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Characteristics in Water Phantom of Epithermal Neutron Beam Produced by Double Layer Beam Shaping Assembly

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ABSTRACT:

A double layer beam shaping assembly (DLBSA) was designed to produce epithermal neutrons for BNCT purposes. The Monte Carlo N-Particle extended program was used as the software to design the DLBSA and phantom. Distribution of epithermal neutron and gamma flux in the DLBSA and phantom and absorbed dose in the phantom were computed using the Particle and Heavy Ion Transport code System program. Testing results of epithermal neutron beam irradiation of the water phantom showed that epithermal neutrons were thermalized and penetrated the phantom up to a depth of 12 cm. The maximum value of the absorbed dose was $2 \times 10^{-3}$ Gy at a depth of 2 cm in the phantom.

KEYWORDS: Absorbed dose, DLBSA, Epithermal neutron, Gamma distribution, Water phantom.
1. INTRODUCTION

Boron neutron capture therapy (BNCT) is a method in cancer therapy that causes minimal damage to normal tissues. This method makes use of the ability of boron to capture the neutron beam, subsequently killing cancer cells (Sauerwein et al. 2012).

In BNCT, highly lethal energetic particles ($\alpha$, $^7$Li) produced after a breakup process of $^{11}$B nuclei when $^{10}$B atom captures a thermal neutron are used. The decay process of the boron–neutron reaction is shown in Equation (Moghaddasi and Bezak 2018):

\[ ^1n + ^{10}B \rightarrow ^{11}B + \gamma \]
\[ ^1n + ^{10}B \rightarrow ^7Li + ^4He + \gamma \]
\[ ^1n + ^{10}B \rightarrow ^7Li + ^4He \]

The products of this reaction have high linear energy transfer characteristics ($\alpha$ particle approximately 150 keV $\mu$m$^{-1}$, $^7$Li-nucleus approximately 175 keV $\mu$m$^{-1}$). The path lengths of these particles in tissues are in the range of 4.5–10 $\mu$m: hence resulting in an energy deposition limited to the diameter of a single cell.

Nuclear reactor neutron sources have long been used for BNCT, because high intensity neutron beams have been supplied only by reactors. However, many of these reactors have been shut down, reducing the number of reactors available for BNCT. Many reactors have been constructed only recently for BNCT. Conversely, accelerator-based neutron sources are becoming popular in neutron application fields (Kiyanagi 2018). Accelerators have several potential advantages related to safety, cost, and high neutron flux in the keV range, compared with reactor-based neutron sources for clinical radiotherapy (Peng et al. 2019).

One of accelerators used in BNCT comes from a cyclotron (Hashimoto et al. 2014). The resulting neutrons from the cyclotron are regulated so as to comply with the requirement set by the International Atomic Energy Agency (IAEA). Based on these criteria, the minimum beam intensity of the epithermal neutrons ($1 \text{ eV} < E < 10 \text{ keV}$) should be $5 \times 10^8$ n/cm$^2$.s, the number for fast neutron ($E > 10 \text{ keV}$) dose rate per epithermal neutrons flux and gamma dose rate per epithermal neutron flux should be less than $2 \times 10^{-13}$ Gy cm$^2$ and the minimum number for the ratio of the epithermal neutrons flux to the thermal neutrons ($E < 1 \text{ eV}$) flux should be 100 (Ganjeh and Eslami-Kalantari 2019). The part of the cyclotron that is capable of processing the neutron beam is a beam shaping assembly (BSA).

Until now, most efforts for cyclotron-based BNCT have been focused on the design of the BSA to investigate the feasibility of clinical neutron beams having the desired characteristics for patient irradiation. To achieve this, many types of BSA have been designed using Monte Carlo N-Particle Transport Code (MCNP) and the Particle and Heavy Ion Transport code System (PHITS) program (Pelowitz 2008; Sato et al. 2013). The design is expected to produce neutrons that meet the IAEA standard of quality.
BSA designs used in neutron sources typically consist of the moderator, filter, reflector, and collimator as their main components (Kasesaz et al. 2014). Each of the components is commonly designed with a single layer configuration, i.e. they only use one type of material. Such a single-layer configuration has a weakness in that the components of BSA are not maximal in processing neutron radiation beams; hence the result is normally not optimal. To overcome this weakness, double layer and even multilayer configurations have been developed. Such configurations are yet to be optimized, to obtain better radiation beams, and tested, to find if the characteristics of the resulting beams meet the requirements for BNCT (Monshizadeh et al. 2015; Tanaka et al. 2011; Kasesaz et al. 2013).

In principle there are two ways to find the quality of beams for BNCT, either by assessing their quality in the air or in a water phantom. Assessment of radiation beams in the air complies with the IAEA standard (International Atomic Energy Agency 2001). As for the assessment in a water phantom, the emphasis is on the ability of radiation beams to penetrate the phantom and the dose of neutrons sustained by a tumor (Ghal–Eh et al. 2017). A water phantom is typically chosen as the testing material because 70% of the human body consists of water (Tsukamoto et al. 2011). This article reports the characteristics of neutron and gamma distribution in a DLBSA and phantom, as well as the absorbed dose in a water phantom.

2. MATERIALS AND METHODS

The proton source was modeled as 30 MeV protons impinging on $^9$Be target with a diameter and thickness of 5 cm and 0.5 cm, respectively. High-energy neutrons are supposed to originate from $^9$Be(p,n) reactions (Hashimoto et al. 2014). They are subsequently processed using a double layer beam shaping assembly (DLBSA) to yield epithermal neutrons. The configuration of the intended DLBSA is shown in Figure 1, and a three-dimensional model is shown in Figure 2. The materials used as the moderator in the design of DLBSA were aluminum (Al) and LiF. The reason for the selection of Al was because of its high scattering cross-section. Aluminum has a high cross-section at energies above 10 keV (Zaidi et al. 2017). The materials used as reflectors were Pb and FeC, which have a high density and ability to scatter fast neutrons extremely well (Sato et al. 2013). Graphite (C) was also used as a reflector for its low cost. Apart from being cheap it also has a high scattering cross-section and low absorption, particularly at energies above 1 MeV (Türkmen et al. 2017).

The collimator component under consideration was made of Ni and borated polyethylene materials. Ni is considered to be a stable element when it interacts with neutrons. For a fast neutron filter, Fe was used. The effectiveness of Fe as a high energy neutron filter owes to its ability to in-elastically scatter high energy neutrons passing through it. Fe is deemed to be superior in filtering fast neutrons. The ability of Fe to filter fast neutrons derives from its resonant cross-section, which is above 10 above 10 keV (Asnal et al. 2015).
Thermal neutrons are filtered using a material with a high atomic number. Among the atoms with a high thermal neutron absorption cross-section is Cd. Cd is frequently used as a thermal neutron filter. A cross-section of 20,600 barn is reasonably effective to absorb thermal neutrons (Osawa et al. 2017; Asnal et al. 2015).

The material used for shielding was Pb. It has a relatively constant attenuation coefficient, i.e. 0.05 cm²/g to be able to absorb gamma rays with energies of 1-10 MeV (Türkmen et al. 2017). Epithermal neutrons leaving the DLBSA were subsequently imposed on the water phantom, shaped as a round ball, which was placed 1 cm at the front of the DLBSA. The composition of the water phantom in this study was 11.2% of H atoms and 88.8% of O atoms, with a density of 1000 kg/cm³ (Raaijmakers et al. 2000).

Monte Carlo simulation was carried out using the MCNPX and PHITS programs. The MCNPX program was used as the software to design the DLBSA and phantom (Pelowitz 2008). The distributions of epithermal neutron and gamma flux in the DLBSA and phantom and neutron absorbed dose in the phantom were computed using the PHITS program (Sato et al. 2013). The track and deposit tally were used in the PHITS calculation. To draw the particle track and visualization geometry of DLBSA, the ANGEL software was used. The transport was based on the cross-section data library JENDL-4.0 for neutrons and photons, and intra-nuclear cascade (INCL4.6) for protons.

3. RESULTS AND DISCUSSION
3.1 Characteristics of neutron and gamma beams in DLBSA and water phantom

Figure 3 shows the distribution of epithermal neutrons in the DLBSA. The fast neutrons mainly resulted from reactions of 30 MeV protons with 9Be target material through 9/4 Be \((p,n)\) 9/5B. Fast neutrons interacted with Al and LiF moderator, producing epithermal neutrons. The flux of epithermal neutrons around the moderator and filter reached \(10^{11} \text{n/cm}^2\text{s}\). The increase in the amount of epithermal neutrons was also sustained by the presence of Fe placed in front of the moderator filtering high energy neutrons. The effectiveness of Fe as a filter for high energy neutrons is due to its ability to inelastically scatter high energy neutrons passing through the Fe material (Asnal et al. 2015). The flux of epithermal neutrons computed at the end of the collimator exceeded \(10^9 \text{n/cm}^2\text{s}\). An epithermal neutron flux entering the phantom continually decreases in energy (as reflected in the change in color from yellow to blue in the phantom). The decrease in epithermal neutron flux is due to epithermal neutrons transforming into thermal neutrons during interactions with hydrogen atoms. This process is called thermalization (Mishima 1996). The neutron flux in the phantom decreased from \(10^9 \text{n/cm}^2\text{s}\) to \(10^6 \text{n/cm}^2\text{s}\).

![FIGURE 3. Distribution of epithermal neutron flux in DLBSA and water phantom.](image1)

![FIGURE 4. Distribution of gamma in DLBSA and water phantom.](image2)
Figure 4 shows the distribution of gamma particles in the DLBSA and water phantom. The gamma particle flux around the beryllium target was $10^{12}$ g/s.cm$^2$. The particles were dominantly produced from interactions of protons with a beryllium target through $^{9/4}$Be(p,a)$^{6/3}$Li. A small fraction of gamma rays was also generated from capture reactions through $^{9/4}$Be(p,g)$^{10/4}$Be reactions and inelastic collision mechanisms in the form of $^{9/4}$Be(n,n'g) reaction (Hu et al. 2016). Gamma particles were produced from the reaction of neutrons with aluminum through $^{27}$Al(n,g)$^{28}$Al reactions (Ma et al. 2015). Gamma particles enter the phantom and interact with H and O, losing their energy through mechanisms of photoelectric effect, Compton scattering, and pair production (Lamarsh and Baratta 2001). These interactions cause gamma ray flux to continually decline in the phantom.

### 3.2 Neutron absorbed dose in water phantom

Figure 5 shows the neutron absorbed dose in the water phantom, the maximum value of which was $2 \times 10^{-3}$ Gy, obtained at a depth of 2 cm from the surface of the phantom. The value is in accordance with the work of Tanaka et al. (2011) and Morcos and Naguib (2012). The deeper the epithermal neutron penetrates the phantom, the greater the increase in thermalization, causing the value of neutron flux to diminish. The decrease in neutron flux is caused by the thermalization of neutrons with H (Mishima 1996).

![FIGURE 5. Neutron absorbed dose characteristic in water phantom.](image)

Based on the neutron characteristics in the phantom, the neutron beams produced by the DLBSA can be considered as a neutron source for BNCT. The ability of epithermal neutrons in a phantom shows that the neutron beams from the DLBSA can be utilized as a neutron source for the treatment of cancerous tumors situated at 2-8 cm. Some of the types of cancers that can be treated using such a neutron source are head and neck cancer, glioblastoma, lung cancer, breast cancer, pancreatic cancer, brain tumors and sarcoma (Moss 2014; Mirzaei et al. 2016).

### 4. CONCLUSIONS

A double layer beam shaping assembly was designed to produce epithermal neutrons for BNCT purposes. The results of modeling of the DLBSA and phantom show that the distribution
of epithermal neutrons in the DLBSA and phantom continually decrease in neutron and gamma flux. The decrease in epithermal neutron flux and gamma is due to the thermalization process. Epithermal neutrons penetrated the phantom up to 12 cm in depth. Meanwhile, the maximum neutron absorbed dose in the phantom was found at a depth of 2 cm, with $2 \times 10^{-3}$ Gy in value. These results show that the neutron beams produced by the DLBSA are adequate as a neutron source for BNCT, particularly in the treatment of deep-seated tumors.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for its BPPDN Scholarship and Doctoral Dissertation Research Grant, as well as BATAN for its consent to the use of the MCNPX and PHITS code. We are also thankful to the late Prof. Dr. Kusminarto for his support in the conducting of this research.

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ABSTRACT:

The oral application of probiotics in the poultry industry is time-consuming and laborious. Therefore, using a powdered Probiotic supplement that can easily mix with feed is important. We investigated the effect of spray drying encapsulation on the viability of indigenous probiotic lactic acid bacteria during production and storage and evaluated broiler chicken performance after providing the supplement. Encapsulated probiotics exhibited >80% survival rates after spray drying. All bacterial species maintained up to 80% cell viability rates after exposure to 80–85°C temperatures for 15 or 30 s. The viable cell number of all encapsulated bacteria decreased over seven weeks of storage. The supplement was mixed with feed at concentrations 0.5 (T1), 1.0 (T2), and 1.5 (T3) g/kg feed and administered to 48 one-day-old Lohmann broiler chickens for 21 days; a T0 group was raised without Probiotic supplementation. Probiotic supplementation affected body weight gain, live weight, and feed conversion ratio. The cecum length and duodenum and cecum weights significantly differed among the treatment groups. Probiotic supplementation was associated with improved villus development in the intestinal epithelium compared with that of the control. Thus, feed supplementation with indigenous probiotic powder stimulates intestinal epithelial proliferation in broiler chickens during the starter phase, improving their performance.

