Journal of Oil Palm Research Vol. 32 (1) March 2020 p. 64-74
DOI: https://doi.org/10.21894/jopr.2020.0015

SCALE-UP STUDY ON THE SUPERCRITICAL CARBON DIOXIDE STERILISATION OF OIL PALM FRESH FRUIT BUNCH

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ABSTRACT

Existing steam sterilisation method of oil palm fresh fruit bunch (OP-FFB) requires huge quantities of water and about 30%-60% of the water results in palm oil mill effluent (POME). In order to circumvent the perennial POME generation problem, it requires a waterless sterilisation system of OP-FFB. The present study was conducted to determine the sterilisation efficiency of OP-FFB using a pilot scale supercritical carbon dioxide (SC-CO_2) technology. The sterilisation efficiency was evaluated based on the inactivation of Bacillus *spp. and* Aspergillus *spp. in OP-FFB with varying SC-CO_2 pressure (10-30 MPa), temperature (40°C-80°C) and treatment time (15-90 min). Complete inactivation of the microorganisms in OP-FFB was obtained after 45-90 min at treatment range of 10-30 MPa and 40°C-80°C. The findings of the present study reveal that the SC-CO_2 sterilisation is a conceivable technology to be used in OP-FFB sterilisation, replacing the current water steam sterilisation technology.*

Keywords: oil palm fruit bunch, supercritical carbon dioxide, steam sterilisation, sterilisation.

Date received: 19 October 2018; **Sent for revision:** 24 December 2018; **Accepted:** 20 May 2019.

INTRODUCTION

Sterilisation of oil palm fresh fruits bunch (OP-FFB) is an important stage for palm oil production from oil palm fruits. The sterilisation process functions in supplying heat for fruits softening and easy detachment of the fruits from fresh fruit bunch (FFB) stalks and inactivate enzymes that are responsible for the rise of free fatty acids (FFA) in the oil (Vincent *et al.*, 2014). Numerous sterilisation methods have been utilised in OP-FFB sterilisation, including steam sterilisation (Junaidah *et al*., 2015), oven dry heating (Abdul Hadi *et al.*, 2012), radio frequency (Liu *et al.*, 2015) and microwave treatment (Sarah and Taib, 2013). Among these technologies, the steam sterilisation is utilised by palm oil industries since other technologies are still in development at pilot scales in order to test their respective capabilities to fulfill the requirements for sterilisation and can only accommodate small amounts of the fruitlets to be sterilised at one time (Vincent *et al.*, 2014; Lai *et al.*, 2012). However, steam sterilisation process requires large amounts of water in OP-FFB sterilisation, resulting in huge amounts of palm oil mill effluent (POME) (Vincent *et al.*, 2014). POME is a thick brownish liquid that contains high biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solid (SS) and thereby treated as a highly polluted wastewater, which requires effective treatment of POME to eliminate impurities prior to discharge into the nearest watercourse (Vincent *et al.*, 2014; Choong *et al.*, 2018). Therefore, palm oil industries are looking for an effective alternative technology to steam sterilisation to minimise wastewater generation. Besides, the level of FFA present in the palm oil extracted from steam sterilised OP-FFB has raised particular concern to the palm oil producers (Mohd Omar *et al.*, 2017). This is

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because of the fact that the FFA present in palm oil can be oxidised, which subsequently reduce palm oil quality and increase in rancidity (Mohd Omar *et al.*, 2018; Man *et al.*, 1999).

The presence of FFA in palm oil is directly attributed to the action of lipase (triacylglycerol acylhydrolase) (Nanssou Kouteu *et al.*, 2016; Taher *et al.*, 2011). Generally, the OP-FFB contains active endogenous lipases and the high oil content in the mesocarp of oil palm fruit makes it a rich substrate for lipase activity (Taher *et al.*, 2011). Benoit Constant *et al*. (2017) observed the lipase activity in palm oil because of the presence of lipophilic microorganisms in OP-FFB. Banani *et al.* (2015) stated that microorganisms secrete lipase during metabolism, which is subsequently activated during the hydrolysis of palm oil triglycerides thus increasing the FFA content. However, Patil *et al.* (2011) reported that the major portion of lipase (about 80%) is produced by microorganisms (bacteria and fungi), and the rest of the lipases are produced by plants and animals. Several investigations have been conducted to determine microorganisms present in OP-FFB responsible for producing lipase enzymes in OP-FFB. Tagoe *et al.* (2012) detected the presence of bacteria and fungi in oil palm fruits and extracted palm oil using Terminal Restriction Fragment analysis and polymerase chain reaction (PCR) amplifications methods. Khan *et al*. (2005) detected lipolytic fungi on oil palm spikelets such as *Aspergillus* spp., *Mucor* spp. and *Penicillium* spp.

Steam sterilisation is unable to completely denature the lipase enzyme and inactivate pathogens present in OP-FFB, as evinced by studies reporting lipophilic activity and the presence of microorganisms in crude palm oil (Ohimain *et al.*, 2013) and refined palm oil (Tagoe *et al.*, 2012). Ohimain *et al.* (2013) have also raised concern on the conventional steam sterilisation method of OP-FFB due to the presence of lipolytic microorganisms in extracted crude palm oil (CPO). Similarly, Hossain *et al*. (2015a, b) observed that steam sterilisation is unable to completely kill the microorganisms in clinical solid waste due to incomplete denaturation of cellular proteins and enzymes. Thus, there is a need to find alternative sterilisation methods for effective denaturation of lipase and microorganisms present in OP-FFB.

