STERILIZATION OF OIL PALM FRUIT USING SUPERCRITICAL CARBON DIOXIDE AS A GREEN TECHNOLOGY

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by

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LIST OF SYMBOLS

˚C	Degree Celcius
μm	Micron
4X; 100X	4 times enlargement; 100 times enlargement
T	Absolute temperature
t:	Treatment time
Tt	Complete inactivation time

LIST OF ABBREVIATIONS

AC	Autoclaved
ANOVA	Analysis of variance
AV	Anisidine value
CFU	Colony forming unit
cm	Centimetre
CO_2	Carbon Dioxide
CPO	Crude Palm Oil
DOBI	Deterioration Of Bleachability Index
FFA	Free Fatty Acid
FFB	Fresh Fruit Bunch
g	Gram
gf	Gram-force
HCL	Hydrochloric Acid
IUPAC	International Union of Pure and Applied Chemistry
kg	Kilogram
kW	Kilowatt
L	Litre
MHz	Megahertz
min	Minute
mL	Mililitre
MPa	Mega Pascal
MPOB	Malaysia Palm Oil Board
N	Newton
NA	Nutrient Agar
NaOH	Sodium Hydroxide
Nm	Newton Meter

OPEFB	Oil Palm Empty Fruit Bunch
OPF	Oil Palm Fruits
OP-FFB	Oil Palm Fresh Fruit Bunch
OPMF	Oil Palm Mesocarp Fibre
PDA	Potato Dextrose Agar
PKO	Palm Kernel Oil
POME	Palm oil mill effluent
ppm	Parts per million
psi	Pound per square inch
PV	Peroxide value
rpm	Revolutions per minute
SC-CO_2	Supercritical carbon dioxide
SEM	Scanning Electron microscope
SHS	Superheated steam
spp.	Species
UV Totox	Ultra Violet Total Oxidation
wt	Weight

PENSTERILAN BUAH KELAPA SAWIT DENGAN MENGGUNAKAN KARBON DIOKSIDA LAMPAU GENTING SEBAGAI TEKNOLOGI HIJAU

ABSTRAK

Pensterilan adalah proses penting dalam pemprosesan minyak sawit bagi menghasilkan minyak sawit yang berkualiti. Tujuan pensterilan buah kelapa sawit (OPF) adalah untuk menyahaktifkan mikroorganisma lipopfilik, melembutkan pulpa buah dan memudahkan peleraian OPF dari tandan. Kini, industri minyak sawit di Malaysia menggunakan kaedah pensterilan stim untuk pemprosesan OPF. Kaedah pensterilan ini melibatkan penggunaan air yang banyak dan menyebabkan penjanaan efluen kilang minyak sawit (POME) yang tinggi. Selain itu, interaksi OPF dengan kelembapan semasa pensterilan wap telah menyebabkan kandungan asid lemak bebas (FFA) yang lebih tinggi dalam minyak yang diekstrak. Oleh itu, terdapat keperluan untuk melaksanakan teknologi sterilisasi tanpa air bagi menghapuskan penjanaan FFA dalam minyak yang diekstrak dan mengelakkan penghasilan POME. Dalam kajian ini, teknologi karbon dioksida lampau genting (SC-CO_2) digunakan untuk mensterilkan OPF dengan tekanan yang berbeza (7.4-50 MPa), suhu (32-80^{o}C) dan masa rawatan (15-60 min). Prestasi pensterilan SC-CO_2 ditentukan melalui penyahaktifan mikroorganisma (bakteria dan kulat) yang menghasilkan lipase dalam OPF dan dibandingkan dengan pensterilan autoklaf stim. Keputusan menunjukkan bahawa SC-CO_2 menyahaktifkan mocroorgansisms pada suhu sederhana (≤80^{o}C) dan tekanan (8-10 MPa) dalam ≤60 minit masa pensterilan. Kecekapan SC-CO_2 dalam pensterilan OP-FFB ditentukan dengan menggunakan *vessel* bersaiz 10 Liter berdasarkan penyahaktifan *Bacillus* spp. dan *Aspergillus* spp. (mikrob dengan kehadiran tertinggi) dalam OP-FFB, berdasarkan tekanan SC-CO_2 yang berbeza (10-30 MPa), suhu (40-80

^{o}C) dan masa rawatan (15-90 min). Penyahaktifan mikroorganisma yang lengkap dalam OP-FFB diperoleh selepas 45-70 minit rawatan pada tekanan 10 MPa dan suhu 80^{o}C. Dari segi struktur pulpa kelapa sawit (OPF), kebolehmampatan pulpa OPF meningkat dengan ketara selepas pensterilan SC-CO_2 iaitu 58.7%. Kadar kekerasan dan "*fracturability*" pulpa OPF dikurangkan ke 46.4% dan 79.2% selepas sterilisasi SC-CO_2. SC-CO_2 menunjukkan bacaan yang sangat baik dari segi kemampatan dan kekerasan pulpa OPF yang membantu memudahkan proses pengekstrakan CPO. Pengurangan kelembapan dalam OPF selepas SC-CO_2 dapat menstabilkan kandungan FFA dan menghasilkan kualiti CPO yang tinggi. Kualiti CPO diukur untuk kedua-dua rawatan pensterilan dan dibandingkan dengan minyak sawit mentah (CPO) yang diperoleh daripada perindustrian komersil. Selepas pensterilan SC-CO_2, CPO menunjukkan kualiti yang setanding dengan kualiti minyak kelapa sawit premium dengan kandungan; 0.33% FFA, 5.15 *Degree of bleachability index*, 1212 ppm karotena, Tiada *Peroxide value*, 0.2 *Anisidine value* dan 0.99 *UV Totox*. Oleh itu, teknologi SC-CO_2 mempunyai potensi untuk digunakan sebagai kaedah pensterilan tanpa air untuk pemprosesan OPF.