KEYWORDS: Broiler performance, Microencapsulation, Poultry Probiotics.
1. INTRODUCTION

The addition of antibiotics in poultry feed has been banned in the European Union (Cogliani et al. 2011), because of concerns regarding increased antibiotic resistance among microorganisms and the accumulation of antibiotic residues in food. Probiotics are thus promoted as an alternative to antibiotics. Probiotics contain one or more strains of microorganisms, and when powdered, can be used as an animal feed supplement (Fuller 1992). Lactic acid bacteria (LAB) is the most commonly used probiotic agent in poultry production. A previous study showed that, when mixed with poultry feed, LAB replaced pathogens via competitive exclusion (Harimurti 2009; van Immerseel et al. 2009; Arsi et al. 2015; Schneitz et al. 2016). In another study that used probiotics as an efficient replacement of antibiotic growth promoters, an increase in intestinal length and liver and gizzard weights along with a decrease in abdominal fat was reported (Harimurti and Hadisaputro 2014). The oral supplementation of broiler chickens with LAB probiotics at dosages as high as 107, 108, and 109 colony-forming units (CFUs)/mL/bird/day increased the total LAB population in the gastrointestinal tract, as well as the villus height and width and crypt depth, thereby improving the performance of chickens (Harimurti et al. 2012, 2013). Thus, these observations show that LAB of poultry origin are ideal probiotics for broiler chickens. Effectiveness and efficiency are the most important factors in oral probiotic supplementation because individual supplementation on an industrial-farm scale would require a large amount of time and manpower.

Probiotics are beneficial microbes, mostly bacteria, that are administered in a prepared form or directly in alive form to poultry or other animals (Fuller 1992). Probiotic bacteria must remain viable to exert their functions (Harimurti and Hadisaputro 2015). Encapsulation protects the bacteria from external damaging factors (Kailasapathy 2002; Soukoulis et al. 2014), and thus maintains their viability and functional activities. In the context of producing probiotic feed supplements for the large-scale poultry industry, encapsulation is required to maintain cell viability during processing and storage. Spray drying is presently one of the most feasible and frequently used methods for bacterial cell encapsulation. This method is rapid, cost-effective, and reproducible. Additionally, the method facilitates size control and is suitable for industrial-scale applications (Burgain et al. 2011; Serna-Cock and Vallejo-Castillo 2013; Mutukumira et al. 2014; Soukoulis et al. 2014). In a previous in vitro study, encapsulated probiotics inhibited the growth of Salmonella enteritidis and Escherichia coli (unpublished data). Therefore, we elucidated the effects of feed supplementation with encapsulated probiotics on the performance of chickens.

2. MATERIALS AND METHODS

2.1. Bacterial strains and growth media

The following indigenous LAB strains were previously isolated from the gastrointestinal tracts of native Indonesian chickens: Lactobacillus murinus strain Ar-3, Streptococcus thermophilus strain Kp-2, and Pediococcus acidilactici strain Kd-6 (Harimurti et al. 2007). Maltodextrin with a dextrose equivalent of 10 and skim milk powder (SMP) were used as carriers during spray drying. Peptone glucose yeast broth (PGYB) and PGY agar (PGYA) were used for bacterial propagation and cell enumeration, respectively. Coconut water and bean
sprout extract, a bacterial enrichment medium, was prepared as follows: 450 g bean sprouts were chopped, mixed with 900 mL coconut water, sterilized at 121°C for 5 min, and filtered. Coconut water, sterilized at 121°C for 15 min, was added to the solution to a final volume of 1,800 ml

2.2 Probiotic starter preparation and biomass production

Each probiotic strain (L. murinus strain Ar-3, S. thermophiles strain Kp-2, and P. acidilactici strain Kd-6) was inoculated in 5 mL PGYB and incubated for 24 h at 37°C. The cells were then harvested and recultivated in 10 mL PGYB for an additional 24 h at 37°C. A subsequent culture was grown in 30 mL coconut water and bean sprout extract and incubated for 24 h at 37°C. Thereafter, 30-mL cell suspension was harvested, transferred into 1,800 mL coconut water and bean sprout extract, and incubated for another 18 h at 37°C. The final cell suspension was transferred into sterile 1-L centrifuge bottles and centrifuged at 1350 x g for 30 min at 4°C. After discarding the supernatant, sterile peptone-buffered saline was added to each pellet and centrifuged. Each harvested pellet was mixed with 10% (w/v) skim milk (SM) and 2% (w/v) sucrose (5:1, v/v ratio) and stored overnight at 4°C before encapsulation.

2.3 Bacterial cell encapsulation and powder evaluation

Maltodextrin and SMP solutions were prepared in distilled water (20%, w/v, each solution) by gentle stirring. The solutions were then sterilized at 121°C for 15 min and 115°C for 10 min, respectively. Subsequently, they were aseptically mixed together with bacterial pellets for 1 h at room temperature. Initial cell viability was counted before spray drying. The mixed solution, comprising cell biomass and carriers, was spray dried using a small-scale spray dryer with inlet and outlet temperatures of 180°C and 60°C, respectively. The spray-dried powder was collected in a sterile plastic clip and stored under aerobic conditions at room temperature (30°C) for 7 weeks. The collected powder was then subjected to cell viability, moisture content, and water activity analyses accompanied with a scanning electron microscopy (SEM) evaluation of cell shape and morphology. Cell viability was analyzed after preparation (day 0) and during storage. The powders were suspended and diluted in 0.85% NaCl solution and constantly mixed by shaking for 10 min at room temperature to ensure complete dissolution. The solutions were then serially diluted, plated on PGYA, and incubated for 48 h at 37°C under aerobic conditions. The observed colonies were counted, and the CFU/g was calculated. The viability of encapsulated probiotic bacteria was determined on days 0, 3, 7, 14, 21, 28, 35, 42, and 49 of storage and calculated according to Equation 1.

\[
\text{Viability(\%)} = 100 \times \frac{N_x}{N_0} \quad (1)
\]

where \(N_0\) is the cell viability after preparation (day 0) and \(N_x\) is the cell viability during storage (x denotes the number of days in storage). Furthermore, cell viability was evaluated after the encapsulated probiotics were exposed to temperatures between 80°C and 85°C for 15 or 30 s. Serially diluted samples (1 mL) were pour plated on PGYA before and after high-temperature exposure. The moisture content of the encapsulated probiotics was measured using a vacuum oven method (Soukoulis et al. 2014) by exposing the powders at 100°C for 4 h. The water
activity was measured at 25°C using a Pawkit water activity meter. The shape and morphology of the powders were observed using SEM, and images were recorded at an accelerating voltage of 10 kV.

2.4 Animal and feed formulation

This experiment was performed at the poultry research facility of the Faculty of Animal Science of Universitas Gadjah Mada, Indonesia, in compliance with the Guide for the Care and Use of Laboratory Animals. A total of 48 one-day-old chicks, vaccinated against Marek’s disease and infectious bursal disease, were used. The chicks were randomly categorized into four treatment groups of four chickens each: T0, T1, T2, and T3. The T0 group was raised without probiotics, whereas the T1, T2, and T3 groups were provided feed supplemented with probiotics at concentrations of 0.5, 1.0, and 1.5 g/kg, respectively. All treatments were performed in triplicates for 21 d. The broiler chicken diet, mainly comprising corn and soybean meal without antibiotics, was formulated to meet the National Research Council’s (National Research Council 1994) recommendations. This diet contained 22.29% crude protein and 3,074.90 kcal/kg metabolizable energy. In addition, it contained 1.15% (w/w) calcium, 0.63% (w/w) available phosphorus, 0.55% (w/w) methionine, 1.23% (w/w) lysine HCl, and 0.24% (w/w) tryptophan. Feed and drinking water were provided ad libitum. The growth performance of chickens was evaluated by recording the weekly feed consumption, body weight gain, live weight, and feed conversion ratio.

2.5 Sample collection

At the end of the starter phase, three birds from each treatment group were randomly euthanized and sacrificed by severing the jugular vein. The intestines were immediately harvested, and the length and weight of the intestinal tract (duodenum, jejunum, ileum, and cecum) were measured. One bird from each treatment group was randomly sacrificed at the third week of age for histological analysis of the intestinal structure. Small sections (2 cm) of the duodenum, jejunum, and ileum were excised and immediately washed with 0.85% NaCl solution. Each sample was placed in 10% formalin buffer and fixed for 48 h before histological processing.

2.6 Histology

The tissue samples were dehydrated using a series of increasing alcohol concentrations (30%, 50%, 70%, 80%, 90%, 95%, and 100%), and embedded in paraffin wax. A microtome was used to obtain 5-μm sections, which were placed on glass slides for hematoxylin and eosin staining. The histological sections were observed using a light microscope.

2.7 Statistical analysis

Data were analyzed using one-way analysis of variance, followed by Duncan’s new multiple range test, and viability data collected before and after spray drying were analyzed using t-test. Differences were considered to be significant at $p < 0.05$.

3. RESULTS
3.1 Cell viability

Before spray drying, cell viability was $10^8$–$10^{10}$ CFU/g. Log reductions of one- to two-fold were observed after spray drying (Table 1), suggesting that the percent viability of all samples exceeded 80%. The total cell viability of all encapsulated bacterial species weekly decreased during storage (Figure 1). A combination of three and one species (P. acidilactici strain Kd-6) exhibited viability rates of up to 70% during the first four weeks of storage, followed by decreases after seven weeks. After four weeks of storage, S. thermophilus strain Kp-2 and L. murinus strain Ar-3 had viabilities of 63% and 40%, respectively. Moreover, the viability of both strains decreased further after seven weeks. The decrease in bacterial cell viability during feed processing is likely caused by heat treatments. Accordingly, encapsulation should protect bacterial cells and promote survival. In the present study, probiotic cells were encapsulated using a mixture of maltodextrin and SMP and collected as described in the Materials and Methods section. The survival ability of encapsulated cells was then measured in response to exposure to temperatures of 80–85°C. All strains, except L. murinus strain Ar-3, exhibited post exposure viability rates of greater than 80% (Table 2). S. thermophilus strain Kp-2 and P. acidilactici strain Kd-6 could maintain the viability rates as high as 80% after exposure to temperatures of 80–85°C for 30 s. Notably, there were no differences ($p > 0.05$) in the survival rate among four samples exposed to high temperatures for 45 s (data not shown). Thus, a combination of maltodextrin and SMP yielded good cell protection against heat exposure for 15 or 30 s (Table 2). After 15 s, P. acidilactici strain Kd-6 was most persistent compared with L. murinus strain Ar-3 and S. thermophilus strain Kp-2. After 30 s, S. Thermophilus strain Kp-2 and P. Acidilactici strain Kd-6 had higher survival rates than that observed in L. Murinus strain Ar-3.

3.2 Moisture content, water activity, and SEM of the encapsulated probiotics

The samples significantly differed with respect to moisture content and water activity (Table 3). The highest moisture content among the encapsulated probiotics (3.99%) was recorded for P. acidilactici strain Kd-6, whereas the lowest (3.60%) was reported for the combined species. The powder containing P. acidilactici strain Kd-6 had the highest water activity (0.20), whereas that containing S. thermophilus strain Kp-2 had the lowest value at 0.17. The high water activity of P. acidilactici strain Kd-6 was in line with the high moisture content. The morphologies of the encapsulated probiotics of spray-dried powders visualized using SEM are shown in Figure 2. These particles had irregular morphological shapes, including deflated, flat, ball-like, and spherical, and their sizes ranged from 2.56 to 27.00 μm.

