

## Oxidative stability of polyunsaturated fatty acids of n-3 designer eggs under different cooking methods

Maroufyan<sup>1</sup>, E., Fadil,<sup>2</sup> M., Bello<sup>3</sup>, A.U., Ebrahimi<sup>1</sup>, M., Goh<sup>1</sup>, Y.M., and Soleimani, A.F.<sup>3\*</sup>

<sup>1</sup>Department of Preclinical Veterinary Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. <sup>2</sup>International Medical School, Management and Science University, Shah Alam, Selangor, Malaysia, <sup>3</sup>Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

\*Corresponding author: abdoreza@upm.edu.my

Received: 9 September 2017. Accepted: 25 November 2017.

### Abstract

Variation in the extent of cooking time, temperature and heating source may greatly affect the polyunsaturated fatty acid (PUFA) double bond stability in eggs. A study was carried out to determine the oxidative stability of PUFA content of designer eggs subjected to different cooking methods. A total of 160 eggs of 4 commercial brands were obtained: A: conventional, B: DHA Gold<sup>TM</sup>, C: LTK<sup>TM</sup>, and D: Safegg<sup>TM</sup>, and equally and randomly assigned to 4 cooking methods: (i) no cooking, (ii) boiling, (iii) frying, and (iv) microwaving. The results showed that brand and cooking method significantly influenced the PUFA content in the eggs. B had the highest n-3 and n-6 PUFA contents, and the lowest n-6/n-3 PUFA ratio compared to brands A, B, and D. The brand B had the lowest malondialdehyde (MDA) concentration compared to other brands. All methods of cooking increased MDA content ( $P < 0.05$ ). The n-6/n-3 PUFA ratio was not affected by cooking method only in brands C and D ( $P > 0.05$ ). In conclusion, boiling appeared to be the most and microwaving the least suitable method of cooking for eggs, as measured by PUFA and MDA content.

**Keywords:** Cooking, designer egg, yolk, PUFA, MDA

### Introduction

It is now common to find designer eggs with enhanced levels of some beneficial nutrients such as n-3 polyunsaturated fatty acids (PUFA), selenium and vitamin E. The n-3 PUFA enhance antibody response, spatial learning and memory compared to n-6 PUFA (Hajjar *et al.*, 2012; Hintze *et al.*, 2016) and n-6/n-3 ratio is increasingly being used as an index in functional nutrition. However, farm to fork stability of PUFA and n-6/n-3 ratio are unclear and vary among products. Oxidative stability of PUFA content in egg is

known to b; Pereira *et al.*, 2011; Meynier *et al.*, 2014; Douny *et al.*, 2015). In general, the more the double bonds possessed by n-3 and n-6 the greater the heat instability and oxidation rate of fatty acids (Torres- Giner *et al.*, 2010).

Previous study by Van Elswyk *et al.* (1992) showed that short boiling and low heat scrambling had no significant effect on the fatty acid profiles of n-3 fatty acid enriched eggs. However, Murica *et al.* (1999) reported that cooking egg by long boiling, scrambling and microwaving methods negatively affected the fatty acid profile and

vitamin E content. More recently, Ren *et al.* (2013) showed that n-6/n-3 ratio only increased in eggs cooked by frying and not boiling. Though, Douny *et al.* (2015) observed no significant effect of hard-boiling and scrambling on PUFA and n-3 fatty acid content of egg. The egg industry, in response, produced eggs with higher oxidative stability by fortifying the eggs with vitamin E, selenium, algae sourced PUFA or pasteurization. Although egg fortification improved the oxidative stability of fresh eggs (Bourre and Galea, 2006; Mohiti-Asli *et al.*, 2008), it is unclear whether it protects the PUFA content of cooked egg, as the final consumed product. Therefore, the current study aimed to determine the oxidative stability and PUFA content in selected designer eggs before and after cooking with different methods.

## Materials and Methods

A total of 160 eggs of 4 different brands of eggs were obtained (A: conventional, B: DHA Gold™, C: LTK™, and D: Safegg™) and randomly assigned to 4 cooking methods: (i) raw or no cooking, (ii) boiling, (iii) frying, and (iv) microwaving. All the eggs were purchased from a local supermarket and had the same manufacturing date. DHA Gold™ eggs are fortified with marine algae and vitamin E, LTK™ eggs are fortified with fish and flaxseed oil and Safegg™ eggs are fortified with fish oil and in-shell pasteurized.