STERILIZATION OF OIL PALM FRUIT USING SUPERCRITICAL CARBON DIOXIDE AS A GREEN TECHNOLOGY

ABSTRACT

Sterilization is a crucial process for palm oil processing to produce quality palm oil. The purpose of oil palm fruits (OPF) sterilization is to inactivate lipophilic microorganisms, soften the fruit pulp and facilitate the OPF detachment from the stalk. Currently, palm oil industries in Malaysia utilizes the steam sterilization method for OPF processing. This sterilization method requires a huge quantity of water, resulting in generation of large quantities of palm oil mill effluent (POME). Besides, the interaction of OPFs with moisture during steam sterilization is denoting higher free fatty acid (FFA) in extracted oil. Therefore, it urges to implement a waterless sterilization technology to eliminate FFA generation in extracted oil and avoid POME generation. In the present study, Supercritical carbon dioxide (SC-CO_2) technology was utilized to sterilize OPF with varying pressure (7.4-50 MPa), temperature (32-80^oC) and treatment time (15-60 min). The performance of SC-CO_2 sterilization was determined based on the inactivation of lipase producing microorganisms (bacterial and fungi) in OPF and it was compared with steam autoclave sterilization. Results showed that the SC-CO_2 inactivated the microorganisms at moderate temperature ($\leq$ 80^oC) and pressure (8-10 MPa) within $\leq$60 min of sterilizing time. The SC-CO_2 efficiency of OP-FFB sterilization was determined using a 10 Liter vessel, based on the inactivation of *Bacillus* spp. and *Aspergillus* spp. (microbes with highest occurrence) in OP-FFB with varying SC-CO_2 pressure (10-30 MPa), temperature (40-80^oC) and treatment time (15-90 min). Complete inactivation of the microorganisms in OP-FFB were obtained after 45-75 min at 10 MPa and 80^oC. In terms of softness of the palm oil pulp structure (OPF), OPF pulp compressibility increased significantly after SC-CO_2 sterilization which is 58.7%. The OPF pulp hardness and fracturability value was reduced to 46.4% and 79.2% after SC-CO_2 sterilization respectively. SC-CO_2 shows excellent reading in terms of compression and hardness of the OPF pulp that facilitates the extraction process of crude palm oil (CPO). The reduction in humidity in OPF after SC-CO_2 could stabilize the FFA content and produce high quality of CPO. CPO quality is measured for both sterilization treatments and compared to CPOs obtained from commercial industrialization. CPO after SC-CO_2 sterilization shows a very good quality comparable with premium palm oil with 0.33% FFA content, 5.15 Degree of bleachability index, 1212 ppm Carotene, null Peroxide value, 0.2 Anisidine value and 0.99 UV Totox. Thus, the SC-CO_2 technology has the potential to be utilized as a waterless sterilization method for OPF processing.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Oil palm fruit mesocarp (palm oil) is the largest source of vegetable oil. In palm oil industries, the major constraint that burdens the millers is the oil degradation, which involves a rapid oil acidation due to lipase activity that releases free fatty acid (FFA) in the mesocarp of the bruised fruits. The palm oil degradation was caused by lipase enzyme. The FFA is the foreign particle formed from lipolysis of fat by lipase and reduce the quality of the oil. The lipase enzyme accumulated at the bruising surface of the fruits and the delay in the fruit processing causing further degradation of the oil by lipase and increased the FFA content. Acidity had a strong impact on the qualities of commodity oil because FFA content of >5% is considered to be unfit for human consumption (Ebongue et al., 2008). The presence of FFA indicates that degradation has occurred through poor handling and ineffective sterilization during processing and is considered a sign of deterioration of oil quality. The oil palm fruit mesocarp have high lipase activity that increase FFA and necessitates post-harvest sterilization (Morcillo et al., 2013). FFA has to be removed through an energy-costly refining process that leads to oil losses.

Palm mesocarp oil processing can be separated into two major sections which are crude palm oil (CPO) extraction (first section) and oil refinery (second section). The sterilization process is the first process of the first section and is crucial in the CPO production. The effectiveness of the sterilization process can highly affect the quality of the extracted palm oil in preventing further oil degradation. The general purpose of sterilization of oil palm fresh fruit bunch (OP-FFB) is to inactivate lipase activity and lipophilic microorganisms, soften the pulp of the fruits and facilitate the stripping process. The current practice of thermal sterilization in the Malaysia palm oil industry is the conventional steam sterilization. The conventional steam sterilization technique operates at temperature 130ºC-160ºC, elevated pressures of 0.15-0.4 MPa, and sterilization time of 60-90 min. It is inconsistent in inactivating lipase and microbes in oil palm fruit (OPF). This situation can be portrayed by the high FFA in CPO, observed in several palm oil mill. The biggest concern in this sterilization method is because it requires huge amounts of water, which in turn demand higher energy usage and generates large quantities of palm oil mill effluent (POME). To overcome this challenge, waterless sterilization seems as a better option in yielding a high quality palm oil while reducing the operational costs for water and effluent treatment as well as protecting the environment.

1.2 SC-CO_2 sterilization

Among the various types of sterilization process, supercritical carbon dioxide (SC-CO_2) is considered as the most effective sterilization technique, which can be operated at low temperature and moderate pressure (Amaral et al., 2017). SC-CO_2 refers to the carbon dioxide gas in a supercritical state when its temperature and pressure are greater than the critical point of CO_2 (Hossain et al., 2016a). Under standard temperature and pressure conditions, the CO_2 behaves as a gas. When the temperature and pressure of CO_2 are increased above its critical points at 31.1°C and 7.4 MPa respectively, its properties change to that of neither a gas nor a liquid (Hossain et al., 2011). At this point, CO_2 is referred to as SC-CO_2. SC-CO_2 sterilization has been demonstrated to be an effective sterilization method and has been widely utilized in various industrial fields (Dillow et al., 1999; Hossain et al., 2011; Balestrini et al., 2016; Hossain et al., 2016b) including food processing and heat sensitive material due to the relatively low operating temperatures and moderate operating pressures (Spilimbergo and Bertucco, 2003; Hossain et al., 2015). The SC-CO_2 sterilization technology is very effective in the inactivation of microorganisms and deactivation of enzymes and proteins (Kim et al., 2007; Hossain et al., 2015a). The SC-CO_2 has gained consideration in the food processing industry for its role in preserving the food taste and quality by preventing unnecessary modification by enzymes that degrades foods, lowering its quality and emit unpleasant odor (Wimmer and Zarevúcka, 2010; Hu et al., 2013). It is suitable for food processing due to its mild operating condition and waterless condition. Waterless condition made it an excellent choice for food processing which prone to spoilage when exposed to excessive moisture. SC-CO_2 is a better option to be imply as an oil palm sterilization technique which aiming for excellent quality of palm oil.

