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Production of trans-fat-free margarine prepared using catfish oil and palm stearin with the addition of avocado waste oil for tocopherol enrichment

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Abstract

Catfish oil (CFO) is rich in unsaturated fatty acids (UFA) which are potentially produced into margarine through the blending method with palm stearin (PS) addition. The high UFA content of CFO causes margarine to be susceptible to oxidative damage, which can also trigger the formation of trans fatty acids (TFA). An antioxidant compound in the form of avocado waste oil (AWO) that contains tocopherol is added during margarine production to inhibit oxidative damage and TFA formation in the final product. Thus, the temperature and duration of CFO extraction, avocado peel and seed ratio of AWO extraction, and CFO:PS:AWO ratio of margarine production were analyzed for their effect on CFO, AWO, and margarine characteristics. This study was conducted by several stages; CFO extraction with temperature (50, 70 and 90°C) and duration (1 and 2 hrs) treatment, AWO extraction with avocado peel and seed ratio treatment (100:0, 75:25, 50:50, 25:75, 0:100), and margarine production with CFO:PS:AWO ratio treatment (41:40:0, 36:40:5, 31:40:10, 26:40:15, 21:40:20). The results showed that the increasing extraction temperature and duration caused the decrease of CFO hydrolytic-oxidative stability. The best CFO extraction treatment was 50°C and 1 hr of extraction. The increasing avocado peel ratio in AWO extraction led to the increase of AWO antioxidant potential. The best AWO extraction treatment was the ratio of peel:seed = 75:25. The addition of AWO in margarine production for tocopherol enrichment was proven to suppress oxidative damage and TFA formation. The best margarine production treatment was the 3rd formulation (10% of AWO) with UFA-rich and trans-fat-free margarine characteristics. It was selected as the most preferred color, taste and flavor of margarine. These studies' results are beneficial for the product development of UFA-rich and trans-fat-free margarine with good oxidative stability prepared using freshwater fish oil and plant byproduct oil.

1. Introduction

Fish oil product is produced from the extraction process of marine or freshwater fish (Bonilla-Mendez and Hoyos-Concha, 2018). Catfish (*Pangasius* sp.) is a freshwater fish that is easily found in Indonesia and its production is increased through aquaculture (Fujiana *et al*., 2020). The catfish production in Indonesia reached 391,151 tons in 2018 and 408,539 tons in 2020 (KKP, 2021). The high yield of CFO caused by fat content in the head (11.2%), 13.10% in the tailbone, 7.90% in the skin, 26.31% in the viscera and 36.21% in the belly flap (Hastarini *et al*., 2012). CFO yield is higher (76.48%) than other freshwater fish oils such as *Oreochromis mossambicus* (8.57%) and *Chanos chanos* (6.73%) (Kamini *et al*., 2016; Maulana *et al*., 2020). The UFA of CFO (63.78%) is higher than saturated fatty acids (SFA) and is dominated by oleic, linoleic, and linolenic acids (Ayu *et al*., 2019).

The high UFA content in CFO makes the product susceptible to oxidative damage. The hydrolytic-oxidative reaction in fat/oil products can be induced by heat treatment (high temperature and longer duration), so the proper techniques of fat/oil processing need to be considered (Suseno *et al*., 2020; Zhuang *et al*., 2022). Radical compounds generated by lipid oxidation may induce the formation of TFA (Vu and

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Boonyarattanakalin, 2013). Natural TFA was found in fresh catfish (0.16% of elaidic acid and 0.03% of linolelaidic acid) and CFO (0.06% of elaidic acid) (Ningsih, 2011; Ayu *et al*., 2019). WHO declared that the safe limit of TFA consumption is <1% of daily energy intake, but TFA consumption in small amounts over a long period increases the risk of cardiovascular disease (Islam *et al*., 2019).

The CFO is attractively used in margarine production but related to the high UFA content, TFA formation induced by radical compounds, and the presence of natural TFA in CFO, it is necessary to determine a process that can suppress oxidative reaction and TFA formation in the margarine production. The blending method is the easiest and safest way in margarine production. It involves low temperature with short production time through the physical mixing of saturated (e.g., PS) and an unsaturated fat fraction (e.g., CFO) (Hasibuan, 2021). The addition of PS in margarine production increases the product quantity and plasticity. Consequently, the exact ratio of saturated and unsaturated fractions is required (Subroto and Nurannisa, 2020).

The inhibition of oxidative reaction and TFA formation can also be done through the addition of antioxidants. Natural antioxidants are preferred for health reasons and their good antioxidant activity. Rich in UFA and containing tocopherol and other bioactive compounds, avocado waste promises to be utilized as an antioxidant agent. Further utilization of tocopherols and bioactive compounds in the avocado waste can be carried out through AWO extraction with the soxhletation method (Paramos *et al*., 2020). The avocado peels and seeds contain different amounts of tocopherol, which causes the level of antioxidant activity will be different. Thus, the proper ratio of avocado peel and seed is needed to optimize the antioxidant potential of AWO (Amado *et al*., 2019; Santana *et al*., 2019).

Tocopherol content is a primary antioxidant that can break the chain of fatty acid-free radical formation reactions and is heat resistant up to 180°C (Bruscatto *et al*., 2019; Rahmiati *et al*., 2020). The addition of tocopherol in margarine progressively increased the antioxidative stability. As a result, the oxidative damage reaction was inhibited (Azizkhani *et al*., 2011). The inhibition of oxidative reaction will be associated with the inhibition of free-radical-catalyzed TFA formation (Hung *et al*., 2016). Although tocopherol was proven to maintain oxidative stability, tocopherol concentration should be a concern considering the pro-oxidant behavior of tocopherol in high concentrations (Cano-Ochoa *et al*., 2022).