The SC-CO_2 sterilisation has been demonstrated to be an effective sterilisation method and has been widely utilised in various industrial fields (Balestrini *et al.*, 2016; Dillow *et al.*, 1999; Hossain *et al.*, 2016b; 2011). Due to the relatively low operating temperatures and moderate operating pressures, the SC-CO_2 sterilisation technology has attracted attention to sterilise heat sensitive materials (Hossain *et al.*, 2015a; Splimbergo *et al*., 2003). Moreover, the SC-CO_2 sterilisation technology has been proven to be very effective in the inactivation of microorganisms and deactivation of enzymes and proteins (Kim *et al.*, 2007; Hossain *et al.*, 2016a). In recent years, SC-CO_2 has gained attention in the food industry for the inactivation of enzymatic activity, as the presence of the active enzyme in food products results in unpleasant change and degrades their quality (Hu *et al.*, 2013; Wimmer and Zarevúcka, 2010). Balaban *et al*. (1991) investigated the inactivation of pectinesterase in orange juice using SC-CO_2 and determined the juice quality and sensory attributes, where the sensory evaluations showed that the colour and cloudiness of treated juice were better than in untreated juice. Furthermore, the flavour and aroma of the orange juice were not affected by the sterilisation (Balaban *et al.*, 1991). The oil palm lipase is located in the mesocarp of the oil palm fruits with an optimal activity at pH 7.5 (Ngando Ebongue *et al.*, 2006). Therefore, it can be denatured by lowering the pH to an acidic environment. Thus, it bears considerable interest to adopt SC-CO_2 sterilisation technology to sterilise OP-FFB in order to effectively inactivate lipase and microorganisms present in OP-FFB, since SC-CO_2 reacts with microorganisms by lowering the pH below 5.0 with the formation and dissociation of H_2CO_3 (Splimbergo *et al.*, 2003).

In a recent study, Mohd Omar *et al.* (2017) studied the possibility of applying the SC-CO_2 in OP-EFB sterilisation at a laboratory scale, wherein the efficiency of the SC-CO_2 sterilisation was determined based on the microbial inactivation in contaminated OP-EFB and stripping oil palm fruits from OP-FFB. It was found that the SC-CO_2 sterilisation was able to achieve complete inactivation of microorganisms at temperature of 80°C and pressure of 10 MPa at 60 min treatment time. They suggested to conduct a further study on the SC-CO_2 sterilisation of OP-FFB in a large scale. Therefore, the present study was conducted to sterilise OP-FFB using SC-CO_2 in a pilot scale. The influence SC-CO_2 sterilisation of OP-FFB was determined based on the inactivation efficiency of *Bacillus* spp. (bacteria) and *Aspergillus* spp. (fungi) with varying SC-CO_2 pressure, temperature and treatment time. The finding of the present study will assist to determine the viability of SC-CO_2 sterilisation as an alternative technology to sterilise oil palm fruits at industrial scale.

EXPERIMENTAL

Sample Collection and Preparations

Ripe OP-FFB was randomly collected from a pile of recently harvested fruits (within a day) to represent actual fruits from the local palm oil mill in Malaysia. Each bunch was cut into 20-30 cm length in order to separate the bunch into smaller spikelets using a mechanical cutter. The spikelets were then stored in the refrigerator at 4°C until further use.

The microorganisms used in the present study were *Bacillus* spp. (vegetative bacteria) and *Aspergillus* spp. (fungi), which were previously isolated from the OPEFB (Mohd Omar *et al*., 2017). The isolated *Bacillus* spp. and *Aspergillus* spp. were re-cultured in nutrient agar media and potato dextrose agar media, respectively, and incubated to obtain fresh culture. A single isolated colony of *Bacillus* spp. and *Aspergillus* spp. was then transferred into nutrient broth and potato dextrose broth; incubated at 37°C for 24 hr and at 28°C for 120 hr, respectively (Sanders, 2012; Mohd Omar *et al*., 2017). Subsequently, bacterial broth (200 ml) and fungal broth (200 ml) were used to contaminate about 2 kg of OP-FFB, individually, by adding the broth in a drop wise manner. Later, the contaminated OP-FFB was taken into the SC-CO_2 reactor (10 litres) for sterilisation. The same step was repeated using autoclaved steriliser.

Supercritical Carbon Dioxide Sterilisation

The influence of the SC-CO_2 sterilisation of OP-FFB was conducted using a SC-CO_2 reactor system with a volume of 10 litre (Nantong Wisdom, China), as shown in *Figure 1*. The sensitivity of *Bacillus* spp. and *Aspergillus* spp. to SC-CO_2 sterilisation was evaluated with varying pressure (10-30 MPa), temperature (40°C-80°C) and treatment time (15-90 min). The purified CO_2 gas was released into the sterilisation chamber and once the set pressure was achieved, the process was left for a specific duration. At the end of the sterilisation time, the chamber was depressurised by releasing the gas. The treated OP-FFB were then collected to determine the number of viable colonies. Experiments were performed in triplicate and the results expressed as the mean value ± standard error.

Autoclave Sterilisation

Autoclave treatment was carried out using a HiClave autoclave (Hirayama, Japan). The parameters of the treatment were temperature at 121ºC, pressure at 0.1 MPa and duration for 60 min, according to the recommended experimental conditions for autoclave sterilisation by Hossain *et al*. (2012). The autoclave process was carried out and samples were retrieved from the autoclave after completing the sterilisation process.

Quantification of Viable Colonies

The viability of studied microorganisms in SC-CO_2 treated and untreated OP-FFB was determined using pour-plate method. Ten individual oil palm fruits of approximately 100 g were randomly taken from the oil palm spikelets and immersed in 900 ml sterile distilled water. The flasks were tightly sealed and agitated on the shaker at 100 rpm for 5 min. An eight-fold serial dilution was carried out by initially taking out 1 ml of the contaminated into 9 ml of sterile distilled water, from which 0.1 ml were seeded on