1.3 Problem statements

The existing steam sterilization used up huge amount of water and energy since vast quantities of steam must be used for complete heating during the lengthy treatment period (Hadi et al., 2012). The use of steam sterilization exerts deleterious effects upon the environment, specifically via the generation of palm oil mill effluent (POME) which require a costly treatment (Alhaji et al., 2016; Vincent et al., 2014). The term "POME" essentially refers to the total liquid waste produced from all the processes which occur within a palm oil mill. Five to 7.5 tons of water is required per ton of crude palm oil produced, more than 50% of which ends up as POME (Ahmad et al., 2003). The current POME treatment process releases greenhouse gases such as methane, hydrogen sulfide and sulphur trioxide (Vincent et al., 2014). Thus, it urges to determine an effective waterless technology for OP-FFB sterilization in order to eliminate POME generation.

Excessive oil degradation by enzymatic lipolysis activity causes low quality CPO. Oil degradation happens due to delay in post-harvest processing and ineffective sterilization. The harvested fruits are subjected to the sterilization process for preventing oil degradation by inactivating lipase-producing microbes (LPM) and lipase enzymes. Lipase enzyme that can be found in the palm oil was commonly produced by the pathogen namely *Bacillus* sp., *Pseudomonas* sp., *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* sp., (Okechalu et al., 2011; Tagoe et al., 2012), *Rhizopus oryzae* (Hiol et al., 2000), *Mucor* sp. (Abbas et al., 2002; Izah and Ohimain, 2013). Inconsistent sterilization effectiveness or in other words, unable to completely remove the LPM, causing high degradation of CPO and lead to the low value of CPO marketability. Therefore, it is important to achieve a complete sterilization by eliminating the LPM through implementation of a mild sterilization technology that are able to attain complete inactivations of LPM and preserve the CPO quality.

The oil extraction rate (OER) is an important parameter in palm oil business as it is related to yield and profitability (Hoong and Donough, 1998). OER is the percentage of the weight of oil physically recovered from a known weight of FFB processed (Chang et al., 2003). The factors that affected OER value including the oil losses from the processing. The total for oil loss in press fibre is the highest with 13.76% followed by 12.92% of oil loss in the wastewater, 10.77% in final effluent and

5.96% from the sterilizer condensate (Zulkefli et al., 2017). The oil loss in press fibre shows that some oil still remains in the fruit mesocarp after oil extractions. The fruits mesocarp softness is important factor contributing to the oil extraction process. Besides, in current steam sterilizations, more oil loss was observed from the wastewater, final effluent and sterilizer condensate which accumulate as much as 26.65% of the total oil loss. By implementing the waterless sterilization, the oil loss could be reduced and save the monetary losses for the company. A gross overview shows that for a 1% reduction in OER is equivalent to a loss of RM 350,000 per month in revenue, based on its average monthly production capacity at the average market price of crude palm oil at RM 2,500.00 per tonne (Zulkefli et al., 2017).

Since steam sterilization requires the use of water, and is thus a wet process, the presence of water and high temperatures actively promotes hydrolysis of the triglycerides within the palm oil into free fatty acids (FFA), which in turn decrease the palm oil quality (Cheng et al., 2011). Ineffective sterilization caused high FFA content in the CPO and causing losses to the millers, which can be removed thru refining. The presence of FFA within any vegetable oil is an indicator of oil quality, since higher percentages of FFA within a vegetable oil will increase the rate of rancidification. Steam sterilization results in adverse effects on the quality of the palm oil, such as over sterilization which causes poor bleach ability of the palm oil. Due to the high temperatures and pressures involved in steam sterilization, secondary products such as peroxide and anisidine are formed within the crude palm oil, thus compromising the chemical quality of the oil. Reduction of beneficial phytonutrients such as carotenoids also occur due to the high temperatures involved within the process, which results in degradation of such compounds (Okogbenin et al., 2014). Evacuation of hot air from the steam sterilizer also increases the risk of palm oil oxidation at high temperatures, further reducing the oil quality. The long treatment times involved in steam sterilization also make it a slow and inefficient process, requiring a lot of manpower during the batch sterilization process (Sivasothy, 2005). Thermal degradation of carotene, previously suspected of giving rise to undesirable chemicals, now is known to furnish mainly harmless hydrocarbons, most of which are removed by the deodorization step in refining (Goh et al., 1985). All the drawbacks of the existing sterilization can be overcome by applying a waterless sterilization. The SC-CO_2 sterilization will be benefited in this context which therefore providing a superior CPO.

The substitution of the steam sterilization with a method of sterilization which preserves the nutritional quality of palm oil, while avoiding the generation of large amounts of liquid waste, is highly desirable from an economic and environmental standpoint. In the present study therefore propose that supercritical carbon dioxide (SC-CO_2) may be applied to the oil palm fruitlet and fresh fruit bunch (FFB) sterilization process.

1.4 Research Objectives

In view of the existing literature review and the problem statements, some important areas for further research on the sterilization of the oil palm fruits are identified. The present work therefore focused on the following objectives:

a. To study sterilization - inactivation of fungi and bacteria from oil palm fruits using supercritical carbon dioxide (SC-CO_2) and the effect of sterilization parameters.

b. To evaluate the SC-CO_2 efficiency on microorganisms inactivation from oil palm fruits in one and ten liter sterilization vessels.
c. To determine the effect of SC-CO_2 sterilization on the physico-mechanical properties of oil palm fruits in one liter vessel.
d. To determine the effect of SC-CO_2 sterilization on the crude palm oil quality.