Previous studies successfully carried out the margarine production prepared using CFO, but no investigation of TFA content or AWO addition for tocopherol enrichment (Hasanah *et al*., 2017; Hastarini *et al*., 2021). Paramos *et al*. (2020) showed AWO production without α-tocopherol analysis based on the avocado peel and seed ratio while Santana *et al*. (2019) produced AWO through different methods. Thus, the investigation on the effect of extraction temperature and duration on CFO physicochemical characteristics, the ratio effect of avocado peel and seed on AWO physicochemical characteristics and antioxidant potential, and the ratio effect of CFO:PS:AWO on physicochemical and sensory characteristics, and antioxidant potential of margarine have not been carried out yet. Hence, the study aimed to evaluate the characteristics of physical, chemical, sensory, and antioxidant potential, as well as fatty acid profiles following each sample treatment and the established research design.

2. Materials and methods

2.1 Materials

This study used raw materials such as fresh catfish (~50 cm) which were obtained from the fish distributor in Balekambang, Surakarta, Indonesia. Fresh avocado waste (peel and seed) which has a length of >13 cm and weight of 250 g was obtained from a fruit distributor in Jebres, Surakarta, Indonesia. The palm stearin was obtained from PT. Asianagro Agungjaya, Jakarta, Indonesia. All chemical materials were analytic grade from Merck, Sigma Aldrich, and Supelco.

2.2 Catfish oil extraction

The extraction of the CFO was done as described by Kamini *et al*. (2016) with slight modifications. Briefly, the fresh catfish was cleaned and cut to a smaller size. Then the fish was heated using an oven (Memmert, UN55, Germany) with the treatment: temperature (50°C, 70°C and 90°C) and duration (1 hr and 2 hrs). The heated catfish was pressed using hand-press (Fomac, Indonesia). The solid residue and oil layer were separated by a filter cloth. The oil layer (crude CFO) was immediately refined through the next procedure.

2.3 Refining of crude catfish oil

The refining process of CFO carried out refers to the method used by Ayu *et al.* (2019) with modifications. The crude CFO was centrifuged using an LC-04S centrifuge (Oregon, China) at 500 rpm for 2 mins. The oil was stirred at 250 rpm, 65°C for 1 min using C-MAG HS7 magnetic stirrer (IKA, Germany). Then, 3% citric acid (3% of the total weight sample) was added to the oil

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and stirred at 250 rpm, 65°C for 5 mins. The pH mixture was checked using a pH meter (Juanjuan, China) to control the pH (3-5) and held for 5 mins at room temperature. The mixture was neutralized with 9.5% NaOH (0.5-0.7% of the total weight sample) at 250 rpm, 65°C for 5 mins. The pH was controlled in the range of 7. The mixture was centrifuged at 1000 rpm for 10 mins. The oil layer was separated from the precipitate and transferred back to Erlenmeyer. The activated carbon (7% of the total weight sample) was added to the oil and stirred at 65°C for 10 mins. Next, the mixture was centrifuged at 1000 rpm for 10 mins. The upper layer (refined CFO) was collected in dark storage and stored at 10°C until further analysis.

2.4 Avocado waste oil extraction

The extraction of AWO was conducted as described by Paramos *et al*. (2020) with slight modifications. The ratio of peel and seed was (%w/w = 1. 100:0; 2. 75:25; 3. 50:50; 4. 25:75; 5. 0:100). The avocado peel and seed were heated using an oven (Memmert, UN55, Germany) at 60°C for 6 hrs. The dried peels and seeds were mashed using a grinder (Advance, Indonesia). Avocado seed and peel powder were sieved with a size of 40 mesh (ABM, Indonesia). The mixture of avocado peel and seed powder according to a predetermined ratio was wrapped in Whatman paper (100 g) and then arranged in a soxhlet extractor. Ethanol 96% was added in a 1:3 ratio (material: solvent) and extracted at 78°C for 6 hrs. The solvent was evaporated at 40°C and 30 rpm (R-300, Buchi, Indonesia). AWO was collected in a dark bottle and stored at 10°C until further analysis.

2.5 Margarine production by the blending method

The production of margarine was done through the blending method as described by Hastarini *et al.* (2021) with modifications. The refined CFO, PS, and AWO were prepared in the ratio of (%w/w) 41:40:0; 36:40:5; 31:40:10; 26:40:15; 21:40:20. The mixture of CFO, AWO, and PS according to a predetermined ratio were heated along with lecithin as much as 0.3% of the total weight sample at 60°C and 250 rpm using C-MAG HS7 magnetic stirrer (IKA, Germany). The mixture was held for 5 mins. Next, water (16% of total weight sample) and salt (2.7% of total weight sample) were added to the mixture and stirred at 500 rpm, 45°C for 15 mins using a magnetic stirrer. The mixture was tempered using a chiller (GEA, Indonesia) at 5-7°C for 2 × 24 hrs. Then, some of the produced margarine was immediately analyzed for sensory characteristics and the rest was stored at 5-7°C until further analysis.

2.6 Physical, chemical, sensory, and antioxidant characterization

Analysis of refined CFO, AWO, and margarine was conducted by several tests: specific gravity using American Oil Chemists' Society (AOCS) Cc 10c-95 method (AOCS, 2017a), yield percentage (Kamini *et al*., 2016), slip melting point using AOCS Cc 3-25 method (AOCS, 1997a), water and fat content using SNI 3541 (National standardization agency of Indonesia, 2014), acid value using AOCS Cd 3d-63 method (AOCS, 1997c), free fatty acids using AOCS Ca-40 method (AOCS, 1996a), peroxide value using AOCS Cd 8-53 method (AOCS, 1996b), iodine value using AOCS Cd 1-25 method (AOCS, 1998), p-anisidine value AOCS Cd 18-90 method (AOCS, 1997b), total oxidation (Shahidi and Zhong, 2005), extraction fat for fatty acids profile identification using 10. Association of Official Analytical Chemists (AOAC) 996.06 method (AOAC, 2001), gas chromatography operation and TFA content identification using AOCS Ce 1j-75 method (AOCS, 2017b), α-tocopherol content (Mubarak *et al*., 2017; Yulianthi *et al.*, 2017), radical scavenging activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Zio *et al*., 2021), reducing power by ferric reducing antioxidant power method (Zouheira *et al*., 2018), and sensory analysis using SNI 2346 hedonic test method (National standardization agency of Indonesia, 2006). The preference scale uses the following parameters: 1 = Dislike very much; 2 = Dislike; 3 = Neither like nor dislike; 4 = Like; and 5 = Like very much.