3.3 Performance of chickens

Based on the data presented in Tables 1–3 and Figure 1, the combined probiotic culture was selected to analyze the effect of probiotic feed supplementation on the performance of chickens. Although probiotic powder supplementation did not affect feed consumption between the treatments groups, it led to significant increases in body weight gain, live weight, and feed conversion ratio (Table 4). Regarding the intestinal segments (e.g., duodenum, jejunum, and ileum), only the cecum length significantly different among the treatment groups, and the
weights of the jejunum and cecum were significantly different (Table 5). The analysis of intestinal histology indicated a greater villus density in the treatment groups than that in the control group, even at the lowest level of supplementation (0.5 g/kg feed; Figure 3). The ileum clearly contained shorter villi in the control group than that in the treatment groups.

| TABLE 1. Viabilities of encapsulated probiotics before and after spray drying (log CFU/g). |
|-----------------------------------------------|
| Time                          | log CFU/g |
| L. murinus\textsuperscript{a} | 9.53 ± 0.21 |
| S. thermophilus\textsuperscript{a} | 9.30 ± 0.30 |
| P. acidilactici\textsuperscript{b} | 8.52 ± 0.45 |
| Mixture\textsuperscript{a}     | 10.10 ± 0.17 |
| Before                        | 9.00 ± 0.00 |
| After                         | 8.42 ± 0.10 |
|                               | 8.00 ± 0.00 |
|                               | 9.10 ± 0.17 |

\textsuperscript{a}Significant (p < 0.05), \textsuperscript{b}Nonsignificant.

| TABLE 2. Viability percentages of encapsulated probiotics after 15 or 30 s exposure to 80–85°C (%). |
|-----------------------------------------------|
| Time                          | L. murinus  | S. thermophilus | P. acidilactici | Mixture |
| 15 s                          | 92.78\textsuperscript{a,y} ± 3.02 | 87.31\textsuperscript{x} ± 4.56 | 98.43\textsuperscript{a,x} ± 0.74 | 97.13\textsuperscript{a,y} ± 0.87 |
| 30 s                          | 77.92\textsuperscript{b,y} ± 1.76 | 85.05\textsuperscript{xy} ± 0.28 | 81.81\textsuperscript{b,y} ± 4.71 | 87.05\textsuperscript{c,x} ± 0.53 |

\textsuperscript{xy,z}Mean values with different superscripts in the same row differ significantly (p < 0.05), \textsuperscript{a,b,c}Mean values with different superscripts in the same column differ significantly (p< 0.05).

| TABLE 3. Moisture content and water activity of encapsulated probiotics. |
|---------------------------------------------------------------|
| Probiotic                    | Moisture content (%) | Water activity   |
| L. murinus                  | 3.77\textsuperscript{c} ± 0.05 | 0.19\textsuperscript{a} ± 0.01 |
| S. thermophiles             | 3.80\textsuperscript{bc} ± 0.02 | 0.17\textsuperscript{c} ± 0.00 |
| P. acidilactici             | 3.99\textsuperscript{ab} ± 0.13 | 0.20\textsuperscript{a} ± 0.01 |
| Combined species            | 3.60\textsuperscript{c} ± 0.12 | 0.19\textsuperscript{a} ± 0.00 |
| Control (without probiotic) | 4.10\textsuperscript{d} ± 0.15 | 0.18\textsuperscript{b} ± 0.00 |

Control: a mixture of maltodextrin and SMP without probiotic addition. \textsuperscript{a,b,c}Mean values with different superscripts in the same column differ significantly (p < 0.05).
4. DISCUSSION

SMP contains lactose and proteins, which minimize the cellular membrane damage during spray drying. According to the water replacement theory, lactose and milk proteins protect cells by interacting with the polar head groups of membrane phospholipids (Crowe et al. 1998; van Oss 2008; Soukoulis et al. 2014). A previous study reported that samples prepared with a mixture of maltodextrin/SMP (1:1) had high viable cell count (8 log CFU/g) after four weeks of storage at 30°C (Mutukumira et al. 2014), including temperature, moisture content, and oxidative stress (Abd-Talib et al. 2013). Because the encapsulated probiotics in the present study were stored at 30°C and under aerobic conditions, exposure to oxygen might have caused oxidative stress in the cells, triggering cell death and a decrease in viability.

Feed manufacturers commonly use heat and moisture to produce dry meal. In Indonesia, feed mills routinely operate at 85–88°C to generate dry feed (Muttaqin 2005). In the present study, viability rates of greater than 80% were noted after exposure to temperatures of 80–85°C in spraydried probiotics, except for L. murinus strain Ar-3. This finding agrees with a previous study, wherein P. acidilactici strain Kd-6 and S. thermophilus strain Kp-2 were thermotolerant under drought and high-temperature (up to 50°C) conditions (Abd-Talib et al. 2013). Another study reported viability rates of greater than 90% for Bacillus subtilis, a heat resistant, spore-forming bacterial strain, exposed to temperatures of 85–90°C (Setlow 2006).

The moisture content data met the acceptable limit of greater than 4% for a dried product (Gharsallaoui et al. 2007). The water activity is a measure of water molecules available in a “free” form. Bound water is the water molecules involved in hydrating hydrophilic molecules and dissolving solutes; it does not contribute to the water activity (Setlow 2006). A previous study found that the three tested strains had different cell surface hydrophobicities: L. murinus strain Ar-3 had a 93.56% hydrophilic cell surface, whereas S. thermophilus strain Kp-2 and P.
acidilactici strain Kd-6 had values of 96.61% and 96.28%, respectively (Harimurti 2011). This result supports the high moisture content observed in S. thermophilus strain Kp-2 and P. acidilactici strain Kd-6 in the present study.

A study on microcapsule powder reported a similar size range of 1–25 μm (Mutukumira et al. 2014). The strong ability of the maltodextrin/SM matrix to preserve cell viability (Table 2) might be attributable to its good encapsulating properties (Figure 2). Maltodextrin alone exhibited poor interfacial properties and would require chemical modification to improve the surface activity. Conversely, SMP is amphiphilic and exhibits the physicochemical and functional properties required to encapsulate materials with hydrophobic cores. Moreover, protein compounds, such as sodium caseinate, soy protein isolate, and whey protein concentrate and isolate, may exhibit good microencapsulating properties (Soukoulis et al. 2014).

In the present study, probiotic powder supplementation led to a significant increase in body weight gain, live weight, and feed conversion ratio, although it did not affect the feed consumption between the treatment groups. This finding is in agreement with that of previous studies (Harimurti et al. 2012; Amerah et al. 2013; Harimurti and Hadisaputro 2014). The complex metabolites produced by complementary probiotic strains may act synergistically to support health (Ray 1996). A study reported that oral probiotic supplementation increased the concentration of short-chain fatty acids (e.g., propionic acid and butyric acid) in broiler chickens (Harimurti and Hadisaputro 2014). Although LAB do not directly produce butyric acid, their consumption indirectly leads to increased butyric acid concentration in the digestive tract, a process known as crossfeeding. In vitro, lactic acid produced by Bifidobacterium adolescentis is used by Eubacterium halii and Anaerostipes caccae (in coculture) to produce large amounts of butyric acid (Anuradha and Rajeshwari 2005), which is required by the intestinal epithelial cells to stimulate the proliferation of normal epithelium and maintain mucosal barrier defenses (Harimurti et al. 2012). This absorptive function by the ratio of villi height to crypt depth in the small intestine.
### TABLE 4. Effects of supplementation with microencapsulated indigenous probiotic lactic acid bacteria on performance parameters.

| Criteria                  | T0 (control) | T1 (0.5 g probiotics/kg feed) | T2 (1.0 g probiotics/kg feed) | T3 (1.5 g probiotics/kg feed) |
|---------------------------|--------------|-------------------------------|-------------------------------|-------------------------------|
| **Feed consumption, g/bird** |              |                               |                               |                               |
| Week 1                    | 108.71 ± 4.66| 112.91 ± 2.48                 | 107.81 ± 1.31                 | 110.70 ± 1.57                 |
| Week 2                    | 230.27 ± 21.57| 215.70 ± 5.90                | 220.77 ± 12.72                | 240.26 ± 5.52                 |
| Week 3                    | 238.60 ± 30.41| 207.65 ± 12.68               | 242.27 ± 6.65                 | 235.98 ± 28.52                |
| Starter phase             | 577.59 ± 54.46| 536.16 ± 19.11               | 576.85 ± 17.44                | 586.94 ± 33.20                |
| **Body weight gain (g/bird)** |              |                               |                               |                               |
| Week 1                    | 76.83² ± 5.57| 96.17² ± 5.44                 | 90.08² ± 4.50                 | 92.75³ ± 3.53                 |
| Week 2                    | 147.69² ± 15.14| 174.83³ ± 9.77               | 175.25³ ± 4.98                | 186.67³ ± 15.18               |
| Week 3                    | 101.42³ ± 3.36| 111.00³C ± 22.66             | 148.42³c ± 8.31               | 172.33³ ± 40.84               |
| Starter phase             | 324.00º ± 21.69| 364.75º ± 34.95             | 402.50º ± 4.27                | 400.17º ± 37.50               |
| **Live weight (g/bird)**  |              |                               |                               |                               |
| Week 1                    | 119.58⁎² ± 6.64| 140.08² ± 5.57               | 133.00⁰² ± 5.11               | 137.17² ± 5.15                |
| Week 2                    | 275.75⁰ ± 17.18| 312.00⁰ ± 15.16              | 306.50⁎ ± 6.34                | 317.17³ ± 12.25               |
| Week 3                    | 366.75⁰ ± 23.01| 408.67⁰ ± 35.50              | 445.42³ ± 5.95                | 444.58³ ± 35.25               |
| Starter phase             | 366.75⁰ ± 23.01| 408.67⁰ ± 35.50              | 445.42³ ± 5.95                | 444.58³ ± 35.25               |
| **Feed conversion ratio (g/g)** |            |                               |                               |                               |
| Week 1                    | 1.5³ ± 0.09  | 1.3³ ± 0.08                   | 1.2⁰ ± 0.08                   | 1.2⁰ ± 0.03                   |
| Week 2                    | 1.5⁰ ± 0.09  | 1.2⁰ ± 0.04                   | 1.3⁰ ± 0.05                   | 1.3⁰ ± 0.02                   |
| Week 3                    | 2.5⁰ ± 0.45  | 2.0⁰ ± 0.16                   | 1.8⁰ ± 0.13                   | 1.9⁰ ± 0.25                   |
| Starter phase             | 1.8⁰ ± 0.07  | 1.4⁰ ± 0.08                   | 1.4⁰ ± 0.05                   | 1.4³ ± 0.07                   |

a,b,c Mean values with different superscripts in the same row differ significantly (p < 0.05).

### TABLE 5. Effect of supplemented microencapsulated indigenous probiotic lactic acid bacteria on intestinal length (cm) and weight (g).

| Treatment   | Duodenum | Jejunum | Ileum | Cecum |
|-------------|----------|---------|-------|-------|
|             | Length10 | Weight10| Length | Weight | Length10 | Weight10 | Length | Weight |
| T0 (control)| 18.50 ± 1.32 | 2.27 ± 0.43 | 41.33 ± 1.04 | 4.10b ± 0.53 | 40.67 ± 3.78 | 2.95 ± 0.45 | 10.45b ± 0.64 | 1.30b ± 0.16 |
| T1 (0.5 g/kg feed)| 18.77 ± 2.58 | 2.09 ± 0.49 | 45.80 ± 4.70 | 4.07b ± 0.80 | 43.43 ± 0.51 | 2.59 ± 0.47 | 9.22b ± 1.09 | 1.12b ± 0.24 |
| T2 (1.0 g/kg feed)| 17.17 ± 0.76 | 2.46 ± 0.29 | 46.00 ± 2.00 | 5.16b ± 0.84 | 43.63 ± 2.90 | 3.40 ± 0.50 | 9.72b ± 0.60 | 1.49b ± 0.03 |
| T3 (1.5 g/kg feed)| 20.67 ± 1.04 | 2.74 ± 0.54 | 46.73 ± 5.37 | 5.65b ± 0.42 | 43.27 ± 3.16 | 4.07 ± 0.85 | 11.93² ± 1.41 | 1.88b ± 0.41 |

a,b Mean values with different superscripts in the same column differ significantly (p < 0.05).

10 Nonsignificant.
5. CONCLUSIONS

In conclusion, the tested maltodextrin/SMP mixture exhibited good encapsulating properties that protected probiotic cells during heat treatment and maintained cell viability during storage. Supplementation with the encapsulated probiotics improved chickens’ live weights, body weight gain, and feed conversion ratios as well as promoted better villus development, which could increase nutrient absorption. Therefore, encapsulated probiotic supplementation could be used as an alternative chicken feed supplement to improve the growth performance.
FIGURE 3. Histologic analysis of duodenal (a–d), jejunal (e–h), and ileal tissues (i–l) from broiler chickens during the starter phase. Scale bars, 20 μm.

ACKNOWLEDGMENTS

The authors acknowledge all the poultry laboratory assistants of the Faculty of Animal Science, Universitas Gadjah Mada, for their roles in study execution. This study was part of Ms. Monica Sonia Indri Pradipta’s thesis work at the Faculty of Animal Science, Universitas Gadjah Mada.

COMPETING INTERESTS

One of the authors, Widodo, also serves as the Editor-in-Chief of AJSTD. To avoid potential conflicts of interest, they were recused from the review and final decision processes.

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Smart Animal Health Monitoring System

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ABSTRACT:

In present scenario, due to the dependency of human life on animals, it is mandatory to monitor animal health on regular basis. Also, regular health check-ups of an animal costs too much which some people will not be able to afford. In addition to it, if the health of the animal is not taken care properly and diagnosed in time, it can be life threatening to animals. Hence, we have proposed and developed a prototype of smart animal health monitoring system which can continuously monitor physiological parameters such as body temperature, environmental humidity, heart rate and position of the animal using LM35, DHT11, Heart rate sensor and accelerometer respectively. These sensors are mounted on animal body and data is obtained and displayed with the help of Wi-Fi module ESP8266 through IoT and LCD display. This system will contribute in the novelty and viability of health care of animals.

KEYWORDS: IoT, Wi-Fi module ESP8266, LM35, DHT11, Heart rate sensor, Accelerometer etc.
1. INTRODUCTION

With rise in population, the requirements of the individuals have increased. Several farmers depend on cattle for milk production. Similarly, the pet adopters and trainers depend on emotional state of the pet for human-canine interaction to achieve effective pet training. Since the animals cannot convey their emotions to the owners, it is the responsibility of every pet adopter or a farmer to monitor the physiological and behavioral parameters of the animals. This paper presents the prototype of the smart animal health monitoring system to continuously monitor the physiological parameters such as body temperature, heart rate, humidity and movements of the animals with the help of sensors. This system is cost effective and can be used by the farmers as well as canine adopters at home.

This system is helpful in detecting the signs and symptoms of illness at early stages and isolating the animal from the herd to prevent spreading of the disease. Also, in canines the disease can be detected at an early stage and can be diagnosed. In order to monitor the physiological parameters, sensors such as temperature sensor, humidity sensor and heart rate sensor has been used. In case if the animal goes out of sight, accelerometer is used to find the position and the movement of the animal. These sensors and accelerometer are interfaced with Arduino UNO and the data is obtained. Since these data has to be sent to graphical user interface (GUI), we use wi-fi module to transmit the data with the help of internet. LCD display is also used to display the data of each sensor.

