

## Effects of *Myristica fragrans* Houtt. (Nutmeg) extract on chemical characteristic of raw beef during frozen storage

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### Abstract

The objective of this study was to investigate the effect of nutmeg (*Myristica fragrans* Houtt.) extract at different concentrations on chemical characteristics of raw beef under frozen storage. Nutmeg extracts at concentrations of 0.25%, 0.65%, 1.25%, 2.50% and 5.00% (g/ml) were used to treat raw beef (2.5 × 2.5 × 1.0 cm; 4 ± 0.5 g) with dilution method. Treated samples were then individually packed in overwrapped trays and stored for 3 weeks at -18 ± 1°C. The effects of the extract on the chemical characteristics such as lipid oxidation, colour, pH, moisture, fat, and protein content of raw beef were evaluated at 0, 4, 7, 10, 14 and 21 days of storage. Lipid oxidation was evaluated based on thiobarbituric acid-reactive substance (TBARS) content. Colour of beef was observed by spectrophotometer in colorimetric parameters CIELabs. Values of pH were measured using pH meter. Moisture, fat and protein content were determined using method by Analysis Association of Official Analytical Chemists (AOAC). The result showed that extract at concentration of 1.25% inhibited TBARS value meaning that extract of 1.25% or more was able to maintain the oxidative stability of beef at -18°C. A 1.25% of extract was also able to maintain the redness (a\*) of treated beef compared to untreated during frozen storage. The pH values of all samples beef decreased starting from 10<sup>th</sup> day of storage. Untreated samples (0.00%) showed the lowest pH values compared to other treated samples at the end day of storage. There was no significant different in term of protein content in all treated or untreated samples. However, fat and moisture content were significantly affected by the concentration of nutmeg extract. Treated beef was able to retain its moisture with only loss of moisture ranging from 0.2% – 2.00% while untreated samples had 5.00% loss of moisture. The fat content of untreated samples (0.00%) showed a reduction of 0.2% of fat content at the end of storage compared to all treated sample with only loss of 0.1% - 0.05%. Overall, nutmeg extract can be used to maintain the chemical characteristics of raw beef during storage for 3 weeks.

### Keywords

Chemical characteristic

*Myristica fragrans*

Extraction

Nutmeg extract

Raw beef

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### Introduction

Frozen storage is one of the major preservation methods for beef product that can extend its shelf life and reduce its physico-chemical changes. However, the quality of the beef will still be affected through this preservation method. Freezing storage is associated with formation of ice crystal on the surface of beef that can cause damage on physical structure and loss of liquid (Mariana *et al.*, 2014). Beef meat is a highly perishable food due to the composition value, where it consists of high polyunsaturated fatty acid that have a high tendency to auto-oxidize during storage (Monahan, 2000). This process leads to loss of several characteristic such as texture, colour and nutritional value (Malikarjunan and Mittal, 1996; Ferguson *et al.*, 2001). Colour perception plays important role in evaluation of meat quality as consumers judge

the colour of meat as an indicator of freshness or wholesome. During storage, distribution and display, beef meat is exposed to the processes of conversion of oxidation; oxymyoglobin to metmyoglobin which leads to the discoloration of beef meat (Mancini and Hunt, 2005).

Nitrate is one of the common additives in beef meat to provide some properties benefits such as reducing microbial growth and enhance the red color of the meat. Regardless of benefits, nitrate can react with amines under circumstances of low pH and high temperature, which then form carcinogenic compound, nitrosamines (Bingham *et al.*, 2002). This has cause an increase of interest by consumer and food industries towards the use natural agents that guarantees on safety and quality in beef storage (Karabagias *et al.*, 2010; Ali and Takwa, 2010).

In recent years, naturally occurring antioxidant

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compounds have been preferably employed in beef because of their potential health benefits compared to synthetic preservatives. Applications of bioactive compounds from plants have shown physicochemical beneficial effect such as; grape seed extract can retard formation of thiobarbituric acid reactive substances (TBARS) in turkey meat (Mielnik *et al.*, 2006), green tea extract can reduce the lipid oxidation in goat meat (Rababah *et al.*, 2011), mint leaves extract has high antioxidant activity in lamb meat stored in chilled temperature (Kanatt *et al.*, 2007).