1.5 Scope of the work

The researches gives emphasis a new waterless supercritical carbon dioxide sterilization (SC-CO_2) technique to be used for OPF sterilization

a. In case of OPF sterilization, a waterless Supercritical carbon dioxide sterilization (SC-CO_2) technique is used to sustain ecosystem by eliminating the water usage as well as solving the effluent (POME) – related issues.
b. For assessing the effectiveness of SC-CO_2 sterilization on the inactivation of microorganisms, the temperature, pressure and sterilization time were adjusted accordingly for achieving complete inactivations.
c. The efficiency of SC-CO_2 was measured by inactivations of selected microorganisms (fungi and bacteria with highest occurrence) using 10 Liter sterilization vessel.
d. The effect of the SC-CO_2 sterilization on the physical properties of OPF is based on the texture analysis. The sterilization effect to the oil palm mesocarp fiber surface condition was observed through scanning electron micrograph (SEM).

e. The CPO that undergo SC-CO_2 sterilization and autoclave will be tested for oil quality based on several parameters such as FFA, degree of bleachability index (DOBI), carotene content, peroxide value, anisidine value and UV Totox. CPO is extracted using manual press to avoid any alteration of the oil properties by chemical etc. The results are compared to the results of the CPO obtained from the palm oil mill.

1.6 Organization of the thesis

The thesis comprises of six chapters dealing with various aspects of OPF sterilization by Supercritical carbon dioxide sterilization (SC-CO_2).

<u>Chapter 1 Introduction</u>

Chapter 1 deals with the general introduction of the conventional oil palm fresh fruit bunch (OP-FFB) sterilization process. The basic features and associated with the disadvantages of steam sterilization are summarized. A brief outline of the Supercritical carbon dioxide sterilization (SC-CO_2) is included. Objectives of the works and scope are incorporated as well. The chapter is concluded with the list of organization of the thesis presented through the study.

<u>Chapter 2 Literature review</u>

The basic aim of this chapter is to critically examine the various aspects of existing sterilization method of OP-FFB. In order to establish the scope of the present study and salient objectives, a comprehensive literature review is carried out with the main focus on the steam sterilization and its drawbacks and the needs for waterless sterilization technique for the OPF processing. This chapter also lists the effectiveness of the supercritical carbon dioxide in sterilization field and its suitability to be used in food sterilization. The overall methodology flow chart for chapter 3, chapter 4 and chapter 5 was shown below:

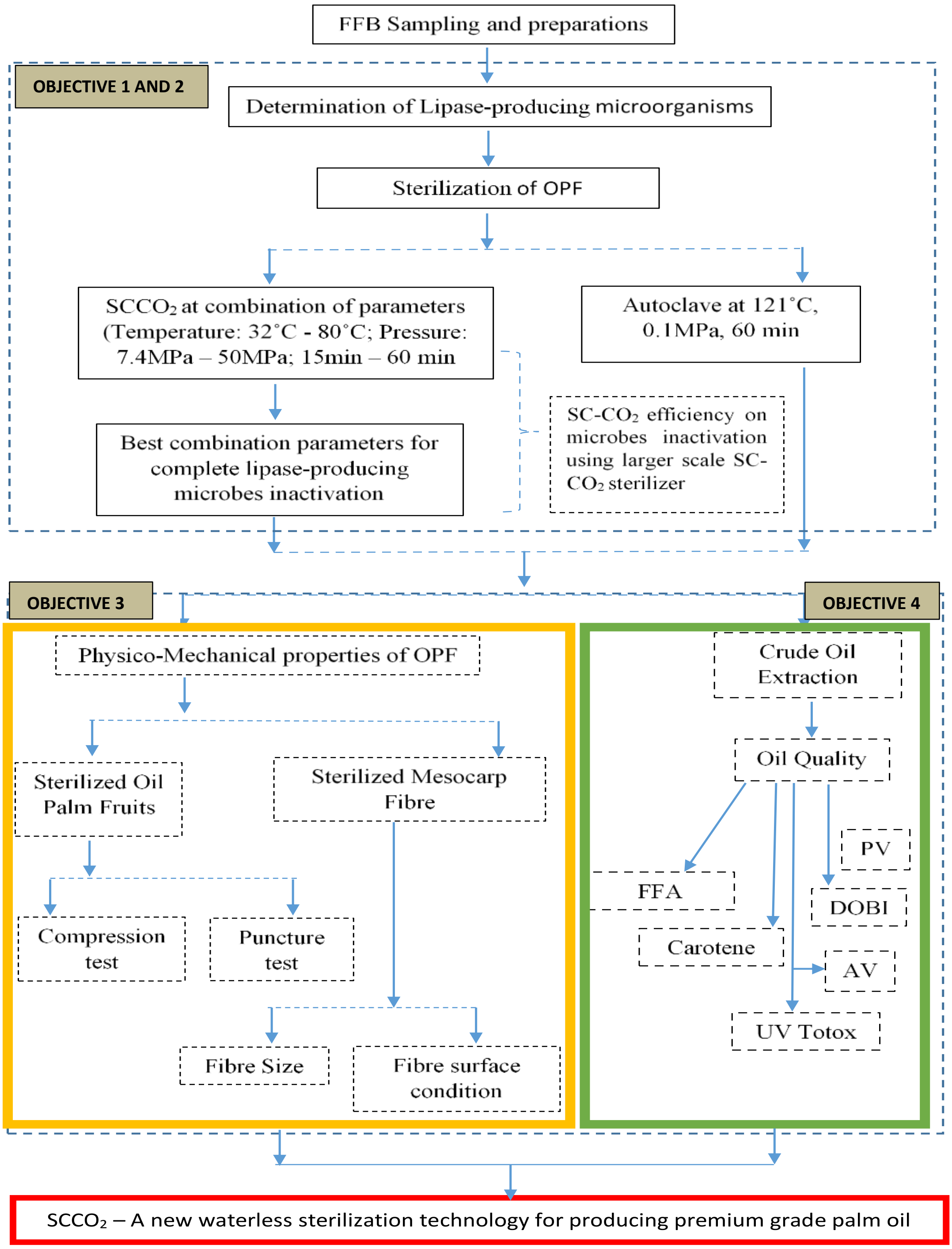

Figure 1.0. Overall Methodology Flow Chart

Chapter 3 Study on Supercritical carbon dioxide (SC-CO_2) sterilization of oil palm fruits and microorganisms inactivation.