### *Cooking of eggs*

The egg cooking method was conducted following the report by Nimalaratne *et al.* (2011). Briefly, for boiling, single layer of eggs were boiled for 10 min in saucepan. For microwaving, eggs were gently cracked in a glass bowl and cooked for 90 sec in a household microwave oven (Panasonic NN-

ST671S, 1100W). A small hole was pierced in yolk membrane to avoid burst. For frying, eggs were fried in non-stick pan preheated to 205°C (Calphalon SK200TY non-stick frying pan) for 6 min (3 min each side). Raw egg yolks were manually separated from whites and wiped using a filter paper to remove adhered albumins. All the yolk samples were frozen immediately at -80°C upon collection until further analysis for fatty acid profile and oxidative status.

### *Fatty acid analysis*

The total fat was extracted from yolk samples and then analyzed using an Agilent 7890A gas chromatograph (Agilent Technologies, Wilmington, USA) as described previously by Maroufyan *et al.* (2012).

### *Malondialdehyde determination*

Malondialdehyde (MDA) was measured as a secondary product of lipid oxidation using thiobarbituric acid-reactive substances (TBARS) assay as described by Jahromi *et al.* (2017).

### *Statistical analysis*

Data were analyzed using the GLM procedure of SAS (SAS version 9.3, SAS Institute, Cary, NC). Data were subjected to 2-way ANOVA with egg brand and cooking method as the main effects and their interactions. Significant differences were separated using Duncan's multiple range tests. The results were expressed as mean ± SEM. Statistical significance was considered at  $P < 0.05$ .

## Results and Discussion

There was no significant brand × cooking method interaction for n-3 and n-6 PUFA

content (Table 1). However, significant interaction ( $P < 0.05$ ) was observed for n-6/n-3 PUFA ratio (Table 1). The results indicated that brands B, C and D had higher n-3 PUFA than brand A (control), while only brands B and D had lower n-6 than control. From functional nutrition point, our result showed that although all of the designer eggs were authentic in their claim of having higher n-3

content, the level of n-3 and n-6/n-3 varied significantly among them. Therefore, it would be greatly beneficial to the egg consumers to have the n-6/n-3 ratio in nutritional fact labels. The cooking method significantly ( $P < 0.01$ ) affected the n-3 PUFA content but not those of n-6 PUFA (Table 1).

Table 1. n-3 and n-6 polyunsaturated fatty acids and their ratio of yolks as affected by egg brand and cooking method (%)

<u>Egg brand</u>	n-3	n-6	n-6/n-3
A	2.12± 0.11 <sup>d</sup>	17.47± 0.36 <sup>a</sup>	8.82± 0.55 <sup>a</sup>
B	2.55± 0.11 <sup>c</sup>	15.62± 0.31 <sup>b</sup>	6.64± 0.35 <sup>b</sup>
C	6.84± 0.22 <sup>a</sup>	17.47± 0.39 <sup>a</sup>	2.63± 0.14 <sup>d</sup>
D	4.38± 0.15 <sup>b</sup>	15.54± 0.29 <sup>b</sup>	3.59± 0.10 <sup>c</sup>
<u>Cooking status</u>			
Raw	4.44± 0.41 <sup>a</sup>	16.64± 0.42	4.49± 0.43 <sup>b</sup>
Boiled	3.99± 0.35 <sup>b</sup>	15.98± 0.39	4.81± 0.43 <sup>b</sup>
Microwaved	3.65± 0.44 <sup>b</sup>	16.55± 0.37	6.31± 0.74 <sup>a</sup>
Fried	3.97± 0.45 <sup>b</sup>	16.89± 0.37	5.71± 0.67 <sup>a</sup>
<u>Effects</u>		<u>Probabilities</u>	
Egg brand	**	**	**
Cooking status	**	NS	**
Egg brand × Cooking status	NS	NS	**

A: conventional, B: DHA Gold<sup>TM</sup>, C: LTK<sup>TM</sup>, D: Safegg<sup>TM</sup>.

NS- Not significant, \*  $P < 0.05$ , \*\*  $P < 0.01$ .

<sup>a-d</sup> Means±SEM within a column-subgroup with no common letters differ at  $P < 0.05$ .