2. LITERATURE SURVEY

![Block diagram of smart animal health monitoring system](image-url)
In this paper [1] the continuous health surveillance system for cattle has been developed to continuously monitor the animal health and to detect the abnormalities by studying the feeding time of the cattle. This system consists of RFID tags, RFID reader and sink node. The RFID tags will be activated and date & time stamp will be displayed when the animal is involved in feeding routine. These data are compared with the database to detect the abnormalities or illness in the cattle.

[2] Prathamesh Khatate, Anagha Savkar and Dr.C.Y. Patil have focused on monitoring various parameters like temperature, respiration, blood pressure, heart rate and cardiac activity using sensors. Temperature sensors DS18B20 is used since it has a wide temperature range. Similarly, the respiratory sensor module is wrapped around animal’s chest. The estimation is obtained by the contraction and relaxation of the lungs. Blood pressure is detected by oscillometric method by obtaining systolic and diastolic pressure. In order to detect the cardiac activity and heart rate the ECG module is used. With the help of Ag-AgCl electrodes the electrical activity of the heart is monitored.

In this paper [3] Athira Vijayan and Manju Suresh have developed wearable sensors for animal health monitoring using ZigBee. This system is used to monitor physiological parameters like temperature, heart rate and posture. These data are fed to micro-controller and transferred to the user end through ZigBee transceiver. The ZigBee transceiver on the other end receives the transmitted data and displays on graphical user interface (GUI). This proposed work is the combination of ZigBee wireless communication technology and embedded technology. This system monitors animal health as well as tracks the movement of animals through GPS. The physiological parameters are measured using the sensors and transmitted through ZigBee transceiver and displayed on the user end device [4].

According to the research [5], Anuj Kumar and Gerhard P. Hancke have focused on the animal health monitoring system which gives the thought to find various parameters concerning animal health. It is easy to detect various health issues related to temperature, rumination, vital sign, humidness & surrounding environmental temperature with the help of this system. To meet society’s concern some milk producers, offer financial help to dairy farmers if they let their cows for daily grazing, but this is difficult for many farmers due to cattle management and control problems.

In this paper [6] the cattle health and environment monitoring system was developed and the main aim of this system is to monitor both environmental parameters and animal health parameters. Various parameters such as heart rate, movement of animals, body temperature, environmental humidness and temperature were monitored with the help of Internet of Things (IoT). Arduino UNO is the basic micro-controller used and various parameters were found and recorded. Alert was sent through GSM module if there were any undesired variations in the parameters. Arduino UNO is interfaced to ESP8266 Wi-Fi module so that the data from the micro-controller is dumped to the cloud and can be accessed using internet. Using Things Speak widget the results were viewed on the user end systems. The livestock are being affected by various issues related to health. These diseases can be detected.

[7] with the usage of cost-efficient, non-invasive, sensor technology. These diseases can be depicted to specific characteristic of animal behavior that have been mapped to the sensors which is necessary to find the health issues. This aid the farmers to observe the activity of cattle.
and detect whether the animal is affected by any disease if there are any variations in the data obtained by sensors. This research paper [8] is based on the animal health and tracking system using IoT. This system monitors the physical as well as movement of animals. The physiological parameters include body temperature, heart rate and respiration rate with the help of sensors mounted on a single module. The future work of this system lies in the application of hardware devices which can be worn by animals and can be connected to any user end devices through Internet of Things (IOT).

3. PROPOSED WORK

In this project we continuously monitor the health of the animal by measuring the physical parameters such as heart rate, temperature, humidity and locomotion of the animal. The sensors are used to gather the information from the animal and the collected data is fed to Arduino UNO. Since the temperature and heart rate are two major parameters that depend on the health condition of the animal; any variation in these two parameters are the reason for the animal to be affected by any kind of diseases. The environmental condition also plays a major role in animal health, so we use humidity sensor DHT11 to measure the environmental temperature and humidity. Also, these parameters affect the locomotion of the animal. Hence, we use accelerometer to track the position of the animal. These data are collected from the sensors and is fed to the Arduino board. The data from Arduino UNO is then transferred to Wi-Fi module and through the internet the data is displayed on the GUI. This proposed system can be used for both cattle and canine. This system is cost effective and diseases can be detected and diagnosed immediately.

4. HARDWARE DESCRIPTION

4.1 Temperature Sensor

Temperature sensor LM35 is used to measure the temperature changes in the animal. The body temperature range of canine ranges from 37.9 to 39.9 degree Celsius and the body temperature of cattle ranges from 35.6 to 38.6 degree Celsius. If the body temperature of the animal exceeds the given range, it denotes abnormality. LM35 consists of 3 pins: Vcc (+5V),
Output, GND. These pins are interfaced with the Arduino UNO and sent to the GUI through Wi-Fi Module.

4.2 Humidity Sensor

Humidity Sensor DHT11 is used to measure the environmental humidity which plays a major role in animal health. If the humidity increases or decrease beyond the limited range, the metabolism in the animal will be affected, which in-turn causes illness.

1.3 Accelerometer

Accelerometer is used to detect the position of animals. It has three axis: X axis, Y axis and Z axis. X axis determines the forward and backward motion of the animal, Y axis determines the leftward and rightward motion of the animal whereas Z axis determines the movement in space. In this system we use only X axis and Y axis.

4.4 Heart rate Sensor

The Heart rate sensor is used to record the number of heart beats per minute in the animals. In cattle the approximate heart rate ranges from 48-84 beats per minute whereas in canine it ranges from 70-120 beats per minute. Some factors such as anxiety and stress causes variation in the heart rate. If the heart rate exceeds the range, then the animal is said to have an abnormality or in some conditions it may also have cardiovascular issues.

4.5 Wi-Fi module

Wi-Fi stands for Wireless Fidelity. Wi-Fi module here is used for the wireless communication between the Arduino and the GUI with the help of internet. ESP8266 is the module used in this system. The data obtained by the sensors is transferred to web by ESP8266 with the help of internet. Through IoT the transferred data is obtained on the GUI for the farmers and canine adopters to monitor animal health.

| Animal | Heart rate (Bpm) | Body Temperature (Celsius) |
|--------|-----------------|--------------------------|
| Cattle | 48-84           | 37-39                    |
| Dog    | 60-140          | 37.6-39.6                |

Table 1: Normal body temperature and heart rate data
5. CONCLUSION

To determine various animal health related problems, the prototype of smart animal health monitoring system was developed which continuously monitor the parameters such as body temperature, heart rate, humidity and movements of the animals. This system is cost effective and consumes very less power. Hence, it can be used efficiently. Since IoT is used in this system the data can also be obtained on the smart phones of the owners.

6. FUTURE WORK

The future of this project lies in adding extra features like GPS and shock generator i.e. a slight shock will be generated when the animal tries to cross the gate of the house or cattle shed and also the system will be programmed in such a way that it automatically calculates and indicates the Thermal Humidity Index (THI).

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Analysis of Radiation Interactions and Biological Effects for Boron Neutron Capture Therapy

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ABSTRACT:

The direct and indirect ionizing radiation sources for boron neutron capture therapy (BNCT) are identified. The mechanisms of physical, chemical and biological radiation interactions for BNCT are systematically described and analyzed. The relationship between the effect of biological radiation and radiation dose are illustrated and analyzed for BNCT. If the DNAs in chromosomes are damaged by ionizing radiations, the instructions that control the cell function and reproduction are also damaged. This radiation damage may be reparable, irreparable, or incorrectly repaired. The irreparable damage can result in cell death at next mitosis while incorrectly repaired damage can result in mutation. Cell death leads to variable degrees of tissue dysfunction, which can affect the whole organism’s functions. Cancer cells cannot live without oxygen and nutrients via the blood supply. A cancer tumor can be shrunk by damaging angiogenic factors and/or capillaries via ionizing radiations to decrease blood supply into the cancer tumor. The collisions between ionizing radiations and the target nuclei and the absorption of the ultraviolet, visible light, infrared and microwaves from bremsstrahlung in the tumor can heat up and damage cancer cells and function as thermotherapy. The cancer cells are more chemically and biologically sensitive at the BNCT-induced higher temperatures since free-radical-induced chemical reactions are more random and vigorous at higher temperatures after irradiation, and consequently them cancer cells are harder to divide or even survive due to more cell DNA damage. BNCT is demonstrated via a recent clinical trial that it is quite effective in treating recurrent nasopharyngeal cancer.

KEYWORDS: Broiler performance, Microencapsulation, Poultry Probiotics.
1. INTRODUCTION

Radiation therapy is a local ionizing-radiation treatment that severely damages cancer cells with as low harm as possible to adjacent normal tissue cells. Cancer cells usually grow and divide much faster than normal tissue cells and are much more sensitive to ionizing radiations. In other words cancer cells are much more susceptible to damage than adjacent normal tissue cells under ionizing-radiation.

High-energy X-ray, g-ray and electron beams have been employed for conventional radiation therapy. This type of radiations have low linear energy transfer (LET) since their energy depositions in tissue as direct radiation interactions (displacements, ionizations, bond-breaks and free-radical formations) and associated indirect interactions (free-radical-induced subsequent chemical reactions and biological responses) are significantly lower than the energy depositions in tissue of high-energy heavier particles such as proton, alpha particle and neutron. Therefore, the radiation absorbed dose and associated biological response, which is characterized by its relative biological effectiveness (RBE), to ionizing radiations depend on the type of radiations.

Boron neutron capture therapy (BNCT) is a therapeutic technique that makes use of energetic alpha particles and back-to-back recoiled $^7$Li ions from boron ($^{10}$B) neutron capture reactions to treat patients with cancer. Both alpha particles and $^7$Li ions have a high linear energy transfer and a combined range in tissue of 12–13 μm (comparable with cellular dimensions) and total kinetic energy of 2.33 MeV. BNCT has been used to treat locally invasive malignant tumors such as primary brain tumors and recurrent head and neck cancer.

The BNCT procedure is normally composed of two-steps: 1) injection via an intravenous process with a tumor localizing drug containing 10B that has a high neutron capture cross section to absorb slow neutrons; and 2) irradiation with epithermal and/or thermal neutrons from either a nuclear reactor or an accelerator. After penetrating through the outer tissue, the neutrons are captured by the $^{10}$B agents in the cancer tumor and then the $^{10}$B neutron-capture nuclear reactions subsequently generate high-energy alpha particles and recoiled $^7$Li ions to damage cancer cells.

2. DIRECT AND INDIRECT IONIZING RADIATION SOURCES

BNCT utilizes energetic alpha particles and recoiled $^7$Li ions from boron ($^{10}$B) neutron capture reactions for the treatment of cancer patients. Therefore, the direct ionizing radiation source to the boron-treated cancer tumor is a neutron beam.

Two kinds of neutron beams are commonly used in BNCT: 1) a thermal beam for treating surface and shallow cancer tumors; and 2) an epithermal beam for treating deeper (e.g., 8 to 10 cm) cancer tumors. Both kinds of beams include contributions from fast, epithermal and thermal neutrons, as well as gamma rays from the neutron source and from the capture and scattering of neutrons in the beam line structures. Consequently, the direct radiation source includes both neutrons and gamma rays in the neutron beam.

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thermal neutrons, as well as gamma rays from the neutron source and from the capture and scattering of neutrons in the beam line structures. Consequently, the direct radiation source includes both neutrons and gamma rays in the neutron beam. During neutron penetration through a boron-treated cancer tumor, the following indirect ionizing radiation sources are generated:

1. Alpha particles and recoiled 7Li ions from boron (10B) neutron capture reactions;
2. Protons from nitrogen neutron capture reactions;
3. Gamma rays from hydrogen neutron capture reactions;
4. Recoiled protons and other recoiled target nuclei from fast neutron collisions;
5. Compton electrons and photoelectrons from gamma ray Compton scattering and photoelectric effect;
6. Electron-positron pair production by the incident gamma ray energy than 1.02 MeV;
7. Bremsstrahlung from deceleration of charged particles;
8. Secondary electrons from charged-particles and target nuclei Coulomb interactions.

3. MECHANISM OF PHYSICAL AND CHEMICAL RADIATION INTERACTIONS

Radiation physical interactions occur very rapidly (up to $10^{-15}$ sec) and the associated chemical reactions occur less rapidly (up to $10^{-6}$ sec), whereas the subsequent biological responses occur rather slowly (in days up to months). The direct ionizing radiation source to boron-treated cancer tumor is neutron beam. Neutrons have no charge and thus are indirectly ionizing. Neutrons can be divided into three energy groups: fast (energy above 5.53 keV), epithermal (energy between 5.53 keV and 0.6825 eV), and thermal (energy below 0.6825 eV) groups. Fast neutrons bombardment can cause displacements and recoils of nuclei in tissue cells during the slowing down process. Epithermal and thermal neutrons are easily captured by odd-neutron-number nuclides such as 5B10. The range of a neutron depends on its kinetic energy; therefore, a thermal neutron beam is used for surface and shallow cancer treatment and an epithermal beam is used for deeper cancer treatment. Typical thermal and epithermal neutron beam ranges are shown in Figure 1 (IAEA 2001).

A neutron beam often also contains gamma rays. In addition to the incident neutron beam, additional ionizing radiations are produced within the body in the form of boron disintegration products, protons from nitrogen neutron capture reactions, gamma rays from hydrogen neutron capture reactions, Compton electrons and photoelectrons from gamma-ray Compton scattering and photoelectric effect, bremsstrahlung from deceleration of charged particles, secondary electrons from charged-particles and target nuclei Coulomb interactions, etc.