Nutmeg, *Myristica fragrans* Houtt., locally named pala is an evergreen tree that produces drupe type fruits, belongs to *Myristicaceae* family. Nutmeg is widely used as spices in cooking and numerous traditional medicines (Dorman *et al.*, 2000). Various researches were discovered on therapeutic values of *M. fragrans*; mace and seed of nutmeg possess anti-inflammatory effect (Murcia *et al.*, 2004), anti-cancer effect (Olajide *et al.*, 1999), anti-glycation (Kazeem *et al.*, 2012), anti-diarrhea (Grover *et al.*, 2002), antioxidant and antimicrobial effect (Suthagar *et al.*, 2012; Kazeem *et al.*, 2012; Ashish *et al.*, 2013).

Numerous active compound have been isolated on nutmeg seed contains  $\alpha$ - pienne, sabinee, safrole, terpinen-t-ol, myristicin,  $\alpha$ - terpine,  $\alpha$  – terpinene (Dorman *et al.*, 2000; Chatterjee *et al.*, 2007), eugonol, isoeugenol (Janssens *et al.*, 1990) lignans (diarylbutane, aryltetraline) (Kwon *et al.*, 2008), macelignan (Chung *et al.*, 2006). To best our knowledge, no report research conducted on effect of nutmeg extract towards chemical characteristics of raw beef during storage. Thus, the aim of this study was to investigate the effect of different concentration of nutmeg extract on colour, pH, lipid peroxidation, and proximate of raw beef during storage for 3 weeks at temperature of  $-18^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

## Material and Methods

### *Nutmeg extraction and preparation*

Nutmegs were collected at Bukit Ekspo, UPM, Selangor. The samples were dried in the oven at  $50^{\circ}\text{C}$  for 3 days. One hundred gram of dried nutmeg was ground and extracted with 400 ml of 99.8% (w/v) absolute methanol (Sigma-Aldrich, Saint Louis, MO, USA) for seven days at room temperature as stated by Rukayadi *et al.* (2008), with some modification. After seven days, the plant material was filtered using Whatman No. 1 filter paper (Whatman International Ltd., Middlesex, England) and concentrated by using rotary vacuum evaporator (Heidolph VV2011, Schwabach, Germany) at  $50^{\circ}\text{C}$  with the speed of 150 rpm for 3 - 4 h. In order to obtain methanol free

extracts, the temperature was increased up to  $85^{\circ}\text{C}$  by two times for 30 seconds. This technique was suggested by Rukayadi *et al.* (2008) that increased temperature  $85^{\circ}\text{C}$  by two times did not affect the active compounds in the extract. The crude extract was then stored at  $4^{\circ}\text{C}$  prior to use.

### *Nutmeg extracts preparation, beef sampling and treatment*

Crude extract of nutmegs were diluted using sterile deionized water (DIW) (B. Braun Medical Industries, Penang, Malaysia) to make 0.00%, 0.25%, 0.50%, 1.25%, 2.50% and 5.00% (w/v). Beef samples were purchased at Pusat Sembelihan Lembu Daging, Shah Alam, Selangor. The samples were then transported to the Food Science Laboratory, UPM in ice box within 40 minutes. Then, the beef meat were cut into  $2.5 \times 2.5 \times 2.0$  cm, average of  $3 \pm 1$  g and divided into 3 groups and randomly selected for homogenous sample. Beef samples were soaked with 5 different concentrations mentioned above; whereas 0.00% (only DIW) was a control for 15 min. Beef samples were then packaged with overwrapped trays with stretched polyethylene permeable film (Polypack Enterprises, Selangor, Malaysia) and stored at  $-18 \pm 1^{\circ}\text{C}$  for 3 weeks. Beef samples were thawed at  $4^{\circ}\text{C}$  for 8 hours before further analysis.

### *Lipid oxidation or thiobarbituric acid-reactive substance (TBARS) analysis*

TBARS values were measured using distillation method of Tarladgris *et al.* (1964). A 10 g of meat was homogenized with 100 ml of distilled water in round bottom flask for 5 min. Thereafter, a few drops of 4 M of hydrochloric acid (Qrec Asia, Selangor, Malaysia) were added to make the mixture pH 1.5. Mixture was distilled through condensing to collect the distillate. A 5 ml of distillate was mixed with 5 ml thiobarbituric acid (TBA) 0.02 M (Sigma Aldrich, Deutschland, Germany) and boiled in water for 35 min. Mixture was cooled for 10 min. Absorbance readings were measured at a wavelength of 538 nm against a blank (5 ml TBA and HCL) using spectrophotometer. The absorbance value was multiplied by a factor of 7.8 (Tarladgris *et al.*, 1964) to obtain TBARS values. The experiments were done two times with triplicates ( $n = 2 \times 3$ ).