Interestingly, n-6/n-3 PUFA ratio was not influenced by all methods of cooking in brands C and D ( $P > 0.05$ ), but for brand B only boiling and frying ( $P > 0.05$ ) and for brand A only boiling ( $P > 0.05$ ) was not detrimental to n-6/n-3 PUFA ratio (Table 2). Regardless of the egg brand, boiling consistently appeared to have no influence on n-6/n-3 PUFA ratio. This was possibly due to lower core temperature of yolks in boiling compared to microwaving and frying. Douny *et al.* (2014) observed a negative relationship

between yolk core temperature and n-3 PUFA content. In their experiment the authors reported that boiling imposed the lowest core temperature to eggs among the different cooking methods. Moreover, the presence of albumen and shell surrounding the yolk might have prevented the interaction of oxygen and light with the lipids during boiling. Similar results were reported by Murcia *et al.* (1999), Cortinas *et al.* (2003), and Ren *et al.* (2013). However, a study by Botsoglou *et al.* (2012) indicated

similar adverse effect on n-6/n-3 PUFA ratio for boiling and scrambling, while Van Elswyk *et al.* (1992) showed no alteration of n-3 content by various cooking methods. The discrepancies between these reports might be attributed to variations in core temperature of yolk caused by differences in cooking protocols. The oxidation level of food is known to be affected by cooking process and length and storage condition (Lee *et al.*, 2006; Soladoyea *et al.*, 2017). It is noteworthy to mention that in the current study the adverse effects of cooking were not evident in egg brands C and D. The exact

reason for such observation is not clear and may be related to composition of feed used in the egg production process. Nevertheless, it is emphasized that designer eggs may vary with respect to farm to fork quality. Among the cooking methods of this study, microwaving appeared to be the most harmful method to n-6/n-3 PUFA content. Similar result was reported by Murcia *et al.* (1999), as one of the related studies in the field. The adverse effect of microwaving may be referred to involvement of high levels of energy in the process, resulting in high core temperature for yolk.

Table 2. n-6/n-3 polyunsaturated fatty acids ratio of the yolks as affected by the egg brand and cooking status

		Egg brand			
		A	B	C	D
Cooking status	Raw	7.27± 0.62 <sup>bx</sup>	5.29± 0.50 <sup>by</sup>	2.39± 0.90 <sup>z</sup>	3.46± 0.25 <sup>z</sup>
	Boiled	6.87± 0.53 <sup>bx</sup>	6.14± 0.71 <sup>bx</sup>	2.51± 0.12 <sup>y</sup>	3.75± 0.16 <sup>y</sup>
	Microwaved	10.52± 1.20 <sup>ax</sup>	8.23± 0.70 <sup>ay</sup>	3.06± 0.51 <sup>z</sup>	3.45± 0.20 <sup>z</sup>
	Fried	10.37± 0.96 <sup>ax</sup>	6.17± 0.39 <sup>by</sup>	2.59± 0.13 <sup>z</sup>	3.71± 0.21 <sup>z</sup>

<sup>a-c</sup> Means±SEM within a column with no common letters differ at  $P < 0.05$ .

<sup>x-z</sup> Means±SEM within a row with no common letters differ at  $P < 0.05$ .

A: conventional, B: DHA Gold<sup>TM</sup>, C: LTK<sup>TM</sup>, D: Safegg<sup>TM</sup>.

n=10

The MDA content of egg yolks as affected by the egg brand and cooking method are presented in Table 3. In general, all cooking methods in the current study resulted in elevation of MDA content ( $P < 0.05$ ). Lipid peroxidation and MDA production increased during heat exposure and thus reducing the nutritional quality of eggs (Nimalaratne *et al.*, 2016, Matumoto-Pintor *et al.*, 2017). Similarly, Ren *et al.* (2013) and Nimalaratne *et al.* (2016) reported elevation in MDA content of eggs following boiling and frying. On the other hand, comparison of different egg brands revealed that brand B was consistently less prone to

lipid peroxidation following boiling, microwaving and frying than the other egg brands. Moreover, brand B had the lowest MDA content among the designer eggs in fresh form ( $P < 0.05$ ). The superior oxidative stability of eggs of brand B might be attributed to its fortification with vitamin E. Dietary supplementation of vitamin E was reported to decrease MDA in serum and egg yolk (Cherian *et al.*, 1996a,b, Grune *et al.*, 2001, Sahin *et al.*, 2008). It is therefore clear that fortification of high PUFA designer eggs with exogenous antioxidants such as vitamin E protects the nutritional quality of eggs both before and after cooking.

