

Effect of the Roselle (*Hibiscus sabdariffa*) extract on oxidation stability of bulk frying oil during open and deep frying: a response surface approach

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Abstract

Synthetic antioxidant such as BHA (butylated-hydroxyanisole), BHT (butylated-hydroxytoluene) and TBHQ (tertiary-butyl-hydroquinone) are usually added into frying oil to prolong its shelf life and reusability. However, its utilization has become more obsolete. Roselle (*Hibiscus sabdariffa* L.) is a tropical plant which has been reported to possess antioxidant, anti-tumor and antihyperlipemic activities that was suspected to be contributed by its phenolic anthocyanins compound. This study was aimed to study the effect of the addition of roselle extract as a natural antioxidant in bulk frying oil (BFO). Central composite design of Response Surface Methodology was employed to measure the trend of roselle extract utilization in open frying and deep frying practice. The results showed that the addition of roselle extract could retard the formation of free fatty acids (FFA) to maximum 0.1226 mg KOH/g oil (4% addition) and lowered hydroperoxide formation as low as 7.47 MeqO₂/kg oil after treated with 20 times of successive frying. The trend study showed that the addition of 0.04% of roselle extract could prolong the usage of oil up to 6 times in the open frying treatment. On the other hand, only 0.01% of the roselle extract was needed to prolong the oil usage up to 20 times in deep frying practice. This result showed that the roselle extract can be used as a substitute for synthetic antioxidant to prolong the shelf life and reusability of BFO.

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Introduction

Bulk frying oil (BFO) is palm oil derived frying oil that is commonly used in household frying because it is cheap and widely available. Besides BFO, refined-bleached and deodorized (RBD) palm olein which is usually sold with branded packaging at higher price are also used. BFO which only undergoes partial purification process still contains soft stearin fraction that makes it cloudier compared with RBD palm oil (Che Man *et al.*, 1999).

Among the various types of frying oil, palm-derived oils are known as oxidative-stable, heavy-duty frying oil contributed by its high proportion of unsaturated and monounsaturated fatty acids (Nallusamy, 2006; Fan *et al.*, 2013). However, high heat treatment during frying process could affect degradation process such as hydrolysis, oxidation and polymerization (Ketaren, 1986). The repeated use of cooking oil has become a common practice in order to reduce frying cost. However, repeated use of frying oil could lead in the development of lipid oxidation, hydrolysis and polymerization product that resulted in rancidity and oil breakdown. Furthermore, these lipid degradation products were also reported could give an adverse effect on human health such as gastrointestinal problem and cancer (Ketaren 1986;

Maillard *et al.*, 1996; Shahidi and Wanasundara, 2008).

In order to prolong the shelf life of frying oil, synthetic antioxidants such as BHA (butylated-hydroxyanisole), BHT (butylated-hydroxytoluene) and TBHQ (tertiary-butyl-hydroquinone) are usually added into the oil to retard the degradation process (McWilliams, 2001; Shahidi and Zhong, 2005). However, despite that the fact that synthetic antioxidant is proven to be quite effective, its usage in food products is becoming more obsolete and under strict regulation due to the potential health hazards caused by such compounds (Hettiarachchy *et al.*, 1996). Several researchers had mentioned that the unnatural molecules of synthetic antioxidant are hazardous to human health (Williams, 1993; Moktan *et al.*, 2008). Therefore, the research attempts towards the utilization of antioxidant from natural sources have been extensively made for possible application to substitute the synthetic antioxidants.

Roselle (*Hibiscus sabdariffa* L) is a tropical plant, which its brilliant-red petals are usually processed into soft drink due to its unique and refreshing sensation (Fernández-Arroyo *et al.*, 2011). According to Wang *et al.* (2000), roselle has also been traditionally used against hypertension, inflammation and liver disorder. Some studies have reported that the petals

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possess anti-tumor, antioxidant and antihyperlipemic activities that was suspected to be contributed by its phenolic anthocyanins compound (Chen *et al.*, 2003; Lin *et al.*, 2005; Segura-Carretero *et al.*, 2008). Fernández-Arroyo *et al.* (2011) previously mentioned that there were at least 17 main phenolic compounds found in roselle extract, in which hibiscus acid, hydroxycitric acid, chlorogenic acid, delphinidin 3-sambubioside, cyaniding 3-sambubioside and 7-hydroxycoumarin were the major components. In lipid body, phenolic compounds are able to retard the lipid oxidation by donating a hydrogen atom or an electron to neutralize the substrate-derived free radicals such as the fatty acid free radicals, hydroxyl and superoxide radicals and alkoxy radicals (Cao *et al.*, 1997; Prior and Cao, 2000; Shahidi and Wanasundara, 2008). The incorporation of such extract in human foods was also reported not only could preserve their wholesomeness, but also able to reduce the risk of developing atherosclerosis and cancer (Ames, 1983; Namiki, 1990; Ramarathnam *et al.*, 1995). This research was designed to evaluate the use the phenolic compound-rich roselle extract to enrich the BFO and to study the effect of its addition to the oil oxidative stability during open and deep frying.

