.000000	*	*	1.00
Oregano	-249565	0	*	*	1.10481E+09
Clove	10510	0	*	*	72691676.08
Soy Bean Oil	265.6	0.0	*	*	2934971.72
Oregano*Clove	925.9	0.0	*	*	487221.23
Oregano*Soy Bean Oil	410364	0	*	*	2.18116E+09
Clove*Soy Bean Oil	-13580	0	*	*	92080263.78
Oregano*Clove*Soy Bean Oil	165947	0	*	*	878467.53
Oregano*Clove*(-)	-28333	0	*	*	58.20
Oregano*Soy Bean Oil*(-)	168724	0	*	*	1.95615E+08

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0	100.00%	100.00%	0.0000000	100.00%

Analysis of Variance for Sensory (component proportions)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	1	0.000	0.000	0.0000	0.01	0.943
Regression	8	665.911	665.911	83.2389	5.30918E+22	0.000
Linear	2	606.480	6.760	3.3800	2.15582E+21	0.000
Quadratic	3	32.517	14.018	4.6727	2.98039E+21	0.000
Oregano*Clove	1	0.256	0.000	0.0002	9.63648E+16	0.000
Oregano*Soy Bean Oil	1	25.033	6.161	6.1612	3.92975E+21	0.000
Clove*Soy Bean Oil	1	7.228	0.160	0.1598	1.01944E+20	0.000
Special Cubic	1	0.001	2.069	2.0687	1.31945E+21	0.000
Oregano*Clove*Soy Bean Oil	1	0.001	2.069	2.0687	1.31945E+21	0.000
Full Cubic	2	26.913	26.913	13.4567	8.58302E+21	0.000
Oregano*Clove*(-)	1	20.174	3.645	3.6450	2.32487E+21	0.000
Oregano*Soy Bean Oil*(-)	1	6.739	6.739	6.7391	4.29836E+21	0.000
Residual Error	8	0.000	0.000	0.0000		
Total	17	665.911				

Estimated Regression Coefficients for Sensory (component amounts)

Term	Coef
Blocks	0.00
Oregano	-2495.65
Clove	105.10
Soy Bean Oil	2.66
Oregano*Clove	0.09
Oregano*Soy Bean Oil	41.04
Clove*Soy Bean Oil	-1.36
Oregano*Clove*Soy Bean Oil	0.17
Oregano*Clove*(-)	-0.03
Oregano*Soy Bean Oil*(-)	0.17

Response Optimization

Parameters

	Goal	Lower	Target	Upper	Weight	Import
SPC	Minimum	1000	1000	10000	1	1
YGC	Minimum	10	10	30	1	1
Sensory	Maximum	60	70	70	1	1

Global Solution

Components

Oregano	=	2.72048
Clove	=	10.9091
Soy Bean Oil	=	86.3704

Predicted Responses

SPC	=	1122.86	,	desirability =	0.986349
YGC	=	9.80	,	desirability =	1.000000
Sensory	=	68.03	,	desirability =	0.802657

Composite Desirability = 0.925096

Optimization Plot

D3. Phase three data statistical analysis.

D3-I. Texture profile normality test.

Sorted by oil types.

Case Processing Summary

		Cases					
		Valid		Missing		Total	
	Oil	N	Percent	N	Percent	N	Percent
Force	Soybean oil	133	100.0%	0	0.0%	133	100.0%
	Essential oil	133	100.0%	0	0.0%	133	100.0%
Distance	Soybean oil	133	100.0%	0	0.0%	133	100.0%
	Essential oil	133	100.0%	0	0.0%	133	100.0%

Descriptive statistics

	Oil			Statistic	Std. Error
Force	Soybean oil	Mean		34.1920	.49528
		95% Confidence Interval for Mean	Lower Bound	33.2123	
			Upper Bound	35.1717	
		5% Trimmed Mean		34.0447	
		Median		34.2340	
		Variance		32.625	
		Std. Deviation		5.71179	
		Minimum		22.79	
		Maximum		54.09	
		Range		31.30	
		Interquartile Range		6.89	
		Skewness		.503	.210
		Kurtosis		.667	.417
	Essential oil	Mean		36.0320	.36617
		95% Confidence Interval for Mean	Lower Bound	35.3077	
			Upper Bound	36.7563	
		5% Trimmed Mean		36.0350	
		Median		36.3980	
		Variance		17.832	
		Std. Deviation		4.22283	
		Minimum		25.95	
		Maximum		46.77	
		Range		20.82	
		Interquartile Range		6.17	

Descriptive statistics

	Oil			Statistic	Std. Error
		Skewness		-.080	.210
		Kurtosis		-.659	.417
Distance	Soybean oil	Mean		-30.4766	.67694
		95% Confidence Interval for Mean	Lower Bound	-31.8157	
			Upper Bound	-29.1375	
		5% Trimmed Mean		-30.3150	
		Median		-30.1850	
		Variance		60.947	
		Std. Deviation		7.80689	
		Minimum		-49.56	
		Maximum		-14.21	
		Range		35.36	
		Interquartile Range		11.19	
		Skewness		-.200	.210
		Kurtosis		-.488	.417
	Essential oil	Mean		-32.7796	.74118
		95% Confidence Interval for Mean	Lower Bound	-34.2457	
			Upper Bound	-31.3134	
		5% Trimmed Mean		-32.5564	
		Median		-32.5450	
		Variance		73.064	
		Std. Deviation		8.54776	
		Minimum		-53.25	
		Maximum		-14.78	
		Range		38.48	
		Interquartile Range		12.71	
		Skewness		-.284	.210
		Kurtosis		-.515	.417

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Oil	Statistic	df	Sig.	Statistic	df	Sig.
Force	Soybean oil	.077	133	.050	.980	133	.143
	Essential oil	.071	133	.093	.987	133	.224
Distance	Soybean oil	.046	133	.200*	.987	133	.249
	Essential oil	.059	133	.200*	.979	133	.240

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Sorted by day.