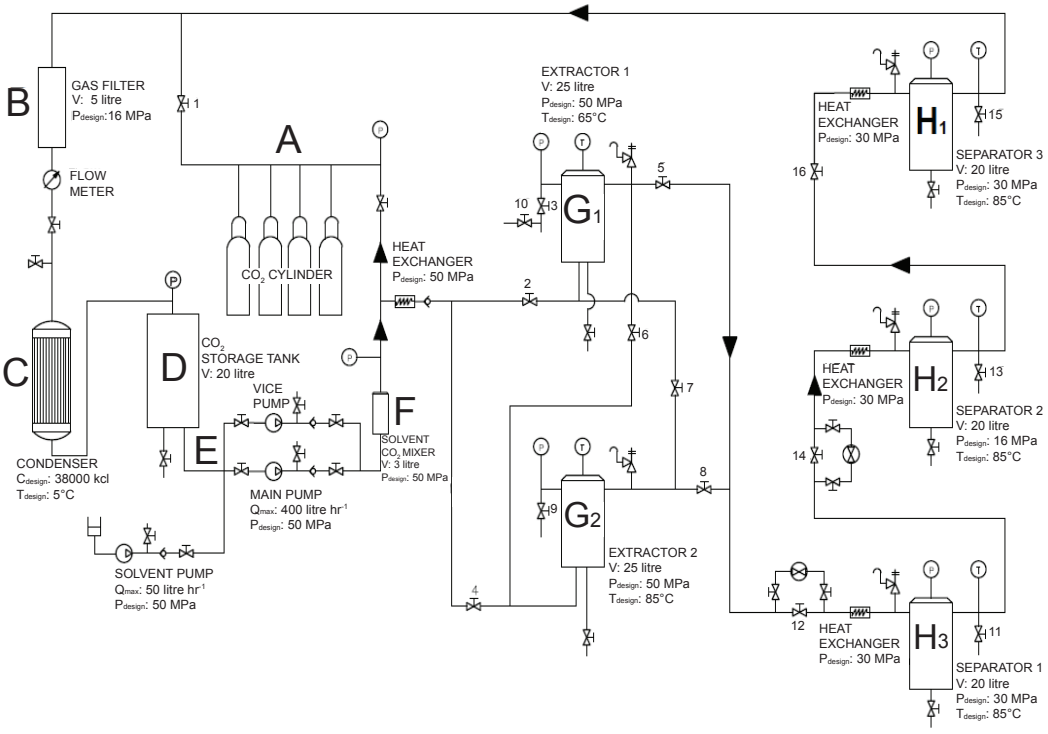


Figure 1. Schematic diagram of pilot scale supercritical carbon dioxide (SC-CO_2) sterilisation system; A - CO_2 tank, B - gas filter, C - condenser, D - CO_2 storage tank, E - pumps, F - solvent and CO_2 mixer, G - G_1 sterilisation vessel 1, G_2 sterilisation vessel 2, H - separator.

Petri dishes containing nutrient agar and potato dextrose agar media for bacterial and fungal growth, respectively. The agar was streaked a few times to obtain colonies with uniform growth. The petri plates were sealed using parafilm and incubated at 37°C ± 1°C for 24 hr and 28°C ± 1°C for 120 hr for bacteria and fungi, respectively. After the incubation period, the number of viable bacterial of fungal colonies were determined as shown in Equation (1) (Hossain *et al*., 2013).

$$\frac{\text{CFU}}{\text{g}} = \frac{\text{Number of viable colonies}}{\text{Agar plating Volume}} \times \frac{1}{\text{Dilution factor}} \times \frac{\text{Volume of contaminate}}{\textit{Mass}\ \text{OPFB}} \quad (1)$$

The initial concentration (log N_0) of *Bacillus* spp. and *Aspergillus* spp. were determined to be 5.67 log colony forming unit per gram (log CFU g^{-1}) and 5.03 log CFU g^{-1}, respectively. The log reduction of the number of viable colonies per gram of OP-FFB was determined as the number of survival colonies before sterilisation (N_0) to the number of viable colonies after sterilisation (N_t), as shown in Equation (2).

$$\text{Log reduction (CFU g}^{-1}) = \log \frac{N_0}{N_t} \quad (2)$$

Morphological Alterations of SC-CO_2 Sterilised OP-FFB

Physical changes of the spikelet fruits were observed based on the fruit surface appearance before and after SC-CO_2 treatment. Further, surface morphology of the oil palm mesocarp fibre (OPMF) of SC-CO_2 sterilised OP-FFB was analysed using a field emission scanning electron microscope (FE-SEM), and compared with the FE-SEM images of OPMF collected from untreated and steam autoclave treated OP-FFB. The OPMF was obtained by cutting treated and untreated OP-FFB manually using a sharp blade with an average length of 20 mm. A piece of OPMF was attached to the FE-SEM stub with a double-sided tape. It was then coated with a thin layer of gold (~ 20 nm) in a Sputter Coater Polar SC515 FISONS. The surface of the sample was viewed in a FE-SEM operated at 5.00 kV.

RESULTS AND DISCUSSION

Inactivation of Microorganisms in Oil Palm Fruits Bunch Using SC-CO_2

The inactivation of microorganisms such as *Bacillus* spp. and *Aspergillus* spp. in OP-FFB using SC-CO_2 with varying pressure and temperature as function of treatment time revealed the reduction of viable cells of both microorganisms. *Figure 2* shows the viable cell reduction of *Bacillus* spp. increased with temperature and pressure from 40°C to 80°C and 10 MPa to 30 MPa, respectively. At 10 MPa pressure, the time required for the complete inactivation (viable cell reduction reached to the maximum at log 5.67 CFU g^{-1}) of the *Bacillus* spp. was determined to be 90 min for 80°C. It was not able to gain the optimal log reduction of the *Bacillus* spp. viable cells at 90 min (maximum treatment time studied) with pressure of 10 MPa and temperature of 40°C and 60°C. It was found that treatment time and temperature required for the complete inactivation of *Bacillus* spp. at 10 MPa was reduced with further increase of pressure. For instance, the treatment time required for the complete inactivation of *Bacillus* spp. were 90 min, 75 min and 60 min at 40°C, 60°C and 80°C, respectively, for 20 MPa. The treatment