This chapter involved the study on the first objective. In this chapter, microbial isolates from OP-FFB were screened for the lipase production. Those lipase-producing fungi and bacteria that possibly be involved in the oil degradation and commonly occurred on the samples were sterilized using supercritical carbon dioxide (SC-CO_2) using different combinations of temperature, pressure and sterilization time. The combinations of parameters are tested until achieving complete inactivation by obtaining zero-colony growth on the culture agar. The microorganism inactivations mechanism by SC-CO_2 is discussed in this chapter. The physical observation on SC-CO_2 treated OPF was also being discussed in here.

Chapter 4 Supercritical carbon dioxide (SC-CO_2) efficiency on fungi and bacteria inactivation of oil palm fresh fruits in ten liter sterilization vessel.

Chapter four explore the efficiency of SC-CO_2 in OP-FFB sterilization in a bigger scale; ten liter sterilization vessel to fulfil the second objective. The inactivation process was tested on specific fungi and bacteria (highest occurrence) for further understanding. In addition, the dependence of the temperature on the inactivation of *Bacillus* spp. and *Aspergillus* spp. in OP-FFB subjected SC-CO_2 pressure (10-30 MPa) were determined.

Chapter 5 Comparative studies on physical properties of oil palm fruits and extracted oil quality using autoclave and SC-CO_2 sterilization.

This chapter lists the third and fourth objectives which is to observed the compressibility and hardness of oil palm fruits and oil palm mesocarp fibers condition before treatment (raw untreated) and after treatment (SC-CO_2–treated). Compressive strength and hardness of the treated OPF have been investigated to check its suitability for oil extraction. In addition, the single fiber taken from the SC-CO_2 treated OPF was compared to the fiber from various OPF treatment from past research including chemical treatment, superheated steam etc. On the other hand, the CPO quality were examined prior to sterilization based on several parameters including Free fatty acid (FFA), Carotene, Deterioration of bleachability index (DOBI), Peroxide value (PV), Anisidine value (AV), and Ultraviolet total oxidation (UV Totox) value. CPO quality will be determine based on the parameters value especially FFA. Furthermore, the results were compared with autoclave results and the commercial CPO results obtained from the palm oil mill. The outcome of the CPO quality will decide the effectiveness of the sterilization process in removing the FFA thus preventing further oil degradation.

Chapter 6 Conclusion and recommendation

Chapter 6 summarized the major conclusion for each objectives. Several important conclusions have been drawn on the Supercritical carbon dioxide sterilization of OPF. Likewise, the chapter outlines the important recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The demand for vegetable oils in food industries has been increasing due to the increase in human populations as well as the importance of its usage in food preparations. In 2016, the world oil palm consumption was about 58.31 million tonnes, comprising the highest consumed vegetable oil in 2016, making oil palm by far the world's number one fruit crop (MPOB, 2017). The oil palm alone is capable to fulfill the continuously growing world demand for vegetable oils that is estimated to reach 240 million tonnes by 2050 (Corley, 2009). A considerable amount of palm oil are used in other products aside from food industry and these include cosmetics, pharmaceuticals, surfactants and washing agents. Oil palm is cultivated mostly in humid tropical countries, namely Indonesia, Malaysia, and Thailand as well as African countries (Basiron, 2005). Malaysian palm oil accounted for 16.8 million tonnes of the total global trade of oil and fats in 2016 with 37% of the world's palm oil production (MPOB, 2017).

It is essential to produce crude palm oil (CPO) with excellent quality and stability, specifically with regards to its acidity and oxidation, in order to achieve good quality refined oils (Basiron, 2005; Pacheco et al., 2017). Previous studies have shown that lipase in oil palm fruits, caused the degradation of triacylglycerols in the palm oils releasing free fatty acids (Junaidah et al., 2015; Kouteu et al., 2016; Pacheco et al., 2017). Lipases or triacylglycerol acylhydrolases, (EC 3.1.1.3) is capable of hydrolyzing ester bonds in triacylglycerols, releasing free fatty acids and glycerols. Since palm oil is basically made up of triglycerides, hydrolysis by lipase causes an increase in the free fatty acid (FFA) levels in CPO (Taher et al., 2011). The major fatty acids predominant in palm oil are oleic and palmitic acids. Aside from the acidity, FFA level is one of the most frequently determined criterion as part of the quality indices during the production, storage, and marketing of palm oil products (Barcelos et al., 2015). At present, CPO produced by Malaysian palm oil mills is required to comply either with the trade specification or the revised quality standard for CPO ex-bulking, as published in Malaysian Standards of MS 814 (Junaidah et al., 2015; MPOB, 2017).

It is evident that sterilization of OP-FFB is a crucial step in palm oil production, the main purpose of which is to inactivate microorganisms with lipolytic activities, in particular lipase activity. The high temperature steam also serves to soften the fruit pulp and facilitate the stripping of the fruits from the OP-FFB (Sukaribin and Khalid, 2009; Junaidah et al., 2015). Studies reported that the quality of palm oil is directly attributed to the degree of lipase inactivation by sterilization (Sukaribin and Khalid, 2009). At present, the most common sterilization method in palm oil industry is using saturated steam at temperatures 130ºC-160ºC (Junaidah et al., 2015). In essence, it has been recognized that the steam sterilization technique employed in palm oil mills to sterilize the oil palm fresh fruit bunches (OP-FFB) is incapable of inactivating lipase producing microbes present in the OP-FFB (Ishikawa et al., 1995; Sukaribin and Khalid, 2009; Hossain et al., 2012). Such weakness in the current sterilization spawns CPO that has higher FFA and is likely to be of lower grade. Hydrolysis by lipase also creates mono- and diglycerides, which greatly affect crystallization and other downstream processing. As a consequence, it has become a concern that the resulting refined oil would not meet the demand of the international premium quality standard (Taher et al., 2011; MPOB, 2017). Hence it is vital that the CPO produced are of the finest quality with low FFA, since low FFA is the major criterion in assigning prices to the CPO (Taher et al., 2011).