Table 3. Malondialdehyde (MDA,  $\mu\text{M}$ ) content of yolks as affected by the egg brand and cooking status

		Egg brand			
		A	B	C	D
Cooking status	Raw	12.2 $\pm$ 0.7 <sup>by</sup>	11.1 $\pm$ 0.9 <sup>by</sup>	19.8 $\pm$ 1.4 <sup>bx</sup>	17.0 $\pm$ 1.2 <sup>bx</sup>
	Boiled	20.5 $\pm$ 2.4 <sup>axy</sup>	19.4 $\pm$ 2.0 <sup>ay</sup>	23.2 $\pm$ 2.0 <sup>abxy</sup>	26.0 $\pm$ 1.3 <sup>ax</sup>
	Microwaved	21.5 $\pm$ 1.5 <sup>ax</sup>	15.3 $\pm$ 0.7 <sup>ay</sup>	26.3 $\pm$ 2.2 <sup>ax</sup>	22.8 $\pm$ 1.4 <sup>ax</sup>
	Fried	23.0 $\pm$ 1.3 <sup>ax</sup>	15.5 $\pm$ 1.2 <sup>ay</sup>	26.1 $\pm$ 2.1 <sup>ax</sup>	24.7 $\pm$ 2.8 <sup>ax</sup>

<sup>a-c</sup> Means $\pm$ SEM within a column with no common letters differ at  $P < 0.05$ .

<sup>x-y</sup> Means $\pm$ SEM within a row with no common letters differ at  $P < 0.05$ .

A: conventional, B: DHA Gold<sup>TM</sup>, C: LTK<sup>TM</sup>, D: Safegg<sup>TM</sup>.

n=10

## Conclusion

The yolk MDA content increased regardless of cooking method, but n-6/n-3 PUFA ratio only increased by microwaving and frying in egg brands A and B. The n-6/n-3 PUFA ratio in egg brands C and D was not affected by cooking.

## Acknowledgements

The research was funded by the Management and Science University under Seed Grant scheme.

## References

- Botsoglou, E.A.; Govaris, A.; Pexara, and Fletouris, D. 2012. Effect of processing and storage on the fatty acid composition of n-3 or n-6 fatty acid-enriched eggs. *Int. J. Food Sci. Technol.* 47: 2388–2396.
- Bourre, J.M.; Galea, F. 2006. An important source of omega-3 fatty acids vitamins D and E carotenoids iodine and selenium: a new natural multi-enriched egg. *J. Nutr. Health Aging* 10(5): 371-6.
- Cherian, G., Wolfe, F.H. and Sim, J.S. 1996b. Dietary oils with added tocopherols: effects on egg or tissue tocopherols fatty acids and oxidative stability. *Poult. Sci.* 75(3):423-431.
- Cherian, G., Wolfe, F.H., and Sim, J.S. 1996a. Feeding dietary oils with tocopherols: effects on internal qualities of eggs during storage. *J. Food Sci.* 61(1): 15-18.
- Cortinas, L., Galobart, J., Barroeta, A.C., Baucells, M.D. and Grashorn, M.A. 2003. Change in  $\alpha$ -tocopherol contents lipid oxidation and fatty acid profile in eggs enriched with linolenic acid or very long-chain  $\omega$ 3 polyunsaturated fatty acids after different processing methods. *J. Sci. Food Agric.* 83: 820–829.
- Douny, C., Khoury, R.E., Delmelle, J., Brose, F., Degand, G., Moula, N., Farnir, F., Clinquart, A., Maghuin-Rogister, G. and Scippo, M.L. 2015. Effect of storage and cooking on the fatty acid profile of omega-3 enriched eggs and pork meat marketed in Belgium. *Food Sci. Nutr.* 3(2): 140–152.