Case Processing Summary

		Cases					
		Valid		Missing		Total	
	Day	N	Percent	N	Percent	N	Percent
Force	Day 0	19	100.0%	0	0.0%	19	100.0%
	Day 8	23	100.0%	0	0.0%	23	100.0%
	Day 16	25	100.0%	0	0.0%	25	100.0%
	Day 24	23	100.0%	0	0.0%	23	100.0%
	Day 32	22	100.0%	0	0.0%	22	100.0%
	Day 40	29	100.0%	0	0.0%	29	100.0%
	Day 45	24	100.0%	0	0.0%	24	100.0%
	Day 50	26	100.0%	0	0.0%	26	100.0%
	Day 55	24	100.0%	0	0.0%	24	100.0%
	Day 60	23	100.0%	0	0.0%	23	100.0%
	Day 65	28	100.0%	0	0.0%	28	100.0%
Distance	Day 0	19	100.0%	0	0.0%	19	100.0%
	Day 8	23	100.0%	0	0.0%	23	100.0%
	Day 16	25	100.0%	0	0.0%	25	100.0%
	Day 24	23	100.0%	0	0.0%	23	100.0%
	Day 32	22	100.0%	0	0.0%	22	100.0%
	Day 40	29	100.0%	0	0.0%	29	100.0%
	Day 45	24	100.0%	0	0.0%	24	100.0%
	Day 50	26	100.0%	0	0.0%	26	100.0%
	Day 55	24	100.0%	0	0.0%	24	100.0%
	Day 60	23	100.0%	0	0.0%	23	100.0%
	Day 65	28	100.0%	0	0.0%	28	100.0%

Descriptive statistics

	Day			Statistic	Std. Error
Force	Day 0	Mean		41.8074	1.04331
		95% Confidence Interval for Mean	Lower Bound	39.6155	
			Upper Bound	43.9993	
		5% Trimmed Mean		41.5171	
		Median		42.1210	
		Variance		20.682	
		Std. Deviation		4.54769	
		Minimum		34.75	
		Maximum		54.09	

Descriptive statistics

Day			Statistic	Std. Error
	Range		19.34	
	Interquartile Range		5.44	
	Skewness		.832	.524
	Kurtosis		1.680	1.014
Day 8	Mean		38.1989	1.28103
	95% Confidence Interval for Mean	Lower Bound	35.5422	
		Upper Bound	40.8556	
	5% Trimmed Mean		38.3782	
	Median		38.0030	
	Variance		37.744	
	Std. Deviation		6.14360	
	Minimum		22.80	
	Maximum		49.73	
	Range		26.93	
	Interquartile Range		6.32	
	Skewness		-.395	.481
	Kurtosis		.617	.935
Day 16	Mean		37.6071	.68942
	95% Confidence Interval for Mean	Lower Bound	36.1842	
		Upper Bound	39.0300	
	5% Trimmed Mean		37.7264	
	Median		38.1750	
	Variance		11.882	
	Std. Deviation		3.44709	
	Minimum		30.22	
	Maximum		42.63	
	Range		12.42	
	Interquartile Range		4.96	
	Skewness		-.526	.464
	Kurtosis		-.423	.902
Day 24	Mean		37.2119	.68126
	95% Confidence Interval for Mean	Lower Bound	35.7991	
		Upper Bound	38.6248	
	5% Trimmed Mean		37.2230	
	Median		37.4640	
	Variance		10.675	
	Std. Deviation		3.26719	

Descriptive statistics

Day			Statistic	Std. Error
	Minimum		30.75	
	Maximum		43.52	
	Range		12.77	
	Interquartile Range		4.19	
	Skewness		-.027	.481
	Kurtosis		.074	.935
Day 32	Mean		35.8136	1.00716
	95% Confidence Interval for Mean	Lower Bound	33.7191	
		Upper Bound	37.9081	
	5% Trimmed Mean		35.7721	
	Median		35.2275	
	Variance		22.316	
	Std. Deviation		4.72401	
	Minimum		27.95	
	Maximum		44.55	
	Range		16.61	
	Interquartile Range		7.16	
	Skewness		.019	.491
	Kurtosis		-.874	.953
Day 40	Mean		35.5550	.72758
	95% Confidence Interval for Mean	Lower Bound	34.0646	
		Upper Bound	37.0454	
	5% Trimmed Mean		35.7925	
	Median		36.4030	
	Variance		15.352	
	Std. Deviation		3.91812	
	Minimum		24.11	
	Maximum		41.35	
	Range		17.25	
	Interquartile Range		4.33	
	Skewness		-1.071	.434
	Kurtosis		1.242	.845
Day 45	Mean		34.0906	.69632
	95% Confidence Interval for Mean	Lower Bound	32.6501	
		Upper Bound	35.5310	
	5% Trimmed Mean		33.8372	
	Median		33.4945	
	Variance		11.637	