In addition to its lack of capacity to completely inactivate lipase activity and lipase producing microbes, use of steam also generates huge amounts of palm oil mill effluent (POME). POME is a brownish liquid, of viscous consistency containing high amounts of organic pollutants needs to be effectively treated before discharged to protect inland waters (Liew et al., 2015). As an alternative approach to steam, supercritical carbon dioxide (SC-CO_2) can be used as a sterilization tool. SC-CO_2 has been vastly used in extraction and to a lesser extent in food and medical device sterilization. Among the potential advantages of the SC-CO_2 sterilization include the inactivation of enzymes and microorganisms at a relatively low temperature and moderate pressure without generating residual waste. The CO_2 at a supercritical fluid state is physiologically safe, inexpensive, non-flammable, non-toxic and has the ability to solubilize lipophilic substances (García-González et al., 2015). Based on these premises, the application of SC-CO_2 bears the potential as a sterilization option for OP-FFB, endowing the process as a waterless sterilization and the exclusion of POME generation. With the annihilation of lipase and lipase producing microbes, the concern on the FFA augmentation in the CPO is abated with the concomitant assurance of the CPO products having the premium quality to compete in a competitive global vegetable oil market.

The present study was conducted to assess the possible use of SC-CO_2 in OP-FFB sterilization in contrast to the existing OP-FFB sterilization method. In conjunction to assessing the use of SC-CO_2 sterilization, this study also appraises the effectiveness of SC-CO_2 in the inactivation of enzymes and microorganisms that are capable of lowering the quality the palm oil. An added feature of this review is the presentation on the details of the SC-CO_2 inactivation mechanisms of the microorganisms.

2.2 Palm oil processing from the oil palm fresh fruit bunch

Oil palm trees in Malaysia were brought in from West Africa with the first commercial oil palm estate in Malaysia set up in 1917 at Tennamaran Estate, Selangor (Basiron and Weng, 2004). The size of the palm fruits is about that of a small plum and a single fruit bunch weighing 10-20 kg can have up to 2000 individual fruits (Basiron and Weng, 2004). Each fruit is made up of fleshy mesocarp within which is a hard kernel (seed) protected by a shell called endocarp. The anatomy of the oil palm fruits can be referred to Appendix 1. Crude palm oil is the primary product of palm fruits obtained from the mesocarp, while palm kernel oil (PKO) is derived from the kernels and is considered a secondary product. Although both oils originate from the same fruit, palm oil is chemically and nutritionally different from the PKO. Palm oil is one of two mesocarp oils available commercially (Sambanthamurthi et al., 2000).

In palm oil production, there are several unifying operations involved, wherein, the sterilization is the first step of OP-EFB processing for palm oil extraction in a palm oil mill. The details of the palm oil processing from fresh fruit bunches in the palm oil industry is presented in Figure 2.1. (Source: Sime Darby Research Sdn Bhd, Carey Island, Selangor, Malaysia).

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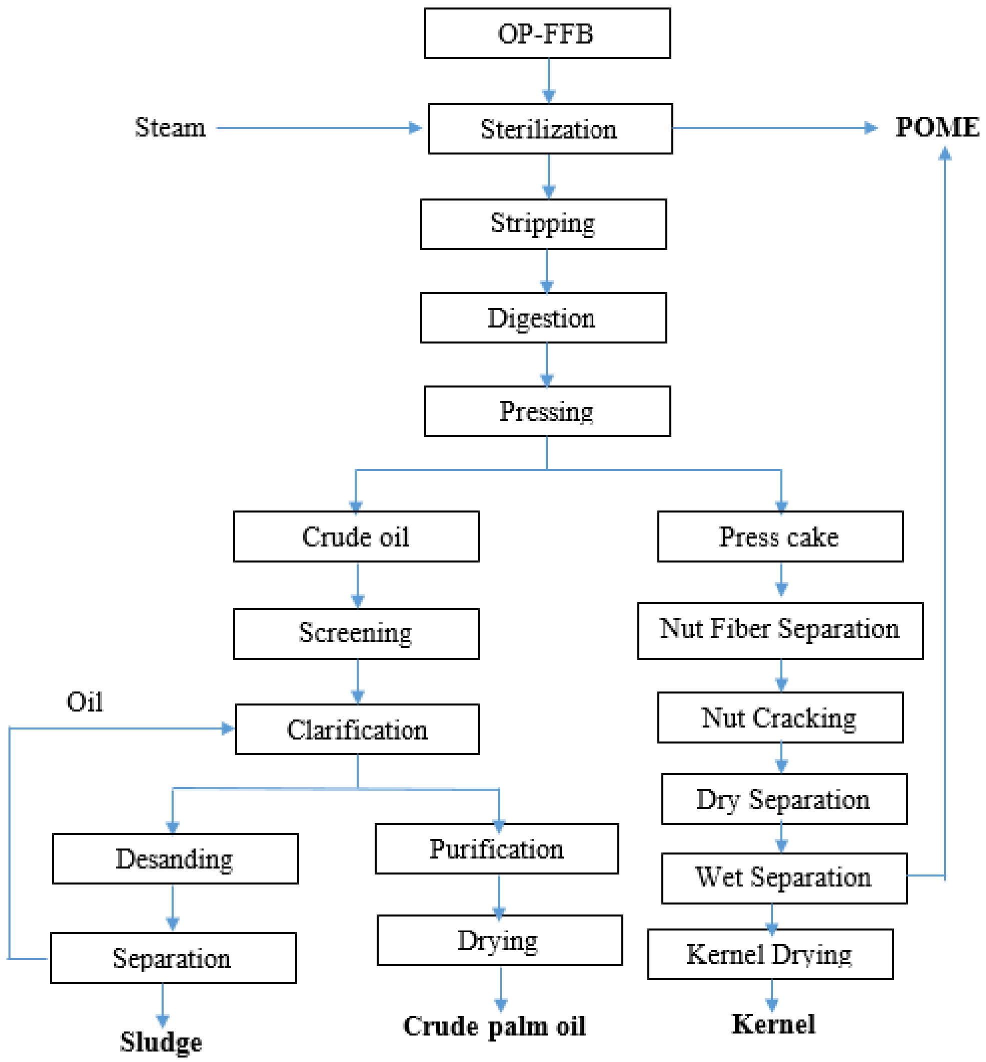

Figure 2.1. Schematic diagram for the palm oil processing from fresh fruit bunch in a palm oil industry (Source: Simedarby Research Sdn Bhd, Carey Island, Malaysia).