### *Beef colour analysis*

Beef colour was estimated using a Minolta CM2002 spectrophotometer (Minolta, Nieuwegein, Netherland) in colorimetric paramaters CIELab space; Lightness  $L^*$  (100= white, 0= black), redness  $a^*$  (+ red – green) and yellowness  $b^*$  (+ yellow – blue). The beef

were measured on four spots on each sample after thawed of the samples. The equipment was calibrated with white plate provided by manufacturer ( $Y=92.6$ ,  $X=0.3136$ ,  $y=0.3196$ ). The experiments were done two times with triplicates ( $n=2 \times 3$ ).

#### *Measuring the pH*

A ten grams of sample from each treatment were mixed with 20 ml of distilled water and was homogenized with a mixer. The pH value was then measured by pH meter (Delta Track Inc., California, USA). The pH meter was calibrated using standard buffers of pH 4.0 and pH 7.0. The experiments were done two times with triplicates ( $n=2 \times 3$ ).

#### *Determination of moisture content*

Moisture content was analyzed to determine moisture loss during storage using oven drying method of Association of Official Analytical Chemists (AOAC). Five grams of each sample were weight in triplicate in dried-clean porcelain crucibles for 24 hours at 100°C oven. After drying, samples together with the crucible were placed in a desiccator and then weighed. Percentage of moisture content was calculated according to formulation by AOAC. The experiments were done two times with triplicates ( $n=2 \times 3$ ).

#### *Determination of fat content*

Beef samples were blended in a blender (Panasonic, Selangor, Malaysia). A 5 g of blended beef were weighted in the thimble and placed into the soxhlet apparatus. Round bottom flask was placed in the oven (105°C) for 30 minutes and the weight was recorded. A 200 ml petroleum ether (Qrec Asia, Selangor, Malaysia) was poured in the flask. The Soxhlet apparatus was connected to the reflux and let the sample refluxed continuously for 8 hours. After 8 hours, the petroleum ether from the round bottom flask was evaporated using rotary evaporator. The round bottom flask was placed again in the oven for 15 min. Then, weights of the flask together with the fat extracted were recorded. The experiments were done two times with triplicates ( $n=2 \times 3$ ).

#### *Determination of protein content*

A 0.15 g of beef sample and 0.8 g of mixed catalyze were added in conical flask. Then, 2.5 ml of concentrated sulphuric acid (Qrec Asia, Selangor, Malaysia) was added to mixture. The mixture was then swirled and boiled in the heating coil. After heated, let it rest until 40°C. A 10 ml of distilled water were added to the mixture and transfer to the into distillation tube. Then, 10 ml of 45% of sodium

hydrochloride (Merck, Washington, USA) were added slowly to separate two layers of solution. Then, the distillation tube was fixed into the condenser. Conical flasks with 10 ml of 2% boric acid (Qrec Asia, Selangor, Malaysia) were used as distillate flask. After ammonia solutions have distilled in the conical flask, it was titrated with unreacted 0.05N sulphuric acid until it become neutral colour. The procedure was repeated for blank (no sample). Percentage of protein was calculated as percentage of nitrogen according to formulation of AOAC (2002). The experiments were done two times with triplicates ( $n=2 \times 3$ ).

#### *Statistical analysis*

Means of color, pH and TBARS value, moisture and fat content value, from each treatment ( $n=2 \times 3$ ) were calculated. Data were analyzed using MINITAB for the analysis of variance (ANOVA), one-way, unstacked, where Tukey's test was used to determine the significance of difference ( $p=0.05$ ) between different treatments.

## **Result and Discussion**

#### *Nutmeg extraction*

The medicinal properties of plant spices have gained wide interest and recognition by researcher due to diversity of phytochemicals that is proven to maintain the quality of food. Besides that, it is safe and has only minimal sides effects compared to synthetic preservatives (Zink, 1997). Results of present research indicate that nutmeg extracts has the ability to control quality of beef; maintain the bright red color, moisture, fat, pH and retard the lipid oxidation at frozen storage.