- Grune, T., Krämer, K., Hoppe, P.P. and Siems, W. 2001. Enrichment of eggs with n-3 polyunsaturated fatty acids: Effects of vitamin E supplementation. *Lipids* 36(8): 833-838.
- Hajjar, T., Goh, Y.M.; Rajion, M.A., Vidyadaran, S., Othman, F., Soleimani, A.F., Li, T.A. and Ebrahimi, M. 2012. Omega 3 polyunsaturated fatty acid improves spatial learning and hippocampal peroxisome proliferator activated receptors (PPAR $\alpha$  and PPAR $\gamma$ ) gene expression in rats. *BMC Neurosci.* 13: 109.
- Hintze, K.J., Tawzer, J., and Ward, R.E. 2016. Concentration and ratio of essential fatty acids influences the inflammatory response in lipopolysaccharide challenged mice. *Prostaglandins Leukot. Essential Fatty Acids* 111: 37-44.
- Jahromi, M.F., Liang, J.B., Ebrahimi, R., Soleimani, A.F., Rezaeizadeh, A., Abdullah, N. and Shokryazdan, P. 2017. Protective potential of *Lactobacillus* species in lead toxicity model in broiler chickens. *Animal* 11(5): 755-761.
- Lee, S., Hernandez, P., Djordjevic, D., Faraji, H., Hollender, R., Faustman, C. and Decker, E.A. 2006. Effect of antioxidants and cooking on stability of n-3 fatty acids in fortified meat products. *J. Food Sci.* 71: 233-238.
- Maroufyan, E., Kasim, A., Ebrahimi, M.; Loh, T.C., Bejo, M.H., Zerihun, H.; Hosseini, F., Goh, Y.M. and Soleimani, A.F. 2012. Omega-3 polyunsaturated fatty acids enrichment alters performance and immune response in infectious bursal disease challenged broilers. *Lipids Health Dis.* 11: 15.
- Matumoto-Pintro, P.T., Murakami, A.E.; Vital, A.C., Croge, C., da Silva, D.F., Ospina-Roja, I.C. and Guerra, A.F. 2017. Effects of storage time and temperature on lipid oxidation of egg powders enriched with natural antioxidants. *Food Chem.* 228: 463-468.
- Meynier, A., Leborgne, C., Viau, M., Schuck, P., Guichardant, M., Rannou, C. and Anton, M. 2014. n-3 fatty acid enriched eggs and production of egg yolk powders: an increased risk of lipid oxidation? *Food Chem.* 153: 94-100.
- Mohiti-Asli, M., Shariatmadari, F., Lotfollahian, H. and Mazuji, M.T. 2008. Effects of supplementing layer hen diets with selenium and vitamin E on egg quality lipid oxidation and fatty acid composition during storage. *Can. J. Anim. Sci.* 88(3): 475-483.
- Murcia, M.A., Martinez-Tome, M., del Cerro, I., Sotillo, F. and Ramirez, A. 1999. Proximate composition and vitamin E levels in egg yolk: losses by cooking in a microwave oven. *Sci. Food Agric.* 79: 1550-1556.
- Nimalaratne, C.; Lopes-Lutz, D.; Schieber, A.; Wu, J. 2011. Free aromatic amino acids in egg yolk show antioxidant properties. *Food Chem.* 129(1): 155-161.
- Nimalaratne, C., Schieber, A. and Wu, J. 2016. Effects of storage and cooking on the antioxidant capacity of laying hen eggs. *Food Chem.* 194: 111-6.
- Pereira, A.L.F., Vidal, T.F., Abreu, V.K.G., Zapata, J.F.F. and Freitas, E.R. 2011. Brand of dietary lipids and storing time on egg stability. *Ciênc. Tecnol. Aliment.* 31(4): 984-991.
- Ren, Y., Perez, T.I., Zuidhof, M.J., Renema, R.A. and Wu, J. 2013. Oxidative stability of omega-3 polyunsaturated fatty acids enriched eggs. *J. Agric. Food Chem.* 61: 11595-11602.

- Sahin, N., Akdemir, F., Orhan, C., Kucuk, O., Hayirli, A. and Sahin, K. 2008. Lycopene-enriched quail egg as functional food for humans. *Food Res. Int.* 41(3): 295-300.
- Soladoyea, O.P., Shanda, P., Duganb, M.E.R., Gariépyc, C., Aalhusb, J.L., Estévezd, M. and Juárezb, M. 2017. Influence of cooking methods and storage time on lipid and protein oxidation and heterocyclic aromatic amines production in bacon. *Food Res. Int.* 99: 660-669.
- Torres-Giner, S., Martinez-Abad, A., Ocio, M.J. and Lagaron, J.M. 2010. Stabilization of a nutraceutical omega-3 fatty acid by encapsulation in ultrathin electrosprayed zein prolamine. *J. Food Sci.* 75(6): 69-79.
- Van Elswyk, M.E., Sams, A.R. and Hargis, P.S. 1992. Composition functionality and sensory evaluation of eggs from hens fed dietary menhaden oil. *J. Food Sci.* 57: 342-344.

