PAPER

EVALUATION OF PHYSICOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF CHICKEN SAUSAGE INCORPORATED WITH DIFFERENT VEGETABLES

S. AHMAD, S. JAFARZADEH, F. ARIFFIN* and S. ZAINUL ABIDIN
Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
*Corresponding author: fazilah@usm.my

ABSTRACT

The physicochemical, antioxidant, antimicrobial and nutritional features of Capsicum, carrot, spinach, purple cabbage and oyster mushroom incorporated in chicken sausages were investigated in this study. The analysis of microstructure showed that sausage with vegetables had a less dense microstructure mainly due to the consequent increase in moisture content, decrease in fat content and decrease in protein density. The highest DPPH value and total phenolic content were demonstrated by the sausages incorporated with purple cabbage while incorporation of spinach gave the highest total flavonoid content. During the end of storage study, the peroxide value in sausages incorporated with purple cabbage significantly ($P<0.05$) delayed lipid oxidation compared to the control from 1.62meq/kg to 0.58meq/kg. The microbial activity was reduced with the incorporation of vegetables, which subsequently would help to extend the shelf-life of the samples. The results suggest that vegetables used are potentially useful in improving the nutritional quality in chicken-based sausage-like product.

Keywords: hysicochemical properties, antioxidant properties, antimicrobial properties, vegetables, chicken sausage

1. INTRODUCTION

Changes in lifestyle and eating habits of human beings, has been shown by researchers and health organizations (World Health Organization (WHO)), Food and Agriculture Organization (FAO) to be the major cause of increases of diseases like, obesity, cancer, cardio vascular failures, (Who and Organization, 2003). Nowadays people are showing greater interest in foods that contain bioactive or functional components, which will give additional benefits to their health status (COFRADES *et al*., 2008b).

Chicken meat and its products have experienced increasing popularity and become widely spread all over the world. Chicken sausage is one of the popular foodstuffs among these products (BARBUT, 2016), while continually eating processed meat products will affect health status and at the same time will increase cancer risk (Chan *et al*., 2008). thus the demands have initiated to an extensive research on the meat industry to incorporate health-enhancing ingredients. In order to achieve this goal, application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat safety status, quality, extending shelf-life and preventing economic loss (YIN and CHENG, 2003).

Many researches have been conducted to explore the feasibility of using non-meat ingredients to promote a healthier meat sausage product and much research has indicated that lipid oxidation and microbial growth in meat products can be controlled or minimized by using either synthetic or natural food additives (COFRADES *et al*., 2008a) Various synthetic antioxidants, are commonly used to delay the development of rancidity in food products (MARTINEZ-TOME *et al*., 2001). However, consumers are concerned about the safety of synthetic food additives. This concern has led to arouse a great interest in natural additives (POKORNÝ, 1991). Natural agents possessing antioxidant and antimicrobial properties have the advantage of being readily accepted by consumers, as they are considered natural.

Capsicum, carrot, spinach, purple cabbage and oyster mushroom can provide several health benefits in daily diet. Capsicum is the second-most consumed vegetable worldwide and is characterized by its high levels of vitamin C (ascorbic acid), pro-vitamin A (carotene) and calcium. In fact, intakes of 50-100 g fresh capsicum could provide 100% and about 60% of the recommended daily amounts of vitamin C and A, respectively. Capsicums are also rich in carotenoids, compounds with antioxidant and anticarcinogenic capacity (Marti *et al*., 2011). The carrots are the unique roots, contain other compounds, such as phenolic compounds and organic acids and have a characteristic flavor due to the presence of terpenoids and polyacetylenes, therefore, it has nutritional property for human health (RAEES *et al*., 2015). Also spinach is an extremely nutritious vegetable, rich both in core nutrients and phytochemicals. The major micronutrients in spinach are vitamins A (from β-carotene), C, K and folate, and the minerals, calcium, iron and potassium. Spinach also provides fibre and is low in calories (HEDGES and LISTER, 2007). Purple cabbage is a rich source of anthocyanins, minerals, vitamins, oligosaccharides and a number of bioactive substances that provides a positive impact on human health (SYUHAIRAH *et al*., 2016). Oyster mushroom has a great nutritional value since they are an excellent source of protein, with an important content of essential amino acids and fiber, and poor in fat and also they provide a nutritionally significant content of vitamins (B_1, B_2, B_{12}, C, D, and E) and often regarded as an ideal and healthy food for people (REIS *et al*., 2012).

To overcome the disadvantages of replacing synthetic antioxidants and colorants with natural ones in meat and meat products, focus of the current study is on antioxidant and antimicrobial effects and color properties of plant based materials such as Capsicum, carrot, spinach, purple cabbage and oyster mushroom. Therefore, the objective of the present study was to determine the physicochemical, nutritional, antioxidant and antimicrobial properties of chicken sausage added with Capsicum, carrot, spinach, purple cabbage and oyster mushroom.

2. MATERIALS AND METHODS

2.1. Materials

Minced chicken meat (MCM) and five types of vegetables; capsicum (*Solanaceae annuum*), carrot (*Daucus carota*), spinach (*Spinacia oleracea*), purple cabbage (*Brassica olerace*) and oyster mushroom (*Pleurotus sajor-caju*) were purchased from a local market in Penang, Malaysia. The trichloroacetic acid was purchased from R&M Chemicals, UK, malondialdehyde, boron trifluoride-methanol (Merck, Germany), thiobarbituric acid, cathechin (Sigma-Aldrich, USA) and all other chemical reagents used for the experiments were of analytical grade.

2.2. Preparation of chicken sausage

Mechanically Deboned Chicken Meat (MDCM) and five types of vegetables; spinach, purple cabbage, carrot, capsicum and oyster mushroom were purchased from a local market in Penang, Malaysia. In our previous research, we were conducted with various percentages (30%, 40% and 50%) of vegetables in the sausage formulations. Based on last study results, sausages formulated with 40% vegetables were selected as the best formulation (SYUHAIRAH *et al*., 2016). The selection of the best formulation was based on the result of physical properties of the sausages prepared. As shown in Table 1 the sausages with the selected formulation were prepared and all ingredients were mixed for 5 min using a mixer (Robot Coupe®, Blixer 3, France).