In this study, crude extract of nutmeg were extracted using methanol absolute. To obtain plants crude extracts, solvent used is crucial. Solvent used must be able to extract as much as possible components of phytochemicals in these plants. The polar methanol solvent is able to produce higher yield with higher bioactivities including antioxidant activities (Hirai, 1986). Pooja *et al.* (2012) and Lan *et al.* (2007) suggested to use methanol as a solvent in order to obtain better major antioxidants present in the nutmeg. In contrast, the use of methanol for the purpose of food applications is not recommended. However, some modifications method was done as described in "Nutmeg extract preparation, beef sampling and treatment" where the temperature was increased up to 85°C by 2 times for 30 seconds during evaporation. At this temperature, the methanol would all be evaporated as its boiling temperature was only

Table 1. Color of beef treated with nutmeg extract during storage at -18°C

Redness a*						
[%] and Day	0	4 <sup>th</sup>	7 <sup>th</sup>	10 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>
0.00%	8.47 ± 0.11 <sup>b</sup>	6.29 ± 0.02 <sup>b</sup>	5.32 ± 0.06 <sup>d</sup>	5.27 ± 0.06 <sup>a</sup>	5.01 ± 0.07 <sup>c</sup>	4.92 ± 0.08 <sup>b</sup>
0.25%	8.53 ± 0.15 <sup>b</sup>	6.49 ± 0.04 <sup>ab</sup>	5.71 ± 0.09 <sup>cd</sup>	5.49 ± 0.04 <sup>ab</sup>	5.49 ± 0.26 <sup>bc</sup>	5.01 ± 0.07 <sup>cd</sup>
0.65%	8.69 ± 0.10 <sup>ab</sup>	6.57 ± 0.06 <sup>ab</sup>	5.90 ± 0.01 <sup>bcd</sup>	5.60 ± 0.03 <sup>abc</sup>	5.28 ± 0.43 <sup>ab</sup>	5.57 ± 0.41 <sup>ab</sup>
1.25%	8.03 ± 0.28 <sup>ab</sup>	6.63 ± 0.14 <sup>a</sup>	6.015 ± 0.07 <sup>bc</sup>	5.89 ± 0.03 <sup>ab</sup>	5.58 ± 0.03 <sup>ab</sup>	6.29 ± 0.17 <sup>a</sup>
2.50%	8.65 ± 0.15 <sup>a</sup>	6.66 ± 0.14 <sup>ab</sup>	6.54 ± 0.35 <sup>ab</sup>	6.18 ± 0.09 <sup>bc</sup>	6.01 ± 0.04 <sup>ab</sup>	6.24 ± 0.19 <sup>a</sup>
5.00%	8.72 ± 0.52 <sup>a</sup>	6.69 ± 0.08 <sup>a</sup>	6.75 ± 0.06 <sup>a</sup>	6.59 ± 0.23 <sup>a</sup>	6.38 ± 0.09 <sup>a</sup>	6.31 ± 0.21 <sup>a</sup>
Lightness L*						
[%] and Day	0	4 <sup>th</sup>	7 <sup>th</sup>	10 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>
0.00%	50.98 ± 1.51 <sup>ab</sup>	46.89 ± 1.08 <sup>b</sup>	49.23 ± 0.57 <sup>a</sup>	55.82 ± 0.71 <sup>c</sup>	54.95 ± 0.82 <sup>a</sup>	66.89 ± 0.82 <sup>a</sup>
0.25%	46.66 ± 0.23 <sup>b</sup>	47.31 ± 1.56 <sup>b</sup>	46.21 ± 0.94 <sup>a</sup>	46.76 ± 0.63 <sup>c</sup>	44.72 ± 0.26 <sup>a</sup>	49.71 ± 0.55 <sup>c</sup>
0.65%	48.44 ± 0.23 <sup>ab</sup>	45.25 ± 1.42 <sup>b</sup>	46.60 ± 0.39 <sup>a</sup>	47.34 ± 0.81 <sup>abc</sup>	46.27 ± 0.07 <sup>a</sup>	49.77 ± 0.63 <sup>c</sup>
1.25%	49.02 ± 0.24 <sup>ab</sup>	46.38 ± 0.59 <sup>b</sup>	48.49 ± 1.65 <sup>a</sup>	47.89 ± 0.81 <sup>abc</sup>	46.33 ± 0.03 <sup>a</sup>	50.84 ± 0.74 <sup>b</sup>
2.50%	50.001 ± 0.44 <sup>a</sup>	49.61 ± 0.14 <sup>ab</sup>	49.23 ± 0.14 <sup>a</sup>	49.61 ± 0.41 <sup>ab</sup>	46.84 ± 0.72 <sup>a</sup>	52.45 ± 0.19 <sup>b</sup>
5.00%	50.98 ± 0.48 <sup>a</sup>	52.25 ± 0.44 <sup>b</sup>	47.93 ± 0.23 <sup>a</sup>	49.92 ± 0.57 <sup>a</sup>	49.77 ± 0.21 <sup>a</sup>	49.28 ± 0.13 <sup>c</sup>
Yellowness b*						
[%] and Day	0	4 <sup>th</sup>	7 <sup>th</sup>	10 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>
0.00%	17.43 ± 0.25 <sup>a</sup>	16.29 ± 0.23 <sup>a</sup>	16.36 ± 0.14 <sup>b</sup>	15.17 ± 0.21 <sup>a</sup>	14.92 ± 0.21 <sup>a</sup>	14.51 ± 0.25 <sup>a</sup>
0.25%	17.18 ± 0.71 <sup>a</sup>	16.38 ± 0.13 <sup>a</sup>	16.68 ± 0.43 <sup>ab</sup>	15.65 ± 0.47 <sup>a</sup>	14.98 ± 1.02 <sup>a</sup>	14.59 ± 0.54 <sup>a</sup>
0.65%	17.32 ± 0.29 <sup>a</sup>	16.66 ± 0.45 <sup>a</sup>	16.08 ± 0.22 <sup>b</sup>	15.46 ± 0.19 <sup>a</sup>	15.32 ± 0.07 <sup>a</sup>	14.28 ± 0.49 <sup>a</sup>
1.25%	17.34 ± 0.48 <sup>a</sup>	16.98 ± 0.35 <sup>a</sup>	16.17 ± 0.31 <sup>b</sup>	15.81 ± 0.23 <sup>a</sup>	15.35 ± 0.52 <sup>a</sup>	14.58 ± 0.14 <sup>a</sup>
2.50%	17.34 ± 0.44 <sup>a</sup>	16.33 ± 0.18 <sup>a</sup>	15.94 ± 0.19 <sup>b</sup>	15.39 ± 0.11 <sup>a</sup>	15.50 ± 0.70 <sup>a</sup>	14.49 ± 0.29 <sup>a</sup>
5.00%	17.71 ± 0.39 <sup>a</sup>	16.55 ± 0.32 <sup>a</sup>	17.24 ± 0.42 <sup>a</sup>	15.65 ± 0.47 <sup>a</sup>	15.27 ± 0.78 <sup>a</sup>	14.67 ± 0.45 <sup>a</sup>