2.7 Statistical analysis

The data of each research stage were displayed as a mean ± standard deviation (SD) from three replications. The statistical analysis of CFO production used a Completely Randomized Design (CRD) factorial, with two treatments such as temperature and duration of extraction. The data were analyzed using the General Linear Model Full Factorial followed by Duncan's Multiple Range Test (DMRT). The statistical analysis of AWO production used a CRD with one treatment, named the ratio of avocado peel and seed. The data were analyzed using the One-Way Analysis of Variance (ANOVA) method and continued by DMRT. The statistical analysis of margarine used CRD with one treatment, named the ratio of CFO:PS:AWO. The data were analyzed using One-Way ANOVA followed by DMRT. A $p<0.05$ was considered a significant difference.

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3. Results

3.1 The physical and chemical characteristics of the catfish oil

The statistical analysis showed that the temperature treatment has a probability score (Sig.) <0.05 for the between-subjects effects test on all parameters while the duration treatment has a probability score <0.05 on all parameters except for the TFA content results. The probability score of temperature*duration treatment was <0.05 on all parameters except for TFA content. This result was indicated that the extraction temperature affected all the parameter values of CFO. The extraction duration and the interaction of temperature and duration affected the yield, specific gravity, free fatty acids (FFA), acid value (AV), peroxide value (PV), iodine value (IV), p-anisidine value (PAV), and total oxidation (TOTOX) value of CFO.

The interaction effect of extraction temperature and duration on the physicochemical characteristics of CFO are presented in Table 1. The highest CFO yield was 15.462% at 90°C and 2 hrs of extraction while the lowest was 8.453% at 50°C and 1 hr of extraction. All CFO extraction treatment has the same slip melting point (SMP) range (28-38°C). The higher the extraction temperature and the longer the heating duration, the lighter the CFO specific gravity would be. The lowest specific gravity was 0.979 g/mL at 90°C and 2 hrs of extraction.

The increase in temperature and duration interaction of CFO extraction coincided with the decrease in the CFO hydrolytic-oxidative stability. The interaction of 50°C and 1 hr of extraction produces the lowest value of FFA (0.262%), PV (0.969 mEq/kg), PAV (10.537 mEq/kg) and TOTOX (12.473 mEq/kg), while the interaction of 90°C and 2 hrs of extraction produces the highest value of FFA (0.840%), AV (0.844 mg KOH/g), PV (3.892 mEq/kg), PAV (14.78 mEq/kg) and TOTOX (22.56 mEq/kg). The highest IV (134.345 g I_2/100 g) at 50°C and 1 hr of extraction, while the lowest IV (127.217 g I_2/100 g) at 90°C and 2 hrs of extraction. The TFA analysis showed that the temperature treatment affected the TFA content, but the duration treatment did not show any effects. The higher temperature treatment generated a higher TFA content. The TFA content of CFO was in the range of 0.003317 g/100 g - 0.005077

Table 1. The interaction effect of extraction temperature and duration on the yield and physicochemical characteristics of catfish oil.

Treatments	Yield (%)	The physical characteristics of CFO: Slip melting point (°C)	Specific gravity (g/mL)
50°C 1 hr	8.453±0.050^a	28-38	0.992±0.0006^e
50°C 2 hrs	8.956±0.051^b	28-38	0.991±0.0006^e
70°C 1 hr	10.453±0.050^c	29-38	0.989±0.0006^d
70°C 2 hrs	10.955±0.051^d	29-38	0.985±0.0006^c
90°C 1 hr	13.456±0.051^e	30-38	0.983±0.0006^b
90°C 2 hrs	15.462±0.054^f	30-38	0.979±0.0006^a

	The chemical characteristics of CFO: Free fatty acids (%)	Acid value (mgKOH/g)	Peroxide value (mEq/kg)
50°C 1 hr	0.262±0.001^a	0.270±0.001^a	0.969±0.008^a
50°C 2 hrs	0.309±0.025^b	0.284±0.008^a	2.000±0.009^b
70°C 1 hr	0.500±0.007^c	0.513±0.013^b	2.428±0.020^c
70°C 2 hrs	0.527±0.006^d	0.541±0.002^c	2.675±0.020^d
90°C 1 hr	0.706±0.003^e	0.800±0.009^d	2.839±0.004^e
90°C 2 hrs	0.840±0.005^f	0.844±0.010^e	3.892±0.019^f

	The chemical characteristics of CFO: P-anisidine value (mEq/kg)	TOTOX (mEq/kg)	Iodine value (g I_2/100 g)	Trans fatty acids (g/100 g)
50°C 1 hr	10.537±0.025^a	12.473±0.021^a	134.345±0.160^f	0.003317±0.000006^a
50°C 2 hrs	11.457±0.025^b	15.457±0.015^b	133.139±0.183^e	0.003317±0.000006^a
70°C 1 hr	12.227±0.025^c	17.080±0.036^c	131.765±0.183^d	0.004887±0.000006^b
70°C 2 hrs	13.157±0.025^d	18.507±0.067^d	130.601±0.183^c	0.004887±0.000006^b
90°C 1 hr	14.197±0.025^e	19.877±0.031^e	129.121±0.317^b	0.005077±0.000006^c
90°C 2 hrs	14.777±0.025^f	22.560±0.063^f	127.217±0.317^a	0.005077±0.000006^c

Values with the same superscripts are not statistically significantly different (p>0.05).

g/100 g. The best CFO extraction treatment based on the highest score of the De Garmo analysis (0.9412) was the interaction of 50℃ and 1 hr of CFO extraction.