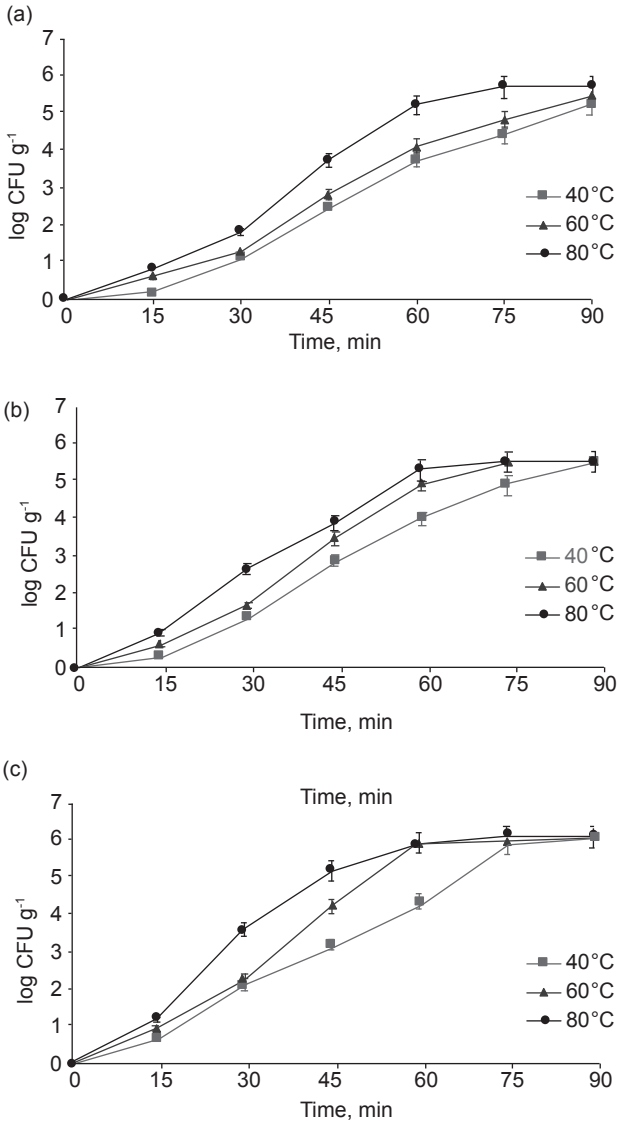


Figure 2. Effect of temperature on the inactivation of Bacillus *spp. in oil palm fruits bunch using supercritical carbon dioxide (SC-CO_2) at pressure 10 MPa-30 MPa. (a) 10 MPa, (b) 20 MPa and (c) 30 MPa.*

time further reduced at 30 MPa to 75 min for 40°C, 60 min for 60°C and 80°C.

The log reduction of the viable cells of *Aspergillus* spp. was determined with varying SC-CO_2 pressure and temperature as a function of time, as presented in *Figure 3*. It was found that the viable cell reduction increased with increasing temperature from 40°C to 80°C and pressure from 10 MPa to 30 MPa. The treatment time required for the complete inactivation of *Aspergillus* spp. in OP-FFB at 10 MPa were 90 min, 90 min and 60 min for 40°C, 60°C and 80°C, respectively. As the pressure increased the degree of *Aspergillus* spp. inactivation also increased, which substantially reduced the required treatment time for the complete inactivation of *Aspergillus* spp. in OP-FFB. The treatment time required for the complete inactivation *Aspergillus* spp. at 20 MPa were 90 min, 60 min and 60 min for 40°C, 60°C and 80°C, respectively. The treatment time for the complete inactivation of the *Aspergillus* spp. were further reduced at 30 MPa to 75 min, 60 min and 45 min at 40°C, 60°C and 80°C, respectively.

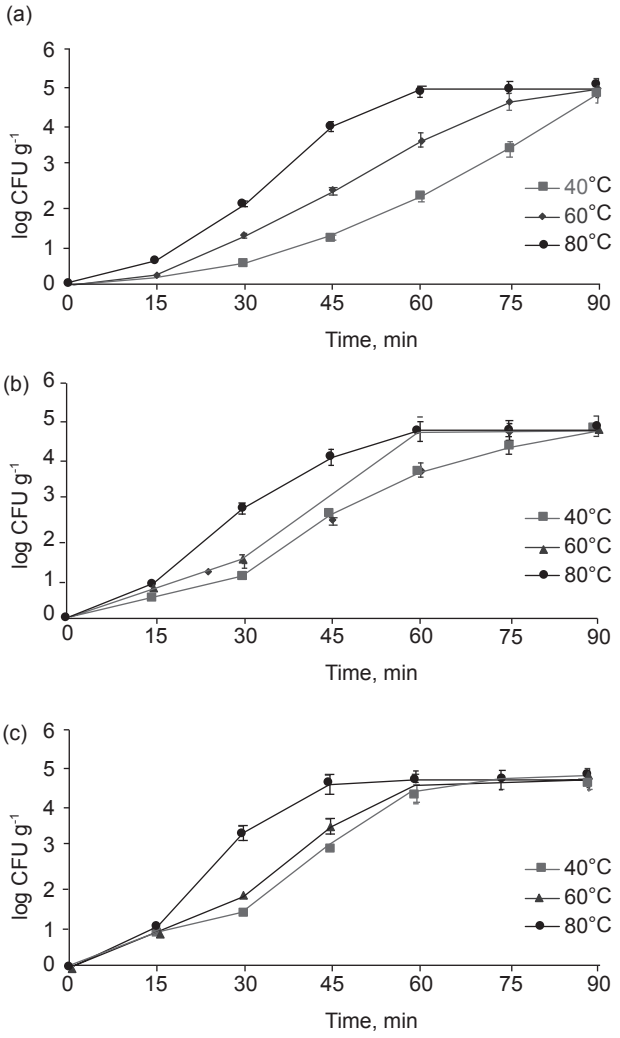


Figure 3. Effect of temperature on the inactivation of Aspergillus *spp. in oil palm fruits bunch using supercritical carbon dioxide (SC-CO_2) at pressure 10 MPa-30 MPa. (a) 10 MPa, (b) 20 MPa and (c) 30 MPa.*

