THE EFFECT OF DIFFERENT TYPES OF OIL AND TEMPERATURE ON THE FORMATION OF HETEROCYCLIC AMINES AND POLYCYCLIC AROMATIC HYDROCARBONS IN REDUCED FAT PORK PATTIES

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Abstract – The effects of partially replacing (40%) pork back fat in cooked pork patties with various types of vegetable oil (olive oil, sunflower oil and grape seed oil) while cooking at temperatures of 180°C and 220°C on the formation of HCAs and PAHs were examined. HCAs and PAHs were extracted by solid-phase extraction and analyzed by HPLC. Control patties contained the highest amount of HCAs and relatively higher PAHs cooked at 180°C and 220°C. All 3 fat modified patties had significantly lower HCAs, it could be attributed to antioxidants in such oils, as a significant negative correlation (r= -0.618, p<0.01) was found between the antioxidant capacity of lipids and the total amount of HCAs. TBARS and protein carbonyls were both positively related (r=0.826 and 0.788, p<0.01) to the amount of total HCAs. Patties modified with olive oil had a lower amount of PAHs compared with others. Type of oil and the interaction of between cooking temperature and type of oil had effects on the formation of HCAs and PAHs, but not the cooking temperature.

Key Words – free radicals, antioxidant capacity and oxidation.

I. INTRODUCTION

It has been established that increasing saturated fatty acid intake can elevate the cholesterol level, this will increase the risk of cardiovascular disease, changing fatty acids profiles by replacing saturated fatty acids (SFA) with unsaturated fatty acids is necessary to make food healthier in the past 50 years [1]. However, recent studies reported that intake of vegetable oils that are abundant in linoleic acid (corn oil and sunflower oil) might increase the risk of heart disease, compared with intake of SFA [2]. They are also increasingly associated with the generation of potentially toxic compounds, such as aldehydes and ketones in domestic cooking [3]. Meanwhile, carcinogens such as heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs) and N-nitrous compounds can be formed in processed meat products. These 5 Aminoimidazoarenes (AIAs) compounds, IQ, 2-amino-3-methylimidazo[4,5-f]quinolone, MeIQ, 2-amino-3,4-methylimidazo[4,5-f]quinolone, MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline and PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine have been classified into human carcinogens. PAHs are hydrocarbons that contain two or more benzene rings, such as pyrene, anthracene and naphthalene. Benz[a]anthracene (BaA) and benzo[a]pyrene (BaP) are PAHs with more potent carcinogenicity [4]. Olive oil and sunflower oil can dramatically enlarge the percentage of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) respectively, also they are both rich in antioxidants such as vitamin E [5]. Grape seed oil has high PUFA with tocopherols and polyphenolic compounds [6]. The formation of HCAs and PAHs are involved in radical reactions at high cooking temperatures. Thus, the objectives of this study were explore the effects of partially replacing pork back fat with sunflower oil, olive oil and grape seed oil on the formation of HCAs and PAHs, and also examine the effect of different cooking temperatures on the formation of carcinogens in fat reduced pork patties.

II. MATERIALS AND METHODS

Preparation of pork patties

Four types of pork patties were prepared with pork back fat, sunflower oil, olive oil and grape seed oil. The formulation of patties per kg: 700g lean pork mince, 180g distill water, 100g pork back fat and 20g salt. For fat modification, 40g fat was replaced by sunflower oil, olive oil and grape seed oil. The remaining ingredients were same. All ingredients were homogenized 5min to reach the uniform emulsion. Each patty was weighed 100g, shaped in

9.0×9.0×2.5cm foil cup. Patties were cooked in an air-forced oven: 180°C 26min and 220°C 22min until the core temperature of patties reached to 73°C. The treatments (4×2) were produced in triplicate.

Table 1: Fatty acid profile and antioxidant containing in 4 types of lipids (per 100g edible portion)

	Pork back fat	Sunflower oil	Olive oil	Grape seed oil
SFA %	40.30	14.3	14.3	12.4
MUFA %	43.4	20.5	73.0	20.2
PUFA %	10.0	63.3	8.2	68.2
Antioxidant	VE 1mg [5]	VE 49.22mg [5]	VE 100-300mg [5]	VE 10-15mg Polyphenols 5.9-11.5mg Gallic acid equivalent[6]

Thiobarbituric acid-reactive substances (TBARS)

5g of ground patty was homogenized with 15mL perchloric acid (3.86%) and 0.5 mL BHT (4.2% in ethanol) in a beaker (ice bath), then filtered and centrifuged (1854rcf for 4min). 2 mL aliquots were added with 2 mL thiobarbituric acid (0.02M) in test tubes. Tubes were then placed in a boiling water bath (100 °C) for 45min. After cooling, the absorbance was measured at 532nm. The standard curve was prepared by 1,1,3,3-tetraethoxypropane solution (0.2268 g) in 3.86% perchloric acid [7].