Palm oil is semi-solid at room temperature; a characteristic brought about by its 50% saturation level (Sambanthamurthi et al., 2000). In its virgin form, the oil is bright orange-red in color due to its high content of carotene. The typical composition of fatty acids in palm oil is 45% palmitic acid, 40% oleic acid, 10% linoleic acid and 5% stearic acid (Sambanthamurthi et al., 2000; Eknæs et al., 2017). Palm oil and its products possess good resistance to oxidation and heat at prolonged elevated temperatures, thus making palm oil an ideal ingredient in frying oil blends (Eknæs et al., 2017).

2.3 Sterilization of oil palm fresh fruit bunch

Sterilization of FFB in palm oil processing typically uses steam at 0.276 MPa for 70 to 90 min mainly to arrest oil quality degradation due to enzymatic activity. It also serves several other reasons, including eliminating auto-oxidation, and facilitating the digestion process by weakening the pulp structure of the fruits (Sukaribin and Khalid, 2009). The sterilization uses a large amount of steam for an extended period to ensure the heat is able to infiltrate the inner layers of the FFB and in between the packed FFB. Larger sized FFB weighing six to seven kilograms, requires an hour of steam treatment and for smaller FFB of three to five kilograms, a half hour exposure is necessary. Not all of the steam are consumed in heating the FFB, since about half of the steam might be lost as condensate and end up as palm oil mill effluent or POME (Tagoe et al., 2012; Ohimain et al., 2013; Umudee et al., 2013). The effectiveness of the sterilization process results in a high-grade CPO. The major purpose of the sterilization process is to eliminate enzymatic activity that leads to the production of the FFA, which causes the CPO to turn rancid (Tagoe et al., 2012) . The rancidity affects taste and odor that decreases the CPO quality and its marketability. The heated steam, helps in softening the fruit mesocarp to enhance the oil extractability and increase yield while at the same time moistens the nuts to prevent kernel breakage (Sukaribin and Khalid, 2009; Tagoe et al., 2012; Ohimain et al., 2013; Eknæs et al., 2017).

There are numerous sterilization techniques utilized in palm oil processing which is subject to the capacity of the production scale. In small-scale palm oil producers, prevalent in many African nations, cooking is the most common sterilization method used. In large-scale palm oil production, where the financial capacity is stronger, steam sterilization is more commonly found as it can accommodate large amounts of oil palm bunches (Umudee et al., 2013). Other sterilization techniques being tested but yet to be adopted on a large scale include oven dry heating (Hadi et al., 2012), radio frequency (Liu et al., 2015) and microwave treatment (Sarah and Taib, 2013; Umudee et al., 2013). These technologies are in development at pilot scale, where the full spectrum of parameters to meet industrial scale are still being investigated.

2.3.1 Steam sterilization

The sterilization method utilized in a palm oil mill affects the quality of the palm oil and process performance. Thus, selection of the sterilization method in the palm oil industry is crucial, which should be operated at the lowest possible sterilization temperature and the shortest possible sterilizing time. In Malaysia, the common sterilization method employed in the palm oil mill industry is steam sterilization. Steam sterilization operates at temperatures of 130°C-160°C, elevated pressures of 0.15-0.4 MPa, and sterilization period of 60-90 min (Chavalparit et al., 2006; Simarani et al., 2009) in steam sterilization process, OP-FFB undergoes treatment using heated pressurized steam as the medium and in this context sterilization is to eliminate lipase producing microbes. The processed steam supplies heat for the sterilization of the OP-FFB and maintains temperatures in the various processes for efficient extraction and purification of palm oil (Chavalparit et al., 2006).

Steam sterilization not only destroys microbes that produce the oil-splitting enzymes and prevent hydrolysis and auto-oxidation, but also denature proteins which allows the oil-containing cells to come together and the oil to flow more easily upon application of pressure. Steam application would also weaken and soften the pulp structure, making it easier for detachment of the fibrous material and its contents during the digestion process. The heat allows the oil to be released more readily by disrupting the oil-bearing cells in the mesocarp cellular structure (Okogbenin et al., 2014). In addition, the increase and subsequent decrease in the pressure causes a contraction of the nuts which assists in the detachment of the oil palm kernel from the nut shells. The moisture in the steam chemically breaks down the gums and resins (Simarani et al., 2009). The presence of these gums and resins causes the oil to foam during frying and they can be removed during oil clarification process. For large-scale installations, where OP-FFB are cooked whole, the wet heat weakens the fruit stem and makes it easy to remove the palm fruits from the OP-FFB (Sarah and Taib, 2013).

The typical steam sterilization process consumes large amounts of water. The sterilization process consumes about 30% to 60% of the total process steam, depending on the type of sterilizer technology used and the sterilization pattern, which is either a single-peak, double-peak or triple-peak cycle (Sarah and Taib, 2013; Okogbenin et al., 2014). The selection of the sterilizer for use in palm oil mills is based on the steam and power consumption because this will affect the energy efficiency of the palm oil extraction process. The use of energy at the palm oil mills must be as efficient as possible since it is intertwined with the reduction of carbon footprints of palm oil and impact on global warming. Conventionally, the sterilization process is carried out in cylindrical pressure vessels, either in horizontal or vertical position. It is filled with steam under pressure as a batch process. Of late, the sterilization process is also conducted in a heating cabin overflowing with steam at atmospheric pressure as a continuous process. Steam consumption for the sterilization process is about 110 kg to

400 kg per tonne OP-FFB, depending on the type of sterilizer technology used (Berger, 1983).

The limitations of various steam sterilization methods utilized in palm oil industries are shown in Table 2.1.