The inactivation of the microorganisms in OP-FFB using SC-CO_2 revealed that the SC-CO_2 is an effective method to sterilise OP-FFB. Both pressure and temperature play important role to inactivate *Bacillus* spp. and *Aspergillus* spp. in OP-FFB using SC-CO_2 sterilisation. The pressure enhances the CO_2 solubilisation in the moisture present in OP-FFB, easing the CO_2 penetration to microbial cell. Wherein, an increase of temperature increases diffusivity of CO_2, at lower pressure, it required higher temperature and treatment time to reach the complete inactivation (Hossain *et al.,* 2016a). However, the treatment time had the minimal effect on the reduction of microbial colony count at 40°C (considered as low temperature). After an hour of SC-CO_2 exposure at 80°C, more than 1 log reduction of the microbes (90% inactivation) was attained for both fungi and bacterial under the three applied pressures. The log reduction increased gradually for the first 15 min and increased rapidly thereafter. The complete inactivation of *Bacillus* spp. was gained at 10 MPa, 80°C for 75 min. With an increase of pressure and under similar SC-CO_2 temperature, the treatment time further reduced to 60 min at 20 MPa and 30 MPa. In our previous study, the complete inactivation of bacteria and fungi in SC-CO_2 treated oil palm fruits was obtained at 10 MPa, 80°C and 60 min (Mohd Omar *et al.,* 2017). Time required for the complete inactivation of *Bacillus* spp. was 75 min under similar pressure and temperature, this happened probably due to the variation of sample size (20 g *vs*. 2 kg) and reactor volume.

The inactivation trend was found almost similar in the case of the *Aspergillus* spp. inactivation in OP-FFB using SC-CO_2 (*Figure 3*). The fungi were being rapidly inactivated after 15 min SC-CO_2 exposure with viability reduction rate of the cells until all the cells became non-viable after 60 min SC-CO_2 exposure at 10 MPa and 80°C. Almost similar characteristics were exhibited in a previous study in the case of inactivation of *Bacillus* spp. in palm fruit fibre (Nik Norulaini *et al*., 2008). Nik Norulaini *et al*. (2008) observed an absence of colonies after formation in the treated palm fruit fibre using SC-CO_2 at 50°C and 20.7 MPa. Meanwhile, Hossain *et al.* (2016a) obtained a complete inactivation of *Bacillus* spp. in clinical solid waste using SC-CO_2 at 10 MPa and 80°C for 60 min. Efaq *et al.* (2017) gained the complete inactivation of *Aspergillus* spp. in clinical waste using SC-CO_2 at 35 MPa, 75°C for 90 min. The complete inactivation of the microorganisms gained in the present study at relatively lower pressure and temperature might be due to the presence of higher moisture content in OP-FFB.

The irreversible inactivation of bacterial and fungal spores in food products should be confirmed for safe consumption. Studies reported that SC-CO_2 inactivates the microorganisms. The SC-CO_2 pressure damaged the cell walls physically and the

extracts cytoplasm materials chemically (Hossain *et al.*, 2016b; 2013). Efaq *et al.* (2017) reported that the SC-CO_2 inactivate *Aspergillus* spp. spores in clinical waste by damaging crust, outer coat and inner coats and the membrane. The CO_2 was able to kill the fungi spores by penetrating the spores, causing the wall damages and spore burst. The effect of SC-CO_2 sterilisation treatment to the bacteria was reported by Hossain *et al.* (2016a). The scanning electron micrographs of SC-CO_2 treated *Bacillus* spp. showed broken cells walls, exposure of cells, cell rupture, cell distortion, punctured holes and loss of the membrane. Microbial cell death can possibly be caused by the application of pressure as it can increase the internal pressure, and when followed by subsequent depressurisation, can interrupt the cellular structure and extraction of cell wall lipids and rupture the microbial cell wall. The sterilisation time found in the present study (60-90 min) is in line with the conventional steam sterilisation process of OP-FFB (70-90 min) (Vincent *et al.*, 2014). However, the sterilisation time of OP-FFB using SC-CO_2 could be further reduced with addition of chemical modifiers (Dillow *et al.*, 1999).

Analysis of the Temperature Dependence by the *Arrhenius* Equation

The dependence of temperature on the inactivation of *Bacillus* spp. and *Aspergillus* spp. in OP-FFB subjected to SC-CO_2 pressure (10 MPa, 20 MPa and 30 MPa) was determined using the following equations (Hossain *et al.*, 2013; Kim *et al.*, 2007).

$$k = a.e^{\frac{-E}{RT}} \quad \text{Equation (3)}$$

$$\ln k = \ln a + \left(\frac{-E}{R}\right)\left(\frac{1}{T}\right) \quad \text{Equation (4)}$$

where k is the inactivation rate (min^{-1}) of *Bacillus* spp. and *Aspergillus* spp. in SC-CO_2 sterilised OP-FFB, a is the pre exponential factor (min^{-1}), E is the activation energy (kJ mol^{-1}), T is the absolute temperature and R is the molar gas constant (8.314 J mol^{-1} K^{-1}). The activation energy (E_a) defines the sensitivity of *Bacillus* spp. and *Aspergillus* spp. to the SC-CO_2 sterilisation. The temperature-dependent inactivation rate (k) was determined by the *Arrhenius*