Table 1. Formulations of sausage preparation.

Ingredients (g)		Samples/100g
	Control	V40
Minced chicken meat	75	45
Vegetables	-	30
Tapioca flour	10	10
Spices	4	4
Palm oil	1.8	1.8
Fresh egg white	3	3
Cold water	3	3
Salt	1.7	1.7
Sugar	1.35	1.35
Sodium thiosulphate	0.15	0.15

Ratio of vegetables: chicken meat = 40:60 Vegetables: Capsicum (CP), carrot (C), spinach (S), purple cabbage (PC), oyster mushroom (OM).

The batter was then stuffed manually into 2.5 cm diameter cellulose casing. Sausages were steamed in a steamer (Electric and Steamer, Model RS-6, 0881, China) until their internal temperature reached 72±2EC (measured using a thermocouple probe) and held for approximately 30 min. The steamed sausages were promptly cooled in ice water for 15 min, the casing was peeled and vacuum-packed. The prepared sausage samples were kept in the freezer at -18EC prior to analyzes.

2.3. Scanning Electron Microscope (SEM)

Scanning electron microscope (SEM) Microstructure characteristics of the samples were measured according to the procedure described by Andres *et al*. (2006). Small pieces of sausages of 0.5cm in diameter and 0.2-0.3cm thick were used for analysis. The samples were dehydrated under vacuum (sputtering), allowing surface and cross-section visualization. Micrographs of the samples were obtained by scanning electron microscope (SEM 505, Philips, Netherlands)

2.4. Chemical composition of sausages

2.4.1 Moisture determination

Moisture content in the samples was determined by using AOAC Method number 960.39 (William 2000) – Oven Drying Method. The moisture content was calculated using the following equation:

$$\text{Moisture content } (\%) = \frac{\text{weight of wet sample (g)} - \text{weight of dried sample (g)}}{\text{weight of wet sample (g)}} \times 100$$

2.4.2 Protein determination

The amount of protein content in the percentage of protein content was determined by multiplying the percentage nitrogen of sample with a factor of 6.25. The percentage of protein was calculated as below:

$$\%(\text{w/v}) \text{ Nitrogen} = \frac{(\text{mL HCL of sample} - \text{mL HCL of blank}) \times \text{HCL molarity} \times 14 \times 100}{\text{Weight of sample (g)}} \times 100$$

$$\%\text{Protein content} = \% \text{ Nitrogen} \times 6.25 \text{ (conversion factor)}$$

2.4.3 Fat determination

The amount of fat content in sausage was determined based on AOAC Method number 960.39 (William 2000) – Soxhlet Method. The percentage of crude fat was calculated as below:

$$\%\text{fat (dry basis)} = \frac{Weight\ of\ flask\ with\ fat\ (g) - Weight\ of\ flask\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

2.4.4 Ash determination

The amount of ash in sausages was determined by using AOAC Method number 945.38 (William 2000) – Dry Ashing Method. Percentage of ash content was calculated using the equation as below:

$$\%(\mathrm{w/w})\ \mathrm{Ash} = \frac{Weight\ of\ crucible\ with\ ash\ (g) - Weight\ of\ crucible\ (g)}{Weight\ of\ sample\ (g)}$$

2.4.5 Crude fiber determination

The amount of crude fiber in sausages was determined by using AOAC Method number 962.09 (William 2000)– Acid/alkaline Hydrolysis Method. Percentage of crude fiber content was calculated using the equation as below:
%(w/w) Crude fiber=
Where, S = Weight of sample (g), C = Weight of filter paper (g), A = Weight of crucible (g) + filter paper (g) + dried precipitate B = Weight of crucible (g) + ash (g)

2.5. Antioxidant composition

2.5.1 DPPH radical scavenging activity

DPPH radical scavenging activity was determined using a method described by Tangkanakul *et al*. (2009). Percentage of DPPH scavenging activity (%SA) was calculated from the equation (1-X/C)*100, where X is absorbance of extract and C is absorbance of control.

2.5.2 Total phenolic content

Total phenolics were determined using the method adapted from SINGLETON and ROSSI (1965). The results were expressed as mg gallic acid equivalent (GAE)/100g food.

2.5.3 Total flavonoids content

Total flavonoid content was determined according to the colorimetric method described by ZHISHEN *et al.* (1999), The total flavonoid content was expressed in mg of catechin equivalent per gram of sample (mg CE/g).

2.6. Storage test

The sausages with the selected formulation were used for the storage test. The samples were stored in a refrigerated incubator at 4±1°C and took three samples from each packaging at pre-determined time (0, 7, 14 and 21days of storage), and evaluated properties of the sausages for lipid oxidation and microbial activity.

2.6.1 Microbial analysis

Bacterial counts were carried out using pour plate method as described by Andrés *et al.* (ANDRES *et al*. 2006) for every seven days of refrigerated storage (4°C) for 0, 7, 14, 21 days. The initial dilution was made by aseptically blending 10g of the sample with 90mL peptone solution (1g/L) in Stomacher at 200 rpm for 1 minute. A series of sample dilution was prepared until 10^{-4} and plated duplicate with Plate Count Agar (PCA) for the total mesophilic aerobic count (incubated at 37ºC for two days), Potato Dextrose Agar (PDA) was used for yeast and mould count (incubated at 30ºC for five days). Data was expressed as log colony forming units (CFU)/g sample.

2.6.2 Peroxide value

Measurement of peroxide value (POV) was determined according to the SALLAM *et al.* (Sallam *et al.* 2004). The POV was calculated and expressed as milliequivalent peroxide per kg of sample:

$$\mathrm{POV\ (meq/kg)} = \frac{S \times N}{W} \times 100$$

Where, S = Volume of titration (mL) N = Normality of sodium thiosulfate solution W = Weight of sample (kg).