3.2 The physical and chemical characteristics, and antioxidant potential of avocado waste oil

The ANOVA test results showed a probability score <0.05 on all parameters, indicated the treatment affected all the characteristics of AWO. The yield, physicochemical characteristics, and antioxidant potential of AWO are presented in Table 2. The highest oil yield was 13.86% at a 75:25 ratio, and the lowest oil yield was 11.14% at a 25:75 ratio. Based on that data, avocado peel oil tends to have a higher yield than avocado seed oil. Both peel and seed waste had the same SMP range (26-33℃). The highest specific gravity at the avocado peel and seed ratio = 100:0 (0.9739 g/mL), and the lowest value at 25:75 of ratio (0.9678 g/mL).

The data in Table 2 showed the lowest FFA content was 0.271% at the ratio of avocado peel and seed = 0:100. The ratio of 100:0 produced the highest AV (0.96 mg KOH/g) and PV (3.25 mEq/kg), while the ratio of 0:100 produced the lowest AV (0.56 mg KOH/g) and PV (0.96 mEq/kg). The ratio of 25:75 had the highest IV result (87.88 g I_2/100 g). Analysis of PAV showed the lowest result at the ratio of 25:75 (5.014 mEq/kg). The ratio of 100:0 had the highest TOTOX value (12.91 mEq/kg) while the ratio of 0:100 had the lowest TOTOX value (7.28 mEq/kg).

The radical scavenging activity is expressed in the percentage of inhibition and showed the highest result was at the ratio of avocado peel and seed = 75:25 (61.097%), and the lowest result was at the ratio of 0:100 (37.55%). The α-tocopherol content of AWO was in the range of 8.723 mg/100 g - 14.14 mg/100 g. The ratio of 75:25 had the highest reducing power result (411.770 mgTr/g), while the ratio of 0:100 had the lowest result (310.320 mgTr/g). Avocado peel oil tends to have a higher antioxidant potential than avocado seed oil. However, the highest ratio of avocado peel oil (100%) had a lower antioxidant potential than the ratio of avocado peel: seed = 75:25. This phenomenon may occur because each avocado waste has its antioxidant

Table 2. The effect of avocado peel and seed ratio on the yield, physicochemical characteristics and antioxidant potential of avocado waste oil.

Ratio of avocado peel:seed (%w:w)	Yield (%)	The physical characteristics of AWO	
		Slip melting point (°C)	Specific gravity (g/mL)
100:0	13.650±0.04^{c}	26-33	0.9739±0.0003^{d}
75:25	13.863±0.05^{d}	27-32	0.9731±0.0004^{c}
50:50	11.498±0.01^{b}	26-32	0.9697±0.0003^{b}
25:75	11.135±0.05^{a}	26-32	0.9678±0.0002^{a}
0:100	11.530±0.09^{b}	26-33	0.9726±0.0004^{c}
	The chemical characteristics of AWO		
	Free fatty acids (%)	Acid value (mgKOH/g)	Iodine value (g I_2/100 g)
100:0	0.534±0.001^{d}	0.963±0.04^{e}	85.658±0.73^{a}
75:25	0.535±0.002^{d}	0.856±0.02^{d}	85.869±0.60^{ab}
50:50	0.530±0.001^{c}	0.757±0.04^{c}	86.715±0.60^{b}
25:75	0.331±0.001^{b}	0.631±0.00^{b}	87.878±0.64^{c}
0:100	0.271±0.001^{a}	0.561±0.00^{a}	85.340±0.64^{a}
	The chemical characteristics of AWO		
	Peroxide value (mEq/kg)	P-anisidine value (mEq/kg)	TOTOX (mEq/kg)
100:0	3.247±0.453^{d}	6.409±0.032^{d}	12.905±0.032^{e}
75:25	2.524±0.221^{c}	6.434±0027^{d}	11.482±0.027^{d}
50:50	2.194±0.221^{c}	5.219±0.027^{b}	9.607±0027^{c}
25:75	1.629±0.234^{b}	5.014±0.032^{a}	8.270±0.032^{b}
0:100	0.957±0.003^{a}	5.368±0.031^{c}	7.282±0.031^{a}
	The antioxidant potential of AWO		
	Radical scavenging activity (%)	α-Tocopherol (mg/100 g)	Reducing power (mgTr/g)
100:0	55.611±0.444^{b}	12.025±1.996^{bc}	401.473±1.23^{c}
75:25	61.097±0.443^{e}	14.14±2.088^{c}	411.770±1.10^{e}
50:50	59.059±0.412^{d}	10.44±2.088^{ab}	406.418±0.72^{d}
25:75	57.326±0.372^{c}	11.50±2.083^{abc}	378.915±1.27^{b}
0:100	37.550±0.373^{a}	8.723±1.753^{a}	310.320±1.19^{a}

Values with the same superscripts are not statistically significantly different (p>0.05).

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potential. Therefore, the combination of a particular ratio displayed a higher value than one of them alone. The best AWO extraction treatment based on the highest score (0.6390) of the De Garmo analysis was the ratio of 75% peel to 25% seed.

3.3 The physical, chemical, and sensory characteristics, and antioxidant potential of margarine

The probability score of the ANOVA test was < 0.05 on all parameters analysis, which explained the treatment affected the margarine characteristics. The physicochemical characteristics and the antioxidant potential of the margarine are presented in Table 3. Based on the results, the SMP of margarine was in the range of 32-43°C. The highest SMP range was 40-43°C at the 1st formulation (0% of AWO) and the lowest SMP range was 32-35°C at the 5th formulation (20% of AWO). The water content of margarine was in the range of 14.45-14.80%. The highest water content was in the 5th formulation (20% of AWO). The fat content of margarine was in the range of 83.039-83.419%.