Total protein carbonyl value

1g of ground patties was homogenized in 10mL 20mM sodium phosphate buffer with0.6M NaCl for 30s. Two equal aliquots of 0.2mL mixture was added with cold 10% TCA (1mL) and centrifuged for 5min at 3090rcf. One pellet was mixed with 1mL 2M HCl and the other with 1mL of 0.2% (w/v) DNPH in 2M HCl. 1mL 10% TCA added into tubes and washed twice with 1 mL ethanol. The pellets were then mixed with 1.5mL of 20mM sodium phosphate buffer containing 6M guanidine HCl centrifuged for 2min at 3090rcf. Result was calculated from absorption at 280nm using BSA as standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein [7].

Separating and analyzing HCAs and PAHs

Each 3g ground pattie surface (2mm) was blended with 12ml 1M NaOH and then transferred into an Extrelut20 column with 17g diatomaceous earth. The HCAs were eluted out by 60ml ethyl acetate, and poured into a PRS cartridge that was conditioned with 7ml ethyl acetate, whereas PAHs were eluted by 60mL of CH_2Cl_2 containing 5% toluene. The PAHs residue was re-dissolved in 1mL n-hexane eluted by 25mL of n-hexane and 60mL of 60:40 (v/v) n-hexane–CH_2Cl_2 mixtures in a glass column with silica gel. For HCAs, the PRS cartridge was then washed by 6ml 0.1M HCl, 15ml methanol/0.1M HCl (45/55, v/v) and 2ml water, then eluted by 20ml 0.5M ammonium acetate (pH 8.5) and transferred into a C18 cartridge which was conditioned with 5ml methanol and 5ml pure water. HCAs were eluted by 1ml methanol/ammonium hydroxide (9/1, v/v). The extract of HCAs and PAHs solvent were dried under a stream of nitrogen. The HCAs were dissolved with 50µl methanol, PAHs were dissolved in 1ml acetonitrile [4, 8].

The 5 HCAs were separated by a reversed-phase Luna 5uC18column (25cm×4.6mm, 5µm) with 0.01M triethylamine pH 3.6(A) and acetonitrile (B) (HPLC). A linear gradient of solutions was carried out that 95%A decreased to 75%A and 5%B increased to 25%B in 30min at a flow rate of 1mL/min. The temperature of column was 40°C. The DAD detector was set at 252nm.

The PAHs were separated by the same column with a mobile phase (the mixture of 84% acetonitrile and 16%HPLC water) by using a HPLC (1mL/min at 40°C). Fluorescence detector was performed by applying the excitation and emission wavelength program: 280/410 nm 0-8.50min (BaA), 376/410 nm 8.50-15min (BaP). The quantity of each individual HCA and PAHs was determined from calibration curves, which were established by the standard solutions of each HCA and PAH at 0.5,5 and 50ng/mL [4, 8].

Total antioxidant capacity (TEAC) of lipids

An ABTS radical solution was prepared by mixing 2.5mM ABAP with 20mM $ABTS^{2-}$ stock solution in 100mM phosphate buffer. The solution was heated at 60°C for 12min, covered with foil and cooled to 22°C. 40µl of the oil/methanol mixture was mixed with 1960µl of the radical solution. Difference of absorbance at 734nm in 6 min was recorded. A calibration curve was made by linear concentrations of Trolox [9].

Statistical analysis

Each sample was triplicated for chemical analysis and HCAs and PAHs determination. The significant difference between means of HCAs and PAHs in 8 samples were carried out by Duncan test. The correlation between total HCAs/PAHs and TEAC/TBARS/protein carbonyl were

examined by Pearson's correlation. The interaction of temperature and type of oil was analyzed by two-way ANOVA (SPSS Statistics 21, $p<0.05$).