2.7. Statistical analysis

The results were analyzed using the two-way statistical analysis of variance (ANOVA), followed by Duncan multiple range test in the experiments of preliminary study. The results of chemical analysis and shelf life determination were compared using one-way ANOVA, followed by Duncan multiple range test. All data were processed using SPSS package (SPSS 21.0 for Windows, SPSS Inc, Chicago, Illinois, U.S.A) and expressed as mean value±standard deviation. Statistical significance was indicated at 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Scanning Electron Microscope (SEM)

The microstructures of sausages incorporated with 40% of vegetables were observed by using scanning electron microscope (SEM). Overall, the microstructures of samples were not visually different for each type of vegetables added as shown in Fig. 1. In general, all sausage samples had the microstructure consist of course protein gel matrix and fat with numerous holes, which formed a porous network structure. The fat globules observed and integrated in the matrix, bound by strands of 75 protein network. Meanwhile, fine strands and sheets within the protein matrix showed the presence of fiber in the sausage samples (Fig. 1e, f). Control sausage presented a denser network structures of a honeycomb-like appearance (Fig. 1a). Similar finding on the microstructure were also reported by AYO *et al*. (2008). Thus, consistent with the results that shows higher hardness and tougher sausage texture as presented in our previous work (SYUHAIRAH *et al*., 2016). According to CARBALLO *et al*. (1996), a less dense matrix microstructure, softer and chewy texture formed by sausage incorporated with vegetable is mainly due to consequent increase in moisture content, decrease in fat content and protein density. Based on the result, sample OM40 as in Fig. 1b shows a packed protein gel matrix compared to the rest of sausages with vegetable. This suggests due to the presence of high fiber and fat content as reported in Table 3.

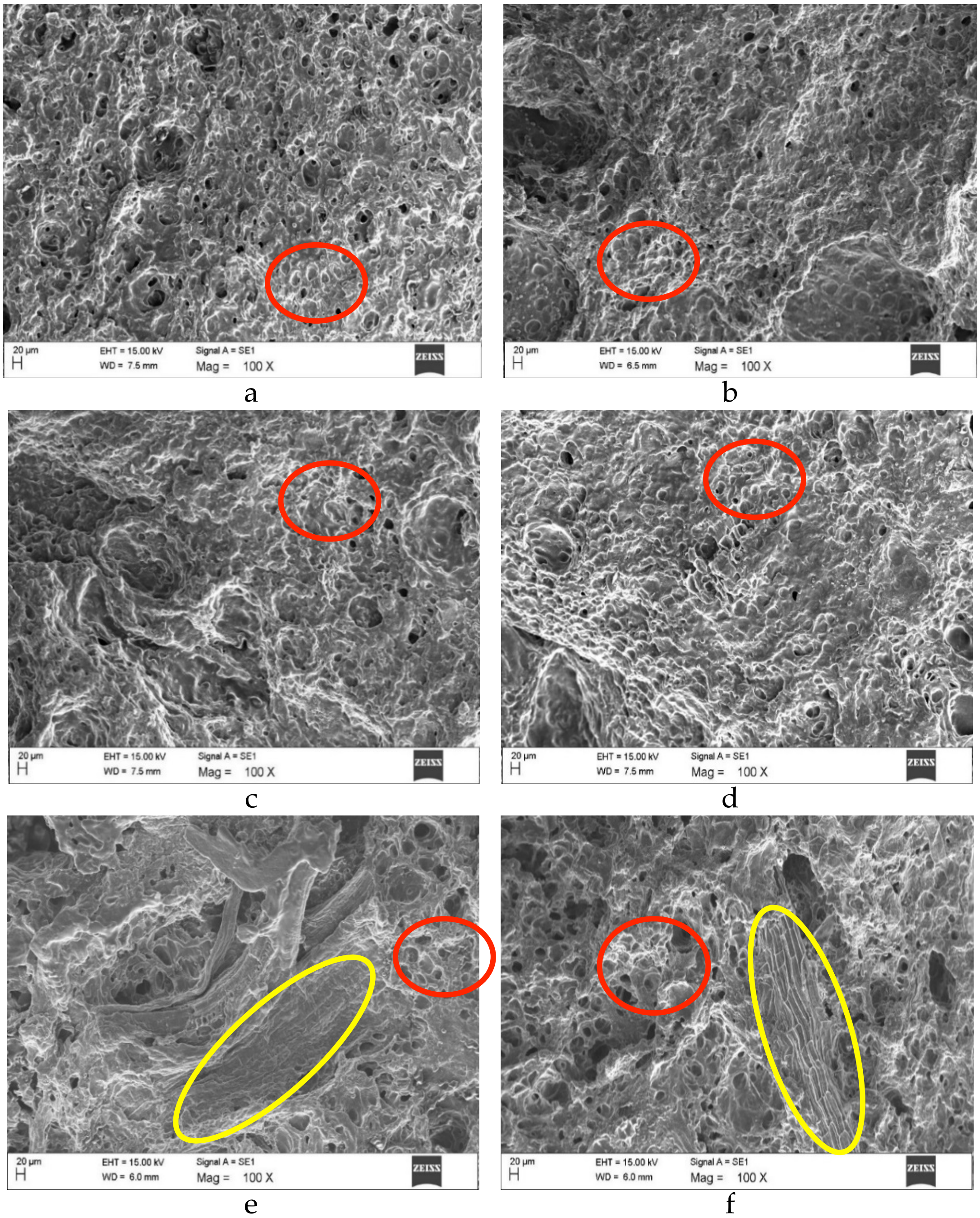

Figure 1. Scanning electron micrograph of sausages with 40% incorporation of vegetables at 100x magnifications: (a) Control (b) Oyster mushroom (c) Capsicum (d) Carrot (e) Spinach (f) Purple cabbage. Fat globules (O), fiber strands (O).

The sample OM40 however, yielded the lowest hardness value (3.73 kg) (SYUHAIRAH *et al*., 2016). This is probably due to smooth and velvety texture of oyster mushroom, as the alignment of myofibrillar proteins induced by incorporation of vegetables is depending on the original texture of vegetables and fiber content. As a result, a varied gel matrix or network is developed and water retained in the network might be different. Thus, the disposition and aggregation of protein filaments contributed to the difference in water-holding capacity as well as the textural properties.