Descriptive statistics

Day			Statistic	Std. Error
	Std. Deviation		3.41127	
	Minimum		29.87	
	Maximum		43.05	
	Range		13.18	
	Interquartile Range		3.47	
	Skewness		1.238	.472
	Kurtosis		1.436	.918
Day 50	Mean		33.2058	.86709
	95% Confidence Interval for Mean	Lower Bound	31.4200	
		Upper Bound	34.9916	
	5% Trimmed Mean		33.3052	
	Median		33.1810	
	Variance		19.548	
	Std. Deviation		4.42133	
	Minimum		24.12	
	Maximum		40.16	
	Range		16.04	
	Interquartile Range		7.84	
	Skewness		-.284	.456
	Kurtosis		-.778	.887
Day 55	Mean		32.7591	.65775
	95% Confidence Interval for Mean	Lower Bound	31.3984	
		Upper Bound	34.1197	
	5% Trimmed Mean		32.7364	
	Median		33.1525	
	Variance		10.383	
	Std. Deviation		3.22230	
	Minimum		27.34	
	Maximum		38.68	
	Range		11.34	
	Interquartile Range		5.54	
	Skewness		-.030	.472
	Kurtosis		-1.081	.918
Day 60	Mean		31.7870	.92173
	95% Confidence Interval for Mean	Lower Bound	29.8755	
		Upper Bound	33.6986	
	5% Trimmed Mean		31.7195	

Descriptive statistics

	Day			Statistic	Std. Error
		Median		31.5010	
		Variance		19.540	
		Std. Deviation		4.42045	
		Minimum		24.04	
		Maximum		40.53	
		Range		16.49	
		Interquartile Range		5.91	
		Skewness		.430	.481
		Kurtosis		-.336	.935
	Day 65	Mean		30.4636	.71187
		95% Confidence Interval for Mean	Lower Bound	29.0029	
			Upper Bound	31.9242	
		5% Trimmed Mean		30.5522	
		Median		31.0360	
		Variance		14.189	
		Std. Deviation		3.76688	
		Minimum		22.79	
		Maximum		36.88	
		Range		14.10	
		Interquartile Range		5.59	
		Skewness		-.516	.441
		Kurtosis		-.357	.858
Distance	Day 0	Mean		-36.7031	2.10028
		95% Confidence Interval for Mean	Lower Bound	-41.1156	
			Upper Bound	-32.2905	
		5% Trimmed Mean		-36.9307	
		Median		-38.3940	
		Variance		83.812	
		Std. Deviation		9.15491	
		Minimum		-49.56	
		Maximum		-19.75	
		Range		29.82	
		Interquartile Range		13.76	
		Skewness		.575	.524
		Kurtosis		-.745	1.014
	Day 8	Mean		-33.9639	1.40660
		95% Confidence Interval for Mean	Lower Bound	-36.8810	
			Upper Bound	-31.0468	

Descriptive statistics

Day			Statistic	Std. Error
	5% Trimmed Mean		-34.2375	
	Median		-34.1140	
	Variance		45.506	
	Std. Deviation		6.74583	
	Minimum		-45.39	
	Maximum		-17.77	
	Range		27.63	
	Interquartile Range		6.87	
	Skewness		.764	.481
	Kurtosis		.741	.935
Day 16	Mean		-35.4854	1.29999
	95% Confidence Interval for Mean	Lower Bound	-38.1684	
		Upper Bound	-32.8024	
	5% Trimmed Mean		-35.5717	
	Median		-34.9240	
	Variance		42.249	
	Std. Deviation		6.49993	
	Minimum		-45.92	
	Maximum		-22.89	
	Range		23.04	
	Interquartile Range		11.18	
	Skewness		-.155	.464
	Kurtosis		-.862	.902
Day 24	Mean		-36.2917	1.89233
	95% Confidence Interval for Mean	Lower Bound	-40.2161	
		Upper Bound	-32.3672	
	5% Trimmed Mean		-36.1983	
	Median		-34.0440	
	Variance		82.361	
	Std. Deviation		9.07527	
	Minimum		-53.25	
	Maximum		-20.59	
	Range		32.67	
	Interquartile Range		12.49	
	Skewness		-.350	.481
	Kurtosis		-.599	.935
Day 32	Mean		-30.3235	1.45436

Descriptive statistics

	Day			Statistic	Std. Error
		95% Confidence Interval for Mean	Lower Bound	-33.3480	
			Upper Bound	-27.2990	
		5% Trimmed Mean		-30.2414	
		Median		-32.7750	
		Variance		46.533	
		Std. Deviation		6.82155	
		Minimum		-43.59	
		Maximum		-18.85	
		Range		24.75	
		Interquartile Range		11.79	
		Skewness		.240	.491
		Kurtosis		-.842	.953
	Day 40	Mean		-28.7737	1.56235
		95% Confidence Interval for Mean	Lower Bound	-31.9741	
			Upper Bound	-25.5734	
		5% Trimmed Mean		-28.2757	
		Median		-27.9650	
		Variance		70.787	
		Std. Deviation		8.41351	
		Minimum		-52.11	
		Maximum		-16.29	
		Range		35.83	
		Interquartile Range		8.78	
		Skewness		-.976	.434
		Kurtosis		.921	.845
	Day 45	Mean		-30.0153	1.76584
		95% Confidence Interval for Mean	Lower Bound	-33.6683	
			Upper Bound	-26.3624	
		5% Trimmed Mean		-29.4799	
		Median		-28.7400	
		Variance		74.837	
		Std. Deviation		8.65084	
		Minimum		-52.56	
		Maximum		-17.67	
		Range		34.90	
		Interquartile Range		11.24	
		Skewness		-.951	.472
		Kurtosis		.933	.918