The basic interaction of a moving charged particle in the body is the Coulomb interaction between the charged particle and the bound electrons in tissue cells. A charged particle traversing tissue loses energy primarily through ionization and excitation of atoms and/or molecules. A moving charged particle exerts electromagnetic forces on tissue-cell molecular electrons and imparts energy to them. Energy transferred may be sufficient to ionize an electron or excite atoms into an excited state. A heavy charged particle can transfer a small fraction of its energy in a single electronic collision and travel essentially straight paths in tissue.
The rate of energy loss along an alpha particle track is shown in Figure 2 (Attix 1986), and the range of the alpha particle track from boron-neutron capture is about 9 μm in body tissue. All forms of ionizing radiations eventually result in a distribution of low-energy ionized electrons. The interactions of ionized electrons within cancer tumors are very important in radiation chemistry and biology. A large difference in mass between electrons and heavy charged particles has important consequences for interactions. Charged particles deposit energy through two mechanisms—collisional and radiative losses. The collisional loss is the energy loss via Coulomb interactions with orbital electrons in tissue, which results in ionization and excitation, and the radiative loss is via bremsstrahlung. Delta ray is a secondary electron generated by incident fast electron or charged particle via ionization. The life history of a fast electron is shown in Figure 3 (Attix 1986).

High energy photons travel considerable distances before undergoing interaction leading to energy transfer to electrons ultimately depositing their energies into tissue. Photons are far more penetrating than charged particles of similar energy. There are three photon energy loss mechanisms after interactions: pair production, Compton scattering and photoelectric effect.

![Figure 1](image_url)

**FIGURE 1.** Typical thermal and epithermal neutron beam ranges.
Ionizing radiations produce a large amount of secondary electrons that rapidly slow down to energies below 7.4 eV, threshold to produce electronic transitions, in body blood and tissue.
Depending on the amount of energy transferred to the bound electrons, the tissue cell molecules can undergo:

- Ionization (threshold in water ~ 13 eV);
- Excitation (threshold in water ~ 7.4 eV);
- H-H Bond broken: 4.51 eV (H₂ - H + H);
- Hydrogen bond broken ~ 0.4 eV;
- Average Dipole-dipole bond broken ~ 0.2 eV.

Free radicals, especially highly reactive HO⁻ and H⁻, form in irradiated body blood and tissue cells as follows (Equation 1):

\[
\text{Irradiated H}_2\text{O} - \text{e}^-_{aq} + \text{HO}^- + \text{H}^- + \text{H}_2\text{O}_2 + \text{H}_3\text{O}^+ \quad (1)
\]

Typical yields produced by absorption of 100 eV x-ray or electron are shown in Table 1 (Attix 1986).

Various free radical species then proceed to react with each other or with other molecules in their vicinity. The chemical reactions between/among the radical species and adjacent molecules lead to the subsequent biological changes observed after cancer tumor irradiation.

### 4. MECHANISM OF BIOLOGICAL RADIATION INTERACTIONS

Chromosomes (microscopic bodies inside the nucleus of each cell) are organized in pairs and are responsible for the function and reproduction of each cell in a living organism. When a cell divides to reproduce, an exact copy of the cell chromosomes are created for the new cell. If the DNAs in the chromosomes are damaged by ionizing radiations, the instructions that control the cell function and reproduction are also damaged. This radiation damage may be: 1) reparable, 2) irreparable, or 3) incorrectly repaired. The irreparable damage can result in cell death at next mitosis, and the incorrectly repaired damage can result in mutation. Cell death leads to variable degrees of tissue dysfunction, which can affect the functions of the whole organism.

Typical damage of ionizing radiations on DNA by alpha (α), beta (β) and γ- or x-ray is shown in Figure 4. An alpha particle, which is a heavy charged particle, has a greater probability of causing damage compared to a beta (β) and γ- or x-ray.

|        | e⁻_{aq} | HO⁻ | H⁻ | H₂ | H₂O₂ | H₃O⁺ |
|--------|---------|-----|----|----|------|------|
| x-ray  | 2.63    | 2.72| 0.55| 0.45| 0.68 |      |
| electron| 1.87   | 1.17| 2.52| 0.74| 1.84 | 4.97 |

**TABLE 1. Typical yields produced by absorption of 100 ev x-ray or electron.**
Cancer cells cannot live without oxygen and nutrients via the blood supply. Cancer cells often send out signals, called angiogenic factors, that encourage the development of new blood vessels (which is called angiogenesis), which enables the further growth of the cancer tumor. A cancer tumor can be shrunk by damaging angiogenic factors and/or micro blood vessels (capillaries) via ionizing radiations to decrease blood supply into the cancer tumor. In addition to preceding direct radiation damage on cancer cells, the collisions between ionizing radiations and target nuclei and the absorption of the ultraviolet, visible light, infrared and microwaves from bremsstrahlung in tumor can heat up and damage cancer cells and functions as thermotherapy (namely, hyperthermia). The cancer cells are more chemical and biological sensitive at the BNCT-induced higher temperatures since free-radical-induced chemical reactions are more randomly and vigorously at higher temperatures after irradiation, and consequently the cancer cells are harder to divide or even survive due to more cell DNA damage. When the ambient temperature is above 45°C for a long period, a cancer cell is often difficult to survive.

5. RELATIONSHIP BETWEEN BIOLOGICAL RADIATION EFFECT AND RADIATION DOSE

The biological radiation effect is closely related to the biological radiation dose. The physical radiation dose is a measure of the amount of energy from all ionizing radiations deposited in irradiated material such as irradiated tumor cells. However, the biological radiation dose must account for the relative biological effectiveness (RBE), defined as the ratio of the doses required by two radiations to cause the same level of biological effect, among different ionizing radiations. Consequently, the biological radiation dose in tissue (HT) is defined as the sum of the products of all individual physical radiation doses in tissue (DT,Ri) and their associated individual RBE weighting factors (wRi), as shown in Equation 2.

\[ H_T = \sum_{R_i} w_{RI} D_{T,RI} \]  

(2)
The biological radiation dose evaluation for BNCT requires careful evaluation on different components of the radiation field, which includes the boron-capture-derivative dose, the fast neutron dose, the nitrogen-capture proton dose, and the sum of the gamma doses. The standard RBE weighting factors for various ionizing radiations are provided in International Commission on Radiation Units and Measurement, Report 60 (Allisy et al. 1998).

Typical BNCT depth-dependent dose curves including total and various components are shown in Figure 5 (IAEA 2001), where the RBE values represent the RBE weighting factors. A recent BNCT clinical trial case is illustrated in Figure 6 (Wang et al. 2016), which shows a set of two F18-labeled-fluorodeoxy-glucose positron emission tomography/computed tomography images of a recurrent nasopharyngeal cancer (indicated by arrows) before BNCT and the other set of cancer-disappeared images three months after BNCT. Two-fraction BNCT with intravenous L-boronophenylalanine (L-BPA, 400 mg/kg) was administered at a 28-d interval. The drug was infused at the rate of 180 mg/kg/h for two hours before neutron irradiation in the first phase, and at the rate of 1.5 mg/kg/min in the second phase, concurrent with neutron irradiation and stopped when the neutron beam was off (Wang et al. 2016).

The epithermal and thermal neutron fluxes at the TRIGA reactor neutron beam outlet were on the order of $10^8$ neutrons/cm$^2$-sec for BNCT. The prescription dose (D80) of 20 Gy-Eq per fraction was selected to cover 80% of the gross tumor volume (Wang et al. 2016).

BNCT may cause specific side radiation effects on various body parts. Getting radiation therapy on the head and neck can cause tooth decay, a stiff jaw, gum sores and difficulty swallowing. Chest radiation treatment can cause shortness of breath, cough and lung inflammation. Exposing the abdomen or stomach to radiation may cause diarrhea, nausea or vomiting. Consequently, BNCT must be carried out very carefully with the radiation dose in tumor adjacent tissue as low as practicable.
FIGURE 6. A typical BNCT clinical trial: F18-labeled-fluorodeoxy-glucose positron emission tomography/computed tomography images of a recurrent nasopharyngeal cancer before (left set) and 3 months after boron neutron capture therapy (right set). The arrows indicate the tumor.

6. CONCLUSIONS

The direct and indirect ionizing radiation sources for BNCT have been identified. The direct ionizing radiation source is neutron beam, which contains neutrons and associated gamma rays from a nuclear reactor or an accelerator. The indirect ionizing radiation sources include:

1. Alpha particles and recoiled 7Li ions from boron (10B) neutron capture reactions;
2. Protons from nitrogen neutron capture reactions;
3. Gamma rays from hydrogen neutron capture reactions;
4. Recoiled protons and other recoiled target nuclei from fast neutron collisions;
5. Compton electrons and photoelectrons from gamma-ray Compton scattering and photoelectric effect;
6. Bremsstrahlung from deceleration of charged particles;
7. Secondary electrons from charged-particles and target nuclei Coulomb interactions.

Fast neutrons bombardment can cause displacements and recoils of nuclei in tissue cells during the slowing down process. Epithermal and thermal neutrons are easily captured by odd-neutron-number nuclides such as $^5\text{B}^{10}$. The thermal neutron beam is used for surface and shallow cancer treatment and the epithermal beam is used for deeper cancer treatment since thermal neutrons have a shorter range.

All forms of ionizing radiations eventually result in a distribution of low-energy ionized electrons. Interactions of ionized electrons within cancer tumor are very important in radiation chemistry and biology. Large difference in mass between electrons and heavy charged particles has important consequences for interactions. Charged particles deposit energy via two mechanisms—collisional and radiative losses. The collisional loss is the energy loss via Coulomb interactions with orbital electrons in tissue, which results into ionization and excitation, and the radiative loss is via bremsstrahlung. Delta ray is a secondary electron generated by incident fast electron or charged particle via ionization.

High energy photons travel considerable distance before undergoing interaction leading to energy transfer to electrons ultimately depositing their energies in tissue. Photons are far more penetrating than charged particles of similar energy. There are three photon energy loss mechanisms after interactions: pair production, Compton scattering and photoelectric effect. Ionizing radiations produce plenty of secondary electrons that rapidly slow down to energies below 7.4 eV, the threshold to produce electronic transitions, in body blood and tissue. Free radicals, especially highly reactive HO$_2$ and H$_2$, form in irradiated body blood and tissue cells. Various free radical species then proceed to react with each other and/or with other molecules in their vicinity. The chemical reactions between/among the radical species and adjacent molecules lead to the subsequent biological changes observed after cancer tumor irradiation.

If the DNAs in the chromosomes are damaged by ionizing radiations, the instructions that control the cell function and reproduction are also damaged. This radiation damage may be: 1) reparable, 2) irreparable, or 3) incorrectly repaired. The irreparable damage can result in cell death at next mitosis while the incorrectly repaired damage can result in mutation. Cell death leads to variable degrees of tissue dysfunction, which can in turn affect the whole organism’s functions.

Cancer cells cannot live without the oxygen and nutrients supplied to them via the blood supply. A cancer tumor can be shrunk by damaging angiogenic factors and/or capillaries via ionizing radiations to decrease blood supply into the cancer tumor. The collisions between ionizing radiations and target nuclei and the absorption of the ultraviolet, visible light, infrared and microwaves from bremsstrahlung in the tumor can heat up and damage cancer cells and therefore function as thermotherapy. The cancer cells are more chemically and biologically sensitive at the BNCT-induced higher temperatures since free-radical-induced chemical reactions occur more randomly and vigorously at higher temperatures after irradiation, and consequently the cancer cells are harder to divide or even survive because of the higher cell DNA damage.
The biological radiation effect is closely related to the biological radiation dose. The biological radiation dose in tissue is calculated as the sum of the products of all individual physical radiation doses in tissue and their associated individual RBE weighting factors. The biological radiation dose evaluation for BNCT requires careful evaluation on different components of the radiation field, which includes the boron-capture-derivative dose, the fast neutron dose, the nitrogen-neutron-capture proton dose, and the sum of the gamma-induced doses. BNCT has been demonstrated via a recent clinical trial that it is quite effective to treat the recurrent nasopharyngeal cancer.

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ABSTRACT:

In this study, the optimum biodiesel conversion from crude castor oil to castor biodiesel (CB) through transesterification method was investigated. The base catalyzed transesterification under different reactant proportion such as the molar ratio of alcohol to oil and mass ratio of catalyst to oil was studied for optimum production of castor biodiesel. The optimum condition for base catalyzed transesterification of castor oil was determined to be 1:4.5 of oil to methanol ratio and 0.005:1 of potassium hydroxide to oil ratio. The fuel properties of the produced CB such as the calorific value, flash point and density were analyzed and compared to conventional diesel. Diesel engine performance and emission test on different CB blends proved that CB was suitable to be used as diesel blends. CB was also proved to have lower emission compared to conventional diesel.

KEYWORDS: biodiesel, transesterification, castor oil, diesel engine, emission; conversion.
1. INTRODUCTION

Petroleum fuels play a very important role in the development of various industries, transportations, agriculture sector and to meet many other basic human needs in modern civilization. These fuels are limited and depleting day by day as the consumption increase very rapidly. Moreover, the use of petroleum fuel has caused a lot of environmental problems by the high emission of harmful gases. A global movement towards generation of environmentally friendly yet renewable fuel is therefore under way to help meet the increased energy demands. Bio fuel had become one of the most promising alternatives for petroleum fuels.