3.2. Chemical composition

3.2.1 Moisture content and Fiber content

The moisture and fiber content of sausages are as shown in Table 2 Based on the results, the moisture content was significantly higher (P<0.05) for all sausages incorporated with vegetables (64.52-66.46%) as compared with the control sample (58.13%). This is most probably due to the incorporation of vegetables in sausage formulation, which provided a significant (P<0.05) amount of fiber content in the products. According to CHOI *et al*. (2010), addition of dietary fiber increases the moisture content of meat emulsion systems, providing higher water retention and improves emulsion stability. Additionally, GARCıA *et al*. (2002) indicated that the high moisture content of sausage by adding dietary fiber is due to high water retention of the fiber. These results agree with those reported by CHOI *et al*. (2012), who found a significant increase of moisture content in chicken frankfurter when added with pumpkin fiber. Similarly, LEE *et al*. (2009) showed that the addition of kimchi fiber also increased the moisture content of sausages produced.

3.2.2 Fat analysis

Based on the fat analysis, as shown in Table 2 control sausage (14.15%) yielded the highest fat content followed by sample OM40 (7.11%) > CP40 (6.79) > C40 (6.52) > S40 (6.41) and PC40 (5.84) significantly. The added vegetables may have affected fat content, as moisture content relatively increased (Choi *et al*. 2012). These findings are in tandem with the idea of Yang *et al*. (2007) who reported that replacing pork loin with hydrated oatmeal and tofu at 15% significantly lowers the amount of fat content. Similar 78 findings were reported by ALESON-CARBONELL *et al*. (2005) and CHOI *et al*. (2012) with addition of lemon albedo in dry-cured sausage and pumpkin fiber in chicken sausage. Moreover, the highest fat content in sample OM40 compared to other sausage samples with vegetable added is probably due to high fat content in oyster mushroom. Based on the USDA National Nutrient Database for Standard Reference (GEBHARDT *et al*. 2008), the comparison between different types of vegetables used in this study indicated that oyster mushroom contains the highest fat content (0.41g/100g) while the raw purple cabbage contains the lowest fat content (0.16g/100g).

3.2.3 Protein content

Table 2 shown Protein content was significantly (P<0.05) higher in control sausage (14.87%) compared to other sausages incorporated with vegetables. The main reason for that is due to the substitution of 40% chicken meat with vegetable, which lead to significant loss of protein and fat in the samples. (TROUTT *et al*. 1992) reported a similar trend in low fat beef patties containing polydextrose and oat flour as texture modifying ingredients. The replacement of lean meat by apple pomace in mutton nugget further supported the results (HUDA *et al*. 2014).

3.3.3 Ash content

Ash content was significantly ($P<0.05$) higher with the incorporation of vegetable which suggested that due to significant ($P<0.05$) increase in fiber content compared with control (Table 2). Studied by FERNANDEZ-GINES *et al*. (2004) observed that the ash content increases significantly with the addition of dietary fiber such as incorporation of dietary fiber from lemon albedo in low-fat sausage. Similar results were obtained by CHOI *et al*. (2012) who studied the physicochemical properties of reduced fat frankfurters by partially substituting pork back fat with a *makgeolli* lees fiber.

Table 2. Moisture, fat, protein and fiber of sausages containing chicken and 40% vegetables.

Sample	Moisture	Fat	Protein	Fiber	Ash
Control	58.13 ± 0.35^{d}	14.15 ± 0.18^{a}	14.87 ± 0.23^{a}	0.80 ± 0.01^{f}	2.90 ± 0.00^{d}
CP_{40}	64.54 ± 0.35^{c}	6.79 ± 0.06^{bc}	9.80 ± 0.35^{c}	1.21 ± 0.02^{e}	2.93 ± 0.01^{c}
C_{40}	64.52 ± 0.50^{c}	6.52 ± 0.32^{c}	10.13 ± 0.12^{bc}	2.46 ± 0.04^{c}	2.96 ± 0.01^{a}
S_{40}	65.56 ± 0.38^{b}	6.41 ± 0.37^{c}	10.13 ± 0.12^{bc}	2.75 ± 0.08^{b}	2.95 ± 0.00^{b}
PC_{40}	66.46 ± 0.18^{a}	5.84 ± 0.34^{d}	9.73 ± 0.23^{c}	2.30 ± 0.03^{d}	2.95 ± 0.00^{b}
OM_{40}	65.50 ± 0.63^{b}	7.11 ± 0.30^{b}	10.33 ± 0.31^{b}	2.94 ± 0.07^{a}	2.96 ± 0.01^{a}

CP= capsicum, C= carrot, S= spinach, PC= purple cabbage, OM= oyster mushroom Mean±SD lowercase within the column indicate significantly different ($P<0.05$).

3.3. Antioxidant composition

3.3.1 DPPH radical scavenging activity, total phenolic content and total flavonoid content

The use of antioxidant helps to minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality and increase the shelf life of food products (Fukumoto and Mazza 2000). Incorporation of capsicum, carrot, spinach, purple cabbage and oyster mushroom would help to deliver the benefits of high antioxidant properties of these vegetables into sausage samples. Therefore, numerous studies have been widely used to determine the antioxidant contents. In this study, DPPH radical scavenging activity, total phenolic content and total flavonoid content of methanolic sausages extract were analysed and the results are tabulated in Table 3. Based on the result, the highest DPPH value was demonstrated with the sausages incorporated with purple cabbage (87.38%SA) followed by capsicum (86.89%SA) with no significant difference. Meanwhile, sausage with spinach, oyster mushroom, carrot and control recorded (63.82%SA), (48.24%SA), (45.28% SA), and (42.21%SA) respectively, with significant differences. The high DPPH value in PC40 and CP40 samples indicates the higher ability of antioxidant compounds in these samples to lose a hydrogen and possibly acting as a primary antioxidant. Both samples also possibly have better reaction with free radicals, particularly of the peroxy radicals, which are the major propagator of the autoxidation chain of fat, thereby, abort the chain reaction. Osman and Milan (2006) reported that the DPPH value in raw purple cabbage was the highest (>80%SA) compared with bitter melon, paprika, mulberry leaves, grape peel, onion peel and red beet. The result found in this study is also in line with MATSUFUJI *et al*. (2007) who reported that the red capsicum has higher DPPH value (>80%SA) compared with green, yellow and green capsicum.