Mean values ± standard deviation with different lowercase letters in the same row have significance different (p<0.05).

at 63°C. Thus, crude extract obtained in this study was free-crude methanol extract.

#### Lipid oxidation or thiobarbituric acid-reactive substance (TBARS) analysis

In this study, the effect of nutmeg extract at different concentration on beef towards TBARS formation during storage at -18°C have been investigated. The result is shown in Figure 1. Untreated samples showed higher in TBARS values than those of all treated samples. Accumulation of TBARS in beef might be caused by the fraction of unfrozen water or releasing of pro-oxidants during thawing-freezing of muscle tissue initiate the lipid oxidation (Coleen *et al.*, 2012). During frozen storage, lipid oxidation is usually slow but does not stop because the reactive species are soluble in the lipid fraction and stable at the low temperatures (Zarzycky and Swiniarska, 1993). Previous report mentioned that the low temperature storage promotes oxygen penetration in meat surfaces which leads to increasing the depth of oxymyoglobin on the surfaces (Hood, 1984). Beef treated with concentration of 5.00% was able to reduce range of 30-40% TBARS value compared to untreated.

All treated sample gives low TBARS values, meaning the extracts used able to maintain the oxidative stability of beef at -18°C. These results are in agreement with Dorman *et al.* (2000), stated that nutmeg has antioxidant properties, where it contain isoeugenol, eugenol and B-carophyllne which are

less polar and have a partition coefficient greater interaction with lipid bilayer leads to high antioxidant content. Moreover, the characteristic of active compound in nutmeg does help antioxidant in the treated beef due to benzyl and aromatic compound of myristicin, eliminein, safrole, that have better abstraction of hydrogen from free radicals formed (Ashish *et al.*, 2013).