Biodiesel is the potential biofuel that can easily being produced from vegetable oil. Biodiesel has become an interesting alternative fuel over conventional diesel for decades. Biodiesel is suitable to be used in diesel engine due to the similar properties to conventional diesel in terms of power and torque and none or very minor engine modification is required (Mushtaq et al. 2011). Moreover, biodiesel is biodegradable which will results in less environmental impact upon accidental release to the environment (Janaun & Ellis 2010).

Biodiesel has many important technical advantages over conventional diesel such as inherent lubricity, low toxicity, derivation from a renewable and domestic feedstock, superior flash point, negligible sulphur content and lower exhaust emissions (Moser 2009). Biodiesel had been used widely as a blend with diesel. The use of biodiesel as diesel blends will promote cleaner emission with less soot particles and whiter smoke. It also helps in reducing engine wear by lubrication and produces less sulphur emission. A biodiesel lifecycle study in 1998 which was jointly sponsored by the U.S. Department of Energy and the U.S. Department of Agriculture concluded biodiesel reduces carbon dioxide emissions by 78 percent compared to petroleum diesel. The CO2 released into the atmosphere when biodiesel burned is recycled by plants, which produce more oxygen (Petracek 2014).

Among the common vegetable oils used as feedstock for the production of biodiesel are soybean, rapeseed, castor, jatropha and palm oil. Castor oil is one of the promising feedstock for biodiesel production. Castor oil is produced by means of extraction from castor bean. Castor oil is distinguished by its high content (over 85%) of ricinoleic acid. No other vegetable oil contains so high a proportion of fatty hydroxyacids. Castor oils have high molecular weight (298), low melting point (5˚C) and very low solidification point (–12˚C to –18˚C) that make it industrially useful, most of all it is has the highest and most stable viscosity of any vegetable oil (Shrirame et al. 2011).

The chemical structure of castor oil is of great interest because of the wide range of reactions it affords to the oleochemical industry and the unique chemicals that can be derived from it. These derivatives are considerably superior to petrochemical products since they are from renewable sources, bio-degradable and eco-friendly (Nielsen et al. 2011). Recent research had concerns in the using of castor oil as a feedstock for biodiesel production. As castor oil is non-edible, there is no issue of competition with the food market and it can be the promising source of feedstock for biodiesel production.

In this study, the acid-based catalyzed transesterification of castor oil was carried out to determine the optimum reaction condition for the production of castor biodiesel. Then, the fuel properties such as density, flash point and calorific value was analyzed and compared to
conventional diesel. Engine performance and emission of castor biodiesel was also tested using various biodiesel blends and compared to the conventional diesel.

2. METHODOLOGY AND MATERIALS

In this study, crude castor oil was extracted from castor bean by using mechanical and solvent extraction. The castor beans used was obtained from a local company. The acid value of the crude castor oil was determined by titrimetry. The castor oil was converted into biodiesel by using two-step transesterification processes. In this process, the first step is acid catalyzed esterification used to convert free fatty acids (FFA) in castor oil to methyl ester, followed by base-catalyzed transesterification using potassium hydroxide as a catalyst with methanol. In the second step, potassium hydroxide was dissolved in methanol and the mixture is then heated up to 60°C to react accordingly to form methoxide. On the other hand, the pretreated oil in step 1 was then heated up to 60°C. The heated oil was mixed with the methoxide and the solution was shaken at 250 r.p.m. for 2 h by using orbital shaker. The volume ratio of methanol to oil used was 1:4.0, 1:4.5 and 1:5.0 while the volume ratio of potassium hydroxide catalyst to oil used was 0.0025:1, 0.0050:1 and 0.0075:1. The volume ratio of alcohol to oil was kept constant when the catalyst amounts were being manipulated. The volume ratio of catalyst to oil was kept constant when the amount of alcohol was being manipulated. After completing the process, the mixture was allowed to settle for 8 h and then the mixture was poured into separatory funnels. The lower layer of glycerol, extra methanol, catalyst and other byproducts were removed. The upper layer of methyl ester or biodiesel was washed several times with de-sterilized water until the washing water become neutral. The biodiesel layer was filtered to remove impurities and then the biodiesel was heated up to 100°C to remove any remaining water. The biodiesel was tightly sealed and kept for storage.

Biodiesel testing was carried out to compare the properties and performances of castor biodiesel and conventional diesel. The density, flash point and calorific value are measured respectively using density meter, multi-flash flash point tester and bomb calorimeter. Emission analyses were carried out using Flue Gas Analyzer. Castor biodiesel and conventional diesel were tested using FT-IR Shimadzu Iraffinity-1 Spectrophotometer for component analysis.

Diesel engine test was performed using Techno-mate, TNM-TDE-700 machine. The diesel engine testing was done three times with each blend of biodiesel. The blending percentage of biodiesel with diesel was set to 0%, 10%, 20%, 30%, 40%, 50% and they are mentioned as B10, B20, B30, B40, B50. Important values such as motor speed, output voltage, output current and time for 20 ml fuel flow were recorded. The brake load for the diesel engine testing was fixed at 120 N and the radius of brake arm was set to 0.5 m.

3. RESULTS AND DISCUSSIONS.

3.1 Measurement of Free Fatty Acid (FFA) in Crude Castor Oil

Measurement of FFA in crude castor oil is essential for the decision of the method of transesterification for biodiesel production. From the titration method, the acid value of FFA in
crude castor oil was determined to be between 20% to 23%, which is higher than 4%. The best conversion method for oil with free fatty acid higher than 4% was two-steps transesterification where FFA value is reduced at the first step (acid esterification) before proceeding to the second step (base transesterification).

3.2 Optimization of Biodiesel Production by Manipulation of Catalyst and Alcohol Amount

For the first set of experiment, the amount of catalyst was set as the manipulated variable while the amount of methanol was set as the constant variable. From Table 1, it is observed that the highest yield of biodiesel was achieved with potassium to oil ratio of 0.0050:1. However, the biodiesel yield before and after the optimal amount of catalyst was noted to be lower. In the case of the catalyst shortage (0.0025:1 ratio), the biodiesel yield percentage was 60% as catalyst was exhausted before all the crude oil was converted to biodiesel while in the case of excess catalyst (0.0075:1 ratio), the yield percentage was at 55% as excess catalyst attributed to soap formation which decreased the production of biodiesel.

For the second set of experiment, the amount of methanol was set as the manipulated variable while the amount of catalyst was set as the constant variable. From Table 2, it is observed that the highest yield of biodiesel was achieved with the 1:4.5 of oil-to-methanol ratio. The biodiesel yield was also affected by the amount of methanol used. The shortage of methanol used will decrease the yield of biodiesel significantly. Excess methanol (1:5.0 ratio) contributed to methanol wastage and difficulty in the end product purification. Excess methanol increased the solubility of glycerol which was the by-product formed in biodiesel transesterification thus, it caused difficulty in the purification of biodiesel. Shortage of methanol (1:4.0 ratio) attributed to the lack of solution for the reaction to take place. Thus, it could be concluded that the optimal reactant proportion used to achieve the highest yield of biodiesel produced by base-catalysed transesterification was 0.0050:1 KOH to oil ratio with 1:4.5 of oil to methanol ratio.

| Table 1. Biodiesel Yield Percentage for Different Amount of KOH (catalyst). |
|-----------------------------|--------------------------|-------------------|
| KOH to oil ratio (v/v) | Castor biodiesel produced (ml) | Biodiesel yield (%) |
| 0.0025:1 | 12 | 60 |
| 0.005:1 | 13 | 65 |
| 0.0075:1 | 11 | 55 |

| Table 2. Biodiesel Yield Percentage for Different Amount of Methanol. |
|-----------------------------|--------------------------|-------------------|
| Oil to methanol ratio (v/v) | Castor biodiesel produced (ml) | Biodiesel yield (%) |
| 1:4.0 | 9.5 | 47.5 |
| 1:4.5 | 10.5 | 52.5 |
| 1:5.0 | 7.0 | 35.0 |
3.3 FTIR Analysis.

Based on the infrared spectrum of castor biodiesel and conventional diesel Figures 1 and 2, both castor biodiesel and conventional diesel showed the alkane C–H bond which lies on the wave numbers from 2800 cm\(^{-1}\) to 3000 cm\(^{-1}\) and alkene C–H bond at 1400 cm\(^{-1}\) to 1500 cm\(^{-1}\). Thus, it could be confirmed that both conventional diesel and castor biodiesel had the same functional group of C–H. However, the conventional diesel had no oxygen group, whereas castor biodiesel showed oxygen functional group of ester C–O bond at 1000 cm\(^{-1}\) to 1300 cm\(^{-1}\) and ester C=O bond at 1735 cm\(^{-1}\) to 1750 cm\(^{-1}\). The presence of oxygen in biodiesel promotes cleaner and complete combustion. On the other hand, the conventional diesel without any oxygen component produced more black smoke and incomplete combustion during burning.

FIGURE 1. FTIR spectrum of castor biodiesel.
Density. Density value must be maintained within tolerable limits to allow optimal air to fuel ratios for complete combustion in diesel engine (Ibeto et al. 2012). For diesel, the standard range for density value is 848 kg/m³. For biodiesel, the standard for density value is in the range of 870 kg/m³– 900 kg/m³. For crude castor oil, the density value is in the range of 956 kg/m³ – 963 kg/m³. As observed from Table 3, the density for conventional diesel, castor biodiesel and crude castor oil are 841 kg/m³, 921 kg/m³ and 950 kg/m³, respectively. It is observed that in comparison to ASTM standards, conventional diesel and crude castor oil conforms to the range while castor biodiesel is sighted to be slightly higher than that of the standard. Higher density value of biodiesel is contributed by the high viscosity of castor oil. The lack of double bond in triglyceride molecule plus long hydrocarbon tail on fatty acid molecule contribute to high viscosity of castor oil (Okullo et al. 2012). High-density biodiesel is not favourable as it can lead to incomplete combustion and particulate matter emissions (Galadima et al. 2008). However, this problem could be solved by blending biodiesel with conventional diesel.
Calorific value. From Table 4, it was observed that conventional diesel had the highest calorific value followed by crude castor oil and castor biodiesel with the calorific value of 44.803 MJ/kg, 38.130 MJ/kg and 37.908 MJ/kg. The calorific value for castor biodiesel was slightly lower than that of the conventional diesel, where more amounts of biodiesel was needed to produce the same thermal energy as conventional diesel. Biodiesel has lower calorific value as its composition comprised of additional oxygen functional group and relatively lower hydrocarbon content compared to that of conventional diesel (Mathiyazhagan & Ganapathi 2011).

Table 3. The density value for crude castor oil, castor biodiesel and conventional diesel.

| Sample            | Density value (kg/m³) |
|-------------------|-----------------------|
| Conventional diesel | 841                   |
| Castor biodiesel  | 921                   |
| Crude castor oil  | 950                   |

Flash point. Flash point is the temperature that indicates the overall flammability hazards in the presence of air; higher flash point makes for safe handling and storage of biodiesel (Mushtaq et al. 2011). The flash point values for conventional diesel, castor biodiesel and crude castor oil are 75.0°C, 130.0°C and 230.0°C, respectively. From Table 5, it was observed that crude castor oil had the highest value of flash point followed by castor biodiesel and conventional diesel. Castor biodiesel had higher flash point value over conventional diesel as biodiesel was more viscous compared to conventional diesel. Flash point was positively correlated with the viscosity of diesel. The higher the viscosity, the higher the boiling point and thus caused higher flash point. Other than that, diesel has branches and lower molecular weight components which lead to a reduction of flash point (Knothe 2010). The high flash points of biodiesel make it suitable to be used as alternative to conventional diesel.

Table 4. The calorific value of crude castor oil, castor biodiesel and conventional diesel.

| Sample            | Calorific value (MJ/kg) |
|-------------------|-------------------------|
| Conventional diesel | 44.803                 |
| Castor biodiesel  | 37.908                  |
| Crude castor oil  | 38.13                   |

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3.5 Engine Performance

Data collected from diesel engine testing were calculated and presented as shown in Table 6. From the results shown, it was observed that the brake horsepower, engine power output and mechanical efficiency were decreasing while the biodiesel blend ratio was increasing. However, the specific fuel consumption increased with the increase of biodiesel blend ratio.

### Table 5. The flash point for crude castor oil, castor biodiesel and conventional diesel.

| Sample             | Flash point (°C) |
|--------------------|------------------|
| Conventional diesel| 75               |
| Castor biodiesel   | 130              |
| Crude castor oil   | 230              |

### Table 6. Engine performance of various castor biodiesel blends.

| Castor biodiesel blends | Fuel consumption rate (ml/s) | Brake horse power, BHP (kW) | Engine power output (kW) | Specific fuel consumption, SFC (ml/kW) | Mechanical efficiency, η (%) |
|-------------------------|------------------------------|-----------------------------|--------------------------|----------------------------------------|-----------------------------|
| B0                      | 0.25                         | 7.527                       | 1.201                    | 16.653                                 | 13.022                      |
| B10                     | 0.247                        | 7.521                       | 1.187                    | 16.849                                 | 13.012                      |
| B20                     | 0.241                        | 7.521                       | 1.165                    | 17.167                                 | 13.012                      |
| B30                     | 0.241                        | 7.515                       | 1.165                    | 17.167                                 | 13.002                      |
| B40                     | 0.241                        | 7.515                       | 1.142                    | 17.513                                 | 13.002                      |
| B50                     | 0.238                        | 7.508                       | 1.142                    | 17.513                                 | 12.99                       |

The decrement percentage of the decrease of engine power output for B10, B20, B30, B40 and B50 relative to B0 (conventional diesel) were 1.17%, 3.00%, 3.00%, 4.91% and 4.91%, respectively. The decrement of engine power output were due to lower energy content per volume of castor biodiesel compared to conventional diesel. However the power output decrement was less than 5% although 50% blends of biodiesel were used. This showed that biodiesel was suitable to be used as diesel blends.