Material and Methods

Preparation of roselle extracts

Roselle petals were obtained from local plantation in Jember, East Java. Only the red roselle petals were chosen as research material. The roselle petals were then sun-dried, continued by oven drying (50-60°C) and then stored in a sealed plastic bag until used. The roselle extract (RE) was prepared by using ethanol extraction according to the method of Misnawi (2003) and Misnawi *et al.* (2014). Powdered dried roselle petals (± 50 g) was mixed with 150 ml ethanol and macerated for 24 hours at room temperature. The separation was carried out in vacuum filtration to obtain the supernatant. The roselle extract was obtained by evaporating the supernatant by using a rotary evaporator at 50-65°C. The extract was then stored in screw capped inert bottle until the experiment.

Determination of total phenolic content

Total phenolic content of roselle extract was determined using Follin-Ciocalteu method following Szydłowska-Czerniak's (2010) with slight modifications. Finely weighted RE (0.2 ml) was transferred into test tube and added with 1 ml ethanol, 4.8 ml distilled water and 0.5 ml Follin-Ciocalteu

(50%) reagent. The mixture was then vortexed and rested for 5 minutes. To the extract, 1 ml of saturated sodium carbonate (Na_2CO_3) was added and the solution was made up to 10 ml with distilled water. After resting for 1 hour in a dark room, the absorbances of the solutions were measured using a UV-160A spectrophotometer (Shimadzu Corp., Nagakyo-Ku, Kyoto, Japan), measured at 725 nm against a reagent blank. (-)-epicatechin (0-200 mg/L) was used as standard in calibration curve preparation.

Experiment condition

The experiment was conducted according the design suggested by Design-Expert software version 7.0 (Stat Ease, Minneapolis, MN, USA) using face centered central composite design (CCD). The independent variables were RE concentration added into the BFO which ranged from 0% (no addition) until 0.04% and the frequency of frying which ranged from 0 (no frying) until 20 times of frying. Frying treatment was carried out using 2 L of Roselle extract enriched-BFO to fry the local tofu with 10 tofu used for each frying. The frequencies of frying was divided into 0, 10 and 20 times of frying. The frying method was divided into open frying (OF) and deep frying (DF).

Free fatty acid

FFA was determined using the method of AOCS Ca 5a-40 (AOCS, 1998). Approximately, 28.2 ± 0.2 g oil was transferred into a flask followed by 50 ml hot neutralized ethanol and 2 ml of phenolphthalein indicator (1%). The solution was then mixed using a magnetic stirrer. The mixture was then titrated against sodium hydroxide solution 0.1 M until a permanent pink color persisted for at least 30 s. The weight percentage of FFA was calculated on oleic acid basis (mg KOH/g oil).

Peroxide value

The peroxide value (PV) was determined using the official method of AOAC 965.33 (AOAC, 2000). Thirty milliliters of chloroform/acetic acid 3:2 (v/v) was used to dissolve a known weight of oil sample (5 ± 0.05 g). Approximately 0.5 ml freshly prepared saturated KI solution was then added to the mixture and then vortexed for exactly 1 min. Distilled water (30 ml) and starch indicator (0.5 ml, 1%) were added and then titrated with sodium thiosulfate (0.1 M) until the blue color was disappeared. The result was expressed as $\text{MeqO}_2/\text{kg fat}$.

Oil clarity

Oil clarity was analyzed employing a UV-160A

spectrophotometer (Shimadzu Corp., Nagakyo-Ku, Kyoto, Japan), measured at 583 nm. The clarity was determined according the percentage of transmittance detected by the spectrophotometer.

DPPH Radical scavenging activity

Radical scavenging activity (RSA) analysis was performed based on the activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH). This analysis was carried out using the method of Gadow *et al.* (1997) with some modification. Approximately 0.2 g of sample was dissolved in 20 ml of ethanol and stirred for 10 minutes. The solution was then centrifuged for 3 minutes at 5000 rpm. Approximately 1 ml of the filtrate placed into a test tube and mixed with 0.5 ml DPPH reagent and then shaken vigorously. The solution was then placed in a dark condition for 20 minutes. After that, the solution was topped up to 5 ml using ethanol. The absorbance of the sample mixture was then measured at 517 nm using a UV-160A spectrophotometer (Shimadzu Corp., Nagakyo-Ku, Kyoto, Japan). Antioxidant activity was presented as a % scavenging activity following the formula below:

$$\%RSA = ((Abs_{blank} - Abs_{sample}) / Abs_{blank}) \times 100\%$$

Statistical analysis and optimization

Face centered central composite design was carried out for the experiment employing 2 variables (three levels of each variable) with five replications at the center points. The levels of variable were coded at -1, 0 and +1. The independent variables were frying frequency (A) and % RE concentration (B). The experiment design is shown in Table 1. Statistical analysis was carried out using Design Expert® version 7.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA). The model was performed at 5% significance level in which $p < 0.05$ was considered as significant parameters and non-significant parameters ($p > 0.05$) were excluded. The response function Y for all responses was fitted to a second-degree polynomial using below equation:

$$Y = b_0 + b_1A + b_2B + b_{12}AB + b_{11}A^2 + b_{22}B^2 + \varepsilon$$

The linear, quadratic and interaction effects were represented along with the coefficient (b_0) by b_1 , b_2 , (linear effects), b_{12} (interaction effects), thus b_{11} and b_{22} for quadratic effects. The optimum condition for the chosen desired goal is decided by using Myers and Montgomery desirability method contained in the software in purpose to obtain minimum FFA and PV and maximize the clarity and DPPH RSA.

Result and Discussion

Our analysis of the roselle extract has found that the total phenolic content was 13.496 mg equivalent (-)-epicatechin/L extract. Compared to other extract such as *G. glabra* (liquorice), the total phenolic content of RE used in this research was higher (liquorice extract 0.117 mg equivalent (-)-epicatechin/kg extract) (Rackova *et al.*, 2007).

The results of quality analyses that were carried out according the design of CCD are presented in Table 1, in which the RSM analysis was done separately between the responses from open frying and deep frying. Summarized ANOVA (analysis of variance) regarding the model fitting (model adequacy, lack of fits, R^2 and adjusted R^2), model equation and significant factors for each response is shown in Table 2. All of the responses showed that the quadratic model was significant, which mean that the additions of quadratic terms significantly improved the model adequacy (Herpandi *et al.*, 2013). The lack of fit analysis results also showed that the quadratic model was suitable for predicting and analyzing the result. Little and Hills (1978) and Koochecki *et al.* (2009) previously mentioned that the R^2 should not be less than 0.80 and must be followed by close value of adjusted R^2 (adj- R^2) to make sure that non-significant terms were excluded and the models were adequate. Our model showed that the values of R^2 were high, ranged from 0.941 to 0.998 and followed by close values of adj- R^2 (0.896-0.996). These results showed that the use of quadratic models for analyzing the responses was adequate and could become good indicators for optimization.

Effect of roselle extract in open frying

Our analysis on free fatty acid content resulted in the values of FFA of frying oil treated with open frying method ranged from 0.1123 to 0.1691% (Table 1). Ketaren (1996) previously stated that the FFA content more than 0.2% could generate undesirable flavor. These values were still lower from the standard applied in Indonesia as stated in Indonesian National Standard SNI 1-3741-1995 which still allowed the FFA content of commercial frying oil up to 0.3% (BSN, 1995). This standard is similar to those of CODEX (CODEX STAN 210-1999) which limits the FFA content up to 0.3% and peroxide value up to 10 meqO₂/kg oil. Despite its acceptable values, the RSM results (Figure 1) showed that the addition of RE significantly lowers the formation of FFA during open frying. However, the retardation of FFA formation as the increase of frying frequency became rather indistinguishable due

Table 1. Face centered experimental design in coded and actual level of variables

Expt. No	Frequency (times)		Concentration (%)		Open Frying Treatment				Deep Frying Treatment			
	Coded Level a	Actual Level A	Coded Level b	Actual Level B	FFA	PV	Clarity	DPPH	FFA	PV	Clarity	DPPH
1	-1	0	-1	0.00	0.1248	1.997	98.250	0.000	0.1021	2.249	98.975	0.000
2	-1	0	0	0.02	0.1226	0.996	95.750	25.670	0.0953	2.988	96.375	30.74
3	-1	0	1	0.04	0.1123	2.739	90.525	35.410	0.0953	2.988	93.025	60.350
4	0	10	-1	0.00	0.1407	18.722	54.425	0.000	0.1339	7.250	56.500	0.000
5	0	10	0	0.02	0.1453	12.923	59.300	17.540	0.1089	5.976	60.700	25.780
6	0	10	0	0.02	0.1362	13.917	58.900	17.540	0.1180	5.976	62.700	25.780
7	0	10	0	0.02	0.1453	16.932	58.900	9.460	0.1084	7.968	62.700	15.970
8	0	10	0	0.02	0.1362	17.928	61.500	9.460	0.1180	6.972	58.700	15.970
9	0	10	0	0.02	0.1430	15.425	60.600	13.160	0.1135	6.723	58.700	20.370
10	0	10	1	0.04	0.1260	12.451	62.950	18.520	0.1134	5.976	60.350	40.300
11	1	20	-1	0.00	0.1691	21.489	33.950	0.000	0.1429	9.245	42.600	0.000
12	1	20	0	0.02	0.1611	22.140	42.700	8.780	0.1271	7.719	44.775	7.1400
13	1	20	1	0.04	0.1623	18.924	51.375	8.890	0.1226	7.470	48.125	5.290