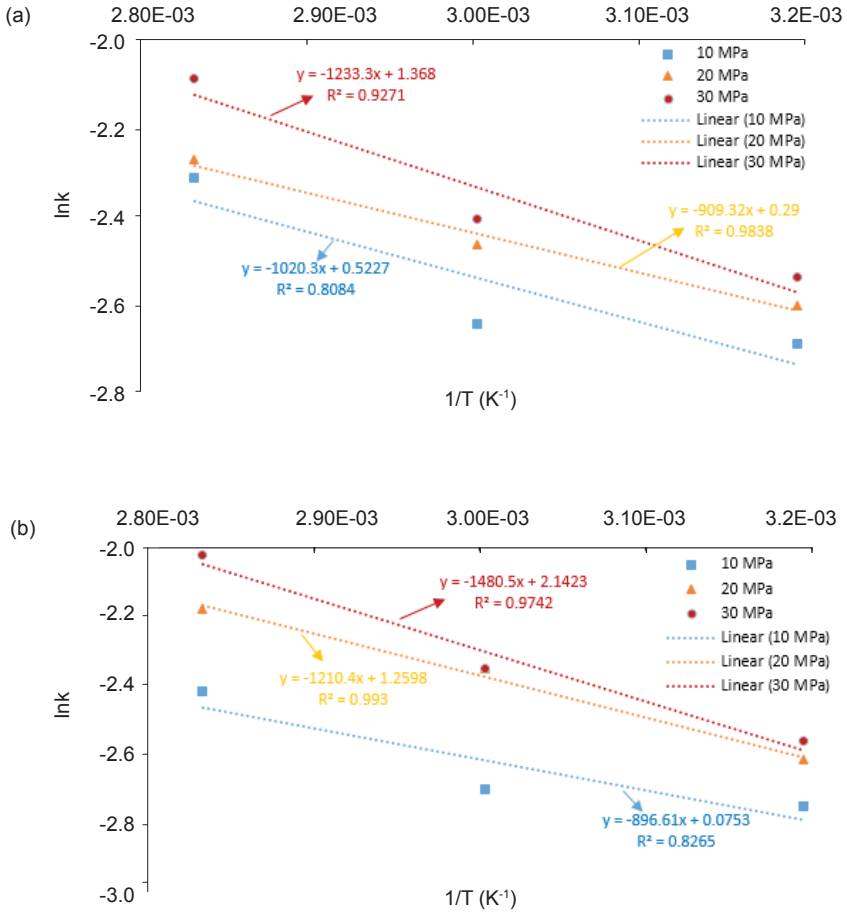


Figure 4. Determination of temperature dependence on the inactivation of Bacillus *spp. (a) and* Aspergillus *spp. (b) in oil palm fresh fruit bunch (OP-FFB) using supercritical carbon dioxide (SC-CO_2) at pressure of 10 MPa-30 MPa.*

plot for the inactivation of *Bacillus* spp. and *Aspergillus* spp. in OP-FFB subjected to SC-CO_2 pressure 10 MPa to 30 MPa, is shown in *Figure 4*. The linear regression curves shown in *Figure 4* reveal the *E* values for the inactivation of *Bacillus* spp. and *Aspergillus* spp. increased with increasing pressure. The E_a values were determined to be 7.56 kJ mol^{-1}, 8.48 kJ mol^{-1} and 10.23 kJ mol^{-1} for the inactivation of *Bacillus* spp.; 7.54 kJ mol^{-1}, 10.1 kJ mol^{-1} and 12.1 kJ mol^{-1} for the inactivation of *Aspergillus* spp. in OP-FFB at SC-CO_2 pressure 10 MPa, 20 MPa and 30 MPa, respectively.

The activation energy (E_a) value for the microbial inactivation depends on types of microorganisms, sterilisation methods and variations of sample and range of temperature studied (Hossain *et al.*, 2016a; Kim *et al.*, 2007). The highest activation energy (E_a) values obtained in the present study were 10.3 kJ mol^{-1} and 12.3 kJ mol^{-1} at pressure of 30 MPa for the inactivation of *Bacillus* spp. and *Aspergillus* spp., respectively. Hossain *et al.* (2016a) obtained the E_a value of 11.64 kJ mol^{-1} for the inactivation of *Bacillus* spp. in SC-CO_2 treated clinical solid waste at pressure of 10 MPa, which is almost similar to the finding of the present study. However, the determined E_a values in the present study were much lower than E_a values reported Liao *et al.* (2007) and Kim *et al.* (2007). Liao *et al.* (2007) obtained the E_a value of 20.6 kJ mol^{-1} for the inactivation of *Salmonella typhimurium* in SC-CO_2 treated carrot juice at pressure of 10 MPa. Kim *et al.* (2007) obtained the E_a value of 110 kJ mol^{-1} for the inactivation of *Escherichia coli* (*E. coli*) in SC-CO_2 treated physiological saline. Hossain *et al.* (2016a) obtained the E_a value of 155 kJ mol^{-1} in steam autoclaved clinical solid waste. Shuler and Kargi (2002) determined the E_a value of 532 kJ mol^{-1} for the thermally sterilised *E. coli*. Thus, it can be postulated that SC-CO_2 is a semi-thermal sterilisation technology, as it requires minimal energy compared to thermal sterilisation method. Although, temperature is an important variable in SC-CO_2 sterilisation technology for the inactivation of microorganisms, but it is not as important as for thermal sterilisation methods (Hossain *et al.*, 2016a).

Morphological Alteration of Sterilised OP-FFB

Figure 5 displays untreated (*Figure 5a*) and SC-CO_2 treated (*Figure 5b*) oil palm spikelet images. As can be seen in *Figure 5*, the SC-CO_2 treatment changed the colour of the oil palm spikelet (*Figure 5b*). Traces of palm oil can be seen on the SC-CO_2 treated oil palm fruit surfaces, probably due to the softening the oil palm fruits by the SC-CO_2. The SEM images of the oil palm fruit fibre surface are illustrated in *Figure 6*.

The oil palm fruit surface image before treatment (*Figure 6a*) shows a clean surface without any signs of oil compared to the images after autoclaved (*Figure 6b*) and SC-CO_2 treatments (*Figure 6c*), which display oil presence on the surface. Steam autoclaved palm fruits show uneven sterilisation on the surface. Certain parts of the fruits fibre surface were damaged and swollen which might be due to the steam absorption and effect from the high temperature. Meanwhile, the SC-CO_2 treated palm fruit surfaces remained intact with oil leaching uniformly. The SC-CO_2 is being treated as mild sterilisation technology and widely utilised in food industry to sterilise heat sensitive food products (Hossain *et al.*, 2016b; Kim *et al.*, 2007; Hu *et al.*, 2013). The SEM analyses showed that the morphology and surface appearance of the SC-CO_2 treated oil palm fruits are not altered or damaged (*Figure 6*). Further, the SEM image of the SC-CO_2 treated fruit surfaces revealed minimal oil leaching compared to the steam autoclaved oil palm fruits. Thus, the application SC-CO_2 sterilisation technology in the OP-FFB sterilisation would minimise oil loss in the palm oil industry.