#### Beef colour analysis

Table 1 shows colour of beef samples treated with nutmeg extracts during frozen storage. The redness and lightness (Table 1) of treated samples had significant differences with untreated samples, meanwhile for the yellowness (Table 1) there were no significant differences. Mancini and Hunt (2005) reported that color stability was correlated to lipid oxidation and natural pigments. Based on Table 1, lightness of untreated beef (0.00%) was increased from 50.98 to 66.89. This may due to scattered light reflection due to oxidized lipids (Coleen *et al.*, 2012; Zaritzky, 2012).

For redness a\*, beef treated with 1.25% and above of nutmeg extract were no significant difference decrease in a\* from the first day regards to maintain the red colour stability of the beef with during frozen storage during 3 weeks (Table 1). However, untreated samples and treated samples with the concentration of 0.25% and 0.65% showed significant different decrease in a\* (redness) from first day may due to the variety of the phenolic acid compounds available in

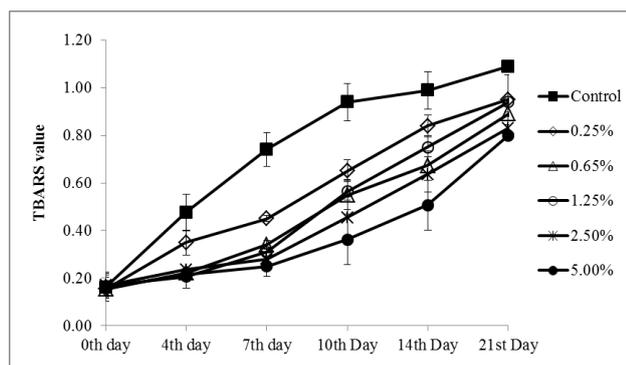


Figure 1. TBARS value formation in beef with treated with nutmeg extract during storage at  $-18^{\circ}\text{C}$ . Beef treated without nutmeg extract (-■-), with 0.25% of nutmeg extract (-◇-), 0.65% of nutmeg extract (- -), 1.25% of nutmeg extract (-○-), 2.50% of nutmeg extract (-x-) and 5.00% of nutmeg extract (-●-). Significantly different ( $p < 0.05$ ) from the control (without extract)

different nutmeg extract concentration which related to the oxidation. Increase of lipid oxidation may reduce the redness of the beef samples. This suggestion was similar as reported by Abdalla and Roozen (1999) and Mariana *et al.* (2014) with reducing colour of frozen meat. Mariana *et al.* (2014) reported that colour of beef frozen patties were significantly loss of redness for first 4 weeks of storage influenced by oxidized meat. Abdalla and Roozen (1999) studied colour of beef muscle frozen at  $-18^{\circ}\text{C}$  were reduce due to metmyoglobin accumulation. This can be explained with cell ruptured by ice crystallization during storage contact with myoglobin leads to myoglobin auto-oxidation and related lipid myoglobin oxidation (Zaritzky, 2012).

#### The pH measurement

Figure 2 shows pH values of beef samples treated with nutmeg extracts during frozen storage. Muscles of beef during frozen storage were frozen and may alter the pH of beef. Usually, muscle tissue starts to freeze at  $-1^{\circ}\text{C}$  and muscle are leaning to supercool, water in tissue start out to freeze without ice crystal formation (Gill and Shand, 1993). Figure 2 indicates pH value of all samples beef decreased ranged from 6.43 to 5.23 starting from 10th day of storage. Untreated samples (0.00%) showed lowest pH values (5.13) compared to all treated samples at the 21st day (5.72 to 6.03). This may due to dissociations of organic acids, lactic acids, acetic acids in muscle tissue. The findings of pH values are in agreement with Leygonie *et al.* (2011) and Jeremiah and Gibson (2001). Leygonie *et al.* (2011) reported that meat that has been frozen and thawed have lower pH values compared to non-thawed freezing. This is due to pH value is measured base on the amount of free hydrogen ion, when the beef samples was freeze, the