Several hydrolytic and oxidative analyses seemed to decrease until reached the lowest point in the 3rd formulation (10% of AWO), but in the 4th formulation (15% of AWO), the value was increased up to the 5th formulation (20% of AWO). The FFA results of the 3rd formulation (10% of AWO) was 0.288% and significantly different from the 1st formulation (0% of AWO) namely 0.553%. The AV results of the 2nd formulation (5% of AWO) was 0.374 mgKOH/g and was significantly different from the 3rd formulation (10% of AWO), which had the lowest value (0.304 mgKOH/g). The 3rd formulation (10% of AWO) had the highest IV results (135.04 $gI_2/100$ g) and the lowest PV (2.25 mEq/kg), PAV (10.57 mEq/kg), and TOTOX (15.07 mEq/kg) results and were significantly different from the others.

Table 3. The effect of CFO, PS, and AWO ratio on the physicochemical characteristics and antioxidant potential of margarine.

Formulations	Ratio of CFO: PS: AWO	The physical characteristics of margarine		
		Slip melting point (°C)	Water content (%)	Fat content (%)
Formulation 1	41: 40: 0	40-43	14.450 ± 0.050^{a}	83.419 ± 0.106^{c}
Formulation 2	36: 40: 5	38-42	14.500 ± 0.050^{a}	83.302 ± 0.131^{bc}
Formulation 3	31: 40: 10	35-39	14.600 ± 0.050^{b}	83.241 ± 0.131^{abc}
Formulation 4	26: 40: 15	34-37	14.700 ± 0.050^{c}	83.091 ± 0.120^{ab}
Formulation 5	21: 40: 20	32-35	14.800 ± 0.050^{d}	83.039 ± 0.141^{a}

		The chemical characteristics of margarine		
		Free fatty acids (%)	Acid value (mgKOH/g)	Iodine value (g I_2/100 g)
Formulation 1	41: 40: 0	0.553 ± 0.043^{c}	0.573 ± 0.012^{d}	133.56 ± 0.317^{a}
Formulation 2	36: 40: 5	0.356 ± 0.108^{ab}	0.374 ± 0.023^{b}	134.03 ± 0.256^{ab}
Formulation 3	31: 40: 10	0.228 ± 0.123^{a}	0.304 ± 0.023^{a}	135.04 ± 0.485^{c}
Formulation 4	26: 40: 15	0.463 ± 0.049^{bc}	0.530 ± 0.014^{c}	134.30 ± 0.183^{b}
Formulation 5	21: 40: 20	0.513 ± 0.022^{c}	0.553 ± 0.013^{cd}	133.56 ± 0.000^{a}

		The chemical characteristics of margarine			
		Peroxide value (mEq/kg)	P-anisidine value (mEq/kg)	TOTOX (mEq/kg)	Trans fatty acids (g/100 g)
Formulation 1	41: 40: 0	3.05 ± 0.087^{c}	11.13 ± 0.010^{e}	17.23 ± 0.165^{d}	0.0090 ± 0.000006^{e}
Formulation 2	36: 40: 5	2.92 ± 0.144^{c}	10.91 ± 0.010^{d}	16.74 ± 0.280^{cd}	0.0061 ± 0.000006^{a}
Formulation 3	31: 40: 10	2.25 ± 0.250^{a}	10.57 ± 0.010^{a}	15.07 ± 0.490^{a}	0.0073 ± 0.000006^{b}
Formulation 4	26: 40: 15	2.58 ± 0.144^{b}	10.77 ± 0.010^{b}	15.94 ± 0.289^{b}	0.0082 ± 0.000006^{c}
Formulation 5	21: 40: 20	2.83 ± 0.144^{bc}	10.83 ± 0.010^{c}	16.50 ± 0.289^{bc}	0.0088 ± 0.000006^{d}

		The antioxidant potential of margarine		
		Radical scavenging activity (%)	α-Tocopherol (mg/100g)	Reducing power (mgTr/g)
Formulation 1	41: 40: 0	55.65 ± 0.075^{a}	1.94 ± 0.300^{a}	219.28 ± 0.341^{a}
Formulation 2	36: 40: 5	57.27 ± 0.075^{b}	4.05 ± 0.306^{b}	309.34 ± 0.341^{d}
Formulation 3	31: 40: 10	60.02 ± 0.069^{e}	9.51 ± 0.525^{c}	330.62 ± 0.341^{e}
Formulation 4	26: 40: 15	58.64 ± 0.075^{d}	10.75 ± 0.306^{d}	283.70 ± 0.347^{c}
Formulation 5	21: 40: 20	57.77 ± 0.075^{c}	13.56 ± 0.306^{e}	278.33 ± 0.347^{b}

Values with the same superscripts are not significantly different (p>0.05).

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The highest TFA content was 0.0090 g/100 g in the 1st formulation (0% of AWO), and the lowest result was 0.0061 g/100 g in the 2nd formulation (5% of AWO).

The higher the addition of AWO, the higher the α-tocopherol content result. The highest result was 13.56 mg/100 g at the 5th formulation (20% of AWO), and the lowest result was 1.94 mg/100 g at the 1st formulation (0% of AWO). The highest result of radical scavenging activity and reducing power in the 3rd formulation (10% of AWO) and the lowest result in the 1st formulation (0% of AWO). The highest percentage of inhibition was 60.02%, and the lowest result was 55.65%. The highest reducing power was 330.62 mgTr/g and the lowest result was 219.28 mgTr/g.

The sensory analysis results are presented in Table 4 and supported by Figure 1. The hedonic test was conducted right after the margarine was produced, and the result was the initial sensory characteristics with limited quality degradation. Twenty-five untrained panelists were involved to estimate the level of consumer preference for the sample. The treatment affected the consumer acceptance level of all sensory characteristics. The color attributes of margarine displayed in the 1st, 2nd, and 3rd formulations were not significantly different and indicated that all three samples as the most preferred color. The taste and flavor attributes at the 3rd formulation (10% of AWO) were selected as the most preferred taste (4.64) and flavor (4.12) characteristics. The best margarine production treatment based on the analysis of the De Garmo was the ratio of CFO:PS:AWO 31:40:10 (3rd formulation) with a score of 0.8899.