One of the major shortcomings of the steam sterilisation method of OP-FFB sterilisation is the generation of the huge amount of POME (Benoit Constant *et al.*, 2017; Mohd Omar *et al.*, 2018).

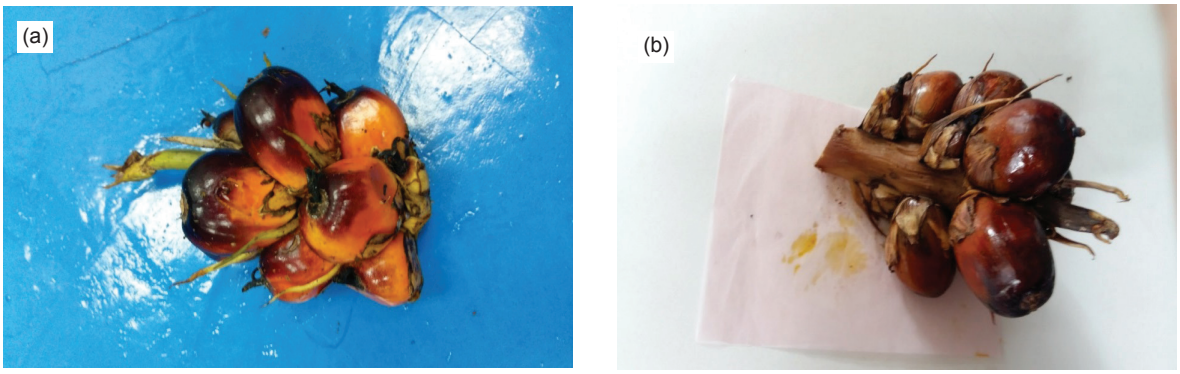


Figure 5. (a) Raw oil palm spikelet and (b) supercritical carbon dioxide (SC-CO_2) treated oil palm spikelet at 80°C, 10 MPa, 60 min.

Figure 7 shows the simplified process flow diagram of OP-FFB sterilisation using steam sterilisation method currently practiced by palm oil mills (a) and proposed supercritical sterilisation (b). It can be observed that steam steriliser uses a lot of steam and produces huge amount of POME amounting to 62.5 kg/100 kg FFB. The amount of CPO obtained is about 22.5 kg/100 kg FFB. The mass balance of the palm oil production is almost similar as reported by other researchers (Ohimain *et al.,* 2013). In comparison with supercritical sterilisation (SC-CO_2), no water was involved in the sterilisation process