FFA: mg KOH/g oil
 PV: MeqO₂/kg oil
 Clarity: Percentage of transmittance
 DPPH: % RSA

Table 2. Summarized ANOVA of the variables according central composite design (CCD)

Parameters	FFA		PV		Clarity		DPPH RSA	
	OF	DF	OF	DF	OF	DF	OF	DF
Model	Quadratic significant	Quadratic significant	Quadratic significant	Quadratic significant	Quadratic significant	Quadratic significant	Quadratic significant	Quadratic significant
Lack of fit	not significant	not significant	not significant	not significant	not significant	not significant	not significant	not significant
R ²	0.951	0.947	0.952	0.940	0.998	0.994	0.941	0.970
Adjusted R ²	0.916	0.908	0.918	0.896	0.996	0.990	0.899	0.945
Equation (Actual levels)	Y=0.126+0.00115A+0.03811B	Y=0.1046+0.00299A+0.978B+18.786B ²	Y=2.416+1.844A-0.041A ²	Y=2.5+0.583A-0.0125A ²	Y=97.78-5.158A-69.094B+31.438AB+0.096A ²	Y=98.05-5.04A+14.344AB+0.11A ²	Y=1.55-0.628A+1367.69B-33.15AB+12817.24B ²	Y=-1.57+0.616A+1725.5B-68.825AB
Significant factor	A, B	A, B, B ²	A, A ²	A, A ²	A, B, AB, A ²	A, AB, A ²	A, B, AB, B ²	A, B, AB

to its slight curve inclination in every point of RE concentration. This phenomenon was suspected to be contributed by the native characteristic of the oil that already resistant to hydrolysis process. On the lipid body, free fatty acids occurred mainly due to the hydrolysis process in occurrence of water. The hydrolysis process breakdown the triglycerides into glycerol and free fatty acids (Melton *et al.*, 1994). Even though the FFA content was reported to be correlated with the oxidation occurred in lipid body (White, 1991; Melton *et al.*, 1994), this condition did not represent the inability of the antioxidant in RE to retard the oxidation process since the result of PV showed otherwise.

The PV results showed that the addition of RE into the BFO could effectively retard the oxidation process. The PV ranged from 0.996 to 22.140 MeqO₂/kg oil. Open frying methods tend to exhibit high PV represented intense oxidation occurred in lipid body during frying. It is suspected to be contributed by the different cooking style and equipment during the experiment. Kalogeropoulos *et al.* (2007) previously mentioned that the cooking oil in the surface area which contact with the atmosphere are more susceptible to oxidation resulted in high content of oxidized fatty acid and polymerized triacylglycerol. Higher food to oil contact surface ratio, higher exposure to atmospheric oxygen and lower temperature control under processing also intensify the degradation rate of oil (Andrikopoulos *et al.*, 2002b). Hence, it is reasonable since we did the open frying using a large shallow pan type fryer which allowed wider contact area with the atmosphere. Moreover, the open frying required stirring process which intensified the contact between oil and atmosphere resulted in higher oxidation rate compared to deep frying, which only

need minimum stirring (Santos *et al.*, 2013).

The addition of RE could suppress the formation of hydroperoxide and increase the oxidative stability of BFO. The BFO without the addition of roselle extract exhibited a high increase of PV (22.140 MeqO₂/kg oil, 20 times frying). In contrast, as the increase of the RE concentration added to the oil, the more resistant the oil to the oxidation, resulting in lower PV up to 18.924 MeqO₂/kg oil (20 times frying with the addition of 0.04% RE) (Figure 2). Shahidi and Warasundara (2008) previously mentioned that the formation of hydroperoxide could be retarded by antioxidant since the antioxidant is able to form a stable phenoxy radical complex with singlet/triplet oxygen radical and propagation step product (radical) which could initiate the oxidation process. With the absence of radicals, the hydroperoxides were forced to go to the termination steps resulting in stable compound which minimize further propagation of oxidation (Bravo, 1998).