Table 2: HCAs and PAHs in cooked pork patties that fat partially replaced with different oils at 180 and 220 degrees

Patties	Cooking temperature	IQ (ng/g)	MeIQ (ng/g)	MeIQx (ng/g)	4,8-DiMeIQx (ng/g)	PhIP (ng/g)	BaA (ng/g)	BaP(ng/g)
Control	180°C	Nd	18.26±14.46^{a}	8.34±1.78^{ab}	25.66±1.51^{b}	11.43±6.33^{a}	0.15±0.01^{a}	2.44±0.37^{c}
	220°C	3.88±3.50^{a}	59.70±0.98^{b}	13.45±7.43^{b}	43.37±15.67^{c}	24.07±1.99^{b}	0.21±0.03^{b}	3.08±0.06^{d}
Olive oil	180°C	0.58±0.01^{b}	Nd	3.50±0.68^{a}	Nd	Nd	0.15±0.02^{a}	2.24±0.40^{bc}
	220°C	1.30±0.42^{b}	Nd	2.52±0.36^{a}	1.31±0.22^{a}	14.78±1.49^{a}	0.15±0.01^{a}	1.44±0.27^{a}
Sunflower	180°C	Nd	Nd	4.32±0.50^{a}	1.02±0.50^{a}	Nd	0.14±0.01^{a}	1.88±0.17^{ab}
oil	220°C	0.64±0.16^{b}	Nd	4.31±0.55^{a}	5.12±0.35^{a}	22.70±1.95^{b}	0.31±0.02^{c}	3.53±0.20^{e}
Grape seed	180°C	Nd	Nd	Nd	Nd	Nd	0.18±0.01^{ab}	3.29±0.15^{d}
oil	220°C	0.59±0.04^{b}	1.31±0.06^{c}	Nd	Nd	Nd	0.18±0.05^{ab}	2.51±0.07^{c}

Results with different letters in the same column are significantly different at the level $p<0.05$. Nd: not detected.

Table 3: Effect of oil and temperature on the TBARS, protein carbonyl, total HCAs and PAHs in cooked pork patties

Fat partially replaced pork patty	TBARS (mg MDA/kg patty)	Protein carbonyl (nmol/mg protein)	Total HCAs (ng/g)	Total PAHs (ng/g)
Control	0.71±0.27^{c}	12.11±1.43^{b}	104.07±43.74^{a}	2.93±0.45^{b}
Olive oil	0.44±0.14^{b}	3.41±0.76^{a}	13.07±7.78^{b}	1.98±0.53^{a}
Sunflower oil	0.25±0.02^{ab}	3.85±0.42^{a}	14.93±9.95^{b}	2.93±1.01^{b}
Grape seed oil	0.19±0.05^{a}	3.69±0.40^{a}	0.95±1.04^{b}	3.09±0.42^{b}
Significance (p-value)	0.001	0.001	0.001	0.031
Cooking temperature				
180°C	0.30±0.13^{a}	5.40±3.70	19.91±29.78	2.61±0.61
220°C	0.50±0.30^{b}	6.12±4.08	46.60±58.11	2.86±0.88
Significance (p-value)	0.044	0.657	0.171	0.433
Oil*temperature	0.001	0.23	0.001	0.001

Results with different letters in the same column are significantly different at the level $p<0.05$.

III. RESULTS AND DISCUSSION

The range of the total HCAs was from Nd to 140.57±22.03ng/g (Table 2). The dominating compounds of HCAs were MeIQ and 4,8-DiMeIQx in C patties, PhIP in S and O patties. The effect of types of oil on the formation of individual HCAs was pronounced. The reduction of 4, 8-DiMeIQx and PhIP were 87%-95%, 8%-42% in S patties and O patties respectively, and they were completely inhibited in G patties. The results indicate that olive oil and sunflower oil, which were abundant in tocopherols, could totally prohibit MeIQ, whereas MeIQx, 4, 8-DiMeIQx and PhIP were successfully inhibited by grape seed oil (tocopherols and polyphenols). The different efficiency of prohibition might be due to antioxidants interacting with various pathways during the development of MeIQ, MeIQx and PhIP. Polyphenolic compounds could efficiently scavenge the carbonyl compounds that are involved in the Strecker degradation of phenylalanine to produce phenylacetaldehyde (intermediate of PhIP) [10]. Total HCAs level was successfully reduced average 86% in O, S and G patties, where a strong negative correlation with TEAC of oils was found. Cooking temperature had no effect on the concentration of total HCAs in patties, but the interaction between types of oil and cooking temperature was notable. The possible reason might be due to lipid perform differently under high temperature.