Descriptive statistics

Day			Statistic	Std. Error
Day 50	Mean		-29.5073	1.26942
	95% Confidence Interval for Mean	Lower Bound	-32.1217	
		Upper Bound	-26.8929	
	5% Trimmed Mean		-29.2576	
	Median		-29.0200	
	Variance		41.897	
	Std. Deviation		6.47279	
	Minimum		-44.37	
	Maximum		-19.41	
	Range		24.97	
	Interquartile Range		9.57	
	Skewness		-.420	.456
	Kurtosis		-.310	.887
Day 55	Mean		-29.8992	1.60710
	95% Confidence Interval for Mean	Lower Bound	-33.2237	
		Upper Bound	-26.5746	
	5% Trimmed Mean		-29.9251	
	Median		-31.5700	
	Variance		61.987	
	Std. Deviation		7.87316	
	Minimum		-44.00	
	Maximum		-14.78	
	Range		29.23	
	Interquartile Range		13.14	
	Skewness		.068	.472
	Kurtosis		-.976	.918
Day 60	Mean		-30.6950	1.93548
	95% Confidence Interval for Mean	Lower Bound	-34.7089	
		Upper Bound	-26.6810	
	5% Trimmed Mean		-30.6245	
	Median		-28.9750	
	Variance		86.160	
	Std. Deviation		9.28223	
	Minimum		-47.71	
	Maximum		-14.21	
	Range		33.51	
	Interquartile Range		11.43	

Descriptive statistics

	Day			Statistic	Std. Error
		Skewness		-.498	.481
		Kurtosis		-.496	.935
	Day 65	Mean		-28.5723	1.34325
		95% Confidence Interval for Mean	Lower Bound	-31.3284	
			Upper Bound	-25.8161	
		5% Trimmed Mean		-28.6298	
		Median		-29.4050	
		Variance		50.521	
		Std. Deviation		7.10783	
		Minimum		-39.61	
		Maximum		-16.09	
		Range		23.53	
		Interquartile Range		13.13	
		Skewness		.071	.441
		Kurtosis		-1.140	.858

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Day	Statistic	df	Sig.	Statistic	df	Sig.
Force	Day 0	.090	19	.200*	.949	19	.376
	Day 8	.129	23	.200*	.980	23	.910
	Day 16	.095	25	.200*	.959	25	.400
	Day 24	.156	23	.151	.962	23	.507
	Day 32	.097	22	.200*	.971	22	.731
	Day 40	.143	29	.133	.924	29	.140
	Day 45	.150	24	.174	.894	24	.116
	Day 50	.097	26	.200*	.968	26	.562
	Day 55	.126	24	.200*	.958	24	.406
	Day 60	.139	23	.200*	.967	23	.626
	Day 65	.112	28	.200*	.957	28	.302
Distance	Day 0	.144	19	.200*	.933	19	.197
	Day 8	.146	23	.200*	.948	23	.262
	Day 16	.113	25	.200*	.951	25	.266
	Day 24	.142	23	.200*	.957	23	.400
	Day 32	.206	22	.016	.920	22	.077
	Day 40	.151	29	.090	.936	29	.080
	Day 45	.137	24	.200*	.929	24	.095
	Day 50	.096	26	.200*	.966	26	.519
	Day 55	.127	24	.200*	.961	24	.463
	Day 60	.117	23	.200*	.942	23	.203
	Day 65	.098	28	.200*	.953	28	.230

D3-II. Texture profile One-way ANOVA.

Descriptive statistics

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Force	Day 0	19	41.8074	4.54769	1.04331	39.6155	43.9993	34.75	54.09
	Day 8	23	38.1989	6.14360	1.28103	35.5422	40.8556	22.80	49.73
	Day 16	25	37.6071	3.44709	.68942	36.1842	39.0300	30.22	42.63
	Day 24	23	37.2119	3.26719	.68126	35.7991	38.6248	30.75	43.52
	Day 32	22	35.8136	4.72401	1.00716	33.7191	37.9081	27.95	44.55
	Day 40	29	35.5550	3.91812	.72758	34.0646	37.0454	24.11	41.35
	Day 45	24	34.0906	3.41127	.69632	32.6501	35.5310	29.87	43.05
	Day 50	26	33.2058	4.42133	.86709	31.4200	34.9916	24.12	40.16
	Day 55	24	32.7591	3.22230	.65775	31.3984	34.1197	27.34	38.68
	Day 60	23	31.7870	4.42045	.92173	29.8755	33.6986	24.04	40.53
	Day 65	28	30.4636	3.76688	.71187	29.0029	31.9242	22.79	36.88
	Total	266	35.1120	5.09733	.31254	34.4966	35.7273	22.79	54.09
Distance	Day 0	19	-36.7031	9.15491	2.10028	-41.1156	-32.2905	-49.56	-19.75
	Day 8	23	-33.9639	6.74583	1.40660	-36.8810	-31.0468	-45.39	-17.77
	Day 16	25	-35.4854	6.49993	1.29999	-38.1684	-32.8024	-45.92	-22.89
	Day 24	23	-36.2917	9.07527	1.89233	-40.2161	-32.3672	-53.25	-20.59
	Day 32	22	-30.3235	6.82155	1.45436	-33.3480	-27.2990	-43.59	-18.85
	Day 40	29	-28.7737	8.41351	1.56235	-31.9741	-25.5734	-52.11	-16.29
	Day 45	24	-30.0153	8.65084	1.76584	-33.6683	-26.3624	-52.56	-17.67

Descriptive statistics

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Day 50	26	-29.5073	6.47279	1.26942	-32.1217	-26.8929	-44.37	-19.41
	Day 55	24	-29.8992	7.87316	1.60710	-33.2237	-26.5746	-44.00	-14.78
	Day 60	23	-30.6950	9.28223	1.93548	-34.7089	-26.6810	-47.71	-14.21
	Day 65	28	-28.5723	7.10783	1.34325	-31.3284	-25.8161	-39.61	-16.09
	Total	266	-31.6281	8.25129	.50592	-32.6242	-30.6319	-53.25	-14.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Force	Between Groups	2456.146	10	245.615	14.140	.000
	Within Groups	4429.298	255	17.370		
	Total	6885.445	265			
Distance	Between Groups	2293.353	10	229.335	3.713	.000
	Within Groups	15748.869	255	61.760		
	Total	18042.223	265			

Force

Tukey HSD[a,b]

Day	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
Day 65	28	30.46					
Day 60	23	31.79	31.79				
Day 55	24	32.76	32.76	32.76			
Day 50	26	33.21	33.21	33.21			
Day 45	24	34.09	34.09	34.09	34.09		
Day 40	29		35.56	35.56	35.56	35.56	
Day 32	22			35.81	35.81	35.81	
Day 24	23				37.21	37.21	
Day 16	25				37.61	37.61	
Day 8	23					38.20	38.20
Day 0	19						41.81
Sig.		.098	.071	.291	.124	.512	.102

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 23.886.