Phenolic compounds such as flavonoids, anthocyanins, and carotenoids are the major antioxidant components found in plants, which are free radical scavengers not only because of their ability to donate hydrogen atoms or electrons but also due to their stable radical intermediates (SHAHIDI *et al*. 1992). It was observed that PC40 recorded the highest phenolic content as well as antioxidant capacity. Meanwhile, control sausage had the lowest phenolic content and antioxidant capacity. The results demonstrated that the highest contribution of phenolic compounds provided by the incorporation of purple cabbage to the sausages, in comparison with the possible contribution provided by the chicken as in the control sausage. This showed that the radical scavenging effect of the sausages' extracts positively correlate with the total amount of phenolic compounds. Previous studies by BOO *et al*. (2012) also present positive correlation between the quantity of phenolic compounds and the DPPH free radical scavenging effect in extracted samples. Therefore, this study stipulated that incorporation of vegetables helps to improve the nutritional quality of new formulated sausages.

Flavonoids can be classified as anthocyanidins, flavanols (catechins), flavones, flavanones and flavonols, which are responsible for the orange, red and blue colour in fruits and vegetables (Lin and Tang 2007). However, to screen phenolic-rich, including flavonoid-rich fruits and vegetables, the value is not dependent on their colour. The total flavonoid content in sausage samples are in the order of S40 (6.24 mgCE/g) > OM40 (5.70 mgCE/g) > PC40 (5.11 mgCE/g) > CP40 (4.63mgCE/g) > C40 (4.30 mgCE/g) > control (2.93 mgCE/g). The highest flavonoid content in sample S40 is probably due to the high concentration of flavonoid in spinach. PANDJAITAN *et al*. (2005) reported that the total flavonoid in mature commercial cultivar of spinach was 12.7mgCE/g. Nonetheless, it is worth to note that the antioxidant in vegetables vary considerably due to several factors; genetics, cultivation practices, environmental, growing conditions, maturation, storage, and processing.

Table 3. Total phenolic content, DPPH and total flavonoid of sausages containing chicken and 40% vegetables.

Sample	Parameters		
	DPPH (%SA)	TPC (GAE/100g)	Flavonoid (mg CE/g)
Control	42.21±1.61^{e}	14.37±0.08^{e}	2.93±0.09^{f}
CP_{40}	86.89±1.33^{a}	21.18±0.02^{b}	4.63±0.03^{d}
C_{40}	45.28±0.41^{d}	15.51±0.13^{d}	4.30±0.06^{e}
S_{40}	63.82±0.79^{b}	17.23±0.33^{c}	6.24±0.27^{a}
PC_{40}	87.38±1.14^{a}	23.17±0.34^{a}	5.11±0.12^{c}
OM_{40}	48.24±2.34^{c}	17.24±0.22^{c}	5.70±0.15^{b}

CP = capsicum, C = carrot, S = spinach, PC = purple cabbage, OM = oyster mushroom.
Mean±SD. Lowercase within the column indicate significantly different (P<0.05).

3.4. Storage test

3.4.1 Peroxide value

The peroxide value (PV) is used as an indicator of the primary oxidation in sausage samples. Based on the result in Fig. 2, a gradual increase in PV was observed for all samples throughout the storage ($P<0.05$) from day 0 until day 21. Control sausage had significant ($P<0.05$) highest PV compared to sausages containing 40% vegetables as the storage time increased from 2.89 at day 0 to 6.00 at day 21, indicating that lipid oxidation was most extensive in this sample. This was expected as the control sample had the highest fat content (14.15%). Moreover, sausages incorporated with vegetables had a lower PV comparative to the control sample probably due to free radical scavenging antioxidants interfere with the initiation or propagation steps of lipid oxidation reactions by scavenging lipid radicals and forming low-energy antioxidant radicals that do oxidation of unsaturated fatty acids (MAQSOOD and BENJAKUL, 2010). Between samples incorporated with vegetable, S40 had the lowest initial PV followed by OM40, C40, CP40 and PC40 respectively. However, at the end of storage period, samples PC40 and CP40 showed the lowest PV. This suggested that possibility of hydroperoxide formed as a primary oxidation product in sample PC40 and CP40 might have undergone decomposition into secondary oxidation products. A decrease in the level of primary oxidation products is associated to hydroperoxide degradation, producing secondary lipid peroxidation products (BOSELLI *et al*., 2005). Overall, the result revealed that incorporation of purple cabbage gave the best antioxidative effect in the sausage and thus reduced the oxidation level. Nonetheless, all samples produced had PV within acceptable limits (3.11-6.00 meq/kg) until day 21 of storage. In all cases, PV was lower than 25 meq of active O_2/kg of fat, which is the limit of acceptability for fatty foods (ABDULHAMEED *et al*. 2014).

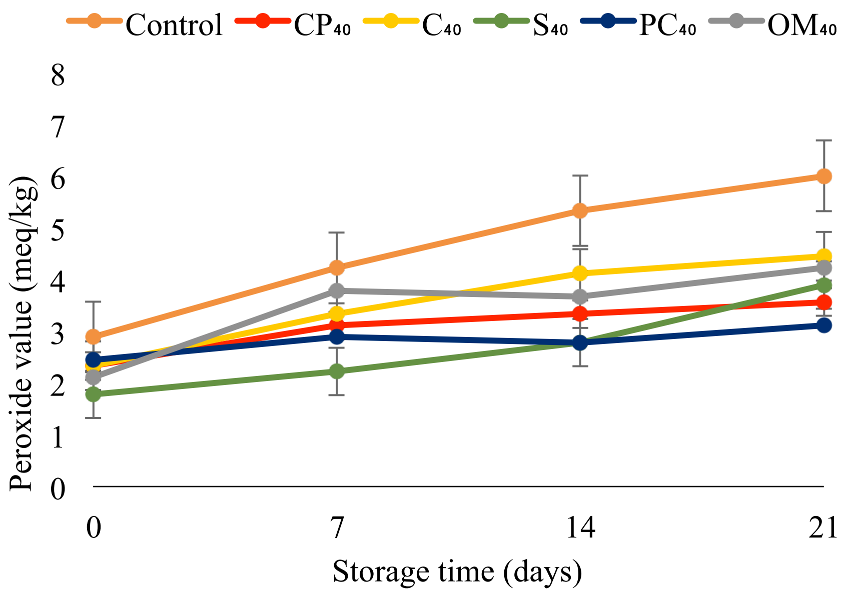

Figure 2. Effects of different vegetable incorporation on peroxide value of sausages stored at 4°C, for 21 days (vertical bars represent standard error).
CP = capsicum, C = carrot, S = spinach, PC = purple cabbage, OM = oyster mushroom.