Table 2.1 Disadvantages of various types of steam sterilizers utilized in palm oil mills (Berger, 1983; Koh and Chooi, 1990; Poku, 2002; Lai et al., 2015; Zainon et al., 2017)

Steam sterilizer	Operation	Steam consumption (kg per ton FFB)	Disadvantage
Horizontal Cylinder	Single-peak, double-peak or triple-peak cycles as batch process.	110-130	Involved saturated steam pressure (0.15 MPa). Oil loss resulting from spilled fruits during loading to cage, transfer to sterilizer and out to thresher, oil dripping from cage and sterilizer condensate Triple or double sterilizing cycle is required for proper cooking Condensate oil cannot be recovered due to iron pickup Reduced cages lifespan Cost for railway, tracks, boogie wheels for cages, overhead crane/tippler, winch/capstan, prime movers, and marshalling yards upkeep.

Vertical Cylinder	Single-peak, double-peak or triple-peak cycles as batch process.	305 – 335	Impedes heat penetration due to considerable stacking height and resulting fruit bunch compression in the vertical vessel. Requires higher steam pressure with multiple-peak cycles and a longer sterilization time for effective heat treatment of the fruit bunches resulting in higher steam consumption. OP-FFB dropping from height causes mechanical damages on to the internal parts Uneven steam distribution affects sterilization Prolonged sterilization cycle time
Continuous Sterilizer	Use mechanical splitter to transport the oil palm fruits by scraper conveyor within the heating cabin exposing the material to steam at atmospheric pressure.	300-360	High steam consumption Requires a second stage of post heating of stripped fruits for effective heat treatment that consumes extra process steam. Power consumption for the bunch splitter machine and material transport scraper conveyor within the heating cabin.

Steam sterilization affects the performance of the oil extraction process and the final quality of the oil. In order to attain good bleachability of the palm oil, air must be properly released before the sterilization, and it is correspondingly crucial to lower the heat exposure time and the temperature.

Among the three types of steam sterilizers, the conventional horizontal sterilization process is the most commonly utilized in palm oil mills (Zainon et al., 2017). Generally, the horizontal sterilization process operates as a batch process in single-peak, double-peak or triple-peak cycles. The pressurized steam can easily reach out into the interior of the horizontally positioned cylindrical vessel from different directions, which facilitates the heat penetration into oil palm fruit bunches in the cages. The inherent setback of the horizontal vessel is the ineffectiveness of air removal during the initial venting. The air can be removed via diffusion through multiple-peak cycles. This demands higher steam consumption and longer sterilization period. The heat treatment under this situation consumes between 360 and 400 kg of process steam per tonne of FFB at steam temperature of 143°C and 0.4 MPa steam pressure in triple peak cycles. However recent improvements in removing the air quickly and completely enhanced the performance of the horizontal sterilizer which is found to be the most energy-efficient sterilization process in palm oil mills. It operates at a pressure 0.15 MPa, temperatures of 121°C to 134°C, and with steam consumption as low as 110 to 130 kg per tonne OP-FFB for a sterilization time of about 40 min (Berger, 1983).

The vertical sterilization process is utilized by the palm oil mills where space is limited. In this process, sterilization of oil palm fruit is conducted as a batch process using steam pressure at 0.4 MPa in either single-peak, double-peak or triple-peak cycles (Koh and Chooi, 1990). However, the vertical sterilization process of oil palm fruit bunches requires multiple-peak cycles with higher steam pressures and longer sterilization time. The operating conditions of vertical sterilizers are steam pressure of 0.4 MPa, temperature of 140°C for a duration of 60 min with steam consumption between 305 and 355 kg per tonne OP-FFB and 200 kW of power (Lai et al., 2015). The high temperature, fruit bunch compression and poor drainage cause oil to be adsorbed into the empty fruit bunches and sterilizer condensate causing a diminished process efficiency.

The third method is the continuous sterilization chamber that uses a heating chamber with a continuous flow of steam at atmospheric pressure brought down from process steam pressure of 0.4 MPa. The FFB would be cut up in half and passed into the heated chamber and a steady stream of steam is allowed in penetrating the bunch. The technique evades the fluctuation in the steam flow that would otherwise affects the stability of the chamber pressure and temperature. However the continuous system does not fit into the current operation of the mills that are subject to the irregularity in FFB supply and the set 16 to 20 hours daily running of the mill operations (Zainon et al., 2017). Its design arrangement also spawns other setbacks that include operating temperature of about 98ºC and high steam consumption due to the continuous flow. The system uses a continuous steam of between 300 to 360 kg/tonne FFB that requires 200 kW of energy (Lai et al., 2015).

2.3.2 Cooking/boiling

Cooking/boiling is the use of high-temperature, wet-heat treatment of loose oil palm fruits. It uses high amounts of water as a medium. Small-scale palm oil mills generally use the cooking and drying technique. Most small-scale processors do not have the capacity to generate steam for sterilization. Therefore, the threshed fruits are cooked in water (Poku, 2002; Okechalu et al., 2011; Vincent et al., 2014; Fahim et al., 2014). A frequent problem which occurs is that the whole fruit bunches which include spikelets absorb a lot of water in the cooking process. So in this case, high pressure steam is more effective in heating fruit bunches without losing much water. The difference between the cooking process in small scale operations and the steam sterilization is that in the former process, bunches are threshed before fruits are cooked, while in the latter process bunches are threshed after heating to loosen the fruits. Small-scale operators use the bunch waste (empty fruit bunch) as cooking fuel. In larger mills the bunch waste is incinerated and the ash, a rich source of potassium, returns to the plantation as fertilizer. The cooking technique is implemented in most palm oil mills in Africa. However, this technique has several disadvantages which include the consumption of huge amounts of water in the cooking process, requires a lot of manpower, and the utilization of high temperatures which causes a reduction in the phytonutrients content as certain nutrients can easily deteriorate at high temperatures (Fahim et al., 2014).

2.3.3 Oven dry heating

The dry heating process is a lengthy heating process which may expose the oil palm fruits to probable burning (Hadi et al., 2012). In addition, hardened mesocarps would only make the depericarping process tougher. Heat penetration in terms of heating duration is the key factor in sterilization of the oil palm fruits utilizing the dry heating method. Moderate heating time of two to three minutes is highly effective to