The fatty acids profile of the standard, the CFO extracted by 50°C and 1 hr of treatment, and the 3rd formulation (10% of AWO) of margarine were presented in Table 5. The TFA content of the fame mix standard was detected at the retention time of 36.077 for elaidic acid and 36.162 for linolelaidic acid. The CFO extracted by 50°C and 1 hr of extraction showed a higher UFA (49.84%) than SFA (46.86%). The best margarine formulation (3rd formulation) displayed the UFA ratio was higher (50.17%) than the SFA (49.57%). The blending method and tocopherol enrichment through AWO addition in margarine production successfully inhibited TFA formation. The highest TFA content in margarine (0.0090 g/100 g) appears to experience a very small increase from the previous TFA content (0.003317 g/100 g) in CFO. Margarine produced in this study proved to be rich in UFA.

4. Discussion

CFO yield in this study was higher than research by Lestari *et al*. (2020), who reported that the oil yield produced by 80-120°C of extraction was 8.73-12.40%. The interaction of higher temperature and longer duration of extraction exhibited a huge heat exposure that facilitates cell wall damage, protein coagulation, and moisture reduction, which optimized the oil yield (Bako *et al*., 2017; Lestari *et al*., 2020). AWO yield in this study concurred with Paramos *et al*. (2020), which showed the oil yield range was 9.18-10.3% for avocado peel oil and 10-14% for avocado seed oil. The avocado peel contains a lower cellulose percentage than the avocado seed. Its peel extraction makes it much easier to produce the optimum oil yield (Qu *et al*., 2010).

The SMP range of CFO in this study (28-38°C) was slightly different from the study of Lestari *et al.* (2020), which showed the SMP of CFO was 37°C. The SMP of lipids generally increases along with the increase in the hydrocarbon chain length. The absence of double bonds results in linear molecular geometry and tight intermolecular interaction. The decrease in SMP is due

Table 4. The sensory characteristics of margarine.

Formulations	Ratio of CFO: PS: AWO	Sensory analysis (Hedonic test)		
		Color	Taste	Flavor
Formulation 1	41: 40: 0	3.28^{b}	1.76^{a}	2.44^{a}
Formulation 2	36: 40: 5	3.88^{c}	3.72^{c}	3.32^{b}
Formulation 3	31: 40: 10	3.68^{bc}	4.64^{d}	4.12^{c}
Formulation 4	26: 40: 15	2.64^{a}	2.64^{b}	3.24^{b}
Formulation 5	21: 40: 20	2.40^{a}	2.82^{b}	3.48^{b}

Values with the same superscripts are not significantly different (p>0.05).

Figure 1. The appearance of margarine produced by various CFO:PS:AWO ratios through the blending method.

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Table 5. The fatty acids profile of FAME MIX standard, CFO (50°C 1 hr), and margarine (3rd formulation).

No	Fatty Acids	Supelco 37 FAME MIX Standard		CFO extraction treatment of 50°C 1 hr		Ratio of margarine CFO:PS:AWO = 31:40:10 (3rd formulation)	
		Retention Time	% Relative Area	Retention Time	% Relative Area	Retention Time	% Relative Area
1	C4:0	2.188	5.83	2.153	0.00		
2	C6:0	4.655	5.75	4.688	0.00	4.602	0.08
3	C8:0	10.003	5.46				
4	C10:0	13.422	2.38	13.428	0.01		
5	C11:0	17.013	5.06	17.023	0.38		
6	C12:0	20.598	2.22	20.607	0.01	20.485	0.01
7	C13:0	23.650	1.90				
8	C14:0	24.113	4.69	24.167	4.34	24.042	0.01
9	C14:1	27.062	1.85	26.262	0.12	26.167	0.04
10	C15:0	27.495	2.08	27.497	0.18	27.402	0.07
11	C15:1	30.042	1.82	30.068	1.08	29.958	0.48
12	C16:0	30.763	6.70	31.018	33.15	30.922	42.19
13	C16:1	33.198	1.77	33.112	0.19	31.752	0.03
14	C17:0	33.873	1.90	33.885	0.18	33.015	0.08
15	C17:1	35.373	1.69	35.273	0.00	33.798	0.09
16	C18:0	35.880	2.03	36.027	8.34	36.147	6.85
17	C18:1*t*	36.077	2.62	36.100	0.07	36.148	0.24
18	C18:1*n9*	36.093	3.94	36.395	37.56	36.243	34.18
19	C18:2*t*	36.162	1.59	36.160	0.10	36.292	0.13
20	C18:2n6	36.272	2.29	37.058	8.26	36.297	11.81
21	C20:0	36.903	3.97			38.100	0.04
22	C18:3n6	41.403	1.65	40.403	0.03	41.297	1.08
23	C20:1n9	41.622	1.70	41.417	0.12		
24	C18:3n3	42.070	1.69	42.095	0.70	41.962	0.28
25	C21:0	42.763	1.65				
26	C20:2	42.982	3.59	42.967	1.25	42.840	0.53
27	C22:0	43.880	3.81	43.872	0.14	43.760	0.19
28	C20:3n6	47.008	1.81	47.583	0.18		
29	C22:1n9	47.577	1.66			47.468	0.18
30	C20:3n3	49.030	1.69			49.037	0.57
31	C23:0	49.128	1.76	49.138	0.12		
32	C20:4n6	49.917	2.79	49.895	0.06	49.787	0.12
33	C22:2	49.940	1.11				
34	C24:0	52.603	1.87	53.053	0.01	52.733	0.05
35	C20:5n3	54.698	1.78	54.058	0.04	54.573	0.21
36	C24:1	55.525	2.71	54.685	0.05		
37	C22:6n3	55.555	1.19	55.505	0.03	55.378	0.20
Monounsaturated Fatty Acids					46.86		49.57
Polyunsaturated Fatty Acids					10.65		14.93
Saturated Fatty Acids					46.86		49.57
Unsaturated Fatty Acids					49.84		50.17

to the unsaturation degree of lipids. The presence of double bonds in the hydrocarbon chain results in bent molecular geometry, which causes the intermolecular interaction to be weak. As a result, the SMP is relatively lower for the UFA and higher for the SFA (Siram *et al*., 2019). The SMP range of AWO in this study (26-33°C) was lower than regular avocado oil (38-42°C) (Ogbuagu and Okoye, 2020) and indicated that the UFA of AWO was higher than regular avocado oil. The SMP of margarine also seemed to decrease with an increase in AWO addition of margarine production. The study by Amado *et al*. (2019) showed the high content of UFA in