3.4.2 Microbial analysis

Besides lipid oxidation, the quality attributes of sausages could deteriorate due to microbial growth. This can lead to major public health hazards and economic loss in terms of food poisoning and meat spoilage. Hence, the incorporation of vegetables into sausage formulation anticipated to serve both functions; antioxidant and antimicrobial properties useful for preserving meat quality, extending shelf-life and preventing economic loss (SALLAM *et al*. 2004). Based on the results obtained as in Fig. 3, the initial total plate count (TPC) in sausages was in the range of 1.91-2.36 log10CFU/g and all the samples showed an increased in the count during storage. Samples incorporated with capsicum, carrot, spinach, purple cabbage and oyster mushroom comparatively had lower microbial count. At day 21 of storage, the count for sample CP40, C40, S40, PC40, OM40 were 4.00, 4.22, 4.30, 3.99 and 4.00 log10CFU/g, respectively, which were lower than that of the control (5.05 log10CFU/g). However, the count in all samples was below 6 log10CFU/g, which is the MPL (Maximal Permissible Limit) for APC recommended by Malaysia Food Regulation (Tee 2011).

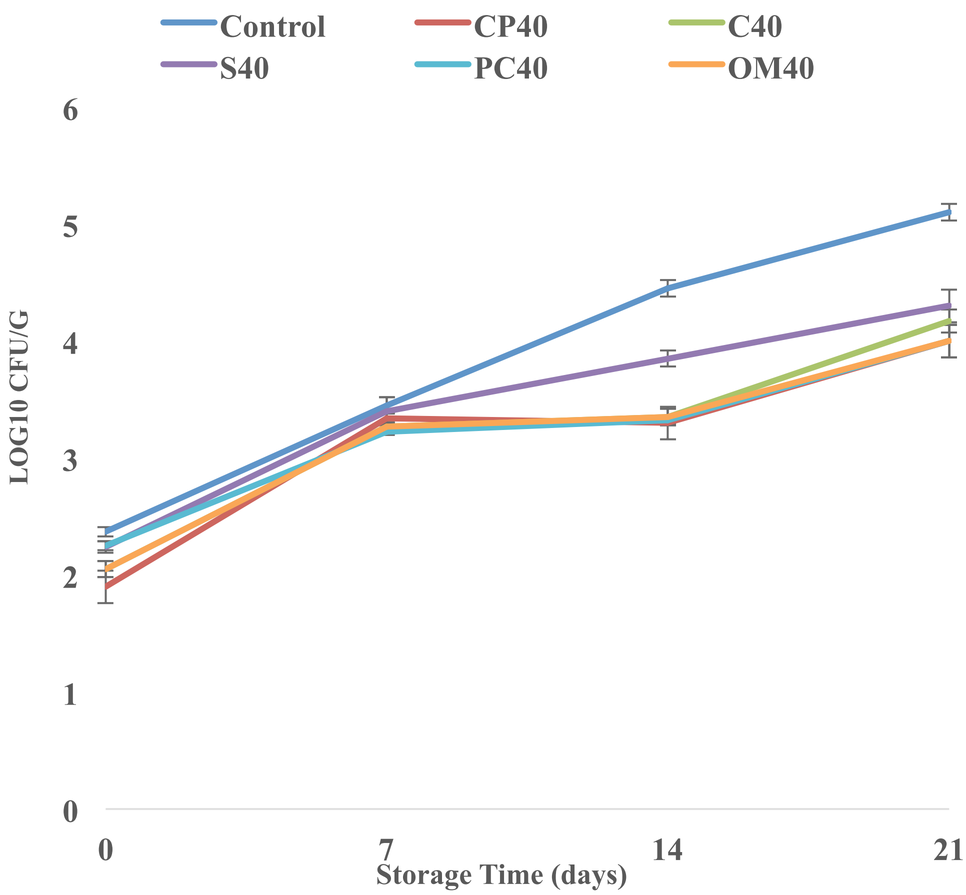

Figure 3. Changes on total plate count in sausage incorporated with different vegetables during storage at 4°C, for 21 days. CP = capsicum, C = carrot, S = spinach, PC = purple cabbage, OM = oyster mushroom.

In this study, PC40, CP40 and OM40 samples had a comparatively lower microbial count than C40 and S40 samples. The difference was probably due the higher phenolic compounds presence in sausage incorporated with purple cabbage, capsicum and oyster mushroom as compared to sausage with carrot and spinach (Table 4). This finding is in agreement with those reported by LIU *et al*. (2009) who found lower TPC in chicken frankfurter added with Chinese mahogany, which has higher phenolic compound than rosemary chicken frankfurter. In addition, the lower TPC in sample PC40, CP40 and OM40 than other sausage samples are probably due to the antimicrobials properties present, which retards bacteria growth. A previous study by VO and Ariyo proved that mushrooms contain some bioactive compounds such as rutin, gallic acid and catechin, which contain a high antimicrobial effect.