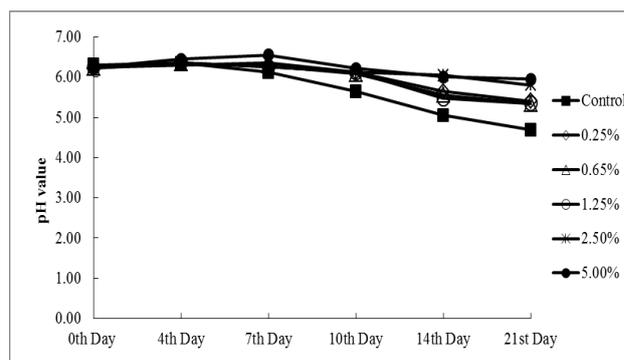


Figure 2. Measurement pH values of beef treated with nutmeg extract during storage at  $-18^{\circ}\text{C}$ . Beef treated without nutmeg extract (-■-), with 0.25% of nutmeg extract (-◇-), 0.65% of nutmeg extract (- -), 1.25% of nutmeg extract (-○-), 2.50% of nutmeg extract (-x-) and 5.00% of nutmeg extract (-●-). Significantly different ( $p < 0.05$ ) from the control (without extract).

proteins may denatured and released the  $\text{H}^+$  ion and leads to give lower pH value. Loss of the fluid during storage may result lower pH (Coleen *et al.*, 2014). This mean beef treated with nutmeg during storage may control loss of fluid as well as maintaining the pH values.

#### Measurement of moisture, protein and fat content

In this study, analyzing moisture, fat and protein was done to determine the effect of nutmeg extract on these nutrition values of beef. The percentage of moisture, protein and fat content of beef samples were shown in Figure 3a, 3b and 3c, accordingly. All beef samples contain protein ranging from 19.00 to 19.08% per 100 g; no significant difference ( $p > 0.05$ ) among them (Figure 3b). This mean there was no effect of nutmeg extract on protein content of beef. Meanwhile, moisture content of beef treated with nutmeg varies significantly difference ( $p < 0.05$ ) among five concentration of nutmeg extract tested.

Figure 3a, shows that starting at the 10<sup>th</sup> day of storage, untreated sample loss moisture content range of 10%-20% compared to treated sample (<10%). The results are in agreement with the results of Mariana *et al.* (2014) reported on increasing of moisture loss in beef patties. She mentioned that frozen storage and thawing process led to moisture loss and decrease water holding capacity. Moreover, Coleen *et al.* (2012) explained that freezing and thawing affect the amount of exudate (thaw or drip loss). Moisture loss is caused by ice crystal formation on the surface of beef during storage as well as thawing process (Zaritzky, 2012). Figure 3a shows reducing of moisture losses starting at beef treatment of 0.65% nutmeg extract which ranging <10% of moisture loss compared to untreated beef 20% of moisture loss. This can be explained by the fact that nutmeg extract is an oil

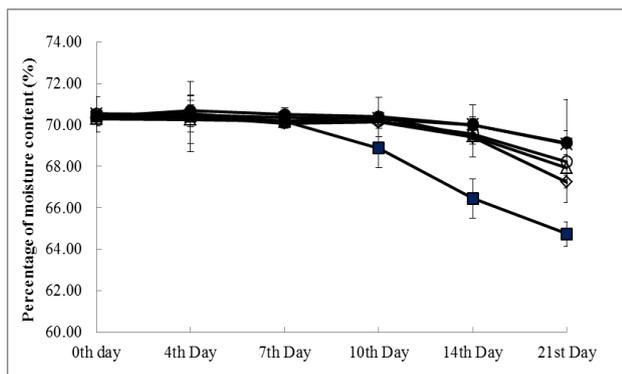


Figure 3a. Moisture content of beef treated with nutmeg extract during storage at -18oC. Beef treated without nutmeg extract (-■-), with 0.25% of nutmeg extract (-◇-), 0.65% of nutmeg extract (- -), 1.25% of nutmeg extract (-○-), 2.50% of nutmeg extract (-x-) and 5.00% of nutmeg extract (-●-). Significantly different (p<0.05) from the control (without extract)