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avocado waste, so its addition to other types of lipid products will decrease the SMP of the final product. The SMP of margarine in this study was also higher than the SMP of CFO because of the PS addition. According to Siswanti *et al*. (2021), the SMP of PS was 52-55°C, and the physical blending of saturated and unsaturated fat caused the SMP on the final product to be between them.

FAO (2012) declared that the specific gravity of fish oil was 0.928 g/mL and vegetable oil was 0.92 g/mL. The specific gravity of CFO and AWO in this study were higher than the FAO results. The increase in temperature and duration of oil extraction triggers the oil structure damage resulting in a density decrease that is related to a specific gravity decrease (Baig *et al*., 2022). The specific gravity of oil is influenced by its molecular weight. The fatty acids that have a lot of branches are known to have a higher density (Arisky *et al*., 2021). Hence, due to the high UFA content in avocado peel (Amado *et al*., 2019), the specific gravity of avocado peel was higher than avocado seed. The specific gravity also depends on the saturation degree, impurities, and polymerization (Perera *et al*., 2020).

The National Standardization Agency of Indonesia through SNI 3541 (National Standardization Agency of Indonesia, 2014) regulated the water content of margarine as <18% and >80% of fat content. Thus, the water and fat content of margarine in this study met the requirements. According to Lestari (2010), the water content of margarine should not exceed the limit to maintain hydrolysis stability and shelf life because microorganisms easily reproduce in high water content. Fat content is known to affect margarine firmness and plasticity. Total fat content depends on the fat phase to the aqueous phase ratio in margarine production.

The 1st (0% of AWO), 2nd (5% of AWO), and 3rd (10% of AWO) formulations were selected as the most preferred color characteristics, and the 3rd formulation was selected as the most preferred flavor and taste characteristics. These indicated that the 3rd formulation of margarine had a better level of consumer acceptance than others. AWO addition was favored to margarine sensory characteristics. The color attribute is a representative factor in determining product quality. Chlorophyll and carotenoid compounds of AWO were estimated to elevate the margarine color acceptance. The flavor and taste attributes were essential to determine the quality and acceptability of the product. As a result of the CFO refining process without deodorization stages, the CFO's distinct flavor appeared in margarine. The addition of AWO with a rich buttery flavor and taste in margarine production was appraised to cover the fishy flavor or taste. Thus, margarine sensory characteristics are accepted at a particular formulation (Winarno, 2002; Wang *et al*., 2010; Hasanah *et al*., 2017; Hastarini *et al*., 2021; Cervantes-Paz and Yahia, 2021).

The FFA, AV, PV, IV, PAV, and TOTOX in all CFO samples were quite low and met the standard of the National Standardization Agency of Indonesia (2018) and Codex (2017b) requirements. Fish oil should have an FFA value of <1.5%, <3 mgKOH/g of AV, <5 mEq/kg of PV, >120 m/iod of IV, <20 mEq/kg of PAV, and <26 mEq/kg of TOTOX value. All AWO samples in this study met the requirements of Codex (2017a) regarding vegetable oil standards. Vegetable oil should have an FFA value of <5%, <4 mgKOH/g of AV, <15 mEq/kg of PV, and 75-94 g I_2/100 g of IV. The PV of margarine in this study was <5 mEq/kg and higher than the study of Hastarini *et al*. (2021), with a result of 1.52-1.75 mEq/kg.

The highest value of AV, FFA, PV, PAV, and TOTOX and the lowest value of IV at 90°C and 2 hrs of CFO extraction proved that the greatest CFO damage occurred in the highest temperature and the longest duration of extraction. This interaction resulting a larger heat energy total that directly influenced the hydrolytic and oxidative rate of CFO, which is indicated by an increase in the hydrolysis products (FFA and AV) and oxidation products (PV, PAV, and TOTOX) (Prive *et al*., 2010; Saeed and Naz, 2019). The result of AV, FFA, PV, PAV, and TOTOX of AWO showed that the greater the ratio of avocado peel, the more likely it will generate AWO with a higher hydrolytic-oxidative analysis value. This means the avocado seed had higher hydrolytic-oxidative stability than the avocado peel. As the avocado peel contains a higher UFA than the avocado seed (Amado *et al*., 2019), the chance of oxidation reaction is higher in the avocado peel.

The results of FFA, AV, PV, PAV, and TOTOX of margarine showed that the addition of AWO was proved to decrease the hydrolytic-oxidative analyses value and reached the lowest point at 10% of AWO addition. This indicated the antioxidant potential of AWO in the inhibition of lipid deterioration. The addition of 15% AWO and so on, tended to increase the results. As a consequence of using the selected AWO with the highest α-tocopherol value for tocopherol enrichment in margarine, the initial FFA and PV of the selected AWO were slightly higher than other treatments. The initial FFA and PV in AWO are thought to have a prooxidant effect at 15% and 20% of AWO addition in margarine. Free fatty acids had a pro-oxidative activity through the catalytic effect of the carboxylate group on the free radicals formation because of the decomposition of the hydroperoxides forming at the early oxidation stage.

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Another mechanism occurs due to an FFA causing the reduction of surface tension, which facilitates the oxygen transfer into the oil. The presence of water in bulk oil causes FFA to act as surfactants to concentrate the surface of the oil-water interface, resulting in lipid decomposition (Auborg, 2001; Kittipongpittaya *et al*., 2014). The hydroperoxides as oxidation reaction products are reactive oxygen species. The metal ions accelerate the breakdown of hydroperoxides into hydroxyl radicals and facilitate the peroxidation of lipids (Desai *et al*., 2014).