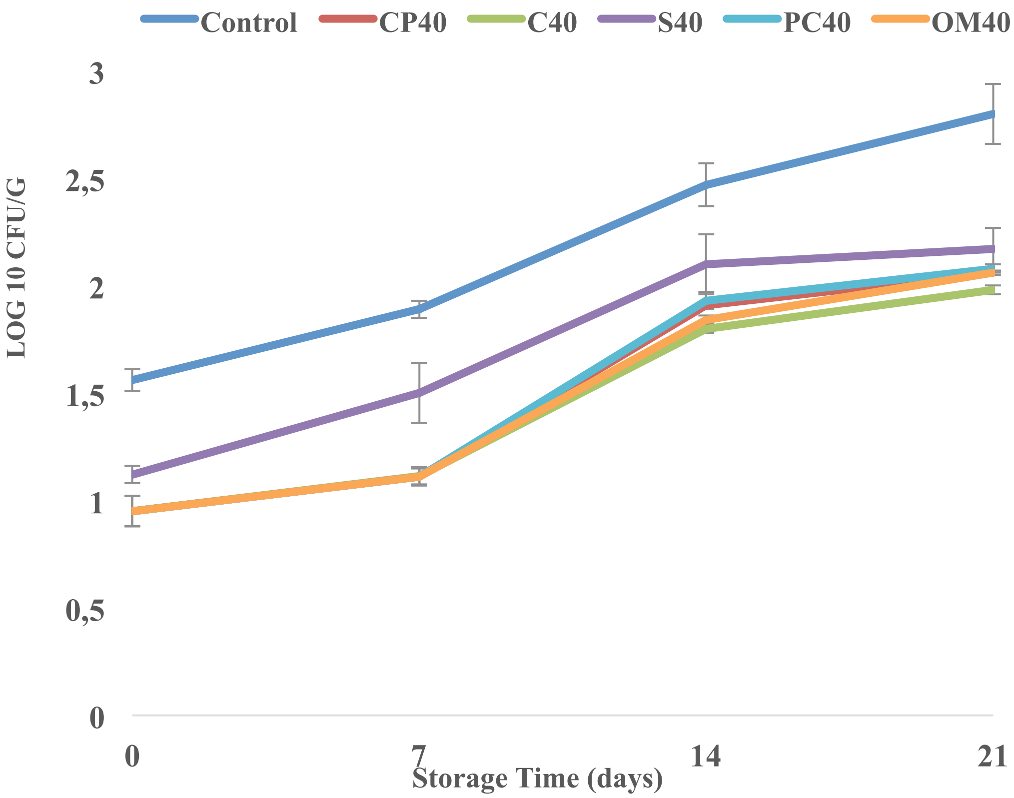

Figure 4. Changes on yeast and mould count in sausage incorporated with different vegetables during storage at 4°C, for 21 days. CP = capsicum, C = carrot, S = spinach, PC = purple cabbage, OM = oyster mushroom.

Yeast and mould results showed an increased in the count during refrigerated storage ranging from 0.95-1.60 log10CFU/g at day 0 to 1.98-2.82 log10CFU/g at day 21 for all sausages formulations (Fig. 4). The trend was similar with the TPC result obtained where incorporation of vegetables in sausage lowered the yeast and mould count. The inhibition of yeast and mould growth was also probably due to the growth of lactic acid-producing bacteria under anaerobic packaging conditions during refrigerated storage (Bradford *et al*. 1993).

4. CONCLUSION

This study unveils that the incorporation of capsicum, carrot, spinach, purple cabbage and oyster mushroom in chicken-based sausage-like product at 30%, 40% and 50% did alter the physicochemical properties of sausages produced. In general, 40% incorporation of vegetables demonstrated more desirable physical properties, which are of particular importance in the case of quality and economic justification of the sausages. The sausages with vegetables had a less dense microstructure with a porous network due to a consequent increase in moisture content, decrease in fat content and protein density. However, the sample with oyster mushroom gave a more closely packed protein gel matrix. The highest DPPH value (87.38%SA) and total phenolic content (23.17GAE/100g) were demonstrated by the sausages incorporated with purple cabbage, which helps to deliver health benefits on human health and retard the formation of oxidation products. Sausage with purple cabbage has the highest stability during 21 days of storage time. The sample had significantly ($P<0.05$) reduced the peroxide value at the end of storage from 1.62meq/kg to 0.58meq/kg compared with the control. Microbial activity results demonstrated that incorporation of vegetables tends to lower the microbial counts, which would help to extend the shelf life of sausages produced. Overall results indicated that the incorporation of vegetables in sausages was potentially helps to deliver both antioxidant and antimicrobial properties, act as a natural preservative and improve quality of the chicken based sausages and reduced production cost.

REFERENCES

Abdulhameed A., Zzamam W., Yang T. 2014. Application of Superheated Steam in Sample Preparation (Chicken Sausage) for Determination of Total Fat , Fatty Acid and Lipid Oxidation. 2:27-33. DOI: doi.org/10.13189/fst.2014.020301

Aleson-Carbonell L., Fernández-López J., Pérez-Alvarez J.A. and Kuri V. 2005. Characteristics of beef burger as influenced by various types of lemon albedo. Innov Food Sci Emerg Technol 6:247-255

Andres S.C., Garcıa M.E., Zaritzky N. and Califano A.N. 2006. Storage stability of low-fat chicken sausages. J Food Eng 72:311-319

Ayo J., Carballo J., Solas M.T., Jiménez-Colmenero F. 2008. Physicochemical and sensory properties of healthier frankfurters as affected by walnut and fat content. Food Chem 107:1547-1552

Barbut S. 2016. Poultry products processing: an industry guide. CRC press

Boo H-O, Hwang S-J, Bae C-S *et al*. 2012. Extraction and characterization of some natural plant pigments. Ind Crops Prod 40:129-135

Boselli E., Caboni M.F., Rodriguez-Estrada M.T. *et al*. 2005. Photoxidation of cholesterol and lipids of turkey meat during storage under commercial retail conditions. Food Chem 91:705-713

Bradford D.D., Huffman D.L., Egbert W.R. and Jones W.R. 1993. Low-fat fresh pork sausage patty stability in refrigerated storage with potassiunn lactate. J Food Sci 58:488-491

Carballo J., Fernandez P., Barreto G. *et al*. 1996. Morphology and texture of bologna sausage as related to content of fat, starch and egg white. J Food Sci 61:652-665

Chan L.W., Cheah E.L.C., Saw C.L.L. *et al*. 2008. Antimicrobial and antioxidant activities of Cortex Magnoliae Officinalis and some other medicinal plants commonly used in South-East Asia. Chin Med 3:15