Distance

Tukey HSD[a,b]

Day	N	Subset for alpha = 0.05	
		1	2
Day 0	19	-36.70	
Day 24	23	-36.29	
Day 16	25	-35.49	-35.49
Day 8	23	-33.96	-33.96
Day 60	23	-30.69	-30.69
Day 32	22	-30.32	-30.32
Day 45	24	-30.02	-30.02
Day 55	24	-29.90	-29.90
Day 50	26	-29.51	-29.51
Day 40	29		-28.77
Day 65	28		-28.57
Sig.		.064	.090

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 23.886.

D3-II. Texture profile Independent sample T-test for D40 & D65 with Fresh sample (D0).

Day-40 E-sample vs Fresh sample

Group Statistics

	Day	N	Mean	Std. Deviation	Std. Error Mean
Force	Day 0	19	41.8074	4.54769	1.04331
	Day 40 Essential Oil	15	36.8373	2.09003	.53964
Distance	Day 0	19	36.7031	9.15491	2.10028
	Day 40 Essential Oil	15	31.2907	9.63249	2.48710

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	4.997	.032	3.910	32	.000	4.97004	1.27115	2.38078	7.55929
	Equal variances not assumed			4.231	26.483	.000	4.97004	1.17461	2.55772	7.38235
Distance	Equal variances assumed	.015	.903	1.673	32	.104	5.41232	3.23527	-1.17771	12.00235
	Equal variances not assumed			1.663	29.442	.107	5.41232	3.25528	-1.24113	12.06577

Day-40 S-sample vs Fresh sample

Group Statistics

	Day	N	Mean	Std. Deviation	Std. Error Mean
Force	Day 0	19	41.8074	4.54769	1.04331
	Day 40 Soybean Oil	14	34.1811	4.94273	1.32100
Distance	Day 0	19	36.7031	9.15491	2.10028
	Day 40 Soybean Oil	14	26.0769	6.11558	1.63446

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	.725	.401	4.590	31	.000	7.62630	1.66156	4.23751	11.01508
	Equal variances not assumed			4.531	26.757	.000	7.62630	1.68331	4.17096	11.08164
Distance	Equal variances assumed	2.560	.120	3.761	31	.001	10.62612	2.82545	4.86358	16.38867
	Equal variances not assumed			3.993	30.775	.000	10.62612	2.66132	5.19672	16.05553

Day-65 E-sample vs Fresh sample

Group Statistics

	Day	N	Mean	Std. Deviation	Std. Error Mean
Force	Day 0	19	41.8074	4.54769	1.04331
	Day 65 Essential Oil	14	31.8266	2.28080	.60957
Distance	Day 0	19	36.7031	9.15491	2.10028
	Day 65 Essential Oil	14	32.0018	6.03445	1.61278

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	3.753	.062	7.522	31	.000	9.98073	1.32681	7.27468	12.68677
	Equal variances not assumed			8.260	27.887	.000	9.98073	1.20834	7.50511	12.45634
Distance	Equal variances assumed	2.283	.141	1.669	31	.105	4.70127	2.81636	-1.04274	10.44528
	Equal variances not assumed			1.775	30.704	.086	4.70127	2.64806	-.70160	10.10413

Day-65 S-sample vs Fresh sample

Group Statistics

	Day	N	Mean	Std. Deviation	Std. Error Mean
Force	Day 0	19	41.8074	4.54769	1.04331
	Day 65 Soybean Oil	14	29.1005	4.50183	1.20316
Distance	Day 0	19	36.7031	9.15491	2.10028
	Day 65 Soybean Oil	14	25.1427	6.57123	1.75624

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	.081	.778	7.966	31	.000	12.70687	1.59504	9.45376	15.95998
	Equal variances not assumed			7.979	28.331	.000	12.70687	1.59252	9.44647	15.96727
Distance	Equal variances assumed	1.789	.191	4.017	31	.000	11.56034	2.87818	5.69025	17.43043
	Equal variances not assumed			4.222	30.992	.000	11.56034	2.73780	5.97650	17.14417

D3-III. Texture profile Independent sample T-test for S-sample and E-sample.

Day-8

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	11	38.2323	7.92732	2.39018
	Essential Oil	12	38.1683	4.28439	1.23680
Distance	Soybean Oil	11	-33.2661	8.83130	2.66274
	Essential Oil	12	-34.6036	4.37920	1.26417

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	3.007	.098	.024	21	.981	.06402	2.62479	-5.39453	5.52258
	Equal variances not assumed			.024	15.089	.981	.06402	2.69121	-5.66922	5.79727
Distance	Equal variances assumed	4.705	.042	.466	21	.646	1.33749	2.86732	-4.62542	7.30040
	Equal variances not assumed			.454	14.353	.657	1.33749	2.94759	-4.96990	7.64488

Day-16

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	12	37.6375	3.57047	1.03070
	Essential Oil	13	37.5790	3.47522	.96385
Distance	Soybean Oil	12	-33.7636	4.58938	1.32484
	Essential Oil	13	-37.0748	7.71295	2.13919