Clarity has been mentioned to be well correlated with the purity and condition of frying oil. Das and Pereira (1990) mentioned that the development of brown color in frying oil is normally associated with oxidation and polymerization occurred during frying. The clarity of treated frying oil (open and deep fried) showed similar results with PV, represent the ability of RE to maintain the clarity of BFO during frying. Open frying-treated oil exhibited clarity drop from 98.250 to 33.95 (Table 1). The increase of frying frequency significantly decreased the clarity of oil, whereas the addition of RE at higher concentration effectively retard the reduction (from 90.53 to 51.38, 0.04% RE) (Figure 3).

The DPPH RSA analysis of RE extract-added BFO exhibited the highest antioxidant activity was shown

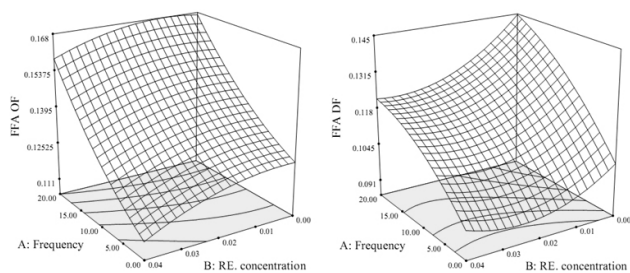


Figure 1. *Left*: Response surface for the effect of RE concentration (%) and frying frequency (times) on the FFA of open frying oil. *Right*: Response surface for the effect of RE concentration (%) and frying frequency (times) on the FFA of deep frying oil.

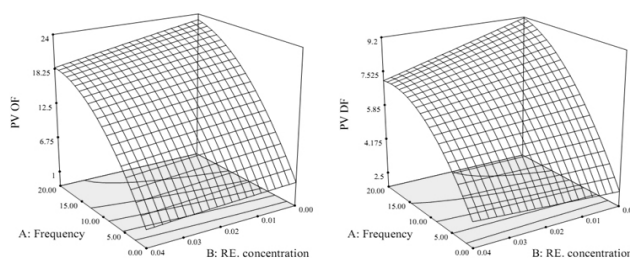


Figure 2. *Left*: Response surface for the effect of RE concentration (%) and frying frequency (times) on the PV of open frying oil. *Right*: Response surface for the effect of RE concentration (%) and frying frequency (times) on the PV of deep frying oil.

by BFO with 0.04% addition of RE and reduced as the increase of frying frequency (from 35.41 to 8.89% DPPH RSA) (Figure 4). As the increase of frying frequency, more of antioxidant of RE is used to retard the oil breakdown during the frying process, thus resulted in a decrease of the DPPH RSA due to the decrease of active antioxidant compound left in the oil. The oil with the addition of 0.04% RE exhibited relatively low RSA when processed up to 20 times frying (8.89% DPPH RSA). It showed that the antioxidant of RE in BFO almost completely used to retard the oil oxidation.

Effect of roselle extract in deep frying

Compared to that of open frying, the oil degradation parameters of deep fried oil such as FFA and PV exhibited lower values. The FFA of oil treated with deep frying was ranged from 0.0953 to 0.1429 mg KOH/g oil. These values were also in the acceptable range of FFA standard of CODEX (CODEX STAN 210-1999) which limits the FFA up to 0.3%. If the retardation of FFA production of open frying-treated oil was rather indistinguishable (as aforementioned), the addition of the roselle extract on the oil for deep frying showed significantly different results. Without the addition of RE, the FFA was increased significantly from 0.1021 to 0.1429 mg KOH/g oil, whereas the addition of 0.04% RE

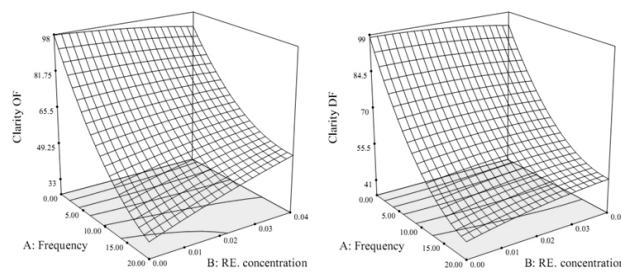


Figure 3. *Left*: Response surface for the relation between RE concentration (%) and frying frequency (times) on the Clarity of open frying oil. *Right*: Response surface for the relation between RE concentration (%) and frying frequency (times) on the Clarity of deep frying oil.

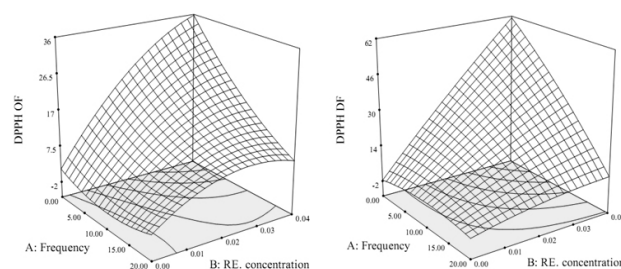


Figure 4. *Left*: Response surface for the relation between RE concentration (%) and frying frequency (times) on the DPPH RSA of open frying oil. *Right*: Response surface for the relation between RE concentration (%) and frying frequency (times) on the DPPH of deep frying oil.

resulting in maximum value of FFA of 0.1226 mg KOH/g oil.