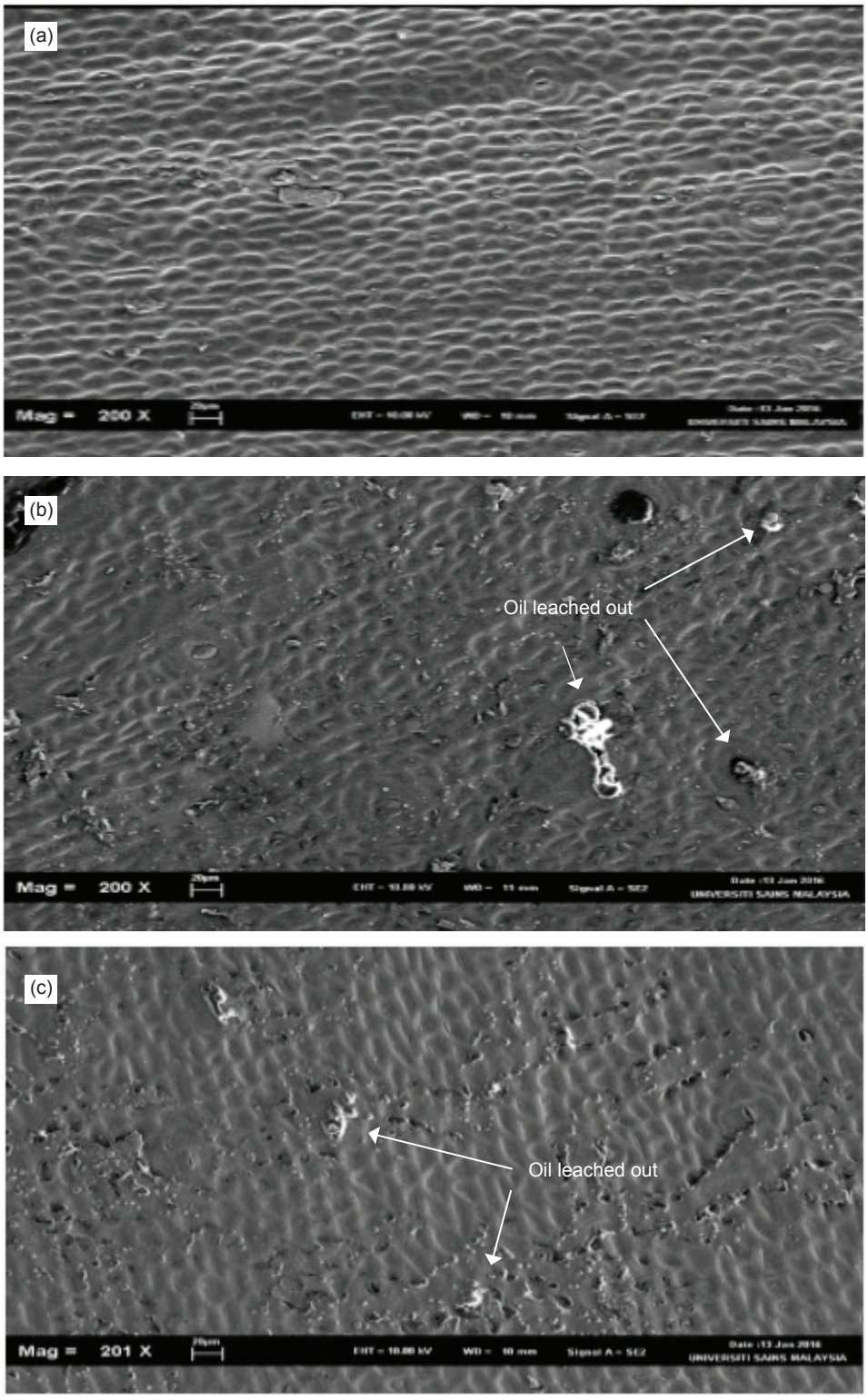
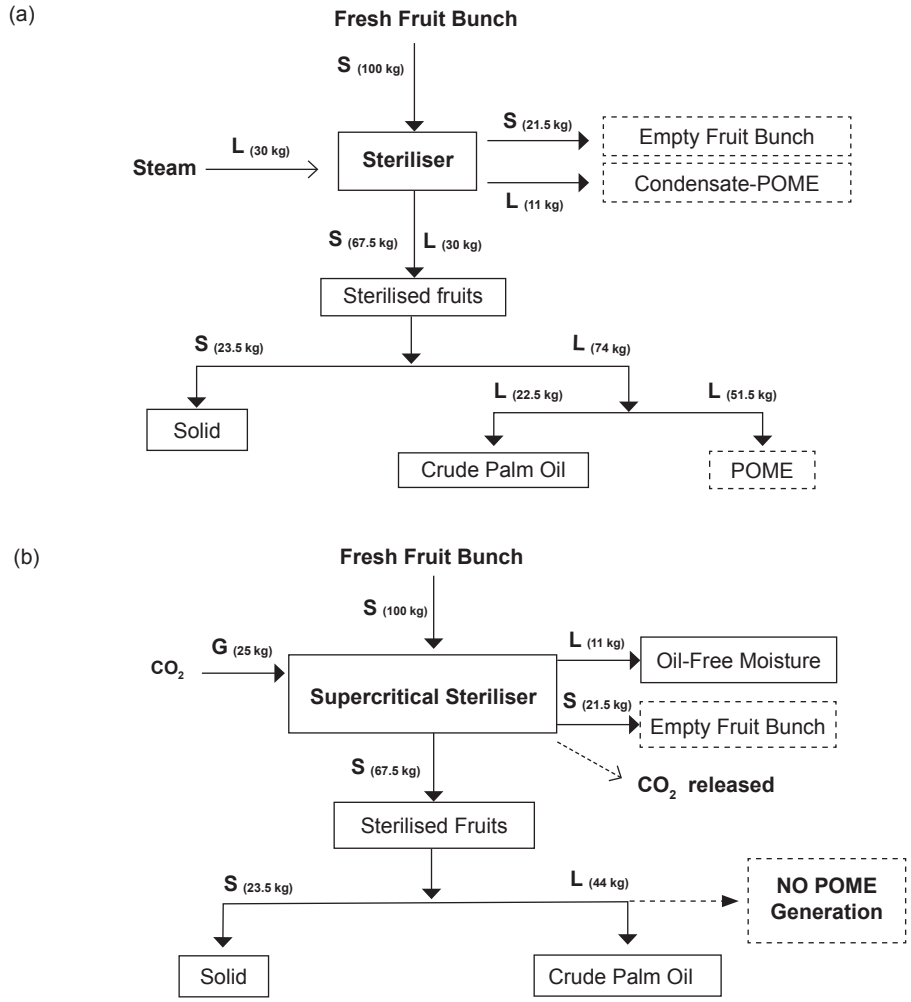


Figure 6. Scanning electron microscopy (SEM) images of treated and untreated oil palm fruit surface (a) untreated oil palm fruits, (b) steam autoclave treated oil palm fruits at 0.1 MPa, 121°C, 60 min and (c) SC-CO_2 treated oil palm fruits at 10 MPa, 80°C, 60 min.



Note: Main waste streams in bold, all percentages on wet FFB basis, S - Solid, L - Liquid.

Figure 7. Simplified process flow diagram of an oil palm mill using (a) steam sterilisation and (b) supercritical sterilisation.

but it was replaced by 25 kg of CO_2 of which 95% can be recycled and reused. More CPO is expected to be produced by using this technology with zero generation of POME. The SC-CO_2 is considered more efficient in preparing the fruits for the oil extraction process without generating any wastewater.

CONCLUSION AND RECOMMENDATION

Sterilisation of OP-FFB using SC-CO_2 technology revealed that the inactivation of *Bacillus* spp. and *Aspergillus* spp. in OP-FFB depends on pressure, temperature and treatment time. The log reduction of the both microorganisms increased with increasing pressure and temperature and treatment time. The treatment time required for the complete inactivation of *Bacillus* spp. and *Aspergillus* spp. were 75 min and 60 min, respectively, at 10 MPa, 80°C. With an increase of pressure and under the similar SC-CO_2 temperature (80°C) the treatment time further reduced to 60 min and 45 min at 30 MPa for the inactivation of *Bacillus* spp. and *Aspergillus* spp. in OP-FFB, respectively. The maximum activation energy (E_a) values were determined to be 10.2 kJ mol^{-1} and 12.1 kJ mol^{-1} for the inactivation of *Bacillus* spp. and *Aspergillus* spp., respectively, which is much lower than steam sterilisation process. The analyses of SEM images revealed minimal oil leaching from the SC-CO_2 treated oil palm fruit. Overall, it can be concluded that SC-CO_2 sterilisation technology has great potential to be utilised for OP-FFB sterilisation. The application of SC-CO_2 in OP-FFB sterilisation would benefit the palm oil industry in many ways including eliminating lipase producing microorganisms, minimise palm oil loss and no POME generation. Moreover, the non-chemical and environmental-friendly characteristics of this treatment give an extra value to this novel method.

ACKNOWLEDGEMENT

The authors would like to thank the Research and Innovation Division, Universiti Sains Malaysia for providing research university grant, No. 1001/RUI-PTEKIND/8011056 as a financial support.

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