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	.105	.748	.042	23	.967	.05850	1.40957	-2.85741	2.97441
	Equal variances not assumed			.041	22.722	.967	.05850	1.41116	-2.86267	2.97967
Distance	Equal variances assumed	5.831	.024	1.290	23	.210	3.31119	2.56678	-1.99861	8.62098
	Equal variances not assumed			1.316	19.794	.203	3.31119	2.51621	-1.94105	8.56342

Day-24

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	10	36.7485	3.70374	1.17123
	Essential Oil	13	37.5684	2.99418	.83043
Distance	Soybean Oil	10	-35.2705	8.73448	2.76209
	Essential Oil	13	-37.0772	9.60410	2.66370

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	.802	.381	-.588	21	.563	-.81988	1.39517	-3.72129	2.08153
	Equal variances not assumed			-.571	17.085	.575	-.81988	1.43575	-3.84791	2.20814
Distance	Equal variances assumed	.004	.949	.465	21	.647	1.80665	3.88715	-6.27712	9.89043
	Equal variances not assumed			.471	20.334	.643	1.80665	3.83724	-6.18926	9.80257

Day-32

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	12	34.6948	5.58347	1.61181
	Essential Oil	10	37.1562	3.20882	1.01472
Distance	Soybean Oil	12	-30.2055	7.85898	2.26869
	Essential Oil	10	-30.4650	5.74878	1.81792

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	2.428	.135	-1.232	20	.232	-2.46137	1.99824	-6.62962	1.70689
	Equal variances not assumed			-1.292	17.993	.213	-2.46137	1.90462	-6.46294	1.54021
Distance	Equal variances assumed	2.756	.112	.087	20	.932	.25950	2.99238	-5.98249	6.50149
	Equal variances not assumed			.089	19.723	.930	.25950	2.90720	-5.81028	6.32928

Day-40

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	14	34.1811	4.94273	1.32100
	Essential Oil	15	36.8373	2.09003	.53964
Distance	Soybean Oil	14	-26.0769	6.11558	1.63446
	Essential Oil	15	-31.2907	9.63249	2.48710

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	14.649	.001	-1.908	27	.067	-2.65626	1.39183	-5.51205	.19953
	Equal variances not assumed			-1.861	17.255	.080	-2.65626	1.42697	-5.66353	.35101
Distance	Equal variances assumed	2.539	.123	1.725	27	.096	5.21380	3.02169	-.98619	11.41380
	Equal variances not assumed			1.752	23.903	.093	5.21380	2.97609	-.92986	11.35747

Day-45

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	13	32.9251	2.26810	.62906
	Essential Oil	11	35.4680	4.09127	1.23356
Distance	Soybean Oil	13	-28.1844	6.22931	1.72770
	Essential Oil	11	-32.1792	10.77271	3.24810

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	3.683	.068	-1.923	22	.067	-2.54292	1.32207	-5.28473	.19888
	Equal variances not assumed			-1.836	15.030	.086	-2.54292	1.38470	-5.49383	.40798
Distance	Equal variances assumed	2.754	.111	1.134	22	.269	3.99480	3.52216	-3.30971	11.29930
	Equal variances not assumed			1.086	15.430	.294	3.99480	3.67900	-3.82783	11.81743

Day-50

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	13	31.5315	4.24168	1.17643
	Essential Oil	13	34.8801	4.08165	1.13205
Distance	Soybean Oil	13	-30.8526	5.46306	1.51518
	Essential Oil	13	-28.1620	7.31567	2.02900

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	.002	.965	-2.051	24	.051	-3.34854	1.63264	-6.71814	.02107
	Equal variances not assumed			-2.051	23.965	.051	-3.34854	1.63264	-6.71841	.02133
Distance	Equal variances assumed	.879	.358	-1.063	24	.299	-2.69062	2.53232	-7.91706	2.53583
	Equal variances not assumed			-1.063	22.209	.299	-2.69062	2.53232	-7.93945	2.55822

Day-55

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	13	32.0178	3.13358	.86910
	Essential Oil	11	33.6352	3.24646	.97885
Distance	Soybean Oil	13	-30.4987	7.51424	2.08408
	Essential Oil	11	-29.1906	8.59030	2.59007

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	.018	.894	-1.239	22	.228	-1.61741	1.30497	-4.32375	1.08892
	Equal variances not assumed			-1.236	21.070	.230	-1.61741	1.30900	-4.33907	1.10425
Distance	Equal variances assumed	.194	.664	-.398	22	.694	-1.30806	3.28610	-8.12301	5.50690
	Equal variances not assumed			-.393	20.114	.698	-1.30806	3.32443	-8.24017	5.62406

Day-60

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	12	30.9193	3.90471	1.12719
	Essential Oil	11	32.7336	4.93237	1.48716
Distance	Soybean Oil	12	-29.1027	8.18281	2.36217
	Essential Oil	11	-32.4320	10.46593	3.15560

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	1.037	.320	-.982	21	.337	-1.81430	1.84666	-5.65464	2.02603
	Equal variances not assumed			-.972	19.069	.343	-1.81430	1.86607	-5.71908	2.09048
Distance	Equal variances assumed	2.703	.115	.854	21	.403	3.32933	3.89868	-4.77843	11.43709
	Equal variances not assumed			.845	18.940	.409	3.32933	3.94178	-4.92267	11.58134

Day-65

Group Statistics

	Oil	N	Mean	Std. Deviation	Std. Error Mean
Force	Soybean Oil	14	29.1005	4.50183	1.20316
	Essential Oil	14	31.8266	2.28080	.60957
Distance	Soybean Oil	14	-25.1427	6.57123	1.75624
	Essential Oil	14	-32.0018	6.03445	1.61278

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	6.824	.015	-2.021	26	.054	-2.72614	1.34877	-5.49858	.04629
	Equal variances not assumed			-2.021	19.261	.057	-2.72614	1.34877	-5.54656	.09428
Distance	Equal variances assumed	.013	.909	2.877	26	.008	6.85907	2.38441	1.95785	11.76030
	Equal variances not assumed			2.877	25.813	.008	6.85907	2.38441	1.95612	11.76202

D3-IV. S-sample SPC data organisation for building Baranyi-Roberts model.