On the other hand, the addition of RE also resulted in a much lower value of PVs, which ranged from 2.249 to 9.245 MeqO₂/kg oil and in acceptable values of CODEX STAN 210-1999 (<10 MeqO₂/kg oil). The addition of RE into BFO treated with deep frying could limit the formation of hydroperoxide up to 7.47 MeqO₂/kg oil after 20 times of successive frying. These results are in agreement with that of Andrikopoulos *et al.* (2002a) who stated that deep frying method resulted in better recoveries of oxidative product compared to open frying. Kalogeropoulos *et al.* (2007) thus mentioned that the higher the surface area which contact with the atmosphere, the higher the oxidation process that resulted in fatty acid and polymerized triacylglycerol, hydroperoxide would be. Even though the peroxide value of BFO without the addition of RE also still in acceptable limit (<10 MeqO₂/kg oil after 20 times frying), the PV of it (0% RE, 20 times frying) was already close to the limit (9.245 MeqO₂/kg oil). This condition suggests that this oil may exceed the limit of PV if used for another successive frying.

Surprisingly, the addition of RE on BFO did not result in statistically significant effect on the retardation of hydroperoxide formation during deep frying, even though it was proved that the addition

of RE could retard the oxidation process. Roselle extract was reported to contain mostly antioxidant from the anthocyanin group (Fernández-Arroyo *et al.*, 2011) which was reported by Idham *et al.* (2012) to have high reactivity towards environmental changes and could be easily degraded in high temperature condition, resulting in undesirable brown-colored compound. This character of RE thus also explains the trend of clarity drop of RE added BFO exhibited in this research. Even though the deep frying treated BFO has less contact with the atmosphere, the drop in clarity was almost similar to that of open frying (Figure 3). The result of DPPH analysis thus confirms this phenomenon. The DPPH of BFO treated for 20 times of successive frying only remaining as much as 5.29% RSA. The decrease of DPPH might be caused by the degradation of its mostly anthocyanins itself due to high temperature condition during deep frying treatment; in which the other antioxidant compound resistant to high temperature were still available and contributed into the retardation of oil oxidation.

Trend study

The addition of RE on BFO had successfully prolonged the shelf life of the BFO both in open frying and deep frying treatment. Among all the indicators of oil degradation that had been measured, PV became a limiting factor due to its significant changes during frying treatment. Based on the limitation of CODEX STAN 210-1999 (FFA <0.3% and PV <10 MeqO₂/kg oil), the addition of 0.04% of RE could prolong the oil reusability up to 6 times of open frying, whereas without the addition of RE, the reusability of BFO in open frying treatment was limited for only five times of frying.

On the other hand, the additions of as low as 0.01% RE was sufficient to prolong the oil usage in deep frying method up to 20 times without exceeding the limitation of the CODEX STAN 210-1999. Even though without the addition of RE the BFO could also stand for 20 successive frying, the PV (as the indicator of oil degradation) of non-added BFO were already close to the limitation (9.245 MeqO₂/kg oil); higher than to that of RE added BFO's (7.47 MeqO₂/kg oil) which show a limited possibility to use the non-added BFO for another successive frying.

Generally, the addition of RE up to 0.04% was found to be insufficient, especially in the BFO treated with open frying. Thus, the possibility to use higher concentrations of RE should be carried out. Based on CODEX STAN 210-1999, the use of synthetic antioxidant such as TBHQ, BHA and BHT is limited up to 120, 175 and 75 mg/kg of frying oil, whereas the use of natural compound such as ascorbyl

palmitate is up to 500 mg/kg. However, the use of other natural antioxidant such as tocopherol was not limited. This condition, thus allows the use of RE as a natural antioxidant on higher concentrations necessary to achieve the desired result, according to Good Manufacturing Practices (GMP) regulations.

Conclusion

The attempt to study the effect of the roselle extract on the oxidative stability of bulk frying oil has been successfully carried out in this study. The addition of roselle extract effectively maintains the quality of bulk frying oil during frying process and prolongs its usage cycle. The addition of 0.04% of the roselle extract in an open frying could prolong the usage of bulk frying oil up to six times successive frying compared to that of without roselle extract addition. On the other hand, only 0.01% of the roselle extract is needed to prolong the oil usage up to 20 times in the deep frying. These results proved that the roselle extract could be utilized as a synthetic antioxidant substitute to preserve the bulk frying oil quality.