From Figure 4, the increment of specific fuel consumption were observed when the blending percentage of biodiesel increased. The increment percentage for for B10, B20, B30, B40 and B50 relative to B0 (conventional diesel) were 1.18%, 3.09%, 3.09%, 5.16%, 5.16% respectively. The higher specific fuel consumption of those higher percentage blends ratio was due to the fact that biodiesel had lower calorific value than the conventional diesel. More fuel was consumed to produce 1 kW of power compared to conventional diesel. Furthermore, higher containment of oxygen in biodiesel was also the cause of the lower calorific value. Despite the better combustion of biodiesel compared to the conventional diesel, the oxygen in biodiesel...
took up space in the blend and slightly increased the fuel consumption rate. Thus, higher oxygen content in biodiesel leads to the low calorific value of Biodiesel (Islam et al. 2014).

![Graph of engine power output versus castor biodiesel blends](image1)

**FIGURE 3.** The graph of engine power output versus castor biodiesel blends

![Graph of specific fuel consumption versus castor biodiesel blends](image2)

**FIGURE 4.** The graph of specific fuel consumption versus castor biodiesel blends.

Based on Figure 5, the mechanical efficiency decreased slightly with the increase of blending percentage of biodiesel. The decrement noted for B10, B20, B30, B40 and B50 were 0.08%, 0.08%, 0.15%, 0.15%, 0.25%, respectively. The decrement was however, insignificant as it was less than 1%. The lower mechanical efficiency of biodiesel is mainly due to the low volatility and high density of ester which affects the automization of the fuel and thus leads to poor combustion (Islam et al. 2014).
3.6 Emission Analysis

From Table 7, it was observed that castor biodiesel had lower emission of carbon monoxide (CO), sulphur dioxide (SO2) and nitrogen dioxide (NO2) compared to conventional diesel. Lower emission of CO and SO2 for biodiesel were due to the additional oxygen content in biodiesel, which improved the combustion in the cylinders of diesel engine. Higher nitrogen oxide (NOx) emission for biodiesel is due to the oxygen concentration in biodiesel causing the formation of NOx in the emission gas (Christopher et al. 2001). NOx emission is primarily a function of total oxygen inside the combustion chamber, temperature, pressure, compressibility, and velocity of sound. Furthermore, the increase of NOx emission is due to the higher cetane number of biodiesel which will reduce the ignition delay (Fazal et al. 2011). The increase of NOx emission is a result of the reduced ignition delay. However, the NOx emissions can be reduced through engine tuning or using exhaust catalytic converter (Leung 2001; Enweremadu Mbarawa 2009). Moreover, the use of exhaust gas recirculation (EGR) can reduce the NOx emission too where the temperature of exhaust gas is reduced when passing through the combustion chamber. In overall, castor biodiesel emitted cleaner gas emissions than conventional diesel and the combustion is more complete. These properties make biodiesel suitable to be used as an alternative to conventional diesel or as a blend to lower the emission of conventional diesel.

Table 7. The emission analysis for conventional diesel and castor biodiesel.

| Sample             | CO (p.p.m.) | SO2 (p.p.m.) | NO2 (p.p.m.) | NOx (p.p.m.) |
|--------------------|-------------|--------------|--------------|--------------|
| Conventional diesel| 79          | 3            | 0.4          |              |
| Castor biodiesel   | 60          | 2            | 0.2          | 3            |
4 CONCLUSION

The optimum reactant proportion for base catalyzed transesterification of castor oil was determined to be 1:4.5 of oil to methanol ratio, and 0.005:1 of potassium hydroxide to oil ratio. The additional content of oxygen in castor biodiesel promoted complete combustion in diesel engine thus, it led to lower emissions. The high flash point of castor biodiesel made it safe for handling and storage. Reduction of mechanical efficiency and power output of castor biodiesel compared to conventional diesel was minor and tolerable. Lower emission of castor biodiesel compared to conventional diesel proved it as a green fuel. Castor biodiesel was a suitable fuel to be used as diesel blends.

ACKNOWLEDGEMENT

This study was supported by the Department of Mechanical and Manufacturing Engineering, Faculty of Engineering, University Malaysia Sarawak, Malaysia.

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Fabrication, Rheology and Antioxidant Activity of Palm Esters-based Emulsions Loaded with Tocotrienol

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ABSTRACT:

Palm oil esters are wax esters derived from palm oil and oleyl alcohol. Palm oil esters have many applications due to their luxurious moisturizing properties, non-greasy and blend easily with fragrances and colours when applied on skin surface. The aim of this research was to fabricate palm esters-based emulsions cream for topical delivery, characterise the rheological properties and in vitro antioxidant activity of the palm esters-based emulsions system.

Emulsions containing palm oil esters loaded with tocotrienol were obtained in two stages, with propagation of rotor stator at 6000 r.p.m. for 5 min and further emulsified using an ultrasound at various acoustic amplitudes for another 5 min. A stress/rate controlled Kinexus Rheometer with a temperature controller was used to measure the rheological properties of the emulsion. Rheology measurements were performed at 25.0°C ± 0.1°C with 4°/40 mm cone and plate geometry. The in vitro antioxidant activity was investigated using UV-Vis spectrophotometer. The yield stress of the emulsions increased with increasing acoustic amplitudes. The viscoelasticity of the emulsions were enhanced by the increase in the oil and surfactant concentrations. The emulsions with higher oil phase concentration [30% (w/w)] showed greater elasticity which implied strong dynamic rigidity of the emulsions. The cohesive energy increased significantly with surfactant concentration especially for the emulsions with 30% (w/w) oil phase concentration. The palm oil esters emulsions containing tocotrienol gave higher Trolox equivalent antioxidant capacity values which implied higher antioxidant capability. The tocotrienol in emulsion with 30% (w/w) dispersed phase showed that they were the most stable with longest shelf life and exhibited greater inhibitory effects on the ABTS•⁺.

KEYWORDS: Emulsion, rheological properties, antioxidant activity, yield stress, cohesive energy, palm oil esters, Tocotrienol.
1. INTRODUCTION

Palm oil is produced from the fruit of oil palm (Elaeis guineensis) which is grown in mass plantations in tropical countries such as Malaysia, Indonesia and Nigeria. The oil consists of 95% triglycerides and 5% diglycerides whereby carbons of the carboxyls range from 10–20 with or without double bonds (Tanaka et al. 2008). Palm oil esters (POEs) are a constituent of modified form of palm olein oil known simply as palm oil. Desirable characteristics of fat esters including non-toxicity, good fat solubility properties and excellent wetting at interfaces (Radzi et al. 2006) but without the greasy feeling when applied on the skin surface; these have attracted the attention of the industry. The emollient effect of POEs had been proven thereby making this oil highly recommendable for its incorporation into the topical preparation as oil phase. Thus, palm oil esters are excellent ingredient to be used in cosmeceutical and pharmaceutical formulations.

Nanoemulsions are emulsion with droplet size in the range 20 nm–200 nm (Solans et al. 2003). They are independent of molecular size of the hydrophilic solute and the nature of the aqueous phase. In addition, nanoemulsions delivery system was independent of animal skin characteristics such as the stratum corneum thickness and the follicle-type (Wu et al. 2001). Thus, nanoemulsions due to their extremely small size are suitable to be used as delivery system in cosmeceuticals. However, nanoemulsions are only kinetically stable and therefore, it is also a very fragile system by nature (Tadros et al. 2004). As they are transparent and usually very fluid, the slightest sign of destabilization easily appears. They become opaque and creaming may be visible. Thus, stability of the nanoemulsion is a critical factor to be analysed. The achievement of developing long time stability of cosmetic products (3-years shelf life) is often difficult and deeply affects costs in the development of new formulations.

Rheology is an independent scientific discipline: studying the deformability, and flow properties of a matter under an applied stress or strain is revealed by McClements (1999). Owing to the fact that rheology can give a better picture of the behaviour of a material, it is therefore widely used as a tool to test the texture and flow behaviour of industrial products especially in the processing industries such as food (Lorenzo et al. 2008), cosmetics (Bummer & Godersky 1999), pharmaceuticals (Zumalacarregui et al. 2004), polymer (Karg et al. 1985), coating (Kikic et al. 1979), and oil processing (Martin et al. 2006). The rheological results also enable scientists to estimate the product’s quality such as elasticity, viscosity, deformability, storage, shelf life including intermolecular interactions due to ultra-sensitivity at microstructure of materials.

Antioxidants neutralize damaging free radicals by quenching reactive molecules and, thus protecting cells from both endogeneous stress (byproducts of cellular energy) and exogenous stressors (ultraviolet light, pollution, cigarette smoke etc.) (Choi & Berson 2006). Tocotrienol are fat-soluble vitamins related to the family of tocopherols. Tocopherol and tocotrienol are well recognized for their antioxidative effect (Kamal 1996). This effect depends primarily on the phenolic group in the chromanol ring, rather than the side chain (Burton & Ingold 1989). The trolox equivalent antioxidant capacity (TEAC) assay is widely applied to assess the amount of radicals that can be scavenged by an antioxidant, i.e. the antioxidant capacity (Lien et al. 1999).
The present investigation was focused on the preparation of palm esters-based emulsions of tocotrienol and to characterise the rheological properties of the emulsion systems. Furthermore, assessment of the in vitro antioxidant activity of esters-based palm was done by the TEAC assay.

2. EXPERIMENTAL

2.1 Materials

POEs was prepared in the laboratory according to the method of Keng et al. (2009) Sorbitan monooleate (Span® 80) and polyoxethylene (20) sorbitan monooleate (Tween®80) were purchased from Merck, Germany. The HLB values of sorbitan monooleate (Span® 80) and polyoxethylene (20) sorbitan monooleate (Tween® 80) are 4.3 and 15.0, respectively. Tocotrienol (Gold Tri. E 70) was from Golden Hope Bioganic, Malaysia. Xanthan gum from Xanthomonas campestris was obtained from Fluka Chemie GmbH, France. Freshly deionized water was obtained from water deionizer, Mili-Q (Milipore, USA).

2.2 Methods

Preparation of emulsions containing tocotrienol. Emulsions were formulated using POEs containing to cotrienol as dispersed oil phase and Mili-Q water as the continuous aqueous phase. Xanthan gum was dispersed in deionized water at 0.8% (w/w). Preparation of dispersed oil phase was performed by homogenizing 5% (w/w) of surfactants into oil phase with a Polytron homogenizer (Kinematica GmbH, Germany) rotor stator. The ratio of Span® 80:Tween® 80 was 1:4. The preparation was continued by adding the oil phase dropwise to the aqueous solution with continuous homogenized at 6000 r.p.m. for 5 min. The temperature was lowered to 40°C. At 40°C, the active ingredient was added. The emulsions were further homogenized using ultrasonic cavitation for 5 min. The sonifier tip horn was adjusted to 2 cm below the surface of a 100 ml sample.

Rheology measurement. A stress/rate controlled Kinexus Rheometer (Malvern Instrument, UK) with a temperature controller, was used to measure the rheological properties of the emulsion. The measurements were performed at 25.0 ± 0.1°C with 4°/40 mm cone and plate geometry. The samples were allowed to relax for 10 min after being loaded to the plate before the measurement was started.

In vitro antioxidant activity. The antioxidant activity was assessed as described below. Experiments were performed on the Varian Cary 50 UV-Vis spectrophotometer (Varian, Australia). Trolox (2.5, 5.0, 10.0, 15.0 μM) was prepared in ethanol. Ascorbic acid was prepared in 18 MΩ water to a concentration of 10.0, 15.0, 20.0 μM and α-tocopherol in ethanol at 10.0, 15.0, 20.0 μM. ABTS, 2,2’-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) di ammonium salt was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS +) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 h–16 h before use. The radical was stable in this form for more than two days when stored in the dark at room temperature. ABTS.+ solution was then diluted with ethanol to an absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30°C (Roberta et al. 1999).
Diluted ABTS.+ solution (1.0 ml) (A734 nm = 0.700 ± 0.020) was added to 10 μl of antioxidant compounds or Trolox standards in ethanol. The absorbance of the sample was taken at 30°C every min after initial mixing up to 6 min. An appropriate solvent blanks were run in each assay. All determinations were carried out three times, and in triplicate, on each occasion and at each separate concentration of the standard and samples. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data (Roberta et al. 1999).

3. RESULTS AND DISCUSSION

3.1 Rheological Properties of Emulsions System

**Steady-state flow:** The sensitivity of emulsions to shearing. The sensitivity of these emulsions to shearing was tested in steadystate flow. The greater the yield stress \( \sigma_Y \), the more brittle the emulsion, and this leads to believe that the emulsion either undergoes disorganization of its structure or takes longer to recover its initial states. Figures 1 and 2 summarize the yield stress data as a function of acoustic amplitudes (%) and surfactant concentration [% (w/w)], respectively. The yield stress of the emulsions increased with increasing acoustic amplitudes. The increase in acoustic amplitudes (20% to 100%) led to decrease in mean droplet size. The decrease of droplet size leads to the increase in the total droplet surface area. When the total surface area of the droplet increased, the strength of the attractive force will also increase. Thus, greater stress is required to initiate flow when high attractive force is holding the droplets resulting high viscosity with high yield stress (Pal 1996). Mean droplet size was another factor affecting the flow behaviour of the emulsion.