Time (Day)	N	ln(N/No)	Square of residual (Average)	Ab(t)	Model Prediction of Ln (N/No)	Log N (predicted)	Square of residual (Model)	Residuals
0	200	1.034	0.204	0.000	0.000	1.852	1.070	-1.034
0	180	0.929	0.310	0.000	0.000	1.852	0.863	-0.929
0	150	0.747	0.547	0.000	0.000	1.852	0.557	-0.747
0	150	0.747	0.547	0.000	0.000	1.852	0.557	-0.747
8	58	-0.204	2.855	0.000	0.000	1.852	0.042	0.204
8	56	-0.239	2.975	0.000	0.000	1.852	0.057	0.239
8	59	-0.187	2.797	0.000	0.000	1.852	0.035	0.187
8	60	-0.170	2.741	0.000	0.000	1.852	0.029	0.170
16	81	0.130	1.838	0.003	0.001	1.852	0.017	-0.130
16	83	0.155	1.772	0.003	0.001	1.852	0.024	-0.154
16	57	-0.221	2.914	0.003	0.001	1.852	0.049	0.222
16	58	-0.204	2.855	0.003	0.001	1.852	0.042	0.204
24	51	-0.332	3.306	0.024	0.006	1.855	0.115	0.338
24	47	-0.414	3.610	0.024	0.006	1.855	0.176	0.420
24	47	-0.414	3.610	0.024	0.006	1.855	0.176	0.420
24	48	-0.393	3.530	0.024	0.006	1.855	0.159	0.399
32	53	-0.294	3.168	0.184	0.047	1.872	0.116	0.341
32	49	-0.372	3.453	0.184	0.047	1.872	0.176	0.420
40	87	0.202	1.649	1.253	0.322	1.992	0.014	0.120
40	84	0.167	1.740	1.253	0.322	1.992	0.024	0.155
40	130	0.603	0.779	1.253	0.322	1.992	0.079	-0.281
40	150	0.747	0.547	1.253	0.322	1.992	0.180	-0.424
45	105	0.390	1.201	3.364	0.865	2.228	0.226	0.475
45	105	0.390	1.201	3.364	0.865	2.228	0.226	0.475
45	108	0.418	1.140	3.364	0.865	2.228	0.200	0.447
45	105	0.390	1.201	3.364	0.865	2.228	0.226	0.475
50	690	2.273	0.619	6.951	1.787	2.628	0.235	-0.485
50	620	2.166	0.462	6.951	1.787	2.628	0.143	-0.378
50	620	2.166	0.462	6.951	1.787	2.628	0.143	-0.378
50	590	2.116	0.397	6.951	1.787	2.628	0.108	-0.329
55	1170	2.801	1.728	11.449	2.944	3.130	0.021	0.143
55	1210	2.834	1.818	11.449	2.944	3.130	0.012	0.110
55	1250	2.867	1.907	11.449	2.944	3.130	0.006	0.077
55	1560	3.088	2.568	11.449	2.944	3.130	0.021	-0.144
60	4800	4.212	7.433	16.298	4.190	3.672	0.000	-0.022

Time (Day)	N	ln(N/No)	Square of residual (Average)	Ab(t)	Model Prediction of Ln (N/No)	Log N (predicted)	Square of residual (Model)	Residuals
60	5100	4.273	7.767	16.298	4.190	3.672	0.007	-0.083
60	5600	4.366	8.297	16.298	4.190	3.672	0.031	-0.176
60	5800	4.402	8.501	16.298	4.190	3.672	0.045	-0.211
65	16800	5.465	15.833	21.255	5.463	4.224	0.000	-0.002
65	14900	5.345	14.892	21.255	5.463	4.224	0.014	0.118
65	13400	5.239	14.085	21.255	5.463	4.224	0.050	0.224
65	12800	5.193	13.743	21.255	5.463	4.224	0.073	0.270
		1.486	153.001				6.344	
		Average log (N/No)	Sum of squared residuals (Average)				Sum of squared residuals (Model)	

D3-V. S-sample YMC data organisation for building Baranyi-Roberts model.