References

- Ames, B. N. 1983. Dietary carcinogens and anticarcinogens: Oxygen radicals and degenerative diseases. *Science* 221 (4617): 1256-1264.
- Andrikopoulos, N. K., Dedoussis, G. V. Z., Falirea, A., Kalogeropoulos, N. and Hatnikola, H. S. 2002a. Deterioration of natural antioxidant species of vegetable edible oils during the domestic deep-frying and pan-frying of potatoes. *International Journal of Food Science and Nutrition* 53 (4): 351-363.
- Andrikopoulos, N. K., Kalogeropoulos, N., Falirea, A. and Barbagianni, M. N. 2002b. Performance of virgin olive oil and vegetable shortening during domestic deep-frying and pan-frying of potatoes. *International Journal of Food Science and Technology* 37 (2): 177-190.
- AOAC. 2000. Official Methods of Analysis of the Association of Agricultural. Chemist 17th edition. Washington DC: Association of Analytical Community.
- AOCS, 1998. Free fatty acids. In: official methods and recommended practices of the American Oil Chemists Society Vol 5a 5th edition. Champaign: American Oil Chemists' Society.
- Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutrition Review* 56 (11): 317-333.
- BSN. 1995. SNI 1-3741-1995 Minyak goreng. Jakarta: Badan Standarisasi Nasional.
- Cao, G., Sofic, E. and Prior, R. L. 1997. Antioxidant and prooxidant behaviour of flavonoids: structure activity

- relationships. *Free Radical Biology and Medicine* 22 (5): 749-760.
- Che Man, Y. B., Haryati, T., Ghazali, H. M. and Asbi, B. A. 1999. Composition and thermal profile of crude palm oil and its products. *Journal of the American Oil Chemists' Society* 76 (2): 237-242.
- Chen, C. C., Hsu, J. D., Wang, S. F., Chiang, H. C., Yang, M. Y., Kao, E. S., Ho, Y. C. and Wang, C. J. 2003. Hibiscus sabdariffa extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *Journal of Agricultural and Food Chemistry* 51 (18): 5472-5477.
- Das, N. P. and Pereira, T. A. 1990. Effects of flavonoids on thermal autoxidation of palm oil: structure-activity relationship. *Journal of the American Oil Chemists' Society* 67 (4): 255-258.
- Fan, H. Y., Sharifudin, M. S., Hasmadi, M. and Chew, H. M. Frying stability of rice bran oil and palm olein. *International Food Research Journal* 20 (1): 403-407.
- Fernández-Arroyo, S., Rodríguez-Medina I. C., Beltrán-Debón, R., Pasini, F., Joven, J., Micol, V., Segura-Carretero, A. and Fernández-Gutiérrez. 2011. Quantification of the polyphenolic fraction and *in vitro* antioxidant and *in vivo* anti-hyperlipemic activities of Hibiscus sabdariffa aqueous extract. *Food Research International* 44 (5): 1490-1495.
- Gadow, A. V., Joubert, E. and Hansman, C. F. 1997. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α tocopherol, BHT and BHA. *Journal of Agriculture and Food Chemistry* 45 (3): 632-638.
- Herpandi, Huda, N., Rosma, A. and Wan Nadiah, W. A. 2013. Optimizing the enzymatic hydrolysis of skipjack tuna (*Katsuwonus pelamis*) dark flesh using Alcalase® enzyme: a response surface approach. *Journal of Fisheries and Aquatic Science* 8 (4): 494-505.
- Hettiarachchy, N. S., Glenn, K. C., Gnanasambandam, R. and Johnson, M. G. 1996. Natural antioxidant extracts from fenugreek (*Trigonella foenumgraecum*) for ground beef patties. *Journal of Food Science* 61 (3): 516-519.
- Idham, Z., Muhamad, I. A., Setapar, S. H. M. and Sarmidi, M. R. 2012. Effect of thermal processes on roselle anthocyanins encapsulated in different polymer matrices. *Journal of Food Processing and Preservation* 36 (2): 176-184.
- Kalogeropoulos, N., Salta, F. N., Chiou, A. and Andrikopoulos, N. K. 2007. Formation and distribution of oxidized fatty acids during deep- and pan-frying of potatoes. *European Journal of Lipid Science and Technology* 109 (11): 1111-1123.
- Ketaren, S. 1996. *Pengantar Teknologi Minyak dan Lemak Pangan*. Jakarta: Universitas Indonesia Press.
- Koochecki, A., Thaerian, A. R., Ravazi, S. M. A. and Bostan, A. 2009. Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from *Lepidium perfoliatum* seeds. *Food Hydrocolloids* 23 (8): 2369-2379.
- Lin, H. H., Huang, H. P., Huang, C. C., Chen, J. H. and Wang, C. J. 2005. Hibiscus polyphenol-rich extract induces apoptosis in human gastric carcinoma cells via p53 phosphorylation and p38 MAPK/FasL cascade pathway. *Molecular Carcinogenesis* 43 (2): 86-99.
- Little, T. M. and Hills, F. J. 1978. *Agricultural experimentation: design and analysis*. New York: John Wiley and Sons.
- Maillard, M. N., Soum, W. H., Boivin, P. and Berset, C. 1996. Antioxidant activity of barley and malt: relationship with phenolic content. *Food Science and Technology* 29 (3): 238-244.
- McWilliams, M. 2001. *Foods: Experimental Perspectives 4th edition*. New Jersey: Prentice Hall.
- Melton, S. L., Jafar, S., Sykes, D. and Trigiano, M. K. 1994. Review of stability measurements for frying oils and fried food flavor. *Journal of the American Oil Chemists' Society* 71 (12): 1301-1308.
- Misnawi. 2003. Influence of cocoa polyphenols and enzyme reactivation on the flavor development of underfermented cocoa beans. Malaysia: Universiti Putra Malaysia, PhD thesis.
- Misnawi, Teguh, W., Susijahadi, Eka N. D. and Noor A. F. 2014. The utilization of cocoa polyphenol extract to improve the shelf life of bulk frying oil used in open and deep frying. *International Food Research Journal* 21 (1): 111-118.
- Moktan, B., Saha, J. and Sarkar, P. K. 2008. Antioxidant activities of soybean as affected by Bacillus-fermentation to kinema. *Food Research International* 41 (6): 586-593.
- Nallusamy, S. 2006. The role of palm oil in the snack food industry. *International Palm Oil Trade Fair and Seminar*, Kuala Lumpur, November 21-24, 2006.
- Namiki, M. 1990. Antioxidants/antimutagens in food. *Critical Review in Food Science and Nutrition* 29 (4): 273-300.
- Prior, R. L. and Cao, G. 2000. Flavonoids: diet and health relationships. *Nutrition in Clinical Care* 3 (5): 279-288.
- Racková, L., Jancinová, V., Petříková, M., Drábíková, K., Nosál, R., Stefek, M., Kostálová, D., Prónayová, N. and Kováčová, M. 2007. Mechanism of anti-inflammatory action of liquorice extract and glycyrrhizin. *Natural Product Research* 21 (14): 1234-1241.
- Ramarathnam, N., Osawa, T., Ochi, H. and Kawakishi, S. 1995. The contribution of plant food antioxidants to human health. *Trends in Food Science and Technology* 6 (3): 75-82.
- Santos, C. S., Cruz, R., Cunha, S. C. and Casal, S. 2013. Effect of cooking on olive oil quality attributes. *Food Research International* 52 (2): 2016-2024.
- Segura-Carretero, A., Puertas-Mejía, M. A., Cortacero-Ramírez, S., Beltrán, R., Alonso-Villaverde, C., Joven, J. Dinelli, G. and Fernández-Gutiérrez, A. 2008. Selective extraction, separation and identification of anthocyanins from *Hibiscus sabdariffa* L. using solid phase extraction-capillary electrophoresis mass spectrometry (time-of-flight/ion trap). *Electrophoresis* 29 (13): 2852-2861.