![FIGURE 1](image_url). The yield stress of the emulsions as a function of acoustic amplitudes and oil phase concentration. Emulsions with 10% ( ), 20% ( ) and 30% ( ) oil phase concentration.
The yield stress of the emulsions increased with surfactant concentration (Figure 2) indicating structural integrity arising from the strong colloidal interaction between the droplets. The yield stress is the stress that has to be overcome before the emulsion starts to flow (Barnes 1999). The system with high surfactant concentration tends to form a denser interfacial layer, which is incompressible (Hamill & Petersen 1966). Hence, the droplets in such sterically stabilized system are usually characterized as ‘hard sphere’ (McClements 1999). The strength of interaction forces (mainly the attractive and repulsive interactions) between the droplets for the hard sphere system (high surfactant concentration system) was relatively greater than the one with lower surfactant concentration. In the absence of the strong sterically repulsive effect, the droplets in the emulsions system with lower surfactant concentration were able to pack more efficiently even at low shear. Therefore, the droplets were aligning themselves easily with the shear field to initiate flow. This explained that the increase in surfactant concentration led to increase in yield stress of emulsion system.

Oil phase concentration in an emulsion system is another factor affecting the flow behaviour (Akhtar et al. 2005). The attractive force is one of the colloidal interactions which play an important role in the increase in viscosity and yield stress. The magnitude of viscosity and yield stress depend on the strength of the attractive force between the droplets (Pal 1996). Higher strength of attractive force between the droplets leads to increase in viscosity and yield stress.

3.2 Shear Stress versus Shear Rate Profile

The shear stress—shear rate profile of stabilized emulsions are depicted in Figure 3. Much like viscosity—shear rate profile, the rate of change of shear stress depends largely on the rate of change of shear rate. As depicted in Figure 3, at zero shear rate the shear stress responses are not zero. This suggested that these emulsions were shear thinning non-ideal plastic-like
material, with yield stress ($\sigma_Y$) response. In other words, they behaved like pseudoplastic material, which implied that flow can only be induced on these emulsions with the application of certain minimum amount of stress called yield stress. Figure 3 shows that above the yield stress these samples assume a linear shear stress—shear rate relationship. This in turn suggested that, these emulsions did not follow ideal Newtonian flow behaviour even at high shear rate domain. By contrast, the shear stress—shear rate relationship increased exponentially with a certain power law exponent at low-shear rate domain below yield stress, suggesting that the flow behaviour of these emulsions resembled that of plastic-like material at these low shearrate domain.

As far as the effect of concentration on yield stress was concerned, these profiles suggested that yield stress increased monotonically with surfactant concentration. This in turn indicated that all samples examined here exhibited non-Newtonian model type fluid behaviours, implying that the viscoelastic force dominated over the elastic force, and that the emulsions under investigation underwent structural deformation with shear rate irrespective of surfactant concentration. The increase in the yield stress as a function of surfactant concentration further indicated that emulsions with higher surfactant concentration possess higher degree of material structuring as opposed to lower surfactant concentration. This also means that emulsions with higher surfactant concentration offer a larger resistance to force before they started flowing. This in turn suggested that emulsions stabilized with higher surfactant concentration undergo a greater degree of deformation under applied shear in comparison to emulsions stabilized with lower surfactant concentration.

### 3.3 Oscillatory Measurements: Strain Sweep Profile

A critical strain ($\gamma_c$) is the maximum applied strain where the emulsion still gives a linear response to shear stress with constant elastic modulus. The critical strain, $\gamma_c$ of emulsions in different oil phase concentration is shown in Figure 4. The $\gamma_c$ of the emulsions increased with the oil volume fraction and surfactant concentration. The $\gamma_c$ was increased 50%, 46% and 86% as the surfactant concentration was increased from 5% to 10% (w/w) for emulsions with 30%, 20% and 10% (w/w) oil phase concentration, respectively. On the other hand, the $\gamma_c$ increased more than 100% when the oil concentration was increased from 10% to 30% (w/w).

The increase of critical strain of emulsion with 30% (w/w) oil phase concentration when the surfactant concentration was increased implied that the highly packed droplets have developed a strong structure due to the high interdroplet interaction between the droplets which corresponded to the droplet size and droplet concentration of the emulsions system. Since the strength of the interdroplet interactions corresponded to the mean separation distance between the droplets, the highly packed emulsion system will therefore has greater interdroplet interaction forces. The high interdroplet interaction strength was able to hold the droplets and withstand the large deformation forces during the strain sweep test.

The strain sweep profiles also provided information about the elastic component of the emulsions. Figures 5 and 6 show increasing trends in the elastic modulus ($G'$) of the emulsions with surfactant and oil concentration indicating that the interactions between droplets are relatively strong. A trend of increasing elastic modulus accompanying the increased of $\gamma_c$ was observed. The cohesive energy ($E_c$) within the linear viscoelastic regime for when $G'$ is in
phase with the applied strain amplitude can also be obtained (Bossard et al. 2007), as shown below:

\[ E_c = \int_{0}^{\gamma_c} \sigma dy \]  

FIGURE 3. Effect of shear rate of emulsions on the shear stress for (a) 10% (b) 20% (c) 30% oil phase concentration. Surfactant concentration: 5% ( ); 6% ( ); 7% ( ); 8% ( ); 9% ( ) and 10% ( ).
FIGURE 4. The critical strain, $\gamma_c$ of the emulsions as a function of surfactant concentration. Emulsions with 10% ($\text{-}$), 20% ($\text{-}$) and 30% ($\text{–}$) oil phase concentration.

When $\sigma$ equals $G'$ in the linear viscoelastic region, the cohesive energy is defined as:

$$E_c = \frac{1}{2} \gamma_c^2 G'$$  \hspace{1cm} (2)

Tadros (2004) explained that the cohesive energy was related to the structure of the emulsion system, which correlated to the droplet size and number of contact area between the droplets. The droplet concentration and the packing of the droplets influenced the strength of the cohesive force. As discussed before, the number of droplets was increased as the oil concentration was increased from 10% to 30% (w/w) at fix surfactant concentration. As result, the number of contacts area within the droplets increased. Thus, increases in the cohesive energy of the emulsions system were observed.

$E_c$ ranged from a low 0.13 J/m$^3$ for emulsion with 5% (w/w) surfactant concentration in 10% (w/w) oil phase concentration to a high of 15.88 J/m$^3$ for emulsion with 10% (w/w) surfactant concentration in 30% (w/w) oil phase concentration. $E_c$ was low for emulsion with 5% (w/w) surfactant concentration in 10% (w/w) oil phase concentration as the elasticity was low (Figure 6). The higher the cohesive energy, the more stable a system was as the elastic strength was basically a measure of the strength of the internal structure. This in turn demonstrated that the emulsion samples under examination were stable systems, and that the stability of these emulsions systems was enhancing with decreasing droplet size.

Figure 7 show that the cohesive energy increased significantly with surfactant concentration especially for the emulsions with 30% (w/w) oil phase concentration. The dramatic increase of cohesive force was due to the highly packed systems related to the interdroplet interactions that had been previously discussed.
FIGURE 5. The linear viscoelastic region of the emulsions with a series of surfactant concentration [5% (■)]; [6% (▲)]; [7% (▲)]; [8% (▲)]; [9% (▲)] and [10% (●)] for (a) 10%, (b) 20% and (c) 30% oil phase concentration.
FIGURE 6. The elastic modulus of the emulsions as a function of surfactant concentration. Emulsions with 10% (▲), 20% (●) and 30% (▼) oil phase concentration.

FIGURE 7. The cohesive energy of the emulsions as a function of surfactant concentration and oil concentration.

Emulsions with 10% (▲), 20% (●) and 30% (▼) oil phase concentration.

Figure 8. Trolox, the water-soluble analogue of α-tocopherol was known for its high radical scavenging activity and was therefore frequently used as a model compound. It was commonly
used as a reference antioxidant, in which the radical scavenging activity was expressed as Trolox equivalents. The higher the concentration of Trolox used, the more the absorbance of ABTS radicals was suppressed. The selected absorption wavelength was at 734 nm because ABTS + has maximum absorption at 734 nm and most antioxidant does not absorb light at 734 nm. Figure 9 illustrates the effects of the duration of interaction of specific antioxidants on the suppression of the absorbance of the ABTS + radical cation at 734 nm for Trolox, the standard reference compound, compared with α-tocopherol, ascorbic acid and emulsion samples containing tocotrienol.

The results demonstrated that the reaction with ABTS + was completed after 1 min. 30% dispersed phase emulsion containing Tocotrienol (15 μM) has minimum absorption than in 20% and 10% dispersed phase emulsion containing tocotrienol (15 μM) with ABTS + within 6 min. The absorption at wavelength 734 nm for ABTS + radical cation was 0.73 followed by ABTS + radical cation in ascorbic acid and tocopherol which gave 0.42 and 0.28, respectively. From the results, 30% dispersed phase emulsion containing tocotrienol (15 μM) revealed better antioxidant properties by presenting lower absorbance value as compared the standard reference compound, Trolox. The extent of inhibition of the absorbance of the ABTS + was plotted as a function of concentration in order to determine the TEAC that can be assessed as a function of time. The dose-response curve obtained by analysis of a range of concentrations of antioxidant compounds and Trolox standards was plotted as the percentage inhibition of the absorbance of the ABTS + solution as a function of concentration of antioxidant (Figure 10). The result showed that 30% dispersed phase emulsion containing tocotrienol (15 μM) has an inhibitory effect, a maximum concentration of 20 μM reducing ABTS + by about 93%.

**FIGURE 8.** Concentration-response curve for the absorbance at 734 nm for ABTS as a function of concentration of standard Trolox solution.

From the result, 20% dispersed phase emulsion containing tocotrienol (15 μM) appears to exert a slightly greater inhibitory effect on the ABTS + than on that of 10% dispersed phase emulsion. The presence of the 30% dispersed phase emulsion containing tocotrienol (15 μM) enhanced its inhibitory effect on the ABTS + since 20 μM of 20% dispersed phase emulsion
FIGURE 9. Effects of time on the suppression of the absorbance of the ABTS; ABTS (+-); nABTS + ascorbic acid (+-); ABTS + tocopherol (+-); ABTS + Trolox (+-); ABTS + 10% disperse phase containing tocotrienol (+-); ABTS + 20% disperse phase containing tocotrienol (+-); ABTS + 30% disperse phase containing tocotrienol (+-). The concentration of the samples used in the determination is 15 μM.

containing Tocotrienol (15 μM) and 10% disperse phase emulsion containing tocotrienol (15 μM) only produced 87.5% and 82.8% inhibition; respectively of the ABTS assay system. The antioxidant activity could be expressed in terms of the total contribution to the antioxidant activity over the time range studied by calculating the TEAC, the gradient of the plot of the percentage inhibition of absorbance vs. concentration plot for the antioxidant in question is divided by the gradient of the plot for Trolox. This gives the TEAC at the specific time points, 1 min, 4 min and 6 min (Figure 11).

FIGURE 10. Effects of time on the suppression of the absorbance of the ABTS; ABTS (+-); nABTS + ascorbic acid (+-); ABTS + tocopherol (+-); ABTS + Trolox (+-); ABTS + 10% disperse phase containing tocotrienol (+-); ABTS + 20% disperse phase containing tocotrienol (+-); ABTS + 30% disperse phase containing tocotrienol (+-). The concentration of the samples used in the determination is 15 μM.

measured at 6 min and expressed as the TEAC value or IC50 were relatively low as compared to Trolox. The higher TEAC values implied higher antioxidant capability. The TEAC at 6 min of 30% dispersed phase emulsion containing tocotrienol (15 μM) was 2.00 whereas the TEAC

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values of tocopherol and ascorbic acid were 1.64 and 1.42 respectively. Tocotrienol in 30% dispersed phase appeared to have a much higher TEAC value than tocopherol and ascorbic acid in 15 μM concentrations, respectively. This means that tocotrienol can be qualified as the best antioxidant compared to tocopherol and ascorbic acid with the TEAC assay.

![Graph showing the variation of gradient of the percent inhibition vs. concentration](image)

**FIGURE 11.** Profile of the variation of gradient of the percent inhibition vs. concentration plot of each antioxidant at 1, 4 and 6 min: ABTS (—); nABTS + ascorbic acid (—); ABTS + tocopherol (—); ABTS + Trolox (—); ABTS + 10% disperse phase containing tocotrienol (—); ABTS + 20% disperse phase containing tocotrienol (—) and ABTS + 30% disperse phase containing tocotrienol (—). The concentration of the samples used in the determination is 15 μM.

### 4. CONCLUSIONS

The results obtained in the present work showed that emulsions containing palm esters-based as oil phase was a suitable carrier system for the incorporation of tocotrienol with reasonably high values of yield stress, suggesting the stabilization of emulsions. *In vitro* antioxidant activity using TEAC assay was applied to assess the total amount of radicals that could be scavenged by antioxidant samples. The findings indicated that tocotrienol in emulsion with 30% (w/w) dispersed phase exhibited greater inhibitory effects on the ABTS⁺. Results were encouraging and it substantiated the role of palm esters-based emulsions containing tocotrienol as an effective antioxidant therapy.

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