Time (Day)	N	ln (N/No)	Square of residual (Average)	Ab(t)	Model Prediction of Log (N/No)	Log N (predicted)	Square of residual (Model)	Residuals
0	89	1.029	1.059	0.000	0.000	1.502	1.059	-1.029
0	89	1.029	1.059	0.000	0.000	1.502	1.059	-1.029
0	63	0.684	0.467	0.000	0.000	1.502	0.467	-0.684
0	65	0.715	0.511	0.000	0.000	1.502	0.511	-0.715
8	21	-0.415	0.172	0.105	0.009	1.506	0.179	0.424
8	22	-0.368	0.136	0.105	0.009	1.506	0.142	0.377
8	31	-0.025	0.001	0.105	0.009	1.506	0.001	0.034
8	30	-0.058	0.003	0.105	0.009	1.506	0.004	0.067
16	17	-0.626	0.392	0.307	0.026	1.514	0.425	0.652
16	17	-0.626	0.392	0.307	0.026	1.514	0.425	0.652
16	17	-0.626	0.392	0.307	0.026	1.514	0.425	0.652
16	17	-0.626	0.392	0.307	0.026	1.514	0.425	0.652
24	36	0.124	0.015	0.692	0.058	1.528	0.004	-0.066
24	35	0.096	0.009	0.692	0.058	1.528	0.001	-0.038
24	36	0.124	0.015	0.692	0.058	1.528	0.004	-0.066
24	35	0.096	0.009	0.692	0.058	1.528	0.001	-0.038
32	26	-0.201	0.041	1.412	0.118	1.554	0.102	0.320
32	26	-0.201	0.041	1.412	0.118	1.554	0.102	0.320
40	34	0.067	0.004	2.705	0.227	1.601	0.026	0.160
40	35	0.096	0.009	2.705	0.227	1.601	0.017	0.131
40	37	0.151	0.023	2.705	0.227	1.601	0.006	0.075
40	38	0.178	0.032	2.705	0.227	1.601	0.002	0.049
45	45	0.347	0.121	3.945	0.331	1.646	0.000	-0.016
45	45	0.347	0.121	3.945	0.331	1.646	0.000	-0.016
50	65	0.715	0.511	5.614	0.471	1.707	0.060	-0.244
50	63	0.684	0.467	5.614	0.471	1.707	0.045	-0.213
50	74	0.845	0.713	5.614	0.471	1.707	0.140	-0.374
50	74	0.845	0.713	5.614	0.471	1.707	0.140	-0.374
55	63	0.684	0.467	7.774	0.652	1.786	0.001	-0.032
55	63	0.684	0.467	7.774	0.652	1.786	0.001	-0.032
60	77	0.884	0.782	10.455	0.877	1.883	0.000	-0.008
60	76	0.871	0.759	10.455	0.877	1.883	0.000	0.006
60	78	0.897	0.805	10.455	0.877	1.883	0.000	-0.020
60	75	0.858	0.736	10.455	0.877	1.883	0.000	0.019
65	94	1.084	1.175	13.640	1.144	1.999	0.004	0.060

Time (Day)	N	ln (N/No)	Square of residual (Average)	Ab(t)	Model Prediction of Log (N/No)	Log N (predicted)	Square of residual (Model)	Residuals
65	87	1.006	1.013	13.640	1.144	1.999	0.019	0.137
65	92	1.062	1.129	13.640	1.144	1.999	0.007	0.082
65	88	1.018	1.036	13.640	1.144	1.999	0.016	0.126
		0.396 Average log (N/No)	12.392 Sum of squared residuals (Average)				5.824 Sum of squared residuals (Model)	

D3-VI. S-sample SPC data prediction base on Baranyi-Roberts model.

Time (Day)	N	ln(N/No)	Square of residual (Average)	Ab(t)	Model Prediction of Ln (N/No)	Log N (predicted)	Square of residual (Model)	Residuals
0	200	1.034	0.173	0.000	0.000	1.852	1.070	-1.034
8	60	-0.170	2.625	0.000	0.000	1.852	0.029	0.170
16	81	0.130	1.743	0.003	0.001	1.852	0.017	-0.130
24	48	-0.393	3.398	0.024	0.006	1.855	0.159	0.399
32	53	-0.294	3.043	0.184	0.047	1.872	0.116	0.341
40	150	0.747	0.496	1.253	0.322	1.992	0.180	-0.424
45	105	0.390	1.125	3.364	0.865	2.228	0.226	0.475
50	590	2.116	0.443	6.951	1.787	2.628	0.108	-0.329
55	1170	2.801	1.823	11.449	2.944	3.130	0.021	0.143
60	5800	4.402	8.708	16.298	4.190	3.672	0.045	-0.211
65	12800	5.193	14.007	21.255	5.463	4.224	0.073	0.270
70				26.243	6.738	4.778		
75				31.240	7.996	5.324		
80				36.239	9.189	5.843		
85				41.239	10.199	6.281		
90				46.239	10.858	6.568		
95				51.239	11.156	6.697		
100				56.239	11.257	6.741		
		1.451 Average log (N/No)	37.584 Sum of squared residuals (Average)				2.043 Sum of squared residuals (Model)	

D3-VII. S-sample YMC data prediction base on Baranyi-Roberts model.

Time (Day)	N	ln (N/No)	Square of residual (Average)	Ab(t)	Model Prediction of Log (N/No)	Log N (predicted)	Square of residual (Model)	Residuals
0	89	1.029	1.059	0.000	0.000	1.502	1.059	-1.029
8	30	-0.058	0.003	0.105	0.009	1.506	0.004	0.067
16	17	-0.626	0.392	0.307	0.026	1.514	0.425	0.652
24	35	0.096	0.009	0.692	0.058	1.528	0.001	-0.038
32	26	-0.201	0.041	1.412	0.118	1.554	0.102	0.320
40	38	0.178	0.032	2.705	0.227	1.601	0.002	0.049
45	45	0.347	0.121	3.945	0.331	1.646	0.000	-0.016
50	74	0.845	0.713	5.614	0.471	1.707	0.140	-0.374
55	63	0.684	0.467	7.774	0.652	1.786	0.001	-0.032
55	63	0.684	0.467	7.774	0.652	1.786	0.001	-0.032
60	77	0.884	0.782	10.455	0.877	1.883	0.000	-0.008
65	88	1.018	1.036	13.640	1.144	1.999	0.016	0.126
70				17.276	1.449	2.132		
75				21.286	1.785	2.278		
80				25.587	2.146	2.434		
85				30.104	2.524	2.599		
90				34.776	2.916	2.769		
95				39.555	3.316	2.943		
100				44.407	3.723	3.119		
110				54.244	4.545	3.476		
120				64.173	5.371	3.835		
130				74.142	6.193	4.192		
140				84.129	6.997	4.541		
150				94.123	7.762	4.873		
		0.504 Average log (N/No)	3.669 Sum of squared residuals (Average)				1.753 Sum of squared residuals (Model)	