The study results showed that a high ratio of avocado peel in AWO extraction resulted in a high radical scavenging activity and reducing power. According to Rojas-Garcia *et al*. (2022), the avocado peel showed large quantities of phenolic compounds and higher polymerization than the avocado seed. Avocado peel consisted of several kinds of glycosidic bonds, phenolic acids, and polymerized procyanidins, which supported the quenching ability and reducing power. The antioxidant compounds lead to the reaction with radical peroxyl and hydroperoxides, then break the chain reaction at the initiation and propagation phase. Hence, the antioxidant addition is expected to maintain the oxidative stability of fat/oil products (Wang *et al*., 2018). This study's results also showed that the AWO addition increased radical scavenging activity and reducing power in margarine. These are related to the phenolic compounds of AWO as described before. However, the addition of 15% and 20% of AWO in margarine decreases the radical scavenging activity and reducing power. These are presumably because the prooxidant effect of the earlier FFA and hydroperoxide compounds in AWO is greater than the antioxidant mechanism of AWO itself.

The α-tocopherol content of all AWO in this study was in line with Woolf *et al*. (2009), that the α-tocopherol of avocado oil is 70-190 μg/g of oil (7-19 mg/100 g of oil). The ratio of avocado peel:seed = 100:0 showed a higher α-tocopherol content than the ratio of 0:100. Study by Amado *et al.* (2019) also showed the α-tocopherol content of avocado peel was greater than avocado seed. As a result of the different α-tocopherol amounts in avocado peel and seed oil, their combination in this study resulted in the highest α-tocopherol content at a certain ratio. This proves the importance of determining the exact ratio of avocado peel and seed in AWO extraction. The increase in the α-tocopherol content of margarine occurred along with the AWO addition. Margarine without AWO addition appeared to have low oxidative stability compared to others. The α-tocopherol enrichment in margarine optimally reduces oxidative damage at a particular AWO ratio (10%). These results are indeed related to the hydrolytic-oxidative characteristics, radical scavenging activity, and reducing power of AWO as explained before. The α-tocopherol reacts with free radicals in an antioxidant mechanism, it causes the compound to be converted into radical tocopherol which is stable and inhibits the peroxidation cycle (Cillard *et al*., 1980).

The TFA compounds and fatty acids profile were analyzed by Gas Chromatography (GC) with flame-ionization detection. As the TFA content of all CFO and margarine samples in this study was <0.1 g/100 g, it can be labeled as a trans-fat-free food product following the rule by the National Agency of Drug and Food Control of Indonesia (2016). The TFA in CFO was much lower than in several studies namely 0.06-0.16% (elaidic acid) and 0.03% (linolelaidic acid) (Ningsih, 2011; Ayu *et al*., 2019). The extraction duration was proven not to affect the TFA content. The increase in CFO extraction temperature influenced the TFA content, but the results were very low. The TFA is produced under extreme temperatures. This study did not involve any extreme temperatures. It makes the TFA presence thought of as a natural TFA in CFO. Li *et al.* (2013) explained that cis-trans isomerization is spontaneous and impossible to occur at room temperature. In a couple of cases, the temperature between 130-160°C for 1-3 hrs increases the trans isomers in small amounts.

Free radicals as lipid oxidation products can react reversibly toward a double bond to radical adduct forming. As a double bond is reestablished, the trans configuration is selected due to a thermodynamically stable characteristic of a trans double bond (Vu and Boonyarattanakalin, 2013). The trans isomerization is possibly induced by free radicals, hence the addition of antioxidants with a mechanism to prevent the new radical formation should be able to inhibit the isomerization. This study's results displayed that AWO addition in margarine production successfully inhibits TFA formation. Tzeng and Hu (2014) explained that antioxidant compounds provided fatty acid protection by intercepting the radicals-induced cis-trans isomerization.

The fatty acids profile of CFO was slightly different from Ayu *et al*. (2019), with 36.22% of SFA and 63.78% of UFA. Meanwhile, the fatty acids profile of the 3rd formulation (10% of AWO) of margarine was in line with the result of the fatty acids profile in commercial margarine by Mannan *et al*. (2015), which reported the UFA content was higher than the saturated one. The dominant SFA content of margarine in this study and the literature was palmitic acid, while the UFA part was dominated by oleic acid. These fatty acids' composition influenced the firmness and plasticity of the margarine because the SFA had a greater than or equal to 10 carbons in chain length, so the SMP of these fatty acids was high (Paduret, 2022).

5. Conclusion

The increased extraction temperature and duration clearly reduce the hydrolytic-oxidative stability of CFO but increase the oil yield. The best CFO extraction treatment which resulted in the best physicochemical characteristics was 50°C and 1 hr of extraction. The avocado peel was proven to produce AWO with high antioxidant potential but lower hydrolytic-oxidative stability than the avocado seed. The ratio of avocado peel:seed =75:25 positively produced AWO with the highest yield and antioxidant potential with quite good hydrolytic-oxidative stability. The addition of AWO for tocopherol enrichment was successfully applied to margarine production prepared with CFO and PS. The formulation of margarine (CFO:PS:AWO) = 31:40:10 appeared with the highest hydrolytic-oxidative stability, the highest antioxidant potential, and free of TFA content. The addition of AWO as much as 10% (the 3rd formulation) optimally elevated the sensory acceptance in margarine and was selected as the most preferred color, taste, and flavor characteristics. Thus, increasing AWO addition in margarine production significantly increases the α-tocopherol content and at a certain amount provides an optimum antioxidant effect in the inhibition of oxidation reaction and TFA formation. These results may lead to trans-fat-free margarine product development prepared using another UFA-rich freshwater fish oil and plant byproducts as an antioxidant agent through a simple method.

Conflict of interest

The authors declare no conflict of interest.

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