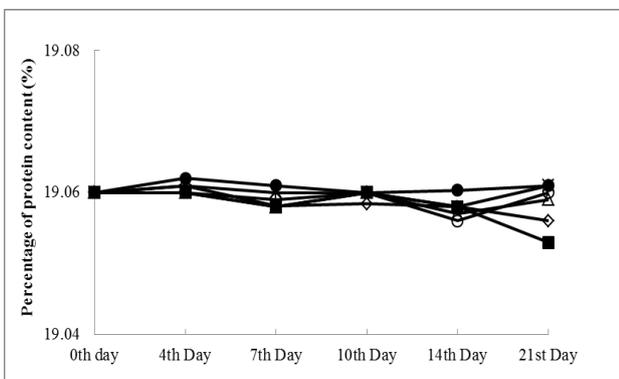


Figure 3b. Percentage of protein content of beef treated with nutmeg extract during storage at -18oC. Beef treated without nutmeg extract (-■-), with 0.25% of nutmeg extract (-◇-), 0.65% of nutmeg extract (- -), 1.25% of nutmeg extract (-○-), 2.50% of nutmeg extract (-x-) and 5.00% of nutmeg extract (-●-). Significantly different (p<0.05) from the control (without extract)

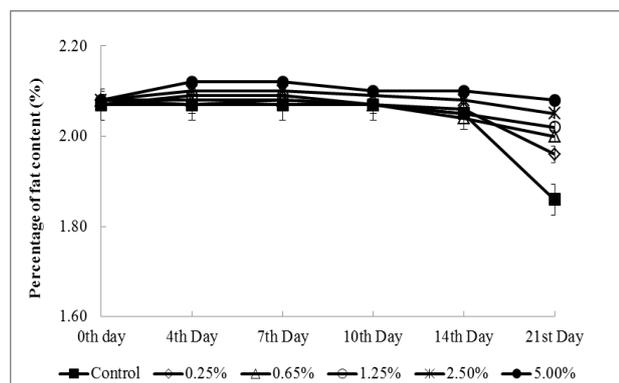


Figure 3c. Percentage of fat content of Percentage of protein content of beef treated with nutmeg extract during storage at -18oC. Beef treated without nutmeg extract (-■-), with 0.25% of nutmeg extract (-◇-), 0.65% of nutmeg extract (- -), 1.25% of nutmeg extract (-○-), 2.50% of nutmeg extract (-x-) and 5.00% of nutmeg extract (-●-). Significantly different (p<0.05) from the control (without extract)

base extract which can act as a protection barrier that covers up the surface area of beef samples preventing from the moisture loss during frozen storage.

Figure 3c shows percentage of fat content of beef samples treated with nutmeg during frozen storage. Untreated samples (0.00%) showed a reduction of fat content (0.2% of loss fat) at 21<sup>st</sup> day of storage whereas for beef treated with 5.00%, 2.50% ,1.25% and 0.65% concentration were only slightly reduced (<0.1% of loss fat). Based on the result, fat content of untreated sample is significant difference (p<0.05) with all treated samples, whereas there are no significant different between with the concentration of extract. The reduction of fat content can be correlated to lipid oxidation which discussed previously.

Research on the effect of different concentrations nutmeg extract on chemical characteristic (oxidative stability, pH, colour, moisture, fat and protein content) of beef at storage at -18°C was done. This research was conducted principally for the achievement on the best combination between different concentrations of extract and the effect of nutmeg extract on chemical characteristic that may serve as potential natural preservatives for beef. Lipid oxidation, pH and colour were analyzed towards beef samples as indicator freshness of beef during storage. Moisture, fat and protein analysis were done to determine effect of the nutmeg extract on moisture loss, protein and fat content changes of beef during storage which contribute to the quality of the beef. This research indicates that the best combination was 1.25% and above of *M. fragrans* Houtt., (Nutmeg) extract slow down the formation of thiobarbituric acid reactive substances, moisture loss and does not affect pH, colour, and fat content of beef during storage at -18°C. However, further research need to be done to compare with normal storage condition since -18°C are known to prolong the shelf life and to effectively compare the performance of the extract.

**Conclusion**

Frozen storage affects the quality of beef and requires a mild preservations technique to maintain the fresh quality of beef. In this research finding, the addition of nutmeg extracts in beef retards the formation of TBARS during 3 weeks of storage at -18°C. *M. fragans* Houtt. extract (1.25% and above) has shown to be able to maintain the redness, and nutritional value (fat, moisture and protein) of beef due to associated oxidative stability and natural pigments in the nutmeg. These findings could give benefits to consumers and food industry especially in natural preservative of beef products and provides an

alternative way to improve the quality of beef during frozen storage. For future research, the isolation and identification of active compounds using gas chromatography could be done.

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