Table 4: Pearson's correlation coefficient (p) between the levels of total HCAs/PAHs and TBARS, protein carbonyl and TEAC

	p_{TEAC}	p_{HCAs}	p_{PAHs}
TBARS	-0.764**	0.826**	-0.154
Protein carbonyl	-0.606**	0.778**	0.019
TEAC	-	-0.618**	0.301

** Significant level p=0.01

Lipid oxidation and protein oxidation could be involved with Maillard reactions in the meat system [11]. However, to our knowledge, few studies have been explored on the correlation between TBARS/protein carbonyl values and total amount of HCAs. All 3 fat modified patties had significantly lower TBARS than control samples (Table 3). This defensive effect again lipid oxidation could belong to the stated antioxidants, since a significantly negative effect was showed

between TBARS values and TEAC of oils (Table 4). The oxidation of protein could be reduced by the presence of antioxidants in oil, as it was contrarily related to the TEAC of oils. However, it might not be dependent on the type of antioxidant, as there was no significant difference among 3 fat modified patties.

Total PAHs were ranged from1.59±0.26ng/g to 3.84±0.21ng/g. The dominating compound was BaP in all samples. The effect of types of oil on the formation of PAHs was significant. O patties had a lower amount of total PAHs. Sunflower oil and grape seed oil are rich in PUFA, especially linolenic acid which could promote the formation of PAHs, compared with olive oil (high in MUFA) [5]. Antioxidants in such oils had no effect on the formation of PAHs, as the relationship between the TEAC of lipids and the amount of total PAHs was not significant. The impact of tocopherols and polyphenolic compounds on the formation of PAHs in processed meat are not well documented. Only the effect of vitamin E on the adverse effect of BaP in human lung epithelial cells has been investigated and confirmed that its antioxidant activity could significantly prohibited free radicle that BaP induced and protect cellular damage [12]. Cooking temperature did not significantly affect the formation of PAHs, but the interaction between oil and temperature was significant.

IV. CONCLUSION

All 3 fat modified patties had significantly lower HCAs. Antioxidants in oils inhibited HCAs might through lipid oxidation and protein oxidation. Type of oil and the interaction of between temperature and oil had effects on the formation of HCAs and PAHs. Overall, replacing fat with olive oil contained lower amount of both HCAs and PAHs in cooked pork patties.

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REFERENCES

1. Sadler, M. J. (2014). Food, Nutrients and Food Ingredients with Authories EU Health Claims. Cambridge, UK, Woodhead Publishing.
2. Ramsden, C. E., Zamora, D, Majchrzak-Hong, S., Faurot, K.R., Broste S. K, Frantz, R.P. et,al. (2016) Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968-73). BMJ 353: i1246.
3. Guillén, M. D. and P. S. Uriarte (2012). Aldehydes contained in edible oils of a very different nature after prolonged heating at frying temperature: Presence of toxic oxygenated α, β unsaturated aldehydes. Food Chemistry 131(3): 915-926.
4. Janoszka, B. (2011). HPLC-fluorescence analysis of polycyclic aromatic hydrocarbons in pork meat and its gravy fried without additives and in the presence of onion and garlic. Food Chemistry 126(3): 1344-1353.
5. McCance, R. A. and E. M. Widdowson (2002). The composition of foods. Section 2.4: Fats and oils. Cambridge, London: The Royal Society of Chemistry, Food Standards Agency.
6. Bail, S., G. Stuebiger, S. Krist, H. Unterweger and G. Buchbauer (2008). Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity. Food chemistry 108(3): 1122-1132.
7. Rodr´ıguez-Carpena, J. G., D. Morcuende and M. Est´evez (2011). Partial Replacement of Pork Back-Fat by Vegetable Oils in Burger Patties: Effect on Oxidative Stability and Texture and Color Changes during Cooking and Chilled Storage. Journal of Food Science 76(7): 1025-1031.
8. Puangsombat, K., P. Gadgil, T. A. Houser, M. C. Hunt and J. S. Smith (2011). Heterocyclic amine content in commercial ready to eat meat products. Meat Science 88: 227-233.
9. van den Berg, R., Haenen, G. and Bast, A. (1999). Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chemistry 66(4): 511-517.
10. Zamora, R. and F. J. Hidalg (2015). 2-Amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine(PhIP) formation and fate: an example of the coordinate contribution of lipid oxidation and Maillard reaction to the production and elimination of processing-related food toxicants. Royal Society of Chemistry 5: 9709-9721.
11. Zamora, R. and F. J. Hidalgo (2007). Coordinate Contribution of Lipid Oxidation and Maillard Reaction to the Nonenzymatic Food Browning. Critical Reviews in Food Science and Nutrition 45(1): 49-59.
12. Zhu, W., Cromie, M.M., Cai, Q., Lv, T., Singh, K., et al. (2014). Curcumin and Vitamin E Protect against Adverse Effects of Benzo[a]pyrene in Lung Epithelial Cells. PLoS ONE. 9(3): e92992.