Choi Y-S, Choi J-H, Han D-J *et al*. 2010. Effects of replacing pork back fat with vegetable oils and rice bran fiber on the quality of reduced-fat frankfurters. Meat Sci 84:557-563

Choi Y-S, Kim H-W, Hwang K-E *et al*. 2012. Effects of pumpkin (Cucurbita maxima Duch.) fiber on physicochemical properties and sensory characteristics of chicken frankfurters. Korean J Food Sci Anim Resour 32:174-183

Cofrades S., López-López I., Solas M.T. *et al*. 2008a. Influence of different types and proportions of added edible seaweeds on characteristics of low-salt gel/emulsion meat systems. Meat Sci 79:767-776

Cofrades S., Serrano A., Ayo J. *et al*. 2008b. Characteristics of meat batters with added native and preheated defatted walnut. Food Chem 107:1506-1514

Fernandez-Gines J.M., Fernandez-Lopez J., Sayas-Barbera E. *et al*. 2004. Lemon albedo as a new source of dietary fiber: Application to bologna sausages. Meat Sci 67:7-13

Fukumoto L.R. and Mazza G. 2000. Assessing antioxidant and prooxidant activities of phenolic compounds. J Agric Food Chem 48:3597-3604

Garcıa M.L., Dominguez R., Galvez M.D. *et al*. 2002. Utilization of cereal and fruit fibres in low fat dry fermented sausages. Meat Sci 60:227-236

Gebhardt S., Lemar L., Haytowitz D. *et al*. 2008. USDA national nutrient database for standard reference, release 21. United States Dep Agric Res Serv

Hedges L.J. and Lister C.E. 2007. Nutritional attributes of spinach, silver beet and eggplant. Crop Food Res Confidential Rep, 1928.

Huda A.B., Parveen S., Rather S.A. *et al*. 2014. Effect of incorporation of apple pomace on the physico-chemical, sensory and textural properties of mutton nuggets. Int J Adv Res 2:974-983

Lee M-A, Han D-J, Choi J-H *et al*. 2009. Physico-chemical and sensory characteristics of semi-dried sausages containing different levels of kimchi powder. Korean J Food Sci Anim Resour 29:557-565

Lin J-Y and Tang C-Y. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem 101:140-147

Liu D-C, Tsau R-T, Lin Y-C *et al*. 2009. Effect of various levels of rosemary or Chinese mahogany on the quality of fresh chicken sausage during refrigerated storage. Food Chem 117:106-113

Maqsood S. and Benjakul S. 2010. Comparative studies of four different phenolic compounds on in vitro antioxidative activity and the preventive effect on lipid oxidation of fish oil emulsion and fish mince. Food Chem 119:123-132

Martí M.C., Camejo D., Vallejo F., Romojaro F., Bacarizo S., Palma J.M. *et al*. 2011. Influence of fruit ripening stage and harvest period on the antioxidant content of sweet pepper cultivars. Plant foods for human nutrition, 66(4), 416-423.

Martinez-Tome M., Jimenez A.M., Ruggieri S. *et al*. 2001. Antioxidant properties of Mediterranean spices compared with common food additives. J Food Prot 64:1412-1419

Matsufuji H., Ishikawa K., Nunomura O. *et al*. 2007. Anti-oxidant content of different coloured sweet peppers, white, green, yellow, orange and red (*Capsicum annuum* L.). Int J food Sci Technol 42:1482-1488

Osman M. Bin and Milan A.R. 2006. Fruits for the future 9: Mangosteen Garcinia mangostana. Crops for the Future

Pandjaitan N., Howard L.R., Morelock T. Gil MI .2005. Antioxidant capacity and phenolic content of spinach as affected by genetics and maturation. J Agric Food Chem 53:8618-8623

Pokorný J. 1991. Natural antioxidants for food use. Trends Food Sci Technol 2:223-227

Raees-ul H. and Prasad K. 2015. Nutritional and processing aspects of carrot (Daucus carota)-A review. South Asian J Food Technol and Env, 1(1), 1-14.

Reis F.S., Barros L., Martins A. and Ferreira I.C. 2012. Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study. Food and Chem Toxicol, 50(2), 191-197.

Sallam K.I., Ishioroshi M. and Samejima K. 2004. Antioxidant and antimicrobial effects of garlic in chicken sausage. LWT-Food Sci Technol 37:849-855

Santana P., Huda N. and Yang T.A. 2015. Physicochemical properties and sensory characteristics of sausage formulated with surimi powder. J Food Sci Technol 52:1507-1515

Shahidi F., Janitha P.K. and Wanasundara P.D. 1992. Phenolic antioxidants. Crit Rev food Sci Nutr 32:67-103

Singleton V.L. and Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16:144-158

Syuhairah A., Huda N., Syahariza Z.A. and Fazilah A. 2016. Research Article Effects of Vegetable Incorporation on Physical and Sensory Characteristics of Sausages. Asian J Poult Sci 10:117-125

Tangkanakul P., Auttaviboonkul P., Niyomwit B. *et al*. 2009. Antioxidant capacity, total phenolic content and nutritional composition of Asian foods after thermal processing. Int Food Res J 16:571-580

Tee E-S (2011) Development and promotion of Malaysian dietary guidelines. Asia Pac J Clin Nutr 20:455-461

Troutt E.S., Hunt M.C., Johnson D.E. *et al*. 1992. Characteristics of low-fat ground beef containing texture-modifying ingredients. J Food Sci 57:19-24

VO O. and Ariyo OO. Antimicrobial and Antioxidant Properties of Pleurotus ostreatus (Jacq: Fries) Cultivated on Different Tropical Woody Substrates

Who J. Organization WH. 2003. Diet, nutrition and the prevention of chronic diseases: report of a joint WH

William H. 2000. Official methods of analysis of AOAC international. AOAC Off method 960.39; 962.09; 945.38

Yang H-S., Choi S-G., Jeon J-T. *et al*. 2007. Textural and sensory properties of low fat pork sausages with added hydrated oatmeal and tofu as texture-modifying agents. Meat Sci 75:283-289

Yin M. and Cheng W. 2003. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat Sci 63:23-28

Zhishen J., Mengcheng T. and Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64:555-559

Paper Received May 13, 2019 Accepted September 2, 2019