- Shahidi, F. and Zhong, Y. 2005. Antioxidant: Regulatory Status. In Shahidi, F. (ed.). *Bailey's Industrial Oil and Fat Products*, Sixth Edition, p. 491-512. New Jersey: John Wiley & Sons, Inc.
- Shahidi F. and Wanasundara, U. N. 2008. Methods for Measuring Oxidative Rancidity in Fats and Oils. In Akoh, C. C. and Min, D. B. (eds). *Food Lipids Chemistry, Nutrition, and Biotechnology*. Boca Raton: CRC Press.
- Szydłowska-Czerniak, Trokowski, A. K., Karlovits, G. and Szlyk E. 2010. Determination of antioxidant capacity, phenolic acids, and fatty acid composition of rapeseed varieties. *Journal of Agricultural and Food Chemistry* 58 (13): 7502-7509.
- Wang, C. J., Wang, J. M., Lin, W. L., Chu, C. Y., Chou, F. P. and Tseng, T. H. 2000. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food and Chemical Toxicology* 38 (5): 411-416.
- White, P. J. 1991. Methods for measuring changes in deep-fat frying oils. *Food Technology* 45 (2): 75-80.
- Williams, G. M. 1993. Inhibition of chemical-induced experimental cancer of synthetic phenolic antioxidants. In Williams, G. M., Sies, H., Erdman, J. W. and Baker, G. T. (Eds.). *Antioxidants: Chemical, physiological, nutritional and toxicological aspects* p. 202-208. Princeton: Princeton Scientific Press.